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A simple extractive technique for honey flavonoid HPLC analysis

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Summary — A simple technique for routine analysis of flavonoids from honey has been described utilising a combination of filtration through the resin Amberlite XAD-2 and extraction with ethyl ether. The proposed method is less complex than other methods for honey flavonoid analysis reported previously. The HPLC conditions for flavonoid analysis have also been improved. This technique was applied to the analysis of flavonoids in 27 honey samples from the La Alcarria region (Spain). The total flavonoid content of the different samples ranged between 5 and 20 µg flavonoid/g honey. The major flavonoids in these samples were the flavanones pinocembrin and pinobanksin and the flavone chrysin. A total of 18 different flavonoids were detected in the honey samples analysed.

honey / flavonoid / botanical origin / geographical origin / HPLC

INTRODUCTION

The occurrence of flavonoids in honey has been reported a number of times over the last 10 yr (Bogdanov, 1984; Amiot *et al,* 1989; Ferreres *et al,* 1991; Sabatier *et al,* 1992). Flavonoid analysis is a very promising technique in studies of the botanical (Amiot *et al,* 1989; Ferreres *et al,* 1992) and geographical (Ferreres *et al,* 1991, 1992; Tomás-Barberán *et al,* 1993a) origins of honey. It is well established that HPLC is a method of choice for analysis of flavonoids. The main problem in the analysis of flavonoids from honey is the latter's very high sugar content, which renders difficult the extraction of these metabolites and sample preparation for HPLC analysis. Liquid–liquid partitions produce inconvenient interphases which do not permit the complete recovery of flavonoids. This problem has recently been solved by using the non-ionic polymeric resin Amberlite XAD-2 (Ferreres *et al,* 1991; Tomás-Barberán *et al,* 1992). In a previous paper we reported the identification of 16 flavo-

noids in honey *via* HPLC analysis of samples prepared with a combination of Amberlite XAD-2 and Sephadex LH-20 column chromatography (Ferreres *et al*, 1991). However, the limitations of this technique were that it was rather complex, especially as regards the Sephadex LH-20 chromatography, and unsuitable for routine analyses in quality control determinations. In addition, it did not allow the quantification of flavonoids, since the recovery of flavonoids from the Sephadex LH-20 column was not accurate owing to the fact that the separation of the flavonoid fraction from the previous eluting phenolic derivatives was not clear-cut. Thus, the aim of the present work was to improve upon this analytical technique, to avoid utilising the expensive Sephadex LH-20 chromatography, and to apply this modified technique to the qualitative and quantitative analysis of flavonoids in La Alcarria honey.

MATERIALS AND METHODS

Honey samples

The different honey samples used in this study came from the La Alcarria region (Cuenca and Guadalajara provinces, Spain) and were directly provided by the bee-keepers. The honey samples had not been industrially processed. To minimize any alterations, the samples were stored at –20°C in the dark. In addition 1 kg commercial honey from La Alcarria was used in the modified technique.

Flavonoid extraction from honey

Two hundred g commercial honey from La Alcarria were thoroughly mixed with 5 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 various phenolic compounds remained in the column while sugars and other polar compounds were eluted with the aqueous solvent (Ferreres *et al*, 1991). The column was washed with acid water (100 ml) and subsequently with distilled water (≈ 300 ml). The whole phenolic fraction was then eluted with methanol (≈ 300 ml until no more colour was eluted) and concentrated under reduced pressure (40°C). Although the main proportion of honey sugars had been removed by filtration through the Amberlite column, some sugars still contaminated the phenolic fraction. This phenolic compounds fraction was analysed *via* HPLC and divided into 4 aliquots. The first aliquot was treated by filtration through Sephadex LH-20 column as reported previously (Ferreres *et al*, 1991). The second aliquot of the eluate from the Amberlite column was redissolved in 10 ml distilled water and filtered through a Maxi-Clean RP-C-18 (900 mg) cartridge to retain the phenolics. The cartridge was washed with 50 ml distilled water and then with 50 ml of the following solutions: 20% methanol, 30% methanol, 40%, methanol, 50% methanol 60% methanol and 80% methanol. The phenolics eluting with the different methanol–water mixtures were analysed *via* HPLC. The third aliquot was redissolved in 4 N NaOH and left overnight in a stoppered test tube under a nitrogen atmosphere to complete saponification. This was then taken to pH 2 with HCl and extracted with ethyl ether (5 ml x 3). The ether extracts were combined, concentrated under reduced pressure and redissolved in 0.5 ml methanol for HPLC analysis. The last aliquot was taken to dryness under reduced pressure and the residue redissolved in 5 ml distilled water. This water extract was partitioned with ethyl ether (5 ml x 3), the ether extracts combined and the ether removed under reduced pressure. The residue was dissolved in 0.5 ml methanol and analysed *via* HPLC.

HPLC analysis of honey flavonoids

This was carried out on a reversed-phase column LiCrochart RP-18 (Merck, Darmstadt) (12.5 x 0.4 cm, 5 μm particle size), using water–formic acid (19:1) (solvent A) and methanol (solvent B) as solvents. Elution was performed at a solvent flow rate of 1 ml/min, starting with

30% methanol which remained isocratic until 15 min, then installing a gradient to obtain 40% methanol at 20 min, 45% methanol at 30 min, 60% methanol at 50 min, and 80% methanol at 52 min, and which then become isocratic until 60 min. Detection was performed with a diode-array detector, and chromatograms were recorded at 340 and 290 nm. The retention times for the different flavonoids identified are shown in table I.

Flavonoid identification and quantification

The different flavonoids were identified by chromatographic comparisons with authentic markers (commercial or previously isolated and identified from honey) (Ferreres *et al,* 1991, 1992) and by their UV spectra. Flavonoids were quantified by absorbance of their corresponding peaks in the chromatograms, the flavanones as pinocembrin detected at 290 nm, the flavones with an unsubstituted B ring (chrysin, galangin and tectochrysin) as chrysin detected at 340 nm and the rest of flavonols and flavones as quercetin detected at 340 nm.

Analysis of flavonoids from La Alcarria honey samples

The different honey samples (50 g each) were filtered through an Amberlite XAD-2 column. The methanolic eluate was then concentrated and extracted with ethyl ether 3 times as described above. The 3 ether extracts were combined, concentrated under reduced pressure and redissolved in 0.5 ml methanol for HPLC analysis.

Table I. Flavonoids from La Alcarria honey.

Compound name	*Structure*	*tR*	*No*
Pinobanksin	3,5,7-Trihydroxyflavanone	15.7	**A**
Quercetin	3,5,7,3',4'-Pentahydroxyflavone	19.7	**B**
Luteolin	5,7,3',4'-Tetrahydroxyflavone	23.6	**C**
Quercetin 3-methyl ether	5,7,3',4'-Tetrahydroxy-3-methoxyflavone	23.6	**C**
8-Methoxykaempferol	3,5,7,4'-Tetrahydroxy-8-methoxyflavone	26.0	**D**
Kaempferol	3,5,7,4'-Tetrahydroxyflavone	27.0	**E**
Apigenin	5,7,4'-Trihydroxyflavone	28.9	**F**
Kaempferol 3-methyl ether	5,7,4'-Trihydroxy-3-methoxyflavone	28.9	**F**
Isorhamnetin	3,5,7,4'-Tetrahydroxy-3'-methoxyflavone	29.7	**G**
Quercetin 3,3'-dimethyl ether	5,7,4'-Trihydroxy-3,3'-dimethoxyflavone	31.5	**H**
Pinocembrin	5,7-Dihydroxyflavanone	33.0	**I**
Quercetin 7,3'-dimethyl ether	3,5,4'-Trihydroxy-7,3'-dimethoxyflavone	34.5	**J**
Quercetin 3,7-dimethyl ether	5,3',4'-Trihydroxy-3,7-dimethoxyflavone	37.7	**K**
Luteolin 7-methyl ether	5,3',4'-Trihydoxy-7-methoxyflavone	38.9	**L**
Chrysin	5,7-Dihydroxyflavone	40.7	**M**
Galangin	3,5,7-Trihydroxyflavone	42.6	**N**
Genkwanin	5,4'-Dihydroxy-7-methoxyflavone	46.5	**O**
Tectochrysin	5-Hydroxy-7-methoxyflavone	56.2	**P**

tR are HPLC retention times in min.

RESULTS AND DISCUSSION

Improvement of the analytical technique

The first step in the extraction of flavonoids from honey is the sorption of the phenolic compounds on a non-ionic polymeric resin Amberlite XAD-2. This was carried out by filtration of a solution of honey in acid water through a column containing the resin, and washing the sugars and other polar compounds with water. The retained phenolics were then eluted with methanol. The recovery of flavonoids in distilled water solutions (pH 5.5) is ≈ 75% (Tomás-Barberán *et al,* 1992), whereas in acid solutions (pH 2) the flavonoid aglycone recovery is > 95% (García-Viguera, 1991). For this reason, the use of acid water (pH 2 with HCl) to dilute honey before passing through the Amberlite XAD-2 column is highly recommended. The HPLC chromatogram of the honey phenolic fraction obtained with the Amberlite XAD-2 column (fig 1) shows that some of the flavonoids previously reported from honey are detected, but that there are also some other non-flavonoid phenolic compounds which contaminate the flavonoids peaks. The overlapping of peaks is clearly observed with a diode-array detector. In addition, there is another problem which makes this extract unsuitable for direct analysis of flavonoids: in spite of the large amount of sugars eliminated in the Amberlite XAD-2 step, some sugars still contaminate the phenolic fraction which is eluted with methanol. When this extract is concentrated to be redissolved in methanol for HPLC analysis, the sugars present give the extract a syrupy texture, rendering difficult its injection in the HPLC. For these reasons additional treatment of the sample is necessary.

A previously described technique (Ferreres *et al,* 1991) was used for this purpose, *ie* a second chromatography on Sephadex LH-20 column with methanol. The purification of the flavonoid fraction by this technique has already been described in detail (Ferreres *et al,* 1991) and deserves no further comment apart from a brief mention of some of its disadvantages. First, it is a time-consuming technique, which is experimentally complex and which requires a UV lamp to follow the chromatography development in a dark room; this step is therefore unsuitable for routine analyses. Secondly, the separation of the flavonoid fraction from the previous eluting phenolic acid derivatives and brown polymers is not clear-cut, and in most cases part of the flavanones pinobanksin and pinocembrin which elute very close to the phenolic acid fraction are discarded; thus the amount of flavanones detected in the chromatograms may not accurately represent the real flavonoid composition of honey. This prevents an accurate quantification of these flavanones from being made. Thirdly, but no less important, is the fact that Sephadex LH-20 is rather expensive, although it can be used for a large number of assays. The chromatogram obtained for the flavonoid fraction of the same honey purified by Sephadex LH-20 chromatography is shown in figure 1. However, there are some advantages to using the Sephadex LH-20 step, since a fraction containing flavonoids only is prepared for analysis, and the chromatograms obtained are clean and suitable for the identification of flavonoid markers which indicate the botanical origin. This step seems to be especially useful in flavonoid analysis of honey samples that are rich in phenolic acid derivatives and brown polymers.

Some attempts were than made the avoid to use of Sephadex LH-20 and to obtain a suitable chromatogram of honey flavonoids.

First, filtration was attempted through a reversed-phase cartridge to retain flavonoids and elute the remaining contaminant sugars which were not eliminated *via* the Amberlite XAD-2 step. In addition, this was used to obtain selective elution of the flavonoid fraction, cleaning the sample of polymeric brown phenolics and other phenolic acid derivatives which appear in the first part of the HPLC chromatogram. The phenolic fraction was passed through a C-18 cartridge and washed with distilled water. The flavonoids were then successively eluted with different methanol–water solutions *ie*: 20% MeOH (only pinobanksin eluted), 30% methanol (pinobanksin and

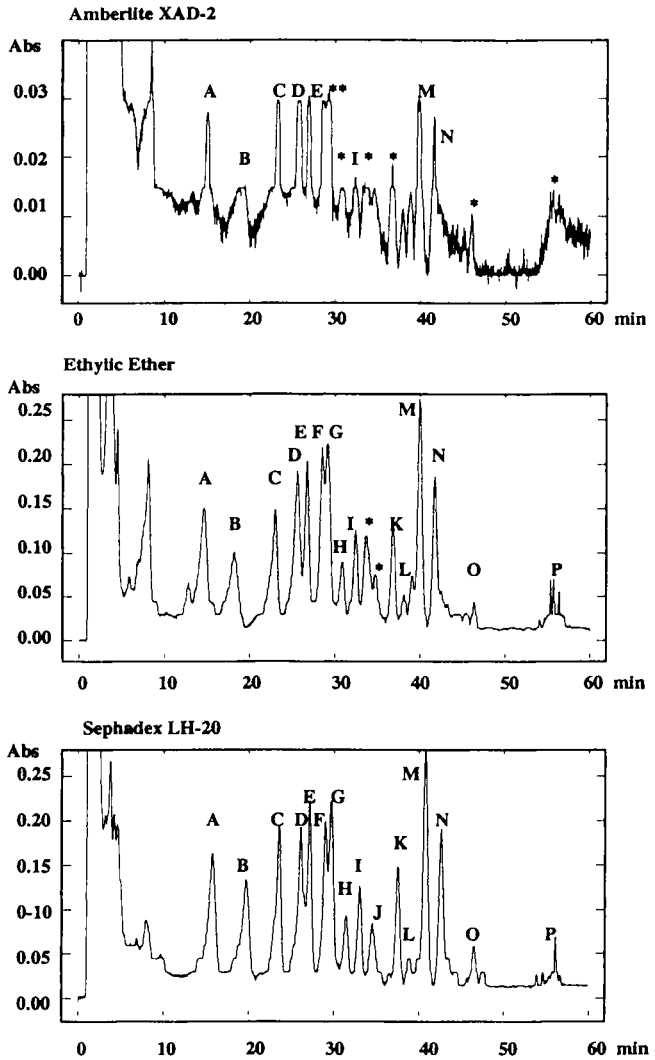

Fig 1. HPLC chromatograms of honey flavonoids recorded at 340 nm. For flavonoid identification see table I; (*) Non-flavonoid phenolics.

quercetin), 40% methanol (quercetin, 8-methoxykaempferol, kaempferol, pinocembrin and chrysin), 50% methanol (quercetin, 8-methoxykaempferol, kaempferol, chrysin and galangin), 60% methanol (quercetin, 8-methoxykaempferol, kaempferol, chrysin and galangin) and 80% methanol (chrysin, galangin, genkwanin and tectochrysin). The results indicated that in order to purify the whole flavonoid fraction, the cartridge should be washed with 50 ml water and 50 ml 15% methanol to remove sugars and phenolic acid derivatives, after which the flavonoids should be eluted with 80% methanol (50 ml). This flavonoid fraction was then concentrated, redissolved in methanol and analysed *via* HPLC. The contaminant sugars had been removed, but the chromatogram obtained still showed some of the lipophilic phenolic acid derivatives and brown polymers which, in some cases, eluted with the same retention times as the flavonoids, rendering this step unsuitable.

A second attempt was made by treating the phenolic fraction with an alkaline solution to hydrolyse the phenolic acid esters which were presumably present in honey as they are important constituents of propolis (Wollenweber *et al,* 1987). This was carried out as described in the *Materials and Methods,* and the ether extracts obtained after acidification of the saponified extract were analysed *via* HPLC but although the chromatograms appeared somewhat cleaner than those obtained for the Amberlite-eluting fraction, they did not show any significant improvement.

The third method attempted was the extraction of flavonoids from the Amberlite XAD-2 purified phenolic compounds fraction with ethyl ether, to preferentially extract the flavonoids and leave dark phenolic polymers and contaminant sugars in the aqueous layer. The partitioning took place 3 times to ensure recovery of the flavonoids, and the extracts were combined, the ether removed under reduced pressure and the residue dissolved in a minimum amount of the methanol to be analysed *via* HPLC. The ether preferentially extracted the flavonoids with a recovery of > 95% after the 3 extractions, and left sugars and other polar compounds in the water layer. In addition, the more lipophilic compounds were eliminated when the dry residue was redissolved in water. This extraction technique was considered suitable, and the chromatogram obtained for honey flavonoids obtained by this method is shown in figure 1. This chromatogram shows no significant differences with that obtained after filtration through Sephadex LH-20, and is the best of the 3 alternatives assayed in this work to avoid the costly Sephadex LH-20 step.

Application of the simplified technique to flavonoid analysis in La Alcarria honey

The flavonoids from 27 honey samples from the La Alcarria region were analysed by the proposed technique in order to validate this procedure and assess its application to the routine flavonoid analysis of honey. Flavonoids were extracted from the samples in only 2 steps: firstly filtration of the honey through Amberlite XAD-2; and secondly, extraction of the flavonoids retained in the Amberlite column with ethyl ether. The flavonoid fractions of the different honey samples were dissolved in methanol (0.5 ml) and analysed *via* HPLC under the chromatographic conditions described in the *Materials and Methods.* The results obtained have been shown in table II. It is interesting that the amount of total flavonoids present in the honey samples analysed ranged between 5 and 20 μg of flavonoid per g honey. In previous studies on the flavonoids from French sunflower honeys, higher amounts (50–100

Table II. Flavonoid content of the different honey samples analysed.

Honey	*A*	*B*	*C*	*D*	*E*	*F*	*G*	*H*	*I*	*K*	*L*	*M*	*N*	*O*	*P*	*Total*
G-16	4.04	0.27	1.37	0.66	0.79	1.13	0.41	0.20	3.78	2.20	0.15	2.82	1.33	–	0.35	19.50
G-33	2.02	0.68	0.82	0.52	0.90	0.95	0.37	–	0.93	0.45	0.05	0.95	0.41	0.07	–	9.12
G-36	1.05	0.25	0.51	0.27	0.43	0.54	0.61	0.12	0.69	0.30	–	0.72	0.25	–	0.09	5.83
G-53	5.56	0.68	1.96	1.06	1.16	1.25	0.83	0.41	3.71	1.10	0.19	2.44	1.09	0.09	0.27	21.80
G-54	4.70	0.95	1.55	0.87	1.16	1.26	0.41	–	2.95	1.11	0.24	2.29	1.07	–	0.38	18.94
G-57	2.05	0.57	0.38	0.55	0.78	0.59	0.26	–	0.43	0.75	0.09	0.56	0.23	–	0.15	7.39
G-62	2.00	–	0.58	0.65	0.20	0.32	0.15	0.09	0.57	0.20	–	0.06	0.21	–	–	5.63
G-101	3.43	–	1.40	0.23	0.30	0.92	0.36	0.15	0.81	0.42	0.12	0.86	0.46	–	–	9.46
G-103	2.12	–	0.96	0.65	0.63	0.91	0.41	0.17	2.38	0.63	–	2.95	0.83	0.10	0.47	13.21
G-105	1.17	0.17	0.42	0.57	0.40	0.63	0.20	–	0.41	0.19	–	0.58	0.18	–	0.14	5.06
G-106	4.11	–	0.66	0.21	0.26	0.68	0.30	–	1.64	0.60	–	1.45	0.39	0.12	0.21	10.63
G-108	2.76	0.29	0.70	0.60	0.57	1.44	0.35	–	0.97	0.40	–	1.00	0.34	0.09	0.18	9.69
G-109	1.51	–	0.58	0.30	0.32	0.81	0.29	–	0.85	0.35	0.07	1.16	0.34	0.07	0.18	6.83
G-110	1.71	0.19	0.44	0.35	0.55	1.06	0.35	–	0.84	0.31	–	1.10	0.37	–	0.24	7.51
G-111	4.43	0.73	0.79	0.65	0.92	2.09	0.41	0.15	2.08	1.14	0.20	2.46	1.14	0.39	0.55	18.13
G-112	3.22	–	1.44	–	0.17	1.04	0.15	0.22	1.86	0.88	–	2.01	0.62	0.12	0.23	11.96
G-113	1.47	–	0.38	–	0.16	0.74	0.29	–	0.42	0.21	–	0.95	0.18	0.07	0.17	5.04
G-114	3.13	0.55	1.03	0.76	0.82	1.46	0.51	0.19	1.66	0.84	0.09	1.77	0.69	0.14	0.29	13.93
G-115	0.75	–	0.43	0.05	0.12	0.76	0.31	–	0.33	0.21	–	0.50	0.19	–	0.04	3.69
G-116	4.60	1.01	2.18	0.82	1.21	1.75	0.87	0.37	2.26	1.04	0.33	2.12	0.66	0.17	0.34	19.73
G-117	0.58	–	0.17	0.09	0.04	0.63	0.31	–	1.07	0.20	–	1.37	0.21	–	0.17	4.84
G-118	4.52	0.19	1.74	0.55	1.14	1.75	0.82	0.21	2.65	1.38	0.27	2.82	1.40	0.35	0.41	20.20
G-119	3.33	0.59	1.44	0.45	0.65	1.01	0.53	0.17	1.31	0.64	0.15	1.25	0.66	0.09	0.12	12.39
G-120	4.59	0.30	0.95	0.31	0.71	1.85	0.85	0.42	2.04	1.10	0.17	2.02	0.37	0.10	0.16	15.94
G-121	2.40	0.56	1.12	0.22	0.70	1.01	0.62	0.21	1.32	0.64	–	1.26	0.68	–	0.21	10.95
G-122	1.41	0.38	0.38	0.90	0.65	0.93	0.21	0.13	0.62	0.31	0.08	0.89	0.28	–	0.15	7.32
G-124	2.33	–	0.26	0.36	0.50	0.72	0.30	0.12	0.75	0.41	–	1.07	0.34	–	0.21	7.37

Values are µg flavonoid/g honey. For flavonoid identification see table I; (-) flavonoid not detected or not possible to quantify.

μg/g honey) were reported (Ribeiro-Campos *et al,* 1990). The main flavonoid components in the honey samples analysed here were the flavanones pinobanksin (**A**) and pinocembrin (**I**) and the flavone chrysin (**M**). Generally, those flavonoids from propolis were present in all the samples analysed (compounds **A**, **I**, **K**, **M** and **N**). However, tectochrysin (**P**), which is also known to be present in propolis, was only detected in some of the samples and in very small amounts. This is probably due to the fact that this is an extremely lipophilic compound which is mainly present in beeswax, and its presence in honey probably depends on the contamination of honey with beeswax (Tomás-Barberán *et al,* 1993b). The occurrence and amount of those flavonoids present mainly in nectar and/or pollen, such as quercetin (**B**), luteolin (C), 8-methoxykaempferol (**D**), kaempferol (**E**), apigenin (**F**) and isorhamnetin (**G**), are much more variable in these honey samples, as could be expected, since their occurrence in honey depends on the latter's botanical origin. It is interesting that quercetin (**B**), which was not detected in 10 of the samples analysed, was present in honeys G-54 and G-116 in amounts of ≈ 1 μg per g honey. Something similar occurred with 8-methoxykaempferol (**D**) which was absent in samples G-112 and G-113 and present in ≈ 1 μg/g honey in samples G-53, G-54 and G-122. This agrees with the different floral origin of these samples collected from the same geographical region. However, all the samples analysed contained apigenin (apigenin + kaempferol 3-methyl ether) in amounts ≥ 1 μg/g honey. On the contrary, quercetin 3,3'-dimethyl ether (**H**), luteolin 7-methyl ether (**L**) and genkwanin (**O**) were detected only in some of the samples, and in very small amounts. Quercetin 3,7-dimethyl ether (**J**), was only detected in trace amounts in some honey samples, but its quantification was not possible and is not included in table II.

CONCLUSION

The proposed simplified technique for honey flavonoid analysis avoids the use of filtration through Sephadex LH-20 by extraction of the flavonoids eluting from the Amberlite XAD-2 column with ether. This is a suitable technique for routine analysis of flavonoids from honey, as has been demonstrated by the successful analysis of a number of honey samples in the present study. The reproducibility of the analysis was ≈ ± 5%. The HPLC chromatographic conditions for the analysis of flavonoids from honey have been improved with respect to other previously reported chromatographic conditions (Ferreres *et al,* 1992), and allows a clear separation between quercetin and pinobanksin, which were eluted together in previous analyses. However, the separation of luteolin and quercetin 3-methyl ether, and apigenin and kaempferol 3-methyl ether, was not possible under these chromatographic conditions (table I).

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Résumé — Technique simple d'extraction pour analyser en HPLC les flavonoïdes du miel. L'analyse des flavonoïdes du miel présente un intérêt pour étudier l'origine géographique et botanique du produit. Nous décrivons ici une technique simple d'analyse des flavonoïdes. Les divers échantillons de miel (50 g chacun) ont été soigneusement mélangés avec 5 parties d'eau (à pH 2 avec HCl) et filtrés à travers une colonne Amberlite XAD-2 qui retient

les flavonoïdes et élimine les sucres. Les flavonoïdes ont été ensuite élués au méthanol et l'éluat a été concentré sous vide. La fraction sortante des flavonoïdes a été analysée par chromatographie liquide haute pression (HPLC), mais on s'est rendu compte qu'une purification plus poussée était nécessaire avant l'analyse en HPLC. Quatre méthodes différentes ont été testées (fig 1). La plus efficace est la méthode déjà décrite de la filtration à travers une colonne Sephadex LH-20, mais elle ne permet pas de quantifier les flavonoïdes, est assez compliquée et ne convient pas pour des analyses de routine. Les 3 autres techniques testées sont plus simples et la meilleure consiste à redissoudre, dans de l'eau additionnée d'éther éthylique, l'éluat obtenu à la sortie de la colonne Amberlite XAD-2. L'éther extrait préférentiellement les flavonoïdes avec un taux de recouvrement supérieur à 95%. Ni la filtration à travers des cartouches à phase inversée ni la saponification de l'extrait avant extraction n'ont donné de meilleurs résultats. Cette technique a été appliquée à l'analyse des flavonoïdes de 27 échantillons de miel de la région de La Alcarria (Espagne) et 18 flavonoïdes différents ont été identifiés (pinobaksine, quercétine, lutéoline, éther de quercétin 3-méthyle, 8-méthoxykaempférole, kaempférole, apigénine, o-méthyl 3 kaempférol, isorhamnétine, o-diméthyl 3,3' quercétine, pinocembrine, o-diméthyl 7,3' quercétine, o-diméthyl 3,7 quercétine, o-méthyl 7 lutéoline, chrysine, galangine, genkwanine et tectochrysine) (tableau I). La quantité totale de flavonoïdes varie entre 5 et 20 µg par g de miel (tableau II). Les flavones pinobanksine et pinocembrine et la flavone chrysine sont les constituants majoritaires des flavonoïdes. Dans l'ensemble, les échantillons avaient tous une composition semblable, avec seulement de petites différences dans les flavonoïdes qui proviennent du nectar et du pollen.

miel / flavonoïde / origine botanique / origine géographique / HPLC

Zusammenfassung — Eine einfache Extraktionsmethode für die HPLC-Analyse von Honig-Flavonoiden. Die Analyse von Flavonoiden im Honig ist für Untersuchungen der geographischen und botanischen Herkunft des Honigs von Interesse. In der gegenwärtigen Studie wird eine einfache Methode zur Analyse der Honig-Flavonoiden beschrieben. Die verschiedenen Honigproben (zu je 50 g) wurden mit fünf Teilen Wasser (bei pH 2 mit HCl) gründlich gemischt und durch eine Amberlite XAD-2-Säule gefiltert, um die Flavonoide zurückzuhalten und die Zucker zu entfernen; die Flavonoide wurden dann mit Methanol herausgelöst und das Eluat im Vakuum konzentriert. Die aus dem Amberlite XAD-2-Filter gewonnene Flavonoid-Fraktion wurde im HPLC analysiert; dabei zeigte sich, daß für die HPLC-Analyse eine weitere Reinigung der Flavonoid-Fraktion erforderlich war. Zu diesem Zweck wurden vier Methoden geprüft (Abb 1). Die wirkungsvollste Technik war die früher beschriebene Filtration durch eine Säule vom Typ Sephadex LH-20, aber bei dieser Methode war keine Quantifizierung der Flavonoide möglich, sie war technisch kompliziert und für Routineuntersuchungen ungeeignet. Die anderen drei geprüften Techniken waren einfacher; als beste Methode erwies sich die neuerliche Lösung des Eluates aus der Amberlite XAD-2-Säule in Wasser mit Äthyläther. Äther extrahierte vor allem Flavonoide, mit einer Rückgewinnungsrate von über 95%. Versuche mittels Filtration durch Patronen in Reverse-Phase oder Saponifizierung des Extraktes vor der Extraktion brachten keine besseren Resultate.

Diese Technik wurde bei der Flavonoid-Analyse von 27 Honigen aus der Region 'La Alcarria' (Spanien) angewendet und es

konnten 18 verschiedene Flavonoide bestimmt werden (Pinobanksin, Quercetin, Luteolin, Quercetin-3-Methyl-Äther, 8-Methoxykaempferol, Kaempferol, Apigenin, Kaempferol 3-Methyl-Äther, Isorhamnetin, Quercetin 3,3'-Dimethyl-Äther, Pinocembrin, Quercetin 7,3'-Dimethyl-Äther, Quercetin 3,7-Dimethyl-Äther, Luteolin 7-Methyl-Äther, Chrysin, Galangin, Genkwanin und Tectochrysin) (Tabelle I). Die Gesamtmenge der Flavonoide in diesen Honigproben schwankte zwischen 5 und 20 µg per Gramm Honig (Tabelle II). Die Flavanone Pinobanksin und Pinocembrin und die Flavone Chrysin waren die Hauptanteile unter den gefundenen Flavonoiden. Im allgemeinen enthielten alle Proben ein ähnliches Flavonoidmuster mit nur geringen Unterschieden bei den Flavonoiden, die aus dem Nektar und Pollen stammen.

Honig-Flavonoide / botanische Herkunft / geographische Herkunft / HPLC

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