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Original article

Methyl anthranilate in *Citrus* honey. Analytical method and suitability as a chemical marker*

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Abstract – The present work describes a method for the determination of methyl anthranilate (MA) in honey, and reports the results of a study carried out on 46 *Citrus* honey samples produced in different countries. The MA content was measured, and the compliance of the samples with the unifloral *Citrus* honey profile was verified, according to the traditional authenticity parameters (physicochemical, sensory and microscopic). The analytical results show that MA values do not significantly differ in unifloral and not unifloral samples. More generally, no relationship could be found between MA content and the level of uniflorality or any single authenticity parameter. The conclusion is that MA content can not be used as a discriminating parameter for *Citrus* honey, and should be used only as a further descriptive element. Among the unifloral samples the MA content was lower in those produced in Italy than in the other countries, and mostly below the 2 mg/kg limit that some European laboratories require to accept *Citrus* honey.

methyl anthranilate / *Citrus* honey / unifloral / authenticity

1. INTRODUCTION

Unifloral *Citrus* honey is a valuable honey variety, particularly appreciated by the consumer for its distinctive, agreeable flavour. It is obtained from cultivated *Citrus* species (botanical family of Rutaceae), mostly orange but also, to a lesser extent, lemon, tangerines, grapefruit, lime, etc. In Europe *Citrus* honey is produced in Mediterranean countries, above all Spain, Italy and Greece, but it is also imported from the largest producers outside Europe, mainly Mexico.

The description of unifloral *Citrus* honey, resulting from the analysis of "traditional" physicochemical, sensory and melissopalynological parameters, was provided by several authors (Peris, 1981; Crane et al., 1984; Serra Bonvehí et al., 1987; Persano Oddo et al., 1995; Serra Bonvehí and Ventura Coll, 1995; Cabrera Ruiz et al., 1997; Mateo and Bosch-Reig, 1998), and recently a collaborative study of the International Honey Commission (Persano Oddo and Piro, 2004) supplied a description of this honey type.

Besides the traditional parameters, *Citrus* honey is characterized by the presence of a volatile compound, methyl anthranilate (MA) (Nelson, 1930; Lothrop, 1932; Deshusses and Gabbai, 1962; White, 1966; Serra Bonvehí, 1988; Ferreres et al., 1994; Serra Bonvehí and Ventura Coll, 1995; White and Bryant, 1996; Nozal et al., 2001), which was proposed as a chemical marker for the evaluation of this honey type. Serra-Bonvehí (1988) proposed a minimum MA content of 0.50 mg/kg, and later the same author reported a MA content of 1.50 mg/kg as characteristic of marketable Spanish *Citrus* honey (Serra Bonvehí and Ventura Coll, 1995).

In this work an HPLC-PDA method for MA determination in honey is presented, and some analytical aspects related to sample

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preparation are examined that were not reported in previous literature. The paper then describes the results of a study on several *Citrus* honey samples to compare the MA content with the traditional authenticity parameters.

2. MATERIALS AND METHODS

2.1. Samples

The study was carried out on 46 *Citrus* honey samples produced in different countries: 36 samples were harvested in 2006 in Italy, and were provided directly by producers; the other 10 samples were imported from other countries and were obtained thanks to the collaboration of France Miel (Mouchard, France), Quality Services International (Bremen, Germany) and CONAPI (Monterenzio, Italy). All the samples were analysed for their MA content and for their compliance with the unifloral *Citrus* honey profile, according to the traditional physicochemical, sensory and melissopalynological parameters.

2.2. Analytical method for methyl anthranilate determination

MA content in honey has been determined via different methods: UV spectrophotometry (White, 1966; Serra Bonvehí, 1988), gas chromatography (Serra Bonvehí, 1988; Serra Bonvehí and Ventura Coll, 1995), HPLC-UV (Nozal et al., 2001), and HPLC-FLD (Beckh, unpubl. data). In the present work a HPLC-UV method, based on Nozal et al. (2001), was used and simplified to be specific for just MA. The method is based on acid extraction, purification on copolymeric cartridge and analysis with an HPLC-PDA system.

2.2.1. Method description

Equipment. The HPLC system employed was manufactured by Varian (model 230 pump, model 330 photodiode array detector, model 400 autosampler). The column was a Varian model Lichrosphere 5RP18 (dimensions 250 × 4.6 mm, 5 μm particle size) and the temperature was kept constant at 30 °C.

Sample preparation. A 5 g aliquot of homogenized honey is weighed (precision 0.01 g) in a disposable polypropylene stoppered centrifuge tube, and 20 mL of 1 M sulphuric acid in distilled water are added. The sample is dissolved by vortexing, mechanically shaken for 20 min and centrifuged (15 min, 4000 rpm); the supernatant is collected.

Table I. Binary gradient elution employed for HPLC-PDA determination of MA in honey extracts.

Time (min)	% Water (1% ACN)	% ACN
0	70	30
10	70	30
15	42	58
18	42	58
18.1	10	90
24	10	90
24.1	70	30
31	70	30

Solid Phase Extraction. The sorbent (Waters Oasis HLB cartridge, 200 mg/6 mL) is conditioned with 2 × 5 mL acetonitrile (ACN) and 3×5 mL 1 M H_2SO_4, the sample extract is loaded at 2 mL/min using a 25 mL sample reservoir fitted above the cartridge, the sorbent is washed with 2 × 2.5 mL distilled water and vacuum dried, MA is eluted with 2 + 3 mL ACN at 1 mL/min. The sample eluate is accurately brought to 5 mL in a volumetric flask and mixed. A 1.5 mL aliquot of the sample eluate is filtered in a syringe through 0.45 μm pores and transferred to a 2 mL autosampler vial.

HPLC-PDA analysis. A 1 mL/min elution was employed (water and acetonitrile, Tab. I). A 20 μL sample volume was injected; the detection wavelength was 218 nm, and the total analysis time was 31 min, considering also the washing and the re-equilibration steps. The calibration employed a linear fit on 3 points, excluding the origin, with an equal weight for every point (0.1, 1, 5 μg/mL of MA, purity > 99%, purchased from Aldrich). The estimated LOQ was 0.05 mg/kg.

The first part of the chromatographic program is an isocratic elution that removes the more polar compounds to reduce interferences in the subsequent part of the chromatogram where MA is to be eluted. The last part of the program removes the most retained compounds with organic solvent and re-equilibrates the column.

Under the specified chromatographic conditions the MA peak was eluted at a retention time of about 17 min (Fig. 1). The UV absorbance spectrum of MA, recorded under the described chromatographic conditions, presented a neat, distinctive peak at 218 nm and another less intense absorbance

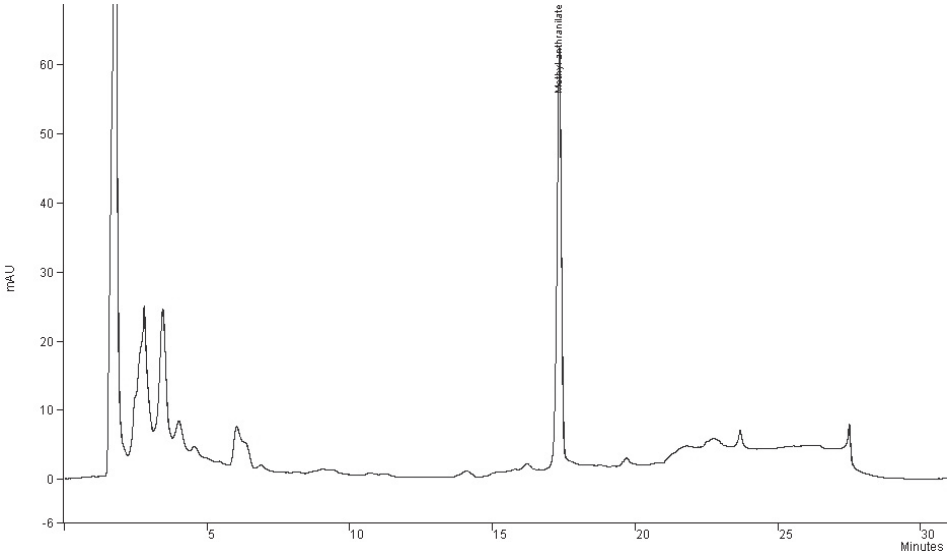

Figure 1. Chromatogram of a honey sample extract acquired at 218 nm.

maximum at 334 nm (Fig. 2). The second wavelength was more selective than 218 nm, as the chromatogram presented less spectral interferences in the near UV. However the sensitivity results were excessively reduced, thus 218 nm was chosen as the optimum wavelength for detection of MA.

2.2.2. Precision and recovery

To assess the repeatability of the entire analytical procedure, four replicate determinations were performed on each of two *Citrus* honey samples at two different MA concentrations. To verify the accuracy of the method, since certified reference materials for MA in honey are unavailable, a MA free sample (Acacia honey) was spiked at three different levels, and three replicate determinations were performed for each level.

2.2.3. Extraction issues

It is important to consider some extraction issues for MA determination in honey.

A simple dissolution in distilled water does not provide an efficient extraction of MA from *Citrus* samples and an effective acid hydrolysis proved to be necessary. In fact the unacidified extracts produced a UV chromatogram (Appendix, Fig. 1) characterized by a big unidentified peak that was eluted about two minutes before MA while a peak much smaller than expected was shown at the MA retention time, often representing less than 0.5 mg/Kg. Dissolving the samples in 1M sulphuric acid produced a decrease in the size of this unidentified peak and a parallel increase in the size of the MA peak.

To explain this observation a liquid chromatographic–tandem mass spectrometric (LC/MS/MS) experiment was set up. A Waters Alliance 2695 HPLC system coupled to a Micromass Quattro Micro triple quadrupole mass spectrometer was used. The optimal ionization conditions for detecting MA were found (capillary 3.5 KV, cone 15 V): the ionization mode that provided the best response for MA was positive electrospray; the quasi molecular ion ([M+H]+, m/z = 152) was chosen as parent ion for three transitions; and the anthranilate fragment ion ([M+H-CH_3OH]+, m/z = 120) was chosen as the parent ion for two additional transitions. The scan spectrum recorded at a 15 V cone voltage is reported in Appendix Figure 2. Five transitions were then selected for MA determination and a multiple reaction monitoring (MRM) method was set up (Appendix Table). A very simple chromatographic method was used for determining MA in unacidified honey samples. The column employed was a Waters X-Terra MS C18 15 cm × 2.1 mm with 5 μm particle size. The flow was kept constant at 250 μL/min, and the column temperature was kept constant at 30 °C.

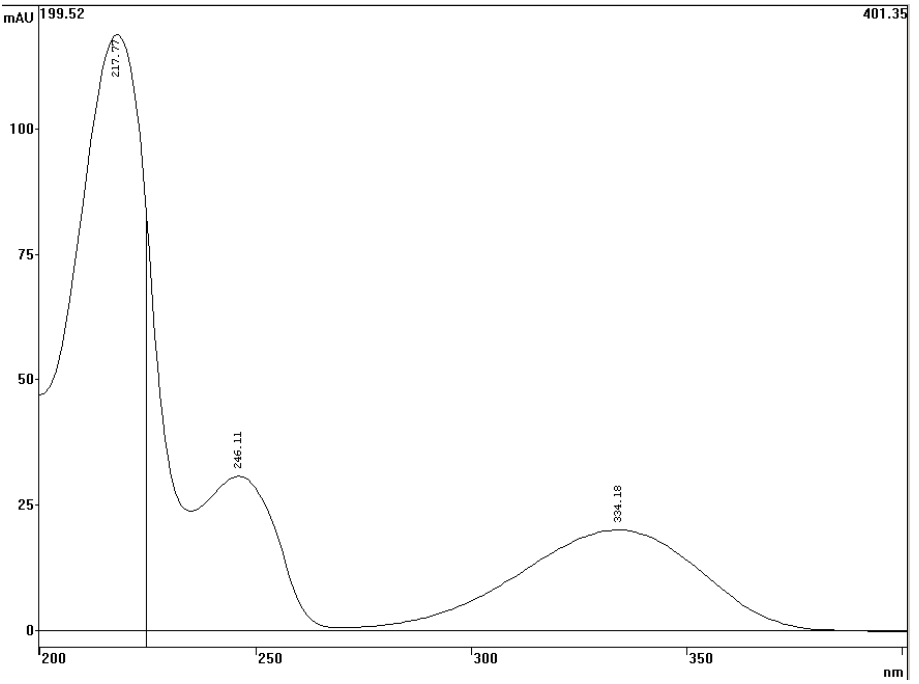

Figure 2. UV spectrum of MA acquired under chromatographic conditions via the photodiode array detector.

The elution employed a binary gradient (water and acetonitrile).

The chromatograms of a MA standard solution (the five selected MRM signals) are shown in Appendix Figure 3. Although it was eluted rather early, MA showed only one peak for each of the five transitions monitored.

The total ion chromatograms resulting from the LC/MS/MS analysis of a MA standard solution and of an unacidified *Citrus* honey solution are displayed for comparison in Appendix Figure 4. The *Citrus* honey extracts obtained without any acid treatment showed more than one peak. Examining the five MRM signals separately (Appendix Fig. 5) it was clear that one of the peaks was the free MA with its expected retention time and ion ratios; another bigger peak (indicated as "A" in Appendix Figs. 4 and 5) eluted earlier than the MA peak but presented only the two transition signals due to the anthranilate fragment ion (m/z = 120) and not due to the entire MA molecule; a third, smaller peak (indicated as "B" in Appendix Figs. 4 and 5) was detected next to, and not completely resolved from, the compound "A" peak. This compound "B" peak presented all the five transitions of MA but was eluted much earlier and was smaller in size. The observations indicated that compound "A" must be an anthranilate ester other than a methyl ester and compound "B" had to be some other form of bound MA.

The hydrolysis proved to be effective in freeing the bound MA and obtaining reproducible results. It is important to stress that added MA, as in spiked samples, remained free and was then detected in the expected amount: thus recovery tests cannot evidence the phenomenon of MA binding and the need for an acid treatment.

The hydrolysis step precluded the use as surrogate standard of a molecule, methyl n-phenyl-n-trifluoroacetyl anthranilate, that was tested with this aim. The molecule was in fact completely hydrolyzed and was not recovered at all. However, given the repeatability and recovery data, the method was considered reliable enough so that the use of a surrogate standard is not required.

Because of the acidity of the sample extract, a copolymeric sorbent was employed for the SPE step. The 5 mL acetonitrile eluate from the Oasis HLB cartridges, representative of a 5 g sample, enables a complete MA recovery and an

adequate sensitivity without the need of further extract concentration.

2.3. Analysis of traditional parameters

The compliance with the unifloral *Citrus* honey profile, according to the traditional authenticity parameters, as reported by the most relevant international literature, was verified through the analysis of the more discriminating parameters: sensory properties, microscopic characteristics, colour and electrical conductivity.

2.3.1. Sensory analysis

The sensory evaluation was performed by a panel of 3 expert assessors; samples were presented anonymously in a little beaker, red coloured in order to mask the honey colour (that may influence the judgement); each assessor worked individually and was asked to evaluate olfactory and gustatory correspondence to the unifloral reference, giving separated scores on a 3-level scale (3 = completely correspondent; 2 = acceptable; 1 = not correspondent), and to note possible defects. The criterion for accepting a sample as unifloral, was an average score ≥ 2.

2.3.2. Microscopic analysis

Qualitative and quantitative melissopalynological analyses were performed according to von der Ohe et al. (2004). The percentage of *Citrus* pollen was calculated excluding the nectarless species and the over-represented pollens, if present. The criteria used for accepting a sample as unifloral, were as follows:

- Percentage of *Citrus* pollen: >10% for Italian honeys (according to Persano Oddo et al., 1995) and >15% for honeys from other countries (according to Serra Bonvehí et al., 1987); the absence of important quantities of other nectariferous species was also considered.
- Total amount of pollen grains (PG/10 g): $< 20 \cdot 10^3$ for Italian honey (Persano Oddo et al., 1995); for the other samples this criterion was not applied, since most of them showed very high values, probably due to the beekeeping techniques (hives with no separation between honey and nest combs).

Table II. Repeatability tests on two *Citrus* honey samples (results are in mg/kg).

	Sample 1	Sample 2
replicate 1	1.897	2.359
replicate 2	1.893	2.397
replicate 3	1.897	2.417
replicate 4	1.916	2.412
average	1.901	2.396
RSD%	0.53%	1.09%

2.3.3. Physicochemical analyses

Colour was determined according to Aubert and Gonnet (1983): the acceptability criterion was $\leqslant 28$ mm Pfund (Persano Oddo and Piro, 2004). Electrical conductivity was measured according to Bogdanov et al. (1997a), and the acceptability criterion was $\leqslant 0.31$ mS/cm (Persano Oddo and Piro, 2004). The HMF content was also measured (Bogdanov et al., 1997b) to ascertain the freshness of the samples, since MA content is reported to decrease with honey aging (White, 1966; Serra Bonvehí and Ventura Coll, 1995; White and Bryant, 1996).

3. RESULTS

The results of the trials for repeatability and accuracy of the method are reported in Tables II and III. Both tests gave very good results.

The analytical results are reported in Table IV, where the 46 samples are grouped according to their compliance with the unifloral *Citrus* honey profile and ranked in order of decreasing MA content. The compliance with the unifloral *Citrus* honey profile, according to the traditional parameters, was synthesized as a global "yes/no" score, assigned on the basis of the analytical results. A "yes" score was attributed to 28 honeys, entirely fulfilling the acceptability criteria described in Section 2.3, and considered completely correspondent to the unifloral *Citrus* honey type. The other 18 samples, presenting one or more values beyond the acceptability limits, were given a "no" score (even if some of these samples had only one irregular value, and possibly could still be marketable as *Citrus* honey).

Table III. Recovery tests for MA determination.

		Determined conc. (mg/kg)	Recovery (%)	Average recovery	Recovery st. dev.
level 1 (expected conc. 0.575 mg/kg)	replicate 1	0.588	102.3	99.0%	3.3%
	replicate 2	0.570	99.1		
	replicate 3	0.550	95.6		
level 2 (expected conc. 1.84 mg/kg)	replicate 1	1.857	100.9	102.5%	1.5%
	replicate 2	1.893	102.9		
	replicate 3	1.910	103.8		
level 3 (expected conc. 3.45 mg/kg)	replicate 1	3.529	102.3	101.2%	1.1%
	replicate 2	3.496	101.3		
	replicate 3	3.450	100.0		

The results of MA determinations showed values between 2.93 and 0.18 mg/kg. The low HMF content (only 2 samples slightly exceeded 10 mg/kg) should ensure that honeys were fresh and MA content was not affected by storage.

The comparison of the MA determinations with the "yes/no" evaluations, showed that the ranges of MA values were largely overlapped between the two groups: 0.65 to 2.86 mg/kg in the "yes" samples and 0.18 to 2.93 mg/kg in the "no" samples. The statistical analysis confirmed that no significant difference in MA content existed between "yes" and "no" groups (t-test = 1.254, df = 44, P = 0.216). More generally, no significant correlation could be found between MA content and any other analytical parameter: sensory score (R^2 = 0.005), percent of *Citrus* pollen (R^2 = 0.027), colour (R^2 = 0.111), electrical conductivity (R^2 = 0.013) or *Citrus* species (orange or lemon, identified through pollen).

In contrast, a noticeable difference could be observed between the samples produced in Italy and in other countries (Fig. 3): the MA values of the Italian "yes" samples were distributed in the range of 0.65–2.58 mg/kg, while the "yes" samples from other countries had values from 2.41 to 2.86 mg/kg. Also the "no" samples from Italy had lower values than the other "no" samples: 0.18 to 1.71 mg/kg, versus 1.25 to 2.93 mg/kg. The statistical analysis confirmed a significant difference between Italian and other samples (t-test = –3.831, df = 44, P = 0.000).

In Table V the average, standard deviation, minimum and maximum MA values of the

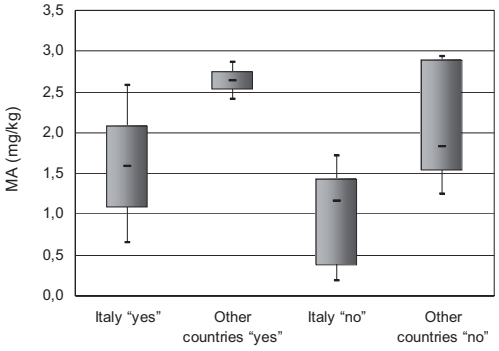

Figure 3. Ranges of MA content in the examined samples. In the box plots medians and quartiles (25–75%) are given.

"yes" samples are reported and compared with those found by other authors: for the imported samples our results are in agreement with previous studies, while the average value of the Italian samples was distinctly lower.

4. DISCUSSION

4.1. HPLC Method

The HPLC-PDA method used in this study attained very good results in terms of sensitivity, recovery and precision.

The use of an acid hydrolysis step proved to be necessary for MA determination by HPLC, and the LC/MS/MS experiment showed that, in the unacidified extracts, the bigger peak that was eluted earlier was due to an anthranilate ester other than free MA. Thus in *Citrus* honey

Table IV. Analytical results of 46 *Citrus* honey samples (in bold: values not complying with the profile of unifloral *Citrus* honey).

Samples' origin	MA (mg/kg)	Traditional parameters							HMF (mg/kg)
		Global evaluation	Sensory score	Citrus pollen (%)	PG/10g (x 10^3)	Other botanical components	Colour (mm Pfund)	El. cond. (mS/cm)	
Mediterranean area	2.86	yes	2.0	40*	10.5		25	0.27	0.7
Spain	2.63	yes	2.2	69	46.1		20	0.17	10.6
Italy (Sardegna)	2.58	yes	2.0	49	4.0		5	0.17	6.5
Spain	2.41	yes	2.0	78*	25.6		15	0.15	4.0
Italy (Calabria)	2.38	yes	2.8	42	19.9		5	0.17	4.0
Italy (Sicilia)	2.34	yes	3.0	68	12.8		10	0.16	2.7
Italy (Calabria)	2.31	yes	2.5	33	6.7		5	0.19	2.7
Italy (Calabria)	2.17	yes	2.7	54	15.0		5	0.15	3.6
Italy (Basilicata)	2.16	yes	3.0	59*	10.7		15	0.22	2.1
Italy (Basilicata)	2.08	yes	2.0	34	7.5		15	0.25	3.3
Italy (Sicilia)	2.06	yes	2.3	24	12.9		20	0.31	5.2
Italy (Sicilia)	1.93	yes	2.0	48	11.6		10	0.26	5.6
Italy (Calabria)	1.91	yes	3.0	56	12.5		5	0.14	0.6
Italy (Basilicata)	1.84	yes	2.8	13	9.9		5	0.17	1.0
Italy (Puglia)	1.61	yes	2.3	48	4.4		15	0.23	5.4
Italy (Calabria)	1.58	yes	2.3	49	7.7		10	0.15	11.1
Italy (Sicilia)	1.48	yes	2.7	72	16.4		5	0.12	5.0
Italy (Calabria)	1.29	yes	3.0	54	9.9		5	0.15	2.9
Italy (Calabria)	1.18	yes	2.6	67	7.3		5	0.12	1.0
Italy (Puglia)	1.17	yes	2.4	28	6.4		5	0.15	2.3
Italy (Calabria)	1.10	yes	2.2	64	10.7		5	0.15	3.1
Italy (Puglia)	1.08	yes	2.8	79	11.1		5	0.12	2.7
Italy (Puglia)	0.93	yes	2.0	50	10.8		5	0.12	3.7
Italy (Calabria)	0.90	yes	2.3	60	16.9		5	0.14	0.8
Italy (Calabria)	0.81	yes	2.4	65	17.8		5	0.13	2.9
Italy (Calabria)	0.77	yes	2.7	50	16.8		5	0.15	4.8
Italy (Basilicata)	0.76	yes	2.5	65	9.5		5	0.13	4.8
Italy (Calabria)	0.65	yes	2.0	52	9.8		10	0.20	5.8
Mexico	2.93	no	**1.2**	15	29.7		**65**	0.25	8.5
Spain	2.93	no	**1.3**	60*	92.5		10	0.16	4.6
Mexico	2.87	no	**1.0**	21	45.5		**60**	0.23	8.5
Mexico	1.83	no	**1.0**	24	66.5		**60**	0.30	8.2
Italy (Lazio)	1.71	no	**1.2**	**5**	**86.0**	Prunus, Pyrus	15	0.23	5.0
Spain	1.62	no	**1.3**	67*	99.0		20	0.20	6.7
Italy (Basilicata)	1.55	no	2.2	19	**35.6**	Hedysarum	10	0.19	4.6
Spain	1.45	no	**1.3**	65*	113.7		20	0.19	4.5
Italy (Puglia)	1.43	no	**1.8**	30	15.9	Trifolium, Vicia	5	0.15	1.7
Italy (Lazio)	1.42	no	**1.3**	**9**	13.0	Hedysarum	10	**0.53**	2.3
Spain	1.25	no	**1.0**	75*	155.1		20	0.20	4.5
Italy (Basilicata)	1.21	no	**1.8**	20	16.8	Hedysarum	15	0.22	6.0
Italy (Basilicata)	1.16	no	**1.5**	28	10.7	Hedysarum, Cruciferae	20	0.24	9.0
Italy (Sicilia)	0.56	no	**1.6**	17	8.1	Carduus	25	**0.44**	4.4
Italy (Calabria)	0.50	no	**1.4**	18	9.3	Robinia	5	0.16	3.5
Italy (Calabria)	0.23	no	**1.3**	**3**	**20.3**	Hedysarum	20	**0.33**	9.0
Italy (Basilicata)	0.22	no	**1.5**	**2**	**31.6**	Hedysarum	5	0.15	7.2
Italy (Sicilia)	0.18	no	**1.4**	15*	13.4	Carduus	25	**0.52**	4.2

** = Prevalence of lemon pollen*

native MA is not completely free but is partially bound to some other molecule that is hydrolyzed by the acid treatment. The acid treatment proved to be effective in freeing the bound MA and obtaining reproducible results.

More LC/MS experiments might be done to better elucidate the structure of the unknown peaks: their pseudo-molecular ions could be found by performing simple scans and parent scans for the fragments observed; daughter scans might then be performed for the parent ions found. A study could be done on the ratio between the area of the earlier eluted peak and the area of the MA peak to assess if it

Table V. Comparison between the results obtained in the present work and those reported by other authors.

Reference	Country	No samples	MA content (mg/kg)			
			Average	St. dev.	Min	Max
White, 1966	USA	21	2.87	1.14	0.84	4.37
Serra Bonvehí, 1988	Spain	12	2.01	1.10	0.57	4.22
Ferreres et al., 1994	Spain	18	2.35	0.54	1.44	3.60
Serra Bonvehí and Ventura Coll, 1995	Spain	15	2.51	0.47	1.78	3.60
White and Bryant, 1996	Florida	63	3.10	0.91	0.68	5.04
Present work	Italy "yes"	25	1.56	0.60	0.65	2.58
	Other countries "yes"	3	2.63	0.23	2.41	2.86

could be of any help in evaluating the honey authenticity.

4.2. Suitability of MA as a chemical marker

From the analytical data, MA was confirmed to be a typical component of *Citrus* honey; however, given the lack of correlation between the MA content and the "yes/no" classification or with any other authenticity parameter, MA can not be considered suitable as a chemical marker to assess the level of uniflorality of this honey type. It may be used only as a further descriptive element to complete the analytical picture of unifloral *Citrus* honey, together with the other authenticity criteria.

Some European laboratories for honey control require a minimum MA content of 2 mg/kg to accept a *Citrus* honey as unifloral. By applying this discriminating limit to our sampling, only 56.5% would be classified coherently with the traditional approach, with 6.5% false positive and 37% false negative results. On the other hand, by assuming a lower MA limit, the number of false negatives would diminish but the false positives would increase. Even by applying more restrictive criteria for the "yes/no" classification (i.e. higher sensory score, higher *Citrus* pollen percent, lower values of colour and electrical conductivity), the situation would not change.

Our results, and the comparison with the literature data, show that Italian *Citrus* honeys have a lower MA content compared to honeys produced in other countries. In Italian samples, MA values lower than 0.5 mg/kg were presented only by "no" samples, and values higher than 2 mg/kg were presented only by "yes" samples; however between 0.5 and 2 mg/kg, "yes" and "no" samples were widely mixed. A minimum content of 0.5 mg/kg could be required for unifloral *Citrus* honeys produced in Italy, however, beyond this value, MA concentration was not correlated with uniflorality. The reason for this difference is not known and would require further investigation, but this feature should be taken into account in the context of the international honey market.

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L'anthranilate de méthyle dans les miels de *Citrus* – méthode analytique et application comme marqueur chimique.

miel monofloral / *Citrus* / anthranilate de méthyle / caractérisation botanique / authenticité

Zusammenfassung – Methylanthranilat in Zitrushonig: analytische Methode und Verwendbarkeit als chemischer Marker. Ein Charakteristikum von Zitrushonig ist die Anwesenheit von Methylanthranilat (MA), von manchen Autoren wurde diese flüchtige Komponente als chemischer Marker für die Authentizität dieses Honigtyps vorgeschlagen. Die vorliegende Arbeit beschreibt eine ausgearbeitete Analyse zur Bestimmung von MA, die auf der Verwendung eines HPLC-PDA Systems

nach einer sauren Extraktion und Reinigung mittels einer Copolymercartridge beruht. Wir berichten die Ergebnisse einer Studie, bei der 46 Zitrushonige aus verschiedenen Ländern untersucht wurden. Diese Proben wurden auf ihren Gehalt an MA und ihre Übereinstimmung mit dem Honigprofil von Zitrussortenhonig entsprechend der überkommenen Parameter (Sensorik, Pollenzusammensetzung und physiochemische Eigenschaften) untersucht. Die Übereinstimmung mit dem Sortenprofil wurde zu einer übergreifenden Ja/Nein Bewertung zusammengefasst.

Die Ergebnisse (Tab. IV) zeigten keinen signifikanten Unterschied des MA-Gehaltes zwischen den „Ja" Proben (0,65 bis 2,86 mg/kg) und den „Nein" Proben (0,18 bis 2,93 mg/kg), und es wurde keine Korrelation zwischen dem MA-Gehalt und irgendeinem der einzelnen analytischen Parameter gefunden. Dagegen wurde ein signifikanter Unterschied zwischen den in Italien und den in den anderen Ländern produzierten Honigen gefunden (Abb. 3), wobei die italienischen „Ja" Proben geringere MA-Werte zeigten als die anderen Proben (im Mittel 1,56 mg/kg ± 0,60 gegenüber 2,63 ± 0,23 mg/kg). Einige Honigkontrolllabors verlangen einen Mindestgehalt an MA von 2 mg/kg, um einen Zitrushonig als sortenrein anzuerkennen. Wenn man diese Unterscheidungsgrenze zu Grunde legt, würden nur 56,5 % der untersuchten Proben übereinstimmend mit der traditionellen Einordnung klassifiziert, mit 6,5 % fälschlich positiven und 37 % fälschlich negativen Ergebnissen.

Wir schließen daraus, dass MA als chemische Bestimmung der Sortenreinheit von Zitrushonig nicht geeignet ist, sondern nur als zusätzliches beschreibendes Element herangezogen werden kann, um zusammen mit den anderen Kriterien das analytische Bild dieses Honigtyps zu komplettieren.

Für italienische Zitrushonige könnte ein Minimalgehalt von 0,5 mg/kg gefordert werden, oberhalb dieser Grenze besteht allerdings kein Zusammenhang zwischen Sortenreinheit und dem Gehalt an MA. Der Grund für das unterschiedliche Verhalten der italienischen Zitrushonige sollte weiter untersucht werden, da dieses im Kontext internationaler Vermarktung bedeutsam ist.

Methylanthranilat / Zitrushonig / Sortenreinheit / Authentizität

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Online Material

ELECTRONIC-ONLY MATERIAL: APPENDIX

Table I. Multiple Reaction Monitoring (MRM) method employed in the LC/MS/MS determination of MA in unacidified honey extracts.

MRM method				
Parent Ion (m/z)	Product Ion (m/z)	Dwell (s)	Cone (V)	Coll (eV)
152.1	120.1	0.2	15	10
152.1	91.0	0.2	15	25
152.1	64	0.2	15	35
120	92	0.2	25	15
120	64	0.2	25	20

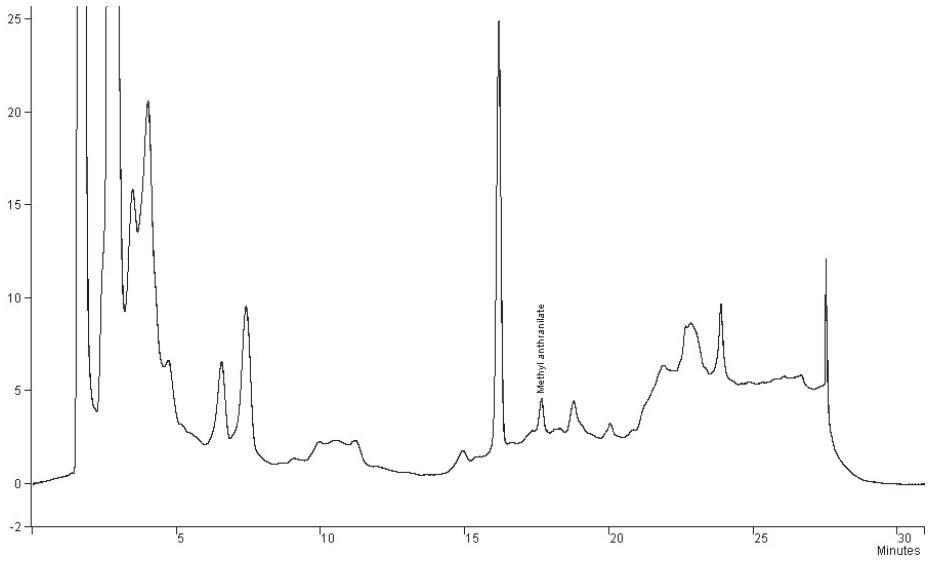

Figure 1. Chromatogram of a *Citrus* honey sample dissolved in distilled water with no acid treatment.

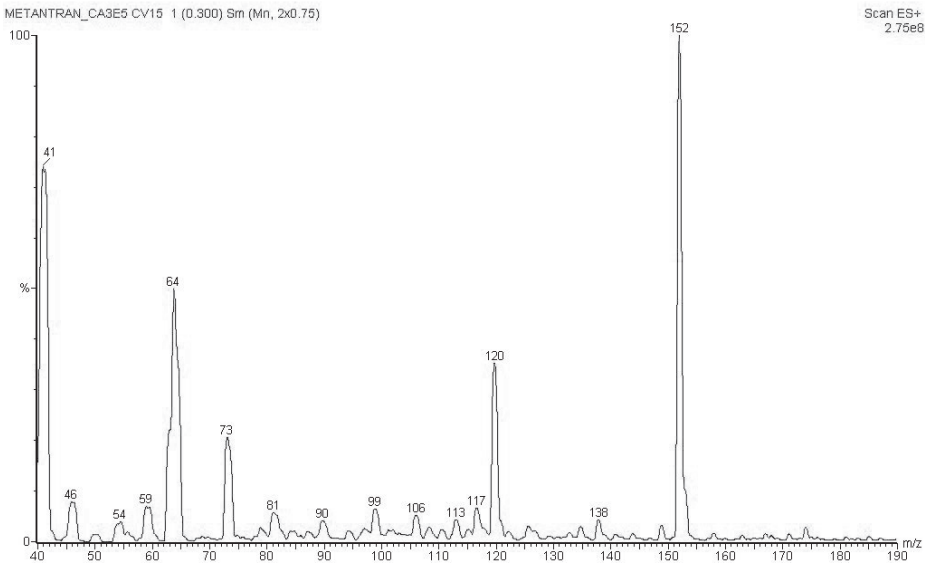

Figure 2. MA scan spectrum (m/z 40–190) recorded in infusion mode at 3.5 KV capillary voltage and 15 V cone voltage.

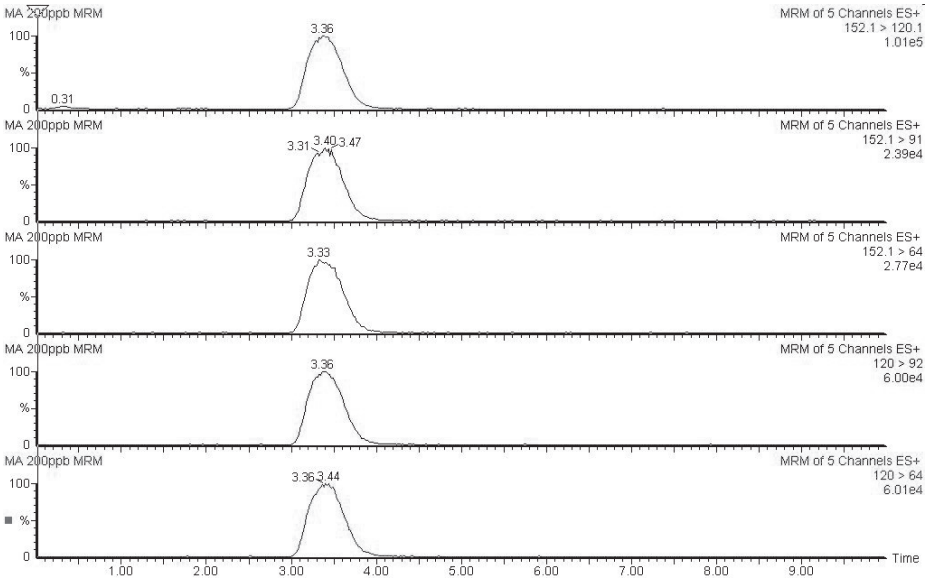

Figure 3. Chromatograms of a MA standard solution: five MRM signals.

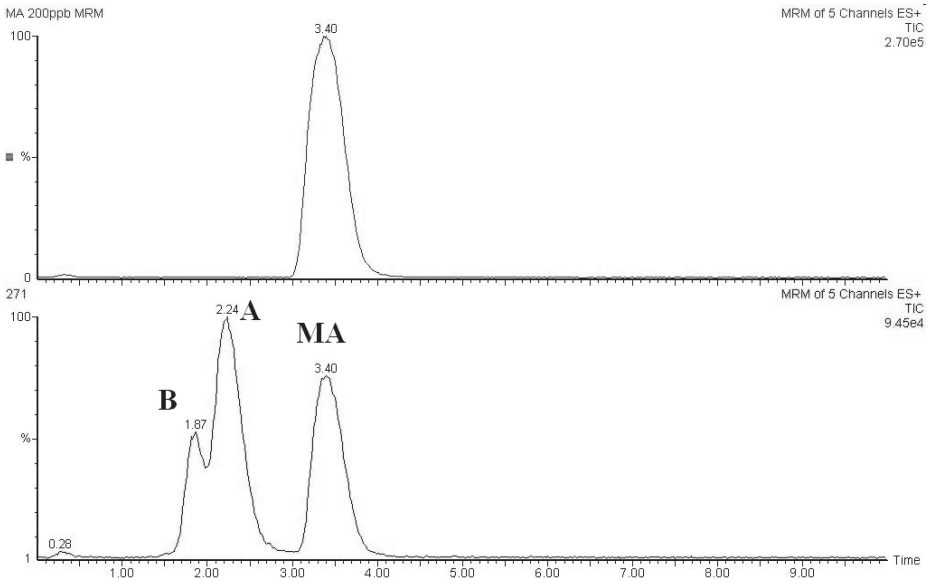

Figure 4. Total ion chromatograms of a MA standard solution (above) and of an unacidified *Citrus* honey extract (below).

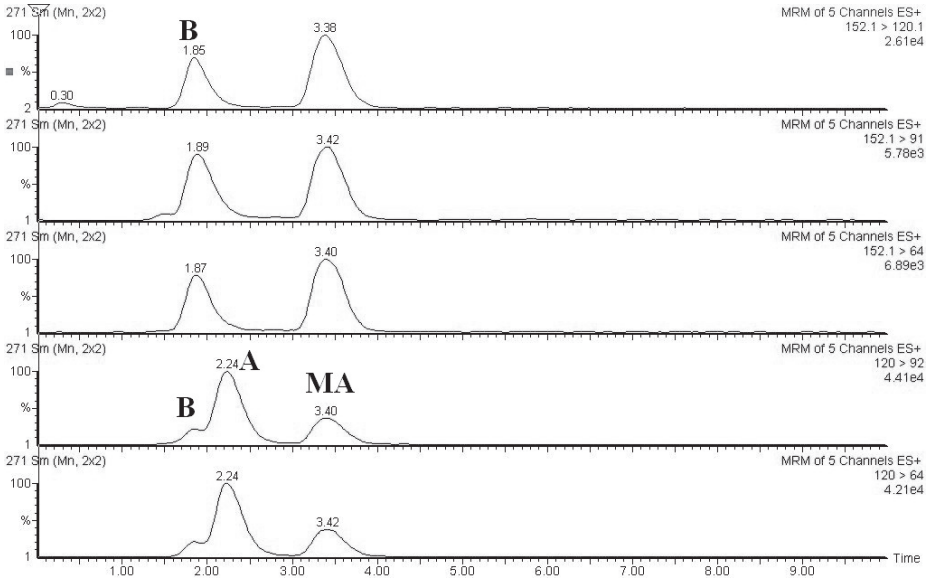

Figure 5. MRM signals of MA for an unacidified *Citrus* honey sample.