DOI: 10.1515/JAS-2015-0015 J. APIC. SCI. VOL. 59 NO. 1 2015

DE GRUYTER OPEN

Original Article

APPLICATION OF GAS CHROMATOGRAPHY WITH THE MASS DETECTOR (GC-MS) TECHNIQUE FOR DETECTION OF BEESWAX ADULTERATION WITH PARAFFIN

Ewa Waś*
Teresa Szczęsna
Helena Rybak-Chmielewska

Research Institute of Horticulture, Apiculture Department, Puławy, Poland

*corresponding author: ewa.was@inhort.pl
Received 01 April 2015; accepted 11 May 2015

Abstract

To detect beeswax adulteration with hydrocarbons of alien origin (e.g. paraffin), gas chromatography with mass detector (GC-MS) technique was used. The method has been verified here on beeswax samples with different addition (3, 5, 10, 30, and 50%) of paraffin and validated under the conditions of repeatability and within – laboratory reproducibility. The addition of paraffin to beeswax can already be detected on the basis of an analysis of the chromatograms. The intensity of individual alkane peaks increased with the increase of the amount of paraffin added to the beeswax. This increase was the mostly visible for the alkanes with even numbers of carbon atoms in the molecule: $C_{24}H_{50}$, $C_{26}H_{54}$, $C_{28}H_{58}$, $C_{30}H_{62}$, $C_{32}H_{66}$, and $C_{34}H_{70}$. These observations have also been proven by quantitative analysis performed using the internal standard method. Adding paraffin to beeswax resulted in an increase in the total contents of n-alkanes as well as individual alkanes, and in particular, of the even-numbered alkanes. The addition of paraffin to beeswax also resulted in the appearance of alkanes containing over 35 carbon atoms in the molecule, which were not detected in beeswax. The method for determination of beeswax hydrocarbons with the GC-MS technique is characterised by satisfactory repeatability and within-laboratory reproducibility. This method can be used for the detection of beeswax adulteration with hydrocarbons of alien origin (e.g. paraffin).

Keywords: adulteration, beeswax, GC-MS, hydrocarbons, n-alkanes, paraffin.

INTRODUDCTION

Due to the insufficient domestic production of beeswax and the lack of obligatory legal regulations concerning beeswax quality, instances of its adulteration are quite frequent. The addition of alien substances such as paraffin, decreases beeswax quality as well as the quality of the foundation made of such adulterated beeswax. As a consequence, it may no longer be possible to use this product in beekeeping. It may also not be possible to use such a product in some branches of industry, for example, the cosmetic, pharmaceutical, and food industry.

The sensory assessment and physico-chemical characterisation are only approximate and do not allow to detect the adulteration of beeswax unambiguously. Beeswax which has paraffin added, becomes china-white and pliable when kneaded with the fingers. What is more, the addition of paraffin causes the beeswax to become smooth and shiny where rubbed (Curyło, 1983). As we mentioned previously (Waś et al., 2014a), a lot of methods so far used for the determination of hydrocarbons in beeswax and for the detection of beeswax adulteration were based on the physico-chemical parameters. These parameters include: melting point, density, solubility, and saponification, acid and ester numbers, and iodine index (Tulloch, 1973; Serra Bonvehi et al., 1989; Serra Bonvehi, 1990; Vit et al., 1992; Poncini et al., 1993). Some interrelations were found between the physico-chemical properties of beeswax and its degree of adulteration with paraffin. With the increase in the amount of paraffin added to beeswax, beeswax density decreases, and the acid and saponification numbers decline. A higher addition of paraffin also results in the decline of the value of the iodine index. The melting point of beeswax increases or decreases, depending on the melting point of the added paraffin (Bernal et al., 2005; Jimenez et al., 2007; Serra Bonvehi and Ornantes Bermejo, 2012).

The methods used in the quality control of beeswax, that are based on the above-mentioned physico-chemical parameters, are still used (Bogdanov, 2004; Bernal et al., 2005; Serra Bonvehi and Ornantes Bermejo, 2012; Maia and Nunes, 2013) but are not very precise. Such methods frequently require ascertaining with the chromatographic methods. Using gas-chromatography in the analysis of the beeswax composition made it possible to identify alien substances used in its adulteration.

So far in Poland, to determine the hydrocarbons of beeswax and to detect its adulteration, column chromatography combined with weight analysis was used (Curyło and Zalewski, 1957a, b; Curyło and Ignaszewska, 1969; PN-R-78890, 1996). The method allows for the quantitative analysis of the total hydrocarbons for which the Polish Standard allows a maximum content of 16.5% (PN-R-78890, 1996). The method was not accurate due to the usage of pharmaceutical gasoline, this method was also dangerous and unhealthy.

Therefore, the aim of the study was to use the gas chromatography with mass detector (GC-MS) technique for the detection of beeswax adulteration with hydrocarbons of an alien origin (e.g. paraffin).

MATERIAL AND METHODS

Reagents

All analytical standards and reagents used in this experiment were for gas chromatography: squalane (99.9% purity) was from Supelco (Bellefonte, PA, USA), the standard mixtures of n-alkanes (C_8H_{18} - $C_{20}H_{42}$ and $C_{21}H_{44}$ - $C_{40}H_{82}$) were obtained from Fluka (Buchs, Switzerland; Saint Louis, MO, USA), hexane SupraSolv® (≥98% purity) from Merck (Darmstadt, Germany), and heptane anhydrous (≥98.5% purity) was from Sigma-Aldrich (Steinheim, Germany). Solid-phase extraction (SPE) cartridges filled with neutral aluminum oxide (Alumina – N, 1000 mg, 6 mL) were purchased from Agela Technologies (Wilmington, DE, USA). Helium (99.9999% purity) was provided by Air Products (Warsaw, Poland).

Research material

The experiment material consisted of technical paraffin (colourless to off-white solidified mass), and beeswax samples with 3, 5, 10, 30, and 50% additions of the paraffin, prepared in the laboratory on the basis of the virgin beeswax, and a sample of beeswax adulterated with paraffin that has been sent by a customer to the laboratory for the analysis. The virgin beeswax used for preparing the samples adulterated with paraffin, was obtained from the cappings and light combs of the so-called „wild construction" which came from the apiaries of the Department of Beekeeping Technology of the Research Institute of Horticulture, Apiculture Division in Puławy, Poland.

Sample preparation

To prepare beeswax samples with 3, 5, 10, 30, and 50% additions of paraffin, 9.7 + 0.3, 9.5 + 0.5, 9.0 + 1.0, 7.0 + 3.0, and 5.0 + 5.0 g of beeswax and paraffin, respectively, was weighed on the technical scales and put into the glass jars. The jars were then tightly closed and placed in an incubator at 70°C and shaken for about 30 minutes until the contents were fully dissolved.

Then, all tested samples were prepared in the same way, 0.05 ± 0.001 g were diluted with 7.5 mL of heptane using an incubated shaker (in 50°C for 12 min). After cooling the solution, 2.5 mL of squalane of 400 mg/L concentration was added. After that, the hydrocarbon fraction was isolated using the SPE technique (Waś et al., 2014a).

Analysis of hydrocarbons in paraffin and beeswax adulterated with paraffin

Analyses of hydrocarbons in the studied samples were carried out using a gas chromatograph with a mass detector (GCMS-QP 2010 Plus, Shimadzu). Chromatographic separation of hydrocarbons was performed on ZB-5HT INFERNO column (20 m × 0.18 mm × 0.18 µm, Phenomenex). Identification of the studied compounds was done based on the mass spectra of the NIST 05 library and on the retention indexes. The quantitative analysis of n-alkanes was performed using the internal standard method - with squalane ($C_{30}H_{62}$) used as an internal standard. The technique is described in more details in the previous paper (Waś et al., 2014a). That method has been proven here on beeswax samples with different additions (3, 5, 10, 30, and 50%) of paraffin, and the method has been validated for determining the hydrocarbons in paraffin and beeswax adulterated with paraffin.

Assay validation

The method previously elaborated by Waś et al. (2014a) was validated here with repeatability, within-laboratory reproducibility and recovery for determination of hydrocarbons in paraffin and beeswax adulterated with paraffin. The repeatability and within-laboratory reproducibility were determined based on the variability coefficients calculated for

the contents of individual n-alkanes in the beeswax sample adulterated with paraffin (with the total of n-alkanes equalling 12.86 g/100 g). For the recovery determination of n-alkanes, two solutions of paraffin with a concentration of 0.5 mg/mL were injected into the chromatographic column, as described previously (Waś et al., 2014a).

RESULTS

Verification of GC-MS method for detection of beeswax adulteration

The use of the GC-MS technique for detection of beeswax adulteration has been verified by the analysis of n-alkanes in beeswax samples with different additions of paraffin. In paraffin and pure beeswax used in our experiment, the same homologous series of linear saturated hydrocarbons (n-alkanes) were identified. However, the n-alkanes found in paraffin had longer carbon chains (from 20 to 40 atoms of carbon in the molecule) in comparison to the n-alkanes determined in beeswax (Fig. 1). Longer carbon chains were also identified in beeswax adulterated with paraffin (Fig. 2). Moreover, based on the comparative analysis of the chromatograms of n-alkanes in pure beeswax (Fig. 1a) and in beeswax with different additions (3, 5, 10, 30, and 50%) of paraffin (Fig. 2), it can be easily noted that the intensity of the peaks of individual alkanes increased when the amount of paraffin added to the beeswax was increased. The increase of the peak intensities was the mostly visible for the alkanes with even numbers of carbon atoms in the molecule: $C_{24}H_{50}$, $C_{26}H_{54}$, $C_{28}H_{58}$, $C_{30}H_{62}$, $C_{32}H_{66}$, and $C_{34}H_{70}$, even with only a 3% addition of paraffin (Fig. 2a). With a 30 and 50% addition of paraffin, the intensity of

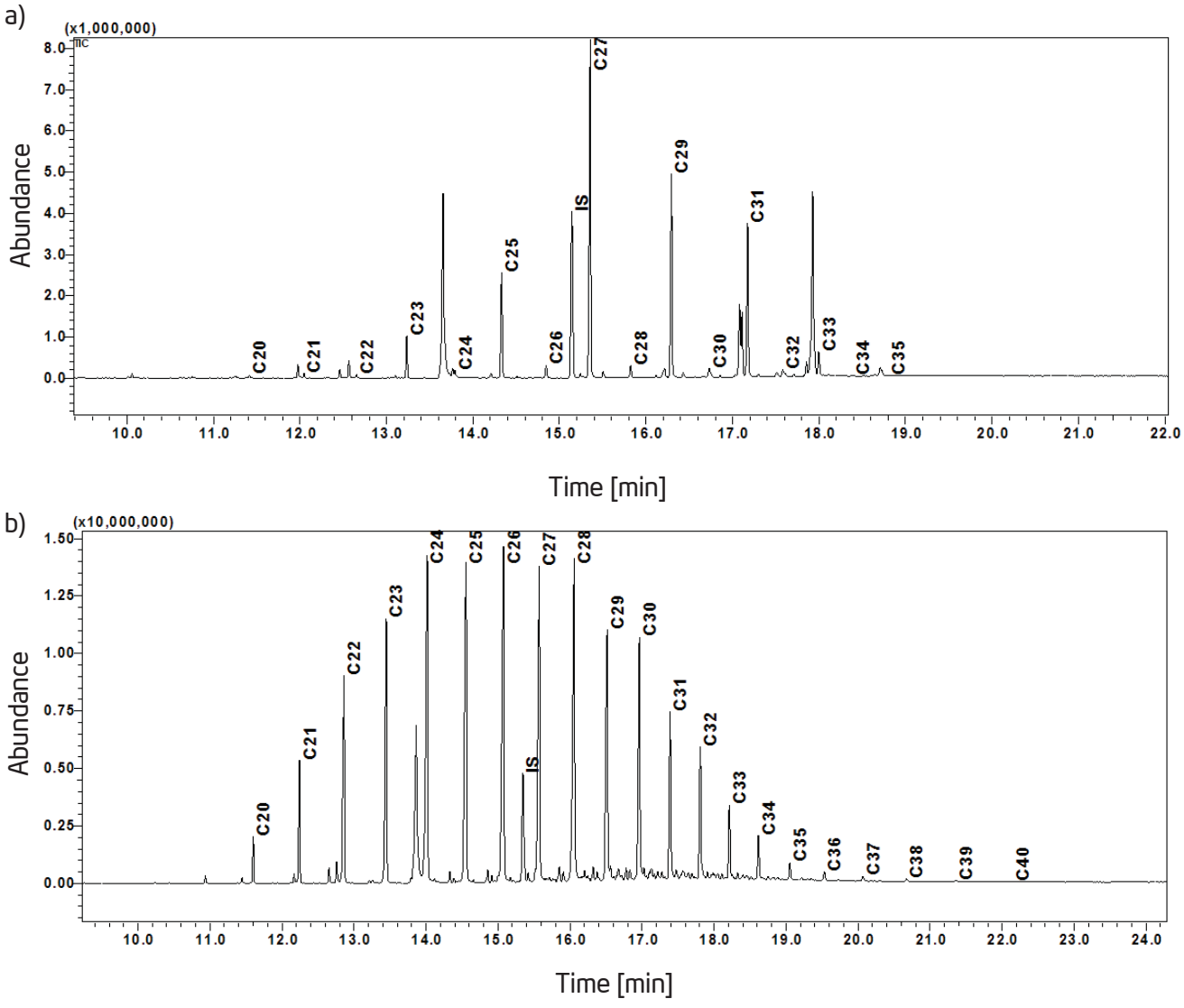

Fig. 1. Chromatograms of n-alkanes in pure beeswax (a) and paraffin (b).
C_{20} – C_{40} - n-alkanes with the formula $C_{20}H_{42}$ – $C_{40}H_{82}$; IS – internal standard - $C_{30}H_{62}$

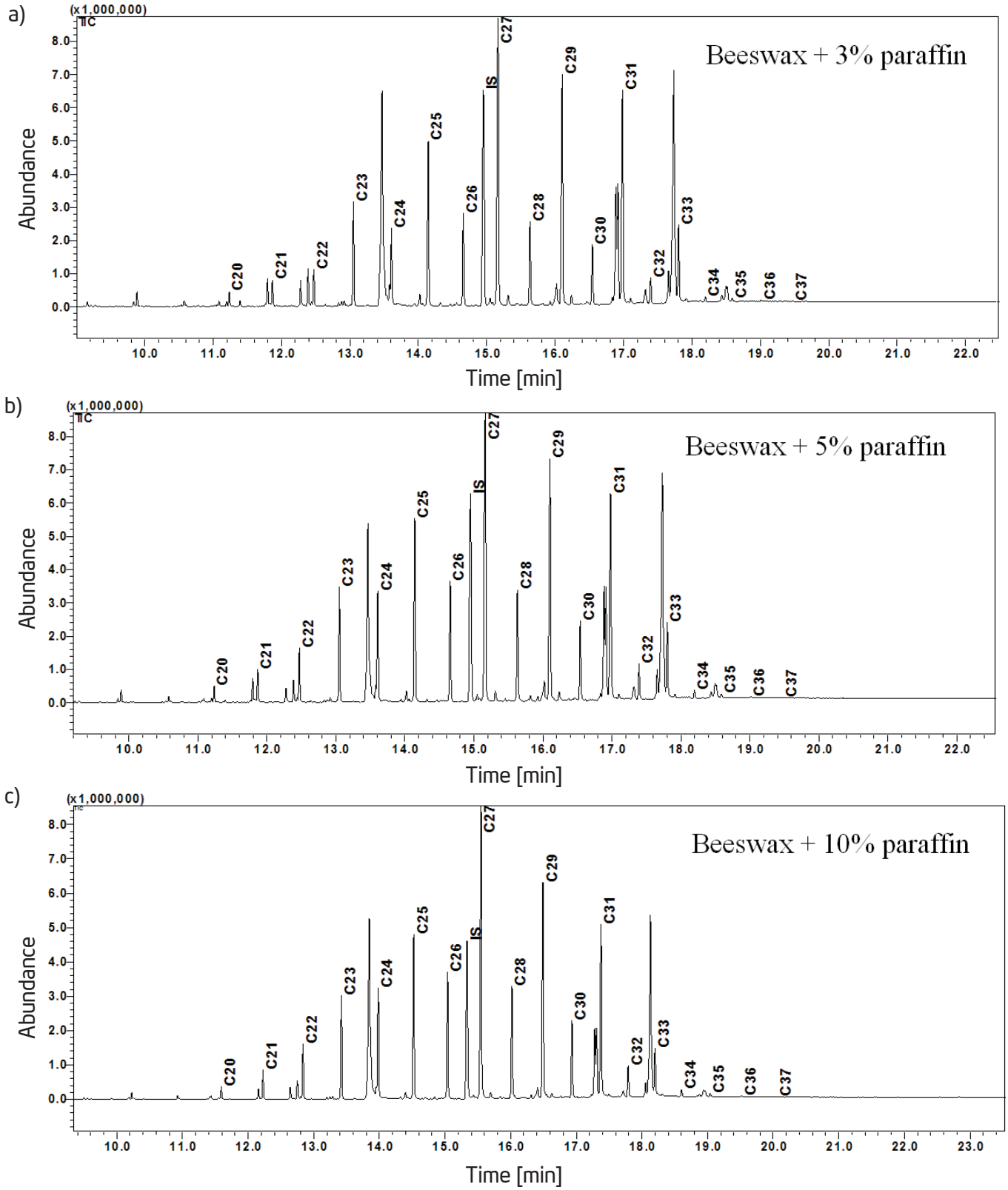

Fig. 2. Chromatograms of n-alkanes ($C_{20}H_{42}$ – $C_{40}H_{82}$) in beeswax with different additions of paraffin a) 3% b) 5% c) 10% d) 30% e) 50%.
IS – internal standard - $C_{30}H_{62}$

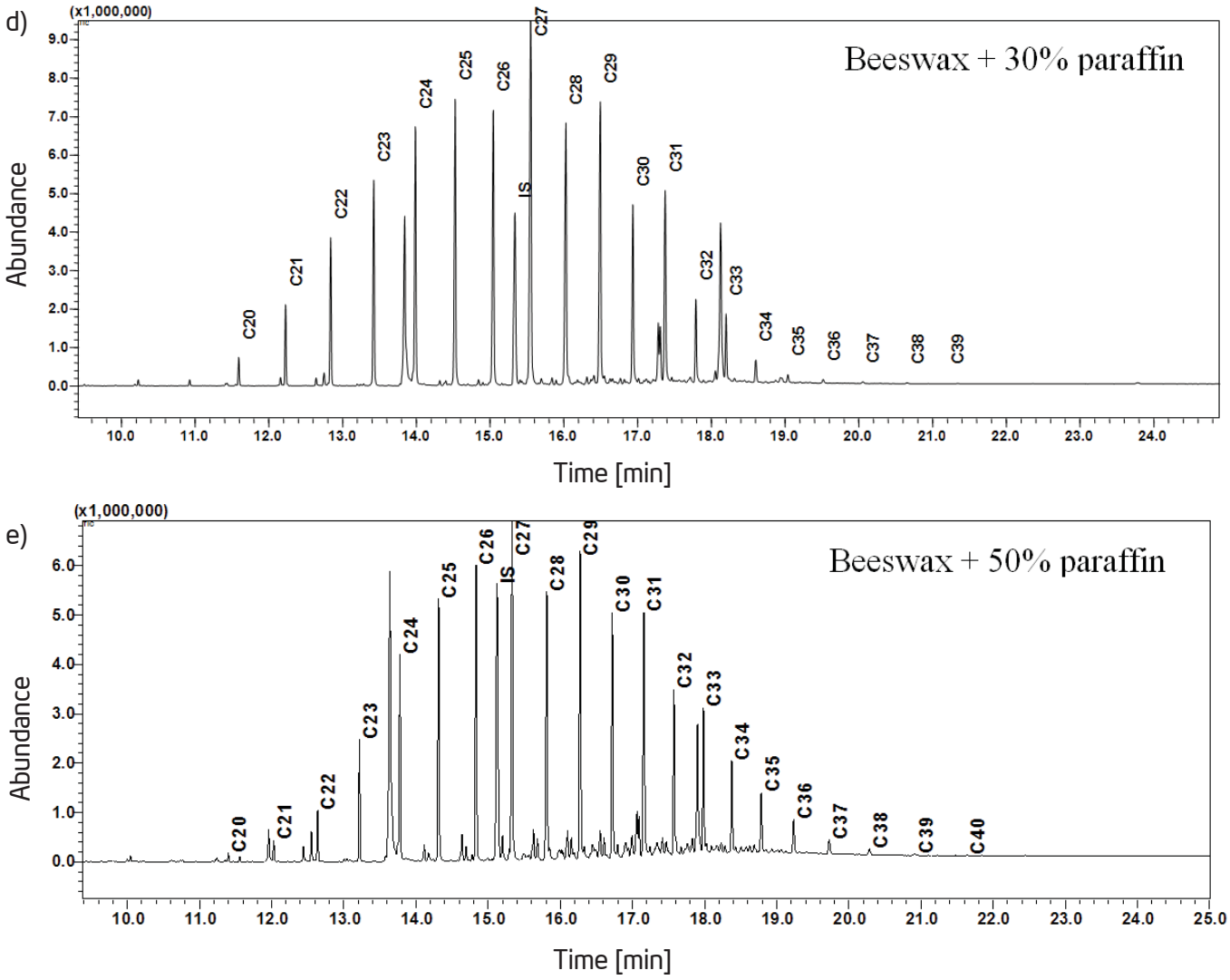

Fig. 2 (Continued). Chromatograms of n-alkanes ($C_{20}H_{42}$ – $C_{40}H_{82}$) in beeswax with different additions of paraffin a) 3% b) 5% c) 10% d) 30% e) 50%.
IS – internal standard - $C_{30}H_{62}$

peaks for the even- and odd-numbered alkanes were comparable (Fig. 2d, 2e). Moreover, a high similarity of the n-alkanes chromatograms for beeswax with a 50% addition of paraffin (Fig. 2e) to the n-alkanes chromatograms obtained for the pure paraffin (Fig. 1b) could be easily noticed. Also, the addition of paraffin to beeswax resulted in the occurrence of alkanes containing over 35 atoms of carbon in the molecule (Fig. 2), which were not detected in pure beeswax (Fig. 1a).

Results of the quantitative analysis also indicated the presence of paraffin in beeswax samples (Tab. 1, Fig. 3). The total content of n-alkanes in pure beeswax was 9.44 g/100 g. A much higher total amount of these compounds was found in paraffin (68.42 g/100 g). Higher contents were also determined in beeswax adulterated with paraffin in comparison to pure beeswax. The total content of n-alkanes in beeswax with a 3, 5, 10, 30, and 50% addition of paraffin were: 13.48, 15.56, 18.63, 31.26, and 42.40 g/100 g, respectively. The addition of paraffin to beeswax also resulted in increased contents of individual alkanes, and in particular, of the even-numbered alkanes (Tab. 1, Fig. 3), which could have been already observed on the chromatograms. Only a 3% addition of the paraffin resulted in more than a five-fold increase in the contents of even-numbered alkanes (from 0.60 to 3.12 g/100 g). In beeswax samples with a 5, 10, and 30% addition, the contents of even-numbered alkanes were much higher and amounted for 4.43, 5.97, 13.88 g/100 g, respectively. The highest content (20.97 g/100 g)

Table 1.

Mean contents of n-alkanes (g/100 g) determined in the studied samples (n = 3)

Formula of n-alkanes	Pure beeswax	Paraffin	Beeswax with different additions of paraffin				
			3%	5%	10%	30%	50%
$C_{20}H_{42}$	0.05	0.74	0.10	0.12	0.22	0.26	0.40
$C_{21}H_{44}$	0.06	1.92	0.20	0.26	0.29	0.75	1.07
$C_{22}H_{46}$	0.05	3.92	0.30	0.43	0.58	1.50	2.19
$C_{23}H_{48}$	0.42	5.34	0.82	0.97	1.14	2.23	3.09
$C_{24}H_{50}$	0.09	7.72	0.62	0.89	1.24	2.90	4.41
$C_{25}H_{52}$	1.02	7.30	1.47	1.65	1.92	3.28	4.40
$C_{26}H_{54}$	0.15	8.21	0.74	1.03	1.44	3.24	4.84
$C_{27}H_{56}$	3.05	7.16	2.96	3.10	3.85	4.58	5.24
$C_{28}H_{58}$	0.12	7.74	0.68	0.96	1.31	2.94	4.56
$C_{29}H_{60}$	2.08	5.39	2.23	2.35	2.67	3.28	3.89
$C_{30}H_{62}$	0.10	4.93	0.46	0.66	0.85	1.94	2.86
$C_{31}H_{64}$	1.78	3.17	2.03	2.12	2.17	2.37	2.58
$C_{32}H_{66}$	0.04	2.34	0.19	0.27	0.36	0.89	1.34
$C_{33}H_{68}$	0.44	1.14	0.63	0.66	0.57	0.72	0.83
$C_{34}H_{70}$	<0.025*	0.71	0.04	0.07	0.10	0.23	0.37
$C_{35}H_{72}$	<0.025*	0.33	0.03	0.04	0.06	0.10	0.17
$C_{36}H_{74}$	-	0.18	<0.025*	<0.025*	<0.025*	0.05	0.08
$C_{37}H_{76}$	-	0.11	<0.025*	<0.025*	<0.025*	0.03	0.05
$C_{38}H_{78}$	-	0.06	n.d.**	n.d.**	n.d.**	<0.025*	0.03
$C_{39}H_{80}$	-	<0.05*	n.d.**	n.d.**	n.d.**	<0.05*	<0.05*
$C_{40}H_{82}$	-	<0.05*	n.d.**	n.d.**	n.d.**	n.d.**	<0.05*
Total	9.44	68.42	13.48	15.56	18.63	31.26	42.40

* limit of determination

** n.d. - not detected at limits of determination 0.025 g/100 g for $C_{38}H_{78}$ and 0.05 g/100 g for $C_{39}H_{80}$ and $C_{40}H_{82}$

of these alkanes was determined in beeswax with a 50% addition of paraffin, which comprised 49.4% of all the alkanes determined in that sample. The content of odd-numbered alkanes increased with an increase of the amount of paraffin added to the beeswax, but the percentage in the total content of all the alkanes decreased (Tab. 1, Fig. 3).

Validation of the method for detection of beeswax adulteration with paraffin

The results obtained for the recovery of individual n-alkanes and for the total n-alkanes in paraffin, are shown in Table 2. The highest recovery (≥100%) was found for $C_{20}H_{42}$, $C_{27}H_{56}$, $C_{29}H_{60}$, $C_{31}H_{64}$, $C_{32}H_{66}$, $C_{35}H_{72}$, and $C_{36}H_{74}$, and the lowest (85.7%) for $C_{38}H_{78}$. The recovery for the internal standard ($C_{30}H_{62}$) was 97.1% and for the total of n-alkanes 98.7%. Results obtained for the within-laboratory repeatability and reproducibility are shown in Table 3. The average coefficients of variation for the total content of n-alkanes was 0.87% and 2.47%, under the conditions of within-laboratory repeatability and reproducibility, respectively.

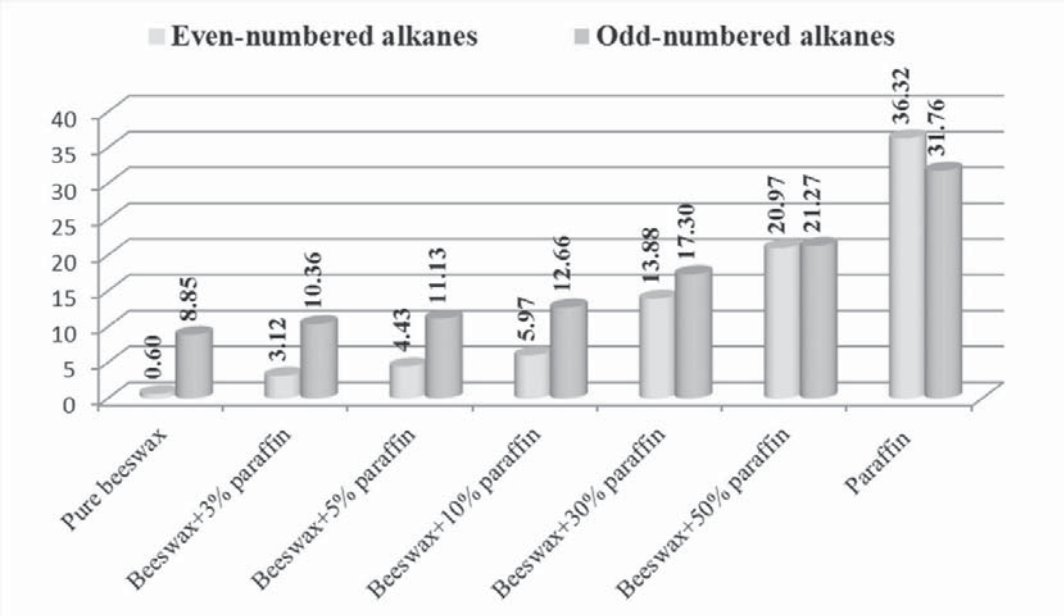

Fig. 3. Average contents of n-alkanes (g/100 g) with even and odd numbers of carbon atoms in the molecule in beeswax with different additions of paraffin.

DISCUSSION

The development of a new method for detection of beeswax adulteration using the GC-MS technique was necessary because of the inaccurate and laborious method (PN-R-78890, 1996) so far used in Poland. Also the methods used in other countries, even chromatographic were incomplete, because they did not provide reliable results of quantitative analysis.

The previously developed method for determination of hydrocarbons of beeswax (Waś et al., 2014a) was validated and used in this work to detect adulteration of this product with paraffin. First of all, the technique was verified by the analysis of n-alkanes in beeswax samples adulterated with paraffin in the laboratory. Qualitative analysis carried out by comparing the chromatograms obtained for pure beeswax and paraffin, allowed to detect addition of paraffin in beeswax. The analysis of chromatograms were also used by other authors (Brüschweiler et al., 1989; Jimenez et al., 2004, 2009; Serra Bonvehi and Ornantes Bermejo, 2012), and such analyses turned out to be very useful in detecting adulteration. The addition of paraffin to beeswax resulted in an increased intensity of peaks. The most visible, even with 3% addition of paraffin, increase of the peaks intensity was observed for the even-numbered alkanes. The intensity of these peaks increased when the paraffin addition to the beeswax was increased. It was also noticed that with the increase of the amount of paraffin added to the beeswax, the peak intensities of odd-numbered alkanes increased as well, while the increase of these peak intensities were significantly lower in comparison with the peaks of even-numbered alkanes. With the 30 and 50% additions of paraffin, the intensity of the peaks for the even- and odd-numbered alkanes were comparable. The addition of paraffin to beeswax also resulted in the appearance of alkanes containing over 35 carbon atoms in the molecule which were not detected in beeswax. Similar observations, basing on the analysis of the chromatograms of the beeswax samples having an addition of paraffin prepared in the laboratory were conducted by Jimenez et al. (2009), and Serra Bonvehi and Ornantes Bermejo (2012).

The efficiency of the GC-MS technique in the detection of beeswax adulteration has been also proven by quantitative analysis. The addition of paraffin to beeswax is indicated by the resulting increase in the total content of n-alkanes, as well as in the contents of individual alkanes and, in particular, the even-numbered alkanes. With the increase in the amount of paraffin added to beeswax, the content of even-numbered alkanes and their share significantly increased. The obtained results of the quantitative analysis are hard to compare with the results shown by other authors (Jimenez et al., 2009; Serra Bonvehi and Ornantes Bermejo, 2012), because of the reasons mentioned already in our previous work (Waś et al., 2014a). These reasons mainly relate to the different calculation methods as well as analytical techniques (differences in detection and sample preparation).

Table 2.
Recovery (%) of individual n-alkanes and internal standard (IS) in paraffin (n = 3)

Formula of n-alkanes	Content of n-alkanes (g/100 g)		Recovery
	without SPE	SPE	
$C_{20}H_{42}$	0.71	0.74	104.2
$C_{21}H_{44}$	1.98	1.92	97.0
$C_{22}H_{46}$	4.09	3.92	95.8
$C_{23}H_{48}$	5.50	5.34	97.1
$C_{24}H_{50}$	7.94	7.72	97.2
$C_{25}H_{52}$	7.44	7.30	98.1
$C_{26}H_{54}$	8.33	8.21	98.6
IS*	-	-	97.1
$C_{27}H_{56}$	7.13	7.16	100.4
$C_{28}H_{58}$	7.86	7.74	98.5
$C_{29}H_{60}$	5.38	5.39	100.2
$C_{30}H_{62}$	4.94	4.93	99.8
$C_{31}H_{64}$	3.13	3.17	101.3
$C_{32}H_{66}$	2.33	2.34	100.4
$C_{33}H_{68}$	1.15	1.14	99.1
$C_{34}H_{70}$	0.72	0.71	98.6
$C_{35}H_{72}$	0.33	0.33	100.0
$C_{36}H_{74}$	0.18	0.18	100.0
$C_{37}H_{76}$	0.12	0.11	91.7
$C_{38}H_{78}$	0.07	0.06	85.7
$C_{39}H_{80}$	< 0.05**	< 0.05**	-
$C_{40}H_{82}$	< 0.05**	< 0.05**	-
Total	69.35	68.46	98.7

* Internal Standard
** limit of determination

Table 3.
Repeatability and within-laboratory reproducibility for determination of n-alkanes in beeswax adulterated with paraffin

Formula of n-alkanes	Coefficients of variation (%)	
	Repeatability (n = 5)	Reproducibility (n = 10)
$C_{20}H_{42}$	5.51	13.86
$C_{21}H_{44}$	3.59	6.93
$C_{22}H_{46}$	3.06	9.15
$C_{23}H_{48}$	1.35	5.85
$C_{24}H_{50}$	2.08	7.80
$C_{25}H_{52}$	1.26	5.10
$C_{26}H_{54}$	1.53	5.42
$C_{27}H_{56}$	0.85	2.59
$C_{28}H_{58}$	1.53	3.47
$C_{29}H_{60}$	1.10	3.02
$C_{30}H_{62}$	1.35	4.89
$C_{31}H_{64}$	1.26	2.63
$C_{32}H_{66}$	1.5	8.18
$C_{33}H_{68}$	1.42	4.25
$C_{34}H_{70}$	2.07	4.78
$C_{35}H_{72}$	3.16	6.09
$C_{36}H_{74}$	2.81	6.62
$C_{37}H_{76}$	2.70	5.80
$C_{38}H_{78}$	1.69	7.82
$C_{39}H_{80}$	3.22	8.61
$C_{40}H_{82}$	4.12	5.93
Total	0.87	2.47

Furthermore, it must be noted that the method reported here with GC-MS is characterised by satisfactory repeatability and within-laboratory reproducibility. The method can be used for determination of hydrocarbons in pure beeswax (Waś et al., 2014a, b) as well as for detection of adulteration of this product with paraffin, which was confirmed in this study. The elaborated method has already been used for assessing the quality of commercial beeswax samples (unpublished data).

CONCLUSIONS

The method for the determination of beeswax hydrocarbons with the GC-MS technique can be used for detection of beeswax adulteration with hydrocarbons of alien origin (e.g. paraffin). This method allows for qualitative and quantitative analysis of foreign hydrocarbons added to beeswax. The addition of paraffin to beeswax can already be detected on the basis of an analysis of the chromatograms.

ACKNOWLEDGEMENTS

The authors wish to thank their colleagues from the Department of Beekeeping Technology, the Apiculture Division, Research Institute of Horticulture, for the virgin beeswax used in the preparation of the samples adulterated with paraffin.
This research was supported in part by the Ministry of Science and Higher Education of Poland, COST ACTION FA0803, grant number 527/N-COST/2009/0.

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