

EFFICIENCY OF GC-MS METHOD IN DETECTION OF BEESWAX ADULTERATED WITH PARAFFIN

Ewa Waś*
Teresa Szczęsna
Helena Rybak-Chmielewska

Research Institute of Horticulture, Apiculture Division in Puławy, Kazimierska 2,
24-100 Puławy, Poland

*corresponding author: ewa.was@inhort.pl
Received 15 April 2016; accepted 10 May 2016

Abstract

The efficiency of the gas chromatography - mass detector (GC-MS) technique for the detection of beeswax adulterated with paraffin, was evaluated. For this purpose, beeswax samples with paraffin additions (3, 5, 10, 30, 50%) were analysed. Since not enough is known about paraffin compositions, and since it is difficult to detect paraffin in beeswax, the aim of our research was also to compare the hydrocarbon composition of different types of paraffin. The analysis showed that the types of paraffin available on the market, differ qualitatively and quantitatively as far as their hydrocarbon compositions are concerned. In all kinds of paraffin, we found homologous series of n-alkanes that were much longer than those in beeswax. In beeswax, the amount of added paraffin that is possible to detect, differs and depends on the kind of paraffin used for adulteration. In this study, the minimum estimated percent that was detected using the GC-MS technique, was 3%. The adulteration is indicated by the presence of hydrocarbons containing over 35 carbon atoms in the molecule, and by the higher contents of n-alkanes ($C_{20}H_{42}$ – $C_{35}H_{72}$), in comparison to the concentration of these compounds determined in pure beeswax. We also presented the results of the quality control of commercial beeswax. Based on our results, it can be stated that beeswax adulteration is currently a problem.

Keywords: adulteration, beeswax, detection, efficiency, GC-MS, paraffin

INTRODUCTION

The recent increased interest in the testing of beeswax composition and in the quality assessment of beeswax, is related to the numerous cases of beeswax adulteration. Substances such as ceresin, stearin, natural plant waxes (e.g. Candelilla wax, Carnauba wax), animal waxes and fats (e.g. lanolin, tallow), and mineral waxes (Montan, ozocerite) have been added to beeswax. It is paraffin, though, that is the product most frequently used for beeswax adulteration. Due to having a similar composition and similar physico-chemical properties, the addition of paraffin to beeswax, especially when added in small amounts, is difficult to detect (Bogdanov, 2009; Jimenez et al., 2009; Serra Bonvehi & Ornantes Bermejo, 2012; Maia, Barros, & Nunes, 2013; Svečnjak et al., 2015). The chemical composition of paraffin has not yet been sufficiently analysed. The information

found in literature mostly concerned physico-chemical properties (Bernal et al., 2005) or concerned the estimated data on the paraffin composition stated by the producers. From these data, it follows that the group of long-straight-chained saturated hydrocarbons (n-alkanes) dominate in paraffin. The content of these hydrocarbons is estimated to be at different levels, and even at levels of up to 90%. Depending on the kind of paraffin (in particular the melting point of paraffin), and the purification level of the obtained product, the paraffin can also contain other hydrocarbons (e.g. branched, cyclic, unsaturated as well as hydrocarbons with long alkyl functional groups). The paraffin available on the market differs in the degree of refining, and the content of oil. The classification of such paraffin mainly depends on the solidification point or melting point. The paraffin properties and compositions may differ depending on the intended purpose of the

paraffin (Szpyrka, 1999).

The chromatographic techniques applied for the evaluation of beeswax quality (Tulloch & Hoffman, 1972; Tulloch, 1973; Serra Bonvehi, 1988; Jimenez et al., 2003, 2004, 2006, 2007, 2009; Serra Bonvehi & Ornantes Bermejo, 2012; Maia & Nunes, 2013; Waś, Szczęsna, & Rybak-Chmielewska, 2014a, 2015) are more efficient. These techniques allow for the detection of smaller amounts of adulterant (e.g. paraffin) added to beeswax, than the methods based on the determination of physico-chemical parameters (White & Kushmir, 1961; Tulloch & Hoffman, 1972; Tulloch, 1973; Serra Bonvehi, Cañas Lloria, & Gomez Pajuelo, 1989; Serra Bonvehi, 1990; Vit et al., 1992; Poncini, Poncini, & Prakash, 1993; Bernal et al., 2005; Serra Bonvehi & Ornantes Bermejo, 2012; Maia & Nunes, 2013) or the gravimetric analysis method (DGF-M-V-6, 1957; PN-R-78890, 1996). The amount of paraffin detected also depends, to a great extent, on the kind of paraffin added to beeswax (Jimenez et al., 2009; Serra Bonvehi & Ornantes Bermejo, 2012). As it has been previously stated, the indicators of beeswax adulteration with hydrocarbons of an alien origin (e.g. paraffin) can be hydrocarbons containing even numbers of carbon atoms in the molecule (these hydrocarbons occur in trace amounts in beeswax), and the presence of hydrocarbons containing over 35 atoms of carbon, which were not found in beeswax (White, Reader, & Riethof, 1960; Downing et al., 1961; Streibl, Stransky, & Sorm, 1966; Tulloch & Hoffman, 1972; Tulloch, 1973; Jimenez et al., 2004, 2006, 2007, 2009; Serra Bonvehi & Ornantes Bermejo, 2012). The addition of paraffin results in an increase in the hydrocarbon content. At the same time, the addition of paraffin dilutes the beeswax and results in a lower content of: the esters of palmitic acid (palmitates), hydroxymonoesters, some fatty acids, and alcohols (Jimenez et al., 2006, 2007, 2009; Serra Bonvehi & Ornantes Bermejo, 2012).

In the current work, we continue our investigations into the detection of beeswax adulterated with paraffin (Waś, Szczęsna, & Rybak-

Chmielewska, 2015). Until now we applied and validated the GC-MS method, previously developed for determination of hydrocarbons of beeswax (Waś, Szczęsna, & Rybak-Chmielewska, 2014a) for detection of the adulteration of this product with paraffin. In the previous work (Waś, Szczęsna, & Rybak-Chmielewska, 2015) we tested just one type of paraffin with the total content of n-alkanes ($C_{20}H_{42}$ – $C_{40}H_{82}$) amounted to 68.42 g/100 g, and beeswax mixture with different additions (3, 5, 10, 30, 50%) of this type of paraffin. Based on the results obtained for the basic parameters of validation (repeatability, within-laboratory reproducibility, and recovery) presented in previous paper (Waś, Szczęsna, & Rybak-Chmielewska, 2015) we found that the GC-MS method can be used for detection of beeswax adulteration with hydrocarbons of alien origin (e.g. paraffin). But because of differences in paraffin compositions available on the market, for there to be an estimation of the minimum percent that can be detected by GC-MS, other types of paraffin and beeswax-paraffin mixtures should be tested.

Thus, the aim of our research was to compare different types of paraffin hydrocarbon compositions, and to evaluate the efficiency of the GC-MS method for detecting paraffin adulteration of beeswax.

MATERIAL AND METHODS

Reagents

All reagents used in this experiment were for gas chromatography ($\geq 98\%$ purity). Squalane from Supelco (Bellefonte, PA, USA) was used as an internal standard. Standard mixtures of n-alkanes were purchased from Fluka (Buchs, Switzerland and Saint Louis, MO, USA). Hexane SupraSolv[®] was obtained from Merck (Darmstadt, Germany), and heptane anhydrous 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

Paraffin samples

Paraffin of different physico-chemical properties ($n = 8$) were obtained from different producers and distributors: Paraffin wax (melting point 58-62°C, Sigma Aldrich), Paraffin LTP 64/25 (solidification point 62.4°C, Lotos Group, Jasło), Paraffin LTP 56H (solidification point 58.0°C, Lotos Group, Jasło), Paraffin LTP 53/35 (solidification point 53.0°C, Lotos Group, Jasło), Paraffin LTP 56/25 (solidification point 56.4°C, Lotos Group, Jasło), Paraffin PW 573 (solidification point 58.5°C, PPH Standard, Lublin), Paraffin P-1/C (Centro-Chem, Lublin), and an unknown type of paraffin (Naftan, Oleksze).

Beeswax-paraffin mixtures

In the laboratory, virgin beeswax was adulterated with 3, 5, 10, 30, and 50% additions of paraffin LTP 56/25 and PW 573, as was described previously (Waś, Szczęsna, & Rybak-Chmielewska, 2015). These samples were used to evaluate the suitability of the GC-MS method for detecting beeswax adulterated with paraffin, and for estimating the minimum percentage of paraffin that can be detected in beeswax by GC-MS.

Real beeswax samples

The research material consisted of two types of materials: beeswax foundation sheets ($n = 28$) and commercial beeswax (in the form of melted chunks, $n = 13$). The foundation beeswax samples ($n = 18$) were obtained during the 2007 to 2014 time period from individual beekeepers cooperating in the study, and from colleagues of other Faculties of the Apiculture Department. The rest of the beeswax foundation ($n = 10$) as well as the commercial beeswax samples ($n = 13$) were sent for testing from 2011 to 2014, to the Bee Products Quality Testing Laboratory, Apiculture Department, Research Institute of Horticulture in Puławy, Poland.

Sample preparation

The preparation procedure applied to all tested samples was as reported previously (Waś,

Szczęsna, & Rybak-Chmielewska, 2014a). An amount of 0.05 ± 0.001 g from the sample, was dissolved in 7.5 mL of heptane. The solution was mechanically shaken for 12 min in 50°C to complete the dissolution of the sample. After cooling, an addition of 2.5 mL of 400 mg/L of squalane (IS) was made. Then, the hydrocarbon fraction was isolated using the SPE technique with cartridges filled with neutral aluminum oxide after elution with 3 mL of hexane.

Gas chromatography - mass spectrometry performance

The gas chromatography - mass detector analysis of hydrocarbons was conducted using the Shimadzu GCMS-QP 2010 Plus system (Shimadzu, Kyoto, Japan) which was equipped with an AOC-5000 autosampler. For chromatographic separation, the ZB-5HT INFERNO column (20 m \times 0.18 mm \times 0.18 μ m, Phenomenex) was used. Identification of the studied compounds was performed based on the mass spectra of the NIST 05 library and on the retention indexes. The quantitative analysis of n-alkanes was made using the internal standard method. The technique was previously described in more detail (Waś, Szczęsna, & Rybak-Chmielewska, 2014a). The gas chromatography - mass detector method has been validated for the determination of hydrocarbons in pure beeswax, and in paraffin as well as beeswax adulterated with paraffin. The recovery of the individual n-alkanes was from 94.4 to 105.9% (in pure beeswax), and from 85.7 to 104.2% (in paraffin). For the total n-alkanes in paraffin and beeswax, the recovery was 98.7 and 100.5%, respectively. The limits of determination of the method defined for each alkane ($C_{20}H_{42}$ - $C_{40}H_{82}$) were from 0.005 to 0.05 g/100 g (Waś, Szczęsna, & Rybak-Chmielewska, 2014a, 2015).

Quality assessment of real beeswax samples

In assessing the quality of commercial beeswax, the following criteria indicating its adulteration with hydrocarbons of alien origin (e.g. paraffin) were used: the presence of hydrocarbons containing over 35 carbon atoms in the molecule, higher contents of individual

n-alkanes ($C_{20}H_{42}$ – $C_{35}H_{72}$) as well as a higher content for the total of these compounds in comparison to the maximum values determined in pure beeswax (Waś, Szczesna, & Rybak-Chmielewska, 2014b). The maximum permissible concentrations accepted for pure beeswax amounted to 11.7 g/100 g (for total n-alkanes $C_{20}H_{42}$ – $C_{35}H_{72}$) and 1.0 g/100 g (for total even-numbered n-alkanes from $C_{20}H_{42}$ to $C_{34}H_{70}$). The uncertainty of the method - 4% for total content of n-alkanes, was estimated based on the coefficient of variation for within-laboratory reproducibility (Waś, Szczesna, & Rybak-Chmielewska, 2014a). The limit of determination for total n-alkanes $C_{36}H_{74}$ – $C_{40}H_{82}$ was established for 0.1 g/100 g.

RESULTS

Hydrocarbon composition of different types of paraffin

All of the tested types of paraffin contained from 20 to 40 atoms of carbon in the molecule. Longer carbon chains were found in the paraffin: LTP 53/35, the paraffin purchased from Naftan (up to 42), Paraffin wax and LTP 64/25 (up to 44), and PW 573 (up to 45). The examples of chromatograms of paraffin hydrocarbons were presented at Fig. 1a,b,c. Diverse paraffin compositions were also confirmed by the quantitative analysis of the linear alkanes (Tab. 1). Depending on the kind of paraffin, the contents of individual alkanes were different and amounted to a few of g/100 g. The coefficients of variation (CV) for the content of individual n-alkanes determined in paraffin were very high, and for some of compounds ($C_{21}H_{44}$, $C_{38}H_{78}$ and $C_{40}H_{82}$) exceeded 100%. The highest average contents (4.99 g/100 g) were found for $C_{26}H_{54}$, and the lowest (below 1 g/100 g) for $C_{20}H_{42}$, $C_{21}H_{44}$, and for the alkanes of the homologous series - from $C_{37}H_{76}$ to $C_{40}H_{82}$. The hexacosane ($C_{26}H_{54}$) turned out to be the dominant alkane for the majority ($n = 5$) of paraffin types. The range for the total content of n-alkanes was from 37.04 to 57.41 g/100 g. The sum contents of alkanes with even and odd numbers of carbon atoms in the molecule (up to 35 atoms of carbon), were almost identical. The

contents, on average, amounted to 23.43 g/100 g for the even-numbered alkanes, and 23.16 g/100 g for the odd-numbered, which comprised 47.0 and 46.4%, respectively, of all n-alkanes detected in the paraffin samples. In all types of paraffin, there were alkanes containing over 35 atoms of carbon in the molecule. The content of these alkanes ranged from 0.26 g/100 g (in P-1/C) to 8.89 g/100 g (in LTP 64/25), on average 3.30 g/100 g. Their average percentage was 6.6% of all the determined alkanes. In the case of paraffin, in which the content of these alkanes was the highest, the percentage even reached 24.0%. In some paraffin samples, the contents of these alkanes would be even higher than stated, but due to the lack of standards it was impossible to quantitatively determine alkanes containing over 40 atoms of carbon in a molecule.

GC-MS analysis of beeswax - paraffin mixtures

Based on the comparative analysis of the chromatograms of n-alkanes in pure beeswax used to prepare beeswax-paraffin mixtures (Fig. 2a) and in beeswax with different paraffin additions of 3% (Fig. 2b,c), 5, 10, 30, and 50%, the paraffin added to beeswax can be easily detected. Obviously, when the amount of paraffin added to the beeswax increased, the intensity of the peaks of individual alkanes also increased. The increase in the peaks' intensity was mostly visible for the alkanes with even numbers of carbon atoms in the molecule even if there was only a 3% addition of paraffin (Fig. 2b,c). Moreover, in the chromatograms of beeswax adulterated with paraffin (Fig. 2b,c) there appeared alkanes $C_{36}H_{74}$ – $C_{40}H_{82}$, which were not found in the pure beeswax (Fig. 2a). Also noteworthy are the higher peak intensities of the unsaturated hydrocarbons (alkenes), and unidentified peaks of compounds belonging to other homologous series (e.g. branched or cyclic hydrocarbons) visible by the peaks which appeared between the identified n-alkanes. This was particularly true in the chromatograms of beeswax with 30 and 50% additions of paraffin. Our observations with the fingerprints of

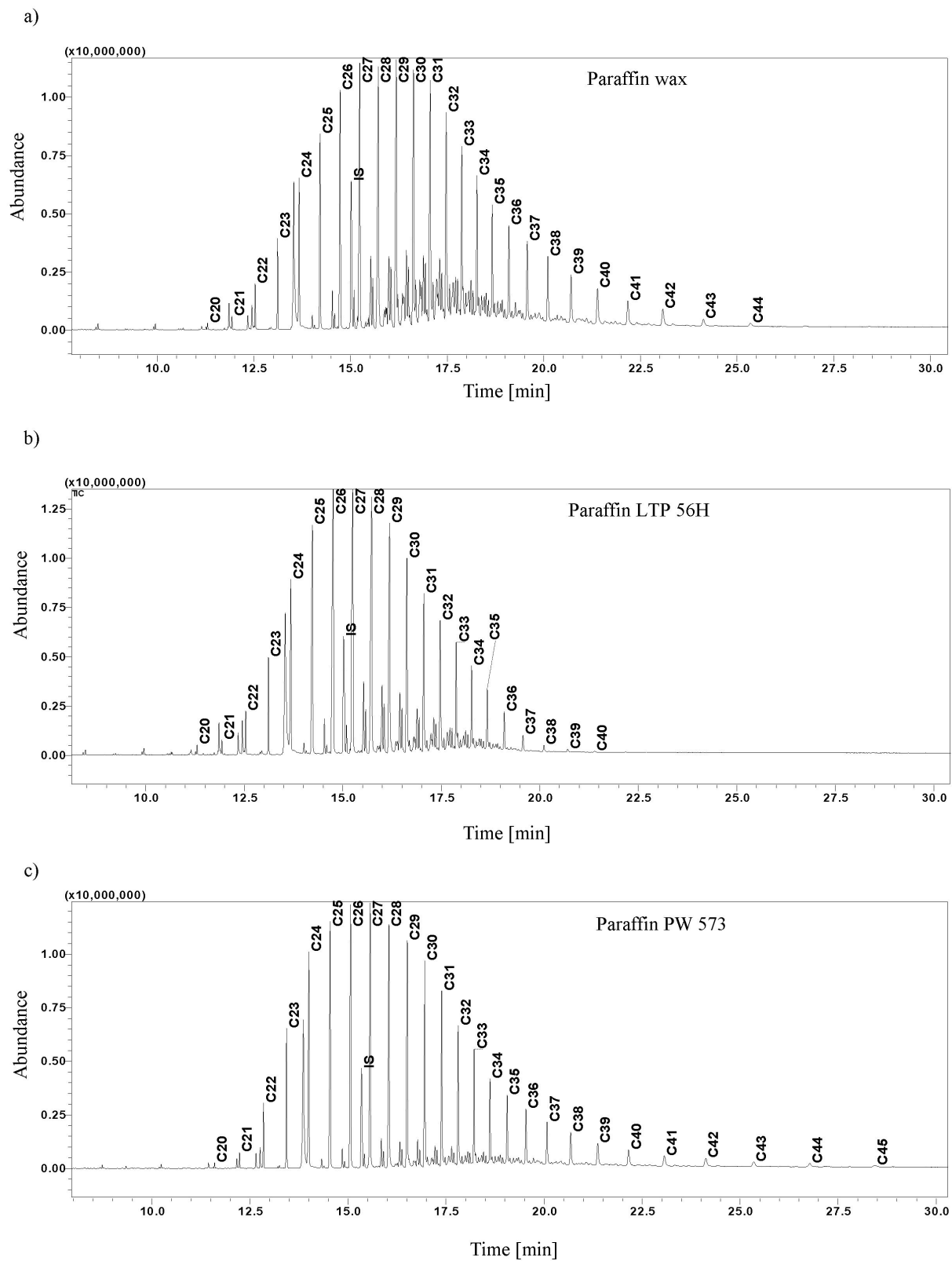


Fig. 1. GC-MS chromatograms of hydrocarbons in different types of paraffin: a) Paraffin wax b) LTP 56H c) PW 573; (C_{20} - C_{45} - n-alkanes with the formula $C_{20}H_{42}$ – $C_{45}H_{92}$; IS - internal standard - $C_{30}H_{62}$)

Table 1
Contents (g/100 g) of n-alkanes in different paraffin types

Formula of n-alkanes	Paraffin wax	LTP 64/25	LTP 56H	LTP 56/25	LTP 53/35	PW 573	Naftan	P-1/C	Min - Max	Mean \pm SD	CV (%)
$C_{20}H_{42}$	0.07	0.08	0.11	0.48	0.36	0.11	0.11	0.36	0.07 - 0.48	0.21 \pm 0.16	77.3
$C_{21}H_{44}$	0.16	0.08	0.16	1.28	1.10	0.24	0.52	2.54	0.08 - 2.54	0.76 \pm 0.85	112.1
$C_{22}H_{46}$	0.54	0.21	0.49	2.47	2.22	1.11	1.33	4.19	0.21 - 4.19	1.57 \pm 1.33	84.8
$C_{23}H_{48}$	1.12	0.33	1.21	3.80	3.59	2.48	2.03	5.20	0.33 - 5.20	2.47 \pm 1.63	66.1
$C_{24}H_{50}$	2.26	0.73	3.04	5.76	5.45	4.46	3.37	5.51	0.73 - 5.76	3.82 \pm 1.79	46.9
$C_{25}H_{52}$	2.87	0.85	4.72	5.77	5.53	5.31	3.60	5.10	0.85 - 5.77	4.22 \pm 1.69	40.0
$C_{26}H_{54}$	4.05	1.16	7.21	6.36	5.83	5.90	4.18	5.22	1.16 - 7.21	4.99 \pm 1.88	37.6
$C_{27}H_{56}$	4.83	1.30	6.61	5.63	5.14	5.87	3.95	4.90	1.30 - 6.61	4.78 \pm 1.61	33.7
$C_{28}H_{58}$	5.48	1.56	6.48	5.71	4.70	5.67	3.99	4.73	1.56 - 6.48	4.79 \pm 1.51	31.6
$C_{29}H_{60}$	5.28	1.92	5.28	4.43	3.79	5.23	4.03	4.05	1.92 - 5.28	4.25 \pm 1.12	26.4
$C_{30}H_{62}$	4.77	2.65	3.76	3.44	3.06	4.20	4.14	3.64	2.65 - 4.77	3.71 \pm 0.67	18.2
$C_{31}H_{64}$	4.52	3.76	2.89	2.63	2.57	3.56	4.10	2.87	2.57 - 4.52	3.36 \pm 0.73	21.7
$C_{32}H_{66}$	3.80	4.17	2.25	1.83	2.00	2.72	3.62	1.94	1.83 - 4.17	2.79 \pm 0.94	33.6
$C_{33}H_{68}$	2.60	3.67	1.58	1.07	1.30	2.26	2.62	0.97	1.07 - 3.67	2.01 \pm 0.94	46.8
$C_{34}H_{70}$	2.05	2.86	1.18	0.77	1.01	1.83	2.21	0.50	0.77 - 2.86	1.55 \pm 0.81	52.5
$C_{35}H_{72}$	1.81	2.81	0.87	0.54	0.80	1.54	1.89	0.24	0.54 - 2.81	1.31 \pm 0.85	65.1
$C_{36}H_{74}$	1.47	2.46	0.49	0.34	0.60	1.25	1.41	0.11	0.34 - 2.46	1.01 \pm 0.78	76.5
$C_{37}H_{76}$	1.38	2.09	0.22	0.21	0.42	1.19	0.90	0.06	0.21 - 2.09	0.81 \pm 0.71	87.6
$C_{38}H_{78}$	1.16	1.70	0.10	0.13	0.28	1.00	0.50	0.04	0.10 - 1.70	0.61 \pm 0.61	98.8
$C_{39}H_{80}$	0.96	1.52	0.05	0.07	0.20	0.83	0.28	0.03	0.05 - 1.52	0.49 \pm 0.55	111.4
$C_{40}H_{82}$	0.81	1.13	0.03	0.04	0.11	0.68	0.14	0.03	0.03 - 1.13	0.37 \pm 0.44	118.2
Total $C_{20}H_{42}$ - $C_{40}H_{82}$	51.99	37.04	48.73	52.77	50.05	57.41	48.92	52.20	37.04 - 57.41	49.89 \pm 5.88	11.8
Total ENA*	23.02	13.43	24.52	26.83	24.63	26.00	22.95	26.07	13.43 - 26.83	23.43 \pm 4.28	18.3
Total ONA**	23.19	14.73	23.32	25.14	23.81	26.47	22.74	25.87	14.73 - 26.47	23.16 \pm 3.67	15.8
Total $C_{36}H_{74}$ - $C_{40}H_{82}$	5.78	8.89	0.89	0.80	1.60	4.94	3.23	0.26	0.26 - 8.89	3.30 \pm 3.03	91.9

* ENA - total content of even-numbered n-alkanes from $C_{20}H_{42}$ to $C_{34}H_{70}$

** ONA - total content of odd-numbered n-alkanes from $C_{21}H_{44}$ to $C_{35}H_{72}$

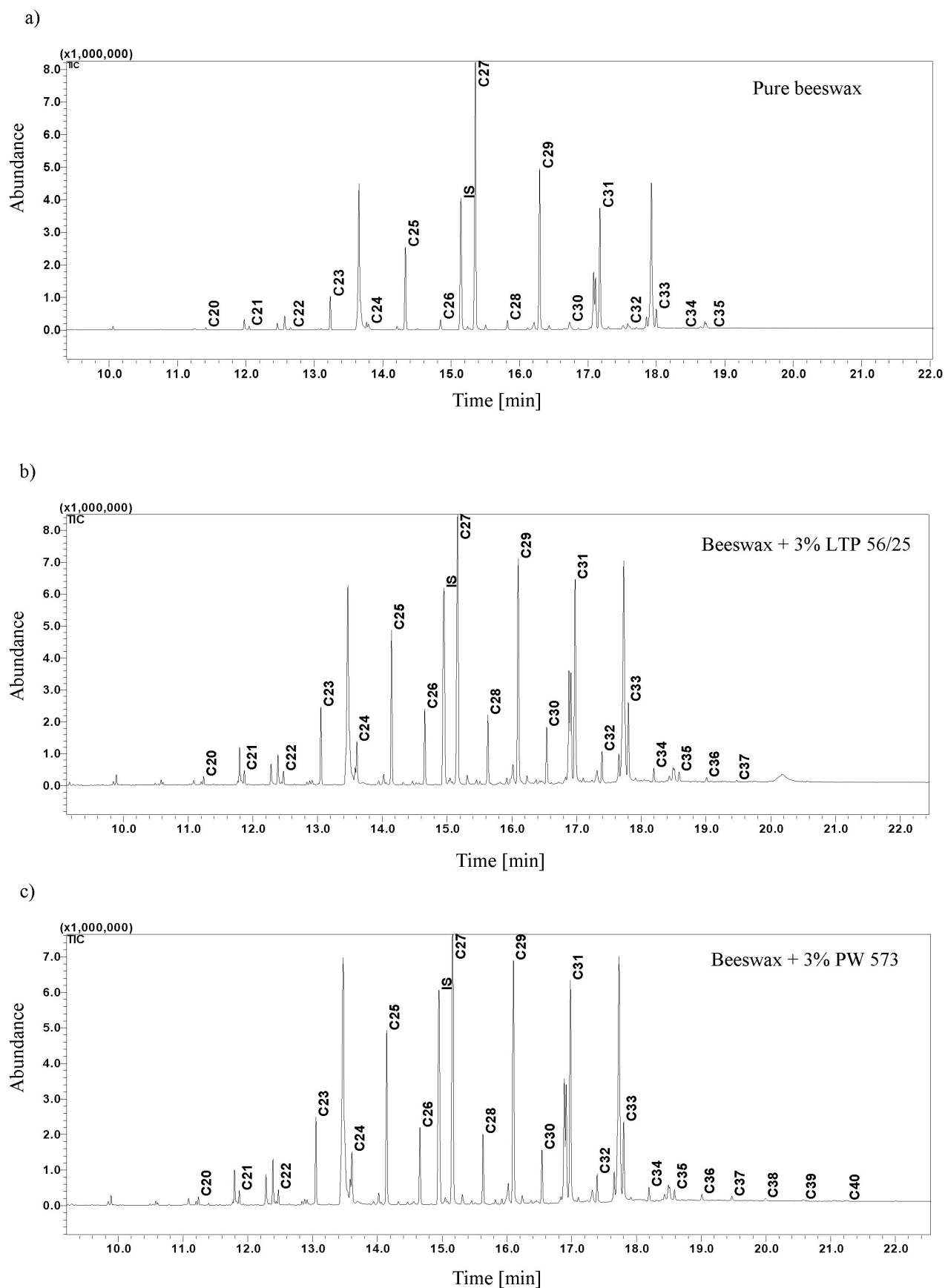


Fig. 2. GC-MS chromatograms of n-alkanes ($C_{20}H_{42}$ – $C_{40}H_{82}$) in pure beeswax (a) and with 3% additions of the paraffin: LTP 56/25 (b) and PW 573 (c); IS – internal standard – $C_{30}H_{62}$

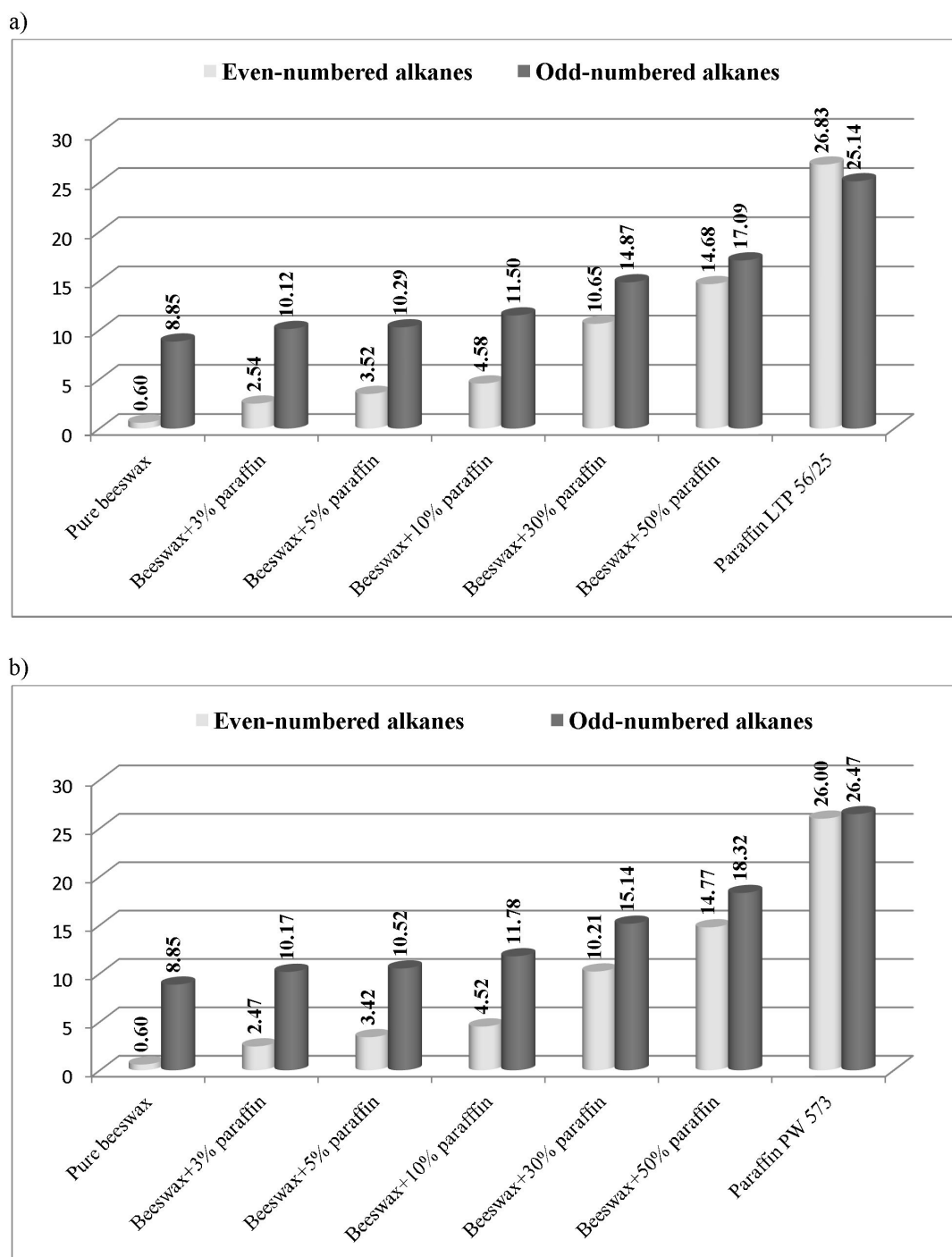


Fig. 3. Contents (g/100 g) of even and odd-numbered n-alkanes ($C_{20}H_{42}$ – $C_{35}H_{72}$) in beeswax with different additions of paraffin LTP 56/25 (a) and PW 573 (b).

beeswax-paraffin mixtures, confirmed the results of the quantitative analysis (Fig. 3a,b). The addition of paraffin to beeswax resulted in increased contents of individual alkanes. There was a particular increase in the even-numbered alkanes and their percentages in the total content of determined alkanes. The content of alkanes with even numbers of carbon atoms in

a molecule of pure beeswax, was 0.60 g/100 g, which comprised only 6.4% of the determined alkanes. The content and percentages of even-numbered n-alkanes significantly increased when the amount of paraffin added to beeswax increased. The percentage of even alkanes in beeswax with 3, 5, 10, and 30% additions of paraffin was calculated for c.a. 20, 25, 30, and

40%, respectively, in the total content of alkanes determined in these mixtures. In beeswax with a 50% addition of paraffin, the percentage of these alkanes was the highest and amounted for 46.2 (in the mixture with LTP 56/25) and 44.6% (in the mixture with PW 573). Moreover, the share of even alkanes in beeswax with a 50% addition of paraffin was comparable to the percentage of even-numbered n-alkanes in pure paraffin (51.6% in LTP 56/25 and 49.6% in PW 573). The content of odd-numbered alkanes also increased when the amount of paraffin added to the beeswax increased, but their percentage in the total content of all the alkanes decreased (Fig. 3a,b). The lowest (3%) addition of paraffin resulted in an increased total content of alkanes; from 9.45 to 12.66 g/100 g (for LTP 56/25) and 12.81 g/100 g (for PW 573). In the case of the 50% paraffin additions, the total content of n-alkanes increased to 32.61 and 35.50 g/100 g, respectively. In the case of the 3% addition of paraffin LTP 56/25, the content of the alkanes $C_{36}H_{74}$ - $C_{40}H_{82}$ was found to be lower than the assumed limit of determination for the total content of these alkanes (0.10 g/100 g). For beeswax with a 3% addition of paraffin PW 573, the content of these n-alkanes was found to be 0.17 g/100 g. It is obvious, that the highest contents of alkanes $C_{36}H_{74}$ - $C_{40}H_{82}$ were found in samples with a 50% addition of paraffin, and comprised 2.6 and 6.8% of the total content of all n-alkanes determined in these samples.

When there is a presence of alkanes containing

over 35 carbon atoms it can indicate beeswax adulterated with hydrocarbons of an alien origin (such as paraffin). This adulteration is also indicated by the higher contents of n-alkanes ($C_{20}H_{42}$ - $C_{35}H_{72}$) than the maximum concentrations determined for these compounds in pure beeswax. In this work, the estimated minimum percentage of paraffin that can be detected with the GC-MS technique, is 3%.

Quality assessment of real beeswax samples

Most of the beeswax foundation samples (25 from 28) had the same homologues series of n-alkanes (from $C_{20}H_{42}$ to $C_{35}H_{72}$) as well as some of the same alkenes ($C_{23}H_{46}$, $C_{25}H_{50}$, $C_{27}H_{54}$, $C_{29}H_{58}$, $C_{31}H_{62}$, $C_{33}H_{66}$, $C_{35}H_{70}$) and dienes ($C_{31}H_{60}$, $C_{33}H_{64}$, $C_{35}H_{68}$) which were found in natural beeswax. The same hydrocarbon composition had 6 samples of commercial beeswax (in the form of melted chunks) obtained for testing. An example of a hydrocarbon profile recorded for a foundation sample made from pure beeswax is presented in Fig. 4. The results of the quantitative analysis were collected and shown in Tables 2 and 3. The total content of n-alkanes in authentic samples were from 8.66 to 11.00 g/100 g (in foundation samples, Tab. 2) and from 8.73 to 11.01 g/100 g (in commercial beeswax samples, Tab. 3). The contents of individual n-alkanes as well as the total content of these compounds found in authentic samples were within the range determined in pure beeswax. There were no hydrocarbons of an alien origin,

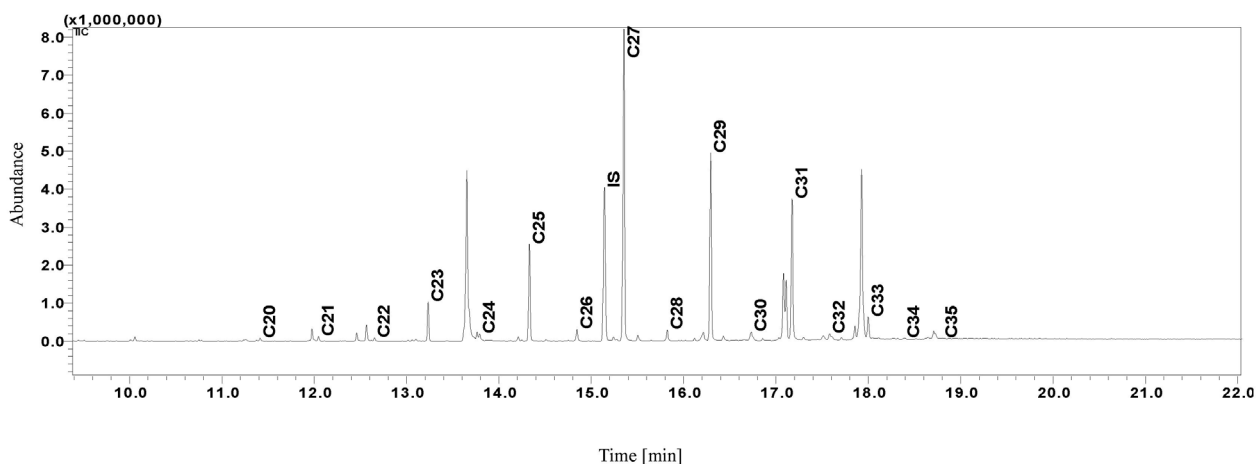


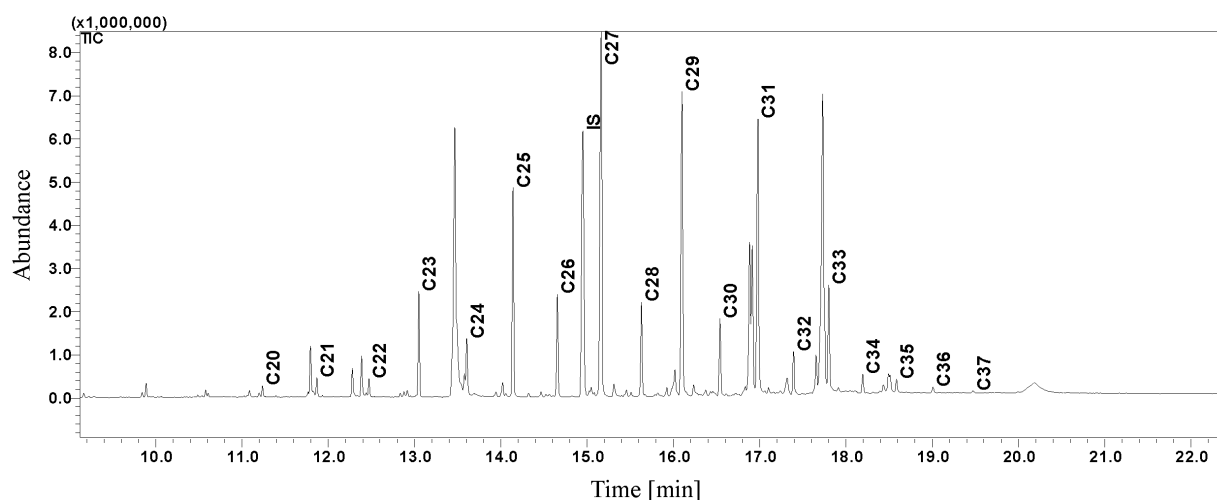
Fig. 4. GC-MS chromatogram of hydrocarbons in pure foundation beeswax (C_{20} - C_{35} - n-alkanes with the formula $C_{20}H_{42}$ - $C_{35}H_{72}$; IS - internal standard - $C_{30}H_{62}$).

containing over 35 carbon atoms.

In some samples (3 samples of foundation and 7 samples of commercial beeswax) sent to the laboratory for testing, hydrocarbons of an alien origin were found (Tab. 2 and 3). In nine adulterated samples, the total content of n-alkanes as well as the contents determined for most individual compounds were higher in comparison to the amounts determined in pure beeswax. The maximum content determined for the total of n-alkanes was 29.31 g/100 g. The ranges for total even-numbered alkanes determined in adulterated samples were from 0.97 to 7.99 g/100 g (in adulterated foundation,

Tab. 2) and from 2.55 to 13.31 g/100 g (in adulterated commercial beeswax, Tab. 3). In one adulterated sample (foundation beeswax), the determined amounts were within the range determined in pure beeswax (except $C_{34}H_{70}$ and $C_{35}H_{72}$), but the presence of hydrocarbons with more than 35 carbon atoms in the molecule assures the adulteration. These hydrocarbons were found in all adulterated samples, and their total contents were from 0.29 to 2.97 g/100 g, with an average of 1.41 g/100 g (in adulterated foundation, Tab. 2) and from 0.23 to 4.53 g/100 g, with an average of 1.26 g/100 g (in adulterated commercial beeswax, Tab. 3).

a)



b)

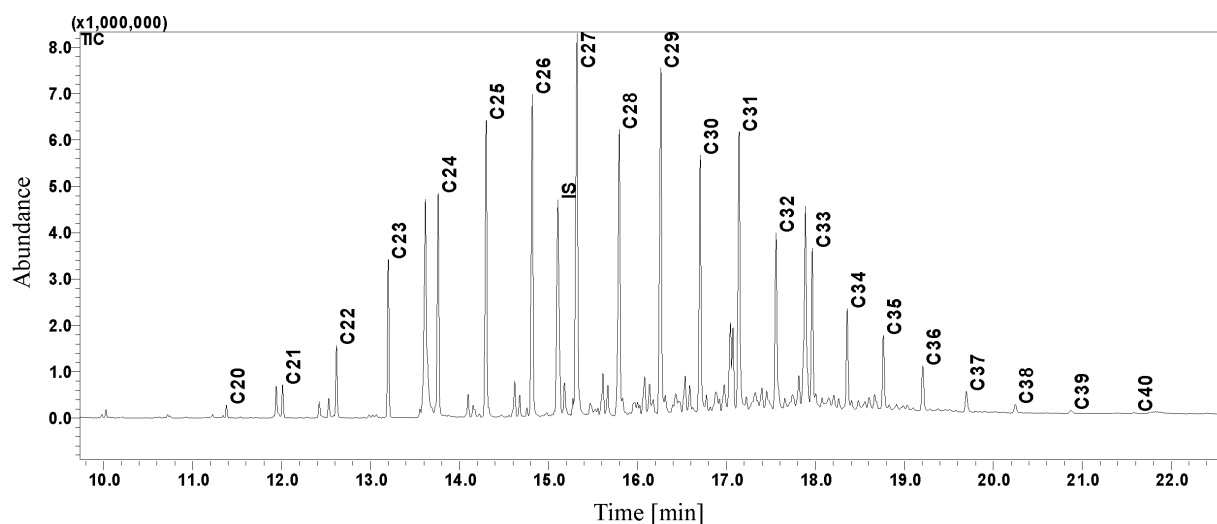


Fig. 5. GC-MS chromatograms of hydrocarbons in beeswax adulterated with paraffin a) total of n-alkanes equal to 12.9 g/100 g; b) total of n-alkanes equal to 29.3 g/100 g ($C_{20} - C_{40}$ - n-alkanes with the formula $C_{20}H_{42} - C_{40}H_{82}$; IS - internal standard - $C_{30}H_{62}$)

Table 2
Contents (g/100 g) of n-alkanes in beeswax foundation samples

Formula of n-alkanes	Authentic foundation (n = 25)			Adulterated foundation (n = 3)			Concentration (g/100 g) in pure beeswax (n = 70)*
	Min – Max	Mean ± SD	Min – Max	Mean ± SD	Min – Max	Min – Max	
C ₂₀ H ₄₂	0.02 – 0.06	0.04 ± 0.01	0.02 – 0.50	0.03 ± 0.01	0.01 – 0.06	0.01 – 0.06	
C ₂₁ H ₄₄	0.03 – 0.10	0.07 ± 0.02	0.08 – 0.14	0.11 ± 0.03	0.03 – 0.10	0.03 – 0.10	
C ₂₂ H ₄₆	0.02 – 0.09	0.06 ± 0.02	0.04 – 0.19	0.11 ± 0.07	0.02 – 0.09	0.02 – 0.09	
C ₂₃ H ₄₈	0.23 – 0.65	0.54 ± 0.11	0.55 – 0.83	0.67 ± 0.14	0.12 – 0.68	0.12 – 0.68	
C ₂₄ H ₅₀	0.05 – 0.13	0.11 ± 0.02	0.11 – 0.73	0.41 ± 0.31	0.03 – 0.13	0.03 – 0.13	
C ₂₅ H ₅₂	0.87 – 1.47	1.28 ± 0.15	1.04 – 1.59	1.37 ± 0.29	0.42 – 1.47	0.42 – 1.47	
C ₂₆ H ₅₄	0.09 – 0.22	0.19 ± 0.04	0.20 – 1.20	0.70 ± 0.50	0.06 – 0.22	0.06 – 0.22	
C ₂₇ H ₅₆	2.64 – 3.67	3.16 ± 0.26	2.50 – 3.29	2.96 ± 0.41	2.44 – 4.40	2.44 – 4.40	
C ₂₈ H ₅₈	0.07 – 0.19	0.16 ± 0.04	0.19 – 1.42	0.81 ± 0.60	0.06 – 0.19	0.06 – 0.19	
C ₂₉ H ₆₀	1.33 – 2.40	2.18 ± 0.23	2.29 – 2.72	2.47 ± 0.22	1.68 – 2.73	1.68 – 2.73	
C ₃₀ H ₆₂	0.07 – 0.19	0.14 ± 0.04	0.19 – 1.64	0.92 ± 0.73	0.05 – 0.19	0.05 – 0.19	
C ₃₁ H ₆₄	1.48 – 2.17	1.90 ± 0.16	2.09 – 2.51	2.24 ± 0.24	1.53 – 2.64	1.53 – 2.64	
C ₃₂ H ₆₆	0.03 – 0.10	0.06 ± 0.02	0.11 – 1.56	0.84 ± 0.72	0.01 – 0.12	0.01 – 0.12	
C ₃₃ H ₆₈	0.15 – 0.72	0.41 ± 0.14	0.74 – 1.63	1.12 ± 0.46	0.31 – 0.76	0.31 – 0.76	
C ₃₄ H ₇₀	<0.025** – 0.03	–	0.08 – 1.20	0.62 ± 0.56	< 0.025** – 0.03	< 0.025** – 0.03	
C ₃₅ H ₇₂	<0.025** – 0.03	–	0.12 – 1.03	0.53 ± 0.46	< 0.025** – 0.03	< 0.025** – 0.03	
C ₃₆ H ₇₄	n.d.***	n.d.***	0.08 – 0.82	0.41 ± 0.38	n.d.***	n.d.***	
C ₃₇ H ₇₆	n.d.***	n.d.***	0.06 – 0.78	0.37 ± 0.37	n.d.***	n.d.***	
C ₃₈ H ₇₈	n.d.***	n.d.***	0.05 – 0.59	0.28 ± 0.28	n.d.***	n.d.***	
C ₃₉ H ₈₀	n.d.***	n.d.***	0.05 – 0.43	0.20 ± 0.20	n.d.***	n.d.***	
C ₄₀ H ₈₂	n.d.***	n.d.***	0.05 – 0.33	0.16 ± 0.15	n.d.***	n.d.***	
Total C ₂₀ H ₄₂ – C ₄₀ H ₈₂	8.66 – 11.00	10.31 ± 0.53	10.70 – 24.48	17.32 ± 6.90	8.27 – 11.66	8.27 – 11.66	
Total ENA****	0.36 – 0.98	0.76 ± 0.16	0.97 – 7.99	4.43 ± 3.51	0.26 – 0.99	0.26 – 0.99	
Total ONA*****	8.15 – 10.01	9.57 ± 0.40	9.43 – 13.53	11.47 ± 2.05	8.00 – 11.18	8.00 – 11.18	
Total C ₃₆ H ₇₄ – C ₄₀ H ₈₂	–	–	0.29 – 2.97	1.41 ± 1.39	–	–	

* contents of n-alkanes determined in pure beeswax collected from light and dark coloured combs (Waś, Szczesna, & Rybak-Chmielewska, 2014b), proposed as concentration guide-values to distinguish between pure and adulterated beeswax

** limit of determination

*** n.d. - not detected at limits of determination 0.025 g/100 g for C₃₈H₇₈ and 0.05 g/100 g for C₃₉H₈₀ and C₄₀H₈₂

**** ENA – total content of even-numbered n-alkanes from C₂₀H₄₂ to C₃₄H₇₀

***** ONA – total content of odd-numbered n-alkanes from C₂₁H₄₄ to C₃₅H₇₂

Table 3

Contents (g/100 g) of n-alkanes in commercial beeswax samples

Formula of n-alkanes	Authentic samples (n = 6)		Adulterated samples (n = 7)		Concentration (g/100 g) in pure beeswax (n = 70)*	
	Min – Max	Mean ± SD	Min – Max	Mean ± SD	Min	Max
C ₂₀ H ₄₂	0.02 – 0.06	0.03 ± 0.01	0.03 – 0.50	0.11 ± 0.17	0.01	0.06
C ₂₁ H ₄₄	0.04 – 0.10	0.08 ± 0.02	0.03 – 0.23	0.12 ± 0.07	0.03	0.10
C ₂₂ H ₄₆	0.04 – 0.08	0.05 ± 0.01	0.05 – 0.90	0.24 ± 0.30	0.02	0.09
C ₂₃ H ₄₈	0.36 – 0.68	0.53 ± 0.14	0.28 – 0.96	0.60 ± 0.20	0.12	0.68
C ₂₄ H ₅₀	0.05 – 0.13	0.11 ± 0.03	0.13 – 1.38	0.53 ± 0.43	0.03	0.13
C ₂₅ H ₅₂	0.99 – 1.45	1.20 ± 0.21	0.80 – 1.59	1.28 ± 0.27	0.42	1.47
C ₂₆ H ₅₄	0.10 – 0.22	0.18 ± 0.05	0.26 – 1.67	0.77 ± 0.48	0.06	0.22
C ₂₇ H ₅₆	2.50 – 3.48	2.97 ± 0.32	1.41 – 2.90	2.55 ± 0.52	2.44	4.40
C ₂₈ H ₅₈	0.08 – 0.19	0.15 ± 0.04	0.40 – 2.00	0.86 ± 0.56	0.06	0.19
C ₂₉ H ₆₀	1.93 – 2.32	2.17 ± 0.15	1.77 – 2.51	2.16 ± 0.27	1.68	2.73
C ₃₀ H ₆₂	0.05 – 0.19	0.15 ± 0.06	0.48 – 2.18	0.88 ± 0.61	0.05	0.19
C ₃₁ H ₆₄	1.37 – 2.19	1.89 ± 0.28	1.82 – 2.30	2.07 ± 0.18	1.53	2.64
C ₃₂ H ₆₆	0.01 – 0.12	0.07 ± 0.04	0.36 – 2.61	0.92 ± 0.89	0.01	0.12
C ₃₃ H ₆₈	0.15 – 0.71	0.53 ± 0.21	0.77 – 2.30	1.16 ± 0.56	0.31	0.76
C ₃₄ H ₇₀	<0.025** – 0.03	–	0.17 – 2.08	0.62 ± 0.74	< 0.025**	– 0.03
C ₃₅ H ₇₂	<0.025** – 0.03	–	0.18 – 1.78	0.59 ± 0.62	< 0.025**	– 0.03
C ₃₆ H ₇₄	n.d.***	n.d.***	0.09 – 1.49	0.42 ± 0.56	n.d.***	
C ₃₇ H ₇₆	n.d.***	n.d.***	0.07 – 0.93	0.29 ± 0.37	n.d.***	
C ₃₈ H ₇₈	n.d.***	n.d.***	0.05 – 0.95	0.25 ± 0.36	n.d.***	
C ₃₉ H ₈₀	n.d.***	n.d.***	0.05 – 0.46	0.15 ± 0.20	n.d.***	
C ₄₀ H ₈₂	n.d.***	n.d.***	0.05 – 0.71	0.21 ± 0.30	n.d.***	
Total C ₂₀ H ₄₂ – C ₄₀ H ₈₂	8.73 – 11.01	10.12 ± 0.86	12.91 – 29.31	16.75 ± 5.88	8.27	11.66
Total ENA****	0.35 – 0.99	0.74 ± 0.22	2.55 – 13.31	4.94 ± 3.86	0.26	0.99
Total ONA*****	8.38 – 10.09	9.38 ± 0.65	10.06 – 11.48	10.54 ± 0.50	8.00	11.18
Total C ₃₆ H ₇₄ – C ₄₀ H ₈₂	–	–	0.23 – 4.53	1.26 ± 1.73	–	–

* contents of n-alkanes determined in pure beeswax collected from light and dark coloured combs (Waś, Szczesna, & Rybak-Chmielewska, 2014b), proposed as concentration guide-values to distinguish between pure and adulterated beeswax

** limit of determination

*** n.d. - not detected at limits of determination 0.025 g/100 g for C₃₈H₇₈ and 0.05 g/100 g for C₃₉H₈₀ and C₄₀H₈₂

**** ENA - total content of even-numbered n-alkanes from C₂₀H₄₂ to C₃₄H₇₀

***** ONA - total content of odd-numbered n-alkanes from C₂₁H₄₄ to C₃₅H₇₂

Chromatograms of hydrocarbons in beeswax samples adulterated with paraffin are shown in Fig. 5a,b. In these chromatograms the increase in the peaks' intensity of individual alkanes (in particular alkanes with even numbers of carbon atoms in the molecule) as well as a higher intensity of the peaks of unsaturated hydrocarbons (alkenes) can be observed. Moreover, in the chromatograms of beeswax adulterated with paraffin, there appeared alkanes $C_{36}H_{74}$ - $C_{40}H_{82}$ and other compounds belonging to other homologous series (e.g. branched or cyclic hydrocarbons). These compounds are visible by the peaks appearing in the chromatograms between the identified n-alkanes.

DISCUSSION

Learning the possible paraffin compositions is important because paraffin is the most frequently used product in adulteration of beeswax. The current studies on paraffin composition have shown that those paraffins available on the market, differ not only in physico-chemical properties but also in the qualitative and quantitative composition of the hydrocarbons. In all types of paraffin, the identified homologous series of n-alkanes were much longer (containing over 35 carbon atoms in the molecule) than in beeswax, as was previously reported (Jimenez et al., 2009; Serra Bonvehi & Ornantes Bermejo, 2012; Waś, Szczęsna, & Rybak-Chmielewska, 2015). We did not find hydrocarbons containing below 20 carbon atoms in the molecule as had been mentioned by other authors, that may be included in some paraffin types (Szpyrka, 1999; Jimenez et al., 2004; Serra Bonvehi & Ornantes Bermejo, 2012). Our studies of paraffin composition proved that an almost identical content of even- and odd-numbered alkanes is a characteristic feature of paraffin. In the case of beeswax, the average percentage of alkanes with even numbers of carbon atoms in the molecule was c.a. 5 and 6% in beeswax obtained from light and dark coloured combs, respectively (Waś, Szczęsna, & Rybak-Chmielewska, 2014b). The higher content of even-numbered alkanes in the paraffin, was

reported in our previous work (Waś, Szczęsna, & Rybak-Chmielewska, 2015) as well as by other authors (Jimenez et al., 2009; Serra Bonvehi & Ornantes Bermejo, 2012). Moreover, the content of linear alkanes determined in the current work suggests that besides this group of hydrocarbons, other compounds belonging to other homologous series (e.g. alkenes, branched or cyclic hydrocarbons) have a significant share in paraffin. The presence of these compounds in paraffin is indicated by the peaks appearing in the chromatograms between the identified n-alkanes. Other authors also mentioned that, in addition to linear alkanes, other hydrocarbons occur in paraffin. These authors stressed, however, that the share of other hydrocarbons can differ depending on the kind of paraffin (Jimenez et al., 2009; Serra Bonvehi & Ornantes Bermejo, 2012).

There are differences in the paraffin composition and its difficult to detect, especially small amounts of paraffin added to beeswax. For these reasons, the studies with beeswax-paraffin mixtures initiated previously (Waś, Szczęsna, & Rybak-Chmielewska, 2015), were extended here to include mixtures with other types of paraffin. Based on the results obtained for beeswax with different additions of varied types of paraffin, we estimated the efficiency of the method for detection of beeswax adulteration with paraffin. We also estimated the minimum percentage of paraffin that can be detected by GC-MS. Moreover, our earlier studies of the composition of beeswax hydrocarbons (Waś, Szczęsna, & Rybak-Chmielewska, 2014a, 2014b) and the results presented here and previously (Waś, Szczęsna, & Rybak-Chmielewska, 2015) for different types of paraffin and beeswax-paraffin mixtures prepared in the laboratory, allowed us to establish criteria for detecting beeswax adulteration with paraffin. The addition of paraffin to beeswax resulted in the occurrence of alkanes containing over 35 atoms of carbon in the molecule, which were not detected in pure beeswax. The beeswax adulteration with paraffin resulted also in increased contents of individual alkanes (in particular, of the even-numbered alkanes) as

well as increased of total contents of hydrocarbons. The maximum limits for the contents of individual compounds and for total n-alkanes were accepted based on the maximum concentrations determined in pure beeswax (Waś, Szczęsna, & Rybak-Chmielewska, 2014b). Similar criteria, but with slightly different critical values for the contents of beeswax hydrocarbons, were also accepted by other authors (Tulloch & Hoffman, 1972; Tulloch, 1973; Jimenez et al., 2004, 2007, 2009; Serra Bonvehi & Ornantes Bermejo, 2012). Some of the authors suggested that unsaturated hydrocarbons (alkenes), and saturated hydrocarbons (n-alkanes) with a number of carbon atoms lower than 17, as well as some branched hydrocarbons, could also be taken into consideration as indicators of beeswax adulterated with paraffin (Jimenez et al., 2004, 2009; Serra Bonvehi & Ornantes Bermejo, 2012). These investigators stressed that minimum detection depends, to a great extent, on the kind of paraffin added to beeswax, which is also confirmed by our studies. Because of the higher content of n-alkanes in paraffin used by us previously (Waś, Szczęsna, & Rybak-Chmielewska, 2015), the same percentage additions of this paraffin to beeswax resulted in a greater increase in the content of these compounds in comparison to the results presented here for other beeswax-paraffin mixtures. Our estimated minimum percentage that can be detected with the GC-MS, is 3%. In our experiment, a 3% addition of different types of paraffin to beeswax already exceeded the maximum total content of n-alkanes (11.66 g/100 g) determined in pure beeswax (Waś, Szczęsna, & Rybak-Chmielewska, 2014b). Moreover, this 3% addition resulted in increased even-numbered alkanes and the appearance of alkanes containing over 35 atoms of carbon in the molecule. However, such an addition of paraffin containing fewer n-alkanes and alkanes with shorter carbon chains would be hard to detect. A similar limit of detection (below 4%) for paraffin in beeswax was estimated by Jimenez et al., 2009. Slightly higher (approximately 5%) values as the minimum detectable amount of paraffin, were stated by Serra Bonvehi &

Ornantes Bermejo (2012). Similarly, the infrared spectroscopy method for detecting adulteration of beeswax, allows for a 5% detection of added paraffin (Maia, Barros, & Nunes, 2013; Svečnjak et al., 2015). Also, although less precise in comparison to the proposed GC-MS method and frequently requiring confirmation using chromatographic methods, is the detection of adulteration on the basis of the values of some physico-chemical parameters. These parameters include the melting point, saponification number or iodine number which differ from the standard (White, Reader, & Riethof, 1960; Tulloch & Hoffman, 1972; Tulloch, 1973; Bernal et al., 2005; Jimenez et al., 2006, 2007, 2009; Serra Bonvehi & Ornantes Bermejo, 2012).

The gas chromatography with mass detector method (GC-MS) was developed previously for determination of beeswax hydrocarbons (Waś, Szczęsna, & Rybak-Chmielewska, 2014a). However, the method has been validated also for the determination of hydrocarbons in paraffin and in beeswax adulterated with paraffin (Waś, Szczęsna, & Rybak-Chmielewska, 2015). In this work, we evaluated the efficiency of the GC-MS technique for detecting beeswax adulterated with paraffin. When using the GC-MS method for this purpose, the presence of hydrocarbons containing over 35 carbon atoms in their molecules needs to be taken into consideration. One also needs to consider the content of the total as well as individual n-alkanes (in particular, even-numbered ones). Then, n-alkane contents must be compared with the maximum contents determined for pure beeswax, proposed as the concentration guide values for distinguishing between pure and adulterated beeswax (Waś, Szczęsna, & Rybak-Chmielewska, 2014b). When detecting adulteration of beeswax with paraffin, it is also necessary to pay attention to other groups of hydrocarbons (e.g. unsaturated, cyclic, and branched hydrocarbons). The above criteria indicating beeswax adulteration were adopted in assessing the quality of commercial beeswax samples. Based on the beeswax quality control results presented in this work and recent reports from other authors (Serra Bonvehi & Ornantes Bermejo, 2012; Maia &

Nunes, 2013; Svečnjak et al., 2015), it must be noted that there is an ongoing beeswax adulteration problem. This may be due to the lack of mandatory regulations regarding beeswax quality, and to an insufficient scale of beeswax production.

In conclusion, the method for the determination of beeswax hydrocarbons using the GC-MS technique, is also suitable for the detection of beeswax adulteration with hydrocarbons of alien origin (e.g. paraffin). The gas chromatography with mass detector technique allows for about a 3% detection of paraffin added to beeswax. The adulteration of beeswax with paraffin can be indicated by the presence of hydrocarbons containing over 35 atoms of carbon in the molecule, and can be indicated by the higher contents of n-alkanes ($C_{20}H_{42}$ – $C_{35}H_{72}$), in comparison to the maximum contents determined for beeswax. The paraffin types available on the market differ as regards quantitative and qualitative hydrocarbon compositions. The linear alkanes occurring in such paraffin types contain more carbon atoms in the molecule when compared to the n-alkanes occurring in beeswax. There is currently a problem of beeswax adulteration, although the scale of the problem in the domestic market is not well-known. Since there are quite a number of cases of beeswax adulteration, and the possibility of beeswax adulteration with other products similar to paraffin (e.g. ceresin, petroleum-derived), the testing of beeswax quality should be continued and intensified.

ACKNOWLEDGEMENTS

The authors are grateful to their colleagues from the Beekeeping Technology and Bee Breeding Faculties of the Apiculture Department, Research Institute of Horticulture as well as the individual beekeepers for the paraffin and comb foundation samples.

This study was partially supported by the Ministry of Science and Higher Education of Poland, COST ACTION FA0803, grant number 527/N-COST/2009/0.

REFERENCES

- Bernal, J. L., Jimenez, J. J., del Nozal, M. J., Toribio, L., & Martin, M. T. (2005). Physico-chemical parameters for the characterization of pure beeswax and detection of adulterations. *European Journal of Lipid Science and Technology*, 107(3), 158–166. <http://doi.org/10.1002/ejlt.200401105>
- Bogdanov, S. (2009). Beeswax: production, properties, composition and control. In *Beeswax Book*. (pp. 1–17). Bee Product Science. Retrieved February 12, 2012 from www.bee-hexagon.net/wax/beeswax-production-composition-control/www.bee-hexagon.net
- DGF-M-V-6. (1957). *DGF - Einheitsmethoden – Abteilung M – Wachse. German Standard – Beeswax*. Retrieved April 4, 2014, from <http://www.dgfett.de/methods/inhaltsverzeichnis.pdf>
- Downing, T. D., Kranz, Z. H., Lamberton, J. A., Murray, K. E., & Redcliffe, A. H. (1961). Studies in waxes. XVIII. Beeswax: A spectroscopic and gas chromatographic examination. *Australian Journal of Chemistry*, 14, 253–263.
- Jimenez, J. J., Bernal, J. L., Aumente, S., del Nozal, M. J., Martin, M. T., & Bernal, Jr. J. (2004). Quality assurance of commercial beeswax – Part I. Gas chromatography – electron impact ionization mass spectrometry of hydrocarbons and monoesters. *Journal of Chromatography A*, 1024, 147–154. <http://doi.org/10.1016/j.chroma.2003.10.063>
- Jimenez, J. J., Bernal, J. L., Aumente, S., Toribio, L., & Bernal, J. Jr. (2003). Quality assurance of commercial beeswax II. Gas chromatography – electron impact ionization mass spectrometry of alcohols and acids. *Journal of Chromatography A*, 1007, 101–116. [http://doi.org/10.1016/S00219673\(03\)00962-2](http://doi.org/10.1016/S00219673(03)00962-2)
- Jimenez, J. J., Bernal, J. L., del Nozal, M. J., Martin, M. T., & Bernal, J. (2006). Sample preparation methods for beeswax characterization by gas chromatography with flame ionization. *Journal of Chromatography A*, 1129, 262–272. <http://doi.org/10.1016/j.chroma.2006.06.098>

- Jimenez, J. J., Bernal, J. L., del Nozal, M. J., Martin, M. T., & Toribio, L. (2009). Identification of adulterants added to beeswax: Estimation of detectable minimum percentages. *European Journal of Lipid Science and Technology*, 111, 902–911. <http://doi.org/10.1002/ejlt.200800263>
- Jimenez, J. J., Bernal, J. L., del Nozal, M. J., Toribio, L., Bernal, J. (2007). Detection of beeswax adulterations using concentration guide-values. *European Journal of Lipid Science and Technology*, 109, 682–690. <http://doi.org/10.1002/ejlt.200600308>
- Maia, M., Barros I.R.N.A., A., & Nunes, F. M. (2013). A novel, direct, reagent-free method for detection of beeswax adulteration by single-reflection attenuated total reflectance mid-infrared spectroscopy. *Talanta*, 107, 74–80. <http://dx.doi.org/10.1016/j.talanta.2012.09.052>
- Maia, M., & Nunes, F. M. (2013). Authentication of beeswax (*Apis mellifera*) by high-temperature gas chromatography and chemometric analysis. *Food Chemistry*, 136, 961–968. <http://dx.doi.org/10.1016/j.foodchem.2012.09.003>
- PN-R-78890. (1996). *Wosk Pszczeli*. Warszawa: Wydawnictwo Normalizacyjne „Alfa”.
- Poncini, L., Poncini, A., Prakash, D. (1993). The effects of washing on the fluorescent impurities and chemical properties of Fijian beeswax from *Apis mellifera* L. *Apiacta*, 2, 42–51.
- Serra Bonvehi, J. (1988). Estudio de la composición de la cera de abejas (*Apis mellifera* L.) española. *Grasas y Aceites*, 39, 334–342.
- Serra Bonvehi, J. (1990). Estudio de la adulteración de la cera de abejas. *Grasas y Aceites*, 41, 69–72.
- Serra Bonvehi, J., Cañas Lloria, S. Gomez Pajuelo, A. (1989). Características físico-químicas de la cera de abejas producida en España. *Alimentación, equipos y tecnología*, 5-6, 213–216.
- Serra Bonvehi, J., Ornantes Bermejo, F. J. (2012). Detection of adulterated commercial Spanish beeswax. *Food Chemistry*, 132, 642–648. <http://doi.org/10.1016/j.foodchem.2011.10.104>
- Streibl, M., Stransky, K., & Sorm, F. (1966). Über einige neue Kohlenwasserstoffe in Wachs der Honigbiene (*Apis mellifera* L.). *Fette Seifen Anstrichmittel*, 68, 799–805.
- Svečnjak, L., Baranović, G., Vinceković, M., Prđun, S., Bubalo, D., Tlak Gajger, I. (2015). An approach for routine analytical detection of beeswax adulteration using FTIR-ATR spectroscopy. *Journal of Apicultural Science*, 59(2), 37–49. <http://doi.org/10.1515/JAS-2015-0018>
- Szpyrka, R. (1999). Parafina skład, właściwości, zastosowania. *Paliwa, oleje i smary w eksploatacji*, 64, 27–34.
- Tulloch, A. P. (1973). Factors Affecting Analytical Values of Beeswax and Detection of Adulteration. *Journal of American Oil Chemists' Society*, 50, 269–272.
- Tulloch, A., & Hoffman, L. L. (1972). Canadian Beeswax: Analytical Values and Composition of Hydrocarbons, Free Acids and Long Chain Esters. *Journal of American Oil Chemists' Society*, 49, 696–699.
- Vit, P., Roldan, S., Tamer, E., Olivo de Acosta, E., Bianchi, M. (1992). Detección de adulteraciones en cera de abejas comercializadas en Venezuela. *Revista del Instituto Nacional de Higiene Rafael Rangel*, 23, 23–27.
- Waś, E., Szczęsna, T., Rybak-Chmielewska, H. (2014a). Determination of beeswax hydrocarbons by gas chromatography with a mass detector (GC-MS) technique. *Journal of Apicultural Science*, 58(1), 145–157. <http://doi.org/10.2478/JAS-2014-0015>
- Waś, E., Szczęsna T., Rybak-Chmielewska H. (2014b). Hydrocarbon composition of beeswax (*Apis mellifera*) collected from light and dark coloured combs. *Journal of Apicultural Science*, 58(2), 99–106. <http://doi.org/10.2478/JAS-2014-0026>

Waś, E., Szczęsna T., Rybak-Chmielewska H. (2015). Application of gas chromatography with the mass detector (GC-MS) technique for detection of beeswax adulteration with paraffin. *Journal of Apicultural Science*, 59(1), 143–152. <http://doi.org/10.1515/JAS-2015-0015>

White, J. W. Jr., & Kushnir, I. (1961). Analysis of Mixtures of Beeswax And Petroleum Waxes. *American Bee Journal*, 101(1).

White, J. W. Jr., Reader, M. K., Riethof, M. L. (1960). Chromatographic Determination of Hydrocarbons in Beeswax. *Journal of the Association of Official Agricultural Chemists*, 43, 778–780.

