Identification of beeswax and its falsification by the method of infrared spectroscopy

Volodymyr Vyshniak[1], Oleg Dimitriev[2], Svitlana Litvynchuk[1], Valeriy Dombrovskiy[3]

1 – National University of Food Technologies, Kyiv, Ukraine
2 – V. E. Lashkaryov Institute of Semiconductor Physics of the National academy of sciences of Ukraine, Kyiv
3 – Bikiper company "Kyivoblbdzholoprom", Boiarka, Ukraine

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Corresponding author:

Volodymyr Vyshniak
E-mail: vladimir_kiev@i.ua

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Abstract

Introduction. The purpose of the research was to determine the characteristics of the infrared spectra of reflection and absorption of beeswax, as well as wax with the content of impurities in it, in particular paraffin and ceresin.

Materials and methods. Natural beeswax, paraffin, ceresin, as well as their mixtures were investigated by infrared spectroscopy of reflection and absorption using Infrapid-61, Luminar 5030 and SPECORD M-80 spectrometers.

Results and discussion. Infrared reflection spectra from smooth surfaces of samples (paraffin, ceresin, wax, a mixture of beeswax and ceresin, a mixture of wax paraffin and ceresin, a mixture of wax and paraffin) have a similar structure. There are two clearly expressed maxima at wavelengths of 1510 and 1581 nm. The ratio $R_w(1581)/R_w(1510)$ varies from 1.115 to 1.265. The smallest value corresponds to natural beeswax, and the maximum value is ceresin. After shredding the sample, the infrared spectral diffuse reflections did not undergo significant changes, the most intense spectral maxima did not change its position, but the redistribution of spectral lines by intensity was happened out. There were pronounced differences in the region from 1723 to 2400 nm. The coefficient α for the reflection spectra from the smooth surface was ~ 1.2, and for the reflection spectra from the crushed samples ~ 1.1. The reflection spectra in the region from 1100 to 1350 nm have a clear maximum at a wavelength of 1212.5 nm.

IR reflection spectra allowed us to clarify the difference between the natural beeswax and ceresin through the ratio of reflection features at 1510 and 1581 nm: the maximal ratio corresponded to the former, while the smallest one to the latter. The different proportion of bands corresponding to CH_2 and CH_3 stretching vibrations suggested that hydrocarbon chains of wax molecules are longer than those of paraffin and ceresin studied. It was found that hydrocarbon contaminants in the bee wax are associated with narrowing of the C=O band at ~1736 cm^{-1}.

Conclusions. The detected spectral laws will enable the identification of natural beeswax and detect its counterfeit.

Introduction

Bee wax is widely used in many branches of the national economy [1, 2, 29], as well as in metallurgical, electrical, chemical, pharmaceutical [3-5], food industry, in particular in the production of cheese and sweets, as well as the coverage of fruits and vegetables in order to increase their shelf life [6, 30]. However, most of the wax is returned to beekeeping for the industrial production of wax.

Waxes with impurities are called counterfeit [7-10]. It poses a serious threat to the beekeeping industry. The falsification of beeswax causes economic and image losses of the industry, worsens the productivity and quality of honey, and also significantly reduces the welfare of honey bees. The most common impurities that are added to beeswax are paraffin, ceresin, microcrystal wax, fat, stearic acid, clay, sand, hard fat, chalk, gypsum.

The high cost of beeswax compared with other waxy products (paraffin, ceresin), as well as the fact that it is quite difficult to distinguish the impurities from natural wax by external signs, making beeswax attractive for falsification [11].

According to the current requirements of quality, the industry is allowed to produce wax with insignificant content of paraffin and ceresin, but in order to improve the quality of beekeeping products and to avoid further complications, it is desirable to avoid the presence of these unnatural impurities.

Currently, there are a number of classic traditional methods for verifying the authenticity of beeswax [11] and controlling its quality: organoleptic, chemical research methods, high temperature gas chromatography and other diagnostic methods. However, such studies are long-term in time and usually lead to the destruction of researched sample.

It should be noted that the existing methods of control – organoleptic, chemical, physical, – although reliable, but morally and technically obsolete [11, 12], and do not correspond to the current level of quality control of products of beekeeping [13, 14]. An urgent need for modern methods of quality control [15, 16] is due to the fact that fake beeswax is a direct threat to the existence of honey bees, and can negatively affect the quality of all products of beekeeping.

The purpose of the research was to determine the characteristics of the infrared spectra of reflection and absorption of beeswax, as well as wax with the content of impurities in it, in particular paraffin and ceresin.

Research of wax raw materials by obtaining IR reflection spectra does not foresee the use of reagents and allows identification of beeswax, as well as draw conclusions about its falsification. This method is fast, direct and non-destructive.

These benefits of the method allow you to save time and money, and also allow you to explore more samples per unit time.

The use of high-quality natural beeswax is a necessary condition for obtaining organic (natural) bee products [17-20].

Materials and methods

Materials

Wax was obtained on the apiary of the Kyiv-Svyatoshinsky district of Kyiv region 20 years ago by melting old honeycomb in a steam kettle. The wax was stored in a dark place at room temperature in the form of blocks of cylindrical shape with a diameter of 30

centimeters and a height of 10 centimeters, in a closed package (sack) of dense fabric. Paraffin used in medicine was used for conducting experiments.

Cerezin was taken at the enterprise of the wax processing industry, which got there in the form of counterfeit raw materials. In the process of chemical analysis, it was found that the test sample is ceresin, and has nothing to do with beeswax. According to external signs (color, plasticity, fragility, structure of breakage, etc.) the sample was identical to natural beeswax, which made it impossible to identify it without the use of physico-chemical methods of analysis.

Before the beginning of the research, the samples were melted to a liquid state at a minimum melting point (~ 65 degrees Celsius). Subsequently, the liquid was poured into a special form, where it was cooled to room temperature, and hardened. Thus, three samples were obtained: natural beeswax, paraffin and ceresin. Similar samples were obtained with different concentrations of paraffin and ceresin in natural beeswax. The relative homogeneity of the samples was obtained by mixing the components in the melting process, their small mass and, as a result, a fairly small hardening time (about a few minutes). The above factors made it possible to avoid explicit stratification of the sample, due to a slightly different density of its components. The diameter of the cylindrical specimens was 5 cm and their height was 5 mm, the dimensions correspond to the cuvette of the Infrapid-61 infrared diffraction reflector. During solidification a smooth (glossy) surface was formed.

Separately, to increase the accuracy of the experiment, new samples were prepared in analogy to the previous one, with only one difference: natural beeswax was taken from the swarm family. As is known, during the rebuilding of honeycombs, bees take wax from the very beeswax, adding only about 20 percent of their wax to newly built honeycombs. That is, if the beeswax, contaminated by impurities, enters the apiary, then after the melting process these impurities will be present in wax. This will greatly complicate the conduct of the experiment [21].

To obtain spectra of diffuse reflection in the near infrared region, each sample was mechanically shredded with the device that is a set of metal equidistant knives of the same size.

For IR absorption spectroscopy, the samples were mixed with the KCl powder and pressed as pellets. The reference samples represented pure wax, cerezine and paraffin material. In order to study the effect of wax contamination by cerezine or paraffin, certain amount of wax was mixed with one or both latter materials in different proportions. The typical spectra here are presented for samples with approximately equal proportions of the components in the mix.

Research methods

The reflection spectra from a smooth surface and shredded samples were researched by using the Infrapid-61 spectrometer in the near infrared range from 1330 to 2370 nm. The spectrometer initially recorded the reflectance spectrum from reference I_0 (component part of the instrument), then a reflection spectrum from the researched sample I was obtained. The spectra are represented as the reflectivity of R in relative units (the ratio of the intensities $I/I_0 = R$), depending on the wavelength in nm.

The Luminar 5030 spectrometer recorded reflection spectra from the unprepared surface of the samples. Each sample was scanned 150 times in 1 nm increments. By averaging the results, reflectance spectra were obtained for each object of the research. The process of scanning one sample lasted about 7 seconds.

IR absorption was studied using a dual-beam SPECORD M-80 spectrophotometer in the 4000 to 800 cm^{-1} range. As all the samples demonstrated similar fingerprints near 3000 cm^{-1} corresponding to CH_3 and CH_2 stretching, the spectra below are given only in the region of 1900-600 cm^{-1}, where characteristic IR vibrations of the polymers are displayed [22-28].

Results and discussion

NIR reflection research

Infrared reflection spectra from smooth surfaces of several samples (P – paraffin, C – Ceresin, W – Wax, WC – a mixture of beeswax and ceresin, WCP – a mixture of wax paraffin and ceresin, WP – a mixture of wax and paraffin) are shown in Figure 1. All the spectra have a similar structure. There are two clearly expressed maxima of 1509 and 1581 nm (features c and e in Figure 1) and more than four minima, in particular at 1415, 1533, 1725, and 2254 nm (features b, d, f, g). The highest intensity in the reflection spectrum corresponds to the wavelength of 1581 nm.

Analyzing the ratio of the maxima of the corresponding spectra for wavelengths of 1510 and 1581 nm (Table 1), we can conclude that the parameter α, which is equal to the ratio R(1581)/R(1510), varies from 1.115 to 1.265. The smallest value $\alpha = \frac{R_{W(1581)}}{R_{W(1510)}} = 1.115$ corresponds to natural beeswax, and the maximum value corresponds to ceresin $\alpha = \frac{R_{W(1581)}}{R_{W(1510)}} = 1.265$. Among the spectral features it is worthwhile to mention the characteristic intervals between extremums (Figure 1, Table 2): BC = 94, CE = 72, EF = 145 nm.

After shredding the sample the infrared spectrum of diffuse reflection did not undergo significant changes, the most intense spectral maxima did not change its position (Figure 4), but the redistribution of spectral lines by intensity was carried out. There were pronounced differences in the region from 1723 to 2400 nm (Figure 4), in contrast to the spectra obtained from the smooth surface of the sample (Figure1).

Analyzing the positions of the spectral line maxima (Figure1) at wavelengths of 1581 and 1510 nm (Table 1), it can be noted that they are located in the following sequence $Rp > R_{WP} > R_{WC} > R_{WCP} > R_C > R_W$.

The coefficient α for the reflection spectra from the flat surface is ~ 1.2, and for reflection spectra from shradded samples is ~ 1.1 (Table 1 and 2).

Figure 5 presents the IR reflection spectra in the spectral range from 1100 to 1350 nm, obtained with the device Luminar 5030. The horizontal axis deferred wavelength in nanometers, while the vertical axis optical density is lg1/R, where R – reflecting the ability of the body.

Differences in intensity of the spectral characteristics of the researed samples at a wavelength of 1212.5 nm allow to make conclusions concerning presence of impurities (wax and ceresin) in the researched samples.

Spectral maxima at the specified wavelength are located in the following sequence $R_P > R_W > R_{WCP} > R_{WC} > R_{WP} > R_C$.

Table 1

Ratio of the intensities of the spectral maxima for 1510 and 1581 nm. Smooth surface

Sample	R_{1510}	R_{1581}	R_{1581}/R_{1510}
W	0.309	0.357	1.155
P	0.318	0.397	1.248
C	0.310	0.392	1.265
WP	0.375	0.448	1.195
WC	0.328	0.394	1.201
WCP	0.318	0.394	1.239

Table 2

Ratio of the intensity of the spectral maxima for 1510 and 1581 nm. Shredded sample

Sample	R_{1510}	R_{1581}	R_{1581}/R_{1510}
W	0.879	0.932	1.06
P	0.743	0.827	1.11
C	0.727	0.806	1.12
WP	0.792	0.857	1.08
WC	0.757	0.824	1.09
WCP	0.799	0.874	1.09

Table 3

Characteristic intervals between spectral extremums (Figure 1)

Extremum	Coordinate		Segment	Length of the segment, nm
A	1397		**AB**	18
B	1415		**BC**	94
C	1510		**CD**	24
D	1533		**DE**	48
E	1581		**EF**	145
F	1726		**FG**	528
G	2254		**GH**	45
H	2299		**HI**	57
I	2356			

The $W_{original}$ spectral line (Figure 5) corresponds to a rough (not molded) sample of beeswax obtained on an apiary 20 years ago. Spectral line W is obtained from a melted sample. The indicated differences in the infrared reflection spectra can be used in obtaining information concerning the history of the production of wax raw materials.

In the article [1] a relatively new direct method for the investigation of wax was proposed, without the use of chemical reagents. The method involves the use of IR spectroscopy in the range from 4000 to 650 cm^{-1}. It allows you to identify beeswax and detect fake (impurities). In the described method, the absorption coefficient was recorded, namely the ratio: $I_{1739\,cm^{-1}} / I_{2852\,cm^{-1}}$, $I_{1714\,cm^{-1}} / I_{2852\,cm^{-1}}$, $I_{1739\,cm^{-1}} / I_{1714\,cm^{-1}}$. The results of the studies were confirmed by high temperature gas chromatography. The difference between this work and the previous one is that the absorption and reflection spectroscopy was used. The registration of absorption spectra took place in the spectral range from 1900 to 600 cm^{-1}, and the reflectance spectra were recorded in the range from 1500 to 2400 nm.

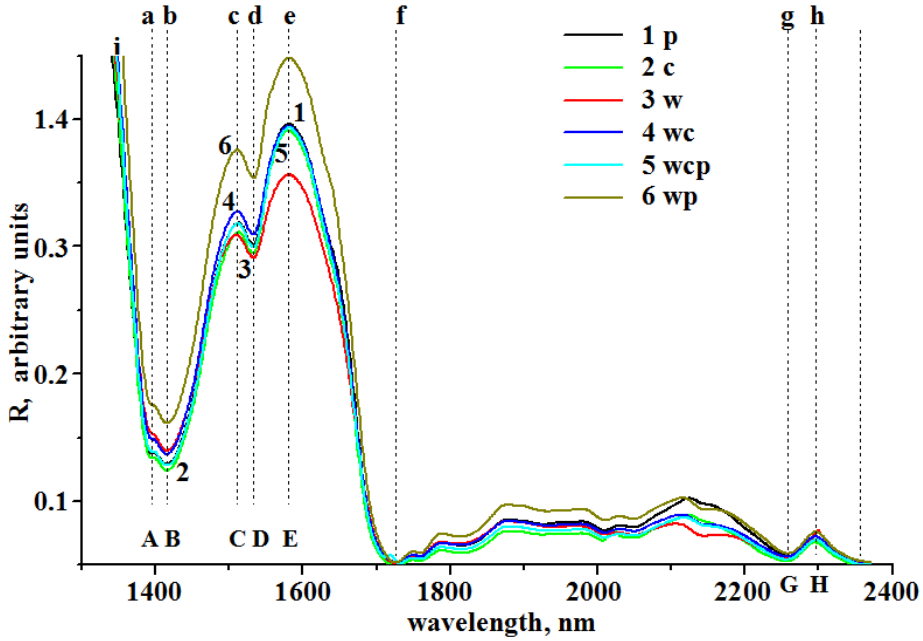

Figure 1. IR reflection spectra. Smooth (glossy) surface of the samples: p – paraffin, c – ceresin, w – natural beeswax, wc – a mixture of wax and ceresin, wcp – a mixture of bee wax, ceresin and paraffin wax, wp – a mixture of wax and paraffin

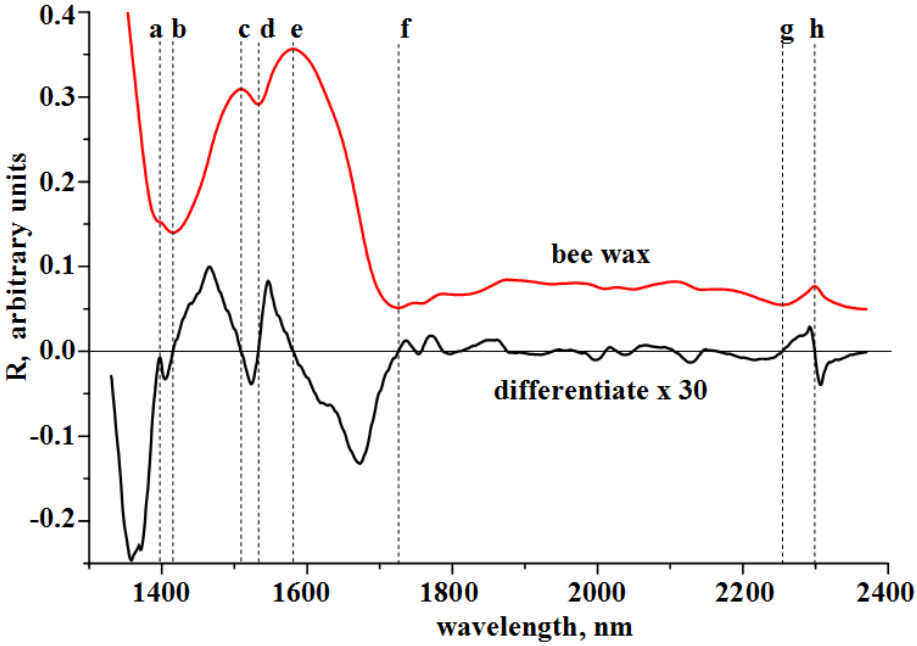

Figure 2. IR reflection spectrum of natural beeswax and its first derivative

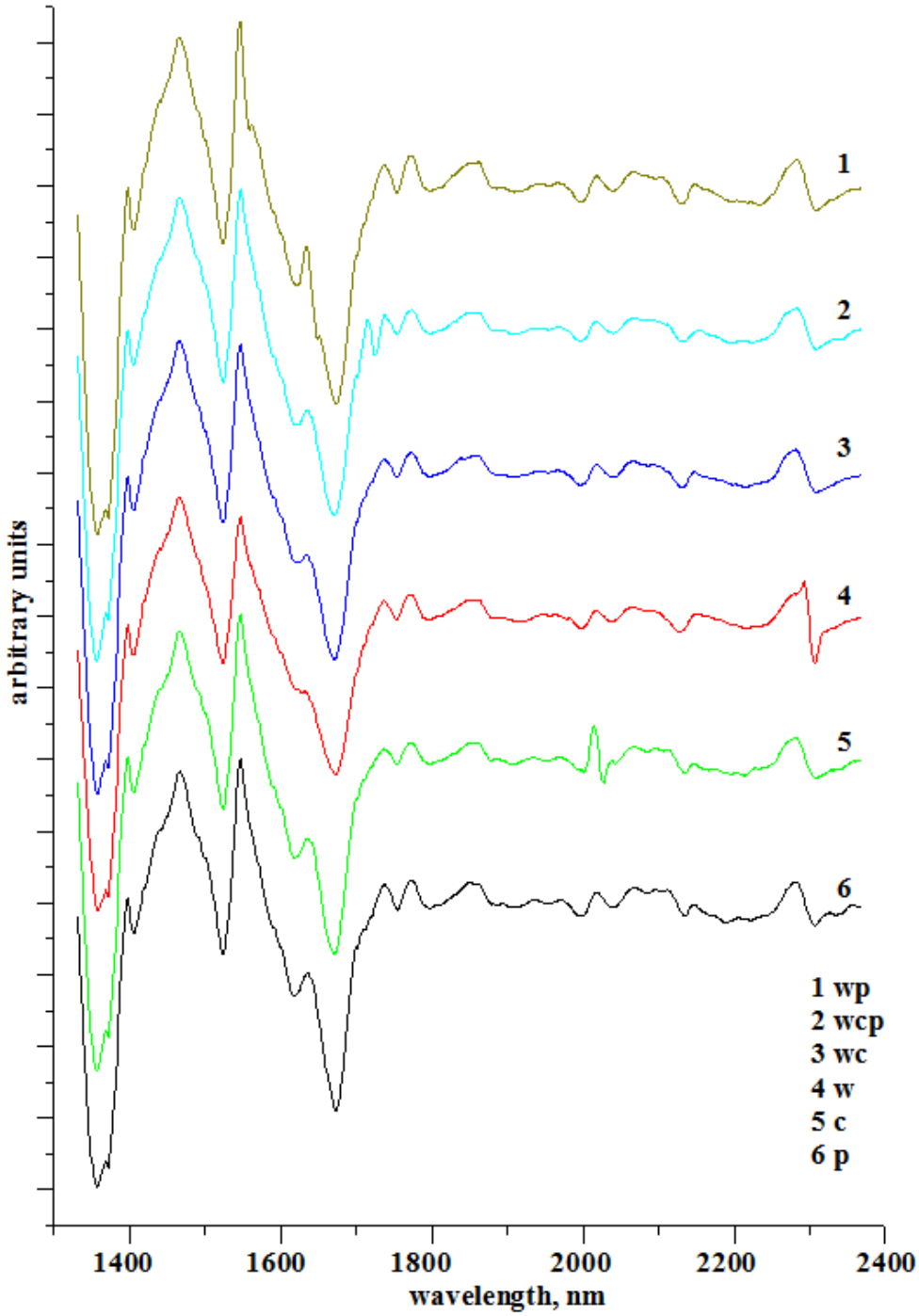

Figure 3. First derivatives of IR reflection spectra from a smooth surface of samples

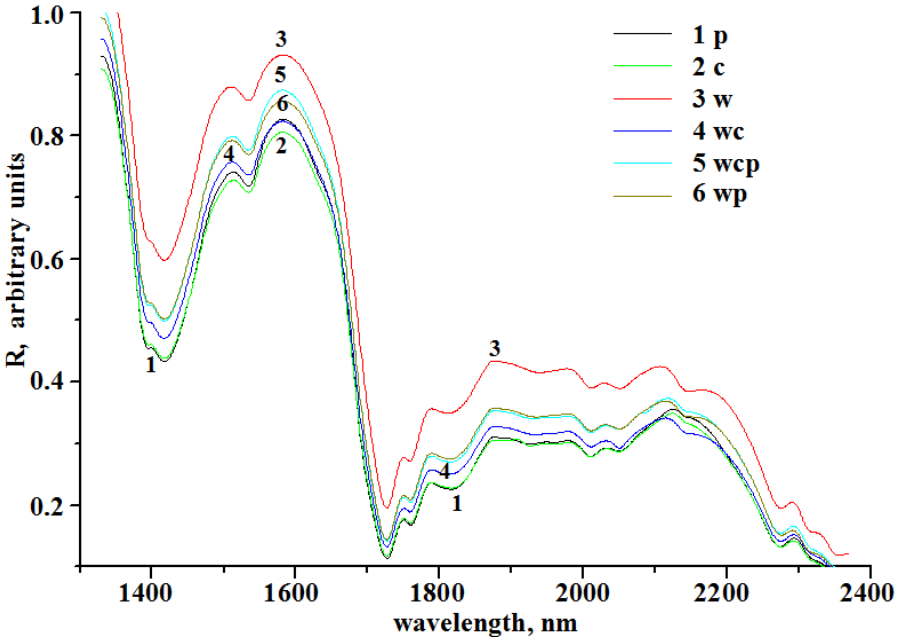

Figure 4. IR spectrum of diffuse reflection of shredded samples

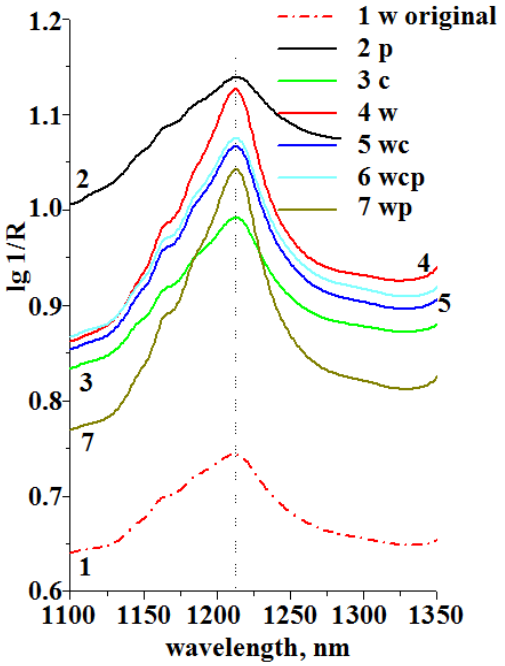

Figure 5. IR reflection spectra of waxy substances

Wax is considered to be a fairly stable compound, with virtually unlimited shelf life, provided that proper storage conditions are met. At the same time, in the long-term storage, especially in non-leakproof containers at high temperatures, the surface of the sample undergoes certain changes in the physical and chemical parameters, which visually manifests itself in the change of color, and as a consequence in the features of the reflection spectra, and so on. This fact needs to be taken into account in optical studies, since the information received with the help of infrared reflection spectroscopy essentially depends on the state of the surface of the sample.

To some inconvenience of the use of IR reflection spectroscopy one can attribute the fact that the spectra have a similar structure, and it is difficult to distinguish them visually from each other (Figure 1, 4). The proposed research methodology needs improvement in the detection of impurities of low concentration (several percent and less). Interpretation of the results may be complicated if there is a large number of impurities of different chemical nature. In studies, it is necessary to take into account the state of the surface of the sample, and if necessary, to shred (break) the sample, or to melt it. In a case of presence of a large amount of water in beeswax (more than 20%) the use of infrared spectroscopy is ineffective.

IR absorption research

Typical spectra of IR absorption of the neat components are shown in Figure 6.

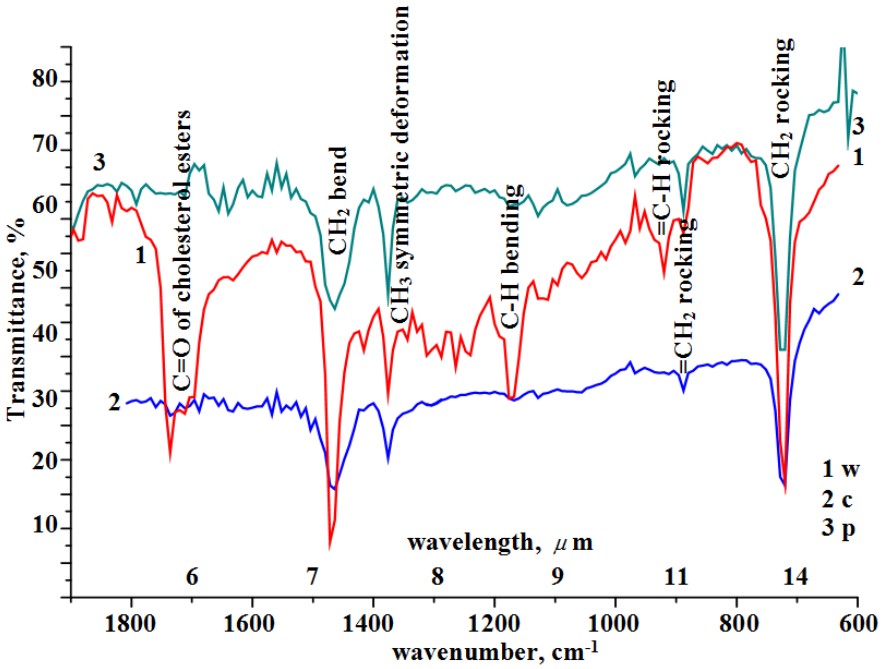

Figure 6. IR spectra of (1) wax, (2) ceresin and (3) paraffin reference samples

All the samples demonstrate similar bands, i.e., at 1470 cm^{-1} corresponding to CH_2 bending, 1376 cm^{-1} corresponding to CH_3 symmetric deformation, 1170 cm^{-1} corresponding to C-H bending, 888 and 720 cm^{-1} corresponding to CH_2 rocking. The above features are typical for hydrocarbon chains and reflect the fact that the compounds have similar chain

fragments. However, one can distinguish different proportion of the above band intensities. Particularly, the different proportion of CH_2 and CH_3 bands suggests that hydrocarbon chains of wax molecules are longer than those of paraffin and cerezine studied. One more difference is that the spectrum of wax is more reach than those of paraffin and cerezine. Particularly, the wax samples demonstrate a strong C=O vibration at ~1736 cm^{-1} corresponding to cholesterol esters. This band is a characteristic feature which allows one to identify wax among other hydrocarbon compounds.

The mixtures of wax with other compounds demonstrate CH, CH_2 and CH_3 bending and rocking vibrations in IR spectra characteristic for all hydrocarbons used (Figure7).

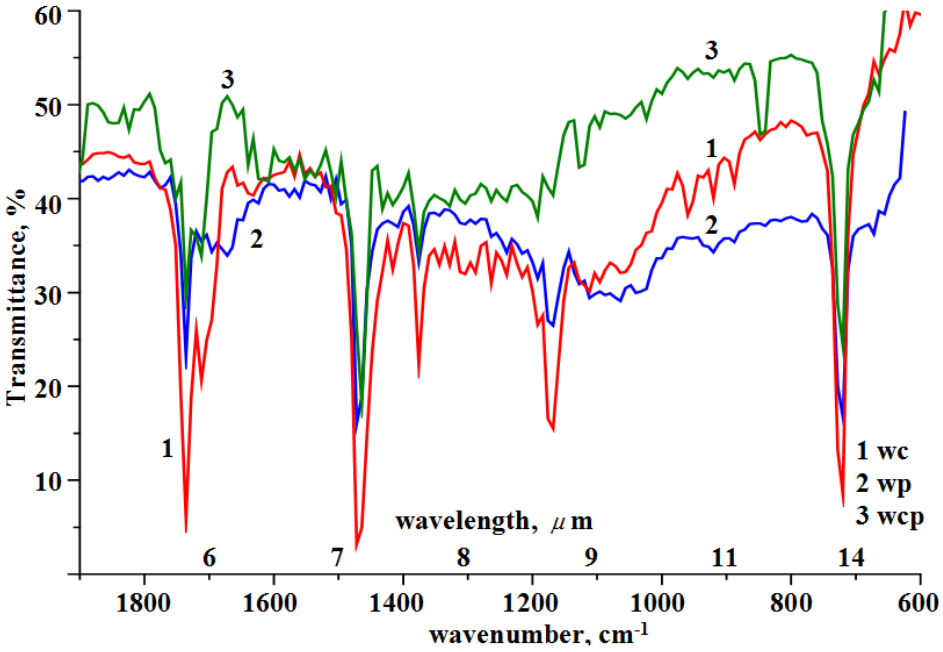

Figure 7. IR spectra of mixtures of (1) wax plus ceresin, (2) wax plus paraffin and

The spectra are also enriched with the strong C=O vibration at ~1736 cm^{-1} indicative of the presence of wax. However, in the mix samples, this band has a more complex structure compared to the neat wax. In fact, the C=O band is splitting in the mixtures, with a strong component at ~1736 cm^{-1} for all the samples accompanied by a smaller red-shifted one. Position of the latter component is dependent on the mixture composition: for the wax-cerezine mixture it is at 1712 cm^{-1}, for the wax-paraffin mixture it is at 1672 cm^{-1}, and for the wax-cerezine-paraffin mixture it is at 1712 cm^{-1} again. The splitting and shift of the C=O band is related to the different environment for the carbonyl group in the mix samples, when part of these groups remains in the native wax environment whereas the other in the foreign paraffin or ceresin environment, respectively. In fact, the spectrum of the neat wax (Figure 6) also contains the component at 1712 cm^{-1}, however, this component is less resolved due to broadening of the C=O band itself, suggesting more polar environment for

the carbonyl group in the wax. The different broadening of the C=O band is shown in Table 4 and the factor can be used for identification of the contaminant in the bee wax itself.

Table 4

Full width at half maximum (FWHM) of the C=O band in the neat wax and mixed samples

Composition	W	WC	WP	WCP
FWHM (cm^{-1})	62	45	20	43

Thus, our results evidence that hydrocarbon contaminants in the bee wax are associated with narrowing of the C=O band at ~1736 cm^{-1}. This spectroscopic feature can be used for further quantitative analysis of the purity of the bee wax itself.

Conclusions

1. Infrared reflection spectra (in the range of wavelengths 1330–2370 nm) from smooth surfaces of samples (paraffin, ceresin, wax, a mixture of beeswax and ceresin, a mixture of wax paraffin and ceresin, a mixture of wax and paraffin) have a similar structure, but there are two clearly expressed maxima of different intensity at wavelengths of 1510 and 1581 nm.
2. The ratio of the intensity of the reflection spectra at characteristic wavelengths $\alpha = R_w(1581)/R_w(1510)$ varies from 1.115 (natural bee wax) to 1.265 (ceresin).
3. The maximum intensity of optical density is observed at a wavelength of 1212.5 nm, which can be used to identify the wax and allow conclusions to be made regarding the aging of the sample.
4. Infrared spectra of diffuse reflection of shredded samples will not undergo significant changes in comparison with spectra from a smooth surface. The most intense spectral maxima do not change their position, but there is a redistribution of spectral lines by intensity.
5. The different proportion of CH_2 and CH_3 bands suggests that hydrocarbon chains of wax molecules are longer than those of paraffin and cerezine studied. One more difference is that the spectrum of wax is more reach than those of paraffin and cerezine. Particularly, the wax samples demonstrate a strong C=O vibration at ~1736 cm^{-1} corresponding to cholesterol esters. This band is a characteristic feature which allows one to identify wax among other hydrocarbon compounds.

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