

An Investigation of Molecular Structure and Dynamics of Crude Beeswax by Vibrational Spectroscopy

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Abstract

Fourier transform infrared spectroscopy and Raman spectroscopy were used to investigate the structure of beeswax combs in native state (crude beeswax) of the Polish bees (*Apis mellifera*) from different country regions. Aliphatic saturated and monounsaturated compounds are major comb wax constituents, mainly esters and hydrocarbons, but also alcohols, free acids and other materials. The long chains of hydrocarbons, with C₂₁-C₃₅, C₂₇ and C₃₁ alkanes predominating, tend to form semicrystal-like local structures during the process of wax aggregation into solid state. In general, this process is quite similar to the one studied in our laboratory for binary n-alkanes mixtures in solid solution.

Keywords: beeswax, FTIR spectroscopy, Raman spectroscopy, hydrocarbons

Introduction

Beeswax is the most important among natural waxes because of its unique properties and useful applications in medicine, cosmetics, food, engineering and industry. Structural and dynamic characterization of beeswax is necessary in order to understand the relationship between its properties and structure and, on the basis of these relationships, to design new application for beeswax, like modern cosmetics, pharmaceuticals etc. Beeswaxes are a complex mixture of hydrocarbons, carboxylic acids, esters and alcohols [1] and have been investigated by many analytical methods. Wax excreted by bees has liquid consistency and later it turns into solid state. The major constituents of beeswax are esters (30-80%), which consist of molecular species of C₄₀ to C₄₆. Analyses of those materials were performed using liquid chromatography [2]. Fröhlich et al. widely performed the quantitative chemical analyses of cuticular waxes of the honeybee by means of gas chromatography and mass spectrometry [3]. Wax esters are chemically diverse, and most are formed from primary or second alcohols [4]. ¹³C measurements were used to

investigate the molecular structure of crude beeswax. In that work chemical shifts, cross-polarization rate and T₁ relaxation were observed [5]. Micro-Raman spectroscopy and Raman mapping were applied to investigate the spectral distribution and chemical composition of wax and propolis in the comb of bees [6]. In this paper we report on changes in the band shape of beeswaxes' IR spectra, which depend on temperature and time. The molecular structure has been investigated by using FTIR and Raman spectroscopy. We tried to compare spectral changes in the composition of the samples which came from three different areas of Poland: Gliwice (industry area –sample 1), Siewierz (forests and green fields -sample 2) and Toszek (agricultural area – sample 3).

Experimental procedures

For the Fourier-transform infrared spectroscopic investigation, samples of waxes combs, which stem from Polish bees species (*Apis mellifera*), were heated to melting point temperature, mixed and then quenched. The combs were placed between KBr windows. In this way, spectra

were recorded at a resolution of 2 cm^{-1} in the mid-IR range. Moreover, a slice of wax had been cut, placed on a transparent plate and then measured by Raman spectroscopy. A LabRam system spectrometer was equipped with an Olympus X-40 microscope with image magnification of 100. The argon laser was operated at a wavelength of 514.4 nm. The spectra were recorded with a resolution of 1 cm^{-1} within the range of $500\text{--}3000\text{ cm}^{-1}$.

Results and discussion

The FTIR spectrum of beeswax which originated from Siewierz is displayed in Figure 1A. It shows the hydrocarbon vibrations ($\sim 2950\text{ cm}^{-1}$ and $\sim 1465\text{ cm}^{-1}$), many bands of esters ($\sim 1700\text{ cm}^{-1}$) and alcohols ($\sim 1053\text{ cm}^{-1}$). Beside these basic compounds, there were bands of amides ($\sim 1327\text{ cm}^{-1}$) and amines (~ 1219 and 728 cm^{-1}) found.

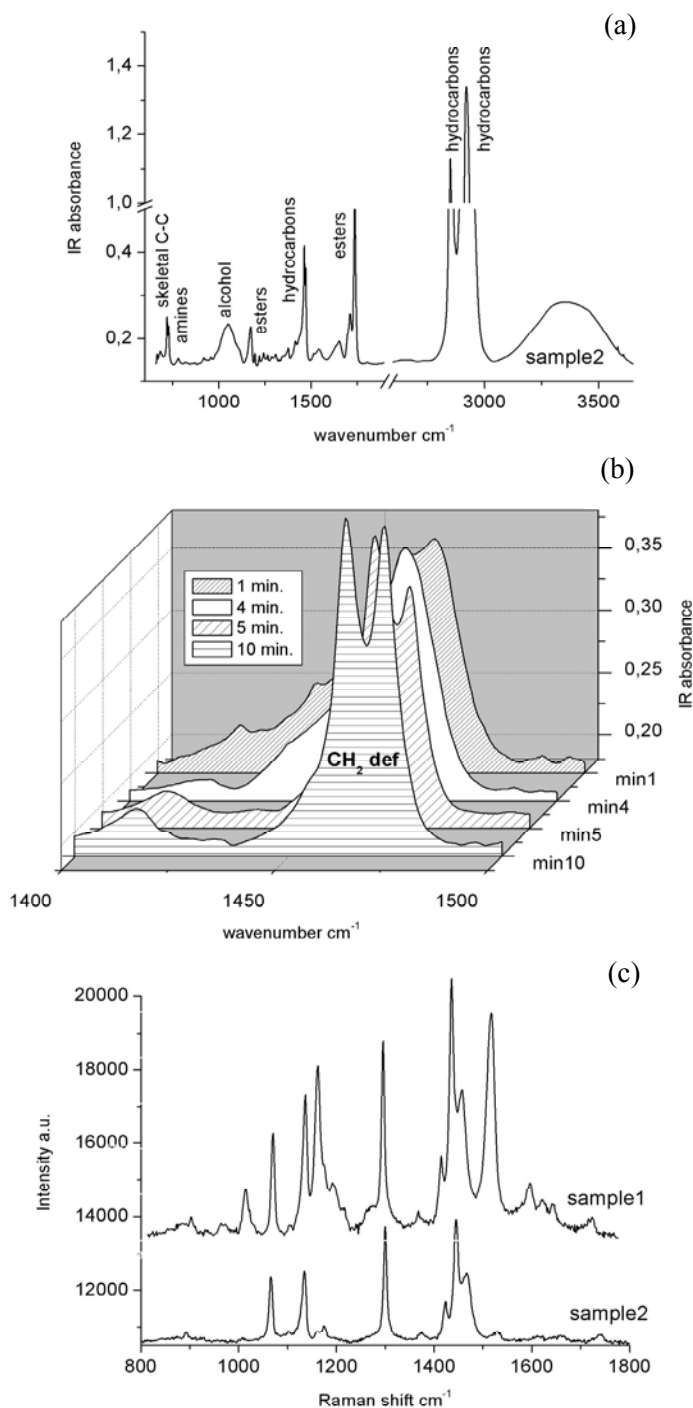


Fig. 1. Vibrational spectra data of beeswaxes from forest and field areas – sample 2 (a, b and c) and from an industry area – sample 1 (c)

Figure 1B shows the interesting part of the FTIR spectra of beeswax from Siewierz. Before the investigation, this sample was heated to melting point temperature and quenched to room temperature. From 1 to 10 minutes after preparation there were changes in the spectral bands of the CH₂-groups observed. At first in this spectral range, there was only one central band whose intensity decreased in time (see Fig. 1B). After 5 minutes, an increase of side shoulders at the finally splitted band arising from CH₂-group vibrations was observed. After 10 minutes, the splitting of this band near to 1460 cm⁻¹ was 8.7 cm⁻¹. It was the same phenomenon of forming a semicrystal local structure of hydrocarbons, as reported for linear n-alkanes in binary mixtures in solid solution [7].

The Raman spectra of three samples of beeswaxes were recorded in the spectral region of 800 - 1800 cm⁻¹. Sample 1, which originated from an industry area, showed a band-rich spectrum (see Fig. 1C) with mainly hydrocarbon components. From our other preliminarily measurements, using X-ray photoelectron spectroscopy, there were found traces of inorganic compounds of Si and also P and S. These elements probably originated from dust and environment pollution. Sulfur compounds could be found in wax because of dusting the combs by a substance, which protects beehives against acarinoses. The characteristic IR spectral bands of ketones were found near 1612 cm⁻¹, they are responsible for the aroma of beeswaxes. The observed esters had the same property of a nice smell. The samples 2 and 3 derived from clean, agricultural or forest places have nearly the same spectrum but being a bit different from sample 1 (see Fig. 1C). They showed bands that originated from hydrocarbons (also cyclic) modes: CH₂-deformation ~1463 cm⁻¹ and CH₃ ~1423 cm⁻¹, and CH₂-twist ~1298 cm⁻¹. The band of various esters of acetic acid, aromatic acids or sulphonic acid, being the main components of beeswaxes was recorded near the wavenumber of 1135 cm⁻¹. The silicon compound bands in the Raman spectra were found at 1066 cm⁻¹. In every sample, there were small intensity bands found that can be assigned to carbo-acids which could be the base material for forming esters. Amines and amides are part of the toxin produced by bees.

Conclusions

The FTIR and Raman method proved to be very quick and useful for obtaining information about the molecular

structure of beeswaxes. Fundamental components such as hydrocarbons, esters, small amount of amides and alcohols were found in beeswax combs. Beside those, there were some impurities detected, which could be attributed to the pollution of bees' environment. The processes of segregation and crystallization into mono-hydrocarbon domains as for n-alkanes, which are also components of beeswaxes, was noticed. This is a phenomenon, by which the formation of semicrystal-like local structures occurs during the process of wax aggregation (solid state). It is worth mentioning, that besides a number of beeswax application such as wax ointment or medicine for curing respiratory illnesses it could be checked whether beeswax can also serve as an environment pollution indicator. The application of X-ray photoelectron spectroscopy, sensitive to the element composition of the samples including heavy metal compounds, may be helpful in this regard.

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