# DETECTION OF BEESWAX ADULTERATION WITH HYDROCARBONS USING GAS CHROMATOGRAPHY WITH MASS DETECTOR (GC-MS)

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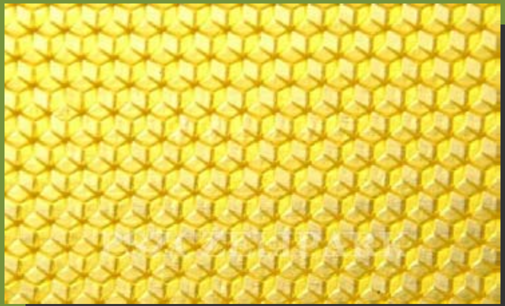
# Beeswax adulteration with hydrocarbons

**For a few years, due to the lack of mandatory regulations concerning beeswax quality, this product has been increasingly adulterated with much cheaper hydrocarbons of alien origin. As a result, the quality of the product is compromised which makes it unusable by the industries.**

**Adulteration of beeswax with hydrocarbons has also a negative impact on the quality of comb foundation. When used on regular basis in bee farming adulterated beeswax interferes with normal brood rearing and development *(Jimenez i in., 2005).***



# Beeswax adulteration with hydrocarbons

According to the requirement laid down by PN-R-78890 „Wosk pszczeli" the content of hydrocarbons cannot exceed 16.5%.

Higher contents of those compounds indicate contamination of that product with alien hydrocarbons.

# Detection of beeswax adulteration with hydrocarbons

**The analysis of the chemical composition of beeswax because of its special nature is exceptionally difficult even if state-of-the-art equipment is used.**

**Until recently, in order to detect adulteration of beeswax with hydrocarbons column chromatography combined with weight analysis was used in Poland (PN-R-78890 „Wosk pszczeli”-„Beeswax”). The method allows only the determination of total hydrocarbons occurring in beeswax.**

# The aim of the study

The aim of the study was to describe hydrocarbons in natural beeswax.

Another objective was to develop a new method to detect beeswax adulteration with paraffin.

# Material and methods

The experiment material was composed of samples of:

- natural beeswax
- commercial paraffin (product most frequently used to adulterate beeswax)
- hydrocarbon-adulterated beeswax
- commercial comb foundation

Hydrocarbon assays were made on a gas chromatograph with a Shimadzu mass detector (Gas Chromatograph Mass Spectrometer, GC-MS-QP 2010 Plus).

# Material and methods

- **Hydrocarbon fraction was isolated on a silica column according to Polish Standard PN-R-78890.**

- **Hydrocarbons were separated on a non-polar ZB- 5HT 30m x 0.25mm x 0.25μm column manufactured by Phenomenex. Chromatography separation of hydrocarbons in temperature program was done with helium as a carrier gas.**

- **An electron source was used to ionize sample molecules in the gaseous phase. The temperature of the electron source was 230$^{0}$C. EI mass spectra were recorded at a standard electron energy of 70eV.**

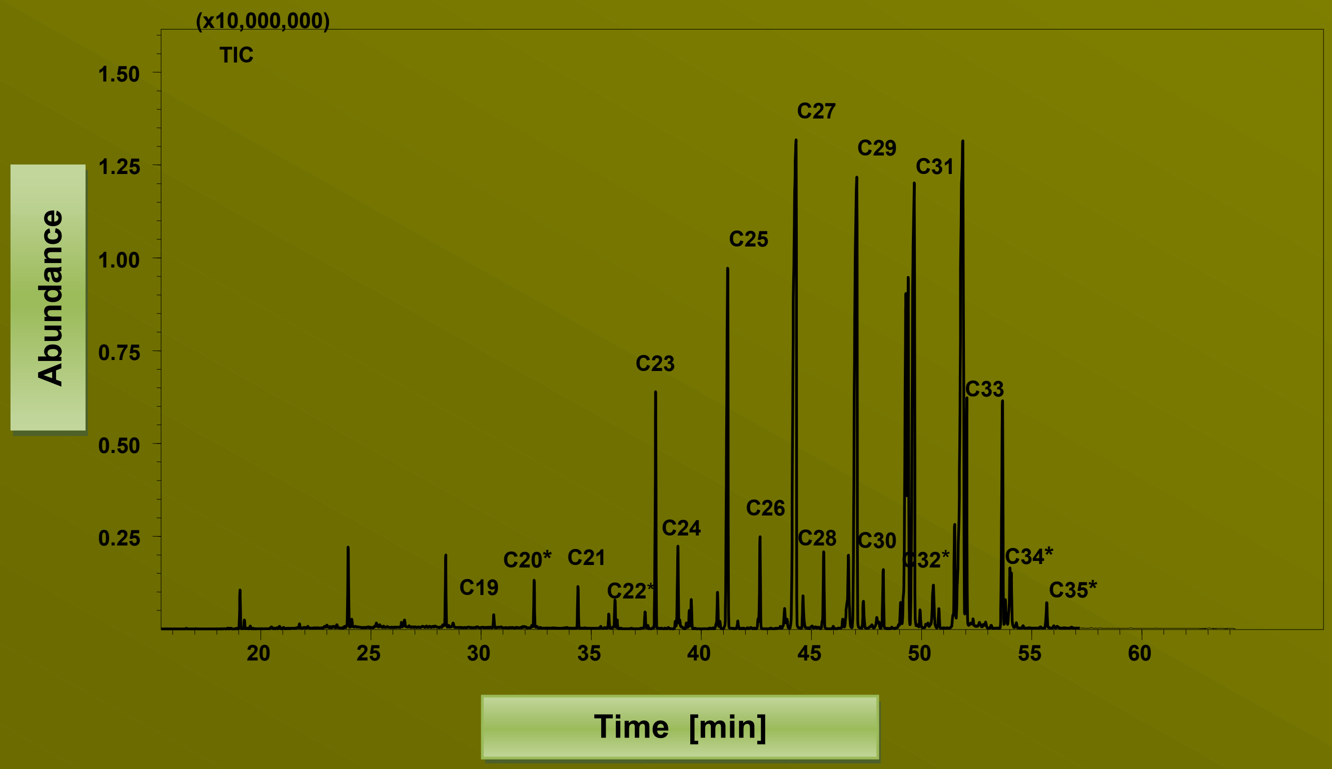
- **Based on the electron spectra of the NIST 05 library and on the retention indexes, identyfication of studied compounds was done.**

## Table 1. The homologous series of saturated hydrocarbons with unramified carbon chains occurring in natural beeswax

| The number of carbon atoms in the molecule | Formula | Compound | Molecular ion |
|---|---|---|---|
| 19 | $C_{19}H_{40}$ | Nonadecane | 268 |
| 20 | $C_{20}H_{42}$ | Eicosane | 282* |
| 21 | $C_{21}H_{44}$ | Heneicosane | 296 |
| 22 | $C_{22}H_{46}$ | Docosane | 310* |
| 23 | $C_{23}H_{48}$ | Tricosane | 324 |
| 24 | $C_{24}H_{50}$ | Tetracosane | 338 |
| 25 | $C_{25}H_{52}$ | Pentacosane | 352 |
| 26 | $C_{26}H_{54}$ | Hexacosane | 366 |
| 27 | $C_{27}H_{56}$ | Heptacosane | 380 |
| 28 | $C_{28}H_{58}$ | Octacosane | 394 |
| 29 | $C_{29}H_{60}$ | Nonocosane | 408 |
| 30 | $C_{30}H_{62}$ | Triacontane | 422 |
| 31 | $C_{31}H_{64}$ | Heneitriacontane | 436 |
| 32 | $C_{32}H_{66}$ | Dotriacontane | 450* |
| 33 | $C_{33}H_{68}$ | Tritriacontane | 464 |
| 34 | $C_{34}H_{70}$ | Tetratriacontane | 478* |
| 35 | $C_{35}H_{72}$ | Pentatriacontane | 492* |

* Molecular ion not observed.

# Chromatogram of alkanes in natural beeswax



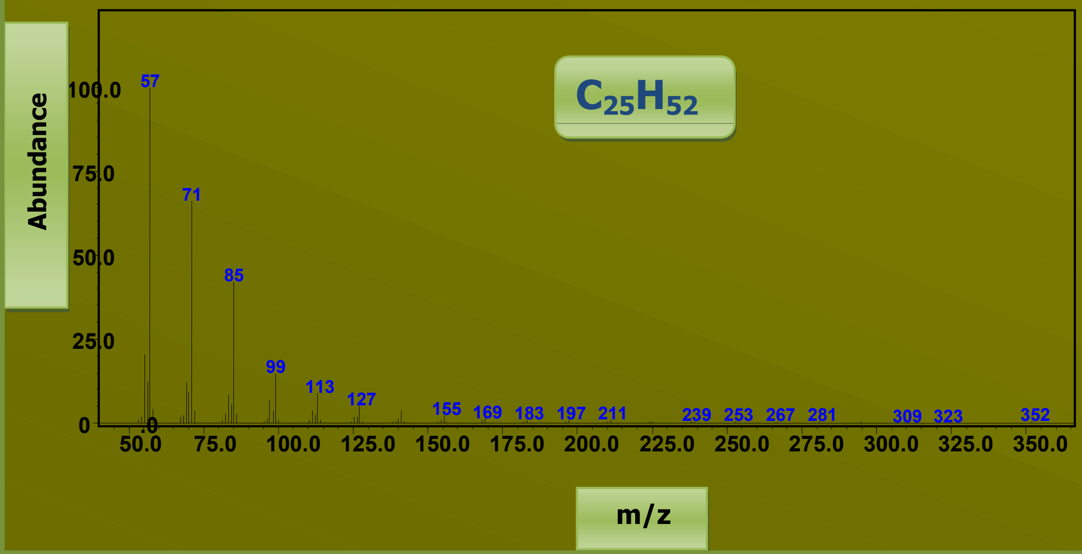
# Electron impact spectrum of alkanes

**Hydrocarbons containing more than eight carbon atoms show very similar spectra and the identification of those compounds depends on the detection of the molecular ion peak.**

**As a rule, a peak of the molecular ion of saturated hydrocarbons with a simple (unramified) chain is visible. However, its intensity decreases with length and ramification degree of the chain. In the spectra of simple-chain alkanes peak groups are observed at m/z values differing by 14 daltons ($CH_2$).**

# Electron impact spectrum of alkanes

**Within each group, the peak of the highest intensity corresponding to a $[C_nH_{2n+1}]^+$ fragment occurs at m/z= 14n+1 (m/z= 29, 43, 57, 71, 85, 99 etc.) and it arises from the breakdown of C-C bonds at different sites of the carbon chain.**



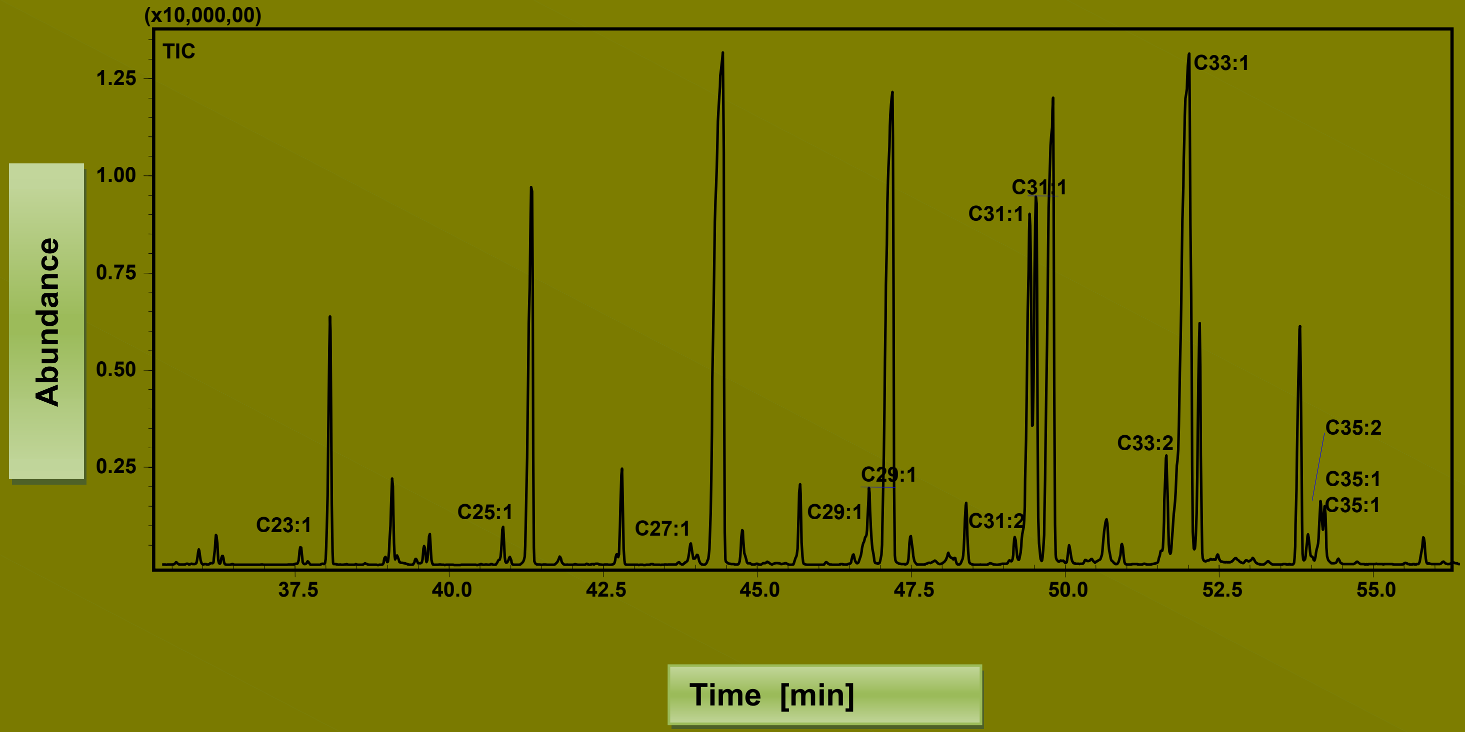

**The $[C_nH_{2n+1}]^+$ ions are accompanied by ions of the $[C_nH_{2n}]^{+\cdot}$ $[C_nH_{2n-1}]^+$ and formulas which arise as a result of the primary fragmentation ions being eliminated.**

## Table 2. Alkenes and dienes occurring in natural beeswax

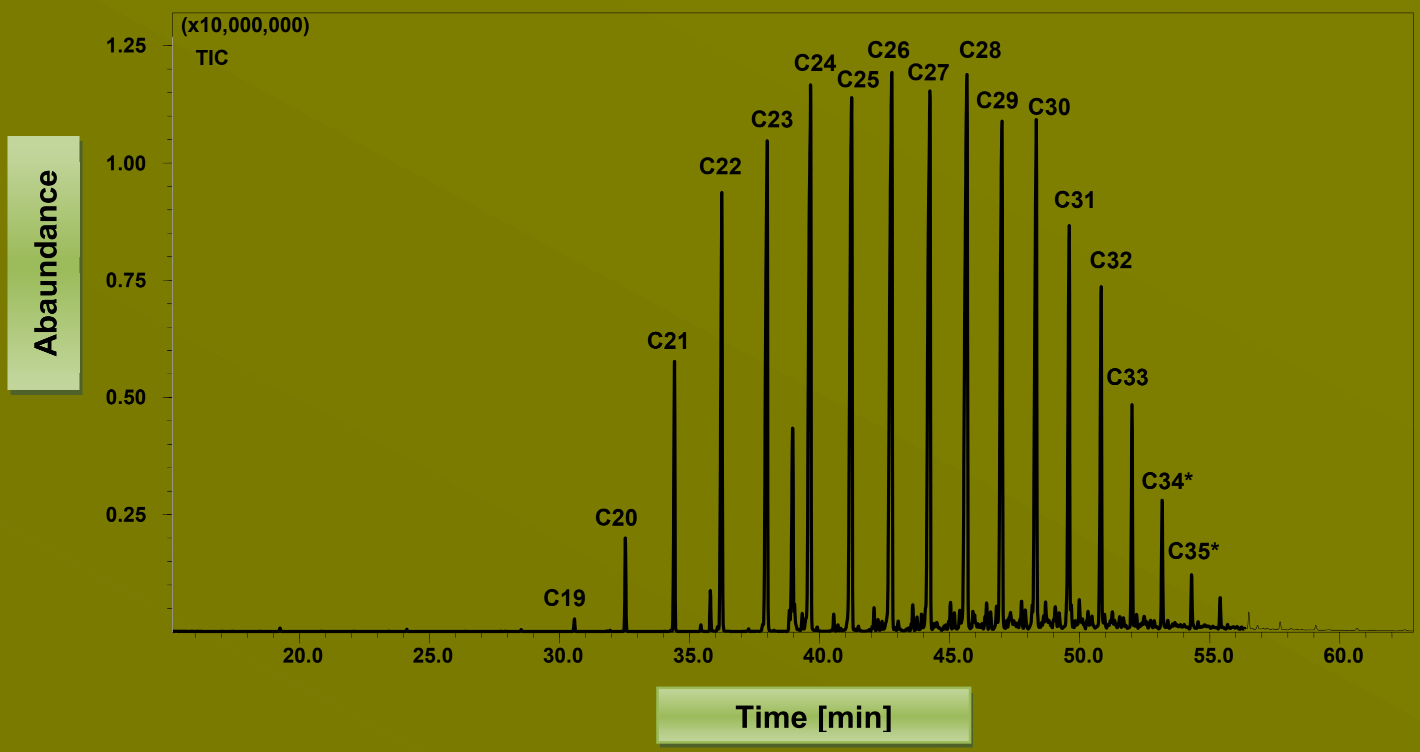
| The number of carbon atoms in the molecule | Formula | Compound | Molecular ion |
|---|---|---|---|
| 23:1* (1)** | $C_{23}H_{46}$ | Tricosene | 322 |
| 25:1 (1) | $C_{25}H_{50}$ | Pentacosene | 350 |
| 27:1 (1) | $C_{27}H_{54}$ | Heptacosene | 378 |
| 29:1 (2) | $C_{29}H_{58}$ | Nonacosene | 406 |
| 31:2 (1) | $C_{31}H_{60}$ | Heneitriacontadiene | 432 |
| 31:1 (2) | $C_{31}H_{62}$ | Heneitriacontene | 434 |
| 33:2 (1) | $C_{33}H_{64}$ | Tritriacontadiene | 460 |
| 33:1 (1) | $C_{33}H_{66}$ | Tritriacontene | 462 |
| 35:2 (1) | $C_{35}H_{68}$ | Pentatriacontadiene | 488 |
| 35:1 (2) | $C_{35}H_{70}$ | Pentatriacontene | 490 |

* :1, :2- the number of double bonds.
** (1,2)- the number of identified isomeres.

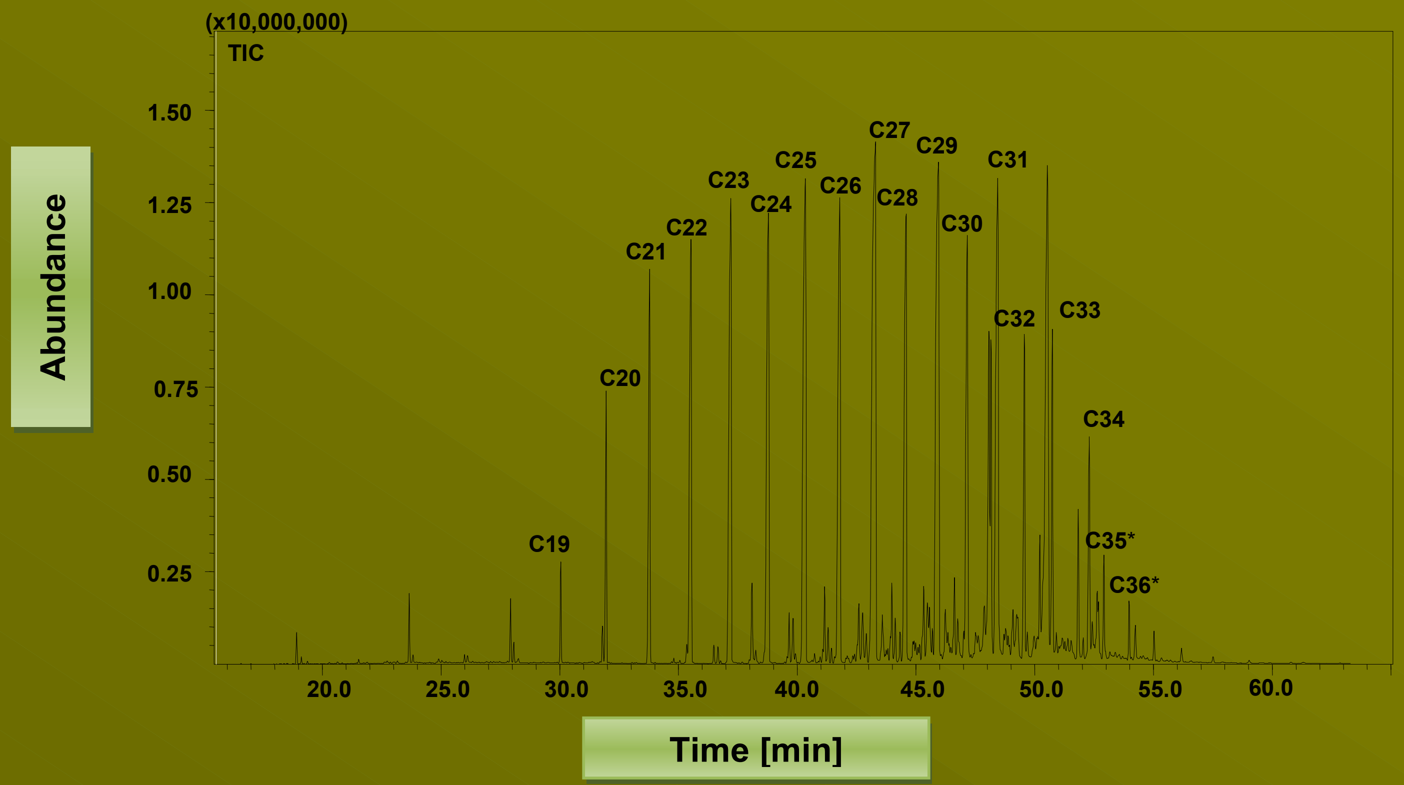
# Chromatogram of alkenes and dienes in natural beeswax



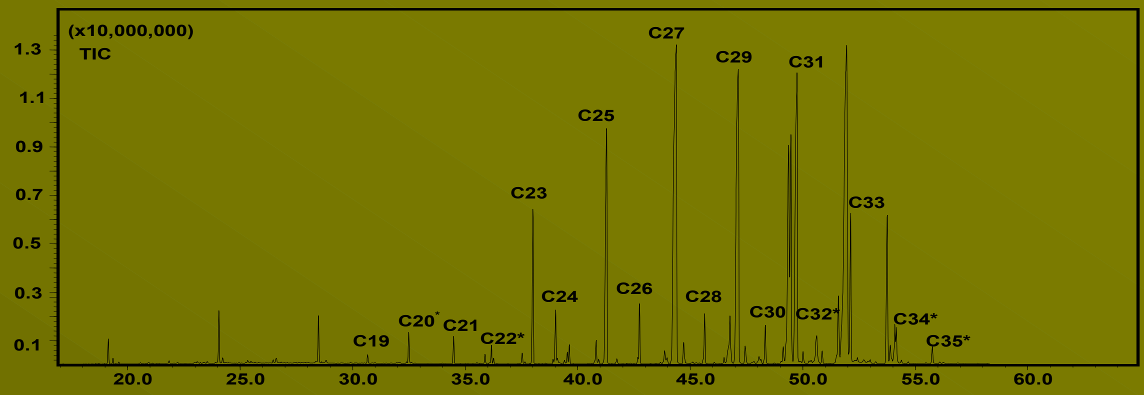
# Chromatogram of alkanes in commercial paraffin

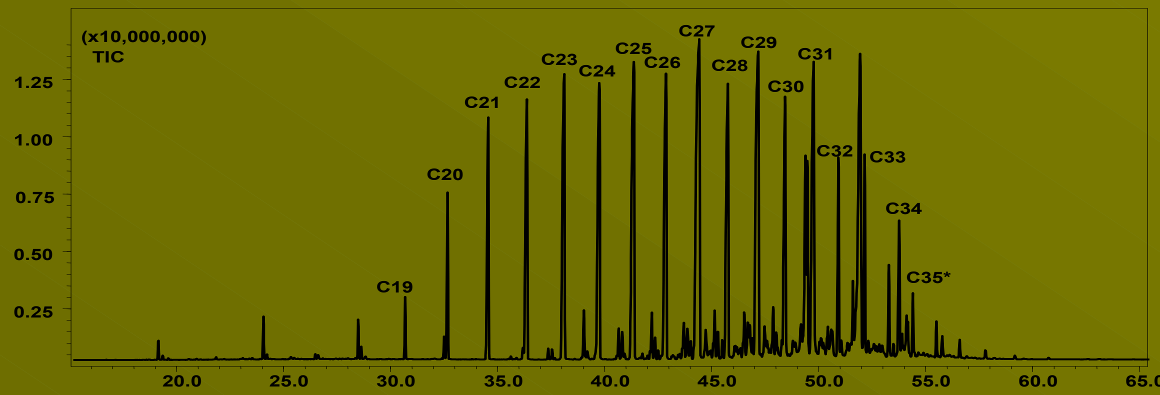


# Chromatogram of alkanes in samples of paraffin – adulterated beeswax

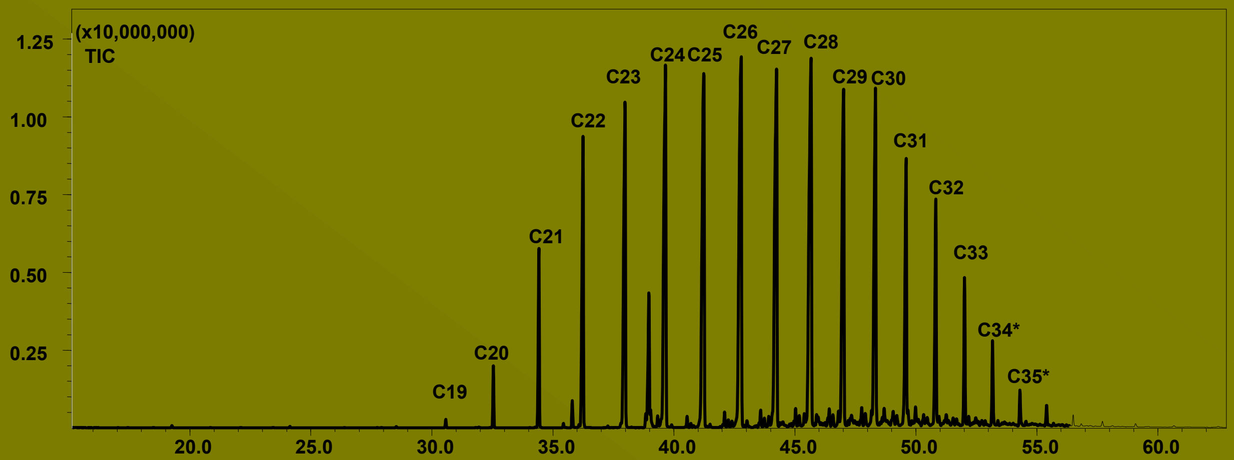


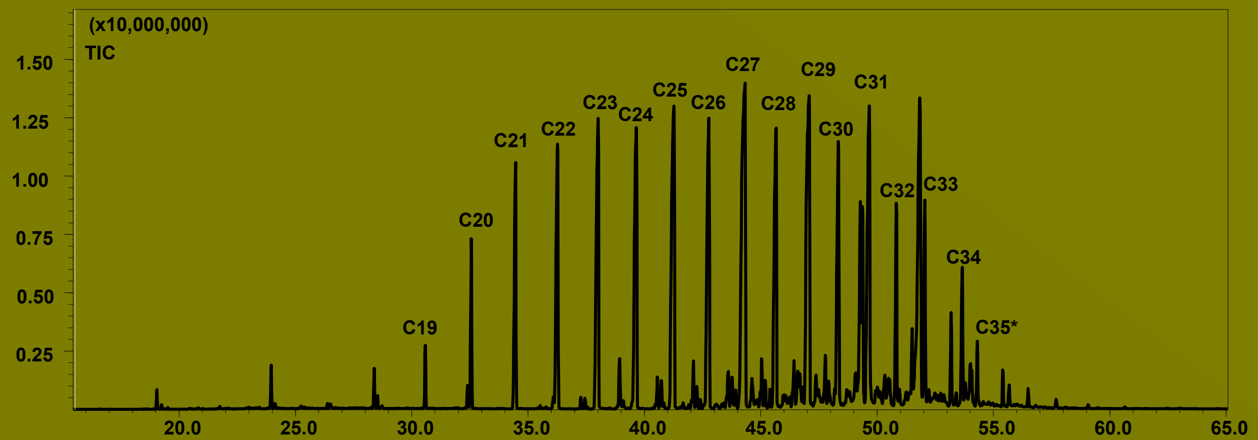
# The comparison of alkanes in natural beeswax and alkanes in commercial paraffin



# The comparison of alkanes in commercial paraffin and alkanes in paraffin – adulterated beeswax



# Conclussions

The homologous series of saturated hydrocarbons with unramified carbon chains occurring in natural beeswax was identified (from $C_{19}H_{40}$ to $C_{35}H_{72}$).

Some of unsaturated hydrocarbons with a single double bond ($C_{23}H_{46}$, $C_{25}H_{50}$, $C_{27}H_{54}$, $C_{29}H_{58}$, $C_{31}H_{62}$, $C_{33}H_{66}$, $C_{35}H_{70}$) and some of unsaturated hydrocarbons with two double bonds ($C_{31}H_{60}$, $C_{33}H_{64}$, $C_{35}H_{68}$) occurring in natural beeswax were also identyfied.

The method to detect beeswax adulteration with hydrocarbons by using the GS-MS technique was elaborated.

# Conclussions

The comparison of hydrocarbon chromatograph spectra in samples of natural beeswax with hydrocarbon chromatograph spectra in samples of commercial paraffin allowed for detection of samples paraffin – adulterated beeswax.

The investigations on the composition of natural beeswax and on the detection of the adulterations of that compound with hydrocarbons will be continued in the forthcoming years.

# Thank You for attention