1st World
Honeydew Honey Symposium
1-3 August, 2008, Tzarevo, Bulgaria
PROGRAM AND ABSTRACTS
Dear honey scientists, dear beekeepers,

Honeydew honey is an important honey type. Research has shown that it has especially high antioxidant and also antibacterial activity. World-wide and from a commercial point of view it is a relatively minor honey type. However, in many European countries like Germany, Switzerland, Austria, Slovenia, Greece, Turkey and others, honeydew honey is harvested in relatively high amounts, achieving very good prices. In other European countries the production levels are lower, rough estimates lie between 1 and 15 % of the total honey harvest.

In the International Honey Commission (IHC) there is a working group on honeydew honey headed by Werner von der Ohe, Germany. The work of this group is culminating now in the organisation of the First World Symposium on Honeydew Honey. The Symposium is a part of the Annual Festival of Honeydew Honey in Tzarevo, Bulgaria, lying in the main honeydew honey producing area in Bulgaria.

The norm for honeydew honey of a minimum value of 0.8 milli Siemens/cm (mS/cm) has been adopted in the Codex Alimentarius Standard and the European Honey Directive. In most countries this honey is labelled as “honeydew” or “forest”. Some countries have specific honeydew denominations such as “fir” or “oak”, based on sensory, microscopic and chemical analysis.

A special Apidologie issue on European unifloral honeys was published in 2004. This publication contains descriptions of European unifloral honeys and other information on unifloral honey characterisation. In this publication all honeydew honeys are regarded as one group. We hope that the work of this symposium will help to increase our knowledge on this valuable type of honey for a better characterisation of specific honeydew honeys, allowing denominations of individual honeydew honey types. It is planned to publish the important results of this conference.

Werner von der Ohe, President of the IHC
Stefan Bogdanov, vice-president of the IHC and Symposium-Coordinator

Scientific Committee
Werner von der Ohe, Germany; Hermann Pechhacker, Austria; Andreas Thrasyvoulou, Greece; Tseko Ivanov, Bulgaria and Stefan Bogdanov, Switzerland.

Program Coordinator and Editor of the Symposium Abstracts
Stefan Bogdanov

Organisers
Strandja Honey Cooperative manov-med@abv.bg

Sponsors and partners
- Tzarevo city administration, www.tzarevo.net
- Ministry of Agricultural and Foods
- Bulmed Honey, Bulgaria, www.bulmed.com
- Balparmak, Turkey, www.balparmak.com.tr
- WWF Danube-Carpatian Programme Bulgaria, www.panda.org/bulgaria
SYMPOSIUM PROGRAM

Thursday, 31.8.2008

- Arrival of participants
- 17.00-20.00 Registration at the venue site: Georgi Kondolov Hall

Friday 1.8.2008

- 08.30 -18.00 Registration
- 9.00-13.00 IHC meeting according to a special program
- 14.30-18.30 1. Tasting Session of Honeydew Honeys for honey specialists with Lucia Piana
  2. Sensory Session for Beekeepers and Guests with Stefan Bogdanov

Saturday, 2.8. 2008

- 8.30 – 12.00 Registration
- 9.00 – 9.45 Official Opening of the Honeydew Honey Festival and the Honeydew Honey Symposium outside the venue with all participants
- 10.00 Begin of the Symposium in the Georgi Kondolov Hall
- 10.00 – 12.45 Lectures
- 10.30 – Press Conference
- 14.30 – 19.00 Lectures
- 20.00-22.00 Cocktail for the Symposium Participants

Sunday, 3.8.2008

- 9.00 – 12.45 Lectures
- 14.30 – 15.30 Poster Session
- 15.30 – 16.30 Discussion and Conclusions

Lectures as Powerpoint Presentations

- Key Note Lectures: 30 minutes
- Research Communications: 15 minutes
- Extended Research Communications: 20 minutes
- Lectures are given by first or underlined author
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**Discussion**

12.25 - 14.30 Lunch

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<td>15.00-15.20</td>
<td>G. Georgiev, G. Tsankov, P. Mirchev, P. Petkov and M. Todorov, Bulgaria</td>
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| 15.45-16.00 | Tseko Ivanov, Bulgaria                       | Chemical composition and Characteristics of Bulgarian Honeydew Honey |
| 16.00-16.15 | Aslı Elif Sunay, Turkey                      | Authenticity and Sensorial Properties of Pine Honey from Turkey |
| 16.15-16.30 | Chrissoula Tananaki and Andreas Thrasyvoulou, Greece | The Physicochemical Characteristics of Greek Oak Honey |
| 16.30-16.40 |                                              |                                                  |

**Discussion**

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| 18.25-18.40 | Rosa Ana Pérez, Maria Montserrat González, María Teresa Iglesias, Encarnación Pueyo and Cristina de Lorenzo, Spain | Analytical, Sensory and Biological Features of Spanish Honeydews Honeys |
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|              | Characterization of Honeydew Honey Blends by Their Volatile and Semivolatile compounds |
| 11.25-11.45  | Miguel A. Fernández-Muiño, M. Teresa Sancho, Spain and M. Gabriela Gutierrez-Cadenas, Venezuela Chrisoula Tananaki and Andreas Thrasyvoulou, Greece |
|              | Determination of Volatile Compounds in of Honeydew Samples from Different Countries in two Laboratories. |
| 11.45-12.00  | Liviu Al. Marghitas, Otilia Bobis, Oltica G. Stanciu, Daniel Dezmiorean, Victorita Bonta, Olimpia Popescu, Romania Chrisoula Tananaki and Andreas Thrasyvoulou, Greece |
|              | Antioxidant Capacity of Honeydew Honey produced in Transilvania |
| 12.00-12.10  | Otilia Bobis and Oltica Stanciu, Romania |
|              | Antioxidant Capacity of Honeydew Honey from Different Countries. |
| 12.10-12.25  | Dinko Dinkov, Bulgaria |
|              | Antibacterial Activity of Bulgarian Honeydew and Blossom Honeys |
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ABSTRACTS

LECTURES

Honeydew Honeys of the World
Werner von der Ohe, Katharina von der Ohe
LAVES -Institut für Bienenkunde Celle, Herzogin-Eleonore-Allee 5, 29221 Celle, Germany
e-mail: Werner.von-der-Ohe@LAVES.Niedersachsen.de

In the first part of the review the quality criteria for honeydew honey as stated in the Codex Alimentarius and the European Honey Directive are discussed.

In the second part the results of the analysis of typical honeydew honey samples sent by the participants in the honeydew symposium are discussed. Samples from different parts of Europe as well as some samples from other continents have been analysed. The origin of the honeydew samples has been described by the participants. The origin goes from fir, spruce to non-coniferous honeydew honey like metcalfa, oak and others.

Honeydew Around the World
Hermann Pechhacker
Institut für Bienekunde, Lunz, Austria
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Up to the 1960s, some European beekeepers did not believe or did not want to believe that honeydew came from insects. But as far back as 1696, Van Leeuwenheek mentioned that honeydew could be produced by insects. Ehrenfels (1829) thought that honeydew was sweated out by plants. Then Stern (1841), Stoehr (1842), Noerdlinger (1854), and Buechen (1891) asserted that honeydew resulted exclusively from insects.

Research work on systematic, physiology, and honeydew production has been done by Noerdlinger, Schmutterer Kloft, Kunkel, Fossel, Scheurer, Pechhacker and many others.

In Europe beekeepers have long been quite familiar with honeydew. Across the entire European continent, honeydew is an important honey flow for beekeepers, especially in all the alpine areas (central Europe). In Greece and Turkey, Marchalina helenica is a very important honeydew producer, whereas in northern Italy and some other countries, Metcalpha pruinosa is an important new honeydew producer.

In Asia, Joshi (2000) found that especially the native bee species collect quite a lot of honeydew. Also in Australia and South America (Chile, Brazil), bees collect honeydew from various insects and trees. In Africa, we observed bees collecting honeydew even on beans, clover, and wheat.

This paper presents details about the amount of honeydew as well as honeydew honey of various plants, honeydew producers and areas.

Authentification of Honeydew Honey: A Review
The authenticity of honey has two different aspects: authenticity in respect of honey production and authenticity in respect of descriptions: geographical and botanical origin, ‘natural’, ‘organic’, ‘raw’ and ‘unheated’ honey. Some of these authenticity issues are the same for all types of honey and were addressed elsewhere (Bogdanov and Martin, 2002). Here only the authenticity of geographical and botanical origin of honeydew honey is addressed. For the authentification of geographic origin microscopic and chemical methods have been used. Microscopic analysis is based on the difference of pollen, originating from the flora of different countries. This method is promising only when the honeys originate from countries with distinctly different flora, while it is more difficult when the flora is similar. In the latter case the use of statistical methods is a promising tool. Chemical analysis can be also used for the differentiation of honeydew honeys. Pine honeys from Greece and Turkey can be differentiated on the basis of the volatile spectra (Tananaki et al. 2007). Also, rapid in-situ techniques such as front phase fluorescence spectrometry and FT-IR (Ruoff et al. 2006) can also be used for this purpose.

Botanical authentification implies mainly the differentiation between blossom and honeydew honey. The most important measurand is electrical conductivity but there are some blossom honeys with high electrical conductivity. Other measurands such as fructose + glucose content, melezitose content, amino acids, pH, acidity etc. can also be used, but there not a single parameter capable of a full differentiation between honeydew and blossom honeys, the best parameter being probably the oligosaccharide melezitose in Swiss honeys (Bogdanov and Gfeller, 2006). Combination of different parameters by discriminant functions was initially used by Kirkwood (1960) by using pH, ash content and reducing sugars and recently was successfully applied by combining pH, fructose, glucose and electrical conductivity (Bogdanov and Gfeller, 2006). Non-routine techniques such as front phase fluorescence spectrometry and FT-IR and volatile analysis (Fernandez et al. 2008) can also be used but require a specific and expensive instrumentation. Sensory analysis is the fastest method but it needs well trained panels. Microscopic analysis can be also used, but only in combination with sensory and physico-chemical measurements.

Typical honeydew honeys such as fir, pine, oak, metcalfa have been characterised in different countries, but there are no internationally valid criteria, similar to unifloral blossom honeys. It is likely, that traditional methods based on chemical, sensory and microscopic will not be successful for the differentiation of honeydew honey types. However statistic approaches based on colour and amino acid profile (Gonzalez-Paramas et al. 2007) and more sophisticated analysis such as front phase fluorescence spectrometry, FT-IR and volatile analysis are promising approaches. Microscopic characteristics have been used for the differentiation of Greek pine and honeydew honey (Dimou et al., 2006) and of spruce and fir honey in Germany and Switzerland (von der Ohe, personal communication).

**Sensory Characterisation of Honeydew Honeys from Different Countries**

Lucia Piana

Apishare, Monterenzio BO, 40050 Italy

e-mail: luciapiana@apishare.it
The methods for honey sensory analysis were specified in Piana et al. Apidologie, 2004. The sensory profile of honeys for honeydew honeys is characterised as follows:

**Visual assessment**: colour: dark to very dark; colour tone: normal honey colour, sometimes with green fluorescence.

**Olfactory assessment**: intensity of odour: medium; description: woody and warm

**Tasting assessment**: sweetness: medium; acidity: week; bitterness: absent; intensity of aroma: medium; aroma description: woody and warm; persistence/aftertaste: medium; other mouth perceptions: sometimes astringent.

Samples of different European countries, originating from different Coniferae (fir, spruce, pine), Latifoliæ (mostly different oak species) and *Metcalfa pruinosa* are characterised with the above method.

**Honeydew: Honey by Another Name?**

Richard Jones
International Bee Research Association, Cardiff, Great Britain

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Scientifically and philosophically precise definitions of honeydew are elusive and references can vary widely. Manna, honeydew and nectar are words used throughout the Ancient World to describe a sweet desirable liquid or crystalline substance sometimes referred to as “food of the gods”. For thousands of years it was thought that honeydew was a product that fell from heaven. This presentation looks at the derivation of the word in a number of languages thereby giving insights into the practical, philosophical and cultural background of a product that has always been surrounded by mystique. It is a substance that figures in most of the world’s major religions; as well as in folklore and legend from the Nordic lands through India to China.

**The Ecology of Aphid Honeydew**

Edward Baker
Barry, Vale of Glamorgan, United Kingdom, CF62 6PR

e-mail: edbaker1975@hotmail.com

Aphid honeydew is consumed by many organisms, either when fresh, when crystallised as manna, or when processed by bees to produce honeydew honey. As such, aphid honeydew is an important energy source in diverse food webs and ecosystems. The ecology of aphid honeydew is outlined by considering its production, composition and utilisation. Information concerning the ecology of aphid honeydew is synthesised from published papers and internet sources.

Aphids feed on plant phloem sap which is generally high in simple sugars and low in essential amino acids. Sap is processed with the aid of symbiotic gut bacteria that help aphids obtain sufficient amino acids to achieve their rapid growth rates. The osmotic concentration of phloem sap is reduced through dilution and conversion into complex sugars. As a result, excreted honeydew is nutritionally distinct from phloem sap, incorporating aphid synthesised sugars and a more balanced essential - non-essential amino acid content.

Amongst the insects, many pollinators, predators, parasitoids and mutualists consume honeydew. Some adult
insects rely on honeydew for survival and as a host location kairomone or oviposition stimulus. Honeydew may be especially important in early spring and autumn when flowers are in short supply and in environments that lack nectar sources. Insect population density and diversity may increase where honeydew is available.

Human consumption of aphid honeydew is in the form of manna or honeydew honey. Manna is collected primarily in dry parts of the world such as the Middle East, but also in the European Alps, and its use by native tribes in North America has been documented.

Production of honeydew honey is known to bees specially important in Central, Southern and Eastern Europe, with aphids of the family Lachnidae particularly important as producers of honeydew collected by bees.

The populations of phyllosphere micro-organisms, such as bacteria and fungi, can increase rapidly when honeydew is available. If this leads to increased immobilisation of atmospheric nitrogen by the micro-organisms, nitrogen inputs to soils may be reduced. The increased carbon input to soils from honeydew may fuel nitrogen immobilisation by soil micro-organisms, possibly leading to reduced soil nitrogen availability.

Whilst honeydew is primarily a waste product to aphids, it can be important to the survival and population dynamics of many other organisms, and consequently is important not only to individual organisms, but also at community and ecosystem levels of interaction.

Producers of honeydew on *Quercus* Spp. in the Strandzha mountain

G. Georgiev¹, G. Tsankov¹, P. Mirchev¹, P. Petkov¹, M. Todorov²

¹Forest Research Institute – Sofia, Bulgarian Academy of Sciences, Bulgaria

²Beekeeper – Tzarevo, Bulgaria

e-mail: ggeorg@bas.bg

The investigation was carried out during 2004–2007 in Strandzha Mountain. Oak forests in radius of 3 km around 14 apiaries in the area of State Forestries Tsarevo, Malko Tarnovo, Zvezdets, Kosti and State Game Station Gramatikovo were studied.

The forests of Bulgarian part of Strandzha are on 219 920 ha, with predominate oak forests, the largest part being *Quercus petraea* Liebl. (47.8 %) followed by – *Q. frainetto* Ten. forests (41.8%).

5 species of honeydew producers were determined: *Lachnus roboris* (Linnaeus 1758), *L. pallipes* (Hartig 1841), *Monelliopsis caryae* (Monell ex Riley & Monell, 1879), *Tuberculatus (Tuberculloides) querceus* (Kaltenbach 1843) and *T. annulatus* (Hartig 1841). The first three ones are new for the fauna of Bulgaria.

The host plant of *T. querceus*, *T. annulatus* and *L. roboris* is *Q. frainetto*, of *L. pallipes* – *Q. hartwissiana* Stev. and of *M. caryae* – *Juglans nigra* L..

A limitation factor for the number of the plant lice is the abundant rains in the beginning of the summer (June – July).

The number of *T. querceus* and *T. annulatus* on the sunlight spots of the crown is 3 times higher than the population on the leaves in relative shadow.
The appearance of first plant lice in regions neighbouring the sea (Ahtopol, Velika and Varvara) could be observed around 15 May and in further beech forests (Brodilovo, Fazanovo and Novite murzovski kolibi) two weeks earlier – at the beginning of May, and about 17 May their number is relatively high and the first cells with honey due appear in the bee hives.

On the leaves with honeydew fungi of the genus *Capnodium* Mont. and *Fumago* Pers. develop.

The electro-conductivity of honeydew honey for the period 1999-2006 varies within the limits of 0.85-1.40 µS. With best qualities was in 2000 when entire quantities were with maximal indicators for honeydew – 1.40 µS.

The data from questionnaires showed that in 2003 in Strandzha were obtained record quantities of honeydew honey (average of 36 kg per hive), in 2004 for the first time since 30 years no honeydew honey was produced.

### Honeydew Honey Control and Legislation

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Quality Services International (QSI), Flughafendamm 9a; D-28199 Bremen, Germany  
e-mail: beckh@qsi-q3.de

The European Commission is focusing on the Quality Control of imported foodstuffs especially of animal origin. Through this the regulations concerning the quality control of honey have strongly increased; the honey market is concerned by an increasing number of problems and the honey trade is influenced by scandals.

Most of European legislation is in force for all honey types also honeydew honey.

The only problem might arise regarding the declaration of honeydew honeys between the different member states of the EU due to their national legal requirements. With new honey producing countries exporting their goods to Europe we are facing the problem that we have only little knowledge of the honeydew producers and the characteristics of these honeys. Up to now honeydew honeys were usually sold as “forest honey”. In some member states the authorities claimed this as illegal. There is as well still the discussion if Eucalyptus honey with high electrical conductivity is really honeydew. Can forest honey only be declared if the honeydew is produced on Picea resp. Abies species? Data and examples from the daily quality control of QSI are presented.

### Chemical composition and Characteristics of Bulgarian Honeydew Honey

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The composition and the properties of 15 samples of honeydew honey from different regions of Bulgaria were studied. The official methods of the International honey commission and the Bulgarian state standard for honey, as well as the methods described by Ivanov were applied.

It was established that honeydew honey differs from nectar honey with higher ash, protein and apparent sucrose content, electroconductivity, pH and invertase and catalase activities. At the same time honeydew honey has lower glucose, fructose, glucose + fructose, apparent reducing sugars and total sugars content as well as lower glucoseoxidase activity. The specific optical rotation is positive in all cases. The other studied parameters of
honeydew honey (water, HMF, acidity and diastase, acid phosphatase, esterase and protease activity) don’t differ essentially from nectar honey parameters.

The obtained results show that Bulgarian honeydew honey has similar composition and properties to honeydew honeys from other countries.

**Authenticity and Sensorial Properties of Pine Honey from Turkey**

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Honey production and trade is one of the important sources of finance in the world. Turkey is one of the 10 largest producers of honey and the largest producer of pine honey with approximately 15,000 tons of production each year. The high capacity of trade opportunity brings problems about authenticity on the agenda on all over the world.

One of the main problems concerning authenticity is adulteration of honey with different types of sugar solutions like high fructose corn syrup, invert sugar syrup, glucose or sucrose solutions. Some beekeepers feed bees with sugar solutions during the nectar flow, so honey is produced from that solution instead of nectar from plants. Addition of syrups directly to honey to increase its quantity and change of geographical or floral origin are other problems for the world honey market. To detect authenticity of honey, the best way is to examine the physical, chemical and sensorial properties of honey together with its microscopic structure (pollens and other particles).

The aim of this study was to collect data on properties of Turkish pine honey to determine its authenticity. For this purpose, a total of 3613 honey samples were collected from beekeepers during a three-year surveillance program (2004-2005-2006) from Muğla region in Turkey and were analysed for HMF (Hydroxymethylfurfural) content, diastase activity, free acidity, water content, conductivity, sugar composition, honeydew elements and stable isotope ratios ($^{13}$C) as part of the project which was also supported by Technology And Innovation Funding Programs Directorate and Foreign Trade Department called “Determination of residues, adulteration and origin of honey according to geographical regions”. Published methods of AOAC (Association of Analytical Communities) and IHC (International Honey Commission) were used and a descriptive sensory analysis was performed to determine the sensorial profile of Turkish Pine Honey. Data were evaluated using different statistical techniques.

As a result of statistical evaluation, 212 samples showed significantly different physical and chemical properties and were considered unauthentic for pine honey. Within those unauthentic samples, results of 41 samples were interpreted as from flower origin and 171 samples as adulteration with sugar solutions. 45 sensory characteristics (7 for texture, 17 for odour and 21 for flavour) were determined for sensorial profile of honey and 8 characteristics were found to be special for pine honey. Results are also discussed by means of specifications described in Turkish Food Codex Honey Regulation (2005/49) for pine honey.

**The Physicochemical Characteristics of Greek Oak Honey**

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In order to characterize the quality properties of the Greek oak honey, the carbohydrate and volatile composition of this honeydew honey were studied. Water content, electrical conductivity, pH, acidity, HMF and diastase activity were also determined. The levels of fructose glucose, sucrose, turanose and maltose were measured by liquid chromatography. The sum of the fructose and glucose contents ranged from 57.0% to 73.8%. Totally 99 volatile compounds were detected in oak honey samples, using a Purge and Trap – GC – MS system. Among the forty compounds, which found in all samples the main substances were: 2-methyl-1-butanol, octane, nonanal, nonanol and decanal. Lilalool, 2-methyl-1-butanol, guaiene, and two hydrocarbons were the characteristic volatile substances of oak honey, which could be used as possible botanical markers.

Examination of Some Quality Parameters of Honeydew Honey from Serbia
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Introduction: Honeydew honeys represent about 1% percent of the total honey production in Serbia (Anđelković & Mladenović, 2003). The majority honeydew comes from Chestnut oak (Quercus petraca).

There are different methods of proving the quality of honey – refraction index, pH value, thickness and sensory features. However, to determine the botanic origin of honey it is recommended to inspect the electrical conductivity (Bogdanov et al. 1997). The purpose of this study was to investigate the electrical conductivity, pH and water content of honeydew specimens sold at beekeepers fairs on the Belgrade market.

Material & Methods: 7 samples were gathered directly from beekeepers mostly from south and west of the country. All honeys had the sensory and physico-chemical investigation. Electrical conductivity was examined by standard method (Bogdanov, 2002) via conductivity meter WTW 340i, pH was measured on equipment pH metter Iskra MA 5735, and water was routinely determined by an Abbe analogue refractometer.

Results and Conclusion: The average of electrical conductivity was 1,077 mS/cm with St. Dev. of 0,24 and coefficient of variation 22,28%, the average value of pH was 4,29 with St. Dev. of 0,30 and coefficient of variation 6,89% and average of water content was 16,26% with St. Dev. of 4,46%. All samples had dark to very dark colour, medium odour, medium sweetness, woody aroma and medium thick consistency.

To conclude all samples of honeydew corresponds to quality criteria of the European Commission (2002) and Persano Oddo & Piro (2004). The production of honeydew in Serbia could be possible, but in the future specific regions with chestnut and oak pasture should be investigated.

Oak (Quercus frainetto Ten.) Honeydew without Mediation of Plant Sucking Insects in Požega basin
(Croatia)
Quercus frainetto Ten. is species oak of the Southeast Europe, part of Italy and the west part of Asia. Western border is in Požega valley region in Croatia, and South Peloponnesus, Greece.

Beekeepers from Požega region record that bees readily collect the plant sap on oak. In this area of distribution of the *Q. frainetto* is recognized as a specific source of honeydew. Growth process of oak fruits is extremely rich in cycles of 5-8 years, and during other years fruit production is standard. Process of balance of the number of fruits on each tree is specific. At the natural reduction of overproduction of fruits, the sweet sap from fruits is noticed. Fruit sap starts to flow over cuticle of the fruit often with the foam appearance. Single fruit produces sap for several days, than process starts on another fruit. Occurrence of fruit sap in wood area can last up to two months.

During honey flow season in 2003 and 2004 year, with medium to low honeydew honey production in Požega basin, 10 samples of honey were taken for the analyses. According to the results these honeys are not typical honeydew honeys. There are no special honeydew sugars and also electrical conductivity is low. There are also only quite less amounts of honeydew elements in the sediment. The sugar spectra of the oak fruits show high amounts of fructose (30,4 %), glucose (17,9 %), trehalose (26,9 %) and one not identified oligosaccharide (24,9 %). So the high fructose/glucose ratio seems to come from these oak fruit excretions. Histological analyses confirmed that mediation of plant sucking insects do not take part in process of honeydew generating. Probably the physiological process of the plant induces this specific honeydew.

**Studies on the Chemical Composition and the Antioxidant Activity of Some Romanian Honeydew Hones**

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**Introduction.** An extensive study on the Romanian honey sorts was undergone during a 2 years period in order to classify them according to their characteristics. Honeydew honey is also common enough but unfortunately not enough studied until now.

**Material and methods.** Several honeydew honey and honeydew honey mixture (mainly consisting of fir, spruce and pine tree) as well as other samples in the plateau and hills areas (with oak, birch species) samples from various regions of Romania were analyzed for their physico-chemical parameters (pH, acidity, water, ash, reducing sugars, diastase number, HMF contents, saccharose and electrical conductivity). The resulting data were used, along with palynological analysis, to characterize the samples in relation to their possible source (nectar, honeydew and
mixture honeys). Some of the most representative samples were also analyzed for their phenolic spectrum using the HPLC method with UV detection as well as for their antioxidant activity against DPPH used as free radical.

Results. The HPLC-UV analysis of Romanian honeydew honey shows a rich profile containing all the polyphenols used as standards. The most representative from the peak height and area point of view seems to be the gallic acid, but all the other phenolic acids are present. Flavonoids make no exception, however their percentages related to the total amount of polyphenols are rather poor. For the AO activity of the honeydew honey samples the results were expressed in mg/g of honey in equivalents Trolox.

Conclusions. In Romania, there is a large diversity of honeydew and nectar mixtures due to the geobotanical conditions and the large diversity of floral species in various regions. The most abundant honeydew honey samples are to be found in the mountain area (conifers) and also in the hills regions under the Carpathians chain. Quite often the plateau samples (Transilvania region) have some accompanying floral species as sun-flower, while the samples in the sub Carpathian regions often contain linden, orchard fruit trees and acacia, species. A special mapping of the main floral and extrafloral honey samples is under development.

Characterisation of Italian Honeydew Honeys

Characterisation of Italian Honeydew Honeys

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In Italy the production of honeydew honey is quite high and widespread around the country. This type of honey comes from the honeydew emitted from a wide variety of sucking insects that are parasites on different plants growing in Italy. Each honeydew is different and each corresponds to a different kind of honey characterized by particular chemical-physical and organoleptic properties. The Italian beekeepers known the honeydew honey well and are not always glad to harvest it. It may cause problems in unifloral honey production and sometimes there are problems in comb extraction. We know that honeydew honey comes from such plants as *abies, picea, citrus, quercus, castanea, tilia*, etc., but only two of this have been studied and characterized in Italy: *abies* honeydew honey and metcalfa honeydew honey.

The production of the abies honeydew honey is limited to the Alps and the Apennines of Tuscany. The colour of the honey is very dark with green tone. The intensity of odour is medium and characteristically warm, vegetal and woody. Sweetness is low and the aroma is characterized by a balsamic and resin sensation. The chemical characteristics are similar to the other honeydew honeys: high colour values, electrical conductivity and pH; positive values of specific rotation and low values of fructose and glucose. Nevertheless in the glucidic spectrum it shows a relatively high content of oligosaccharides like melitizite, erlose, raffinose, trealose and a low content of maltotriose.

The name of the metcalfa honeydew honey comes from the insect that produces the honeydew: *Metcalfa pruinosa*. This insect was introduced to Europe at the end of the 1970s and has now spread to Italy, Slovenia and France where it attacks many plants giving rise, in summer, to significant quantities of unifloral honey. The colour of the honey is very dark, the intensity of odour is medium with warm and vegetal characteristics, sweetness is low or medium and the aroma is warm, vegetal and fruity (cooked fruit). With regard to its physico-chemical properties
this honey displays high values in: terms of colour; electrical conductivity; pH and acidity and positive value of specific rotation. In the glucidic spectrum it shows a relatively high content of maltotriose and a low value of melezitose.

**Honeydew and Honeydew Honey in France**

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In France, the main producing species of honeydew are fire tree (*Abies alba*) and spruce (*Picea abies*). Other species, Scots pine (*Pinus sylvestris*), oaks (*Quercus sp.*), beech (*Fagus sylvatica*), larch (*larix deciduas*) can produce honeydew too but in lesser quantity. Since 1985, it is possible to harvest honeydew from *Metcalfa* (in valley of Rhône). Lime tree, chestnut, maple, poplar and willow produce honeydew but often mixed with nectar. With a forest surface of 155 000 km², France ranks fourth amongst European countries. The franck forests covers 28.2% of the total country: oaks represent 25% of this surface, beech 11%, pruce 8%, maritime pine 8%, fir tree 7%, Scots pine 6%. In order of importance, the producing regions of honeydew are: Vosges, Jura, Alsace, the Alps, the Pyrenees, Massif Central, Haute Loire, South East and Corsica. The potential production of honeydew is enormous but little exploited by beekeepers because of unpredictable productions and risks.

In France, few studies were dedicated to honeydew, its characterization and its production conditions. As a result a first study, conducted in 1975, standards were set up for fir tree honeydew by I.T.A.P.I. (Institute Technical of Apiculture). This honey has a good name among consumers. It is appreciated for its pleasant taste and its particular sticky texture. For the designation “fir tree”, the authorities accept mix honeydew honeys from the genus *Abies* and *Picea*.

Since 1999, there is a Protected Geographical Indication (PGI) for fir tree honeydew originating from Vosges (*Miel de sapin des Vosges*). Specifications define the geographical areas of harvest, the botanical origin (only *Abies pectinata Lmk*), and physico-chemical criteria: HMF, moisture, colour, conductivity and taste. To assist beekeepers to forecast “honeydew flows”, I.N.R.A. (National Institute of Agricultural Research) of Colmar with Mr Yves Bouchery, elaborated a system to support transhumance with reference manual to recognize trees and aphids producing honeydews.

**Analytical, Sensory and Biological Features of Spanish Honeydews Honeys**

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Introduction. Honey composition and properties depend on the plants visited by bees, and thus, indirectly, on soil and climate conditions. Different studies (physico-chemical characterization, sensory features and biological activities) have been carried out to assess the differences between (i) blossom, (ii) “blends” and (iii) honeydew Spanish honeys.

Material & Methods. Melissopalinological analysis was made by use of a non-acetolytic technique to preserve honeydew elements. Physico-chemical parameters were determined according to the Harmonized Methods of the IHC. In addition, total phenols content was assayed by Folin-Ciocalteau; Net absorbance was measured as analytical measure of honey colour; free primary amino acids were quantified by HPLC. Quantitative descriptive sensory analysis was developed and applied. Total antioxidant potential was determined by in vitro (i) radical scavenging capacity and (ii) anti-browning activity. Antimicrobial activity was measured as inhibition of growth of selected Spanish Type Collection bacterial strains.

Results and Conclusion. When compared to nectar honeys, Spanish honeydews showed higher pH, electrical conductivity (E.C.) and acidity, and darker colours. They also possessed higher antioxidant activities. Best indicators of the honeydew origin were (i) colour, (ii) polyphenol content and (iii) E.C. Additional studies taking into account the composition of free amino acids suggested that this profile is a very good indicator of the radical scavenging capacity of honeys. Regarding antimicrobial activity, no generalisation could be obtained. Sensory analysis offered a longer persistence of aroma in honeydews than in floral or “blend” honeys, together with higher perception of bitterness, darker colours and very low perception of granularity in mouth.

Slovakian Honeydew Honeys – Types and Sources
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In 2006 forest area in Slovakia reached 2 007 thousands hectares, actual forest coverage of the country is 41% and is still on increase. Coniferous forests share to this acreage 30,8%, deciduous forest 49,7% and mixed ones 18,9%. Protected areas including buffer zones compose 1 135 thousands ha (23% of the country area). Most abundant species are beech (31,2%), followed by spruce (26,1%), oak (13,4%), pine (7,2%), hornbeam (5,7%) and fir (4%). Sustained areas of spruce, fir and oak reduced to monoculture creates around 520 000 ha.

Good honeydew flow appears irregularly, approximately in four years periodicity. Average amount of honeydew per 1 ha is app. 500 kg, honeybees are able to exploit app. 100 kg. Available honeydew storages in Slovakian forests are thus around 10 400 tons annually, in reality production of pure dark honeydew honeys is a max. 500 tons from a total Slovakian annual honey production of around 3 400 tons, even certain tonnage of mixed dark colored floral-honeydew honeys are produced as well. Average honeydew honey yield per honeybee colony placed in mountainous area is around 20 kg. Most demanded honeydew honeys on the market are pure spruce and fir, other honeys are marketed usually as mixed „forest” honeys. In 2007 year 74 samples of Slovakian honeydew honeys were measured with average values of water content 16,07%, HMF 5,36 mg/kg and sucrose 1,1%.

In Slovakia around 800 species of Aphidoidea and Coccoidea species are presented, from those around 40 species are important as honeydew producers. Monitoring in past 10 years shows that most important honeydew producing species includes 11 aphids and 1 scale insect on coniferous trees as well as 8 aphids and 3 scales on deciduous trees.

Most important honeydew producers by host tree species in Slovakia are:

Coniferous trees:
Fir – Cinara pectinatae, Todolachnus abieticola (sometimes are overpopulated by non-honeydew producer Dreyfusia nordmanniana). In 2006 invasive species Cinara curvipes was found in huge amount on white fir Abies concolor. Spruce – Cinara piceae, Cinara pilicornis, Cinara bogdanowii, Todolachnus abioticola, Physokermes piceae. Pine – Cinara pini, Cinara nuda, Cinara escherichi (on Black pine Pinus nigra also Cinara Beauni). Larch (Larix decidua) – Cinara laricis, Cinara kochiana. Juniper – Cupressobium juniperi. Deciduous trees:
Production of Honeydew Honey in Nepal

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General. In Nepal though people prefer to consume honey produced from forest, but there is a general lack of awareness about honeydew honey. The laboratories facilities are also not available to make chemical and melissopalynological analysis of honey.

Nepal’s diverse climatic conditions and abundance of flora make it host to five species of honeybee that include indigenous honeybees *Apis cerana*, *A. dorsata*, *A. florea*, and *A. laboriosa* and exotic *A. mellifera*. Many plant species, which are reported as good source of honeydew honey in other parts of the world, are found in Nepal (e.g. *Pinus* spp, *Picea* spp, *Quercus* spp, *Populas* spp). However, the species of insects that produce honeydew and extent to which they excrete honeydew is largely unexplored. A very little work has been done in honeydew honey of Nepal. So far, *Cinara eastopi* on *Pinus wallichiana*, *Cinara Camatar* on *Picea smithiana* and *Tinocolloides montanus* on *Prunus cerassoides* have bee found producing an enormous quantity of honeydew.

The measurements of electrical conductivity indicates that about 30-40% of honey harvested from high altitude areas (above 1500 metre above sea level) is produced all or partly from honeydew. Data obtained for electrical conductivity of honey samples collected from *Apis dorsata*, *Apis cerana* and *Apis mellifera* from low altitude area (below 500 masl) suggest that the native bees collect more honeydew than Apis mellifera bees. The average electrical conductivity reported as 0.96mS/cm for *Apis dorsata*, 0.65mS/cm for *Apis cerana* and 0.31 for *Apis mellifera*. As reported in other parts of the world, Nepali honey samples that have high electrical conductivity contain high number of fungal spores and di-trisaccharide sugars such as raffinose, *oligosaccharides* etc.

The study. The samples analysed for the present study were collected from different bee hives located in different villages of Kaski district central Nepal. All of these samples had typical characteristics of honeydew honey. Minimal and Maximal values for *Apis cerana* honey samples are (values in g /100 g honey where not indicated): Moisture: 14.9-21.6; sum of fructose and glucose 66.9-71.2; fructose: 36.2-39.5; glucose: 29-34; sucrose: 2.4-4.6; maltose: 0-1.5; electrical conductivity: 0.59-1.52; HMF, mg/kg: 0-67.3; free acidity, milli equivalents per kg: 3.2-16.3; pH: 4.2-6.0.

A first approach to the characterization of Portuguese Honeydew Honeys

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At the moment, the Portuguese honey sector is mostly focused on the production of monofloral nectar honeys that simultaneously have a greater market demand and commercially represent higher incomes for beekeepers. In Portugal honeydew honey production is frequently associated with lower quality product and therefore, in regions appropriated for this type of production (mountain regions of northeast with an abundant presence of *Quercus pyrenaica* and *Quercus rotundifolia*), the beekeepers use to post the harvesting season for latter summer, mixing nectar and honeydew, but guarantee the minimum physical-chemical parameters that would allow them to sell it as blossom honey. It is therefore uncommon to find pure Portuguese honeydew on the market. Even so, in Central Europe regions this type of honey is highly appreciated due to its strong flavour and taste associated recently with a higher content in antioxidants. This work, focused on physical-chemical analyses of honeydew honeys, aim to contribute for the valorisation of this production of honey among Portuguese beekeepers. The honeys studied were chosen from a wide group of 180 samples produced in 2007 by beekeepers of Northeast of Portugal and collected by the local beekeepers association, AAPNM. 30 samples were selected based on the apiaries locations and surrounding flora together with the organoleptic properties. These samples were analysed for humidity, pH, free acidity, lactones, conductivity, colour and content in phenolic compounds, following IHC methods. Colour was measured at 635 nm and converting to Pfund. The phenolic compounds were analysed for its total content and in terms of flavone and flavanone individual content using the Folin-Ciocalteau, the AlCl₃, and the 2,4-DNP methods. Additionally, a melissopalinology qualitative analyse was made using the method described by Von-Der-Ohe. The pollen analysis revealed, not surprisingly, ratios of HDE/P lower than 3 what could reflect the floral origin of the honeydew and the low humidity conditions, but probably the presence of blend honeys. For other side the results for the physical-chemical parameters showed high values of pH and conductivity, 4,7-5,2 and 0,8 to 1,2 mS/cm, respectively. In terms of free acidity these honeys varies from 25-39 meq/kg, and were classified mostly as dark amber honeys. The amount of phenolic compounds is considerably high in these honeys, mostly due to the presence of flavone/flavonol rather than flavonone.

**Some Physicochemical Properties of Polish Honeydew Honey**

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In Poland, within the habitat range of some coniferous species, especially of fir and spruce, the honeydew of those trees may be the main forage for bees. In certain years Polish beekeepers signal also the occurrence of the so-called “cement” honey in their apiaries. This honey crystallized in the combs into firm, hard mass difficult to remove and impossible to centrifuge. Most frequently, they point to honeydewing of larch trees as the cause behind that phenomenon. The honeydew of not-coniferous species, especially of lindens, oaks, plum-trees, may be also considerable forage for bees in Poland.

The aim of our study was to determine some physicochemical properties of honeydew honey harvested in Poland in the respect of quality parameters. Study material was provided by samples of honeydew honey from coniferous and non-coniferous trees, and also honeydew honey mixed with blossom honey (so-called blossom-honeydew honey).
The samples were originated mainly from south-east part of Poland from 2007. Organoleptic properties, water content, acidity, electrical conductivity, HMF content, diastase activity (DN) as well as quantitative and qualitative sugar composition (HPLC method) were tested in the honey samples.

The results from the study showed that honeydew honey harvested under Polish conditions was characterized by high quality parameters, in all cases compiled general requirements of COUNCIL DIRECTIVE 2001/110/EC relating to honey. Polish honeydew honey stands out by considerable low sucrose content, exceptionally only higher than 2g/100g and considerable high content of sum of glucose and fructose, always above 55g/100g. Electrical conductivity and diastase activity were also on a high level.

**Characterisation of Honeydew Honey in Switzerland**

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**Introduction.** In Switzerland about 2/3 of the honey is of honeydew origin. There are two main groups of honeydew producing trees: coniferous, mainly spruce and fir, besides some pine and larch, and non-coniferous, mostly linden, maple, oak and sweet chestnut. Most frequently Swiss beekeepers do not label their honey as blossom or honeydew, the label Swiss Honey being most frequent. Labels such as “forest honey” or “summer honey” are most usual.

**Material and Methods.** The samples were gathered from beekeepers in different parts of Switzerland, harvested between 1995 and 2003. The physico chemical methods used were those specified in the Swiss Food Manual, which are the same as the ones of the European Honey Commission (1997). Microscopic analysis was carried out according to von der Ohe et al., 2004, descriptive sensory analysis was carried according to Piana et al. 2004. In addition, FT-IR was applied for the determination of the physico-chemical parameters (Ruoff et al. 2005) and for the botanical authentification of honeydew honey (Ruoff et al. 2006).

**Results and Discussion.** The specific characteristics (minimum and maximum values) of Swiss Abies honey (fir and spruce, n=64) are: water content: 13.2 – 17.2 g/100 g; glucose/water ratio: 1.45-2.13; fructose/glucose ratio: 1.07-1.44, electrical conductivity 0.96-1.33 mS/cm; free acidity: 17.2-46.0 milli equivalents/kg; melezitose content: 0.0-8.2 g/100 g. The corresponding values for the non-coniferous honeydew honey, n=48 are: 13.5-18.9; 1.01-2.18; 1.09-1.42; 0.8-1.03; 8-42; 0.0-5.0. The two honey types cannot be differentiated by their physico-chemical characteristics. The coniferous honeys had a slightly higher electrical conductivity and melezitose content and a more resinous taste than the non-coniferous ones, the latter having a more malty taste. The range of the sensory characteristics of non-coniferous honeys is wider than that of the coniferous honey. The sensory difference between fir and spruce honey is less pronounced than the one between coniferous and non-coniferous honeys, the first two honey types can be differentiated by sugar and microscopic analysis. Swiss fir honeys could be distinguished from non-Swiss metcalfa and oak honeydew honeys by FT-IR. The above honey measurands can be determined also after FT-IR calibration. FT-IR is very rapid and not labour-intensive, and is thus a promising tool for the
determination of the important honeydew honey parameters and also for the botanical authentification of honeydew honey.

**Classification tool for Honeydew Honey**

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Beekeeping practice in Slovenia calls all honey types derived from honeydew as a forest honey. Along general type forest honey Slovenia beekeepers define two another honeydew type honeys: fir and spruce honey. During several years of studies of honey samples from different parts of Slovenia we built up a database of physical, chemical, microbiological and sensory characteristics of different types of honey. The major conclusion is that it is not possible to find margins between different type of honey. Quality characteristic of specific type of honey has to be determined subjective. These criteria can favor and stimulated production of major single source of honey. We have prepared a tool to help in decision usually between special type and general type of honey. The tool is prepared based on experience with comparison of the biodiversity between different assesment of species in sampled areas. Based on the calculated similarity indexes we can classify investigated sample according to the similarity to the well defined reference samples marked in the data base. Reference samples can be added or replaced which can be done based on reanalysis of all assest samples. Analysis of Slovene honeydew honeys reviles high diversity in all measured parameters. Fir honey is more uniform as spruce honey. In case of spruce we could expect several types of honey based on high diversity of honeydew producers and partially also related to the geographical region. Along with these two coniferous based honey, we have neglected contribution of honeydew producers in some traditionally claimed unifloral honey from the forest, like maple and lime honey. In case of general floral and forest honey it would be important to use also oligosaharide analysis along for electric conductivity to make decision between floral and honeydew source.

**Volatile Compounds as a Mean to Determine the Botanical and Geographical Origin of Greek Honeydew Honey**

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Pine (*Pinus sp.*), fir (*Abies cephalonica*) and oak (*Quercus Spp*) honeys were analyzed and their characteristic combinations of volatile compounds were found. These combinations were used to find out the botanical origin of honeydew honeys. Analyses of characteristic honey samples from chestnut (*Castanea sativa*), citrus (*Citrus sp.*),...
cotton (*Cossypium hirsutum*), erica (*Erica* sp.), knotgrass (*Paliurus acuteata*), thorn (*Polygonum aviculare*) and thyme (*Thymus* sp.) plants and also from honeydew secretion were also used for the comparison.

The effect of geographic origin on the volatile compounds of pine honey was also studied. Samples from 5 different areas (Rhodes, Crete, Evia, Chalkidiki and Thassos) were collected during a time period of two years. The above areas were selected because they present the highest proportion of the annual Greek production of pine honey. Honey from Crete and Rhodes had different volatile profile from the two areas. Moreover, pine honey from Mugla in southwest Turkey were analysed in comparison to pine honey from Chalkidiki Greece. The conclusion was that samples of the same botanical honeydew origin can be discriminated when they are produced from regions, which are situated far away from each other.

### Characterization of Honeydew Honey Blends by their Volatile and Semivolatile Compounds

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**Introduction:** Flavour compounds have proved to be useful markers to outline the botanical origin of honey. Honeydew honeys are well appreciated by the consumers because of the fact that they are strongly flavoured, among other reasons. The purpose of this work has been to study the flavours’ profiles of both floral and blends of honeydew and floral honeys, trying to find particular markers that could be distinctive of each group of honeys.

**Material & Methods:** 20 samples of honeys coming from a continental climate area were collected. According to melissopalinology, 10 samples were floral honeys and 10 samples were blends of honeydew and floral honeys (HDE/P: 1.5-2.99). Aroma-compounds isolation was performed by ethyl acetate extraction and the concentrated unmethylated extracts were analyzed by GC-MS. Statistical determinations have been carried out by applying one-way ANOVA, cluster and discriminant analyses of Statgraphics Plus 5.1.

**Results:** In the samples analyzed, more than 80 volatile and semivolatile compounds were found. The compounds whose profiles were clearly different in blends of floral and honeydew honeys were the following: Among aliphatic compounds: meso-butane-2,3-diol. Among monoterpenes: hotrienol. Among benzene derivatives: phenylacetic acid and pyrocatechol, and among flavonoids: 5-hydroxy-7-methoxyflavone and pinocembrin. Cluster analysis clearly grouped all the samples according to their botanical origin. Discriminant analysis correctly classified 100% samples, making two well-separated groups.

**Conclusion:** Honeydew honeys could be characterized by their profiles of meso-butane-2,3-diol, hotrienol, phenylacetic acid, pyrocatechol, 5-hydroxy-7-methoxyflavone and pinocembrin.

### Antioxidant Capacity of Honeydew Honey produced in Transilvania

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Introduction: In the present study, antioxidant capacity of honeydew honeys from Transylvania were evaluated, in comparison with the level of total phenolic content.

Materials and Methods: Seven samples of honeydew honeys, nine samples of mixed honeydew-polifloral honeys and 12 of polifloral honeys were analyzed. The botanical origin of honeydew samples was verified by melissopalynological analysis and certified by physicochemical parameters. Total phenolic content was estimated according to the Folin-Ciocalteau spectrophotometric method (Singleton et al., 1999). The scavenging activity (H/e-transferring ability) against 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) was evaluated spectrophotometrically by a slightly modified method of Brand-Williams (1995). Total antioxidant potential of the sample was evaluated using the ferric reducing ability (FRAP) assay as a measure of “antioxidant power” (Benzie & Strain, 1996). For Trolox Equivalent Antioxidant Capacity assay, the procedure followed the method of Re R. et al. (1999) with some modifications.

Results and Conclusions: The total phenolic content of the honeydew honeys studied varied between 120.82 - 109.15 mg GAE g⁻¹. The methanolic solutions (5%) of honeydew honeys demonstrate highly antioxidant potential, the values obtained ranged between 147.08-279.24 mM Trolox equivalent /100 g⁻¹ honey (DPPH scavenging activity), 16.34-27.56 mM Trolox equivalent/100 g⁻¹ honey (TEAC assay) and 35.11-70.71 mmol FeII/100 g⁻¹ honey (FRAP assay). Positive correlations between antioxidant content and their antioxidant capacity was observed (R² = 0.638). The results suggest that the honeydew honey is a good source of antioxidants.

Antibacterial Activity of Bulgarian Honeydew and Blossom Honeys
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In the study were presented data for comparison of antibacterial (agar well diffusion method by Molan, 1992), and antioxidant activity (Al-Mamary et al., 2002), in Bulgarian honeydew (n=20), multifloral (n=10) and acacia honeys (n=10). The influence of antibacterial activity of this type of honey on isolated from wound Staphylococcus aureus, resistant to amoxicillin, kanamycin, lincomycin, erythromycin, doxycyclin and chloramphenicol was also investigated. The results, showed that the antibacterial activity of honeydew honeys was the highest, while the acacia honeys had the lowest antibacterial activity. In most honeys no antibacterial activity was present after adding of catalase solution in most samples. In the honeydew honey sample with the highest antioxidant activity there was remaining specific activity towards Staphylococcus aureus. It was concluded the greater part of the antibacterial activity detected by the agar well diffusion method is due to hydrogen peroxide, but that in the honeydew honeys there can be some non-peroxide antibacterial factors.

Potential and Perspectives on the Medicinal Uses of Honeydew Honey. A Review
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In the study were presented data for comparison of antibacterial (agar well diffusion method by Molan, 1992), and antioxidant activity (Al-Mamary et al., 2002), in Bulgarian honeydew (n=20), multifloral (n=10) and acacia honeys (n=10). The influence of antibacterial activity of this type of honey on isolated from wound Staphylococcus aureus, resistant to amoxicillin, kanamycin, lincomycin, erythromycin, doxycyclin and chloramphenicol was also investigated. The results, showed that the antibacterial activity of honeydew honeys was the highest, while the acacia honeys had the lowest antibacterial activity. In most honeys no antibacterial activity was present after adding of catalase solution in most samples. In the honeydew honey sample with the highest antioxidant activity there was remaining specific activity towards Staphylococcus aureus. It was concluded the greater part of the antibacterial activity detected by the agar well diffusion method is due to hydrogen peroxide, but that in the honeydew honeys there can be some non-peroxide antibacterial factors.
**Introduction** The origin and composition of honeydew honey was and is still studied by many scientists from all the world, especially from Europe. Based on the similarities and differences on the composition of honeydew honey in comparison with the floral honey, we can expand the list of uses and indications of honeydew honey.

**Material & Methods** Available literature on floral and honeydew honey has been studied in order to create simple but clear comparative tables between these two major types of honeys.

**Results and Conclusion** Honeydew honey best medicinal use should be in the prevention or treatment of at least the following conditions found in human and veterinary medicine.

- Infectious diseases (bacterial and viral related diseases), immune system diseases, large intestine diseases
- Diseases related to the presence of various toxins in the body, skin diseases, rheumatic and other degenerative diseases (like osteoporosis) and inflammation related diseases.

This theoretical comparative study is the first of its kind it needs of course to be proved in practice, through detailed, practical, clinical studies.
Preliminary study on honeydew honey from the Bulgarian market

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Honeydew is a sugar-rich sticky substance, secreted by aphids and some scale insects as they feed on plant sap of different coniferous and deciduous species.

Samples of honey labeled Honeydew honey from the Bulgarian market were studied. Among the investigated samples 10 were honeydew honeys produced in the year 2007, and 2 samples in the year 2006. Water content was determined by measuring refractive index with a refractometer, provided at temperature 20 °C. Electrolytic conductivity and pH of a honeydew solution at 20% (dry matter basis) in double distilled water were measured at 20 °C using Multiline P3 (WTW, Germany) and pH Meter 3310 Jenway (England), respectively. Total concentrations of 18 elements (Al, As, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, S, V, and Zn) were determined, after wet digestion of honey sample, by atomic emission spectrometry with inductively coupled plasma (ICP–AES) using VARIAN VISTA-PRO instrument. A microscopic analysis -identification of honeydew elements and pollen was performed. The ratio honeydew elements (HDE) to total frequency of pollen grains (P) calculated after Louveaux et al. (1998).

All honey samples were of dark to very dark brown honey. The average pH value was found 4.27 (20 °C), the mean conductivity was 1.023 mS/cm (20 °C). The influence of geographic regions to element concentrations in honeydew honey is discussed. A honeydew honeys inter comparison was performed. The ration HDE/P was found between 0.22 and 1.16, i.e. few.

Antioxidant activities of Bulgarian Acacia, Multifloral and Honeydew Honeys

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In the study were evaluated antioxidant activities of three different types of honey from different Bulgarian regions: multifloral (n=10), Acacia (n=10), and honeydew honeys (n=10). Percentage antioxidant activities (% AA) of different honeys were assayed in vitro by the method of Al-Mamary et al., 2002. % AA was measured in diluted honey samples by calculating the inhibition of pig liver homogenate oxidation mediated by the FeSO4 ascorbate system. The antioxidant activity of different honeys increased with increasing the levels of honey samples solutions (50 µl, 100 µl and 200 µl). In all cases the highest values of antioxidant activity was found in honeydew honeys. The honeydew honeys harvested near town Madzharovo had the highest antioxidant activity - 9,91% with 50 µl, 35,64% with 100 µl to nearly two times higher (63,33%) with 200 µl from sample solution. The honeydew honeys harvested near town Zarevo had values from 8,32 % with 50 µl, 13,48 % with 100 µl and
15.17% with 200 µl from sample solution. The mean values of antioxidant activity with 200 µl of the other honey types was for multifloral honeys - 13.41±2.14, and Acacia (Robinia pseudoacacia L.) - 10.17±0.39.

Content of Nine Trace Elements in Honeydew Honey from Strandja, Bulgaria

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A study of nine trace elements in six samples honeydew honeys, obtained from the region of Zarevo (Bulgaria) in 2006, were performed. Minerals were determined by Atomic Absorption Spectrophotometer (Model 380, PERKIN ELMER). It was found out that the amount of Mn (10.09±0.25 mg.kg⁻¹), predominated in these type of bee honey. The content of toxic heavy metals - Pb (0.21±0.01 mg.kg⁻¹) and Cd (0.0071±0.0002 mg.kg⁻¹), were within the limits of data for other European honeydew honeys (Altmann, 1983; Bogdanov et al., 1986; Bogdanov, 2006; Bogdanov et al., 2007; Uren et al., 1998). The quantity of other investigated elements – Fe (5.19±0.06 mg.kg⁻¹), Zn (1.01±0.04 mg.kg⁻¹), Cr (0.09±0.002 mg.kg⁻¹) and Co (0.026±0.001 mg.kg⁻¹), were comparable to the values found in other studies (Mladenov, 1978; Dinkov et al., 2000). Data for Cu (0.24±0.02 mg.kg⁻¹) and Ni (0.0045±0.0003 mg.kg⁻¹) were lower in comparison with Swiss honeydew honeys (Bogdanov et al., 2007).

Investigations on the Composition of Honeydew Honeys Collected from the Region of Strandja

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Honeydew honey is honey obtained mainly from excretions of plant sucking insects (Hemiptera) on the living part of plants or secretions of living parts of plants. This type of honey is more preferable for human consumption and used for apitherapy. The main production of honeydew honey in Bulgaria is in the region of Strandha. The information of honeydew honey composition from this region is scanty.

This work was performed to establish the characteristic of chemical composition of honeydew honey from the region of Strandha (Bulgaria).

Twenty seven honeydew honey samples from the mentioned region were analyzed for content of the following physicochemical parameters: apparent reducing sugars, apparent sucrose, moisture content, water-insoluble content, electrical conductivity, free acid, diastase activity, hydroxymethylfurfural content (HMF), ash. The analytical methods, used for the determination of honey quality are according to the current standards, based on Bulgarian government legislation, harmonized with the European Honey Regulation rules.

The resulting data we obtained, characterized investigated honeydew honey samples in relation to their origin. The range values of some parameters were as follows: free acid – from 16.09 to 53.93 meq/kg (one sample > 50);
moisture content – from 15.24 to 17.88%; ash – from 0.365 to 0.709% and water-insoluble content – only one sample > 0.1% (0.107%).

The results in most of the samples showed the specific quality criteria, which responded to the Council Directive 2001/110/EC requirements.

Honeydew as the Source of Honey on the Territory of the Czech Republic
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The European legislation differentiates between nectar honeys (up to 0.8 mS/m) and honeydew honeys (more than 0.80 mS/m). The designation of one of these two types must be written on the label.

In the Czech Republic there occur nectar as well as honeydew honeys, but most honeys are natural mixture of nectar and honeydew honey. We present results of the electric conductivity measurement at the set of 1000 honey samples delivered by beekeepers from the Czech Republic territory from a period of several years.

The results show that authentic honeys form a continuous row from pure nectar to honeydew honeys without any border between both groups.

The mixed honey has a tradition in the Czech Republic. Consumers prefer mixed honeys because of their well-balanced rich taste and nice honey color.

Characterization of Honeydew Honey Blends by Quality Control Parameters
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Introduction: Honeydew honeys are known and appreciated by their particular characteristics of strong colours and flavours. The composition criteria for honeydew honeys in respect of fructose and glucose content, as well as with regard to electrical conductivity are different than the composition criteria for floral honeys. The purpose of this work has been to study some quality control and routine parameters in both floral and blends of honeydew and floral honeys, trying to look for other compositional criteria that could be distinctive of each group of honeys.

Material & Methods: 20 samples of honeys coming from a Continental Climate area were collected. According to melissopaliniology, 10 samples were floral honeys and 10 samples were blends of honeydew and floral honeys (HDE/P: 1.5-2.99). We determined electrical conductivity, moisture, sum of fructose and glucose contents, free acid, lactones, total acidity, formol number, proline, hydroxymethylfurfural content, diastase activity, and invertase activity by the AOAC, harmonized methods, or equivalent and reliable procedures set up by us. Statistical determinations have been carried out by applying one-way ANOVA, cluster and discriminant analyses of Statgraphics Plus 5.1.

Results: As it was expected, at the 95.0% confidence level there was a statistically significant difference between the means and medians of electrical conductivity of floral honeys, and blends of honeydew and floral honeys. Other
parameters whose means and medians proved to be significantly different in both groups of samples were pH, free acid, total acidity, lactones/free acid, and formol number. Cluster analysis did not show a clear separation between the two groups of honeys. Discriminant analysis correctly classified 100% samples, but both groups were very close to each other.

**Conclusion:** Apart from electrical conductivity, other particular composition criteria that honeydew honeys should meet are pH, free acid, total acidity, lactones/free acid, and formol number.

**Characterisation of Honeydew Honeys Produced in Tenerife (Canary Islands)**

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**Introduction** In 2005, 2006 and 2007 honey seasons some batches of Tenerife honeys shown specific characteristics after the physicochemical, melissopalynological, and sensory analysis that made these products different from the other types of local honeys currently characterized at the Casa de la Miel de Tenerife.

In 1991 arrived to Tenerife with ornamental palms from the Caribbean the whitefly *Aleurodicus dispersus* Russell that have been affecting to different crops and ornamental plants since then. These insect produce a typical white cotton-like secretions and abundant molasses, that spots and damage the host leaves and surrounding areas; branches, other plants, floor, etc., specially in banana trees and different kind of palms.

At latter summer, when there is no big nectar flow, some beekeepers started to collect honeys when they normally in the past they didn’t do. These honeys have a common sensory pattern, with dark colour, with caramelized and salty sensations, characteristics that we haven’t found in other known Tenerife honeys in the past. These honeys have been normally collected after August, near banana tree planting and palm parks. With this information we suspect the relationship between this honey and the whiteflies.

The aim of this study is to define the main characteristics of these *special* honeys, and discuss the existence of honeydews in Tenerife produced after the whitefly attack.

**Material & Methods** The study was carried out on 21 batches of honey produced in different places from Tenerife, samples were collected directly from bulk containers of honeys ready for bottling. All the samples were unheated. Some analyses were carried in samples kept at -18ºC in our bank of honeys.

Samples were analyzed by duplicate to determine: moisture (water content), water activity, electrical conductivity, pH, free acidity, hydroxymethylfurfural (HMF), Pfund colour and diastase activity.

Moreover, we made for every sample qualitative and quantitative melissopalynological analysis, according Loveaux et al., and sensory analysis following our own methodology developed in our unit based in routine method of sensory evaluation proposed by IHC. All these analysis are routine control in the certification process of the Quality brand “Miel de Tenerife”.

**Results and Conclusion** The results of the analysis are statistically analyzed, describing basic statistics for the group, variance and multivariate analysis. These honeys have high electrical conductivity (1.334 mS/cm), and higher pH (4.67) and free acidity (35.6 meq/kg) than the rest, with dark colour (123,86 mm Pfund). Pollen analysis
didn’t find a common pattern, and honeydew elements are down the counts reported for other honeydews, most likely due to lack of moisture and high temperatures in summer time in the coast areas of Tenerife where the plants grow, but all of them had low pollen density (median 9800 PK/g).

The results obtained show that the physicochemical, sensorial, and melissopalynological characteristics of these honeys are compatible with honeydew honeys. This is the first time that the existence of this type of honey in the Canary Islands and Tenerife has been reported.

**Effect of Honey Addition to Ground Beef Stored in Refrigeration**

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**Introduction.** Ground meat is likely to suffer a quicker degradation than the whole muscle. Oxidation reactions, and the subsequent decomposition of oxidation products, are considered an important factor in spoilage. Some of the undesirable effects of this process are (i) the development of off-odours, (ii) off-flavour and (iii) discoloration. Analysis of thiobarbituric acid reactive substances (TBARS) is a commonly used technique when meat lipoxidation is evaluated. Volatiles analysis is an interesting tool to provide information about the characteristics of the meat products or their deterioration.

In this work the effect of the addition of honey as preservative to ground beef meat has been evaluated. The parameters colour, pH, TBARS and volatile compounds of the meat sample, with and without honey, were analysed at different times of the refrigerated storage.

**Material & Methods.** *In vitro* scavenging capacity of honey samples were previously evaluated using the free radical DPPH. The assays with ground beef were carried out using two different honeys with very different scavenging capacity: i) a honeydew honey (scavenging activity higher than 60%) and a floral honey (scavenging activity less than 10%). Volatile compounds of ground beef meat were determined by SPME and GC, according to previously established methodology. pH was measured by means of a penetration electrode (Crison 52-329). CIE-L*a*b* colour parameters were used to evaluate colour differences (∆E=√((L*)2+(a*)2+(b*)2)), Chroma (C*=√((a*+b*)2)) and Hue (h*=tan^-1(b*/a*)) values. Secondary products of lipid oxidation were measured by TBARS assay, and volatile compounds were analyzed by SPME and GC-MS with a CAR/PDMS fibre.

**Results and Conclusions.** Honey addition significantly changed pH value and colour of meat samples. TBARS of the ground beef meat containing honey showed significantly lower values, after 3 and 6 days of storage, than the meat control. In general, both honey types delayed lipid oxidation, with this delay being slightly longer for the honeydew honey.

Changes in the volatile profile, during the refrigerated storage, were related to meat degradation. The most representative compound generated during the storage was 3-hydroxy-2-butanone (butter off-flavour). It was observed that both honeys gave an important reduction in the chromatographic signal of this volatile with respect to the control samples after 3 and 6 days of storage. It is suggested that the observed changes in the volatile profile seem to be more closely related to a microbial spoilage that to lipooxidation processes.
Correlation between Ash Content and Electrical Conductivity in Honeydew Honey from Romania
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Introduction Electrical conductivity and ash content represent very important characteristics for honeydew honey and are widely used for discrimination between honeydew and blossom honeys. Electrical conductivity is a good criterion of the botanical origin of honey. Blossom honey should have less than 0.8 mS/cm and honeydew should have more than 0.8 mS/cm.

Material & Methods Ash content and electrical conductivity were determined by the methods of Bogdanov et al. (1997). Ash content was determined by heating 5 g of honey at 400°C in a muffle furnace. Electrical conductivity was measured in a 20% (w/v) solution of honey in deionized water with low electrical conductivity (<14 µS/cm) using Lovibond model conductimeter.

Results and Conclusions The ash content of the 25 studied honey samples differs widely. It ranged from 0.10% to 0.55%. These differences in mineral content are depended on the type of soil in which the original nectar bearing plant was located (Anklam, 1998). The ash content is a quality criterion for honey botanical origin: the blossom honeys have lower ash content than honeydew honeys. According to IHC the value of ash content for blossom honey is ≤ 0.6 g/100g and ≤ 1g/100g for honeydew honey. This parameter is generally replaced by the measurement of electrical conductivity (Bogdanov et al., 1999). Popek (2002) demonstrated that ash content honeydew is 0.56%. The electrical conductivity of honeydews analyzed is less than 0.92 mS/cm and the maximum value accepted by the IHC for this characteristic is ≥0.7 mS/cm. This parameter depends on the ash, organic acids, proteins, some complex sugars and varies with botanical origin (Terrab et al., 2003; Ouchemoukh et al., 2007). A correlation of 0.83 was found between ash content and electrical conductivity for Romanian honeydew honey.

High-Performance Liquid Chromatography Analysis of Sugars in Transilvanian Honeydew Honey
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Introduction. 28 declared honeydew honey samples were collected from different Transilvanian areas. Origin of the samples was verified by physical-chemical parameters and pollen analysis. Their sugar profile was analyzed by means of HPLC with refractive index detection.

Material and methods. 24 honey samples were obtained directly from beekeepers and 4 samples were purchased from the supermarket. 9 sugars were determined according to the method described in Harmonised Methods of the European Honey Commission. Sugar concentrations were expressed in g per 100 g honey (%).

Results and Conclusions. We were able to identify and quantify two monosaccharides (glucose and fructose), four disaccharides (maltose, trehalose, sucrose, turanose) and three trisaccharides (melezitose, erlose and raffinose). All
samples purchased from supermarket were indeed honeydew honey. Different results were obtained for honey samples acquired directly from beekeepers: 3 of them were honeydew honey (electrical conductivity higher than 0.8 mS/cm), the remaining had electrical conductivities lower than 0.8 mS/cm: 9 samples were of mixed origin (blossom and honeydew) and 12 samples were blossom honey. The honeydew honeys (7 samples) present the following sugar profile: fructose and glucose ranged between 33.15 – 37.03% and 24.65 – 30.19%, respectively. The sum of fructose and glucose had an average of 62.71% and a minimum of 57.8%. The content of maltose, the most abundant disaccharide, ranged between 2.04 and 3.14%. Turanose present values between 1.47 and 2.89%. Trehalose was detected in 6 samples, with values ranging between 0.23 and 1.23%. The concentration of sucrose did not exceed 0.4% in any samples. The content of melezitose varied between 1.03 and 6.76%. The percent of erlose was subunitary in all the samples (max. 0.9%). Raffinose was identified only in 3 samples at low percentage (max. 0.67%). The results obtained showed that only 25% of the samples correspond to the requirements of honeydew honey.

**Determination of Physicochemical Parameters in Honeydew Honey in View of Quality and Authenticity**

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**Introduction.** Honeydew honey refers to honey produced by the bees from the secretions of aphides and other bugs, feeding on plant sap. This honey is considered to be a speciality or a delight in some areas of the world because of its taste and because its rarity. 28 declared honeydew honey samples were collected from different Transilvanian areas directly from the beekeepers (24 samples) or from supermarket (4 samples). Physico-chemical parameters as well as microscopic analysis were done in order to authenticate the honeydew honey from this part of Romania.

**Material and Methods.** Honey samples from different locations in Transylvania were analysed for water, pH, free acidity, lactone and total acidity, electrical conductivity, HMF, ash and diastase activity, following the Romanian Standard Analysis Methods and International Honey Commission Methods.

**Results and Conclusions.** After performing all the set of physico-chemical analysis and microscopic examination of the sediment, we are able to say that all the samples purchased from the supermarket are real honeydew honey (electrical conductivity higher than 0.8 mS/cm, the HDE/NPGN higher than 3 and the lowest fructose + glucose content). The remaining 24 samples (from the beekeepers) present different values for these characteristics concluding that: 3 samples are honeydew honey, 9 samples were of mix origin (blossom and honeydew) and 12 samples were blossom honey. Electrical conductivity for honeydew honeys ranged between 0.803 and 0.924 mS/cm, for mix samples between 0.534 and 0.807 mS/cm and for blossom honeys between 0.324 and 0.623 mS/cm. Honeydew honey samples present the lowest water content (14.3 – 16.7%), and the lowest lactone acidity / free acidity ratio (0.24 – 0.48). The sum of monosaccharides (glucose + fructose) for real honeydew honey samples ranged between 57.8 and 66.36% and the ratio between fructose and glucose had a mean value of 1.31. Many of
the analysed samples (75%) declared and sold as honeydew honey do not correspond to the EU requirements for honeydew honey. To improve this situation, a better control by the authorities and certified laboratories, is needed.

Mineral Content of Honeydew Honey Produced in Transilvania

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Introduction: In this work, alkaline earth (Ca, Mg) and trace elements (Fe, Zn) were determined in different honeydew honey samples from Transylvania, by flame atomic absorption spectrometry after dry ashing.

Materials and Methods: Seven samples of honeydew honeys, nine samples of mixed honeydew-polifloral honeys and 12 of polifloral honeys were analyzed. Honey samples were purchased on the markets or obtained from beekeepers, from different regions of the Transylvania. The botanical origin of honeydew samples was verified by melissopalynological analysis and certified by the physicochemical parameters. The spectroscopy measurements were performed using an Atomic Absorption Spectrophotometer AA-6300 produced by Shimadzu-Japonia.

About 10 g of honey were first burned on the flame for avoiding foaming and the weight loses, then calcinated in a muffle furnace at 450°C over the night. Afterwards, 5 ml hydrochloric acid 6M was added and then evaporated. The resulting white ash was dissolved with 30 ml nitric acid 0.1 M (SR EN 14082, 2003).

Results and Discussion: In all studied honeydew honeys, Ca was the most abundant of the elements determined with average concentrations ranging between 54.75-102.6 mg/kg, followed by Mg (52.01-78.4 mg/kg), Fe (3.09-6.27 mg/kg) and Zn (1.54-4.65 mg/kg). Good positive correlation was found between total mineral content and electrical conductivity (R²=0.755). Honeydew honey was found to have a high mineral content, which reflects sources from which the honey is composed, being a good source of minerals.

A Three Year Program for Monitoring Residues of Naphthalene in Pine Honey from Turkey

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Since the year 2000 when a maximum residue limit (MRL) of zero for residues of naphthalene in honey was set by Turkish Food Codex Honey Regulation Nr.2000/39, usage of chemicals for beekeeping has become a more sensitive subject for consumers, honey packers and food control authorities. In the year 2005, MRL of naphthalene, was revised to 10 µg/kg (ppb) by The Ministry of Agriculture and Rural Affairs. Turkish beekeepers used to place naphthalene on honeycombs as an agent for repelling wax moth. However it is absorbed easily by the comb and leaves residues in honey. It was noticed that naphthalene can end up in honey not only through direct use but also as an environmental contaminant and especially by the usage of old contaminated combs. Residues less than 25 ppb is considered to be a result of contaminated honey combs.

The aim of this study was to determine the situation about naphthalene residues in Turkish pine honey. For this purpose, honey samples were collected from beekeepers during a three-year surveillance program from Muğla
region in Turkey and were monitored for residues of naphtalene as part of the project which was also supported by Technology And Innovation Funding Programs Directorate and Foreign Trade Department called “Determination of residues, adulteration and origin of honey according to geographical regions”. The samples were analyzed by a SPME (Solid Phase Micro Extraction) method followed by GC-FID (Gas Chromatograph-Flame Ionization Detector). The detection limit of the GC-FID method that we used was 5 ppb which is enough according to set MRL.

A total of 3199 samples collected from beekeepers, were analyzed for naphthalene. The percentage of samples which has naphthalene residues over 25 ppb was determined as 17% in 2004, 1% in 2005 and 0% in 2006. The residue levels decreased each year as new clean combs entered production as shown by the analysis results. In 2006 only 2% of the samples were over the set MRL, which indicates that the problem is solved for Turkish beekeeping.
Co-organizers of the Symposium

ORGANIC BEEKEEPING ASSOCIATION

www.bio-bees-bulgaria.org

The “Organic Beekeeping Association” (OBA) was created for the development of organic beekeeping in Bulgaria. General objectives of the Association:

- Development of national, regional, and individual programs for development of organic beekeeping in the country
- Providing of information and opportunities for related to that sector training and qualification;
- Preparation of norms and assistance in the registration of organic beekeeping farms;

The main forthcoming tasks are:

- Organisation of training of apiarists in organic beekeeping and creation of national or regional training centers for organic beekeeping;
- Development of perspective plan for pre-equipping of apiary farms with organic beekeeping equipment and machinery, and preparation of such apiaries in compliance with the EU.requirements

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Strandja Honeydew Honey

The co-operative Strandja Honeydew Honey was created om 2007 by 20 beekeepers, producing honeydew honey in the Oak forests of the Strandja mountain situated on the Southern Black Sea Coast. The honeydew honey is popular in Bulgaria due to its favourable organoleptical and biological properties. The beekeepers use mostly Dadant Blatt beehives in stationary apiaries. 20 to 120 tones of honey per year are produced. The harvested honey is mostly sold as a whole to trade partners. The co-operative works on the creation of Protected Geographical Origin Denomination for the Strandja honey and on other bee hive products. The Honeydew Honey Festival is organised by local beekeepers and is celebrated traditionally in the beginning of August, including also beekeepers from other regions and countries.

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