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# The Development of a Biochemical Profile of Acacia Honey by Identifying Biochemical Determinants of its Quality

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**Abstract**

Codex Alimentarius Standard, EU Legislation and National Standards state honey authenticity. Authenticity in respect of production (to prevent adulteration) and authenticity in respect of geographical and botanical origin are the two main aspects of general honey authenticity. Quality of honey depends on the plant source, the chemical composition of these plants as well, as on the climatic conditions and soil mineral composition. Romanian acacia (*Robinia pseudoacacia*) honey that came from the most important Transylvanian massif (Valea lui Mihai, Bihor County, Romania) was evaluated for authenticity by pollen-analysis, several physico-chemical analyses, including sugar profile and mineral content. As polyphenolic content could be also an important factor for botanical authentification, HPLC-DAD-MS analyses were performed to assess the fingerprint of this important secondary plant metabolite. Statistical data were processed in order to develop a biochemical profile of this type of honey and the main quality categories identification. The results of physico-chemical analysis demonstrated that the tested honey samples could be framed into monofloral type of acacia honeys. The analysis of acacia honeys originating from Valea lui Mihai, Romania, showed that polyphenolic profile (phenolic acids and flavonoids) could be used as a complementary method for authenticity determination together with pollen analysis and other physico-chemical analysis.

***Keywords:*** development, profile, acacia honey, biochemical determinants, quality

## Introduction

Beekeeping as a branch of agricultural production has been, since ancient times, one pursuit valued by human society, initially for its products (honey, pollen, royal jelly, propolis, beeswax and bee venom) and later, including in present, for the contribution of these insects to the increasing of crops, fruit, vegetables and seeds production through pollination. Currently, in the context of globalization, beekeeping gains new valences, the target of its practice being not only getting high production, but also significant efficiency. This is achieved through diversification, getting quality products, practicing the organic beekeeping, choosing the most effective marketing channels; promote consumption in compliance with health and environmental protection. In the context of the agro-food chain, beekeeping chain is a very complex one, its study rendering not only specialists in beekeeping and food but also in economic, medical and social sectors, the approach of this scientific research being characterized by a high level of interdisciplinary (Vural and Karaman, 2009; Pocol, 2010).

Honey is considered a potential complete food, regarding nutritional standards, being a natural product, rich in simple, easy assimilable sugars (fructose, glucose), enzymes (invertase, glucose oxidase, catalase, phosphatase), amino and organic acids (proline, gluconic acid, acetic acid), vitamins (ascorbic acid niacin, riboflavin), volatile oils, phenolic acids and flavonoids, minerals and carotenoid like substances (Sudhanshu *et al*., 2010). Phenolic phytochemicals are important aromatic secondary metabolites in plants (Kim *et al*., 2003), which occur as single aglicones or bonded with sugars like glucose, arabinose, galactose, rhamnose (glycosides).

Several studies were carried out on many types of honey and some polyphenolic compounds were found to be markers for authenticity and botanical origin of honey (Martos *et al*., 2000a; Martos *et al*., 2000b; Tomas-Barberan *et al*., 2001; Persanno Oddo and Bogdanov, 2004). The analysis of flavonoids and phenolic acids in honey was used in the last years, in addition to pollen analysis, to evaluate correctly the botanical origin of monofloral honey, knowing that many monofloral types of honey have increased prices and have limited production and availability, so there can be more often subject to falsification. Authenticity testing of honey is one issue closely observed and followed in honey analysis.

High consumer demand and cost of production make this product susceptible to fraud by adulteration with sugars (invert sugar syrups) or by false declaration of floral

origin. Council Directive 2001/110/EC related to honey (2002) includes general and specific characteristics to test the authenticity of botanical origin (Chudzinska and Baralkiewicz, 2010). Until now, many physicochemical parameters were analyzed for testing honey adulteration like: melisopalinological profile, sugar profile, amino acid profile, volatiles, organic acids, mineral content and enzyme activities (Persano Oddo and Piro, 2004). Comparing the results with naturally occurring values can lead to the possible conclusion of adulteration. Melisopalinological analysis requires well-qualified personnel, this method being also time consuming, although it has its advantages. Physicochemical parameters determination of honey became a routine and the most used method for detection of the origin of honey. There are a great number of studies used to detect the botanical origin of different types of honey (Corbella and Cozzolino, 2006; Serrano *et al*., 2004; Singh and Bath, 1997). Flavonoids apigenin, quercetin, luteolin, kaemferol and galangin were reported in honey (Gheldorf *et al*., 2002; Yao *et al*., 2004; Kenjerić *et al*., 2007) while pinocembrin, pinobanksin and chrysin, flavonoids derived from propolis, were determined in many European monofloral types of honey (Sabatier *et al*., 1992, Tomas-Barberan *et al*., 2001).

It is known that honey is made without human intervention; mineral determination is a good procedure to detect honey authenticity, because it is linked to the soil and vegetation from the area where it is produced, being beside a method for botanical authenticity, also a method for geographical authentication. Especially major minerals, but also minor ones are affected by soil composition and therefore are determined in the analysis of authenticity of geographical origin (Pisani *et al*., 2008; Fernandez Torres *et al*. 2005; Nozal Nalda *et al*., 2005). Correlations between soil composition in minerals and honey minerals have been done by Bordean *et al*., 2010; Bordean *et al*., 2007), including building a mathematical model for environmental contamination for heavy metal evaluation.

## Materials and methods

*Chemicals*

Ultra pure water and organic solvents (methanol, acetonitrile, ethyl acetate, acetic acid) were analytical, HPLC or MS grade (Sigma Aldrich Co.). Standards of flavonoids: quercetin, catechin, luteolin, apigenin, naringin, kaempherol, galangin, pinocembrin, crysin, acacetin and phenolic acids: p-hydroxybenzoic, caffeic, ferulic, chlorogenic, p-coumaric, t-cinnamic, o-coumaric, vanillic, homovanilic, protocatecuic and siringic, were purchased from Sigma Aldrich and Fluka Chemie.

*Honey samples*

*Robinia pseudoacacia* honey samples (n=10) were collected from Valea lui Mihai, Bihor County, Romania and were stored at 4°C until analysis. The botanical origin was confirmed by the combination of classical quality determinations and pollen analysis. Given the sample size, significance testing was made by using Kolmogorov-Smirnov normality test and Shapiro-Wilk test, results being presented in Tab. 1 and 2.

Kolmogorov-Smirnov normality test and Shapiro-Wilk test applied to all parameters indicate almost unanimously (with one exception, the variable sucrose) that data are from a normally distributed population and confirm that the sample is a good choice.

*Determination of physiochemical quality*

Selective physicochemical parameters were determined according to the Romanian standard (STAS 784/1...3-89) and Harmonized Methods of International Honey Commission. Water content was determined refractometrically (Abbe digital refractometer WYA-S Selecta Spain). Then the content was expressed in mg/100g; electrical conductivity was measured at 20°C in a 20% (w/v) honey solution in water with a KIT Consort conductometer (CONSORT nv, Belgium) and expressed as μS/cm, pH, free acidity, lactone acidity. Total acidity was determined by automatic

Tab. 1. One-sample test

| Parameters | t | df | Sig. (2-tailed) | Mean Difference | Lower | Upper |
|---|---|---|---|---|---|---|
| Water content (g/100g) | 89.65 | 9 | 0.000 | 17.94 | 17.48732 | 18.39268 |
| Electrical conductivity (mS/cm) | 15.034 | 9 | 0.000 | 0.1547 | 0.131422 | 0.177978 |
| pH | 129.748 | 9 | 0.000 | 3.997 | 3.927312 | 4.066688 |
| Total acidity (meq/kg) | 13.759 | 9 | 0.000 | 14.302 | 11.95058 | 16.65342 |
| Free acidity (meq/kg) | 8.615 | 9 | 0.000 | 6.453 | 4.758615 | 8.147385 |
| Fructose (g/100g) | 111.957 | 9 | 0.000 | 42.65 | 41.7882 | 43.5118 |
| Glucose (g/100g) | 53.921 | 9 | 0.000 | 28.483 | 27.2881 | 29.6779 |
| F/G ratio | 49.412 | 9 | 0.000 | 1.502 | 1.4332 | 1.5708 |
| F+G content (g/100g) | 112.049 | 9 | 0.000 | 71.133 | 69.6969 | 72.56911 |
| Maltose (g/100g) | 16.041 | 9 | 0.000 | 2.696 | 2.315802 | 3.076198 |
| Sucrose (g/100g) | 3.642 | 9 | 0.005 | 1.545 | 0.585401 | 2.504599 |

Test Value = 0; Confidence Interval of the Difference 95%; Source: own processing after experimental data

Tab. 2. Tests of normality

| Parameters | Kolmogorov-Smirnova | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|
| | Statistic | df | Sig (2-tailed) | Statistic | df | Sig (2-tailed) |
| Water content (g/100g) | 0.203 | 10 | ,200* | 0.905 | 10 | 0.247 |
| Electrical conductivity (mS/cm) | 0.233 | 10 | 0.132 | 0.93 | 10 | 0.451 |
| pH | 0.173 | 10 | ,200* | 0.952 | 10 | 0.69 |
| Total acidity (meq/kg) | 0.125 | 10 | ,200* | 0.956 | 10 | 0.743 |
| Free acidity (meq/kg) | 0.195 | 10 | ,200* | 0.943 | 10 | 0.586 |
| Fructose (g/100g) | 0.143 | 10 | ,200* | 0.93 | 10 | 0.444 |
| Glucose (g/100g) | 0.081 | 10 | ,200* | 0.986 | 10 | 0.99 |
| F/G ratio | 0.115 | 10 | ,200* | 0.973 | 10 | 0.918 |
| F+G content (g/100g) | 0.142 | 10 | ,200* | 0.982 | 10 | 0.976 |
| Maltose (g/100g) | 0.183 | 10 | ,200* | 0.881 | 10 | 0.135 |
| Sucrose (g/100g) | 0.29 | 10 | 0.017 | 0.813 | 10 | 0.021 |

Lilliefors Significance Correction*. This is a lower bound of the true significance; Source: own processing after experimental data

titration (TitroLine Easy Schott, Germany). Sugar profile was determined with the help of HPLC method on a Shimadzu system. The system is equipped with a LC-10AD pump, DGU-14A degasser, SIL-10AV VP auto sampler, RID-10A refractive index detector, thermostated at 30°C with CTO-10AS VP temperature controller of separation column (Altima Amino 100 Å 5 μm, 250 mm x 4,6 mm) with a mixture of acetonitrile/water as mobile phase with 1.3 ml/min flow rate. For the quantification of main sugars, a calibration curve in the range 40-0.5 g/100g, with regression coefficient of $R^2$=0.9982 for a mixture of standards (glucose, fructose and saccharose) was obtained. The results were expressed in g/100g honey.

*Pollen analysis*

Pollen is the bee's major source of protein, fat, minerals and vitamins (Adekanmbi and Ogundipe, 2009). Honey samples were subjected to pollen analysis according to Louvreaux *et al*. (1978), and the confirmation of honey type has been made according to Romanian honey quality standards, which states 30, 25 and 20% acacia pollen grains for superior, quality I and quality II honey.

*Mineral content*

Mineral content of honey samples was determined using atomic absorption spectrometry (ContrAA300, Analitik Jena) in air-acetylene flame. The equipment was calibrated using different working standards, concentration of minerals in the samples being measured individually.

*Polyphenols (flavonoids and phenolic acid) isolation*

The main problem when analyzing the flavonoids from honey is the high content of sugars, but using a non-ionic polymeric resin Amberlite solved the inconvenient of recovery of flavonoids after liquid-liquid extraction. Flavonoid and phenolic acid extracts were obtained following a modified method used by Ferreres *et al*. (1994). Honey samples (40 g) were diluted with 5 parts of acidified water (adjusted to pH 2 with HCl) and mixed on a magnetic stirrer for 30 minutes. The honey solution was then, put through a glass column filled with Amberlite XAD-4 resin (Fluka Chemie, Germany). Phenolic compounds remain bounded to Amberlite particles, while sugars were washed with distilled water (pH 2) and neutral distilled water. The phenolic fraction (flavonoids and phenolic acids) was eluted with 400 ml methanol, than collected and evaporated to dryness. The residue was redissolved in water, partitioned with ethyl acetate (4 x 20 ml) and extracted in a separation funnel. The extracts were collected, evaporated to dry and kept in the freezer until analysis. After using this quantity of solvent, the recovery percent of original standards of phenolic compounds as well as the compounds from honey, from the Amberlite column, was situated between 83,09 and 98,08% (very good recovery).

*Abscisic acid analysis*

Abscisic acid is not a phenolic acid, but has very similar behavior, presenting strong UV absorbencies at 248 and 270nm. This plant hormone is known to be present in floral nectar, and the presence in honey is also expected. It is related to plant protection against dryness and environmental stress.

*HPLC analysis of flavonoids and phenolic acids*

Flavonoids and phenolic acid determinations were performed under conditions described by Andjelkovic *et al*. (2006), on an Agilent 1100 LC-MSD system. The system consists of: vacuum degasser, quaternary pump, autosampler, DAD variable wavelength detector, 1100 6-port autoinjector valve (20-μL loop) controlled by Agilent software v. A.09.03 (Agilent Technologies, Waldbronn,

Germany), Phenomenex C18 (ODS, Octadecyl) security guard column, Phenomenex Luna C18 100 Å column (4,6 mm i.d. x 250 mm; particle size 10 μm), maintained at 35°C. Elution was performed at a flow rate of 1.0 ml/min, using as mobile phase a mixture of 0.2% acetic acid in water (solvent A), methanol (solvent B) and acetonitrile (solvent D). Chromatograms were registered at 280 and 340 nm. An Agilent G1946D (SL) mass detector with an ion-trap mass spectrometer equipped with an electro spray ionisation (ESI) system was used to perform the mass spectrometric measurements. As the nebulizing gas, nitrogen at a pressure of 50 psi and the flow was adjusted to 13 l/min was used. The full scan mass spectra of the phenolic compounds were measured from m/z 100 up to m/z 1000. Mass spectrometry data were acquired in the negative ionisation mode. Calibration curves in the range 0.01-0.1 mg/ml of 26 standards of phenolic acids and flavonoids, with the regression coefficients of $R^2$ between 0.9705 and 0.9999 were obtained. The results were expressed in mg phenolic compound/kg honey.

**Results and discussion**

*Honey quality parameters*

The results of physico-chemical analysis presented in Tab. 3 demonstrated that the tested honey samples could be framed into monofloral type of acacia honeys. All samples exhibit a F/G ratio above 1.3, with a maximum value of 1.64 and a sum of reducible sugars around 70 g/100g. The high content of fructose, together with the above two indicators, confirm once more the purity of honey samples and their classification in the monofloral acacia honeys. The pollen content varied between 36 and 21% *Robinia pseudoacacia* pollen grains (in accordance with Romanian standard STAS784/1...3-89). Mean value of acacia pollen grains was 29.2%, this value being in accordance also with the descriptive sheets of unifloral honeys (Persanno Oddo and Piro, 2004).

*Phenolic acids and flavonoid content*

The phenolic acids and flavonoids separated by LC-MS from acacia honey (Tab. 4), comprises 3 phenolic acids (p-hydroxybenzoic, ferullic and t-cinnamic acid), abscisic acid and 5 free flavonoids (pinobanksin, apigenin, pinocembrin, crysin and acacetin). The content of identified phenolic acids was 12.11 mg/kg honey with ferulic acid identified in all analysed samples (0.72-8.66 mg/kg), representing 29% from total amount. The phenolic acid profile of acacia honey also includes p-coumaric, p-hydroxybenzoic, t-cinnamic and vanillic acid quantified in different amounts, but not in all the analyzed samples.

Ferulic acid and p-coumaric acid was quantified by Tomas-Barberan *et al*. (2001) in samples originating from Germany and Italy. Beside these two phenolic acids, Pulcini *et al*. (2006) identified in acacia honey from Italy vanillic acid also, like in the samples originating from Romania.

In all tested samples were identified and quantified pinobanksin (0.64-2.28 mg/kg), pinocembrin (0.38-1.38 mg/kg) and crysin (0.61-1.21 mg/kg). These flavonoids originating from propolis were also described in the studies of Tomas-Barberan *et al*. (2001) and Pulcini *et al*. (2006). The amounts quantified in our studies were although smaller than in the mentioned literature, but higher than the amounts quantified in Croatian honey (Kenjeric *et al*., 2007). The observation for acacia honey is that there are high differences between quantities of phenolic in the samples originating from different geographic areas and not between the profiles of these compounds. This could be due to the climatic conditions, purity of the sample, method of analysis, and sensitivity of the apparatus.

This common and individual polyphenolic profile was also pointed out by Tomas Barberan *et al*. (2001), where

Tab. 3. Selected physico-chemical parameters and sugar profile from acacia (*Robinia pseudoacacia*) honey

| Sample code | Water content (g/100g) | Electrical conductivity (mS/cm) | pH | Total acidity (meq/kg) | Free acidity (meq/kg) | Fructose (g/100g) | Glucose (g/100g) | F/G ratio | F+G content (g/100g) | Maltose (g/100g) | Sucrose (g/100g) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| AH-01 | 17.4 | 0.140 | 4.15 | 8.35 | 6.23 | 42.6 | 28.63 | 1.49 | 71.23 | 2.20 | 2.25 |
| AH-02 | 17.8 | 0.212 | 4.09 | 11.50 | 7.13 | 42.21 | 29.14 | 1.45 | 71.35 | 2.05 | 2.34 |
| AH-03 | 17.4 | 0.148 | 3.87 | 14.94 | 6.39 | 41.99 | 29.74 | 1.41 | 71.73 | 2.28 | 1.13 |
| AH-04 | 19.0 | 0.098 | 4.07 | 12.71 | 10.87 | 41.12 | 31.41 | 1.31 | 72.53 | 2.72 | 1.18 |
| AH-05 | 18.2 | 0.175 | 3.94 | 13.34 | 6.69 | 44.42 | 30.04 | 1.48 | 74.46 | 2.70 | 0.06 |
| AH-06 | 17.4 | 0.152 | 3.93 | 16.29 | 6.65 | 41.45 | 26.00 | 1.59 | 67.45 | 2.06 | 1.24 |
| AH-07 | 17.8 | 0.146 | 3.86 | 19.10 | 5.82 | 44.52 | 28.30 | 1.57 | 72.82 | 3.34 | 0.74 |
| AH-08 | 18.4 | 0.197 | 4.07 | 19.04 | 8.54 | 41.62 | 27.23 | 1.53 | 68.85 | 3.37 | 0.72 |
| AH-09 | 18.8 | 0.136 | 3.99 | 14.27 | 4.37 | 43.01 | 27.83 | 1.55 | 70.84 | 3.31 | 4.83 |
| AH-10 | 17.2 | 0.143 | 4.00 | 13.48 | 1.84 | 43.56 | 26.51 | 1.64 | 70.07 | 2.93 | 0.96 |
| Mean | 17.9 | 0.150 | 4.00 | 14.30 | 6.45 | 42.65 | 28.48 | 1.50 | 71.13 | 2.70 | 1.55 |
| SD | 0.63 | 0.030 | 0.09 | 3.29 | 2.37 | 1.20 | 1.67 | 0.10 | 2.01 | 0.53 | 1.34 |

Tab. 4. Total polyphenolic, flavone/flavonol content and individual phenolic acids and flavonoidic aglicones content from acacia honeys

| Sample | Content of phenolic acids (mg/kg honey) | | | | | AbA | Content of flavonoids (mg/kg honey) | | | | | | Total polyphenols (mg GAE /100g) | Flavone/ flavonols (mg QE/100g) (mg QE/100g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | p-Hyb | Van | p-Cou | Fer | t-Cin | | Pinb | Api | Kem | Pinc | Cry | Aca | | |
| AH-01 | 0.28 | - | - | 2.28 | - | 2.85 | 2.09 | - | 0.49 | 0.38 | 0.94 | 0.15 | 15.0 | 2.1 |
| AH-02 | 0.51 | - | - | 0.72 | - | 15.81 | 2.28 | - | 1.12 | 1.38 | 1.21 | 0.49 | 19.0 | 1.66 |
| AH-03 | - | - | - | 1.25 | 1.38 | 12.93 | 0.64 | 2.44 | | 0.56 | 0.69 | 1.14 | 14.7 | 1.6 |
| AH-04 | 0.48 | 2.91 | 6.36 | 8.66 | 1.75 | 33.05 | 1.06 | 1.55 | 1.55 | 0.64 | 0.61 | 1.20 | 18.0 | 3.0 |
| AH-05 | 0.65 | - | 4.25 | 6.51 | 1.51 | 6.51 | 0.89 | 2.06 | 0.85 | 0.85 | 1.05 | 0,.9 | 26.05 | 3.0 |
| AH-06 | 0.25 | - | - | 1.89 | 0.85 | 2.95 | 2.15 | 0.89 | - | 1.20 | 1.20 | 0.75 | 16.9 | 1.13 |
| RH-07 | - | - | - | 4.25 | 1.40 | 15.25 | 1.90 | - | 0.65 | 0.96 | 0.86 | 1.12 | 12.0 | 1.2 |
| AH-08 | - | 1.85 | 2.51 | 1.15 | - | 7.25 | 2.02 | - | - | 1.06 | 0.98 | 0.95 | 19.5 | 3.2 |
| AH-09 | - | - | - | 6.02 | - | 4.98 | 0.85 | 1.68 | - | 1.35 | 1.15 | 0.24 | 14.0 | 1.33 |
| AH-10 | - | 2.45 | - | 2.45 | - | 18.55 | 0.94 | - | - | 0.79 | 0.85 | 1.05 | 17.1 | 1.08 |
| Mean | 0.43 | 2.40 | 4.37 | 3.52 | 1.38 | 12.01 | 1.48 | 1.72 | 0.93 | 0.92 | 0.95 | 0.80 | 17.27 | 1.93 |
| SD | 0.17 | 0.53 | 1.93 | 2.71 | 0.33 | 9.31 | 0.65 | 0.58 | 0.42 | 0.34 | 0.21 | 0.38 | 4.00 | 0.84 |

Source: own processing after experimental data; Legend: p-Hyb : p-hydroxybenzoic acid; Van : Vanillic acid; p-Cou : p-coumaric acid; Fer : Ferulic acid; t-Cin : t-cinnamic acid; AbA:Abscisic acid; Pinb : Pinobanksin; Api : Apigenin; Kem : Kaemferol; Pinc : Pinocembrin; Cry : Crysin; Aca : Acacetin

Tab. 5. Major minerals (Na, K, Ca, Mg) and minor metals (Cu, Zn, Fe, Mn) for the analyzed honey samples

| Sample code | Na (ppm) | K (ppm) | Mg (ppm) | Ca (ppm) | Cu (ppm) | Zn (ppm) | Fe (ppm) | Mn (ppm) |
|---|---|---|---|---|---|---|---|---|
| AH-01 | 7.66 | 146.66 | 6.15 | 5.12 | 0.611 | 1.666 | 0.435 | 0.000 |
| AH-02 | 20.65 | 209.21 | 6.72 | 2.55 | 0.869 | 2.988 | 1.773 | 0.048 |
| AH-03 | 12.44 | 176.43 | 6.08 | 1.02 | 0.417 | 3.671 | 0.969 | 0.069 |
| AH-04 | 5.06 | 170.05 | 6.69 | 3.59 | 0.305 | 1.195 | 0.498 | 0.092 |
| AH-05 | 14.29 | 168.22 | 3.25 | 1.97 | 0.405 | 0.764 | 2.028 | 0.058 |
| AH-06 | 8.36 | 181.19 | 6.43 | 1.70 | 0.586 | 1.400 | 0.641 | 0.084 |
| AH-07 | 24.32 | 244.58 | 4.57 | 1.64 | 0.536 | 1.064 | 4.027 | 0.294 |
| AH-08 | 14.56 | 169.35 | 5.36 | 6.94 | 0.514 | 1.872 | 0.352 | 0.000 |
| AH-09 | 10.28 | 175.64 | 5.32 | 2.98 | 0.56 | 2.03 | 0.68 | 0.12 |
| AH-10 | 12.56 | 189.23 | 6.48 | 3,02 | 0.38 | 1.86 | 1.56 | 0.68 |
| Mean | 13.02 | 187.10 | 5.70 | 3,05 | 0.52 | 1.85 | 1.30 | 0.16 |
| SD | 5.89 | 25.10 | 1.11 | 1,80 | 0.16 | 0.89 | 1.13 | 0.22 |

Source: own processing after experimental data

not all phenolic acids were identified and quantified in all analyzed samples of the same type.

In all analyzed acacia samples originating from Romania, we have identified a flavonoid compound, specific only for this type of honey: acacetin. The amount of this compound varied between 0.15 and 1.2 mg/kg honey. The total amount of identified flavonoids in acacia honey was 6.81 mg/kg honey, with apigenin and pinobanksin quantified in amounts, representing 24 and 22% from the total amount of identified flavonoids and a lower content for the rest of flavonoids (crysin, kaemferol, acacetin and pinocembrin) as 12 - 12% from total (Tab. 4).

*Abscisic acid in acacia honey*

A high amount of abscisic acid (12.01 mg/kg honey) has been quantified in analyzed acacia honey samples. Abscisic acid has been found also by Ferreres *et al.* (1996) in acacia honey from Spain.

*Minerals in acacia honey*

The main concentrations of minerals in honey derive from soil. Those are transported to honey plants through the root system, reach in nectar; bees collect this nectar and produce honey. The content of minerals of analyzed samples is presented in Tab. 5.

Tuzen *et al*. (2007) determine the mineral content of Turkish honey, finding good correlations between the content of minerals and the botanical and geographical origin. Pisani *et al*. (2008) studied the mineral composi-

tion of some Italian honeys. Many other studies confirm the correlation between mineral composition and botanical and geographical origin of honey (Fernandez Torres *et al*., 2005; Nozal Nanda *et al*., 2005; Lachman *et al*., 2007; Corbella and Cozzolino, 2006).

**Conclusions**

Physico-chemical and mineral content analysis show clearly that the samples taken for analysis, were pure *Robinia pseudoacacia* honeys, the amount and distribution of phenolic acids and flavonoids is affected by the floral origin of different honeys. The analysis of acacia honeys originating from Transylvania, Romania, showed that polyphenolic profile (phenolic acids and flavonoids) could be used as a complementary method for authenticity determination together with pollen analysis and other physico-chemical analysis. Further studies have to be made on the mineral content of soil and nectar producing plant for each type of honey that will be investigated, in order to correlate the above parameters with authenticity and quality of honeys.

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