# MELISOPALINOLOGICAL ASPECTS REGARDING THE COMMERCIAL *ROBINIA PSEUDOACACIA* NATURAL HONEY

## A.A. Prelipcean, Anca Teuşan

*Faculty of Animal Sciences, University of Agricultural Sciences and Veterinary Medicine*
*„Ion Ionescu de la Brad" Iasi, Romania*

**Abstract**

*Robinia pseudoacacia honey is one of the most appreciated and sold honeys on the Romanian market. The study wants to highlight some melisopalinological aspects of Robinia pseudoacacia honeys from some manufacturing companyes that act on the Iaşi commercial market. Depending on the specific pollen granite percent, the quality class can be set, and the possible forgery. The methods used are microscopically analysis on the centrifuged sediment, counting of the determinate pollen grains and nonpollenical impurities. Reporting the pollen grains number to the used honey amount used provides information on the botanical and geographical origins, quality class and purity of the honey. The robinia pollen percentage from the analized samples vary from 12,31% and 52,76,including the honey in all the quality classes for Romania. The nonpollinical impurityes vary from 2627 to 15744 in 10 grams honey. Overprocessing increases the honey quality class, subtracting the impurities but also makes authenticity testing more difficult, makeing this honey easier to forge. In order to have an objective romanain honey quality, a processing and labeling alignment with the european standards is necessary.*

**Key words:** *Robinia pseudacacia* honey, quality, melisopalinology, pollen, standard

## INTRODUCTION

Due to the different polen/nectar and manna proportion contained, from a great variety of vegetal sources, the honeys differ among themselves. This variety can have benefical effects offering products with specific proprieties, according to the particularities of each botanical source, but also negative effects, for the market demands who wants a standardized product. The international normes [7] allow different product categories for the honeys that have particular botanical sources, if those honeys are from the indicated origin and have the properly phisico/chemical, organoleptical and microscopical proprieties. These rules specify certain compositional limits for floral and extrafloral honey, but do not establish any criteria for unifloral honey so that the rules do not guarantee effective control of the categoryes. Limits vary from country to country,  and may lead to some difficulties in international trade in unifloral honey. New and promising analytical methods have been already developed but before being used as ways of routine control, they can be still tested and improved [1]. Knowledge of the *Robinia* honey on the Iasi market, can highlight how the current standards are met. However, the undertaken researches on microscopic polinical and nonpolinical impurities from honey, will highlight some important aspects of honey processing in line with current European standards [6].

## MATERIAL AND WORK METHODS

For analysis were used 6 types of *Robinia* honey marketed in the city of Iasi. Honey has been marked with M1, M2, .., M6. Stated that honey M1, M2 and M3 originated from the same producing company, only the form of presentation being different. Also M4 and M6 honey came from the same company, just different packaging type. All honeys were processed in the laboratory to obtain permanent microscopic blades. The method used was the qualitative analysis proposed by Laveaux [3,4]. For each type of honey samples, 10 grams have been taken. They were placed in centrifuge tubes of 50 ml, above which were added 20ml of distilled water of 20-40°C. Mixed solutions were made, and then centrifuged for 5 minutes at 2500 rpm (1000G).The supernatant liquid was

removed leaving an amount of liquid as safety. Quantities of sediment obtained were filled again with distilled water up to 50 ml. Subsequently the solution was homogenized by mixing. The solution tubes were centrifugated again at 2500 - 3000rpm for 5 minutes. The aim of the operation was to better remove the remaining carbohydrates. Supernatantul was removed with absorbent paper and pipette leaving a quantity of liquid. The centrifuge tubes with sediment were weighed so that it can determined the exact solution existing in the sediment. An analytical balance with an accuracy of 4 decimal places has been used.

Six clean microscopic blades were tagged and weighed, noting the obtained values. Each blade containd two drops of sediment, weighing the blades after each stage. Thus was determined the exact sediment solution on the blades. Blades were left to dry in the drying stove at 30ºC until complete evaporation of water. To achieve permanent preparations, Canada balm has been used. No dye was used whereas pollen granite were well preserved in honey and so

well visible. Microscopic analysis was made with an optical microscope with mobile board, I.O.R. type (MC3).

All the vegetal impurities found have been counted. Also, the pollen granite have been counted and identified.

For a detailed analysis of pollen and its counting, photos were made with a digital camera, Samsung Digimax 420 type.

Determination of pollen was performed using both romanian determinators [5] and the one offered online by the Palinological Research Society from Austria [8]. The *Robinia* pollen granites and nonpolinical impurities were counted with a computer software (Image Tool v.3.00) for the comparative assessment of honey purity.

The polinical and nonpolinical impurities were reported to the entire sediment solution, representing the amount of honey used in the centrifuge. The number of contained items were reported at the total honey quantity (tab. 1). Similarly we determined the amount of pollen and *Robinia* pollen, and we reported it to the entire amount of honey used.

Table 1.
Data centralization

| Blades | M1 | | M2 | | M3 | | M4 | | M5 | | M6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | P1 | P2 | P1 | P2 | P1 | P2 | P1 | P2 | P1 | P2 | P1 | P2 |
| Weight sediment on blades (g) | 0,0122 | 0,0298 | 0,0117 | 0,0336 | 0,032 | 0,03 | 0,0353 | 0,0422 | 0,02 | 0,035 | 0,02 | 0,0359 |
| Nonpolinical impurities contained (particles) | 163 | 229 | 214 | 176 | 122 | 152 | 156 | 66 | 160 | 146 | 122 | 98 |
| Nonpolinical impurities contained, calculated for all the honey used (particles) | 21169 | 44038 | 27436 | 9167 | 18485 | 7958 | 8667 | 3929 | 13223 | 10139 | 13261 | 5904 |
| The impurities and nonpolinical elements mean calculated for the entire honey quantity used (particles) | 32603 | | 36603 | | 13222 | | 6298 | | 11681 | | 9583 | |
| **Impurities in 10 g honey (particles)** | **14016** | | **15744** | | **5586** | | **2627** | | **5213** | | **3971** | |
| Total pollen found on blade (granites) | 63 | 22 | 103 | 333 | 58 | 167 | 169 | 153 | 58 | 104 | 51 | 110 |
| *Robinia* pollen found on blades (granites) | 35 | 11 | 55 | 104 | 23 | 22 | 39 | 13 | 9 | 23 | 7 | 12 |
| Pollen deducted from the total *Robinia* honey used in the centrifuge | 4546 | 2115 | 7051 | 5417 | 3485 | 1152 | 2167 | 774 | 744 | 1597 | 761 | 723 |
| **Average percentage of *Robinia* pollen from the total pollen found (%)** | **52,76 %** | | **42,31 %** | | **26,41 %** | | **15,78 %** | | **18,81 %** | | **12,31 %** | |

## RESULTS

After counting the impurities found in the analyzed samples and reporting it to a quantity of 10 grams honey, we obtained values between 2627 and 15744 impurity particles (fig. 1). Honey M1, M2 and M3 had the highest content of impurities: 14016 for M1, 15744 for M2 and 5586 for M3. The M4 and M6 honeys had the lowest amount of impurities: 2627 for the first one (M4) and 3971 for the second one (M6). M5 honey had a number of 5213 impurities.

The presence of impurities in the honey, when they belong to the visited plant species or when they are in small quantities are not a problem. When their number increases that lead to the deepen of the honey color, loss of translucence and decreased aesthetic qualities of honey. A honey with a lower quantity of impurities (using fine filtration) is quantified as qualitatively superior in terms of aesthetic view,and it represents the evidence of a more efficient processing. Besides the use of fine filters may aggravate the melisopalinological determination of honey origin.

The pollen granites counting was carried out in the first phase by counting total pollen granites, regardless of the plant species found. Subsequently we determined the pollen number of *Robinia* genus.
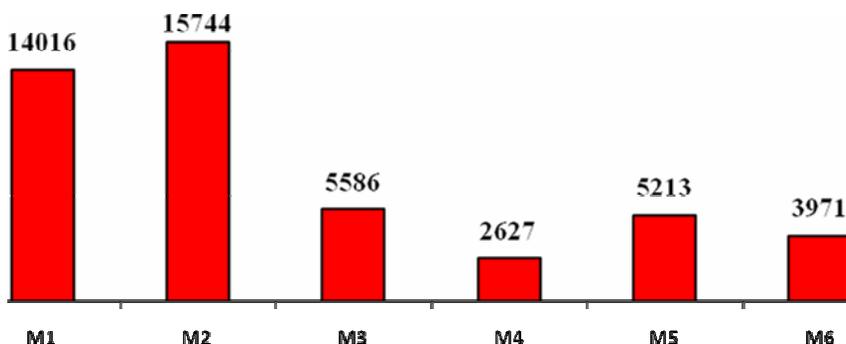


Figure 1.  The amount of nonpolinical impurities calculated for 10 grams honey

We counted 1391 samples of pollen granites, of which 353 belong to the *Robinia* genus (tab.1 and fig. 2). Thus the average of all analyzed honey contain a percentage of about 25% *Robinia* pollen, placing the pollen as frequently. The *Robinia* pollen mean, places the honey in normal limits of *Robinia* honey for Romania: 20-30% [2] (tabelul 2). The situation of the *Robinia* pollen content is different but for each sample of honey taken separately.

Table 2.
Extract from STAS 784/1-89 - honey classification into classes - taking from producers

| Features | | Quality class | | |
|---|---|---|---|---|
| | | Superior | I | II* |
| Specific granules of pollen reported to the total number of pollen grains examined (% min.) | *Robinia* (*Robinia pseudoacacia*) | 30 | 25 | 20 |

*At sales there is no class II

By counting the *Robinia* pollen grains, and reporting to all the pollen in the sample, we established that honey from a single commercial source (M1, M2, M3) have the highest proportion of *Robinia* pollen respectively 52.76% for M1 honey, 42.31% for M2 honey and 26.41% for M3 honey.

The lowest values were recorded for M4 with 15.78% *Robinia* pollen and M6 with

12.31% *Robinia* pollen. The M5 honey had a rate of 18.81% of *Robinia* pollen (Fig. 2).

Besides the first three honey samples (M1, M2 and M3), the *Robinia* pollen proved to be really under-represented even with under the minimum standards of this type of honey.

Graphic representation of the data on the amount of impurities of honey and the *Robinia* pollen, highlight the fact that honey M1, M2 and M3 were not as effective as the ones centrifugated from other companies, having the largest quantities of sediment, default nonpolinical impurities and pollen.
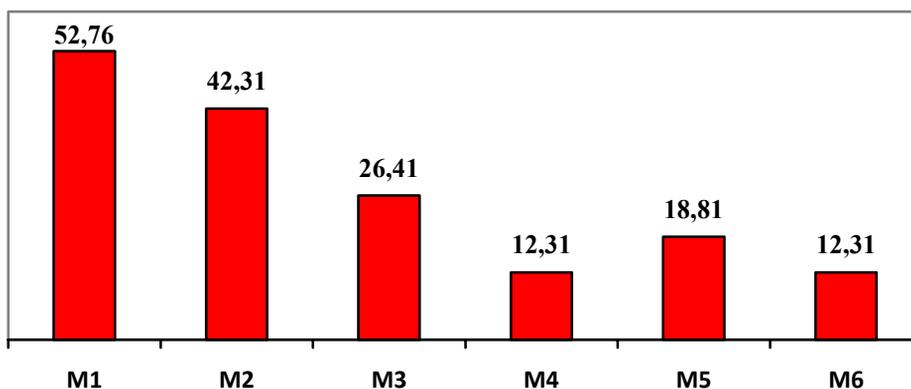


Figure 2. The percentage of pollen of *Robinia pseudoacacia* reported to total residue for the police of each analized sample

In terms of determining the origin of plant in accordance with the quality standards (STAS 784/1-89), the analyzed honey were classified into the following categories as:
-   M1 - 52.76% *Robinia* pollen – superior quality
-   M2 - 42.31% *Robinia* pollen – superior quality
-   M3 - 26.41% *Robinia* pollen – $1^{st}$ quality class
-   M4 - 15.41% *Robinia* pollen - under $II^{nd}$ quality class
-   M5 - 18.81% *Robinia* pollen - under $II^{nd}$ quality class
-   M6 - 12.31% *Robinia* pollen - under $II^{nd}$ quality class

From the analyzed data, we established that the honey with high percentage in *Robinia* pollen, were classified in the superior and first quality classes in terms of percentage of pollen, also have a larger quantity of impurities then those honey that were not classifiable in the quality classes (under $II^{nd}$ quality class).

**DISCUSSION**

The problem of impurities, is questionable when they are macroscopic and affect the quality and aesthetic aspects of the honey trade. A assessment in this direction can be put in evidence only if it is a honey with secure origin. In the large consumption commercial framework, honey does not require a supra-processing to a microscopic level especially if it comes from a processing company with multiple manufacturers. The advantages of supra-procesing is that of producing a clear and aesthetically attractive honey. Instead prolonged procesation has the disadvantage of eliminating microscopic particles used to determine the origin of honey.

In making the honey, the blendering technique is used that consists in mixing several sorts of honey in different sweepers (homogenization devices), in order to obtain some color, taste or flavor. If the honey comes from various collecting sources then there is the possibility that the determination of honey origin to be compromised. It is therefore recommended that this way of conditioning to be used only for polifloral

honey for which the standards for the pollen residue are not very demanding.

## CONCLUSIONS

Under melisopalinologic aspect we noticed that the percentage of *Robinia* pollen varies between 12.31% and 52,76% with an mean within the normal range for Romania.

The amount of impurities is high for samples of honey that presents an increased pollen percentage of *Robinia* pollen, highlighting differences in conditioning levels of honey.

The percentage of *Robinia* pollen content of honey places the analized honeys in the superior and first quality classes. Three samples of honey are below the lower limits of the second quality class.

## REFERENCES
*Journal articles*
[1] Bogdanov S., Gallmann P., Authenticity of honey and other bee products state of the art, ALP Science Nr. 520, 2008
[3] Persano Oddo L., S. Bogdanov, Determination of Honey Botanical Origin: Problems and Issues. Apidologie 35/2004, pg. 2–3
[4] Persano Oddo L., Piro R. and colab., Main European unifloral honeys: descriptive sheets, Apidologie 35/2004, pg. 38-81
[6] Directiva Consiliului European 2001/110/EC regarding honey, Official Journal of the European Communities, 2001
[7] Codex Standard for Honey, Codex Stan 12-1981, Adopted in 1981. Revisions 1987 and 2001
[8] http://paldat.botanik.univie.ac.at/
*Books*
[2] Lazăr Şt. and col., Apicultura, Alfa 2007, Iaşi
[5] Tarnavschi I.T. and col., Monografia polenului florei din România, vol. III, 1990, pg. 28, 74