Scientific report of the PN-II-RU-TE-2014-4-0110 project - *Development and implementation of instrumental techniques for honey authentication and adulteration detection*

Activities carried out according to the contract for 2015: Physicochemical parameters determination of honey (pH, electrical conductivity, free acidity, colour, ash, glucose, fructose and sucrose content).

Abstract

The aim of this study was to analyse 50 samples of honeys of five botanical origins (acacia, tilia, sunflower, polyfloral and honeydew) from physicochemical point of view (pH, free acidity, electrical conductivity, moisture content, water activity, colour, glucose, fructose and sucrose content). The honey classification has been made using the melissopalynological analysis and electrical conductivity.

1. Introduction

Honey is defined by Codex Alimentarius (2001) as "the sweet substance produced by honey-bees from nectar of blossoms or from secretions on living plants, which the bees collect, transform and store in honey combs". Honey composition does not depend only on botanical and geographical origin but also in processing and storage conditions (Lazaridou et al., 2004, Nayik & Nanda 2015). The honey composition is based mainly on monosaccharides (almost 70% of it), in specially glucose and fructose, and disacharides (de La Fuente et al, 2006). Honey contains, beside sugars, moisture and other valuable nutrients (minerals, enzymes, vitamins, amino acids (Baroni et al., 2006) and different classes of phenolic compounds (Kassim et al., 2010). The botanical and geographical origins of honey are influencing the composition and sensory attributes of honey (Gheldof et al., 2002).

According to EU Directive 110/2001 (Council Directive, 2001), the botanical and geographical origins of honey must be declared on the package label. Such regulations aim to guarantee product quality, authenticity and to protect consumers from a fraud (Karabagias et al., 2014). The studies which combines melissopalinological, physicochemical and sensory parameters consider that the botanical and geographical origin of honey may be established using them (de Sousa et al., 2016).

The aim of this study is to classify (using melissopalinological and physicochemical properties (pH, free acidity, ash content, moisture content, water activity, colour, glucose, fructose and sucrose content)) 50 samples of honey purchased from local beekeepers.

2. Materials and methods

Materials

In this study were analysed 50 honey samples from local beekeepers. The samples were of five different types: acacia, tilia, sunflower, polyfloral and honeydew.

Melissopalynological analysis

The analysis was made based on a method described by Louveaux et al. (1978). 10 g of honey was homogenised with 40 ml of water and centrifugated for 15 min at 3000 rpm. The supernatant is removed, and the residue is dissolved in water and centrifugated for 15 min more. The sediment is analysed at microscope (40 x objective).

Physicochemical analysis

The pH, free acidity, moisture content, electrical conductivity and ash content were determined according to the Harmonised methods of the International Honey Commission (Bogdanov 2002). The water activity was measured using a water activity meter AquaLab Lite (Decagon, USA).

Colour has been determined using a Konica CR400 cromameter (Konica Minolta, Japonia). The samples have been placed in 20 mm vat and have been measured to a white spectrum. The colour intensity, hue angle and yellow index (YI) have been computed as:

$$c^* = \sqrt{a^{*2} + b^{*2}} \quad (1)$$

$$h* = tan^{-1}\left(\frac{b*}{a*}\right) \quad (2)$$

$$YI = \frac{142.86 \cdot b*}{L*} \quad (3)$$

The determination of glucose, fructose and sucrose in honey samples was made by a HPLC 10ADVP-SHIMADZU, with RI-detector, according to a method described by Bogdanov (2002) The compounds were separated on a amino column, 250×4.6 mm i.d. and particle size 5μm. The samples were prepared as: 5 g of honey were dissolved in water (40 ml) and transferred quantitatively into a 100 ml volumetric flask, containing 25 ml methanol and filled up to the volume with water. The solution was filtered through a 0.45 μm membrane filter and collect in sample vials. Flow rate 1.3 ml/min, mobile phase: acetonitrile/water (80:20, v/v), column and detector temperature 30 °C, sample volume 10 μl. Sugars were quantified by comparison of the peak area obtained with those of standard sugars. The results for each sugar were expressed as g/100 g honey.

4. Results and discussions

For the honey classification according to the botanical origin have been used the melissopalynological analysis and the electrical conductivity. According to the beekeepers which gave the honey samples 41 were acacia, tilia, sunflower and polyfloral and 9 samples were honeydew. The classification of honey into monofloral (tilia, acacia and sunflower) had in view the quantification of the pollen grains, so: the acacia honey must contain minimum

30% *Robinia pseudoacacia* pollen grains reported to the all pollen grains presented, tilia honey must contain minimum 30% *Tilia europea* pollen grains reported to the all pollen grains presented, and the sunflower honeys must contain at least 40% *Helianthus annuus* pollen grains reported to the all pollen grains presented, respectively (Popescu & Meica, 1995).

Honey classification

Melissopalynological analysis

In figures 1-3 are presented the *Helianthus annuus, Robinia pseudoacacia* and *Tilia europea* pollen grains presented into the monofloral (sunflower, acacia and tilia) and polyfloral honeys.

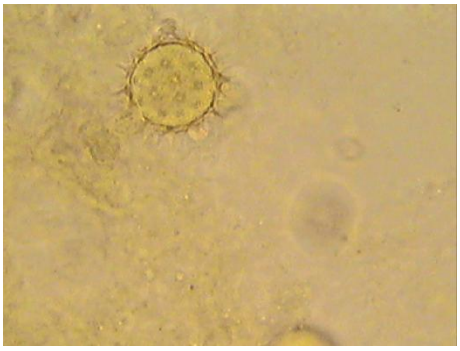

Fig. 1. *Helianthus annuus* pollen grain

Fig. 2. *Robinia pseudoacacia* pollen grain

Fig. 3. *Tilia europea* pollen grain

The pollen content of the three types of honey ranged between 620 and 6598 pollen grains. According to the classification made up by Maurizio (1993), the honey samples

analyzed can be classified in the 1st (less than 2000 pollen grains per gram) and 2nd class (between 2 000 – 10 000 pollen grains per gram). According to the number of pollen grains it seems that the acacia honey had the smallest number (the number of pollen grains per gram ranged between 620 and 5389). In the case of tilia honey ranged between 825 and 5231, while in the case of sunflower ranged between 784 and 6598 pollen grains per gram. The monofloral honey samples have been classified, acorrding to the melissopalynological analysis, into three main classes as acacia (*Robinia pseudoacacia)*, sunflower (*Helianthus annuus)* and tilia (*Tilia europea*) (the major pollen are represented in figure 1).

The pollen grains presented into the acacia honeys were: *Robinia pseudoacacia, Brassica napus, Plantago, Prunus, Trifoloium and Rubus.* The *Brassica napus* pollen had been the main pollen. The pollen grains of *Robinia pseudoacacia* were placed in the 2nd place as frequency; the percentage of this type of pollen ranged between 7% and 37 %.

In the sunflower honeys were presented the next type of pollen grains: *Helianthus annuus, Taraxacum officinale, Trifolium, Fragaria, Tilia, Brassica napus and Robinia pseudoacacia.* The major type of pollen was *Helianthus annuus,* ranging between 52.5 and 67.2%.

In the case of tilia honey, there were observed: *Tilia europea, Brassica napus, Helianthus annuus, Galium and Trifolium pollen grains*. The major pollen was *Tilia europea* (31.2 – 87.4%).

Regarding the polyfloral honeys were identified pollen grains as follows: *Robinia pseudoacacia, Brassica napus, Plantago, Prunus, Trifoloium, Rubus, Taraxacum officinale, Fragaria, Tilia europea, Galium* etc.

After the melissopalynological analysis, the 41 samples have been classified as: 10 samples of acacia, 8 samples of tilia, 11 samples of sunflower and 12 samples of polyfloral.

The classification of honeydew samples

The honeydew honeys must have the electrical conductivity at least 800 μS/cm (Bogdanov et al., 2004). All the honeys had a electrical conductivity higher than this value. It can be concluded that the samples provided by the local beekeepers are authentic.

In table 1 are presented the physicochemical parameters investigated for the 50 samples of honey.

Table 1. Physicochemical parameters of honeys

Parameter	Honey type – mean (standard deviation)					*F-ratio*
	Sunflower	Honeydew	Polyfloral	Acacia	Tilia	
pH	4.18 (0.26)c	4.85 (0.42)b	4.37 (0.42)c	4.45 (0.27)c	5.51 (0.53)a	17.17***
a_w	0.55 (0.03)a	0.54 (0.02)a	0.54 (0.02)a	0.53 (0.04)a	0.54 (0.02)a	0.35ns
Free acidity (meq acid/kg)	13.02 (2.95)bc	16.08 (2.57)ab	20.83 (10.48)a	9.08 (7.54)cd	6.62 (3.97)d	7.31***
Moisture content (%)	18.16 (1.65)a	16.31 (1.10)c	17.05 (1.10)bc	17.02 (1.31)abc	17.81 (1.55)ab	2.95*
Electrical	346.1	1007.94	431.44	156.58	549.31	48.77***

conductivity (μS/cm)	(109.7)c	(147.83)a	(139.88)bc	(28.52)d	(222.09)b	
Ash (%)	0.17 (0.54)c	0.49 (0.07)a	0.21 (0.07)bc	0.08 (0.01)d	0.27 (0.11)b	48.61***
L*	41.22 (2.27)bc	21.64 (1.93)d	39.79 (2.68)c	45.64 (1.47)a	42.18 (1.44)b	58.42***
a*	1.75 (1.29)c	5.77 (1.81)a	3.35 (2.07)b	-1.02 (0.65)d	0.73 (0.95)c	34.27***
b*	15.66 (1.84)a	6.61 (1.81)e	13.95 (2.07)c	11.96 (2.52)d	14.88 (0.78)bc	32.87***
C*	15.81 (1.77)a	8.86 (1.62)c	14.54 (1.60)a	12.03 (2.47)b	14.93 (0.76)a	24.42***
h*	3.46 (5.61)a	0.48 (0.46)ab	-0.60 (2.59)b	-0.64 (4.92)b	-1.60 (3.00)b	2.65*
Yellow index	57.03 (7.38)a	43.03 (7.91)b	49.79 (5.28)a	37.64 (8.67)b	52.34 (5.39)a	29.63***
Fructose (g/100g)	33.52 (1.92)c	35.71 (2.31)c	34.51 (3.28)c	42.81 (3.51)a	39.80 (1.40)b	50.89***
Glucose (g/100g)	31.56 (1.98)b	34.81 (1.68)a	31.98 (2.62)b	28.71 (2.54)c	31.62 (1.89)b	13.12***
Sucrose (g/100g)	1.3 (0.6)a	0b	1.8 (0.9)a	1.20 (0.5)a	1.4 (0.5)a	10.21***

a,b,c – statistical groups, ns - not significant P >0.05, * - P <0.05, ** - P <0.01, *** - P < 0.001

Physicochemical properties

The honey moisture content varied from 14.44 to 19.89 %, meeting the threshold requirements established by the Codex Alimentarius at 20% (Codex Alimentarius, 2001). It can be observed that the sun flower honeys have the highest moisture content while the honeydew honeys the smallest one. The difference of moisture content according to their origin is a significant one ($P < 0.05$). A moisture content higher than 20% accelerates the fermentation process during storage (Oroian 2012). The moisture content of the honeys analysed are in the same range with those reported in the case of Spanish honeys (Oroian et al. 2013, Escriche et al. 2011)

The honey acidity is characterized by the free acidity. This parameter indicated if the honey started to ferment. The maximum allowable value for free acidity is 40 meq acid/kg in the case of mono and polyflora honeys and 50 meq acid/kg in the case of honeydew honeys. In all the cases the honeys free acidity was lowest than the regulation limit.

The honey samples are acid in their nature, the values of pH ranged in this case between 3.88 and 6.39. The pH values are in the same range with those reported for honeys from Algeria (Ouchemoukh et al., 2007), India (Ahmed et al., 2007) and Spain (Oroian et al., 2013).

The electrical conductivity is used often for the classification of honeys into floral and honeydew, a value higher than 800 μS/cm is specific for honeydew honeys (Bogdanov et al., 2004). The values are presented in table 1. The highest values were observed in the care of honeydew honeys (1007.94 μS/cm), while acacia had the lowest electrical conductivity (156.58 μS/cm). The difference of electrical conductivity according to their origin is a

significant one ($P < 0.05$). The values are in the same range with those reported in the case of Spain (Escriche et al., 2011).

Ash content is a quality parameter that expresses the honey mineral content. In the Codex Alimentarius standards (2001) are not established any standard value, but the average content in honey, according to scientific literature, ranges between 0.02% - 1.03% (Chakir et al., 2011). The ash content ranged between 0.17 -0.49%. The high ash contents are presented in the honeydew samples, while acacia honeys have the lowest concentrations.

In the case of honey, water activity is influenced by the molar concentration of the soluble chemical species, and for these reason, the substances which have a high molecular mass or which are presented in small quantities like compounds with nitrogen (proteins, enzymes, aminoacids), acids, vitamins, aroma compounds or minerals do not contribute to the magnitude of water activity (Ruegg & Blanc 1981, Chirife et al. 2006). So it can be concluded that the water activity of honey is influenced more by the glucose and fructose content, and in a little influence by the sucrose (Chirife et al., 2006). In the case of the honeys analysed, the water activity ranged between 0.476 – 0.603. The values are not influenced by the honey origin ($P > 0.05$). The values are in the same range with those reported in the case of honeys from Argentina (Chirife et al., 2006).

Colour represents the first attribute of a honey, and for this reason this parameter is an important one for its comercialization and authentication. Is one of the parameters most used by the consumers for the quality appreciation and acceptability (da Silva et al. 2016). The colour parameters, in CIEL*a*b* coordinates, are presented in the table 1.

In figures 4-8 are presented the honeys; they were grouped according to their origin. It can be observed a great difference between the colour between the different honey types. The acacia samples were pale yellow, while the honeydews were yellow brown.

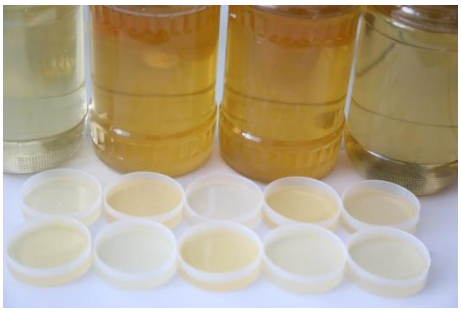

Fig. 4. Acacia honeys

Fig. 5. Polyfloral honeys

Fig. 6. Tilia honeys

Fig. 7. Honeydew honeys

Fig. 8. Sunflower honeys

The highest L* was observed in the case of acacia honeys, followed by tilia, sunflower, polyfloral and honeydew. The acacia and tilia honeys were clearer (highest L* values) than the other honey types, while the honeydew was the darkest one (lowest L* values). The highest intensity of colour (C*) was observed in the case of sunflower and tilia samples, while the honeydew honeys presented the lowest values. In the case of yellow index the sunflower honeys presented the highest values, while the acacia samples the smallest one. There was a significant difference ($P<0.001$) among the honey samples in term of color parameters. The differences in terms of colour between the different honey types are due to the chemical composition and variety (Oroian 2012).

According to the Codex Alimentarius standards (2001), the concentration of glucose and fructose in honeys must be higher than 60 g/100 g honey. All the honeys analysed met this requirements. According to the data presented in table 1, acacia honeys presented the highest values of fructose, and sunflower the lowest. In the case of glucose, the highest concentration was observed in the case of polyfloral honeys. The honeydew samples do not presented sucrose.

5. Conclusions

The 50 honeys samples analysed were of five botanical origins (acacia, polyfloral, tilia, sunflower and honeydew). All the samples have an acidic pH, and their free acidity and

moisture content do not exceed the maximum allowable level. The concentrations of glucose and fructose of each honey comply the Codex Alimentarius regulations.

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