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 - 2016:

We start by recalling the objectives proposed in Annex IV of Contract No. 22/01.10.2015 and in Annex IV of Additional Contract 1/22.01.2016 and Additional Contract 2/26.05.2016.

Year	Stage	Objective	Activities
2015	Stage 1	Physicochemical characterization of honey for achieving its authenticity	Sample and reagents acquisitions
			Physicochemical parameters of honey determination (pH, conductivity, acidity, colour, ash content, glucose, fructose and sucrose content)
2016	Stage 2	Electronic rheometer acquisition	For the rheological studies of the project will be bought an electronic rheometer with different geometries (con-plate, plate-plate)
		Honey authentication using instrumental techniques	Studies regarding the phenolic profile of honey. It will be used an HPLC Shimadzu coupled with DAD dector. The substances wich will be determined are: quercetin, apigenin, myricetin, isorhamnetin, kaempherol, caffeic acid, chrysin, galangin, luteolin, p-coumaric acid, pinocembrin and gallic acid
			Studies regarding the honey authentication using the rheometric and textural techniques. The sample (should be liquid without any crystals because they can influence the determination) will be placed into the plate-plate system of the rheometer which are thermostated at a specific temperature. The texture profile will the determined using a texturemeter having into account the next parameters: hardness (H), viscosity (V), adhesion (A), cohesiveness (Co), springiness (S), gumminess (G) and chewiness (Ch).
			Studies on honey authentication based on spectrometric methods will be focused on portable Raman Raman spectrometer using a high precision. For this analysis it is necessary to sample a small amount (2-3 g) is placed in a quartz cell and the spectral profile is obtained in a few seconds. This profile is processed and stored by a computer. The obtained spectra are analyzed to identify areas that may be used for spectral discrimination of the range of honey according to their origin using statistical

			analysis.
			Studies regarding the authentication of honey using electrochemical methods as an "electronic tongues". In this study will be used for electrochemical techniques a single sensor composed of metal and / or metal oxide electrodes, which are immersed in the matrix to be tested.
		Checking of the proposed instrumental methods using samples from the market to establish its authenticity	In this activity will be acquired various samples of honey on the market (different botanical origins) and analyzed to determine their authenticity using instrumental methods proposed.
		Experimental studies for the honey adulteration detection using Raman spectrometry	Studies regarding the honey adulteration detection using the Raman Spectroscopy. The Raman spectroscopy will be used for the authentic and adulterated samples. It will be focused on the identification of new peaks or distinct zone

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. The honey authentication has been made using physicochemical parameters, texture parameters, Raman spectroscopy, phenolic profile and electrochemical measurements while the honey adulteration detection has been based on Raman spectroscopy. The authentication of honey based on rheology measurement has not been made because the acquisition procedure has ended on November 25, 2016.

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)), Raman spectroscopy, textural profile, phenolic profile and electrochemical measurements 50 samples of honey purchased from local beekeepers. Another goal of this project was to evaluate the usefulness of Raman spectroscopy on honey adulteration detection.

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.

Raman spectroscopy

The spectra were recorded using an i-Raman spectrometer (EZM-A2-785L, B&W TEK Inc. USA) equipped with a fiber-optic Raman probe, a thermoelectric cooled CCD detector with 2048 pixels and a 785 nm laser with a maximum output power of 495 mW in the signal range of 250 – 2339 cm^{-1} and a spectral resolution of 3 cm^{-1}. The samples were placed into a quartz cell with 1 cm path (the quartz cell is placed into a cuvette holder) scanned at an increment of 10 nm. Integration time was of 15s. Before being used they were warmed up to 55 °C to dissolve any crystals, and kept in flasks at 30 °C to remove air bubbles that could interfere with spectra studies.

Texture profile

The texture parameters of honeys, like the rheological parameters, can be influenced by the presence of crystals and air bubbles (Bhandari et al. 1999, Mossel et al. 2000). Before being used they were warmed up to 55 °C to dissolve any crystals, and kept in flasks at 30 °C to remove air bubbles that could interfere rheological/textural studies (Oroian 2012).

The TPA was carried out at 25 °C with Mark 10 Texture Analyzer (Mark 10 Corporation, USA) equipped with a 50 mm disc probe, the flask diameter was 70 mm. The TPA was operated at a constant speed of 150 mm/min, until a depth of 12.5 mm (the honey column had 25 mm). The TPA can offer a great number of texture parameters, as: hardness (H), viscosity (V), adhesion (A), cohesiveness (Co), springiness (S), gumminess (G) and chewiness (Ch) (Chen & Opara 2013).

Phenolic profile determination

The phenolics extraction was made using the method described by Baltrušaitytė et al. (2007) and Escriche et al. (2011). The phenolics compounds were separated and quantified using the method described by Coneac et al. (2008).

Electrochemical measurements

The cyclic voltammetry has been made using a PGSTAT 204 (Autolab, Germany) with an electrochemical cell with three electrodes: reference electrode (Ag/AgCl), counter electrode (Glassy Carbon Electrode Rod) and working electrode (Au, Ag, Pt and glass electrode). The electrochemical data have been collected using a Nova 2.0 software (Autolab, Germany).

Statistical analysis

Statistical analysis was performed using The Unscrambler X 10.1 software (Camo, Norway).

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).

4.1. 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.

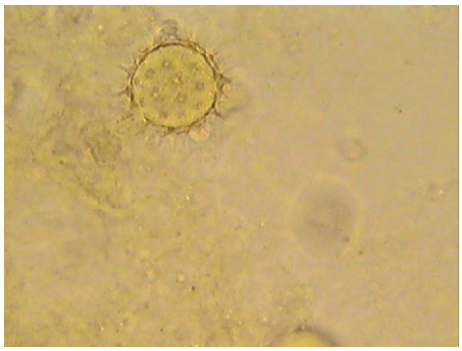


Fig. 1. *Helianthus annuus* pollen grain

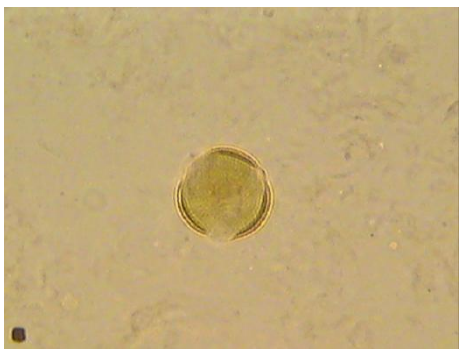


Fig. 2. *Robinia pseudoacacia* pollen grain

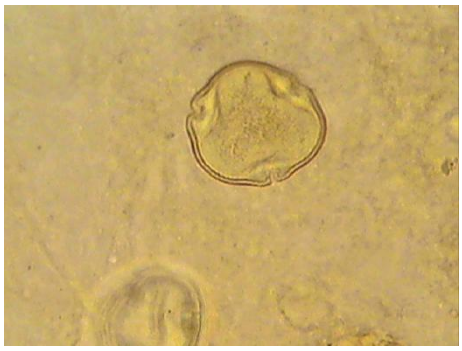


Fig. 3. *Tilia europea* pollen grain

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 conductivity (µS/cm)	346.1 (109.7)c	1007.94 (147.83)a	431.44 (139.88)bc	156.58 (28.52)d	549.31 (222.09)b	48.77***
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

4.2. 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.

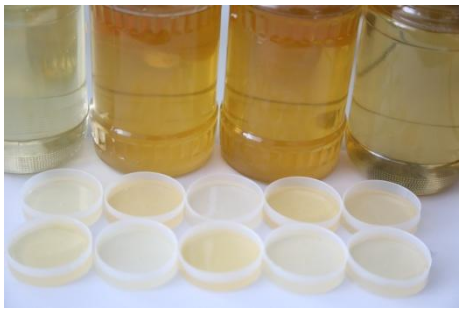


Fig. 4. Acacia honeys

Fig. 5. Polyfloral honeys

Fig. 6. Tilia honeys

Fig. 7. Honeydew honeys

Fig. 8. Sunflower honeys

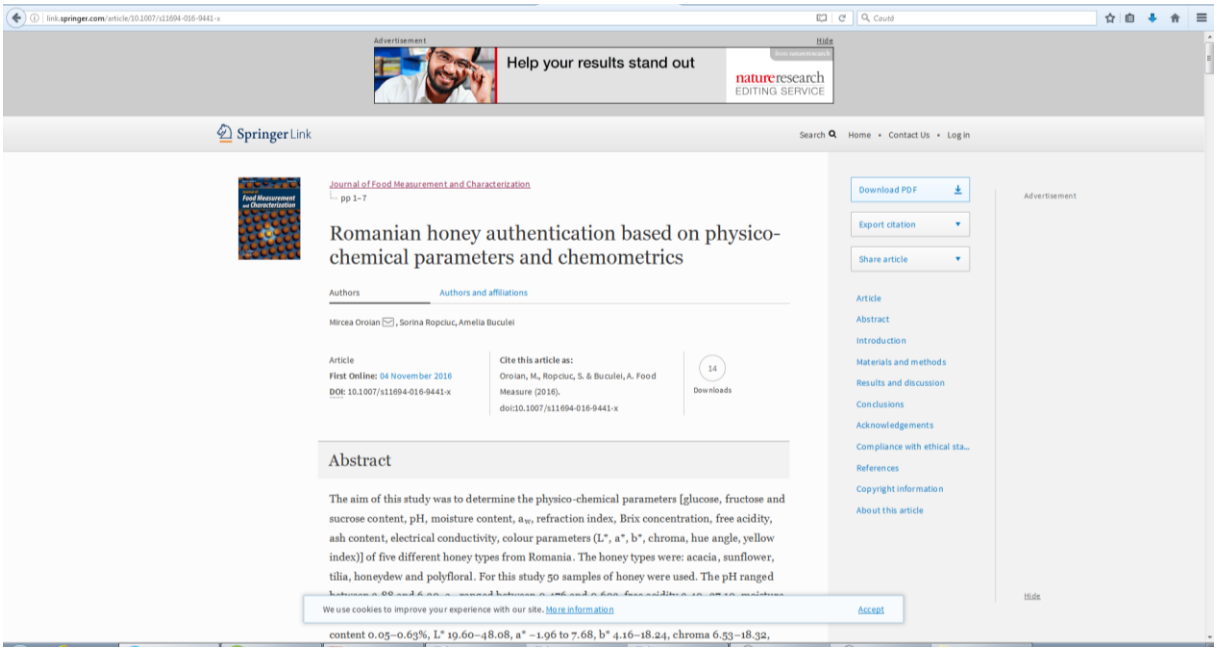
The highest L* was observed in the case of acacia honeys, followed by tilia, sunflower, polyfloral and honeydew. The acacia and tilia honeys were clearer (highest L* values) than the other honey types, while the honeydew was the darkest one (lowest L*

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.

Dissemination result – It was accepted the next article:

Oroian, M., Ropciuc, S., & Buculei, A., 2016, Romanian honey authentication based on physico-chemical parameters and chemometrics. *Journal of Food Measurement and Characterization*, 1-7. http://link.springer.com/article/10.1007/s11694-016-9441-x



4.3. Raman spectroscopy

The study involved the analysis of the honey (76 samples) using a Raman spectroscope and the recorded spectra data were submitted to a linear discriminant analysis (LDA) with cross validation step. The Raman spectra analysis has been proved to be an excellent tool (simple, rapid and non destructive method) for honey authentication; by the linear discriminant analysis (LDA) applied 83.33 % of the honey has been correctly cross validated.

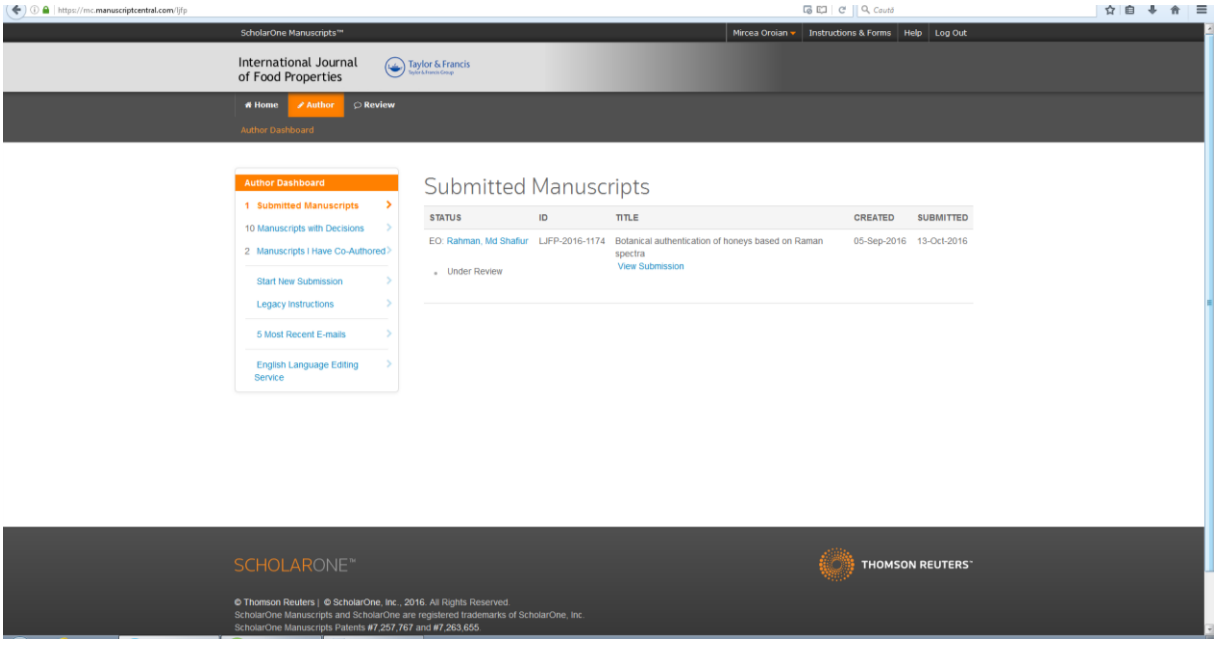
In the table 2 are presented the classification of honey based on Raman spectra using LDA

Tab. 2. Classification of honey based on Raman spectra using LDA

Model	Honey type	Acacia	Tilia	Polyfloral	Honeydew	Sunflower	Total	% correct
Original	Acacia	15	0	0	0	0	15	100%
	Tilia	0	12	0	0	0	12	100%
	Polyfloral	0	0	18	0	0	18	100%
	Honeydew	0	0	0	15	0	15	100%
	Sunflower	0	0	0	0	16	16	100%
	Total	15	12	18	15	16	76	100%
Cross validation	Honey type	Acacia	Tilia	Polyfloral	Honeydew	Sunflower	Total	% correct
	Acacia	5	0	0	0	1	6	83.33%
	Tilia	0	5	0	1	0	6	83.33%
	Polyfloral	0	0	4	1	1	6	66.67%
	Honeydew	0	0	0	6	0	6	100.00%
	Sunflower	1	0	0	0	5	6	83.33%
	Total	6	5	4	8	7	30	83.33%

Dissemination result: it has been submitted an article to ***International Journal of Food Properties*: Botanical authentication of honeys base on Raman spectra** – which is *Under review*

In this scientific report there are no many information because they will be presented in the article as soon as it is published.



4.4. Phenolic profile

The aim of this study is to assess the usefulness of physicochemical parameters (pH, water activity, free acidity, refraction index, Brix, moisture content and ash content), colour parameters (L*, a*, b*, chroma, hue angle and yellow index) and phenolics (quercetin, apigenin, myricetin, isorhamnetin, kaempherol, caffeic acid, chrysin, galangin, luteolin, *p*-coumaric acid, gallic acid and pinocembrin) in view of classifying honeys according to their botanical origin (acacia, tilia, sunflower, honeydew and polyfloral). Thus the classification of honeys has been made using the principal component analysis (PCA), linear discriminant analysis (LDA) and artificial neural networks (ANN). The multilayer perceptron network with 2 hidden layers classified correctly 94.8% of the cross validated samples.

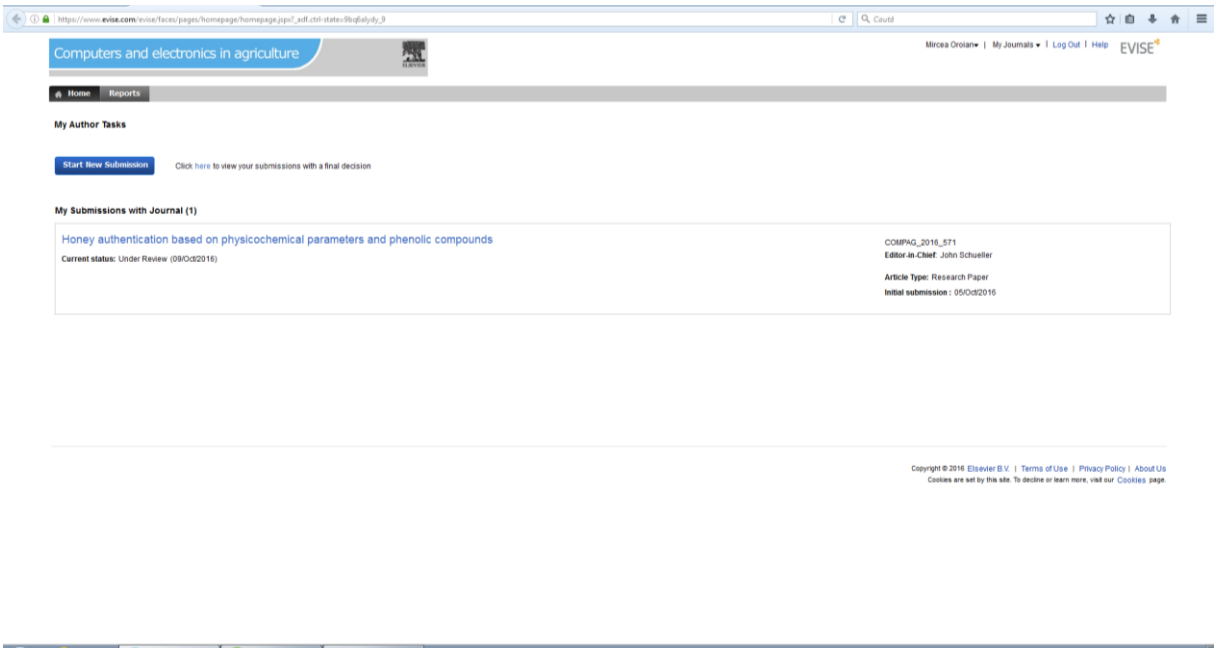
In the table 3 is presented the classification of honey based on phenolics and physicochemical parameters using LDA

Tab. 3. Classification of honey based on phenolics and physicochemical parameters

Validation – cross validation	Original group						Corect, %
		Acacia	Tilia	Polyfloral	Honydew	Sunflower	
Phenolics	Acacia	5	0	2	2	1	50.00%
	Tilia	2	5	0	0	1	62.50%
	Polyfloral	1	0	10	0	1	83.33%
	Honeydew	0	0	1	6	2	66.67%
	Sunflower	2	0	5	1	3	27.27%
Physicochemical parameters and phenolics	Acacia	9	0	0	0	1	90.00%
	Tilia	0	8	0	0	0	100%
	Polyfloral	0	0	11	0	1	91.67%
	Honeydew	0	0	0	9	0	100%
	Sunflower	1	0	1	0	9	81.82%

Dissemination result: it has been submitted an article to ***Computers and electronics in agriculture*****: Honey authentication based on physicochemical parameters and phenolic compounds – which is** *Under review*

In this scientific report there are no many information because they will be presented in the article as soon as it is published.



It has been presented an article at the The 15th International Symposium PROSPECTS FOR THE 3rd MILLENNIUM AGRICULTURE, 29th September – 1st October, 2016, Cluj-Napoca, Romania – Oroian, M., Ropciuc, S., Buculei, A., Pădureț, S., Todosi, E., 2016, Phenolic profile of honeydew honeys from the north-east part of Romania.

4.5. Texture analysis

The aim of this study was to investigate the physicochemical properties (pH, a_w, free acidity, ash content, moisture content, colour (L*, a*, b*, chroma, hue-angle, yellow index (YI)), fructose, glucose and sucrose content) and textural parameters (hardness (H), viscosity (V), adhesion (A), cohesiveness (Co), springiness (S), gumminess (G) and chewiness (Ch)) of 50 samples of honey of different botanical origin (acacia, tilia, sunflower, polyfloral and honeydew). In order to achieve the authentication of the honey samples analysed, their data have been submitted to principal component analysis (PCA) and linear discriminant analysis (LDA). According to the PCA, it can be observed the distribution of the five different types of honeys in five different zones, while the LDA has classified correctly 92.0% of the honeys according to their botanical origin, using the cross validation, and 96.0% using the original group. In the LDA projection, the textural parameters (chewiness, hardness, cohesiveness, springiness) dominated the two functions. In the table 4 are presented the classification result of the analysed honeys in function of their botanical origin based on physicochemical and texture parameters.

Tab. 4. Classification of honey using LDA

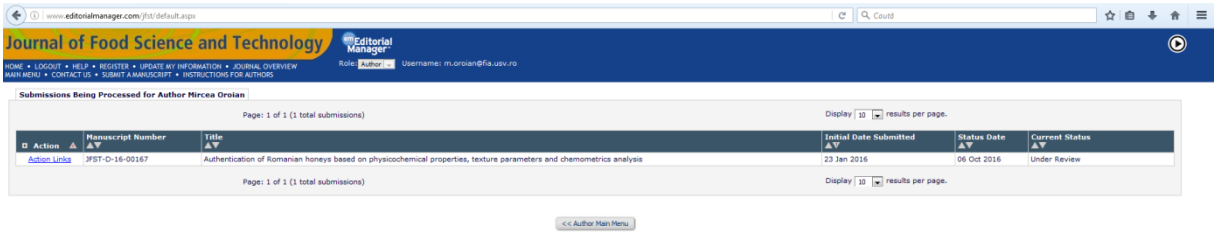
Model	Honey type	Predicted group membership, %					Total
		Acacia	Tilia	Polyfloral	Honeydew	Sunflower	
Original group	Acacia	100	0	0	0	0	100
	Tilia	0	100	0	0	0	100
	Polyfloral	0	8.30	83.30	0	8.30	100
	Honeydew	0	0	0	100	0	100
	Sunflower	0	0	0	0	100	100
Cross-	Acacia	100	0	0	0	0	100

validated	Tilia	0	75.0	12.5	0	12.5	100
	Polyfloral	0	8.30	83.3	0	8.3	100
	Honeydew	0	0	0	100	0	100
	Sunflower	0	0	9.09	0	90.91	100

Dissemination result

It has been submitted an article to ***Journal of Food Science and Technology*: Authentication of Romanian honeys based on physicochemical properties, texture parameters and chemometrics analysis** – which is Under review

In this scientific report there are no many information because they will be presented in the article as soon as it is published.



It has been published the next article: Oroian, M., Todosi Sănduleac, E., Pădureț, S., 2016, Physico-chemical and textural properties of honeys from north east part of romania, Food and Environment Safety, 15(3), 234-239.

4.6. Electrochemical measurement for honey authentication

The electrochemical measurement has been made on an electrochemical cell with three electrodes: reference electrode (Ag/AgCl), counter electrode (Glassy Carbon Electrode Rod) and working electrode (Au, Ag, Pt and glass electrode).

In the figure 9 are presented typical cyclic voltammograms for acacia honey using different working electrodes (Au, Ag, Pt and glass electrode).

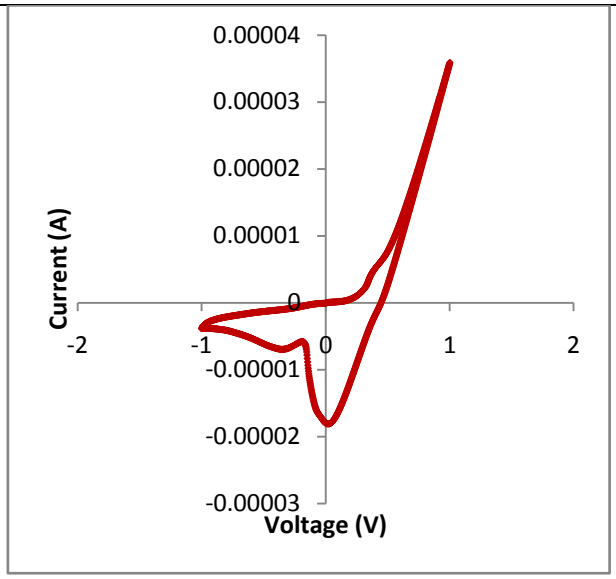


Fig.9. Cyclic voltamograms for acacia honey using Ag as working electrodes

The current data for each honey were submitted to linear discriminant analysis using the Unscrambler X 10.1 software (Camo, Norway). The data are presented into the tables 5-8. It can be observed that in the case of gold, platinum and glass electrode the classification of honey according to their botanical origin is 100% correctly, while in the case of silver electrode the percentage of correct classification is 96.08% (polyfloral and sunflower honeys have some samples which were classified into other groups).

Tab.5. Classification of honey based on silver electrode

	Honey type					Total	Percentage
Honey type	Tilia	Acacia	Polyfloral	Honeydew	Sunflower		
Tilia	8	0	0	0	0	8	100.00%
Acacia	0	10	0	0	0	10	100.00%
Polyfloral	0	0	11	0	1	12	91.67%
Honeydew	0	0	0	10	0	10	100.00%
Sunflower	0	0	1	0	10	11	90.91%
Total	8	10	12	10	11	51	96.08%

Tab.6. Classification of honey based on gold electrode

	Honey type					Total	Percentage
Honey type	Tilia	Acacia	Polyfloral	Honeydew	Sunflower		
Tilia	11	0	0	0	0	11	100.00%
Acacia	0	10	0	0	0	10	100.00%
Polyfloral	0	0	12	0	0	12	100.00%
Honeydew	0	0	0	8	0	8	100.00%
Sunflower	0	0	0	0	10	10	100.00%
Total	11	10	12	8	10	51	100.00%

Tab.7. Classification of honey based on platinum electrode

	Honey type					Total	Percentage
Honey type	Tilia	Acacia	Polyfloral	Honeydew	Sunflower		
Tilia	11	0	0	0	0	11	100.00%
Acacia	0	10	0	0	0	10	100.00%
Polyfloral	0	0	12	0	0	12	100.00%

Honeydew	0	0	0	8	0	8	100.00%
Sunflower	0	0	0	0	10	10	100.00%
Total	11	10	12	8	10	51	100.00%

Tab.8. Classification of honey based on glass electrode

	Honey type					Total	Percentage
Honey type	Tilia	Acacia	Polyfloral	Honeydew	Sunflower		
Tilia	11	0	0	0	0	11	100.00%
Acacia	0	10	0	0	0	10	100.00%
Polyfloral	0	0	12	0	0	12	100.00%
Honeydew	0	0	0	8	0	8	100.00%
Sunflower	0	0	0	0	10	10	100.00%
Total	11	10	12	8	10	51	100.00%

Dissemination result: an article is in writing at the moment and it will be submitted to an ISI quoted journal.

In this scientific report there are no many information because they will be presented in the article as soon as it is published.

4.7. Rheology

The authentication of honey based on rheology measurement has not been made because the acquisition procedure has ended on November 25, 2016.

4.7. Checking of the proposed instrumental methods using samples from the market to establish its authenticity

In order to establish the authenticity of different honey types from the Suceava market as: 3 samples of acacia, 3 samples of sunflower, 3 samples of honeydew, 3 samples of tilia and 3 samples of polyfloral. The samples have been submitted to the Raman analysis, texture analysis, electrochemical and antioxidant profile determination.

In the article submitted regarding the texture and antioxidant profile determination, the authentication cannot be made only using this analysis, so the honey samples have been submitted to physicochemical analysis. The resulting data (texture data and physicochemical parameters, and antioxidant profile and physicochemical parameters) have been submitted to validation procedure of the proposed model based on these parameters. In the table 9 are presented the results regarding the authenticity of the samples.

Tab.9. Validation of the models regarding the texture data and physicochemical parameters, and antioxidant profile and physicochemical parameters, respectively

Honey	Texture data and physicochemical analysis		Antioxidant profile and physicochemical parameters	
	Authentic	Non-authentic	Authentic	Non-authentic
Acacia	3	0	3	0
Tilia	2	1 – polyfloral	2	1 – polyfloral
Sunflower	2	1 – polyfloral	2	1 – polyfloral

Polyfloral	2	1 - sunflower	2	1 - sunflower
Honeydew	3	0	3	0

The resulting data (Raman spectroscopy and electrochemical parameters) have been submitted to validation procedure of the proposed model based on these parameters. In the table 10 are presented the results regarding the authenticity of the samples.

Tab.11. Validation of the models regarding the Raman spectroscopy and electrochemical parameters, respectively

Honey	Raman spectroscopy		Electrochemical parameters	
	Authentic	Non-authentic	Authentic	Non-authentic
Acacia	3	0	3	0
Tilia	2	1 – polyfloral	2	1 – polyfloral
Sunflower	2	1 – polyfloral	2	1 – polyfloral
Polyfloral	2	1 - sunflower	2	1 - sunflower
Honeydew	3	0	3	0

All the four methods of authentication validated the same samples. The honeys which were classified of other origins were in the same groups. It is very common the wrong authentication of tilia, sunflower or polyfloral honey because their colour are in the same range and the beekeepers do not make any authentication prior to the honey commercialization.

4.8. Adulteration detection using the Raman spectra

The honey adulteration has been made using glucose, fructose, inverted sugar, concentrated malt must and hydrolysed inulin syrup. The honey samples (acacia, tilia, sunflower, polyfloral and honeydew) have been adulterated with the adulteration agents in different percentages (5, 10, 20, 30, 40 and 50%).

The Raman spectra of the honeys presented two different section, represented by the wave numbers 400 - 640 cm^{-1} and 1200-1430 cm^{-1}. The most prominent peaks were specific to the sugars, which are presented into high concentrations in honey. The vibrations of the pollen, proteins and other floral compounds of the honey are covered by the vibrations of the major compounds (Goodacre, Radovic & Anklam, 2002). In the table 12 are presented the main vibrations of the adulterations agents used in this study.

Tab. 12. Raman vibrations according to different adulteration agents (Dollish et al., 1980, Schrader & Meier, 1989, Lin-Vien et al., 1991, Degen 1997, Goodacre, Radovic & Anklam, 2002)

Raman Band	**Possible identities of the vibration**	**Adulteration agent**				
		Glucose	**Inverted sugar**	**Fructose**	**Hydrolyzed inulin syrup**	**Malt must**

430 cm^{-1}	Skeletal vibration	+	–	+ +	+ +	-
460 cm^{-1}	Skeletal vibration	–	+ +	+	+	++
523 cm^{-1}	Skeletal vibration	+ +	+	+	+	+
600 cm^{-1}	Skeletal vibration	–	+	–	–	-
631 cm^{-1}	Ring deformation	–	+	+ +	+ +	-
709 cm^{-1}	Skeletal vibration	–	–	+ +	–	-
781 cm^{-1}	Ring deformation	+	–	+	+ +	+
825 cm^{-1}	C-OH stretch	–	–	+ +	+	-
870 cm^{-1}	C-O-C cyclic alkyl ethers	–	–	+ +	–	-
918 cm^{-1}	CH, COH bend	+ +	+	+	–	++
983 cm^{-1}	Ring "breathing"	–	–	+	–	-
1074 cm^{-1}	C-O-C cyclic alkyl ethers	+	+	+ +	+ +	+
1127 cm^{-1}	C-OH deformation	+ +	+ +	–	+	++
1267 cm^{-1}	C-O-C deformation	+	+	+ +	+ +	+
1368 cm^{-1}	CH bend + OH bend	+ +	+ +	–	–	++
1460 cm^{-1}	CH_2 bend	+	+	+ +	+ +	+
1640 cm^{-1}	O-H bend from H_2O	+	+	+	+	+

"–" absent, "+" - medium strength vibration, "+ +" – strong vibration

Adding glucose, fructose, inverted sugar and hydrolysed inulin syrups into the honey there are obtained similar spectra to the authentic honeys. Because the syrups have great concentrations of water, the adulteration can be observed in function of the intensity of the peak at 1600 cm^{-1}.

In figure 10 - 13 there is presented the spectra of acacia honey adulterated with glucose syrup.

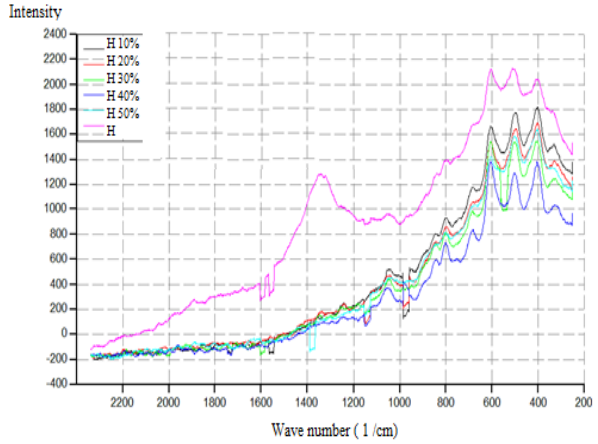


Fig.10. Raman spectal profile of tilia honey adulterated with fructose (H - original honey, H10% - honey adulterated with 10%, H 20% - honey adulterated with 20%, H30% - honey adulterated with 30%, H40 % - honey adulterated with 40%, H 50% - honey adulterated with 50%)

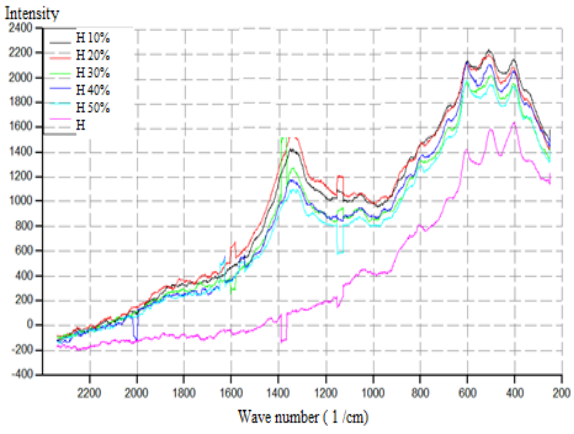


Fig.11. Raman spectal profile of tilia honey adulterated with inulin syrup (H - original honey, H10% - honey adulterated with 10%, H 20% - honey adulterated with 20%, H30% - honey adulterated with 30%, H40 % - honey adulterated with 40%, H 50% - honey adulterated with 50%)

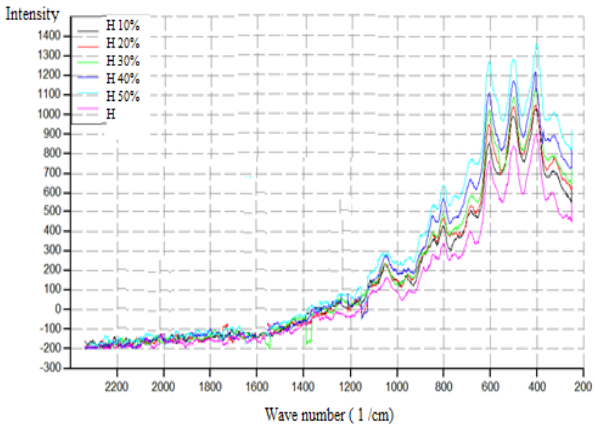


Fig. 12. Raman spectal profile of polyfloral honey adulterated with fructose (H - original honey, H10% - honey adulterated with 10%, H 20% - honey adulterated with 20%, H30% - honey adulterated with 30%, H40 % - honey adulterated with 40%, H 50% - honey adulterated with 50%)

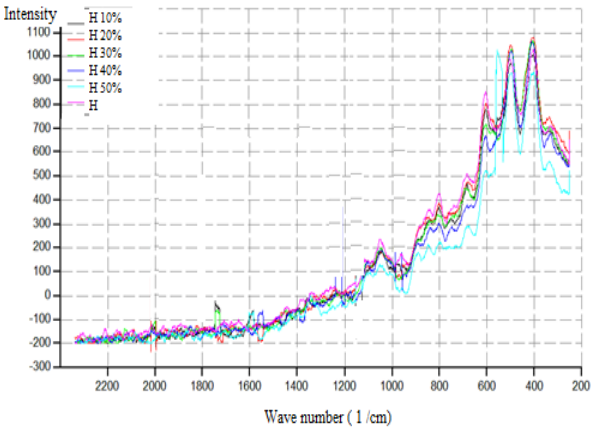


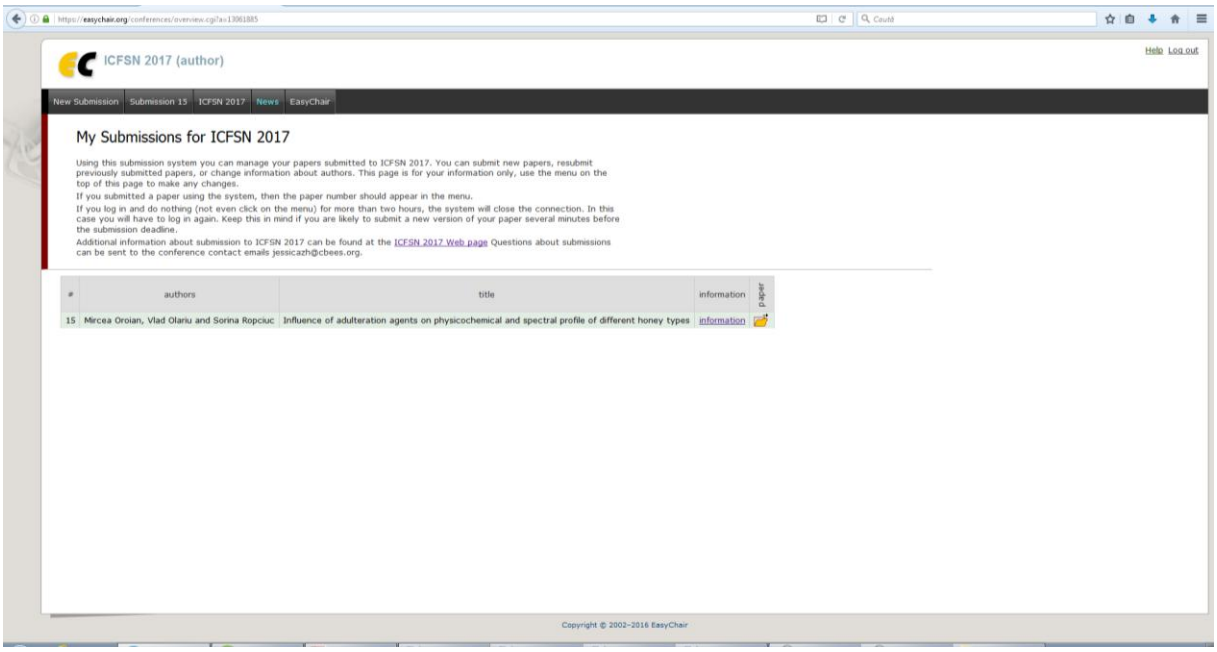
Fig.13. Raman spectal profile of polyfloral honey adulterated with inulin syrup (H - original honey, H10% - honey adulterated with 10%, H 20% - honey adulterated with 20%, H30% - honey adulterated with 30%, H40 % - honey adulterated with 40%, H 50% - honey adulterated with 50%)

The addition of inverted sugar, fructose, glucose, malt must and inulin into the honey leads to the reduction of skeleton vibrations and ring vibrations of the natural sugars presented into the honey. Adding inverted sugar into honey the Raman band from 800 to 1127 cm^{-1} have a higher intensity; this bands are attributed to the C-OH deformation vibrations.

The adulteration with glucose can be observed at 523 and 1368 cm^{-1} (skeleton vibrations and CH bend + OH bends). The fructose can be observed at 807 and 1074 cm^{-1}. The inulin addition into the honey reduces the skeleton and ring vibrations (400-600 cm^{-1}) of the authentic honeys. The addition of malt must into honey increases the intensity of the Raman band at 460 cm^{-1}, 918 cm^{-1} and 1127 cm^{-1}.

Dissemination results: it has been ICFSN 2017 (2017 4th International Conference on Food Security and Nutrition) which will be held in Prague, Czech Republic during March 13-15, 2017 the article Oroian, M., Olariu, V., Ropciuc, S., Influence of adulteration agents on physicochemical and spectral profile of different honey types

In this scientific report there are no many information because they will be presented in the article as soon as it is published.



5. Conclusions

The 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 complies the Codex Alimentarius regulations. The Raman spectra analysis has been proved to be an excellent tool (simple, rapid and non destructive method) for honey authentication; by the linear discriminant analysis (LDA) applied 83.33 % of the honey has been correctly cross validated. LDA of the physicochemical and texture parameters has classified correctly 92.0% of the honeys according to their botanical origin, using the cross validation, and 96.0% using the original group. In the LDA projection, the textural parameters (chewiness, hardness, cohesiveness, springiness) dominated the two functions. The multilayer perceptron network with 2 hidden layers classified correctly 94.8% of the cross validated samples of honeys using the physicochemical and antioxidant profile.

The botanical authentication of honeys using the electrochemical methods was 100% correctly in the case of gold, platinum and glass electrode the classification of honey according to their botanical origin is 100% correctly, while in the case of silver electrode the percentage of correct classification is 96.08% (polyfloral and sunflower honeys have some samples which were classified into other groups).

The identification of the honey adulterated with different agents (inverted sugar, glucose, fructose, hydrolysed syrups and malt must) can be identified using different Raman band specifics to the adulterating agent.

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