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PHYSICAL-CHEMICAL AND BIOCHEMICAL CHARACTERIZATION OF SELECTED ROMANIAN AND POLISH HONEYDEW HONEY

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ABSTRACT: Honey dew is collected by honey bees, which transform it into a strongly flavoured dark colour honey less sweet than floral honey. It is prized in many parts of Europe which often cause increased prices. It is quite common in a number of countries from Europe, including Romania and Poland. Our study aimed to realise a comparative study in terms of physical-chemical and biochemical characteristics of five honeydew honey samples from the western part of Romania and five honeydew honey samples from Podkarpackie regions of Poland. Physical-chemical routine or basic parameters, like ash, humidity, pH, acidity, electrical conductivity and biochemical parameters, like sugar profile, proline and protein, were analysed according to the Romanian Standard Analysis Methods and Harmonised methods of the IHC, or with specific methods.The results obtained show that all the tested samples were authentic honeydew honey, in respect to the legislative requirements.The tested Polish honeydew honey samples had significantly higher value for ash, water, proline, fructose, sucrose, fructose+glucose and for fructose/glucose, in comparison with tested Romanian honeydew honey samples.

KEYWORDS: honeydew, physical-chemical parameters, proline, protein, sugars.

INTRODUCTION

The honeydew is a clear and viscous, sometimes solid sweet substance which in some periods of the year is found on leaves, limbs or stalk plants. It can be of animal origin, when produced by insects, or vegetal, when secreted by leaves, buds or other parts of the plants (Mărghitas, 2002). From this source bees produce the honeydew honey.

In Europe beekeepers have long been quite familiar with honeydew. Across the entire European continent, honeydew is an important honey flow for beekeepers, especially in all the alpine areas (central Europe) (Pechhacker H., 2008). In many European countries (Germany, Switzerland, Austria, Slovenia, Greece, Turkey and others), honeydew honey is harvested in relatively high amounts, reaching very good prices. In other European countries the production levels are lower, rough estimates lie between 1 and 15 % of the total honey harvest. Honeydew honey is highly appreciated for its strong flavour and healthful properties.

Romania's honey market is continuously developing. The favourable geographical conditions, higher export requirements in the EU market and export price are major factors stimulating Romanian beekeeping (Pîrvuţoiu *et al*, 2011). The most abundant honeydew honey samples are to be found in the mountain area and also in the hills regions under the Carpathians chain (Mateescu C., and Antonescu C., 2008).

Poland honey market in the EU is dominated by domestic beekeepers offering traditional products, (http://www.cbi.eu. 2011). In Poland, within the habitat range of some coniferous species, especially of fir and spruce, the honeydew of those trees may be the main forage for bees (Szczęsna *et al*, 2008) .

Many studies on physicochemical properties of honeydew honey are reported in Romania (Mateescu C., and Antonescu C., 2008; Bobiş *et al*, 2008; Bonta *et al*, 2008) and in Poland (Szczęsna *et al*, 2008; Zielińska *et al*, 2014), but comparative studies between Romanian and Polish honeydew honey are sporadic.

Generally honeydew honeys have common physical-chemical characteristics like: high electrical conductivity, ash content, free acidity and pH, positive values of specific rotation, low values of fructose, glucose, fructose + glucose and higher rates of oligosaccharides, while organoleptic characteristics are more variable (Persano Oddo and Piro, 2004; Ruoff K., 2006).

Sugars profiles of different types of honey have been reported by many scientists using different chromatographic techniques (Cotte et al.2004, Sanz, Sanz and Martınez-Castro, 2004).

The content of amino acids and proteins is relatively small. Honey contains almost all physiologically important amino acids. The main amino acid is proline, a measure of honey ripeness (Von der Ohe *et al.*,1991). The proline content of normal honeys should be more than 200 mg/kg. Values below 180 mg/kg mean that the honey is probably adulterated by sugar addition (Bogdanov, 2011).

The aim of our study was to realise a physical-chemical and biochemical characterization of some honeydew honey from Romania and Poland, to compare the obtained values for those routine/basic parameters with the limit values imposed by EC Directive (Council Directive 2001/110/EC, 2001), and to compare them with each other for all studied parameters.

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MATERIAL AND METHODS

Samples

Five honeydew honey samples were obtained from western part of Romania and five honey samples from different Podkarpackie regions of Poland in 2012 and 2013 harvest year. Honey samples were heated in a water bath at temperature below 45°C, degrees until a fluid consistency.

Samples from Romania were purchased from beekeepers from Bihor County – R1, R3, R5 from 2012 and R2, R4 from 2013.

Samples from Poland were collected directly from beekeepers from Podkarpackie County. One sample from 2012 (P1) and four from 2013. Two of this last samples ware labelled as ecological honeydew (P2 and P3) and two usual honeydew (P4 and P5).

Physical-chemical and biochemical characteristics

Physical-chemical parameters, like ash, humidity, pH, acidity, electrical conductivity and biochemical parameters, like sugar profile, proline and protein, were analysed according to the Romanian Standard Analysis Methods (National Standard, 2009) and Harmonised methods of the IHC (Bogdanov, 2009), or with specific methods.

Humidity and total sugar - of the tested samples were determined with digital refractometer KRUSS model AR 2008.

Ash content -The honey is ashed at a temperature no higher than 600°C and the residue weighed.

pH – The sample is dissolved in water, and the pH was measured with HATCH SensyION 378 multiparameter meter.

Free acidity - by titration method (Bogdanov, 2009)

Electrical conductivity – Currently the measurement of electrical conductivity is the most useful quality parameter for the classification of honeys which can be determined by relatively inexpensive instrumentation. This has been confirmed by the data published by Persano Oddo and Piro, 2004. This parameter was measured with HATCH SensyION 378 multiparameter meter.

Sugar profile was determined using high-performance liquid chromatography (HPLC) separation. The chromatographic separation of sugars was achieved in an amine bonded phase column (Luna 5µ NH_2 100A), using acetonitrile (MERCK Germany) /water (80:20) as mobile phase, at a flow rate of 1,0 cm^3/min and refractive index detection, in a MERCK-HITACHI HPLC equipped with L-6200A Intelligent Pump, AS-4000 Intelligent Auto Sampler and D-7000 Chromatography Data Station Software. Fructose, glucose, sucrose (SIGMA-ALDRICH, USA), were used as standards.

Proline -The content of proline is defined as the colour developed with ninhydrin compared with a proline standard (SIGMA-ALDRICH, USA) and expressed as a proportion of the mass of honey in mg/kg. Proline and ninhydrin (REDOX, Romania) form a coloured complex. After adding 2-propanol (MERCK, Germany), the extinction of the sample solution and a reference solution at a 510 nm wavelength is determined (Bogdanov, 2009).

Proteine - To 0.1 ml solution of protein extract (honey sample 50% w/v), were added 5 ml of Coomassie Brilliant Blue 200 mg of Coomassie Brilliant Blue G-250 (MERCK, Germany) dissolved in 100 ml 95% ethanol (SCHARLAU, Germany) and, finally, 200 ml 85% phosphoric acid (MERCK, Germany) added. The resulting solution was diluted to a final volume of 2000 ml). The Coomassie Brilliant Blue forms a blue complex with the proteins. After 2 min of incubation, the absorbance was measured at 595 nm, against an albumin standard solution of bovine serum (10–100 mg/0.1 ml) in 0.15M NaCl (CHIMOPAR, Romania) Bradford method (Bradford, 1976) adapted for honey (Azeredo, 2003).

Statistical analysis

All the analytical measurements were performed in triplicate and are presented as mean ± SD. The means of the determinate parameters for Romanian - Bihor County and Polish - Podkarpackie County honeydew were compared on the one hand with limit values of EU directives and on the other hand by T-test for independent samples, between each other. Differences between means at 95% ($p<0.05$) confidence level were considered statistically significant.

RESULTS AND DISCUSSION

The physical-chemical and biochemical parameters were collected in table 1 and 2, for Romanian and Polish tested honeydew honey samples. Mean values and standard deviations separate for every 5 samples, and the mean values and standard deviations for the 5 samples are given.

Table 1.

Physical-chemical and biochemical parameters for tested Romanian honeydew honey

Parameters	R1	R2	R3	R4	R5	RO honey
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Ash %	0.726±0.02	0.86 ±0.032	0.85±0.07	0.826±0.065	0.87 ± 0.045	0.814± 0.057
El. Conduct. mS/cm	0.853± 0.045	0.953± 0.041	0.89±0.05	0.97±0.04	1.05±0.055	0.942±0.0769
Free acidity meq/kg	33.6±0.458	24.9±0.45	44.3±0.556	29.3±0.75	25.06±0.55	31.432±8.032
pH	3.5±0.1	2.8±0.057	4.3±0.1	3.0±0.1	2.52±0.064	3.224±0.709
Water %	19.0±0.611	18.3±0.888	18.6±0.585	18.6±0.462	18.2±0.305	18.54±0.313
Total sugar %	79.6±0.404	80.1±0.655	79.2±0.709	78.7±0.85	78.0±0.2	79.12±0.81
Fructose %	38.02±1.238	35.98±0.662	36.41±1.14	36.27±1.381	35.1±1.18	36.356±1.060
Glucose %	29.38±0.737	29.96±1.20	30.8±1.599	24.09±1.939	28.06±1.553	28.458±2.637
Sucrose %	1.44±0.055	1.29±0.036	1.26±0.145	1.26±0.073	1.5±0.03	1.35±0.112
Fru+Glc %	67.44±0.525	65.95±0.54	67.21±1.086	66.36±0.825	63.1±2.653	66.012±1.737
Fru/Glc	1.29±0.076	1.2±0.072	1.18±0.089	1.21±0.121	1.25±0.036	1.226±0.0439
Proline mg/kg	721.97±17.07	715.95±17.12	618.18±14.78	847.64±20.2	407.8±6.91	662.308±163.94
Protein mg/kg	676.23±84.01	787.88±63.18	834.12±54.34	518.34±69.7	487.3±13.06	660.774±155.58

Table 2.
Physical-chemical and biochemical parameters for tested Polish honeydew honey

Parameters	P1	P2	P3	P4	P5	Polish honey
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Ash %	0.93±0.045	1.02±0.045	0.95±0.065	1.04±0.073	1.04±0.096	0.996±0.052
El.Conduct. mS/cm	1.06±0.051	1.05±0.064	0.94±0.075	1.09±0.075	1.0±0.097	1.028±0.058
Freeacidity meq/kg	31.6±1.253	41.5±0.971	44.0±1.096	39.7±0.8	32.7±1.814	37.9±5.480
pH	2.9±0.1	3.9±0.15	4.0±0.1	3.96±0.065	3.33±0.208	3.618±0.484
Water %	18.8±0.737	19.9±0.152	20.3±0.4	19.3±0.404	19.1±0.416	19.48±0.609
Total sugar %	79.0±0.555	78.0±0.3	78.0±0.776	79.1±0.2	79.6±0.351	78.74±0.712
Fructose %	45.71±1.697	42.93±1.475	45.6±1.13	43.05±1.622	42.07±1.857	43.872±1.671
Glucose %	25.16±1.704	26.79±1.342	24.81±1.026	26.85±2.375	24.14±0.885	25.55±1.216
Sucrose %	1.97±0.08	2.01±0.085	1.96±0.118	1.85±0.097	2.09±0.121	1.976±0.080
Fru+Glc %	70.87±0.026	69.72±0.141	70.45±0.105	69.9±0.755	66.21±2.335	69.43±1.856
Fru/Glc	1.82±0.185	1.64±0.057	1.83±0.120	1.6±0.207	1.74±0.08	1.726±0.103
Proline mg/kg	1000.23±23.9	1070.92±25.6	976.17±23.33	985.94±23.56	868.3±28.57	980.312±72.80
Protein mg/kg	897.92±67.21	827.75±79.91	698.56±55.18	802.23±26.35	724.4±9.26	790.172±80.44

Table 3 contains the results of routine/basic physical-chemical and biochemical parameters and the legislative requirements in according with Council Directive 2001/110/EC, 2001. Mean values and confidence interval are given.

Table 3.
Physical-chemical routine/basic parameters in comparison with EC Directive limit

Parameters	EC Directive limit	Romanian honey			Polish honey		
		Mean	Confidence interval		Mean	Confidence interval	
			Min	Max		Min	Max
Ash %	≤1.2	0.814	0.7429	0.8851	0.996	0.9311	1.061
El. conduct mS/cm	≥0.8	0.9420	0.8465	1.0375	1.028	0.9549	1.1011
Free acidity meq/kg	≤50	31.432	21.458	41.406	37.9	31.0952	44.704
Water %	≤20	18.54	18.151	18.929	19.48	18.723	20.237
Proline mg/kg	≥180	662.3080	401.5	865.867	980.312	889.9131	1070.711
Sucrose %	≤ 5	1.35	1.2106	1.4894	1.9760	1.8679	2.0841
Fruct + Gluc %	≥ 45	66.012	63.8543	68.1697	69.43	67.1248	71.7352
TotSug %	≤ 80	79.12	78.114	80.126	78.74	77.855	79.625

Table 4 shows the values for "p" obtained after applying T-test for independent samples, and the Statistic significance

Table 4.
Case study -Comparative study of some physical-chemical and biochemical parameters for some Romanian (Bihor-county) and Polish (Podkarpackie County) honeydew honey

Parameters	Romanian honey	Polish honey	p	Statistic significance
	Mean ± SD	Mean ± SD		
Ash %	0.814± 0.057	0.996±0.05225	0.01	**
El. Conduct. mS/cm	0.942±0.07694	1.028±0.05891	0.082	ns
Free acidity meq/kg	31.432±8.03275	37.9±5.48042	0.175	ns
pH	3.224±0.70928	3.618±0.48499	0.313	ns
Water %	18.54±0.313	19.48±0.6099	0.015	*
Total sugar %	79.12±0.8106	78.74±0.7127	0.454	ns
Fructose %	36.356±1.0604	43.872±1.6714	≤0.001	***
Glucose %	28.458±2.6376	25.55±1.216	0.056	ns
Sucrose %	1.35±0.11225	1.976±0.0806	≤0.001	***
Fru+Glc %	66.012±1.73775	69.43±1.8565	0.017	*
Fru/Glc	1.226±0.04393	1.726±0.1038	≤0.001	***
Proline mg/kg	662.308±163.9409	980.312±72.804	0.004	**
Protein mg/kg	660.774±155.5830	790.172±80.445	0.137	ns

p ≤ 0.05 * - significant; p ≤ 0.01 ** distinct significant; p ≤ 0.001*** very significant; p≥0.05 ns – non significant

For all investigated honey samples it was found that the results complied with international regulation (Council Directive 2001/110/EC, 2001).

Ash content and *Electrical conductivity* represent very important characteristics for honeydew honey. In our study ash content has higher values than <0.8% but not exceeded 1.2%. Polish samples had significantly higher values, between 0.931 and 1.061% than in case of Romanian samples, range between 0.743-0.885%. Values in the same ranges were found by Persano Oddo *et al,* 1995, in case of Italian honeydew honey (0.47 -1.09%).

Values for electrical conductivity, pH and free acidity has non significant differences in case of Romanian and Polish honeydew honey.

Electrical conductivity of tested honey samples ranged between 0,8465mS/cm, the minim value, and 1,1011 mS/cm the maximum value. Those values were higher than the minimum level 0.8 mS/cm limit from EC Directive. Similar results were mentioned for honeydew honey from Switzerland 0.71-1.09mS/cm (Ruoff, 2006), for Polish honeydew honey: 0.74 - 1.30mS/cm (Piazza *and* Odo, 2004) and between 0.324-0.927 mS/cm for Romanian honeydew honey (Mărghitaş *et al*, 2008).

pH-values were within 2.4 -4.4 for Romanian samples and within 2.8 and 4.0 for Polish samples, lower than values obtained by Persano Oddo *et al*, 1995, closer to values found by Ruoff, 2006, and similar with those found by (Mărghitaş *et al*, 2008).

The minim level of *Free acidity* was 21.458 meq/kg, for Romanian honey and the maximum level was 44.704 meq/kg, for Polish honey, this values being lowest than 50meq/kg, imposed by EC Directives, and in the same range as those found by Pavelková *et al.* 2013; Primorac *et al.*, 2009.

Water contents of studied honey samples ranged from 18.15-18.92% for Romanian honeydew honey and from 18.72-20.23%, which were lower or very close to the imposed limit of 20%. Similar result were obtained by Piazza *and* Odo, 2004 (18.5-21.9%) and Zielińska *et al*, 2014 (18.4-20.7%). The water content of honey can naturally be as low as 13.6% and as high as 23% depending on the source of the honey, climatic conditions and other factors (Bogdanov *and* Martin, 2002). There was statistically significant difference between water content of Romanian and Polish honeydew honey.

Proline content was higher than 180mg/kg in all tested samples, lower value was 401.5mg/kg for Romanian honey and higher value was for Polish 1070.711mg/kg. In sugar adulterated honeys proline content are lowered. This value can be higher for certain honeys. The proline content depends on the honey type (Bogdanov *and* Martin, 2002). Those values comply to those found by Ruoff, 2006; Primorac *et al.*, 2009. The values found for Polish honeydew honey were distinct significantly higher than those found in Romanian samples.

Protein content is no limited value about EC Directive. Protein content range between 487.3-834.12 mg/kg for Romanian samples and 698.56-897.92 mg/kg for Polish honeydew honey. The difference between the values obtained for the tested samples from Romania and Poland are statistically non significant. Azeredo *et al.*, 2003, realised a proteic classification of different floral origin honey commercialized inBrasil. According to this classification the samples with more than 1000mg/kg was considered *High content*; between 500-1000mg/kg – *Medium content*; less than 500 mg/kg *Low content*. Thus, the honeydew honey samples from Romania and Poland has medium content protein.

Sucrose, Fructose, Fructose and glucose ratio, were biochemical parameters, very significantly higher for Polish honeydew honey in comparison with Romanian honeydew honey samples. *Fructose and Glucose sum* was significantly higher for Polish honey in comparison with Romanian honeydew honey, while *Total sugar* values present non significant difference between studied samples.

Biochemical parameters, representing the sugar profile, limited by EC Directives were *Sucrose, Fructose and Glucose sum* and *Total sugar*. The values for sucrose range between 1.21-1.48% for Romanian samples, respectively 1.86-2.08% for Polish samples, those values were lower than 5%, imposed by EC. Fructose and glucose sum range between 63.85-68.16% for Romanian honey and 67.12-71.73% for Polish honey, all those values were higher than 45% imposed by EC.

Total sugar values were lower than 80% imposed by legislative requirements, respectively, 79.12% representing the mean value for Romanian samples and 78.74%, the mean value for Polish sample. The difference between fructose, glucose and sucrose sum to the total sugar is represented by oligosaccharides (maltose, melesitose, turanose, raffinose, etc), parameters that we could not determined due to lack of standards. Similar with our results, Polish honeydew honey (Szczęsna *et al.*, 2008) stands out by considerable low sucrose content, exceptionally only higher than 2g/100g and considerable high content of sum of glucose and fructose, always above 55g/100g. Zielińska *et al*, 2014, found for Polish honeydew fructose and glucose sum, values in the same area, 67.8-72.4%.

Fructose and *Glucose* range between 35.28 -37.4% and 25.82-31.08% for tested Romanian honeydew honey, respectively 42.20-45.54% and 24.34-26.76% for Polish honeydew honey. Those values comply with those found by Zielińska *et al*, 2014; Bonta *et al.*, 2008.

Fructose and Glucose ratio mean value was 1.22 for Romanian honey and 1.72 for Polish honey. Our results comply with those found by Persano Oddo *et al*, 1995; Ruoff, 2006; Primorac *et al.*, 2009. Due to its high fructose content, higher than glucose, honeydew honey crystallizes slowly.

CONCLUSION

The results obtained for the basic physical-chemical and biochemical parameters analysed in our study show that all the tested honey samples from Romania and Poland were authentic honeydew honey, in respect to the legislative requirements. Polish honeydew honey samples analysed in our research have a significantly higher content of ash, water, proline, fructose, sucrose, fructose and glucose sum and fructose and glucose ration, than in case of Romanian samples. Very significant differences were found for parameters from sugar profile. Further studies are needed on a larger number of samples, both for Romanian and Polish honeydew honey, so that the results can be generalized. It is also necessary to extend the range of analysis in order to have a guaranteed authentication and to be able to specify the type of forest where honeydew was collected.

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