Influence of Adulteration Agents on Physico-Chemical and Spectral Profile of Different Honey Types

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***Abstract*—The aim of this study is to evaluate the influence of some adulteration agents (fructose and hydrolysed inulin syrup) on physico-chemical parameters (pH, electrical conductivity, water activity and CIEL*a*b* parameters) and Raman spectra of some honey types (acacia, tilia and polyfloral) from the North East part of Romania. The physico-chemical parameters (pH, water activity, electrical conductivity and color) of the honey adulterated varied depending on the degree of substitution of honey by adulteration agent. Unlike physico-chemical analyses and color analysis, which determine only the degree of falsification of honey, Raman analysis enables identification of falsification agent based on specific vibrational bands recorded.**

***Index Terms*–honey, physico-chemical parameters, spectral profile, adulteration**

I. Introduction

Honey is defined as the natural sweet substance produced by bees [1]. Food fraud, in particular, adulteration, is a practice which is in steady progress. Adulteration consists in adding external chemicals in food that contain naturally similar substances. Adulterated honey appeared on the world market in the 1970s, when corn syrup and high fructose were introduced in honey. Honey has a high potential to be deliberately adulterated because it has a high cost, being produced in large fluctuations of weather and its harvesting is particularly sensitive. Although honey adulteration is not harmful to health, it affects adversely the market growth by influencing consumer's confidence. Lately, certified quality of honey has become increasingly important for consumers, producers and regulators so that the European Commission encourages the use of analytical methods and modern classics to determine the authenticity of honey [2].

Honey is subjected to cheap adulteration with sweeteners such as aspartame, saccharin, cyclamates, molasses, corn syrups, high fructose corn syrup, invert syrup and inulin syrup with high fructose. This form of adulteration is made use of in order to correct the sweet taste after water addition and to maximize the profit. Honey adulterated by sugar or inverted sugar cannot be easily detected by direct analysis of sugars, because its components are the main components of honey and so the altered product may also have similar physical properties as the natural honey. Depending on their origin, added sugars are divided into two types: C3 and C4. Sugars and sucrose are of C3 type, while the sugar cane sugar and corn starch hydrolysis products are of C4. There have been proposed various methods for detecting counterfeit honey sugar, but most of them do not have utility in practice. Corn and sugar cane metabolism is on the Hatch - Slack or C4 pathway. As a result, sugar syrups derived from cereals shall report a ^{13}C / ^{12}C, expressed as the value of G, which is different from the honey value, where the sugar is derived via a C_3 pathway. The G value for C_4 syrups C4 value is lost to 10 ‰, while the average value of honey is 25.4‰. The original method of measuring the ratio 13C / 12C^{13}C / ^{12}C has been improved by the introduction of the intern protein test. The method currently used enables the detection of honey with 7-10% syrups from sugar cane or corn. In addition to measuring the ratio ^{13}C / ^{12}C, the NMR method with deuterium can be decisive in achieving greater certainty in the interpretation of measurement ratio 13C / 12C [2].

The aim of this study is to investigate the possibility to discriminate the adulterated honey based on the physico-chemical parameters (pH, electrical conductivity, water activity and CIEL*a*b* parameters) and Raman spectra.

II. Materials and Methods

A. Materials

Honey samples (tilia, acacia and polyfloral) have been purchased from local beekeepers of Suceava County.

The samples have been liquefied at 50 °C prior the decrystallization process and normalised at 60 °Brix by water to reduce the spectral interference normally occurring in sugar concentration. The adulteration agents (fructose and hydrolysed inulin syrup) were prepared at 60 °Brix, too. The honeys were adulterated in different concentration as follows: 10%, 20%, 30%, 40% and 50%, respectively.

B. Physico-Chemical Properties Determination

Moisture content, pH, refraction index, Brix concentration and electrical conductivity have been determined using the Harmonised methods of the international honey commission [3]. Water activity was

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measured using a water activity meter AquaLab Lite (Decagon, USA).

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

$$C^* = (a^*)^{0.5} + (b^*)^{0.5} \quad (1)$$

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

$$YI = 142.86\ b^*/L^* \quad (3)$$

C. Raman Spectra Acquistion

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 50 ℃ to dissolve any crystals, and kept in flasks at 30 ℃ to remove air bubbles that could interfere with spectra studies.

D. Statistical Analysis

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

III. Results and Discussion

A. Physico-Chemical Parameters

The physico-chemical parameters of the analysed honeys (original and adulterated ones) are shown in the Table I, Table II and Table III.

TABLE I. Physico-Chemical Parameters of Acacia Honey Adulterated by Fructose and Hydrolyzed Inulin Syrup

Sample	**Proportion**	**CIE L*a*b***					**pH**	a_w	**Electrical conductivity µS/cm**
		L*	a*	b*	c*	H			
1	0%	36.13	0.20	7.90	1.33	-85.53	4.11	0.801	59.40
Fructose syrup									
2	10%	38.80	1.33	-7.63	7.56	-80.20	4.16	0.804	41.90
3	20%	39.50	1.26	-6.43	6.66	-79.00	4.22	0.812	36.00
4	30%	40.06	1.90	-0.53	1.93	-9.60	4.28	0.812	32.40
5	40%	40.33	1.36	-7.16	9.70	-81.33	4.35	0.813	27.80
6	50%	52.53	0.90	-4.13	4.30	-77.86	4.42	0.816	24.30
Hydrolyzed inulin syrup									
7	10%	36.53	1.03	-8.93	9.00	-85.60	2.02	0.801	286
8	20%	36.60	0.96	-7.06	6.56	-85.26	< 2	0.801	871
9	30%	36.63	1.10	-10.56	10.83	-83.83	< 2	0.807	1352
10	40%	38.46	1.00	-9.46	9.43	-84.23	< 2	0.808	1921
11	50%	41.23	1.03	-5.23	5.40	-78.71	< 2	0.823	2710

TABLE II. Physico-Chemical Parameters of Tilia Honey Adulterated by Fructose and Hydrolyzed Inulin Syrup

S	**Proportion**	**CIE L*a*b***					**pH**	a_w	**Electrical conductivity µS/cm**
		L*	a*	b*	c*	H			
1	0%	48.96	0.10	1.80	1.86	-87.70	3.89	0.764	113.50
Fructose syrup									
2	10%	52.10	1.20	1.06	1.56	39.06	3.90	0.788	97.60
3	20%	52.13	2.10	0.73	2.20	19.36	3.91	0.794	83.20
4	30%	53.60	1.76	3.73	4.30	63.03	3.94	0.795	79.70
5	40%	54.23	0.10	0.66	0.73	79.83	3.95	0.803	72.10
6	50%	55.76	1.86	4.50	4.80	67.80	4.04	0.803	60.70
Hydrolyzed inulin syrup									
7	10%	50.66	-0.10	-2.56	2.56	-92.73	3.18	0.801	164
8	20%	52.70	0.56	0.50	0.70	42.20	2.02	0.804	465
9	30%	54.30	1.66	3.40	3.76	64.70	< 2	0.805	1074
10	40%	54.23	1.46	3.15	3.40	65.50	< 2	0.809	1699
11	50%	56.16	2.50	5.55	6.00	65.60	< 2	0.813	2920

TABLE III. PHYSICO-CHEMICAL PARAMETERS OF POLYFLORAL HONEY ADULTERATED BY FRUCTOSE AND HYDROLYZED INULIN SYRUP

Sample	Proportion	CIAL*a*b*					pH	a_w	Electrical conductivity µS/cm
		L*	a*	b*	c*	H			
1	0%	37.90	0.30	3.40	3.40	85.10	3.72	0.806	107.10
Fructose syrup									
2	10%	38.73	2.16	8.80	9.10	76.26	3.82	0.806	91.50
3	20%	41.70	1.43	8.03	8.16	80.16	3.83	0.807	79.20
4	30%	42.30	1.90	9.90	10.10	79.06	3.84	0.810	67.90
5	40%	42.33	1.03	7.53	7.52	82.10	3.86	0.810	57.30
6	50%	43.30	0.26	3.33	3.33	85.56	3.88	0.811	46.60
Hydrolyzed inulin syrup									
7	10%	38.73	1.33	3.66	3.90	69.43	2.82	0.808	178
8	20%	38.96	1.40	3.36	3.63	67.46	2.01	0.810	501
9	30%	39.83	1.93	4.76	4.96	69.36	< 2	0.810	1045
10	40%	40.16	3.46	6.30	7.26	61.63	< 2	0.814	1565
11	50%	40.80	1.36	4.46	4.66	73.26	< 2	0.816	2250

B. Water Activity

Water is the second component as importance for honey. Water activity is a proportional unit of the free water in food products; in the case of lower water activity than 0.60, the food product involved can be considered microbiologically stable. At present, water activity is considered a better parameter than the moisture content for honey. Accordingly to Chirife, Zamora & Motoo [4] there is a linear correlation between the moisture content and water activity. Thus, for the honey samples adulterated by fructose and hydrolyzed inulin syrup, an increase in water activity proportionally with the proportion of the adulteration agent can be observed.

The water activity was of 0.801 for acacia honey, 0.764 for the tilia honey and 0.806 for the polyfloral honey, respectively. There can be observed in the tables 1-3 that water activity is increasing together with the proportion of the adulteration agent. Ribeiro *et al*. [5] observed similar values for the honey adulterated by corn syrup which is reach in fructose.

C. pH

The acidic content of honey is relatively low, but it is important for taste, stability and microbiological resistance. This parameter is important for the honey extraction and keeping, influencing the honey texture, stability and term of validity (Gomes *et al.*, 2010) [6]. A low pH ensures the microorganisms inhibition and prevents their development.

Honey acidity is given by the presence of organic acids, as gluconic acid and other anorganic acids, as chloride. The values of pH are lower than 4.57 (Table I-Table III). The values are in agreement with those reported by Cimpoiu *et al*. [7], Oroian [8] and Oroian *et al*. [9].

The adulteration of honey with fructose increased the pH values of the samples, while the adulteration of honey with hydrolyzed inulin syrup decreased the pH values of the samples. The decreasing of pH values of the samples adulterated with hydrolyzed inulin syrup is as the result of the acidic nature of the syrup (the syrup is hydrolyzed with HCl at pH between 1 - 2). Ribeiro *et al*. [5] observed the same evolution of the pH.

D. Electrical Conductivity

The electrical conductivity is influenced by the botanical origin, and is depending on the ash content, organic acids, proteins, some sugars and poliols. The values of the electrical conductivity ranged between 24.30 – 2920 µS/cm (tab. 1 -3). The samples adulterated by fructose have lower electrical conductivity than the original ones because fructose decreases conductivity, while the samples adulterated by hydrolyzed inulin syrup have higher electrical conductivity than the original ones because of the acidic nature of the syrup.

E. Colour

Honey colour is influenced by the phenolic compounds, pollen and minerals [11]. The acacia honey had the highest colour purity (Table I), the same finding was observed by the Kadar *et al*. (2010) [10] for acacia honey from Romania and Spain. The polyfloral honey presented the highest yellow components (high values of b*) (tab. 3), and by the hue angle. The adulteration of honey by hydrolyzed inulin syrup is decreasing the values of b*. The highest luminosity was observed in the case of tilia honey, and is increasing with the adulteration percentage (Table II). The increasing of the adulteration agent is increasing the values of chroma (c*). The luminosity is influenced by the addition of fructose.

F. Raman Spectra

Fig. 1 - Fig. 4 show the spectra of a sample adulterated by fructose and one adulterated by hydrolyzed inulin syrup. The principal bands are in the wave numbers 400 - 640 cm^{-1} and 1200-1430 cm^{-1}.

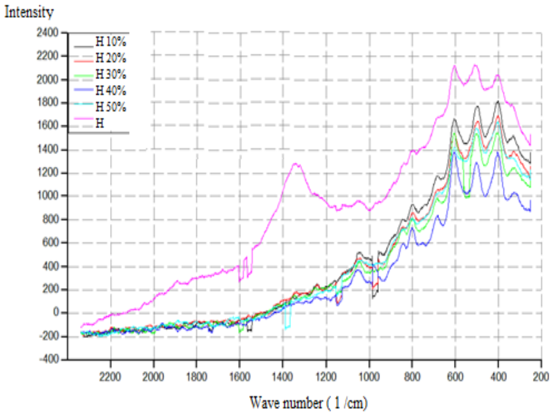

Figure 1. Raman spectal profile of tilia honey adulterated by fructose (H - original honey, H10% - honey adulterated by 10%, H 20% - honey adulterated by 20%, H30% - honey adulterated by 30%, H40 % - honey adulterated by 40%, H 50% - honey adulterated by 50%)

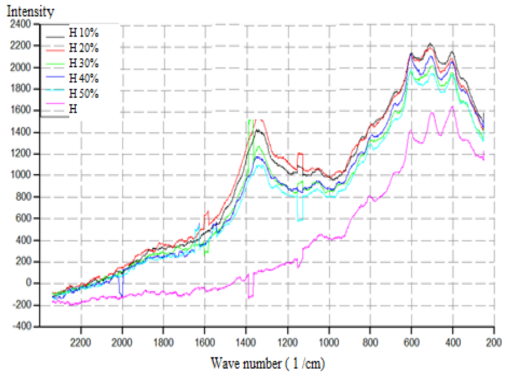

Figure 2. Raman spectal profile of tilia honey adulterated by inulin syrup (H - original honey, H10% - honey adulterated by 10%, H 20% - honey adulterated by 20%, H30% - honey adulterated by 30%, H40 % - honey adulterated by 40%, H 50% - honey adulterated by 50%)

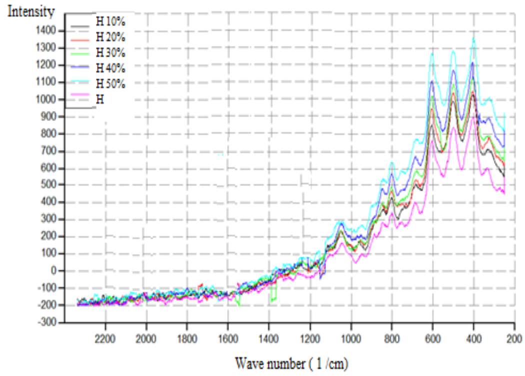

Figure 3. Raman spectal profile of polyfloral honey adulterated by fructose (H - original honey, H10% - honey adulterated by 10%, H 20% - honey adulterated by 20%, H30% - honey adulterated by 30%, H40 % - honey adulterated by 40%, H 50% - honey adulterated by 50%)

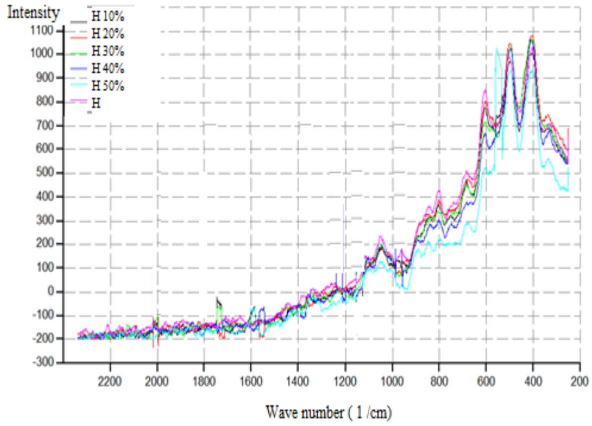

Figure 4. Raman spectal profile of polyfloral honey adulterated by inulin syrup (H - original honey, H10% - honey adulterated by 10%, H 20% - honey adulterated by 20%, H30% - honey adulterated by 30%, H40 % - honey adulterated by 40%, H 50% - honey adulterated by 50%)

TABLE IV. VIBRATIONS SPECIFIC TO EACH ADULTERATION AGENT [12]

Raman band	Vibration	Adulteration agent	
		Fructose	Hydrolyzed inulin syrup
430 cm-1	Skeleton vibration	+ +	+ +
460 cm-1	Skeleton vibration	+	+
523 cm-1	Skeleton vibration	+	+
631 cm-1	Deformation vibrations of the ring	+ +	+ +
709 cm-1	Skeleton vibration	+ +	–
781 cm-1	Ring vibration	+	+ +
825 cm-1	Stretch vibration C-OH	+ +	+
870 cm-1	C-O-C cyclic in alkyl ethers	+ +	–
918 cm-1	Bending vibration CH, COH	+	–
983 cm-1	Ring stretching vibration	+	–
1074 cm-1	C-O-C cyclic in alkyl ethers	+ +	+ +
1127 cm-1	C-OH deformation vibrations	–	+
1267 cm-1	C-O-C cyclic in alkyl ethers	+ +	+ +
1460 cm-1	CH2 bending vibrations	+ +	+ +
1640 cm-1	O-H from H2O bending vibrations	+	+
2893 cm-1	CH bending vibrations	–	+
2940 cm-1	CH2 bending vibrations	+	+

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

The prominent peaks are specific to carbohydrates, this fact being justified by the high content of these compounds in honey. Even proteins, pollens and other components given by the floral origin of honey present vibration bands, but they are covered by the vibrations of the major components [12]. By using fructose or hydrolysed inulin syrups one can observe similar spectra to the authentic honey, whose principal bands can be attributed to carbohydrates. The possible vibrations of fructose and inulin syrup are presented in Table IV.

The addition of fructose and hydrolyzed inulin syrup in acacia honey is reducing the skeleton vibrations and ring vibrations of carbohydrates present naturally in honey. Moreover, the honey substitution is intensifying the vibrations specific to the carbohydrates presented in the adulteration agents. The addition of fructose syrup to honey is highlighted by the wave numbers 807 cm^{-1} and 1074 cm^{-1}. The adulteration of honey by hydrolyzed inulin syrup is reducing the skeleton and ring vibrations intensities (400 – 600 cm^{-1}) specific to the authentic honey.

IV. Conclusions

The physico-chemical parameters (pH, water activity, electrical conductivity and color) of the adulterated honey varied depending on the degree of substitution of honey by adulteration agent. The honey adulteration can be evidenced by CIEL*a*b* method, based on brightness and color saturation. The parameters are reduced by the increase in the syrup added. Differences between the original and adulterated samples were observed by the Raman analysis. Since the Raman method is simple and effective, without requiring preprocessing, it is suitable for onsite testing to field applications. Also, the addition of fructose and inulin reduces the vibration intensity of ring and skeleton of carbohydrates naturally present in honey, seen by analyzing the spectra obtained by Raman spectroscopy. Unlike physico-chemical analyses and color analysis, which can determine only the falsification degree of honey, Raman analysis enables identification of falsification agent based on specific vibration bands recorded.

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