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 13C / 12C, expressed as the value of G, which is different from the honey value, where the sugar is derived via a C3 pathway. The G value for C4 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 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 13C / 12C, 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 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
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:

$$C^* = (a^*)^{0.5} + (b^*)^{0.5}$$  
$$h^* = \tan^{-1}(b^*/a^*)$$  
$$YI = 142.86 \cdot b^*/L^*$$  

$$C = \sum_{i=1}^{c} P_i \cdot f_i$$

$$h = \sum_{i=1}^{c} P_i \cdot f_i$$

$$YI = \frac{\sum_{i=1}^{c} P_i \cdot f_i}{\sum_{i=1}^{c} P_i}$$

C. Raman Spectra Acquisition

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 spectrometer (EZM-A2-785L, B&W TEK Inc. USA) 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°C to dissolve any crystals, and kept in flasks at 30°C 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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Proportion</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>c*</th>
<th>H</th>
<th>pH</th>
<th>$a_*$</th>
<th>Electrical conductivity μS/cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0%</td>
<td>36.13</td>
<td>1.20</td>
<td>7.90</td>
<td>1.33</td>
<td>-85.53</td>
<td>4.11</td>
<td>0.801</td>
<td>59.40</td>
</tr>
<tr>
<td>2</td>
<td>10%</td>
<td>38.80</td>
<td>1.33</td>
<td>-7.63</td>
<td>7.56</td>
<td>-80.20</td>
<td>4.16</td>
<td>0.804</td>
<td>41.90</td>
</tr>
<tr>
<td>3</td>
<td>20%</td>
<td>39.50</td>
<td>1.26</td>
<td>-6.43</td>
<td>6.66</td>
<td>-79.00</td>
<td>4.22</td>
<td>0.812</td>
<td>36.00</td>
</tr>
<tr>
<td>4</td>
<td>30%</td>
<td>40.06</td>
<td>1.90</td>
<td>-0.53</td>
<td>1.93</td>
<td>-9.60</td>
<td>4.28</td>
<td>0.812</td>
<td>32.40</td>
</tr>
<tr>
<td>5</td>
<td>40%</td>
<td>40.33</td>
<td>1.36</td>
<td>-7.16</td>
<td>9.70</td>
<td>-81.30</td>
<td>4.35</td>
<td>0.813</td>
<td>27.80</td>
</tr>
<tr>
<td>6</td>
<td>50%</td>
<td>52.53</td>
<td>0.90</td>
<td>-4.13</td>
<td>4.30</td>
<td>-77.86</td>
<td>4.42</td>
<td>0.816</td>
<td>24.30</td>
</tr>
<tr>
<td>7</td>
<td>10%</td>
<td>36.53</td>
<td>1.03</td>
<td>-8.93</td>
<td>9.00</td>
<td>-85.60</td>
<td>2.02</td>
<td>0.801</td>
<td>286</td>
</tr>
<tr>
<td>8</td>
<td>20%</td>
<td>36.60</td>
<td>0.96</td>
<td>-7.06</td>
<td>6.56</td>
<td>-85.26</td>
<td>&lt; 2</td>
<td>0.801</td>
<td>871</td>
</tr>
<tr>
<td>9</td>
<td>30%</td>
<td>36.63</td>
<td>1.10</td>
<td>-10.56</td>
<td>10.83</td>
<td>-83.83</td>
<td>&lt; 2</td>
<td>0.807</td>
<td>1352</td>
</tr>
<tr>
<td>10</td>
<td>40%</td>
<td>38.46</td>
<td>1.00</td>
<td>-9.46</td>
<td>9.43</td>
<td>-84.23</td>
<td>&lt; 2</td>
<td>0.808</td>
<td>1921</td>
</tr>
<tr>
<td>11</td>
<td>50%</td>
<td>41.23</td>
<td>1.03</td>
<td>-5.23</td>
<td>5.40</td>
<td>-78.71</td>
<td>&lt; 2</td>
<td>0.823</td>
<td>2710</td>
</tr>
</tbody>
</table>

### TABLE II. PHYSICO-CHEMICAL PARAMETERS OF TILIA HONEY ADULTERATED BY FRUCTOSE AND HYDROLYZED INULIN SYRUP

<table>
<thead>
<tr>
<th>S</th>
<th>Proportion</th>
<th>CIE L<em>a</em>b*</th>
<th>pH</th>
<th>$a_*$</th>
<th>Electrical conductivity μS/cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0%</td>
<td>48.96</td>
<td>0.10</td>
<td>1.80</td>
<td>1.86</td>
</tr>
<tr>
<td>2</td>
<td>10%</td>
<td>52.10</td>
<td>1.20</td>
<td>1.06</td>
<td>1.56</td>
</tr>
<tr>
<td>3</td>
<td>20%</td>
<td>52.13</td>
<td>2.10</td>
<td>0.73</td>
<td>2.20</td>
</tr>
<tr>
<td>4</td>
<td>30%</td>
<td>53.60</td>
<td>1.76</td>
<td>3.73</td>
<td>4.30</td>
</tr>
<tr>
<td>5</td>
<td>40%</td>
<td>54.23</td>
<td>0.10</td>
<td>0.66</td>
<td>0.73</td>
</tr>
<tr>
<td>6</td>
<td>50%</td>
<td>55.76</td>
<td>1.86</td>
<td>4.50</td>
<td>4.80</td>
</tr>
</tbody>
</table>

Fructose syrup

<table>
<thead>
<tr>
<th>S</th>
<th>Proportion</th>
<th>CIE L<em>a</em>b*</th>
<th>pH</th>
<th>$a_*$</th>
<th>Electrical conductivity μS/cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0%</td>
<td>50.66</td>
<td>-0.10</td>
<td>-2.56</td>
<td>2.56</td>
</tr>
<tr>
<td>2</td>
<td>10%</td>
<td>52.70</td>
<td>0.56</td>
<td>0.50</td>
<td>0.70</td>
</tr>
<tr>
<td>3</td>
<td>20%</td>
<td>54.30</td>
<td>1.66</td>
<td>3.40</td>
<td>3.76</td>
</tr>
<tr>
<td>4</td>
<td>30%</td>
<td>54.23</td>
<td>1.46</td>
<td>3.15</td>
<td>3.40</td>
</tr>
<tr>
<td>5</td>
<td>40%</td>
<td>56.16</td>
<td>2.50</td>
<td>5.55</td>
<td>6.00</td>
</tr>
</tbody>
</table>
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\(^{-1}\).
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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Mircea Oroian – Prof, PhD eng., Dean of the Faculty of Food Engineering, Stefan cel Mare University of Suceava. Is one of the most prolific young scientists in Food Science & Technology area from Romania. He published more than 20 ISI articles into highly quoted journals from the world. His main expertise areas are: Food Rheology, Food authentication and adulteration detection, Food Chemistry. Mircea Oroian is project manager of 4 research projects and is member of 5 research projects into Food Science & Technology area.

Vlad Olariu – Master Degree in Food Science, Faculty of Food Engineering, Stefan cel Mare University of Suceava. Vlad Olariu was one of the best students of the Faculty of Food Engineering with very good skills into spectrometry and physicochemical analysis of honey and in the same time with statistical analysis.

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