Alternative Analytical Approaches For Detecting Adulteration Of Honey

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Outlines of presentation

1. Introduction
2. Current methodologies used to detect honey adulteration
3. Chemometric applications in honey authentication
4. Objectives
5. Experimental methods / Instrumental analytical techniques
6. Results and discussions
7. Conclusions
Introduction

- Bee products are natural product of high quality and medicinal properties

- HONEY has been considered to have therapeutic properties since ancient times and the factors responsible for such activity are bioactive compounds

- There are many types of honey: acacia, sunflower, linden, rape, polyfloral and honeydew
Honey adulteration
Economically Motivated Adulteration

Intentional substitution or addition of a substance for the purpose of increasing the apparent value of the product or reducing the cost of its production, i.e., for economic gain

The Different Forms of Honey Frauds

- **Addition of sugar syrups**: Honey can be easily directly or indirectly adulterated with inexpensive sugar syrups (from C3 or C4 plants), thus negatively affecting its quality and composition
- **False declaration of botanical or geographical origin**
- **Ultrafiltration of honey**
- **Unripe honey**

**Top 10 products that are most at risk of** food fraud

1. Olive oil
2. Fish
3. Organic foods
4. Milk
5. Grains

6. **Honey and maple syrup**
7. Coffee and tea
8. Spices (such as saffron and chili powder)
9. Wine
10. Certain fruit juices

Evaluation of honey quality is a topical and a significant problem of the food industry

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Direct or indirect adulteration of honey with sugar syrups represents a serious problem which affects its therapeutic effect through the modification of the biologically active compounds content.

Differences in carbon isotope signature in plants

C₃ plants: $\delta^{13}C = -26\%$ (Calvin Benson)

C₄ plants: $\delta^{13}C = -12\%$ (Hatch-Slack)

Range of $\delta^{13}C$ values

-22 to -30 \%

-8 to -13 \%

Feeding bees with sugar syrup during main nectar flow period
# Current methodologies used to detect honey adulteration

<table>
<thead>
<tr>
<th>Direct adulteration</th>
<th>Indirect adulteration</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Starch syrups</strong></td>
<td><strong>Starch or inverted syrups feed to bees</strong></td>
</tr>
<tr>
<td>High-fructose corn syrup (HFCS 42, 55, 90)</td>
<td>Honey with high level of indirect sugar</td>
</tr>
<tr>
<td>Corn syrup</td>
<td></td>
</tr>
<tr>
<td>Rice syrup (42, 55, 90)</td>
<td></td>
</tr>
<tr>
<td>Inverted syrups</td>
<td></td>
</tr>
<tr>
<td>Inverted syrup from sugar cane/sugar beet</td>
<td></td>
</tr>
<tr>
<td>Low quality honey added to high price honey</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Markers</th>
<th>Detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>oligosaccharides, polysaccharides, difructose anhydrides (DFAs)</td>
<td>HPAEC-PAD (down to 1% level)</td>
</tr>
<tr>
<td>δ¹³C isotopic signature</td>
<td>UHPLC/Q-TOF-MS (&gt; 10% level)</td>
</tr>
<tr>
<td></td>
<td>SCIRA (&gt; 7% level)</td>
</tr>
<tr>
<td>2-acetylfuran-3-glucopyranoside (AFGP)</td>
<td>HPLC-DAD (&gt; 10% level)</td>
</tr>
<tr>
<td>difructose anhydrides (DFAs) inulotriose</td>
<td>UHPLC/Q-TOF-MS (&gt; 10% level)</td>
</tr>
<tr>
<td>δ¹³C isotopic signature</td>
<td>GC-MS (&gt; 5% level)</td>
</tr>
<tr>
<td></td>
<td>SCIRA(&gt; 7% level), SNIF-NMR (&gt; 20% level)</td>
</tr>
<tr>
<td>honey bioactive constituents specific for each honey type</td>
<td>HPLC-DAD, HPLC-MS, GC-MS</td>
</tr>
<tr>
<td>δ¹³C isotopic signature</td>
<td>AOAC official method detects the presence of more than 10% of HFCS in honey</td>
</tr>
<tr>
<td>fructosyl-fructose from HFCS Polysaccharides, DFAs and AFGP</td>
<td>GC-MS</td>
</tr>
<tr>
<td></td>
<td>identification of residual syrup in honeys produced before 3 days of bee feeding</td>
</tr>
</tbody>
</table>

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Chemometric applications in HONEY authentication

- Building mathematical–statistical models based on quantitative and qualitative information about the natural constituents
  - Mineral and trace elements
  - Isotopic ratio
  - Volatile compounds
  - Amino acids
  - Sugars
  - Organic acids
  - Phenolics

- Statistical analysis
  - Similarity/Disimilarity
  - Cultivar discrimination
  - Geographical location

Botanical origin
- Natural organic constituents

Adulteration
- Isotopic signature, impurities from syrups

Geographical origin
- Elemental and isotopic ratios ($^{13}$C/$^{12}$C, $^{18}$O/$^{16}$O and D/H) determinations

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Objectives

- Highlight the common analytical methods used to confirm the authenticity of honey in reference to detection of direct and indirect adulteration of honey;
- Characterization of different authentic honey types: physic-chemical properties, bioactive composition and biochemical properties, $\delta^{13}$C signature;
- Investigate the effect of direct incorporation of different percent of sugar syrups in honey or indirect adulteration of honey by bee feeding with sucrose syrup on honey bioactive constituents and biochemical properties;
- Discrimination between pure and adulterated honey by bee feeding with cakes and sugar syrups prior to, or within the main nectar periods;
- Establish the minimal amount of added sugar syrup in honey, by which bioactive compounds profile and honey biochemical properties are definitively modified by the incorporation of syrups;
- Development of appropriate methodologies for objective verification of honey authenticity.
Experimental methods

Samples:

- **Pure honeys**: acacia, rape, linden, sunflower, polyfloral and honeydew
- **Direct adulteration**
- **Indirect adulteration**
- **Commercial honey samples**
Analytical investigations

- basic physicochemical parameters (moisture, pH, electrical conductivity, HMF) - methods proposed by International Honey Commission

- chromatographic methods for fingerprinting low molecular organic compounds and sugar content:
  - phenolic compounds by UHPLC-ESI/MS
  - organic acids and vitamins by UHPLC-DAD
  - sugar composition by HPLC-ELDS

- biochemical properties: total polyphenols (mg GAE/100g), total flavonoids (mg/100g quercetin) and antioxidant activity (DPPH %)) - UV-Vis spectrophotometric methods

- Determination of $\delta^{13}$C in honey and $\delta^{13}$C in protein extracted from honey by SCIRA method was used in order to establish the percent of adulteration of honey with C4 plants sugar (e.g. cane, corn syrup)

- Screening spectroscopic UV-Vis and NMR approaches were tested as alternative analytical methods used for honey authentication

- Statistical tools like ANOVA, PCA, LDA, AHC were used for data processing
Results and discussions

Direct adulteration of honey

- Direct incorporation of sugar syrups in honey led to the change of honey physico-chemical parameters, but within the range of variation for pure honeys, which makes that the single use of basic physico-chemical parameters to be inappropriate for a correct authenticity decision.

- LDA analysis allows the discrimination of adulterated honeys starting from 30-40% sugar syrup addition in honey, maltose, F/G ratio and conductivity can be considered as possible markers.

- Combination with other representative parameters such isotopic fingerprint, elemental composition or bioactive composition would be more suggestive.

Basic physic-chemical investigations

Scatter plot of the first two LDA discriminant functions showing separation between honey samples.
Comparison of sugar composition between pure and adulterated honeydew honeys with sugar syrups

Dendrogram of pure and adulterated honeydew honey samples represented by sugar composition obtained by Ward’s hierarchical clustering method (Hierarchical Cluster Analysis)

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Phenolic compounds variation in pure and adulterated honeys

<table>
<thead>
<tr>
<th>Phenolic compounds mg/kg</th>
<th>Sunflower</th>
<th>honeydew</th>
<th>polyfloral</th>
<th>rape</th>
<th>acacia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pure honeys (n=9)</td>
<td>Adulterated honeys</td>
<td>Pure honeys (n=4)</td>
<td>Adulterated honeys</td>
<td>Pure honeys (n=7)</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>0.000-0.036</td>
<td>0.000-0.008</td>
<td>0.912-4.645</td>
<td>0.566-1.119</td>
<td>0.000-0.520</td>
</tr>
<tr>
<td>3,4- dihydroxibenzoic acid</td>
<td>0.148-2.885</td>
<td>0.117-0.266</td>
<td>1.764-6.053</td>
<td>4.146-5.163</td>
<td>0.273-3.070</td>
</tr>
<tr>
<td>4-hydroxybenzoic acid</td>
<td>0.567-1.601</td>
<td>0.323-0.702</td>
<td>0.474-1.515</td>
<td>0.601-0.943</td>
<td>0.904-3.116</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>0.353-1.884</td>
<td>0.128-0.334</td>
<td>0.235-0.550</td>
<td>0.181-0.279</td>
<td>0.431-1.261</td>
</tr>
<tr>
<td>Syringic acid</td>
<td>0.006-0.101</td>
<td>0.006-0.05</td>
<td>0.106-1.113</td>
<td>0.068-0.173</td>
<td>0.032-0.237</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>0.917-2.702</td>
<td>0.580-1.238</td>
<td>0.817-2.105</td>
<td>0.977-1.621</td>
<td>0.403-2.499</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>0.731-2.786</td>
<td>0.317-0.748</td>
<td>0.523-2.469</td>
<td>0.381-1.212</td>
<td>0.317-1.504</td>
</tr>
<tr>
<td>Cinnamic acid</td>
<td>0.044-0.254</td>
<td>0.012-0.05</td>
<td>0.031-0.398</td>
<td>0.033-0.103</td>
<td>0.053-0.977</td>
</tr>
<tr>
<td>Rutin</td>
<td>0.009-0.171</td>
<td>0.002-0.006</td>
<td>0.006-0.664</td>
<td>0.127-0.262</td>
<td>0.007-2.131</td>
</tr>
<tr>
<td>Quercetin</td>
<td>0.395-0.982</td>
<td>0.272-0.641</td>
<td>0.132-0.765</td>
<td>0.300-0.478</td>
<td>0.192-2.099</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>0.100-0.669</td>
<td>0.041-0.097</td>
<td>0.116-0.554</td>
<td>0.147-0.256</td>
<td>0.269-1.964</td>
</tr>
<tr>
<td>isorhamnetin</td>
<td>0.065-0.323</td>
<td>0.033-0.083</td>
<td>0.050-0.331</td>
<td>0.063-0.109</td>
<td>0.067-0.421</td>
</tr>
<tr>
<td>Apigenin</td>
<td>0.124-1.168</td>
<td>0.003-0.066</td>
<td>0.100-1.047</td>
<td>0.219-0.360</td>
<td>0.218-3.451</td>
</tr>
<tr>
<td>Pinocebrin</td>
<td>0.861-4.492</td>
<td>0.000-0.675</td>
<td>0.011-2.823</td>
<td>0.207-0.372</td>
<td>0.031-5.314</td>
</tr>
<tr>
<td>Galangin</td>
<td>0.316-1.394</td>
<td>0.000-0.259</td>
<td>0.309-0.845</td>
<td>0.072-0.145</td>
<td>0.114-2.316</td>
</tr>
<tr>
<td>Chrysin</td>
<td>0.975-3.352</td>
<td>0.327-0.657</td>
<td>0.005-2.627</td>
<td>0.248-0.444</td>
<td>0.000-4.865</td>
</tr>
<tr>
<td>Pinostrobin</td>
<td>0.000-1.628</td>
<td>0.000-0.072</td>
<td>0.000-1.107</td>
<td>0.005-0.042</td>
<td>0.000-1.913</td>
</tr>
</tbody>
</table>

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LDA discrimination between pure and adulterated honeys based on phenolic composition

When compared to the references, it is possible to differentiate the adulterated honeys from pure honeys and sugar syrups.
## Phenolic compounds profile of sugar syrups

<table>
<thead>
<tr>
<th>Phenolic compounds</th>
<th>Min</th>
<th>Max</th>
<th>Average (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic acid</td>
<td>0.000</td>
<td>0.071</td>
<td>0.035</td>
</tr>
<tr>
<td>3,4- dihydroxibenzoic acid</td>
<td>0.003</td>
<td>0.109</td>
<td>0.056</td>
</tr>
<tr>
<td>4-hydroxibenzoic acid</td>
<td>0.000</td>
<td>0.304</td>
<td>0.152</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>0.000</td>
<td>0.103</td>
<td>0.051</td>
</tr>
<tr>
<td>Syringic acid</td>
<td>0.001</td>
<td>0.683</td>
<td>0.342</td>
</tr>
<tr>
<td>P-coumaric acid</td>
<td>0.001</td>
<td>1.205</td>
<td>0.603</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>0.001</td>
<td>0.822</td>
<td>0.412</td>
</tr>
<tr>
<td>Cinnamic acid</td>
<td>0.000</td>
<td>0.157</td>
<td>0.078</td>
</tr>
<tr>
<td>Rutin</td>
<td>0.003</td>
<td>0.067</td>
<td>0.035</td>
</tr>
<tr>
<td>Quercetin</td>
<td>0.002</td>
<td>0.063</td>
<td>0.033</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>0.002</td>
<td>0.026</td>
<td>0.014</td>
</tr>
<tr>
<td>Isoquercetin</td>
<td>0.000</td>
<td>0.024</td>
<td>0.012</td>
</tr>
<tr>
<td>Apigenin</td>
<td>0.001</td>
<td>0.043</td>
<td>0.022</td>
</tr>
<tr>
<td>Pinocembrin</td>
<td>0.002</td>
<td>0.084</td>
<td>0.043</td>
</tr>
<tr>
<td>Galangin</td>
<td>0.003</td>
<td>0.059</td>
<td>0.031</td>
</tr>
<tr>
<td>Chrysin</td>
<td>0.001</td>
<td>0.046</td>
<td>0.024</td>
</tr>
<tr>
<td>Pinostrobin</td>
<td>0.001</td>
<td>0.003</td>
<td>0.002</td>
</tr>
</tbody>
</table>

## Biochemical properties of sugar syrups

<table>
<thead>
<tr>
<th>Sugar syrup</th>
<th>TP (mg GAE/100g)</th>
<th>TF (mg QE/100g)</th>
<th>DPPH%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar syrup</td>
<td>29.47</td>
<td>0.15</td>
<td>0.16</td>
</tr>
<tr>
<td>Sugar syrup</td>
<td>14.94</td>
<td>0.12</td>
<td>0.71</td>
</tr>
<tr>
<td>Sugar syrup</td>
<td>24.63</td>
<td>0.59</td>
<td>1.49</td>
</tr>
<tr>
<td>Sugar syrup</td>
<td>26.42</td>
<td>0.56</td>
<td>2.37</td>
</tr>
<tr>
<td>Sugar syrup</td>
<td>29.64</td>
<td>0.20</td>
<td>0.94</td>
</tr>
</tbody>
</table>

TP – total polyphenols
TF – total flavonoids
Honey biochemical properties

Biochemical properties of pure and adulterated honeys

<table>
<thead>
<tr>
<th>Honey type</th>
<th>Total polyphenols (mg GAE/100g)</th>
<th>Total flavonoids (mg QE/100g)</th>
<th>DPPH %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pure honey</td>
<td>Adulterated honeys</td>
<td>Pure honey</td>
</tr>
<tr>
<td>sunflower</td>
<td>56.6</td>
<td>27.9-54.77</td>
<td>4.8</td>
</tr>
<tr>
<td>honeydew</td>
<td>128.7</td>
<td>65.2-122.6</td>
<td>8.5</td>
</tr>
<tr>
<td>linden</td>
<td>58.9</td>
<td>39.0-57.7</td>
<td>4.8</td>
</tr>
<tr>
<td>polyfloral</td>
<td>80.1</td>
<td>48.5-88.0</td>
<td>6.0</td>
</tr>
<tr>
<td>rape</td>
<td>41.0</td>
<td>32.6-46.5</td>
<td>4.0</td>
</tr>
<tr>
<td>acacia</td>
<td>50.5</td>
<td>37.2-55.5</td>
<td>2.0</td>
</tr>
</tbody>
</table>

- Biochemical properties of authentic honeys were higher than in the adulterate honeys:
  - 56-6-128.7 mg GAE/100g, 4.8-8.5 mg QE/100g and 5.8-29.5 % DPPH in pure honeys
  - 27.9-122.6 mg GAE/100g, 0.05-8.3 mg QE/100g and 1.4-27.9 % DPPH in adulterated honeys
Due to the fact that sugar syrups used for direct adulteration of honey shows bioactive properties, direct incorporation of sugar syrups in honey has produced an average decrease of 17.0 % for TP content, 34.4% for TF content and 27.7% for % DPPH.
UV-Vis fingerprinting of pure and adulterated honeys

UV-Vis spectral characterization of pure and adulterated sunflower honeys
Discrimination of pure and adulterated honeys based on UV-Vis spectral information
NMR fingerprinting of pure and adulterated honeys

$^1$H-NMR spectra of pure honeydew honey (red), adulterated honeydew honey with 30% sugar syrup (green) and sugar syrup (blue)
Indirect honey adulteration

Isotopic and physicochemical parameters of pure and adulterated honeys

<table>
<thead>
<tr>
<th></th>
<th>Pure honeys</th>
<th>Adulterated honeys</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min</td>
<td>Max</td>
</tr>
<tr>
<td>δ¹³C honey (%)</td>
<td>-27.68</td>
<td>-23.19</td>
</tr>
<tr>
<td>A (%) (adulteration with C4 sugars)</td>
<td>0.07</td>
<td>6.72</td>
</tr>
<tr>
<td>δ¹³C difference protein-honey (%)</td>
<td>0.01</td>
<td>0.90</td>
</tr>
<tr>
<td>nD 20 °C</td>
<td>1.48</td>
<td>1.51</td>
</tr>
<tr>
<td>Moisture</td>
<td>13.00</td>
<td>22.20</td>
</tr>
<tr>
<td>°BRIX</td>
<td>76.31</td>
<td>87.25</td>
</tr>
<tr>
<td>pH</td>
<td>3.60</td>
<td>4.53</td>
</tr>
<tr>
<td>Conductivity (µS/cm)</td>
<td>91.3</td>
<td>943.3</td>
</tr>
<tr>
<td>Acidity (mL NaOH 0.1 N)</td>
<td>0.49</td>
<td>5.45</td>
</tr>
<tr>
<td>HMF (mg/kg)</td>
<td>0.59</td>
<td>39.40</td>
</tr>
<tr>
<td>Fructose (g/100g)</td>
<td>31.70</td>
<td>49.25</td>
</tr>
<tr>
<td>Glucose (g/100g)</td>
<td>18.72</td>
<td>40.43</td>
</tr>
<tr>
<td>Sucrose (g/100g)</td>
<td>0.00</td>
<td>5.12</td>
</tr>
<tr>
<td>Maltose (g/100g)</td>
<td>0.49</td>
<td>4.76</td>
</tr>
</tbody>
</table>

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Principal component analysis (PCA) scores plot showing separation between pure (blue) and adulterated honey (red) samples.

- $\delta^{13}C$ honey signature represent a reliable criteria for identification of honey adulterated with sugars syrups originating from C4 plants (corn-sugar cane), but fails to detect the adulteration of honey with C3 (beet sugar) sugar syrup.
Honey isotopic signature and biochemical properties

<table>
<thead>
<tr>
<th></th>
<th>(\delta^{13}C_{\text{honey}}) (%o)</th>
<th>(\delta^{13}C_{\text{protein}}) (%o)</th>
<th>C4 sugar (%)</th>
<th>mg GAE/100g</th>
<th>mg QE/100g</th>
<th>DPPH %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acacia</td>
<td>-24,49</td>
<td>-24,61</td>
<td>0,80</td>
<td>50,53</td>
<td>2,01</td>
<td>7,70</td>
</tr>
<tr>
<td>Acacia</td>
<td>-23,3</td>
<td>-23,25</td>
<td>0,37</td>
<td>29,83</td>
<td>2,57</td>
<td>1,26</td>
</tr>
<tr>
<td>Acacia</td>
<td>-23,19</td>
<td>-22,34</td>
<td>6,72</td>
<td>31,36</td>
<td>2,53</td>
<td>2,69</td>
</tr>
<tr>
<td>Acacia</td>
<td>-23,46</td>
<td>-23,45</td>
<td>0,07</td>
<td>42,70</td>
<td>3,24</td>
<td>3,94</td>
</tr>
<tr>
<td>Rape</td>
<td>-22,12</td>
<td>-24,95</td>
<td>18,56</td>
<td>55,74</td>
<td>5,31</td>
<td>9,51</td>
</tr>
<tr>
<td>Rape</td>
<td>-26,08</td>
<td>-26,26</td>
<td>1,09</td>
<td>33,33</td>
<td>5,29</td>
<td>9,15</td>
</tr>
<tr>
<td>Rape</td>
<td>-27,09</td>
<td>-26,59</td>
<td>-2,96</td>
<td>40,98</td>
<td>4,02</td>
<td>2,69</td>
</tr>
<tr>
<td>Polyfloral</td>
<td>-22,11</td>
<td>-24,65</td>
<td>16,99</td>
<td>48,13</td>
<td>5,33</td>
<td>8,42</td>
</tr>
<tr>
<td>Polyfloral</td>
<td>-17,84</td>
<td>-24,49</td>
<td>44,96</td>
<td>28,70</td>
<td>5,90</td>
<td>3,45</td>
</tr>
<tr>
<td>Polyfloral</td>
<td>-25,5</td>
<td>-24,93</td>
<td>-3,74</td>
<td>70,11</td>
<td>6,01</td>
<td>11,78</td>
</tr>
<tr>
<td>Sunflower</td>
<td>-25,12</td>
<td>-25,42</td>
<td>1,91</td>
<td>46,60</td>
<td>4,77</td>
<td>7,31</td>
</tr>
<tr>
<td>Sunflower</td>
<td>-26,6</td>
<td>-25,48</td>
<td>7,1</td>
<td>59,11</td>
<td>8,61</td>
<td>9,24</td>
</tr>
<tr>
<td>Honeydew</td>
<td>-26,85</td>
<td>-25,88</td>
<td>-6,00</td>
<td>90,93</td>
<td>5,55</td>
<td>21,09</td>
</tr>
<tr>
<td>Honeydew</td>
<td>-25,04</td>
<td>-24,88</td>
<td>-1,05</td>
<td>98,68</td>
<td>7,07</td>
<td>29,45</td>
</tr>
<tr>
<td>Linden</td>
<td>-26,09</td>
<td>-25,36</td>
<td>-4,66</td>
<td>58,91</td>
<td>4,82</td>
<td>8,42</td>
</tr>
</tbody>
</table>

AOAC 991.41-1996:
• \(\delta^{13}C\) difference protein-honey \(\%o\) < max 1
• C4 sugar \(\%\) < max 7%

Indirect adulteration of honey

Intensive bee feeding with sugar syrups from C4 plants conduct to a decrease of honey bioactive properties and this adulteration can be easily identified by \(\delta^{13}C\) isotopic investigations.

Baile Govora, 24-26 October 2018
Honey authentication based on physicochemical properties, sugar content and $\delta^{13}$C isotopic signature – commercial honeys

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Honey type</th>
<th>Commercially honeys</th>
<th>Sugar syrups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acacia (n=16)</td>
<td>Rape (n=14)</td>
<td>Poliflora (n=3)</td>
</tr>
<tr>
<td>IR</td>
<td>1.495±0.0055</td>
<td>1.493±0.0054</td>
<td>1.494±0.0106</td>
</tr>
<tr>
<td>Moisture</td>
<td>17.0±2.4</td>
<td>17.22±2.1</td>
<td>17.04±2.1</td>
</tr>
<tr>
<td>° BRIX</td>
<td>81.68±2.09</td>
<td>81.16±2.05</td>
<td>81.27±2.02</td>
</tr>
<tr>
<td>pH</td>
<td>3.940±0.214</td>
<td>3.952±0.092</td>
<td>4.251±0.428</td>
</tr>
<tr>
<td>Conductivity (μS/cm)</td>
<td>165.28±49.88</td>
<td>175.46±29.24</td>
<td>600.60±157.45</td>
</tr>
<tr>
<td>Acidity (mL)</td>
<td>0.95±0.262</td>
<td>1.058±0.269</td>
<td>2.949±1.266</td>
</tr>
<tr>
<td>HMF (mg/kg)</td>
<td>7.04±36.29</td>
<td>1.067±35.56</td>
<td>1.914±4.155</td>
</tr>
<tr>
<td>Fructose (%)</td>
<td>41.92±2.61</td>
<td>34.07±7.04</td>
<td>37.77±6.45</td>
</tr>
<tr>
<td>Glucose (%)</td>
<td>25.58±2.78</td>
<td>38.42±3.98</td>
<td>27.46±6.63</td>
</tr>
<tr>
<td>Sucrose (%)</td>
<td>0.00±0.16</td>
<td>0.00±0.21</td>
<td>n.d.</td>
</tr>
<tr>
<td>Maltose (%)</td>
<td>2.39±1.09</td>
<td>1.96±2.58</td>
<td>1.48±0.27</td>
</tr>
<tr>
<td>F/G</td>
<td>1.65±0.16</td>
<td>0.90±0.19</td>
<td>1.44±0.48</td>
</tr>
<tr>
<td>$\delta^{13}$C protein</td>
<td>-25.09</td>
<td>-23.82</td>
<td>-27.61</td>
</tr>
</tbody>
</table>

ICSI Rm. Valcea
Investigation of commercial honeys

HCA analysis of tested honeys based on SCIRA method

Baile Govora, 24-26 October 2018
Botanical origin of commercial honeys based on physic-chemical and isotopic parameters
Honey botanical origin discrimination based on phenolic compounds

Scatter plot of the first two discriminant functions showing separation between honey types

Correlation between the analysed parameters and the factors in discriminant analysis of honeys

Baile Govora, 24-26 October 2018
Conclusions

- Detection of honey adulteration with sugar syrups obtained from C3 plants (beet, wheat, rice) is a challenge.
- Spectroscopic techniques (UV-Vis, NMR) coupled with multivariate statistical analysis of the data can be considered as valuable fingerprinting methodologies used to detect adulteration of honey with C3 sugar syrups.
- Direct incorporation of sugar syrups in honey has produced a decrease of honey biochemical properties compared to the reference honey sample, but within the range of variation for pure honeys.
- Intensive bee feeding during a long period of time conduct to the modification of honey chemical composition and quality similar to direct insertion of sugar into honey.
- Multicomponent analysis, which involves the investigation of numerous parameters is necessary for quality control and authentication of honey.
Acknowledgments

This work was supported by the project:
PN-III-P2-2.1-PED-2016-1656 - Alternative analytical approaches for detecting adulteration of honey with emphasis on its biologically active compounds
SAFE-HONEY, 194PED/2017

Thank you for your attention!

Baile Govora, 24-26 October 2018
Alternative Analytical Approaches For Detecting Adulteration Of Honey

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Honey was introduced in the list of products that are most exposed to the risk of adulteration, in the most cases by the addition of sugars, as well as by false declarations of botanical or geographical origin (Khan et al., 2018). In addition to sensory and nutritional values, one of the most valuable characteristics of honey is the therapeutic potential given by low-molecular-weight bioactive compounds like phenolic compounds, organic acids, amino acids, vitamins, and trace elements. Direct interpretation of sensory sugar syrup or indirect adulteration of honey by beekeeping with industrial sugars leads to determination of bioactive compound fingerprint (SOSha, Analit, Oliveira, & Mafra, 2017). Ensuring procedure of the product on the supermarket or household level is of a basic and therefore, development of simpler, more accurate, and more economical analytical methods able to determine the authenticity and quality of honey represent an important issue (European Commission, 2016).

The objectives of this study were to highlight the common analytical instruments used to verify the authenticity of honey in reference to the detection of direct and indirect adulteration of honey by addition of sugar syrups and to develop cost-effective methodologies based on fingerprinting honey bioactive compounds. For this purpose, different authentic honeys and adulterated honeys produced by direct incorporation of different percent of commercial sugar syrups (from corn, rice, and beet) in honey or produced by bees that has fed supplementary with sugar syrup were analyzed.

Authenticity of different floral honeys (oak, maple, limet, sage, poplar, and honeydew), in terms of adulteration identification, was investigated using basic physicochemical parameters (humidity, pH, electrical conductivity); chromatographic methods for fingerprinting major molecular organic compounds (phenolic compounds by HPLC-CESI/MS, organic acids and vitamins by HPLC-DAD) and sugar composition by HPLC-ELSD, while bioproperties (total polyphenols – TP, total flavonoids – TF and antioxidant activity – % DPPH) were evaluated by UV-VIS methods. Determination of δ13C in honey and δ18O in proteins extracted from honey by SCRA method was used in order to establish the percent of adulteration of honey with C3 plants sugar (e.g. corn, beet syrup). Screening spectroscopic UV-VIS and NMR approaches were tested as alternative analytical methods used for honey authentication. Statistical tools like ANOVA, PCA, LDA, AHC were used for data processing.

Routine quality control methods for honey analysis in combination with chemometric tools were found to be able to classify the pure honeys and adulterated honeys resulted from deliberate addition of more than 10-40% sugar syrups. Authentic honeys samples and commercial sugar syrups shows similar sugar (fructose, glucose) content for that sugar profile cannot be used to distinguish between adulterated and pure honeys than starting from 50% added sugar syrup to honey.

δ13C honey signature represent a reliable criteria for identification of honey adulterated with sugars originating from C3 plants. By coupling δ13C honey isotopic signature with some physicochemical parameters and sugar composition a possible prediction of honey adulteration with sugars originating from C3 plants can be assured. Honey resulted by bee feeding with sucrose originating from C3 plants can be discriminated based on physicochemical investigations mainly based on sugar content.

Direct incorporation of sugar syrups in honey or intensive bee feeding with sugar syrups has produced a decrease of honey bioactive compounds composition and bioactive properties, but, within the natural range of variation for pure honeys, some commercial sugar syrups showing bioactive compounds from added plant extracts (Figure 1).

Figure 1. Variation of bioactive properties in pure and adulterated sunflower honeys.

Screening spectroscopic UV-VIS and NMR measurements coupled with chemometric processing of the data seems to be a more accessible approach used for rapid and economical honey authenticity assessments.

Keywords: honey adulteration, sugar syrups, analytical methods, chemometrics

Acknowledgements

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References
