**Introduction**

Polyphenols are natural phytochemical products of secondary plant metabolites playing a major role as potentially functional components. Depending on the season, floral type and geographical origin, honey samples show different phenol contents. Total phenolic content (TPC) of honey has been widely determined by spectrophotometric methods that are simple, rapid and cost-effective. These methods are not selective, likely providing overestimated values, in particular, when using crude honey. The most common method for TPC estimation is based on a modification of the Folin-Ciocalteu procedure [1], which is very unspecific.

**Materials and methods**

Ninety six samples of *Apis mellifera* honey of different regions were acquired in the area of South Europe (Northern Italy, Slovenia, Hungary, Croatia and Serbia) in 2017. The share of nectar of individual plant species in honey varies depending on the type of vegetation, the flowering period of plant species, as well as the time when the beekeeper produced honey. Absolute uni-floral honey does not exist. Therefore, the chemical composition and organoleptic properties between honey samples vary significantly. Samples were prepared according to the slightly modified method proposed by Meda et al. (2005) [2]. Each honey sample (5g) was mixed with 15 ml ultrapure water, homogenized in ultrasonic bath for 15 min at room temperature, transferred to 50 ml volumetric flask, and filled with ultrapure water. This solution (0.5 ml) was then mixed with 2.5 ml of 0.2 N Folin-Ciocalteu reagent (Kemika, Zagreb, Croatia) for 5 min and 2 ml of 75g/L sodium carbonated (Na₂CO₃) (Kemika, Zagreb, Croatia) was then added. After incubation at room temperature for 2 h, the absorbance of the reaction mixture was measured at 760 nm against a methanol blank. TPC of honey was expressed in mg of gallic acid (GA) per kg of honey (GAE, Gallic Acid Equivalent) using GA calibration curve in the range (0-400 mg/L) (Figure 1).

**Results and discussion**

Table 1. Total phenolic contents reported by different groups for the same types of honey, expressed as GAE (mg GA/kg honey)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Acacia</th>
<th>Chesnut</th>
<th>Sage</th>
<th>Lime</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meda et al. (2005)</td>
<td>27.8±13.4</td>
<td>44.8±14.8</td>
<td>199.9±31.1</td>
<td>-</td>
</tr>
<tr>
<td>Lachman et al. (2012)</td>
<td>99.2±12.7</td>
<td>160.2±27.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lachman et al. (2016)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>82.5±7.4</td>
</tr>
<tr>
<td>Nacoulma et al. (2015)</td>
<td>39.1±5.8</td>
<td>162.1±21.6</td>
<td>96.5±7.4</td>
<td>85.8±7.4</td>
</tr>
</tbody>
</table>

In the analysis of the measured TPC values in Table 1, it can be observed that there are significant differences for the same type of honey. In the finding of the same uni-floral types of honey, the differences can be attributed to the different time of the beekeeper, the different conditions and the length storage, as well as the methodology of beekeeping and the geographical origin. However, the differences may also be due to inconsistencies in the application of measurement methods [3]. Some authors state that they modified the method, but do not state what and which part they modified, with the result that the results cannot be compared. Our experience so far indicates that different authors took different amounts of honey for analysis. In constructing the gallic acid calibration curve, some used a range of 0–200 mg/mL while other authors used 0–400 mg/mL (Figure 1). By taking a larger amount of honey, higher absorbances are also obtained, which is evident in Figure 1 that the calibration line become nonlinear, which can also lead to GAE concentration reading error.

**Summary**

The method for the determination of the polyphenols by the Folin-Ciocalteu method should be described in detail so that they all use the same methodology when preparing the samples so that the results can be compared. It is also necessary to state the time when the honey was extracted and in what conditions it was stored, as well as the location where the honey was extracted. Namely, these are all factors that affect the content of TPC. Adjust the amount of samples used in analyses, if possible, so that the all absorbances are in the part of the calibration curve which is: (1) linear and (2) between the minimal and maximal concentration of GA used for determination of calibration line.

**References:**