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Nine-year statistics of Czech honey carbohydrate profiles in the Czech Republic

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Abstract: Honey is composed mainly of carbohydrates which are represented by mono-, di-, tri-, tetra-, pentasaccharides, and oligosaccharides. The content and proportions of individual carbohydrates reveal information about the origin and technological properties of honey. A total of 5 987 samples of natural honeys originating from the Czech Republic and harvested during a period of nine consecutive years were analysed to research their carbohydrate content, sum of fructose and glucose (Fru + Glc), fructose and glucose ratio (Fru/Glc), and electrical conductivity (Ec). Monosaccharides, melezitose (Mel), and Ec varied according to the source of nectar or honeydew. Sucrose (Suc) content was low 0.87 ± 1.26 g (100 g)$^{-1}$ and did not exceed 15 g (100 g)$^{-1}$.

Keywords: sugars; sucrose; melezitose; HPLC/RI

Honey is a complex natural sweetener produced by the honey bee *Apis mellifera* L. from plant nectar or honeydew. The basic components of honey are clearly defined, but their representation is relatively variable and depends on the botanical source of nectar or honeydew, the environmental and seasonal conditions of honey production as well as the processing and storage conditions to some extent.

Honey is composed mainly of carbohydrates, smaller amounts of water (15–20%) and a large number, up to several hundreds, of minor compounds. Carbohydrates which make up approximately 95% of honey dry matter are mainly represented by monosaccharides (specifically hexoses), i.e. fructose (Fru) [30–45 g (100 g)$^{-1}$ honey] and glucose (Glc) [24–40 g (100 g^{-1}) honey]. In addition, about 25 different oligosaccharides were detected in honey. In addition to monosaccharides, blossom honey contains a significant portion of disaccharides, such as sucrose (Suc), maltose, and turanose. Honeydew honey also contains trisaccharides, such as melezitose (Mel), erlose, and raffinose. Traces of tetra- and pentasaccharides were also isolated from honey (Bogdanov et al. 2004, 2008).

Due to the high content of carbohydrates in honey, most of its properties are affected by these substances. These are mainly sweet taste, high viscosity, susceptibility to crystallization, hygroscopicity, and energy value [average 1 300 kJ (100 g)$^{-1}$]. Mono- and disaccharides are among the osmotically active substances that reduce the

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water activity of honey (a_w = 0.50 to 0.65) and ensure its good microbial stability (Machado De-Melo et al. 2018). The glycaemic index of honey, which depends on the ratio of individual carbohydrates, ranges from 85 (for honeydew honey) to 32 (for acacia honey, which has a high proportion of Fru) (Machado De-Melo et al. 2018).

The content and proportions of individual carbohydrates bring a lot of information about the origin, textural properties, while atypical values may indicate adulteration of honey.

The presence of Glc and Fru is useful for the classification of monofloral honeys. There are significant differences between the sugars of blossom and honeydew honeys which contain higher amounts of oligosaccharides; in particular, the already mentioned Mel and raffinose that do not occur in blossom honeys at all or in low concentrations only (Bogdanov et al. 2004; Pospiech et al. 2021)

The greater the proportion of Glc in it, the sooner the liquid honey crystallizes. The content of Mel is technologically important because this trisaccharide causes rapid crystallization of honey in higher concentrations. The content of Mel is variable: in honeydew honey 0.3–22%, in blossom honey less than 0.1% (Bogdanov et al. 2008).

According to the current legislation, honey with more than 5% Suc is considered to be adulterated if is not demonstrably derived from the listed plant species of the genera of *Robinia*, *Medicago*, *Banksia*, *Hedysarum*, *Eucalyptus*, *Eucryphia*, *Citrus*, *Lavandula*, and *Borago*. Another legislative requirement is that the total Fru and Glc content be at least 60% (blossom honeys) or 45% (honeydew honeys) (European Union Council Directive No. 2001/110/EC; Decree No. 76/2003 Coll., Laying Down Requirements for Natural Sweeteners, Honey, Confectionary, Cocoa Powder And Mixtures of Cocoa Powder With Sugar, Chocolate and Chocolate Pralines; The Ministry of Agriculture of the Czech Republic).

The aim of the study was to describe the saccharide profile of Czech honeys in a nine-year period, including monosaccharides, Suc, Mel, and to discuss the main factors influencing their content in honey.

MATERIAL AND METHODS

Samples. The samples included in this study were exclusively honeys from hobby beekeepers sent to the laboratory in order to obtain the 'Český med' certificate (confirming the authenticity of Czech honey) in compliance with the standard of the Czech Association of Beekeepers (ČSV 1/1999) or a report confirming compliance with Decree No. 76/2003 Coll. (The Ministry of Agriculture of the Czech Republic). Honey from the commercial network was not among the samples. A total of 5 987 samples were examined over a nine-year period.

Physicochemical analysis. The test procedures are based on the Harmonised Methods of the European Honey Commission (Bogdanov 2009).

A sample of honey was dissolved in a 25% aqueous methanol solution. The content of individual saccharide components of honey specified above was determined by high-performance liquid chromatography (HPLC) with refractometric detection ECP2000 (Ecom, Czech Republic), Separon SGX NH2 column (5 µm, 3 × 150 mm; Tessek Ltd., Czech Republic), tempering at 35 °C, refractometric detector RI2012 (Ecom, Czech Republic). The relative standard deviation (RSD) were Suc 9.4%, Fru 9.7%, Glc 11%, Mel 21%.

Qualitative determination of analytes was performed by comparing the retention time of the carbohydrate in the standard and in the sample. Quantification of individual carbohydrates in a honey sample was performed by the method of a calibration line.

The electrical conductivity (Ec) was determined as the conductivity of an aqueous solution of honey containing 20% of the dry matter of the examined honey using a GRYF 156 conductometer with VEL 356/tD probe (Gryf, Czech Republic).

Statistical analysis. Since none of the measured parameters follows Gaussian distribution, it was necessary to use non-parametric statistical tests, or in some cases to transform the data, to allow rigorous analysis. Statistical evaluation of Glc, Fru, Fru + Glc, Fru/Glc and Ec was done by Kruskal-Wallis test. The Dunn-Šidák method was used for post hoc analysis. Due to the large number of samples without Suc or Mel present, it was decided to handle those variables as binary for the purpose of statistical analysis. Therefore Pearson's test for association was used to determine whether the presence of Suc or Mel changes through time. Standard two proportions tests (adjusted by Šidák's correction to control the familywise error rate) were used for post hoc analysis in this case. Every statistical test was computed in Matlab R2020a (Mathworks, US).

RESULTS AND DISCUSSION

Of the main carbohydrates, the Fru monosaccharide was the most frequently represented in the 2012–2020 seasons with an average of 35.43 ± 3.44 g $(100\ g)^{-1}$. Glc averaged at 32.48 ± 4.5 g $(100\ g)^{-1}$. Of the di- and trisac-

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charides, Suc was the most commonly represented with 0.87 ± 1.26 g (100 g)$^{-1}$ and Mel with 1.96 ± 2.95 g (100 g)$^{-1}$. The average conductivity for the observed period was 56.77 ± 32.98 mS m^{-1}. The results for each year are summarised in Table 1, where the botanical type of honey is not distinguished. Most honey can be characterised as multifloral blossom or honeydew honey. The content of monosaccharides is in accordance with other authors, while the average content of Suc is below the data from the literature (Sanz et al. 2005; Kaškonienė et al. 2010; Tomczyk et al. 2019). The presumed reason for the low Suc content is the origin of honey, as in this study authentic honey from hobby beekeepers was used that has a lower Suc content than commercial honeys, especially with regard to the high activity of the invertase enzyme.

The highest average content of Fru 37.12 g (100 g)$^{-1}$ and Glc 34.89 g (100 g)$^{-1}$ was confirmed in 2012. The content of both of these carbohydrates varied over the years. Statistically significant differences were found between the observed seasons ($P < 0.05$), while in addition to 2012, years with comparable content of Glc and Fru were also confirmed. This finding is consistent with Pauliuc et al. (2020), who also did not confirm the differences between the two monitored seasons. The highest proportion of Glc and Fru in 2012 was at the expense of other carbohydrates, which are represented in this study by Mel (Table 1). This year was characterised by low honey production, which also manifested itself in low conductivity (Table 1) and thus in a low content of higher carbohydrates which originate mainly from sucking insects (Shaaban et al. 2020). Year-over-year differences are summarised in the violin plot (Figure 1). Two local modes are shown in the years 2013, 2014, and 2017. The samples show three local modes in 2019 and 2020. The reason for this division is different botanical sources of Fru in this period with a high frequency of occurrence because individual botanical sources have different concentrations of Fru in nectar, which classify the population within the violin plot into more local modes.

The Glc median has fluctuated considerably over the years (Figure 2), which confirms the variability of Glc content in honey over the years. In 2012 and 2019, honeys from the Czech Republic show two local modes, i.e. with low and high Glc content. The year 2012 is also statistically different in Glc content from all other periods ($P < 0.05$). In 2014, 2017, and 2020, the Czech honeys showed three local modes, and this period also differed statistically from the other years ($P < 0.05$). On the other hand, the Fru median has lower variability (Figure 1), which manifests itself in a wider area near the median

Table 1. Carbohydrate content and electrical conductivity (Ec) in 2012–2020 seasons (mean ± SD)

Parameter	2012	2013	2014	2015	2016	2017	2018	2019	2020
n	581	721	563	736	867	815	753	748	203
Glc [g (100 g)$^{-1}$]	34.89 ± 3.55^{a}	30.71 ± 4.25^{bc}	33.73 ± 4.51^{d}	31.33 ± 4.86^{bce}	32.15 ± 5.08^{efg}	33.27 ± 4.82^{d}	32.57 ± 3.34^{fg}	31.65 ± 3.79^{bef}	33.70 ± 3.55^{d}
Fru [g (100 g)$^{-1}$]	37.12 ± 2.29^{a}	35.02 ± 3.13^{bc}	35.72 ± 3.32^{def}	34.90 ± 3.77^{bcd}	34.42 ± 3.95^{bc}	35.36 ± 3.69^{bdef}	36.39 ± 2.93^{eg}	34.93 ± 3.28^{bc}	36.14 ± 2.40^{defg}
Fru + Glc [g (100 g)$^{-1}$]	72.00 ± 5.23^{a}	65.73 ± 6.97^{bc}	69.45 ± 7.28^{de}	66.23 ± 8.18^{bcf}	66.57 ± 8.62^{bf}	68.63 ± 8.01^{deg}	68.96 ± 5.31^{dg}	66.58 ± 6.40^{bcf}	69.84 ± 5.52^{deg}
Fru/Glc ratio	1.07 ± 0.09^{a}	1.15 ± 0.09^{b}	1.07 ± 0.10^{a}	1.13 ± 0.10^{c}	1.08 ± 0.10^{a}	1.07 ± 0.10^{a}	1.13 ± 0.11^{c}	1.11 ± 0.10^{c}	1.08 ± 0.08^{a}
Suc [g (100 g)$^{-1}$]	0.41 ± 0.60^{a}	0.70 ± 0.89^{bc}	0.77 ± 1.22^{a}	1.01 ± 1.18^{bd}	1.26 ± 1.56^{bcd}	1.12 ± 1.69^{bc}	0.95 ± 1.41^{bc}	0.65 ± 0.83^{bcd}	0.45 ± 0.62^{a}
Mel [g (100 g)$^{-1}$]	0.65 ± 1.63^{a}	4.33 ± 4.12^{b}	1.11 ± 2.12^{a}	2.84 ± 3.73^{c}	1.35 ± 1.98^{c}	1.32 ± 1.96^{b}	0.99 ± 1.42^{b}	3.08 ± 3.27^{d}	0.96 ± 2.08^{a}
n	369	567	433	626	716	658	648	631	142
Ec (mS m^{-1})	46.68 ± 24.27^{abcd}	71.51 ± 32.47^{e}	57.13 ± 37.13^{abcf}	61.49 ± 34.03^{afg}	55.86 ± 33.98^{abc}	50.84 ± 31.75^{abcd}	46.99 ± 25.39^{bd}	64.98 ± 34.14^{fg}	42.50 ± 26.01^{bd}

[a–g]Different letters in the superscript indicate statistically significant differences ($P < 0.05$); SD – standard deviation; Glc – glucose; Fru – fructose; Suc – sucrose; Mel – melezitose; Ec – electrical conductivity

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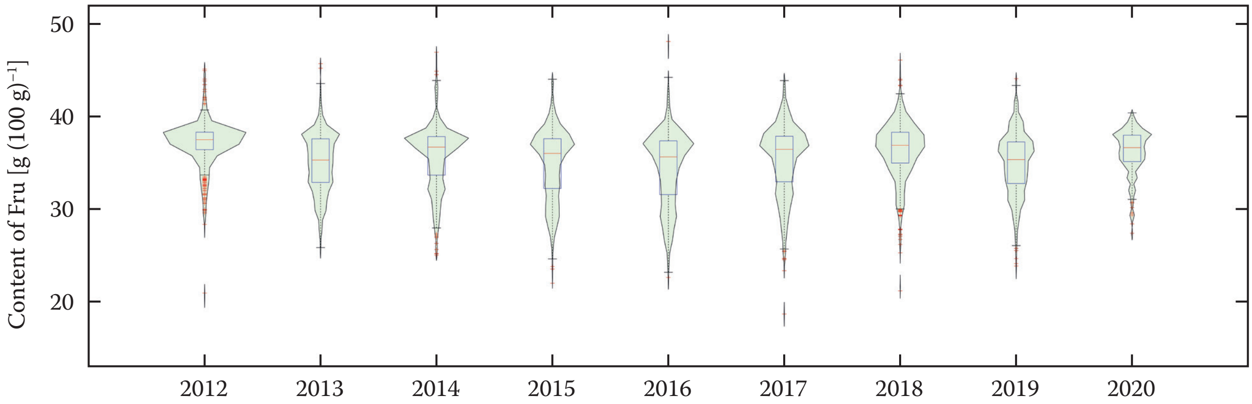

Figure 1. Violin plot of fructose (Fru) content in 2012–2020 periods [g (100 g)$^{-1}$]

of the violin plot. Variability in Glc content is caused by climatic and agrotechnical conditions. An important source of nectar for bees is rape (*Brassica napus*), the nectar of which has a higher Glc content compared to the Fru content (Pierre et al. 1999) and its availability, whether geographical or climatic, for hives, therefore, affects the presence of Glc in honey. Another important source of Glc for honey is dandelion (*Taraxacum officinale*) (Cavia et al. 2002) and lime tree (*Tilia* sp.) (Kaškonienė and Venskutonis 2010) that may also have a year-on-year effect in areas with lower agricultural intensity. A further source of Glc is sunflower (*Helianthus* sp.) (Persano Oddo and Piro 2004) that may also be affected within the context of agricultural activity.

Accompanying parameters for the characterisation of honey are also the sum of Fru and Glc (Fru + Glc) and the Fru and Glc ratio (Fru/Glc). Both parameters are in accordance with the already mentioned content of Glc and Fru. The highest value for Fru + Glc was therefore reached in 2012, specifically 72 ± 5.23 g (100 g)$^{-1}$. This year was statistically significantly different from the other years (P < 0.05). Year-over-year differences in median values indicate that in 2012 and 2018 the content of these carbohydrates was high in most of the analysed samples (Figure 3). On the contrary, the year 2013 was characterised by a large extent, and thus a higher content of other carbohydrates in Czech honey. The years 2014, 2016, 2017, and 2019 are characterised by a high content of Fru + Glc, but also by the content of other carbohydrates which reduce their total sum (Figure 3). The low sum of Fru + Glc is characteristic of honeydew honeys (Sanz et al. 2005), which had a rather large representation in the Czech Republic in the observed period.

The lowest average value of the Fru/Glc ratio was the same in 2012, 2014, and 2017, namely 1.07, although with different variability. The year 2013 was statistically significantly different in this parameter from the other years (P < 0.05). Figure 4 confirms the differences in medians for 2014 and 2017 when honeys in the Czech Republic show two local modes with different Fru/Glc ratio compared to 2012. A similar division was made in 2015 and 2016. On the contrary, the Gaussian distribution was typical of 2012, 2018, and 2019. The year 2020 differed from the others, honeys in the Czech Republic show three local modes according to the Fru/Glc ratio, although with

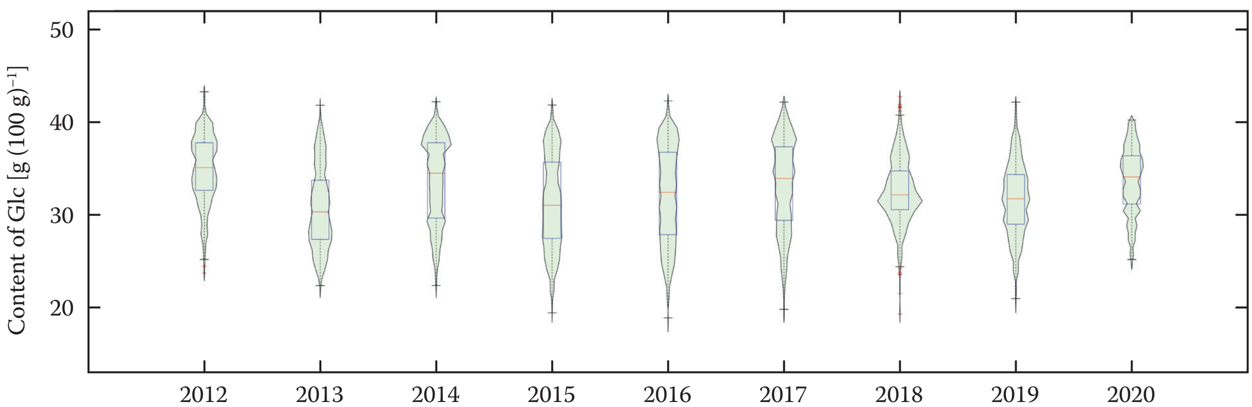

Figure 2. Violin plot of glucose (Glc) content in 2012–2020 periods [g (100 g)$^{-1}$]

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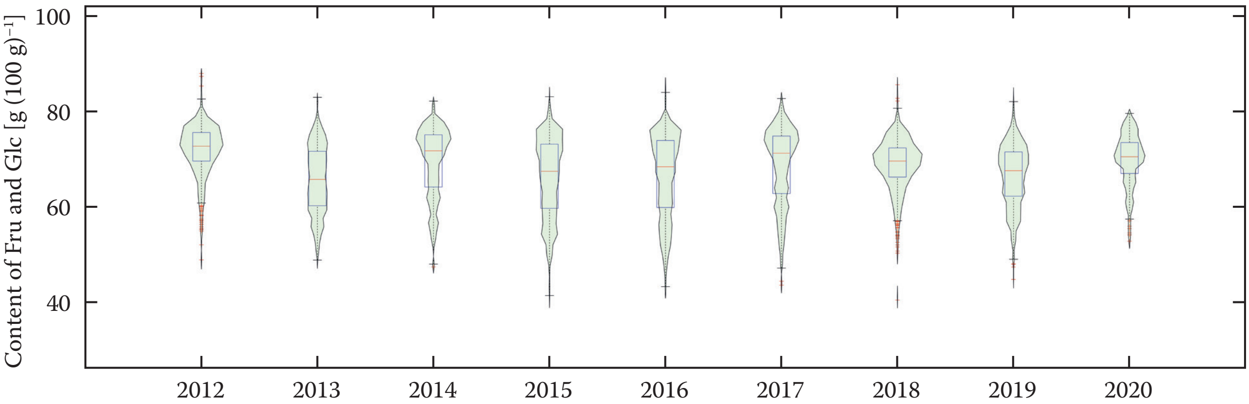

Figure 3. Violin plot of the sum of fructose (Fru) and glucose (Glc) in 2012–2020 periods [g (100 g)$^{-1}$]

a predominance of samples from Fru/Glc < 1. It is confirmed in the literature that the Fru/Glc ratio is low for some monofloral honeys. For example, for dandelion, rape, and willow (*Salix* sp.) the Fru/Glc ratio < 1 was confirmed (Horváth and Molnár-Perl 1997; Cavia et al. 2002; Kaškonienė et al. 2010). The source of nectar can therefore be considered as the reason for the year-over-year differences in the Fru/Glc ratio. Even for related botanical taxa, some variability can be expected in willow honey from Spain, the Fru/Glc ratio was > 1 (de la Fuente et al. 2007) compared to willow honey from Lithuania, where the Fru/Glc ratio was < 1 (Kaškonienė et al. 2010).

The average content of Suc in Czech honeys in the observed period was low, ranging from 0.41 g (100 g)$^{-1}$ in 2012 up to 1.26 g (100 g)$^{-1}$ in 2016. Statistically significant year-over-year differences were confirmed between some years. The years 2012, 2014, and 2020 were characterised by a low Suc content, which was in the range of 0.41–0.77 g (100 g)$^{-1}$; these years also differed statistically significantly from the other years ($P < 0.05$). Figure 5 confirms the uniformly low proportion of non-zero values over the years. The Suc content is an important parameter for proving the adulteration of honey, in particular by adding sugar or feeding the bees during their collection period. The Suc content of honey must not exceed 5 g (100 g)$^{-1}$ [European Union Council Directive No. 2001/110/EC; Decree No. 76/2003 Coll. (The Ministry of Agriculture of the Czech Republic); Thrasyvoulou et al. 2018], apart for exceptions such as certain monofloral honeys. For the Czech Republic, these are mainly false acacia (*Robinia pseudoacacia*), lucerne (*Medicago sativa*), French honeysuckle (*Hedysarum*) where 10 g (100 g)$^{-1}$ is allowed, or lavender (*Lavandula* spp.) and borage (*Borago officinalis*) where 15 g (100 g)$^{-1}$ (European Union Council Directive No. 2001/110/EC) is allowed.

For this reason, honeys with an above-limit value of Suc are also included in the work. The maximum value varied from year to year and ranged from 5.92 g (100 g)$^{-1}$ to 12.83 g (100 g)$^{-1}$. Specifically 5.92, 6.13, 10.20, 10.70, 9.38, 12.83, 12.11, 10.44, 5.86 g (100 g)$^{-1}$ for the years 2012 to 2020. These values are within the limit values according to the legislation [European Union Council Directive No. 2001/110/EC; Decree No. 76/2003 Coll. (The Ministry of Agriculture of the Czech Republic)] and confirm the low occurrence of honeys with a naturally above-

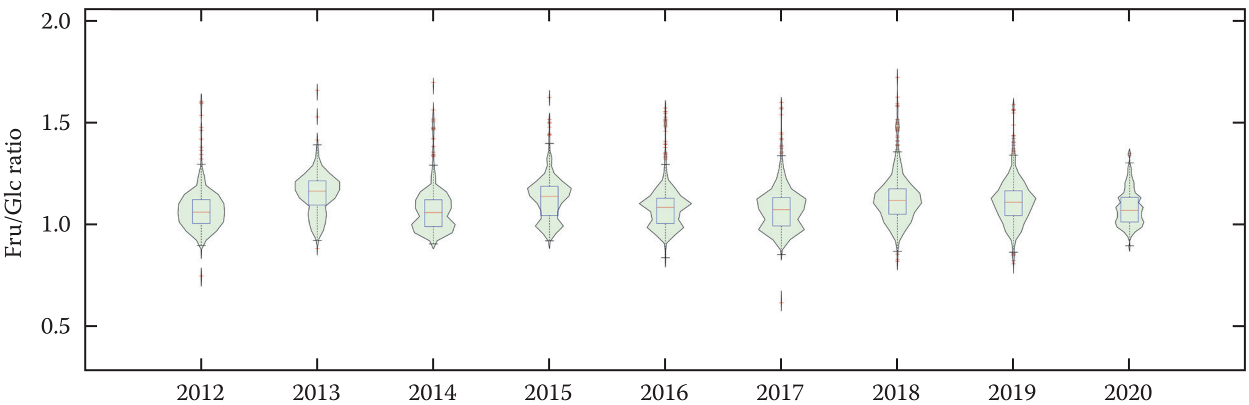

Figure 4. Violin plot of fructose/glucose (Fru/Glc) ratio in 2012–2020 periods [g (100 g)$^{-1}$]

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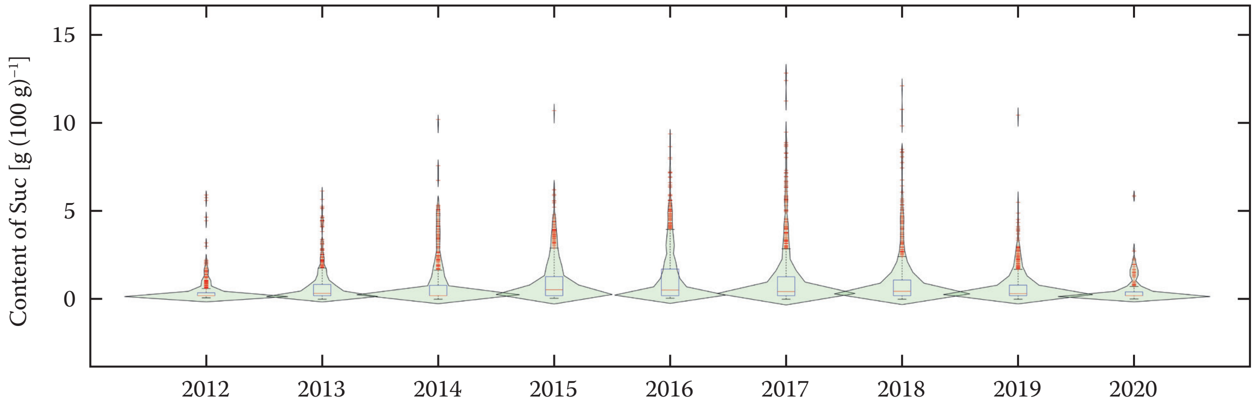

Figure 5. Violin plot of sucrose (Suc) content in 2012–2020 periods [g (100 g)$^{-1}$]

-limit Suc content. During the observed nine-year period, the Suc content of 2.1% of the samples was above 5%.

The average content of Mel in the studied period ranged from 0.65 ± 1.63 g (100 g)$^{-1}$ in 2012 up to 4.33 ± ± 4.12 g (100 g)$^{-1}$ in 2013. Highly statistically significant year-over-year variability was confirmed (P < 0.05). In 2012, 2014, and 2020 with a low Mel content, a statistically significant difference from the other years was confirmed (P < 0.05). The years 2015 and 2016 also differed statistically significantly from the other years (P < 0.05). Figure 6 confirms the occurrence of honeys with a high content of Mel in 2013, 2015, and 2019. However, in 2013, which also has the highest average Mel content, they show four local modes. The year 2019 did not show any local modes (Figure 6) but based on the Mel content, a statistically significant difference from the other years was confirmed (P < 0.05). This finding can be explained by different content of Mel in honeydew honeys from individual localities in the Czech Republic. Different Mel content is caused by different species of sucking insects and by different botanical taxa on which the insect occurs (Persano Oddo et al. 2004; Seijo et al. 2019; Shaaban et al. 2020). Higher content of Mel can also be caused by nectar from some botanical taxa, which is typical e.g. of chestnut [0.1 g (100 g)$^{-1}$] and heather honey [0.3 g (100 g)$^{-1}$] (Rodríguez-Flores et al. 2016, 2019). However, the content of Mel is also significantly lower in blossom honey than in honeydew honey; thus with regard to the achieved values, we assume honeydew to be the source of Mel for the Czech Republic. For honeydew honey, Mel is present in a higher concentration, for oak honeydew honey [0.1 g (100 g)$^{-1}$], evergreen oak honeydew honey [0.9 g (100 g)$^{-1}$], and for coniferous honeydew from *Pinus* sp. [0.6 g (100 g)$^{-1}$] (Pita-Calvo and Vázquez 2018), *Abies alba* [3.2 g (100 g)$^{-1}$] (Rybak-Chmielewska et al. 2013) and for botanically mixed honeydew honeys [4.9 g (100 g)$^{-1}$]. On the contrary, the years 2012, 2014, and 2020 were characterised by a low Mel content in most honeys. This finding is typical of blossom honeys, which in addition to low conductivity are also characterised by a low content of trisaccharides, including Mel (Bogdanov et al. 2004).

The average conductivity in the analysed samples did not exceed 80 mS m^{-1}. The highest average conductivity of 71.51 ± 32.47 mS m^{-1} was reached in 2013. Ec in this year was statistically significantly different (P < 0.05)

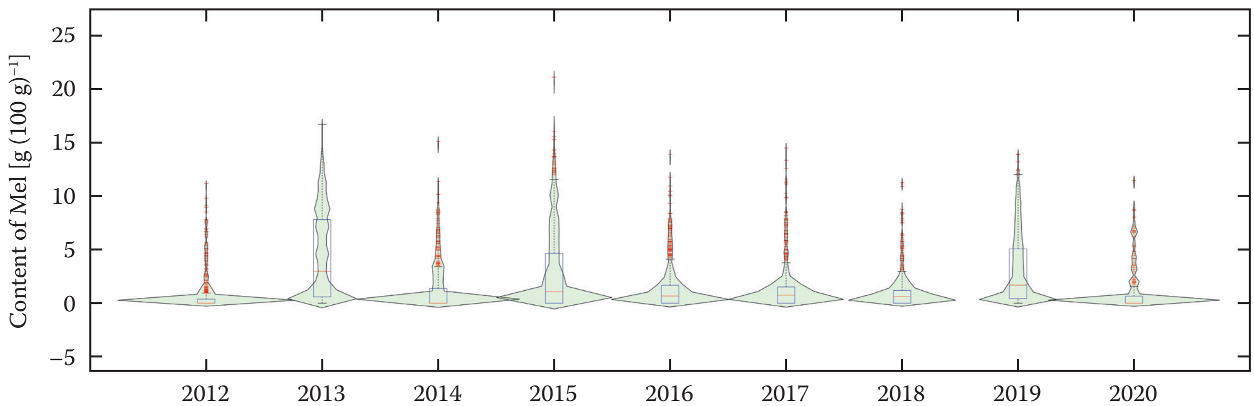

Figure 6. Violin plot of melezitose (Mel) content in 2012–2020 periods [g (100 g)$^{-1}$]

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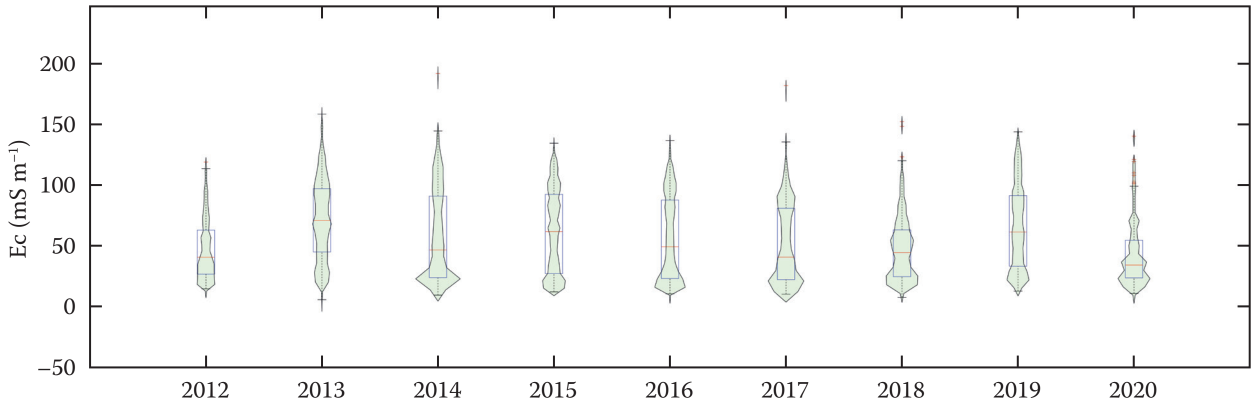

Figure 7. Violin plot of electric conductivity (Ec) in 2012–2020 periods (mS m^{-1})

from the other years (Table 1, Figure 7). High Ec was also confirmed in 2015 and 2019. Ec corresponded to the Mel content, $R = 0.65$ ($P < 0.05$). Both of these parameters are typical of honeydew honey, where honey is a product of suckling insect excrements and therefore has a different chemical composition compared to blossom honeys. Ec in honey is affected by minerals, organic acids, and proteins. For this reason, it has also been confirmed as one of the options for demonstrating the botanical origin or geographical origin of honey (Terrab et al. 2004; Acquarone et al. 2007), although there are also year-over-year differences (Vranić et al. 2017). Ec is also affected by other factors that affect the composition of nectar and honeydew. The literature describes the differences between spring and summer honeys (Yadata 2014), but also the differences between honeys depending on their botanical origin are worth mentioning like lime honey (62 mS m^{-1}), rape honey (19 mS m^{-1}), and dandelion honey (51 mS m^{-1}) (Persano Oddo and Piro 2004). Some monofloral honeys are therefore exempted from the condition of < 80 mS m^{-1} (Pita-Calvo and Azquez 2017). Potential botanical species for the Czech Republic covered by this exemption are bell heather (*Erica*), lime tree (*Tilia* sp.), ling heather (*Calluna vulgaris*).

CONCLUSIONS

Within the monitored nine-year period, a total of 5 987 samples of natural honeys originating from the Czech Republic were analysed. The average Glc content reached 32.48 ± 4.50 g (100 g)$^{-1}$, Fru 35.43 ± ± 3.44 g (100 g)$^{-1}$, Fru + Glc 67.92 ± 7.38 g (100 g)$^{-1}$, Fru/Glc 1.10 ± 0.11 g (100 g)$^{-1}$, Suc 0.87 ± 1.26 g (100 g)$^{-1}$, Mel 1.96 ± 2.95 g (100 g)$^{-1}$, and Ec 56.77 ± 32.98 mS m^{-1}. Fru is the majority carbohydrate in honey. Another highly abundant carbohydrate was Glc. These findings are consistent with other authors. The results of the study confirmed the year-over-year variability of most physicochemical parameters. The Fru/Glc ratio was the least variable, for this parameter the year 2013 was statistically different from the other years. A statistically significant difference was further confirmed for 2012 in the content of Glc and Fru compared to the other years. The content of Suc was low in the monitored period, but honeys with a content of more than 10 g (100 g)$^{-1}$ were also recorded. The average content of Mel also indicates the occurrence of honeydew honeys in the Czech Republic, while years with a very low occurrence of honeydew were confirmed in comparison with years with a rich honeydew collection. The measured parameters did not follow a Gaussian distribution. Presented analyses clearly show significant differences in all measured parameters throughout the years. However, it is not feasible to predict future values of measured parameters based on time alone. It would be necessary to gather data for multiple predictors, as was extensively discussed through this text.

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