CARBOHYDRATE COMPOSITION OF MONOFLORAL WILLOW (*SALIX ALBA* SPP.) HONEY

Vilma Kaskoniene[1], Petras Rimantas Venskutonis[1], Violeta Ceksteryte[2]

[1]*Kaunas University of Technology, Department of Food Technology, Radvilenu road, 19, LT–50254, Kaunas, Lithuania, e-mail: rimas.venskutonis@ktu.lt*

[2]*Lithuanian Institute of Agriculture, Instituto av. 1, LT–58344, Dotnuva, Kėdainių dist., Lithuania*

Abstract

The main task of this study was to determine the carbohydrate composition of willow honeys. The fructose, glucose and the minor oligosaccharide content of seven monofloral willow honeys collected in Lithuania in 2006 during flowering season were analyzed by gas chromatography with flame ionization detector (GC-FID) after the trimethylsilylation of carbohydrates. Fructose, glucose, sucrose, maltose, isomaltose, turanose, trehalose, palatinose, celobiose, raffinose and panose were identified and quantified in all samples. Glucose was predominant in 6 out of 7 samples. The mean values of fructose and glucose varied form 32.92 to 38.88 and from 35.27 to 42.29%, respectively. The ratio of fructose/glucose varied from 0.78 to 1.10. The amount of sucrose was 0.12–0.25%. Data obtained was thoroughly compared with previously published results and it was found that the characteristics of Lithuanian honeys in most cases meet international requirements for natural honey. Some correlations between sugar concentration and the content of willow pollen in the honey were established. However, the information on honey sugar composition is not sufficient for the reliable determination of the botanical origin of honey.

Key words: honey, carbohydrates, willow, gas chromatography

Introduction

In general, honey is a supersaturated sugar solution; sugars are the main constituents of honey accounting for about 95% of dry matter. Fructose and glucose are the major sugars, the former one being a major component almost in all honey types, except for some honeys of rape (*Brassica napus*), dandelion (*Taraxacum officinale*) and blue curls (*Trichostema lanceolatumi*) origin, when glucose is present in higher amounts (Cavia *et al.*, 2002). In addition, disaccharides, trisaccharides and other oligosaccharides are present in honey in small concentrations. The concentration of fructose and glucose as well as their ratio are useful indicators for the classification of unifloral honeys (Persano Oddo *et al.*, 1995; Persano Oddo and Piro, 2004).

It is known that there are compositional differences between honeydew and blossom honeys. Honeydew honey is characterised by a higher concentration of oligosaccharides, mainly trisaccaharides melezitose and raffinose, which usually are not found in blossom honeys (Bogdanov *et al.*, 2004).

Honey has been produced in Lithuania from the ancient times. Detailed carbohydrate composition of Lithuanian honey was not analyzed until now, except for routine measurements of glucose, fructose and saccharose for a standard quality assessment. The main honey plants in Lithuania is spring rape (*Brassica napus* L. ssp.) and in honey from those plants spring rape pollen are over-represented in honeys, but monofloral willow honeys also are collected. Monofloral willow honey medium are found in Spain, Croatia and Scandinavia countries (Persano Oddo and Piro, 2004). Moreover, there is a lack of the data in the literature about willow honey. Therefore, willow honey was selected for the analysis. The main task of this study was to comprehensively characterize carbohydrate composition of monofloral willow honey samples and to determine if there is any dependence between pollen content and the amount of corresponding carbohydrate in the honey. Such data may facilitate faster characterization of honey botanical source; it may also provide the information for the identification of adulteration and incorrect labelling.

Materials and Methods

Honey samples. Seven honey samples of willow origin were collected during 2006 years flowering season in Kėdainiai district, except one sample – in Vilkaviškis district (Lithuania).

The botanical origin of the samples was analysed by melissopalynology method (Baltrušaitytė *et al.,* 2007). Willow pollen content from total content of pollen in honey samples varied from 54.10 to 92.90%. In tested samples small amounts of rape, dandelion, white and red clover, cornflower, raspberry pollen also presented.

Gas chromatography analysis. Honey samples were diluted with ultra-pure water to a final Brix value 5–6. 140 µl of this solution and 10 µl of internal standard were transferred to a GC autosampler vials and freeze-dried in a Maxi-Dry Lyo (Heto-Holten, Allerød, Denmark) for 4 h. *β*-Gentiobiose was selected as an internal standard, because it was not found in honey. Freeze-dried samples were derivatized by the addition of 150 µl of 1-(trimethylsilyl) imidazole and 1 ml of pyridine. The vials were capped and the solutions heated at 80 ºC for 1 h. Trimethylsilylated carbohydrates are sufficiently volatile compounds and can be analyzed by gas chromatography. The stock solutions of reference compounds were prepared in the same way as the samples, from 5.0% (w/v) standard solutions in water.

Trimethylsilylated carbohydrates were analyzed on a GC 8000 series gas chromatograph with a flame ionization detector (Fison Instruments, Milan, Italy) by injecting 0.2 µl of the mixture to the capillary column ZB-5 (30 m×0.25 mm id×0.25 µm) coated with 5% phenylmethylpolysiloxane. The injector and detector temperatures were 260 ºC and 300 ºC, respectively. After testing several programs the most efficient separation was achieved when the temperature was raised from 100 ºC to 180 ºC at 4 ºC/min (5 min hold), increased to 215 ºC at 2 ºC/min and finally raised to 325 ºC/min at 3 ºC/min (10 min hold).

All reference compounds were analyzed in the same way. Identification of honey carbohydrates was achieved by comparing their retention times with those of reference compounds. The concentration was calculated using internal standard and expressed in % (w/w). The analyses were repeated three times.

Materials. All chemicals and solvents were of analytical grade. Ultra-pure (18.2 mΩ) water was used (Millipore, Simplicity, Canada). Pyridine and 1-(trimethylsilyl) imidazole, fructose (99.0%), glucose (99.5%), sucrose (99.5%), maltose (98.0%), isomaltose (98.0%), turanose (98.0%), trehalose (99.5%), *β*-gentiobiose (98.0%), palatinose (99.0%), celobiose (99.0%), raffinose pentahydrate (99.0%) and panose (98.0%) were from Sigma-Aldrich (Steinheim, Germany).

Statistical analysis. Standard deviations were calculated using spreadsheet software (Excel®). Correlation coefficients (R) to determine the relationship between several carbohydrates and the amount of the willow pollen in the honey, as well as between different sugars were calculated using SPSS statistical software.

Results and Discussion

GC-FID chromatograms indicate that sugar composition in the tested willow honey samples is similar, however some variations in the content of individual carbohydrates were observed. Three regions, representing mono-, di- and trisaccharides may be distinguished in the chromatographic profile of honey sugars (Figure 1).

In total, eleven carbohydrates were identified in the studied samples. Some separated peaks were not identified because reference compounds were not available. The amounts of the quantified sugars were within the limits established by Codex Alimentarius Commission (2001).

As it was expected (it is usual to the honey) fructose and glucose were dominant in willow honeys. As it was mentioned above, there are only few kinds of honey (honey from rape, dandelion and blue curl) when glucose is dominant (Cavia *et al.,* 2002). Our results do not totally coincide with this finding, because glucose was dominant in six out of seven samples. The mean values of fructose and glucose varied from 32.92 to 38.88 and from 35.27 to 42.29%, respectively (Table 1). Honey was intensively analysed for sugars, particularly in Southern European countries. For instance, 34.3–39.4% of fructose and 25.8–35.2% of glucose were reported in Spanish honeys (Mateo and Bosch-Reig, 1998); 31.4–39.8%

(fructose) and 27.4–36.3% (glucose) in honeys from Portugal (Mendes *et al.*, 1998). It was found that the percentage of carbohydrates also depends on the bee species (*Apis dorsata, A. cerana, A. millifera*) and concentration of the main sugar may vary in the wide range: 42.3–54.2% of fructose and 33.1–52.2% of glucose were determined in the honeys from Nepal (Joshi *et al.*, 2000).

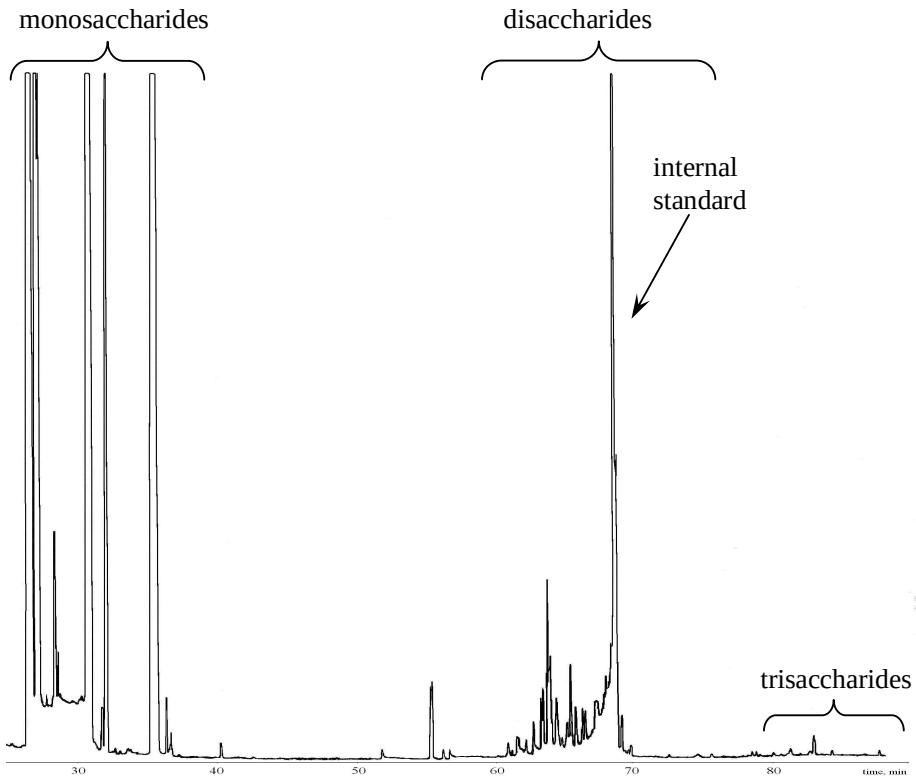

Figure 1. Typical chromatogram of the trimethylsilylated carbohydrates in willow honey

Table 1

Amount of pollen and the main carbohydrates in the tested willow honeys and fructose/glucose ratio

Pollen amount, %	Fructose, %	Glucose, %	F/G
92.90	34.27±0.27	41.55±0.03	0.82
75.80	32.92±0.22	42.29±0.08	0.78
73.20	34.74±0.14	42.23±0.18	0.82
70.20	38.88±0.24	35.27±0.09	1.10
65.60	34.12±0.10	42.21±0.06	0.81
57.50	36.11±0.06	37.80±0.18	0.96
54.10	34.67±0.11	41.86±0.23	0.83

Due to the low fructose value, the fructose/glucose ratio (F/G) was below 1. The ratio of F/G was 0.78–1.10; for comparison, in other studies of honey it was reported 0.84–1.89 (Persano Oddo *et al.*, 1995), 0.86–1.6 (Horváth and Molnár-Perl, 1997), 0.78–1.77 (Costa *et al.*, 1999), 0.99–1.77 (Mateo and Bosch-Reig, 1998).

Seven disaccharides and two trisaccharides were identified in the tested willow honey samples (Figure 2). Maltose was the most abundant disaccharide constituting from 1.14 to

1.85%. Maltose, which is usually present in honey in low quantities (to 3%), was suggested as a marker of natural honey (Persano Oddo *et al.*, 1995; Joshi et al., 2000; Cotte *et al.*, 2003).

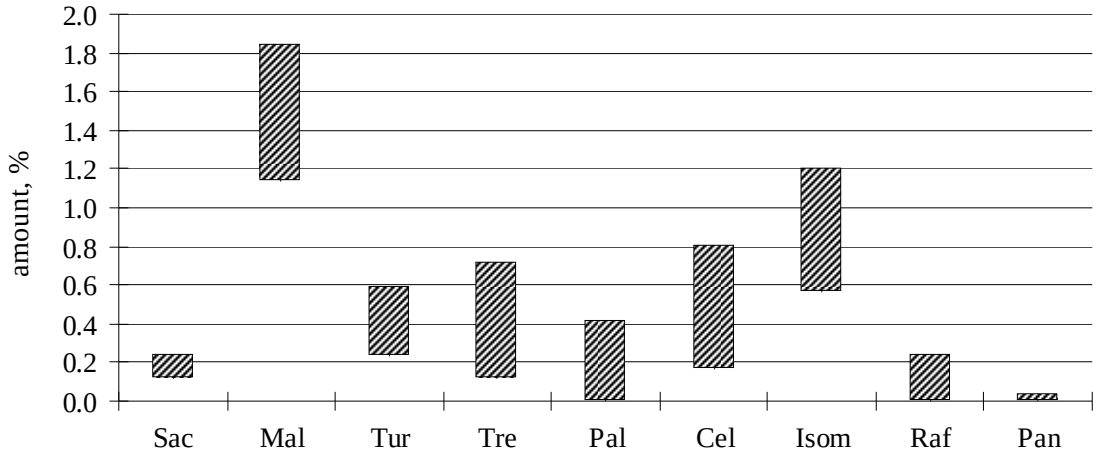

Figure 2. Distribution of disaccharides and trisaccharides in willow honey (Sac–saccharose; Mal–maltose; Tur–turanose; Tre–trehalose; Pal–palatinose; Cel–cellobiose; Isom – isomaltose; Raf – raffinose; Pan – panose)

The content of sucrose (which is the main source of the adulteration of honey) in the all tested samples was in conformity with the limits established by the European Codex Honey Standards, which are ≤5 g 100 g^{-1} for honeys in general and up to 10 g 100 g^{-1} for *Citrus* honeys (Codex Alimentarius, 2001).

Small amounts of two trisaccharides, panose and raffinose were found in the tested willow honeys (Figure 2). Raffinose was not find in one sample, while panose – in two samples. The origin of raffinose in floral honey is not clear; it is suggested that raffinose could be nectar constituent or could get in with honeydew contamination (Da Costa Leite *et al.*, 2000).

The total GC area percent of unidentified compounds in the chromatogram varied from 2.04 to 3.01%. The majority of the unidentified peaks eluted from the column at the end of GC run, i.e. in the region characteristic to trisaccharides (Figure 1). Therefore, it is likely that the majority of these peaks are representing various trisaccharides or other oligosaccharides.

The correlation coefficients between willow content and corresponding sugar, as well as between individual sugars were calculated. The correlation between willow pollen content in the honey and identified carbohydrates was weak; the calculated coefficients were not significant statistically. In the willow honey the correlation between glucose and fructose was strongly negative (R=–0.94, significant at the 0.01 level), while the correlation between some disaccharides was strongly positive: saccharose and maltose (R=0.88, significant at the 0.01 level), turanose and trehalose (R=0.93, significant at the 0.01 level). Medium correlation was noticed between saccharose and turanose (R=0.76, significant at the 0.01 level), and saccharose and trehalose (R=0.76, significant at the 0.01 level). It can be seen, that botanical source of honey cannot be concluded from honey sugar. All observed data were normally distributed (Gaussian distribution law).

Conclusions

The mono-, di- and trisaccharide composition of 7 honeys from willow origin were studied by GC–FID, after the trimethylsilylation of the carbohydrates. Fructose and glucose are dominant in all samples, their amount varied from 32.92 to 42.29% of honey. Fructose glucose ratio was below 1 in six samples, due to the higher amount of glucose in these samples. The amount of sucrose, like monosaccharides, coincides with the recommendations of European Codex Honey Standards; it varied from 0.12 to 0.25%.

The strong correlation between the willow pollen content in the honey and identified carbohydrate was not observed.

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