# **USING RATIO OF THE MAIN SUGARS AND SOME OLIGOSACCHARIDES CONTENT TO INDICATE MARKET'S HONEY AUTHENTICITY**

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### **ABSTRACT**

The ratio of main sugars and oligosaccharides content present in honey, are used nowadays successfully to determine honey authenticity. Therefore, their quantification by instrumental analysis so-called HPAEC-PAD, has been the main aim of our study. Based on the complexity of sugars present in honey, there exists a varying change of their ratio, based on the botanical source, season, and so, needs to be considered. Through the results of the tests performed, we have been able not only to identify the content of main sugars, but also the effect of the specific ratio between some of them, for the characterization or using it as an indicator of honey genuineness. In the present paper it is shown the influence of the sugars ratio on the physical properties of honey such as crystallization, which makes the authentication process easier. As an evidence based procedure presented here, it can be declared that, if the ratio of sugars that produce easily crystallizable media is taken in consideration, it can be served as a selection criteria for the evaluation process or as a characterization factor of authenticity. For the same reason, it can be used also the concentration of some oligosaccharides in honey. Since their content in honey is very complex, although we have considered only five of them, their concentration and the ratio between them were compared with values referred in the specialized literature for genuine honey. Our study is a modest contribution in the field of honey authenticity, using the described procedure presented.

**Keywords:** Honey, sugars, ratio, adulteration, authenticity.

#### **INTRODUCTION**

Honey is a complex mixture of carbohydrates and other minor components such as minerals, proteins etc. Its dry matter is 95% sugars, where fructose and glucose (both monosaccharides) are the predominant sugars. Honey composition is variable due to weather condition, season, bee management and floral source (Wang & Li, 2011). Fructose and glucose are the two main carbohydrates, but there are other minor components consisting of about 25 oligosaccharides quantified in honey (Gallego-Picó, Garcinuño-Martínez, & Fernández-Hernando, 2013). Blossom honeys and honeydew can differ in sugar composition due to the fact that honeydew honey contains higher amount of oligosaccharides, mainly trisaccharides such as: raffinose and melezitose, which are not found in blossom honeys (Bogdanov, Ruoff, & Oddo, 2004). 75% of all sugars in honey are represented from monosaccharides and 10-15% by disaccharides and other higher oligosaccharides (Gallego-Picó et al., 2013). In literature is reported that honey oligosaccharides profile have a potentially valuable role to play in detecting adulteration of honey with industrial syrups (Morales, Corzo, & Sanz, 2008), and the assessment of floral origin of honey (Goodall et al., 1995).

Botanical and geographical origin influence the sugar composition in honey. The sum of fructose and glucose and their ratio, are found useful and are used as indicators to discriminate between honeydew and blossom honey. Generally, honeydew honey represents low values of fructose and glucose and their sum, but it has high fructose/glucose ratio. Although there are some exclusion, because some blossom honeys such as: Heather (*Calluna vulgaris*), Acacia (*Robinia pseudoacacia* L.) and Thymus (*Thymus* spp.) show high ratio of fructose/glucose too (Santos-Buelga & González-Paramás, 2017). The appreciation of honey is recorded since ancient times, till the 18<sup>th</sup> century, due to the fact that it was the only concentrated sweetener available. The adulteration of natural honey with artificial honey is very common and it has been reported for many centuries (Prodolliet & Hischenhuber, 1998).

Currently, inexpensive sugars or industrial syrups are used to adulterate honey, with wellknown adulterants being sugar syrups, such as corn syrup (CS), high-fructose corn syrup (HFCS), glucose syrup (GS), sucrose syrup (SS), inverted syrup (IS), or high-fructose inulin syrup (HFIS), which are produced from sugar cane or sugar beet (Molan, 1996; Singhal et al., 1997; Ruoff & Bogdanov, 2004; Soares et al., 2017; A. J. Siddiqui et al., 2017). Sugar syrups are cheap and are commercially available for fraudulently practices, because they imitate the natural composition of honey, in specific the main sugars found (Prodolliet & Hischenhuber, 1998). Fructose is the major component found in honey, except for some honeys originating from dandelion (Taraxacum officinale), rape (Brassica napus) and blue curls (Trichostema lanceolatumi), where the content of glucose is found to be higher (Cavia et al., 2002). In general, industrial syrups have a high content of maltose (around 29 g/100g), which in natural honey it doesn't exceeds 5 g/100g, and it is used as indicator to assess honey adulteration. Values of ratio maltose/isomaltose in honey above 0.51 (but below 1) are suggested as indication of adulteration with high fructose corn syrup (HFCS) (L W Doner, White, & Phillips, 1979). Also, in literature ratios of sucrose/turanose, maltose/turanose are used to evaluate potentially adulteration of honey with industrial syrups (Horváth & Molnár-Perl, 1997; Cotte et al., 2003; Ruiz-Matute et al., 2010).

In most of the cases, industrial syrups are produced by chemical and enzymatic transformations, which leads to the formation of different oligosaccharides not present in natural honey, and their amount is used as a key criterion in assessing possible mixture of pure honey with industrial syrups (Wang & Li, 2011). Sugars present in honey are described in different works published (I. R. Siddiqui, 1970; Landis W. Doner, 1977) and carbohydrate analysis it is used to authenticate honey, in regard to composition and botanical origin (W White, 1980; Cotte et al., 2003). Also, crystallisation phonemena that occurs naturally in honey could be used as indicator of honey authenticity. In literature values of Glucose/Water  $(G/W) \le 1.7$  are reported when the process of crystallisation is delayed or will not occur, when this ratio is between 1.7 and 2.0 honey will crystallise slowly within one year and values above 2.1 in honey, will generate a fast crystallisation (Ruoff et al., 2007). Also, the ratio of Fructose/Glucose (F/G) it is reccomended to be used as indicator of crystallisation, and values above 1.3, will mean that honey will remain liquid during storage or will crystallize very slowly (Pita-Calvo, Guerra-Rodríguez, & Vázquez, 2017). However, the tendency of honey to crystallise is also influenced by the size of the sugar crystals, the presence of pollen and wax, thermal treatment and storage conditions (Hamdan, 2010). For the determination of honey oligosaccharides the most used technique is HPAEC-PAD, where sugars are separated by anion-exchange chromatography, due to the fact that they are very

weak acids pKa = 12-13, and at high pH values 12-14 they partially or totally ionize (Pita-Calvo et al., 2017), but also other techniques such as GC-FID are used (Kaškonienė, Venskutonis, & Čeksteryte, 2011).

### METHODOLOGY

#### Honey samples

For realizing the objective of our work, we have carried out tests for identification, evaluation and characterization of important sugars in some honey random samples taken from local markets in Tirana, Albania. 11 samples were involved in the study, including local brands and imported ones, where the major part of them were multifloral, and three samples were unifloral: Acacia, Strawberry tree and Chestnut. The samples were classified based on the information given on the label.

#### Moisture determination

Moisture was determined by measuring the refractive index at 20°C using Digital refractometer, according to the International Honey Commission Methods (Bogdanov, 2009).

#### **Sugar Analysis**

#### Chemicals and materials

Glucose , fructose, sucrose, trehalose and maltose, were purchased from Tokyo Chemical Industry, TCI (Europe, Belgium); turanose, isomaltose, melesitose and melibiose, were obtained from Tokyo Chemical Industry, TCI (Tokyo, Japan). All chemicals used whose purity were of analytical purity grade. Ultra-pure water (MicroPure water purification system, 0.055  $\mu$ S/cm, TKA, Thermo Fisher Scientific, Niederelbert, Germany) was used to prepare standard solutions and blanks. Syringe filters (13 mm, PTFE membrane 0.45  $\mu$ m) were purchased from Supelco (Bellefonte, PA). Filter paper (Whatman No. 1) was supplied by Merck (Darmstadt, Germany).

*High-performance anion-exchange chromatography with pulsed amperometric detection* (*HPAEC/PAD*)

0.3 g of honey was weighted and dissolved in 1000 mL of ultra-pure water, and the solution was filtered through a 0.45  $\mu$ m filter before analysis. Chromatographic experiments were performed using DIONEX ICS 3000 DP liquid chromatography system (Dionex, Sunnyvale, CA, USA) equipped with a quaternary gradient pump (Dionex). The carbohydrates were separated on a Carbo Pac®PA100 pellicular anion-exchange column (4×250 mm, particle size - 8.5  $\mu$ m, pore size – microporous,<10 Å, (Dionex) at 30 °C. The mobile phase consisted of the following linear gradient (flow rate, 0.7 mL/min): 0–5 min, 15% A, 85% C; 5.0–5.1 min, 15% A, 2% B, 83% C; 5.1–12.0 min, 15% A, 2% B, 83% C; 12.0–12.1 min, 15% A, 4% B, 81% C; 20.0–20.1 min 20% A, 20% B 60% C; 20.1–30.0 min 20% A, 20% B 60% C; where A was 600mM sodium hydroxide, B – 500mM sodium acetate and C was ultrapure water. Before the analyses, the system was preconditioned with 15% A, 85% C, for 15 min. Each sample (25  $\mu$ L) was injected with an ICS AS-DV 50 autosampler (Dionex). The electrochemical detector consisted of gold as the working and Ag/AgCl as the reference electrode.

#### **RESULTS & DISCUSSIONS**

In Table 1 is shown the sum of fructose and glucose, which is a criteria established in standard of honey, related to honey quality, where for blossom honey the sum must be higher than 60 g/100g honey and for honeydew higher than 45 g/100g (Codex Alimentarius, 2001; EU Directive 2001/110/EC). Also, in Table 1 are shown the ratio of fructose/glucose and

glucose/water that give indication related to honey crystallization process and determination of botanical origin.

Sample	Botanical origin	F+G (g/100g)	Ratio F/G	Ratio G/W	Ratio S/M
S1*	Mixture of honey from EU and non EU	62.41	1.27	1.83	4.83
S2 <sup>a</sup>	Multifloral honey 'Raw Honey'	71.19	1.31	2.77	12.21
<b>S</b> 3	Strawberry tree honey (Arbutus unedo L.)	60.81	1.36	1.39	9.43
<b>S4</b>	Multifloral honey	67.31	1.43	1.51	4.29
S5 <sup>a</sup>	Multifloral honey	62.70	1.14	1.93	4.86
<b>S6</b>	Acacia honey (Robinia pseudoacacia L.)	61.24	1.28	1.68	25.56
<b>S7</b>	Multifloral honey	60.00	1.17	1.76	2.39
S8 <sup>a</sup>	Chestnut honey (Castanea sativa Mill.)	67.66	1.31	1.76	1.52
<b>S9</b>	Multifloral honey	68.88	0.93	2.10	2.72
<b>S10</b> * <sup>a</sup>	Multifloral honey	65.94	1.30	1.60	0.80
S11	Multifloral honey	66.29	2.21	1.16	0.53

F-fructose; G-glucose; S-sucrose; M-maltose; W-water

\*imported sample; <sup>a</sup>crystallised sample

None of the samples was labelled as '*Honeydew Honey*' and from the results was observed that in all samples analyzed, the sum of fructose and glucose was higher than 60 g/100g, which is in accordance with the limit for blossom honey set in honey standard. Sample S2 had the highest amount 71.19 g/100g of honey. Must be mentioned the fact that the ratio of fructose/glucose (F/G) and glucose/water (G/W), are indicators of honey crystallization, which is a natural process in pure honey, and can be used as indicator of honey authenticity toward composition and processing.

In Table 1, it is shown that sample S1 has a F/G ratio 1.27 and G/W 1.83, and theoritacally this sample should had been crystallised, but it is liquid, which arises doubts related to thermal processing, that can delay or inhibit crystallisation by melting glucose crystals (Subramanian, Umesh Hebbar, & Rastogi, 2007). Samples S2, S3, S4, S5 and S6 show normal behaviour related to ratio F/G and G/W and their physical state. Sample S6, which is labelled as '*Acacia Honey*' is liquid and that is typical for Acacia honey (Livia Persano Oddo et al., 2004). Samples S7 and S8 show a strange behaviour, because sample S7, theoritacally had to be crystallised (judging from F/G and G/W ratio), but this sample is liquid, which it can be assumed that the sample has been processed with heat treament or addition of chemical substances such as isobutyric acid or sorbic acid to inhibit granulation (Singhal et al., 1997). While sample S8, that is labelled as '*Chestnut Honey*', judging by values in Table 1 of F/G and G/W ratio, corresponds to physical state that is crystallised in form of granules, but in fact shuold be mentioned that in general honey from sweet chestnut during storage remains liquid.

Also, sample S9, had the lowest value of F/G ratio 0.93, where in general fructose is the predominant monosaccharide in honey, except honey from rape, dandelion where glucose is found in higher content (Cavia et al., 2002). The sample is labelled as '*Multifloral Honey*', and also has a higher ratio of G/W 2.1, and this sample had to be crystallized, but it is liquid, which gives indication of being potentially adulterated with invert sugar or glucose syrup, as the latter does not crystallize. The process of crystallisation in sample S10 (from the ratios it

should be liquid), it can be assumed that the crystallisation process was induced during processing, to produce cream honey, which is a product in high demand by consumers. Also, it is evident that the ratio of S/M is below 1, and it indicates that maltose is in higher concentration. Sample S11, shows the highest ratio of F/G 2.21 in all analyzed market samples, which in fact is a large deviation from normal value that is 1.14, and it is not normal for natural honey. The ratio G/W 1.16 is also relatively very low. Also, the value S/M 0.53 indicate higher amount of maltose. High values of fructose are typical for corn syrups with high fructose, and sample S11 should be regarded as potentially adulterated with this type of syrup.

At the same time the ratio of fructose/glucose and glucose/water are used as indicators to identify and discriminate unifloral honeys (L. Persano Oddo, Piazza, Sabatini, & Accorti, 1995; Thrasyvoulou & Manikis, 1995; Livia Persano Oddo et al., 2004). For Acacia honey, the ratio of F/G above 1.55 is determinant in acacia's honey donomination (Livia Persano Oddo et al., 2004). Sample S6, labelled as '*Acacia Honey*', shows a F/G ratio 1.28, which can be obtained that glucose is in higher amount than usual, and these honey sample will not be accepted to be donominated as '*Acacia Honey*', when trading in EU countries.

Table 2, shows the oligosaccharides: trehalose, melibiose, isomaltose, melesitose and turanose, quantified in honey market samples and the ratio of maltose/isomaltose, sucrose/turanose, maltose/turanose which are used to evaluate honey adulteration by industrial syrups, like high fructose corn syrup and corn syrup, that are obtained by enzymatic reactions, which involves the formation of oligosaccharides.

Sugars	<b>S1</b>	<b>S2</b>	<b>S3</b>	<b>S4</b>	<b>S5</b>	<b>S6</b>	<b>S7</b>	<b>S8</b>	<b>S9</b>	<b>S10</b>	<b>S11</b>
Trehalose	0.093	0.028	0.047	0.007	0.012	0.336	0.336	0.080	0.365	0.024	0.018
Melibiose	0.662	0.047	0.147	0.267	0.178	0.012	0.012	0.856	0.166	0.174	0.014
Isomaltose	2.743	1.835	0.002	2.837	3.179	0.426	2.404	1.236	0.365	3.987	2.973
Melesitose	3.087	0.872	0.001	0.931	0.408	2.825	0.324	0.126	0.001	3.676	6.031
Turanose	1.695	0.709	0.743	3.280	3.453	1.591	0.647	0.986	3.217	2.46	4.043
Ratio M/I	0.236	0.238	230.211	0.406	0.342	0.789	0.607	3.683	11.311	1.557	3.399
Ratio S/T	1.844	7.510	5.549	1.505	1.530	5.393	5.400	6.996	3.492	2.026	1.319
Ratio M/T	0.382	0.615	0.589	0.351	0.315	0.211	2.256	4.617	1.284	2.523	2.500

**Table 2:** Oligosaccharides (%) quantified in honey market samples and some of their ratio

M/I – Maltose/Isomaltose; S/T- Sucrose/Turanose; M/T - Maltose/Turanose

The values of trehalose and turanose were in the range 0.007-0.365% and 0.647- 4.043%, respectively. Trehalose content was in accordance with data reported in literature (Kaškonienė et al., 2011), and the content of turanose, also reported in literature for unifloral honeys from Spain ranged from 0.89 to 4.56% (De la Fuente et al., 2007). Melebiose content was in the range 0.012-0.865%, isomaltose 0.002-3.987% and melesitose 0.001-6.031%. 8 samples out of 11 result to have more than 1% of isomaltose, which is the maximum amount reported in literature for pure honey (Kaškonienė et al., 2011). From the data, it can be assumed that high melesitose concentration in samples S1, S6, S10 and S11 are not typical for blossom honey, because its presence is used to discriminate honeydew from blossom honeys, but also high amount may be present in industrial syrups.

The authentic honey samples are reported to have ratio of oligosaccharides as follows: maltose/isomaltose = 0.5-21.8; maltose/turanose = 0.43-6.7 and sucrose/turanose = 0.05-1.9 (Horváth & Molnár-Perl, 1997), and judging from the results in Table 2, sample S3 show a

M/I ratio 230.211, which is very high, and it can be assumed that starch syrup may have been used to adulterate honey (Kaškonienė et al., 2011). The mixture of honey with high amount of maltose syrups and sucrose syrup, may be identified by the ratios of S/T and M/T (Horváth & Molnár-Perl, 1997), and values in bold show deviation from the reported data in literature.

# CONCLUSIONS

From this work can be clearly proved that the sum of fructose and glucose, the ratio of main sugars in honey (*fructose, glucose, sucrose and maltose*) and the ratio glucose/water, being an important indicator, can be used to discriminate and verify the authenticity of honey. The ratio fructose/glucose and glucose/water could be used to evaluate honey crystallization and from that have more information regarding processing (which may be used to delay crystallization when applying heat treatment, or filter out pollen, wax, which can influence and serve as initiator to start crystallization). In this case it would be suitable to adopt and characterize sugar profile in honey by food control authorities, due to the sophisticated techniques and methods used nowadays from fraudsters to adulterate honey composition, but also misrepresent the botanical source.

In real terms, it was observed that the sum of the fructose and glucose content in all analyzed samples was higher than 60 g/100g of honey, which is the minimum threshold for blossom honey officially referred in Codex Alimentarius standard of honey (Codex Stan 12-1981) and EU Directive 2001/110/EC. A discrepancy of the fructose/glucose ratio in tested samples S9 (0.93) and S11 (2.21) was observed, indicating potentially adulteration with sugar syrups.

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