

## DETECTION OF HONEY ADULTERATION USING HPLC METHOD

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### ABSTRACT

This work deals with the determination of undeclared or illicitly added sugar content in honey samples evaluated using the High-Performance Liquid Chromatography or HPLC with refractive index detection. Labelling of samples was also evaluated in accordance with current legislation. In a total of 21 samples of honey purchased in the fall of 2013, 13 samples were obtained from the regular shopping network, 2 samples were purchase in Health Food stores and 6 samples came directly from local beekeepers and were purchased at the Christmas Markets in Brno.

We have determined the contents of fructose, glucose, sucrose, and oligosaccharides using the HPLC method. We have calculated the basic statistics such as the mean and standard deviation for each sample. Samples have been evaluated according to the Council Directive 2001/110/EC, which lays down limit values for the parameters of honey.

Only four out of 21 honey samples complied with the requirements of Council Directive 2001/110/EC. These were three samples obtained from the regular shopping network and one obtained directly from the local beekeeper. Six samples did not meet the requirements for the sum of fructose and glucose, two samples could not be determined due to the failure to specify the honey type, and fourteen samples failed the requirement of sucrose content.

We have further assessed whether honey samples comply with legislative requirements relating to this product or consumer misleading practices take place. Our analysed samples often lacked indication whether it is a floral honey or honeydew honey; this information was missing in eight out of 21 samples. Samples 5 and 9 did not mention the name of manufacturer. Sample 10 did not mention the country of origin.

**Keywords:** honey; HPLC; labelling; fructose; glucose; sucrose

### INTRODUCTION

Honey is a natural product produced by bee workers from the nectar or honeydew, without any human interference (Roman and Popiela, 2011). It is an easily digestible, energetically valuable food of natural carbohydrate character. It is composed mainly of sugar and water, and also contains other ingredients such as vitamins and minerals (Vallianou, 2014). Honey as a natural food of carbohydrate nature composed mainly of glucose, fructose, organic acids, enzymes (Kňazovická et al., 2011) and solid particles captured by bees while collecting sweet flower juice (nectar), excretions of insects on plant surfaces (honeydew) or on living parts of plants. Bees (*Apis mellifera*) collect the components, transform them, combine them with their own specific substances, store them and let them dehydrate and mature in combs (Decree no. 76/2003 Coll.). "Codex Alimentarius" (2001) defines honey as a non-fermenting sweet substance produced by honey bees from the nectar of plants or from secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, and leave in the honey comb to ripen and mature. According to Council Directive 2001/110/EC (2001), honey is 100% bee product, to which nothing can be added and from which nothing can be removed. Therefore in order to maintain its therapeutic values, it is necessary to deliver it to the consumer in its natural form without any additives and major technological modifications.

Honey is among the most adulterated food products, as it is a natural product with limited production and relatively high cost (Megherbi et al., 2009). Honey adulterations can take place by substitution of botanical and geographical origin, confusion of honeydew honey with floral honey, selling of artificial honey (flavoured sugar solutions), and failure to comply with quality and hygiene requirements (unauthorized quantities of residues of antibiotics and sulphonamides). Honey adulterating may include even heating or storage under unsatisfactory conditions (Čížková et al., 2010). Freshly bottled honey contains virtually no hydroxymethylfurfural (HMF), but its content may increase during storage (Kalábová et al., 2003; Frank, 2010; Bogdanov, 2014). The presence of HMF thus becomes an indicator of food quality deterioration caused by excessive heating during thermal treatment as well as improper and long-term storage, and is also an indicator of possible adulteration (Borkovcová, 2011). Also honey made by feeding bee colonies sugar syrup in the summer and declared as a pure honey, can be judged as adulterated (Titěra, 2006). Honey flavour can be modelled by heating a solution of a monosaccharide with phenylalanine, since almost all phenyl acetic esters are known for possessing honey flavour (Kolínek, 2007).

The natural content of sucrose in honey is (with some exceptions) to 5% (Kameník, 2013). Sugar content of honey depends on its botanical and geographical origin, weather, storage conditions and processing technology (Dobre et al., 2012). Honeydew honey is lower in sugar

than the nectar (floral) honey (Bentabol et al., 2011; Escuredo et al., 2013). Harvesting of honey with high moisture content, or subsequent addition of water to honey can result in honey fermentation and spoilage (Šroll, 2012). Sometimes honey is artificially coloured, because darker honey can give consumers the impression that it is forest honey (Přidal, 2005).

The aim of this study was to detect illegal or undeclared addition of sugar in honey using high performance liquid chromatography (HPLC) and determine whether the honey vendors comply with legal requirements applicable to their product and whether consumers are not deceived.

## MATERIAL AND METHODOLOGY

We have analysed a total of 21 honey samples. Thirteen honey samples came from the regular shopping network, two samples were purchase in Health Food stores and six samples were obtained directly from local beekeepers (Table 1). We have prepared 10% solution from the relevant sample of honey. After mixing, two parallel samples were prepared, centrifuged at 18000 rpm for 5 min and analysed by HPLC. Conditions of analysis: column: steel 7.8 x 300 mm, packing: Rezex RCM-Monosaccharide Ca<sup>2+</sup> (8%), temperature: 80 °C, mobile phase: deionized water, flow rate: 0.8 mL / min, injection volume: 5 µL, pressure: 2.6 MPa, detection: refractometric, detector sensitivity: 0.32. Equipment:

double piston pump LCP 4000, dispense valve D, column oven LCO 101, columns supplied by Phenomenex, differential refractive index detector, laboratory instruments Praha RIDK-102nd. Further, deionized water was used for HPLC, standards used were of HPLC grade, and laboratory centrifuge was Hobbolab 2110 (France). Evaluation software was Clarity.

Calibration: 0 - 1 - 2 - 5 g/100 mL of maltose, sucrose, glucose, fructose, glycerol, methanol, ethanol (Merck, Germany).

Samples were evaluated according to the Council Directive 2001/110/EC, which lays down limit values for the parameters of honey.

We have calculated the basic statistics such as the mean and standard deviation for each sample (n = 3).

## RESULTS AND DISCUSSION

We have determined the amounts of fructose, glucose, sucrose, and oligosaccharides using the HPLC method. Besides floral honeys, samples contained also honeydew honey, for which different values apply than those listed in Council Directive 2001/110/EC. Table 2 shows that only four out of 21 samples met the requirements of **Council Directive 2001/110/EC of 20 December 2001 relating to honey**. These were three samples from regular shopping network and one obtained directly from the beekeeper.

**Table 1** List of honey samples.

Sample no.	Honey type	Purchased from	Country of origin	Additional information
1	Not stated	Beekeeper	Czech Republic	Honey from the Moravian Karst
2	Floral	Beekeeper	Czech Republic	Czech forest honey, KLASA
3	Floral	Shopping network	Blend of EU and non-EU honeys	Meadow
4	Not stated	Shopping network	Blend of EU and non-EU honeys	Mixture of floral and honeydew honey in certain proportions
5	Not stated	Shopping network	Blend of EU and non-EU honeys	Forest honey, Mixture of floral and honeydew honey in certain proportions
6	Floral	Shopping network	Blend of EU and non-EU honeys	Meadow
7	Not stated	Health Food	Czech Republic	Bio buckwheat honey
8	Floral	Shopping network	Blend of EU and non-EU honeys	Meadow
9	Floral	Shopping network	Blend of EU and non-EU honeys	-
10	Floral	Shopping network	Not stated	Bio
11	Floral	Shopping network	Cuba, Mexico, Nicaragua	-
12	Honeydew	Shopping network	Blend of EU and non-EU honeys	Forest
13	Floral	Shopping network	Blend of EU and non-EU honeys	„Honey bear“- honey in bear-shaped bottle
14	Not stated	Beekeeper	Czech Republic	Acacia
15	Floral	Beekeeper	Czech Republic	-
16	Not stated	Beekeeper	Czech Republic	Blended honey
17	Floral	Beekeeper	Slovak Republic	Slovak honey
18	Not stated	Shopping network	Czech Republic	Blend of honeydew and floral honeys
19	Floral	Shopping network	Czech Republic	Bio meadow honey
20	Not stated	Health Food	Greece	-
21	Floral	Shopping network	Czech Republic	-

**Table 2** The average content of carbohydrates (g/100 g) provided by HPLC method (n = 3); Oligosaccharides I (maltosa), Oligosaccharides II (maltotriosa).

Sample no.	Fructose [g/100 g]	Glucose [g/100 g]	Sucrose [g/100 g]	Oligosaccharides I [g/100 g]	Oligosaccharides II [g/100 g]
1	31.2 ±0.62	28.4 ±0.57	7.9 ±0.16	2.1 ±0.04	10.8 ±0.21
2	38.2 ±0.76	37.9 ±0.76	6.7 ±0.13	0.5 ±0.01	0.7 ±0.01
3	38.4 ±0.62	41.6 ±0.89	5.4 ±0.35	0.2 ±0.25	0.40 ±0.21
4	39.9 ±0.68	39.6 ±0.79	5.4 ±0.68	0.3 ±0.56	0.4 ±0.58
5	38.1 ±0.46	39.2 ±0.82	5.9 ±0.59	0.3 ±0.35	0.4 ±0.36
6	41.4 ±0.32	32.0 ±0.45	3.8 ±0.55	0.2 ±0.37	0.3 ±0.78
7	36.9 ±0.55	33.5 ±0.52	8.6 ±0.79	1.2 ±0.56	5.1 ±0.88
8	42.1 ±0.76	32.2 ±0.78	4.5 ±0.46	0.3 ±0.51	0.6 ±0.49
9	34.7 ±0.45	31.2 ±0.57	8.8 ±0.32	0.6 ±0.32	1.2 ±0.78
10	26.6 ±0.51	29.9 ±0.61	2.3 ±0.58	0.2 ±0.11	0.5 ±0.68
11	34.2 ±0.48	29.0 ±0.85	8.5 ±0.87	0	1.1 ±0.23
12	36.6 ±0.73	30.1 ±0.55	12.7 ±0.87	0.7 ±0.58	2.0 ±0.46
13	45.0 ±0.67	33.7 ±0.32	3.9 ±0.98	0.2 ±0.69	0.4 ±0.28
14	43.9 ±0.69	27.9 ±0.66	7.9 ±0.58	0.1 ±0.89	2.3 ±0.25
15	32.8 ±0.85	33.9 ±0.67	6.4 ±0.69	0.9 ±0.25	1.2 ±0.89
16	24.7 ±0.88	22.4 ±0.42	8.0 ±0.85	0.5 ±0.39	1.1 ±0.82
17	27.3 ±0.69	26.0 ±0.51	5.6 ±0.25	0.1 ±0.54	1.3 ±0.78
18	30.7 ±0.89	23.1 ±0.78	4.3 ±0.34	0.9 ±0.65	3.6 ±0.71
19	28.0 ±0.76	27.7 ±0.45	5.7 ±0.54	0.4 ±0.23	1.4 ±0.59
20	23.5 ±0.47	18.1 ±0.58	3.9 ±0.48	1.0 ±0.66	1.2 ±0.53
21	24.4 ±0.65	22.6 ±0.36	3.9 ±0.35	0	0.2 ±0.48

Six samples did not meet the requirements for the sum of fructose and glucose, two could not be included because failing to specify the type of honey and fourteen samples failed the requirement of sucrose content.

The above mentioned directive states that honey if placed on the market or used in any product intended for human consumption must meet the following criteria: the sum of fructose and glucose in floral honey should be no less than 60 g / 100 g and no less than 45 g / 100g for honeydew honey and blends of honeydew honey with floral honey.

Sucrose content: generally, not more than 5 g / 100g; not more than 10 g / 100 g for acacia honey (*Robinia pseudoacacia*), alfalfa honey (*Medicago sativa*), banksia honey (*Banksia menziesii*), sulla honey (*Hedysarum*), eucalyptus honey (*Eucalyptus camadulensis*) leatherwood honey (*Eucryphia lucida*, *Eucryphia milliganii*) and citrus honey (*Citrus spp.*), and not more than 15 g / 100 g for lavender honey (*Lavandula spp.*) and starflower honey (*Borago officinalis*).

Limits for the oligosaccharides are not determined by legislation, but their value should be around 10 % in honeydew honeys and between 2 and 3 % in floral honeys.

Honey samples have been further assessed for their compliance with legislative requirements relating to the product or whether the consumers are not deceived. As reported by Titěra and Vořechovská (2010), the compulsory indications on the label include manufacturer's business name and address, quantity, date of minimum durability or 'best before' date (just the month and year),

the country of origin (CR, EC or non-EC). According to Horňáčková (2009), the most frequently reported minimum durability of honey is two years. Yet we can see much longer minimum durability indicated on honey labels. Horňáčková (2009) further states that each beekeeper can determine the minimum durability of his/her own honey based on laboratory tests, when honey even after his/her determined period of minimum durability meets all requirements for wholesomeness and quality. For products intended to be supplied into stores outside the local district, it is important to indicate the registration number assigned by the competent State Veterinary Administration. Other important information, which must be included on the label, is the type of honey, i.e. whether it is floral (nectar) or honeydew honey. According to Decree no. 113/2005 Coll., the label indications must not include words like true, fresh, pure, home-made, high-quality, natural or healing. It addition, it must not give any information about the preventive effects or healing power of honey. The compulsory indications on the label of honey from the beekeeper include the name and address of the beekeeper, quantity, date of minimum durability or 'best before' date (just the month and year) and information referring to its floral or honeydew origin.

Such label is not necessary, if honey is sold e.g. at 'yard sale'. Our analysed samples often had no indication about the honey origin (floral or honeydew). Specifically, this information was missing on eight out of 21 samples. Samples 5 and 9 lacked any indication about the manufacturer. The country of origin was absent only on

sample 10. The study by **Vlkovič, Vorlová and Přidal (2011)**, which dealt with the issue of proper honey labelling, reported that the most common deficiency in the labelling of honey in stores is the absence of honey type indication.

**Přidal (2012)** therefore suggests that the use of the word 'forest' and the like be restricted by law, thereby to ensure that the label is not misleading and ambiguous. Another possible solution **Přidal (2013)** sees in the absence of any identification of the honey type on the label. Honey type could be indicated only if specific properties prevail which are characteristic of a given generic honey (e.g. sunflower honey, acacia honey, honeydew honey, etc.).

## CONCLUSION

The HPLC method was used to determine the amounts of fructose, glucose, sucrose and oligosaccharides in samples of honey purchased in the Czech Republic. Only four out of 21 samples complied with the requirements set out in Council Directive 2001/110/EC relating to honey. These were three samples from the regular shopping network and one purchased directly from the beekeeper.

The most common deficiency in evaluating the compliance with labelling requirements was the absence of honey type identification. In total, this information was missing in 8 samples. Often, the type of honey was replaced by the word 'forest', which is optional and can mislead the consumer into believing that the product is honeydew honey. This absence of type identification caused also difficulties in assessing the content of sugar, as it was not clear into which category the honey should be classified. Two samples do not identify the manufacturer.

Manufacturers often inundate labels of their products with optional information, which can create a feeling of product exceptionality among the customers and mislead them. While honey adulteration usually does not endanger consumers' health, in any event such practice deceives consumers because instead of natural honey with many favourable properties they consume factory product based on sucrose and starch.

Existing legislation provides for the evaluation of honey rather inaccurately as it does not define the generic status of honey, does not distinguish between floral and honeydew honeys and does not limit the use of the word 'forest' in labelling of honey. The results show that the consumer cannot entirely rely on always buying properly identified honey of the highest quality in specialized stores or regular shopping network. Possible improvements can be brought about by the Regulation no. 1169/2011 of the European Parliament and of the Council, which specifies the mandatory particulars that must be included on packaging.

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