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DETERMINATION OF ORGANIC ACIDS IN HONEY SAMPLES FROM LATVIAN MARKET BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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Abstract
Honey is a naturally sweet product, which is produced by honeybees (*Apis mellifera*). Honey is a natural source of antioxidants and has been known to mankind since ancient times. Honey contains approximately 200 different compounds. Organic acids can be used as an indicator to detect the freshness, authenticity and acidity of honey. The aim of this research was to determine and quantify organic acids such as oxalic, L-tartaric, D-quinic, L-malic, L-ascorbic, citric, fumaric and succinic in honey samples from Latvian market u sing high-performance liquid chromatography. The chromatographic separation of organic acids was carried out with PerkinElmer C18 (4.6 mm × 250 mm I.D, particle size 5 mm) analytical column at the temperature of 35 °C in wavelength at 210 nm. The obtained results showed that the analysed honey samples contain L-tartaric, D-quinic, L-malic, L-ascorbic, citric, fumaric and succinic acids. The concentration of these acids was found to be variable. Oxalic acid was not detected in the analysed honey samples. L-tartaric acid was the main acid in all analysed honey samples.
Key words: honey, organic acids, high-performance liquid chromatography.

Introduction

Honey has been used in traditional medicine since ancient times due to its nutritional value and therapeutic effect (Conti *et al.*, 2018). Honey is a complex natural mixture, which mostly consists of carbohydrates, but it also contains around of 200 components such as enzymes, amino acids, organic acids, vitamins, phenolic compounds and minerals. The qualitative and quantitative content of chemical compounds, organoleptic properties of honey depend on many factors such as floral origin, geographical and climate conditions (Da Silva *et al*., 2016).

The content of organic acids in honey is approximately 0.5% of the fresh weight of honey (Mato *et al.*, 2003). Despite their low quantity, organic acids play an important role to many properties of honey such as organoleptic, physical and chemical (Chakir *et al.*, 2016; Aljohar *et al.*, 2018). Organic acids can also be used as indicators to detect the freshness and authenticity of honey (Tezcan *et al.*, 2011). It has been reported that organic acids are the contributors to antibacterial and antioxidant activities in honey (Alonso-Torre *et al.*, 2006).

Organic acids such as acetic, citric, formic, glutaric, fumaric, succinic, D-gluconic, oxalic, D-glucuronic, L-malic, propionic, D-quinic, L-tartaric and many others are present in honey (Mato *et al.*, 2003; Nozal *et al.*, 2003; Tezcan *et al.*, 2011). The prevailing organic acid in honey is gluconic acid. Gluconic acid is synthesized from glucose oxidase, which honeybees supply during the ripening process. As predominant organic acid, gluconic acid is present in all types of honey. The quantity of gluconic acid depends on the activity of glucose oxidase (Karabagias *et al.*, 2014). Citric acid also has been found in all types of honey. The concentration of citric acid can be used to distinguish floral honey from honeydew. The content of citric acid in floral honey is noteworthy lower than in honeydew honey (Mato *et al*., 2003; Da Silva *et al*., 2016). Some organic acids can be used to detect the authenticity of honey, for example, 2-methoxybutanedionic and 4-hydroxy-3-methyl-*trans*-2-pentenedioic acids mostly prevail in honey, which is harvested in New Zealand (Shamsudin *et al*., 2019).

It has been reported that organic acids such as oxalic, lactic and formic can be used as an effective treatment against ectoparasitic mite (*Varroa)*. It has been stated as a very serious problem in apiculture in Europe and the USA (Norain Sajid *et al*., 2019). The use of synthetic pesticides can negatively affect human health, that is why the organic acids are used as a treatment to the infestation (Bogdanov *et al*., 2002). It has been found out that the concentration of formic acid in honey can be elevated as it is used as a treatment against the ectoparasitic mite (Matysiak, Balcerzak, & Michalski, 2018).

Organic acids can be used as indicators of aerobic or anaerobic fermentation in honey. These organic acids, which occur in honey during fermentation process, can negatively affect the quality of honey (Boussaid *et al*., 2018).

The qualitative and quantitative content of honey can be dependent on many factors. Mostly it depends on botanical origin, geographical and environmental conditions. Also, the duration of storage can impact the content of organic acids.

The aim of this research was to determine and quantify organic acids such as oxalic, L-tartaric, D-quinic, L-malic, L-ascorbic, citric, fumaric and succinic in honey samples from Latvian market using high-performance liquid chromatography.

Materials and Methods

Experiments were carried out at the laboratories

of the Department of Chemistry, the Faculty of Food Technology at the Latvia University of Life Sciences and Technologies. The object of the research was nine multifloral honey samples, which were purchased from Latvian market. Four honey samples HEU1, HEU2, HEU3, HEU4 were commercially available and bought from distributors. The information on the product labels marked the samples HEU1, HEU2, HEU3, HEU4 as blends of honey from the European Union and non-European Union countries. The production year of the four honey samples was not shown on the product labels. Another five honey samples HLV1, HLV2, HLV3, HLV4, HLV5 were bought directly from Latvian beekeepers. These honey samples were collected in different regions of Latvia in the year of 2018. The honey sample HLV1 was collected from beehives in the northern part of Latvia (Vidzeme). The samples HLV2, HLV3 were harvested from beehives in the central part of Latvia (Zemgale). The samples HLV4, HLV5 were collected from beehives placed in the southern part of Latvia (Latgale).

Determination of organic acids

Preparation of standard solution: analytical standard-grade oxalic, L-tartaric, D-quinic, L-malic, L-ascorbic, citric, fumaric and succinic acids were purchased from Flukaä and Sigma-Aldrich. The mixture of 0.0500 ± 0.0001 g oxalic, 0.1000 ± 0.0001 g L-tartaric, 0.1000 ± 0.0001 g D-quinic, 0.1000 ± 0.0001 g L-malic, 0.0500 ± 0.0001 g L-ascorbic, 0.1000 ± 0.0001 g citric, 0.0205 ± 0.0001 g fumaric and 0.2000 ± 0.0001 g succinic acids was weighted in 50 mL volumetric flask with narrow neck, slowly dissolved in a small portion of deionized water and filled with deionized water till the mark and mixed.

Sample preparation: honey samples were diluted to 50% (w/v) with deionized water, homogenized, and centrifuged (Pro-Research, Centurion Scientific Ltd.) for 10 minutes at 3200 rpm.

Detection of organic acids: chromatographic analysis was carried out using a Shimadzu LC-20 Prominence liquid chromatograph, (Shimadzu USA Manufacturing Inc, Canby, USA), detector DAD SPD-M20A, Solvent Delivery Unit LC-20AD, Column Oven CTO-20A, Autosampler SIL-20A, System Controller CBM-20A and data system LCsolution software. The analytical column PerkinElmer C18 (4.6 mm ´ 250 mm I.D., particle size 5 mm) and temperature of column +35 °C were used for separation of organic acids in wavelength at 210 nm. All analyses of the samples were carried out in gradient conditions. The mixture of acetonitrile (HPLC grade) and 0.05 M KH_2PO_4 (1:99) was used as the mobile phase. Starting flow rate was 1.25 mL min^{-1}. Injection volume was 10 mL. The retention times in the analysed honey samples were compared with the retention times of standards to determine organic acids in the samples.

Statistical analysis

The determination of organic acids was carried out in triplicate. The mean ± standard deviations were used to express the obtained data of this study. The calculations were carried out using Microsoft Office Excel 2016.

Results and Discussion

The obtained results of the study showed that organic acids such as L-malic, L-ascorbic, citric, fumaric and succinic acids were not detected in all analysed honey samples (Tables 1, 2). Oxalic acid was the only organic acid, which was not detected in the honey samples. L-tartaric acid was present in all analysed samples. The determined concentration of L-tartaric acid was very high in all samples. The amount of this acid ranged from 0.508 to 0.698 g 100 g^{-1}. The highest concentration of this acid was found in the sample HLV1, but the lowest concentration of L-tartaric acid was found in the sample HLV3. The concentration of L-tartaric acid could be variable, as the samples were harvested from different floral origin and different regions.

D-quinic acid also was detected in the analysed samples. The range of the acid was from 0.002 to 0.447 g 100 g^{-1}. The highest concentration of D-quinic acid was found in the sample HLV4. The lowest concentration of this acid quantified in the sample HEU4. It has been previously reported (Shamsudin *et al.*, 2019) that a high concentration of D-quinic acid could indicate that the sample HLV4 could be *Erica* sp. honey.

Succinic acid was present in seven of nine honey samples (HLV1, HLV2, HLV3, HLV4, HLV5, HEU2, HEU3). The detected concentration of succinic acid ranged from 0.003 to 0.139 g 100 g^{-1}. The highest concentration was found in the sample HLV5. It was found that the concentration of succinic acid in floral honeys from different cities of Santa Catarina state in Brazil ranged from 0.013 to 0.096 g 100 g^{-1}. The highest content of succinic acid was found in bracatinga honeydew honey, where it ranged from 0.484 to 0.672 g 100 g^{-1} (Brugnerotto *et al*., 2019). The high concentration of succinic acid was found to be characteristic of *Quercus* sp. honey (Mato *et al*., 2003).

L-ascorbic acid, which is well known as an antioxidant compound, also was found in the analysed honey samples. The concentration of the acid was lower than the concentration of L-tartaric, D-quinic and succinic acids. The detected content of L-ascorbic acid in the analysed samples ranged from 0.001 to 0.020 g 100 g^{-1}. Among all analysed samples, the sample HLV1 exhibited the highest concentration of L-ascorbic acid. The presence of L-ascorbic acid

Table 1

Oxalic, L-tartaric, D-quinic and L-malic acid content in the analysed honey samples

Sample	Oxalic acid, g 100 g^{-1}	L-tartaric acid, g 100 g^{-1}	D-quinic acid, g 100 g^{-1}	L-malic acid, g 100 g^{-1}
HLV1	ND	0.698 ± 0.04	0.029 ± 0.01	0.012 ± 0.02
HLV2	ND	0.608 ± 0.03	0.268 ± 0.02	ND
HLV3	ND	0.508 ± 0.02	0.221 ± 0.02	0.040 ± 0.001
HLV4	ND	0.627 ± 0.03	0.447 ± 0.03	ND
HLV5	ND	0.624 ± 0.03	0.245 ± 0.02	ND
HEU1	ND	0.611 ± 0.02	0.002 ± 0.001	0.017 ± 0.02
HEU2	ND	0.636 ± 0.03	0.006 ± 0.001	0.060 ± 0.005
HEU3	ND	0.666 ± 0.02	0.018 ± 0.010	ND
HEU4	ND	0.583 ± 0.03	0.004 ± 0.001	0.015 ± 0.03

ND – not detected

Table 2

L-ascorbic, citric, fumaric and succinic acid content in the analysed honey samples

Sample	L-ascorbic acid, g 100 g^{-1}	Citric acid, g 100 g^{-1}	Fumaric acid g 100 g^{-1}	Succinic acid, g 100 g^{-1}
HLV1	0.020 ± 0.005	0.028 ± 0.002	0.001 ± 0.0005	0.011 ± 0.04
HLV2	0.007 ± 0.01	0.015 ± 0.003	0.001 ± 0.0002	0.024 ± 0.02
HLV3	0.001 ± 0.02	0.012 ± 0.002	ND	0.012 ± 0.002
HLV4	0.005 ± 0.002	0.043 ± 0.02	0.001 ± 0.0002	0.087 ± 0.003
HLV5	0.006 ± 0.003	0.092 ± 0.03	ND	0.139 ± 0.05
HEU1	0.004 ± 0.001	0.011 ± 0.002	ND	ND
HEU2	0.001 ± 0.0001	ND	ND	0.017 ± 0.003
HEU3	0.008 ± 0.0005	0.030 ± 0.02	0.001 ± 0.0003	0.003 ± 0.0005
HEU4	ND	0.014 ± 0.005	ND	ND

ND – not detected

and its content in honey could be dependent on many factors such as floral sources and geographical origin (Moniruzzaman *et al.*, 2013; Strelec *et al*., 2018). It was reported that the content of L-ascorbic acid in Romanian honeydew honey was up to 0.013 g 100 g^{-1}, but in Polish honeydew honey it was up to 0.014 g 100 g^{-1} (Chis *et al.*, 2016), but the highest concentration of L-ascorbic acid was found in thyme honey, where the concentration was up to 0.057 g 100 g^{-1} (León-Ruiz *et al.*, 2011).

The presence of citric acid was found in eight of nine honey samples. The content of citric acid in those honey samples ranged from 0.001 to 0.092 g 100 g^{-1}. The highest concentration of citric acid was quantified in the sample HLV5, which was higher than it was found in multifloral honeys from the north-western Spain. The amount of citric acid in the Spanish honeys ranged from 0.007 to 0.014 g 100 g^{-1} (Mato *et al*., 2006). According to other authors, the highest concentration of citric acid was common in honeydew honey (Suárez-Luque *et al*., 2002; Serra Bonvehí, Bentanol Manzanares, & Santos Vilar, 2004).

L-malic and fumaric acids were not found in all analysed samples. The highest concentration of L-malic acid was found in the commercially available honey sample HEU2, where it was 0.060 g 100 g^{-1}. The content of fumaric acid in the honey samples was not found higher than 0.001 g 100 g^{-1}. It had been reported that L-malic and fumaric acids were detected in honey in small quantities (Mato *et al*., 1998; Serra Bonvehí, Bentanol Manzanares, & Santos Vilar, 2004; Mato *et al*., 2006; Tezcan *et al*., 2011).

The observed results (Tables 1, 2) showed that there was a variability in the presence of organic acids in honeys. Comparing the analysed honey samples obtained from Latvian beekeepers to commercially available honey samples, which were the blends of honeys from the European Union and non-European Union countries, the honey samples harvested from Latvian beehives were richer of organic acids. Other authors (Suárez-Luque *et al.*, 2002; Matysiak, Balcerzak, & Michalski, 2018) previously had reported that the content of organic acids were variable. The content of organic acids in honey was dependent on many factors such as floral source, the type of honey, geographical conditions (Siddiqui *et al*., 2017). Also, the duration of storage could induce a decrease in the concentrations of organic acids in honey.

Conclusions

The obtained results of this research showed that honey is a natural source of organic acids. The concentrations and presence of analysed organic acids varied in each honey sample. Honey samples, which were purchased from Latvian beekeepers, had a higher diversity of analysed organic acids than the honey samples from the European Union and non-European Union countries. Even the concentration of analysed organic acids was found to be higher in honeys from Latvia than in honeys, which consisted of honeys produced in the European Union and non-European Union countries.

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