Organic Acid Profiles and Contents of Turkish Honeydew and Floral Honeys

Mohammed Ishaq Haroun1, Nevzat Konar2, Ender S. Poyrazoglu2, Iraz Hospolat3, Nevzet Artuk2

Abstract— Organic acid contents and profiles were studied to characterize Turkish honeys. In the case of organic acids, Pine honey was the richest in organic acids, showed organic acid profile composed of tartaric, malic, maleic, citric, succinic and fumaric acids. Oak (Quercus) honeydew honey was similar to pine honeydew honey in containing highest concentration of citric acid. Only three organic acids (malic, citric and fumaric) were identified in cotton and yayla honeys. To some extend, chestnut honeys showed organic acid profile similar to that of pine honey. A profile containing five organic acids (malic, ascorbic, maleic, citric, succinic and fumaric acid) was found in sunflower honey. Multifloral honey was characterized by containing four (malic, ascorbic, citric and fumaric acid) acids.

Keywords— Honey, floral, honeydew, organic acids, HPLC.

I. INTRODUCTION

Honey contains a number of acids which include amino acids (0.05 – 0.1 %) and organic acids (0.17 – 1.17 %) [1, 2]. The total acidity can be used as an indicator of deterioration due to storage, aging or even to measure the purity and authenticity [1, 3]. The acidity of honey inhibits the presence and growth of microorganisms [2]. A number of organic acids are known to occur in honey, including acetic, butyric, oxalic, citric, formic, gluconic, lactic, malic, citramalic, maleic, pyroglutamic, succinic and fumaric acid [4, 5]. The major organic acid is gluconic acid. Gluconic acid is produced in honey by the action of the enzyme glucose-oxidase on glucose. The organic acids present in honey are significant because they interact with other flavors. Gluconic acid has flavor enhancing properties [2]. However, the extraction and identification of organic acids of honeys is difficult task, since they are present in low concentrations. In order to determine honey organic acids many HPLC methods were developed and many organic acids such as D-glucuronic, citric, galacturonic, propionic, pyruvic, butyric, malic, citramalic, quinic, D-glucronic, lactic, formic, glutaric, fumaric, pyruvic, maleic, citric, succinic and fumaric acids were identified in honeys [4, 5, 6, 7]. Some organic acids such as citric acid are very important. Its concentration is used as a reliable parameter for the differentiation of two main types of honey; floral and honeydew honey [8].

II. MATERIALS AND METHODS

A. Honey samples

Total of 30 honey samples of the main two types of honeys, namely, honeydew and floral honeys produced in different regions of Turkey were used. The honeydew honeys were pine (Pinus sp.) (n = 7) and oak (quercus) (n = 1) honeys. While the floral honeys were cotton (Gossypium barbadense) (n = 5), chestnut (Castanea sp.) (n = 4), sunflower (Helianthus annuus) (n = 3), yayla (mountain pasture) (n = 3) and multifloral (n = 4). These samples were obtained from different sources; 10 honey samples from BALPARMAK Company, 9 honey samples from FER Company, two honey samples chestnut (CNH) and citrus (CHR35) were purchased directly from Ankara market, one sample Oak (Quercus) (OKH25) honey was provided from Agriculture Faculty, University of Ankara. The remaining samples were obtained from Honeybee Breeding Association (Table I). The honey samples were stored under in refrigerator until they were used in the analysis.

B. Reagents

Analytical standard grade of tartaric, malic, maleic, citric, ascorbic, succinic and fumaric acids (Merek, Darmstadt, Germany) dissolved in HPLC grade water were used as standard organic acids (200 ppm). Analytical grade of metaphosphoric acid, sulfuric acid and sodium hydroxide pellets were used (Merk, Darmstadt, Germany)

C. Sample Preparation

Sample was prepared according to Suarez-Luque et al. [3,4]. Amount of 7.50 g honey sample was dissolved in 75 ml of HPLC grade water. The pH was adjusted to 10.5 using 0.1 M NaOH and the mixture was stirred for 15 minutes using magnetic stirrer. The pH was then adjusted to 5.00 using 0.1 M H2SO4. The mixture was transferred to a 100 ml volumetric flask, filled up to the mark and mixed. A volume of 10 ml of this solution was filtered through 0.45 μm cellulose acetate membrane. Then solid phase extraction was applied. Solid Shase Extraction cartridge (SB, Chromabond, Machery - Nagel) was activated with 10 ml of 0.1 M NaOH (3 ml / minute). A volume of 10 ml of honey solution was passed through at a flow rate of 0.5 ml / minute. The cartridge was washed with 10 ml of water (3 ml / minute) and organic acids

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were eluted with 4 ml of 0.1 M H2SO4 (0.5 ml / minute). This solution was injected directly in the HPLC.

D. Chromatographic Analysis

Chromatographic analysis were carried out Shimadzu class VP HPLC system (LC-10AD VP) using Diode Array Detector (DAD (SPD-M10A VP) at 215 nm, Teknorama TR-416056, Tracer Extrasil ODS2 5 μM, 25 x 0.4 cm (Teknorama, Barcelona, Spain) column. An HPLC analysis was performed according to the following conditions: mobile phase: 4.5 % metaphosphoric acid (filtered through 0.45 μm membrane, prepared daily), flow rate 0.7 ml / minute, column oven temperature 25oC, injection volume 20 μL. Honey organic acids were determined by external standard method. Retention time and peak areas of the honey sample chromatograms were compared to that of standard organic acids and the organic acids contents were calculated as μg / 100g honey.

TABLE I

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E. Statistical analysis

The means of organic acid contents of the different groups of honey samples were compared by descriptive analysis and one-way analysis of variance using SPSS (IBM, New York, USA) software program.

TABLE II

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III. RESULTS AND DISCUSSION

The organic acids identified in some Turkish floral and honeydew honeys are presented in Table III. It is clear that the pine (group A) honeydew honey was the richest in the organic acids, agreeing with the findings of Nozal et al. [5]. Pine honey showed organic acid profile composed of tartaric, malic, maleic, ascorbic, citric, succinic and fumaric acids, with citric acid dominating (87.22 – 139.42 mg / 100 g), and then followed by succinic acid. It is the first time that tartaric acid was identified and quantified in honey. Like pine honey, oak (Quercus) (group G) honeydew honey also was richest in citric acid (142.28 mg / 100g). But, it was different from pine honeydew honey in that it contained higher concentration of malic acid (113.43 mg / 100g) and devoid of tartaric, maleic and succinic acids. In the case of citric acid content, these results are in agreement with the findings of Talpay [8], who reported that citric acid concentration is used as a reliable parameter for the differentiation of two main types of honey; floral and honeydew honey. Group B (cotton) honey was poor in the number of organic acids. Only malic, citric and fumaric acids were determined, with malic acid dominating (41.51 – 180.64 mg/100g). Among the honey samples used in this study, the highest concentration of malic acid was determined in this type of honey. Therefore, malic acid could be floral marker for cotton honeys. There is no information concerning organic acids of cotton honeys in the available literature. Most of the honey samples of group C (chestnut) contained malic, ascorbic, citric and fumaric acids, with relatively higher concentrations in citric acid (45.18 - 118.44 g / 100g) and half of the honey samples in this group contained tartaric, maleic and succinic acids. Some samples in this group showed organic acid profile similar to that of pine honey, but relatively in lower concentrations than that of pine honeys. Sunflower honeys showed organic acid profile composed of malic, ascorbic, maleic, citric and fumaric acids, with relatively higher concentrations in citric acid (55.64 – 85.54 mg / 100g). Yayla honeys showed organic profile similar to that of cotton honeys (malic, citric and fumaric acids). But, they could be differentiated from each other easily by the amount of malic acid. The amount of malic acid in yayla honeys was too lower (5.91 – 11.77 mg / 100g) in comparison to that of cotton honeys (41.51 – 180.64 mg / 100g). A different organic acid profile containing four organic acids (malic, ascorbic, citric and fumaric) was determined in multifloral honey samples.
IV. CONCLUSION

Pine honey was the richest in organic acids. It contained all the organic acids tested (tartaric, malic, maleic, ascorbic, citric, succinic & fumaric). Oak (Quercus) honeydew honey was similar to pine honeydew honey in containing highest concentration of citric acid. But, it was different from pine honeydew honey in containing higher concentrations of malic acid and devoid of tartaric, maleic and succinic acids. Cotton and yayla honeys were poor in organic acids. The amount of malic acid was too high in cotton honeys. To some extend, chestnut honeys showed organic acid profile similar to that of pine honey, but relatively in lower concentrations than that of pine honeys. A profile containing five organic acids (malic, ascorbic, maleic, citric, succinic and fumaric) was found in sunflower honey. Multifloral honey was characterized by containing four (malic, ascorbic, citric and fumaric) acids. According to these results, honey organic acids are good parameters for the differentiation of honeys of the different botanical origin.

REFERENCES

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