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# ORGANIC ACID COMPOSITION OF NATIVE BLACK MULBERRY FRUIT

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*Using HPLC, organic acids from the fruit of black mulberry (*Morus nigra *L., Moraceae) were quantified. Malic acid was predominant with a range of 35.4–198.5 mg/g. Citric acid was the second in abundance, with a range of 5.5–23.4 mg/g, followed by tartaric, oxalic, and fumaric with an average of 4.16, 0.62, and 0.019, respectively.*

**Key words**: black mulberry, *Morus nigra* L., organic acids.

Black mulberry, a member of the genus *Morus*, grows wildly in Turkey [1, 2]. Black mulberry fruits are consumed as the fresh fruit or in the form of various confectionary products such as jam, marmalade, frozen desserts, pulp, juice, paste, ice cream, and wine. It is expected that the consumption of black mulberry fruit, with its delicious slightly acid flavor, extraordinary taste, and medicinal use will increase in the near future. In addition, almost all parts of the tree (fruit, leaves and bark of stem and root, etc.) are used for pharmacological actions all over the world, especially in Chinese medicine [3, 4]. The fruit has a tonic effect on kidney energy, and it is used as an anthiphlogistic, diuretic, and expectorant [5, 3]. Fruits of black mulberry are also used to treat mouth lesions in Turkey [6, 7].

Among the constituents of biological samples, organic acids are of increasing interest because of their role in plant physiology as cofactors, buffering agents, and intermediates of the most important metabolic pathways of carbohydrates, lipids, and proteins. Fruit plants contain different organic acids. They are also used extensively as additives, namely antioxidant (tartaric, malic, and citric), acidulants (tartaric, malic, citric, and ascorbic acids), or preservatives (sorbic and benzoic acids) [8–10]. Berries are known to be rich in organic acids [11, 12]. Interest in the composition of berry fruits has also intensified because of increased awareness of their possible health benefits. The nature and concentration of organic acids are important factors influencing the organoleptic properties of fruit [13, 14]. In the pharmaceutical industry organic acids are used as antioxidants, preservatives, acidulants, and drug absorption modifiers. Organic acids can maintain the quality and nutritive value of fruit [8].

Recently there have been studies on its dry matter content, total acidity, ash content, anthocyanin content and flavor characteristics [15–18]. As far as we know, there is no study on the organic acid composition of black mulberry despite the importance of the organic acids in black mulberry. So, the work herein represents a contribution to the definition of the organic acid profile of fruits.

The aim of this research was to investigate the organic acid composition of black mulberry fruit wildly grown at two different locations (Mahmatlar and Sutculer) in Isparta, Turkey. The organic acid composition of the 14 selected black mulberry genotypes characterized by HPLC analyses are presented in Table 1.

All samples presented a similar profile composed of, at least, five identified organic acids: malic, citric, tartaric, oxalic, and fumaric acids. The highest total organic acid content (as the sum of individuals acids), 218.57 mg/g, was found in M-18 and the lowest, 48.91 mg/g in S-10. Both individual and total acid contents of the Mahmatlar group were higher than the Sutculer group (Table 2). As can be seen in Table 2, the amount of total organic acids was dependent on the geographical origin. Especially, differences in the climate are likely to explain these distinctions. Similar reports have been found in the literature relative to other berry fruits [11, 19]. The data also showed the genetic variability among the genotypes.

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TABLE 1. Organic Acid Composition of Black Mulberry Genotypes, mg/g

| Genotypes | Malic acid | Citric acid | Tartaric acid | Oxalic acid | Fumaric acid | Total |
|---|---|---|---|---|---|---|
| | | | Mahmatlar location | | | |
| M-5 | 35.55±0.7 | 10.75±0.5 | 5.25±0.09 | 0.611±0.03 | 0.017±0.00 | 52.18 |
| M-8 | 68.35±0.4 | 8.80±0.4 | 4.90±0.08 | 0.813±0.02 | 0.016±0.00 | 82.88 |
| M-11 | 72.20±0.75 | 12.05±0.55 | 4.85±0.08 | 0.763±0.02 | 0.017±0.00 | 89.88 |
| M-14 | 68.30±0.8 | 9.95±0.5 | 3.35±0.08 | 0.491±0.03 | 0.015±0.00 | 82.11 |
| M-16 | 146.50±1.5 | 17.60±0.9 | 5.95±0.20 | 0.793±0.02 | 0.023±0.00 | 170.87 |
| M-17 | 96.45±0.9 | 12.05±0.5 | 3.80±0.08 | 0.615±0.03 | 0.020±0.00 | 112.94 |
| M-18 | 198.50±1.6 | 15.50±0.7 | 4.20±0.08 | 0.348±0.05 | 0.020±0.00 | 218.57 |
| M-19 | 78.00±0.8 | 9.60±0.5 | 3.05±0.05 | 0.669±0.02 | 0.017±0.00 | 91.34 |
| M-22 | 51.75±0.7 | 10.05±0.5 | 3.60±0.08 | 0.433±0.04 | 0.020±0.00 | 65.85 |
| M-28 | 172.00±1.6 | 23.40±1.1 | 5.55±0.20 | 1.176±0.01 | 0.033±0.00 | 202.16 |
| | | | Sutculer location | | | |
| S-4 | 93.90±0.85 | 9.60±0.5 | 3.20±0.08 | 0.406±0.04 | 0.010±0.00 | 107.12 |
| S-6 | 56.20±0.7 | 8.40±0.4 | 3.35±0.08 | 0.847±0.02 | 0.018±0.00 | 68.81 |
| S-9 | 44.40±0.7 | 5.50±0.4 | 2.95±0.08 | 0.378±0.04 | 0.017±0.00 | 53.24 |
| S-10 | 35.40±0.7 | 9.10±0.5 | 4.05±0.08 | 0.344±0.05 | 0.018±0.00 | 48.91 |
| Max | 198.50 | 23.40 | 5.55 | 1.176 | 0.033 | 218.57 |
| Min | 35.40 | 5.50 | 2.95 | 0.344 | 0.015 | 48.91 |
| Mean | 86.96 | 11.60 | 4.146 | 0.620 | 0.019 | 103.35 |

Data are expressed as mean ±SD.

TABLE 2. Mean Organic Acid Content of Black Mulberry Fruit from Two Locations, mg/g

| Locations | Malic acid | Citric acid | Tartaric acid | Oxalic acid | Fumaric acid | Total |
|---|---|---|---|---|---|---|
| Mahmatlar | 98.760±0.98 | 12.975±0.62 | 4.450±0.10 | 0.671±0.03 | 0.020±0.00 | 116.88 |
| Sutculer | 57.475±0.74 | 8.150±0.45 | 3.388±0.08 | 0.493±0.04 | 0.016±0.00 | 69.52 |

Data are expressed as mean ±SD.

Malic acid was the predominant acid with an average of 86.96 mg/g. In the black mulberry fruit malic acid comprised up to 84% (35.4–198.5 mg/g) of average total acids. Citric and tartaric acids were the second abundant organic acid in the fruits, constituting 11.60 and 4.146 mg/g, respectively. The minor acids quantified were oxalic and fumaric acids with levels of 0.62 and 0.019 mg/g, respectively, in the fruit. On the other hand, M-18 showed the highest malic acid (198.5 mg/g) followed by M-28 (172 mg/g), while S-10 and M-5 had the lowest (around 35 mg/g). The highest citric acid content was found as 23.4 mg/g in M-28, and the lowest in S-9 (5.5 mg/g). Tartaric acid content ranged from 5.95 mg/g (M-16) to 2.95 mg/g (S-9). Oxalic and fumaric acids showed very few differences. This study is the first report on the organic acid composition of black mulberry using HPLC. Therefore, there are no satisfactory data on the compositional analysis of *M. nigra* fruits to compare them with the present results.

The results on the composition of black mulberry fruits might be of use to consumers and food technologists.

## EXPERIMENTAL

**Sampling**. Samples were obtained from native black mulberry (*M. nigra* L.) genotypes which have been evaluated by the author as promising in the previous research in the Mahmatlar and Sutculer districts of Isparta (Turkey). The fruits were harvested at ripeness stages on 5 and 11 August, 2002 from Mahmatlar and Sutculer, respectively. Harvested fruits were brought immediately to the laboratory in iceboxes and stored in a freezer at –80°C until use.

**HPLC Analysis**. Organic acids were analyzed using a Shimadzu class LC VP HPLC system with class LC-VP software, a pump (LC-6AD), and a UV-VIS detector (SPD-10AV VP). YMC Pack-ODS-AM (250 mm × 4.6 mm I.D., 5 μm) columns were used. The flow rate was 0.4 mL $min^{-1}$ and the column temperature was ambient. The mobile phases were prepared in distilled water with 0.05 M $H_3PO_4$, and adjusted to pH 2.2 with NaOH. The UV detector was set at 210 nm. Quantifications were based on the peak area measurements. Black mulberry fruits (10 g), which are diluted with 10 mL distilled water adjusted to pH 1.5 with $H_3PO_4$, were homogenized (Ultra-Turrax). Samples were filtered through filter paper. One-ml samples with 0.5 ml $CH_3OH$ were centrifuged at 3500 rpm for 10 min. Samples diluted with mobile phase were filtered through a 0.45 μ membrane, and the filtrate was injected into the HPLC column.

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