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INVESTIGATION OF THE AMINO ACID COMPOSITION IN BLACK AND WHITE MULBERRY (MORUS NIGRA L. AND MORUS MULTICAULIS PERR.) VARIETIES

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Abstract: This article presents a scientific analysis of the chemical composition and biological significance of mulberry fruits. It specifically focuses on the amino acid and flavonoid content of Morus nigra L. (black mulberry) and Morus multicaulis Perr. (black pearl mulberry) varieties grown in Uzbekistan. The compounds were identified using the high-performance liquid chromatography (HPLC) method. The obtained results are presented and analyzed in tables.

Keywords: Morus nigra L., Morus multicaulis Perr., phenolic compounds, anthocyanins, flavonoids, black mulberry, amino acids, flavonoid, chromatography, apigenin, rutin, hyperoside, robinin, black pearl.

Introduction. Morus nigra is a tree belonging to the Moraceae family, growing up to 10 meters in height. It is widely distributed in the subtropical regions of Asia (including Korea, Japan, China, and India), as well as in North America and Africa [1]. In Asian countries, mulberry trees are mainly cultivated for silkworm breeding, as their leaves serve as the primary and essential food source for silkworms [2].

Mulberry trees grow very rapidly. They require full sunlight and sufficient spacing (at least 4.5 m between trees). Minimal fertilization provides good results. Although mulberry trees are drought-resistant, they must be watered regularly during dry seasons. If the roots become too dry and lack moisture, the fruits may fall off before ripening. Mulberries grow best in slightly acidic to alkaline soils with a pH of 5.6–6.5. The optimal temperature for growth is between 24°C and 34°C.

In regions such as India, China, and Japan, mulberry trees are mainly grown for their leaves, which play an important role in sericulture. Silkworms feed exclusively on mulberry leaves, which provide the nutrients necessary for their growth and silk production [3]. In contrast, European countries, including Turkey and Greece, focus mainly on mulberry fruit cultivation. Anatolia is well known for producing high-quality mulberries, and Turkey is a leading country in this field between Central Anatolia and the Black Sea regions [4].

Mulberry fruits are approximately 2.5 cm long, ripening from red to dark purple, resembling raspberries in appearance. Harvesting usually takes place in July and August. These fruits are consumed fresh or processed into dried fruit, molasses, and vinegar [5]. Due to their pleasant taste, they are also used in liqueur production and as natural coloring agents. Additionally, they are valued in the cosmetics industry for their beneficial properties [6].

Among fruits and vegetables, mulberries are considered one of the richest sources of antioxidants [7]. They contain high levels of phenolic compounds, including anthocyanins, flavonoids, and other polyphenols, which contribute to their strong antioxidant activity [8].

Anthocyanins are glycosides in which sugar groups are attached to anthocyanidins. They are divided into two types: anthocyanidin aglycones (without sugars) and anthocyanin glycosides (with sugars). More than 500 types of anthocyanins have been identified, with the six most common anthocyanidins in fruits and vegetables being cyanidin (50%), delphinidin (12%), pelargonidin (12%), peonidin (12%), petunidin (7%), and malvidin (7%). Among them, 3-glycosides, 3,5- and 3,7-diglycosides or esters are the most frequent, with 3-glycosides being about 2.5 times more common than 3,5-diglycosides. The most widespread anthocyanin in nature is cyanidin-3-glycoside [9].

The color of plants depends not only on anthocyanins but also on the amount of liquid in the cells and the type of metal ions bound with anthocyanins. For example, anthocyanins form red complexes with iron, blue or pink with molybdenum, and white with nickel or copper.

When anthocyanins are boiled with acid (or under enzyme action), they break down into sugars and anthocyanidins. The sugar portion is often glucose, rhamnose, or galactose. Anthocyanins are responsible for the bright colors of many fruits and vegetables and play a vital role in preventing or treating health problems related to inflammation and oxidative stress. They have recognized therapeutic potential against oral diseases, hypertension, diabetes, anemia, and cancer [10].

These compounds exhibit interesting color changes depending on their concentration, pH level, and the presence or absence of co-pigments [11]. In acidic environments, anthocyanins appear red; in neutral conditions, violet; and in alkaline environments, they range from blue-green to purple [12]. Due to their stability and beneficial health effects, these pigments are used as natural colorants in food products [13].

The antioxidant content of mulberries varies depending on soil composition, geographic location, and growing conditions. For instance, soils rich in humus enhance nutrient and water transport through plant roots, redox reactions, chelate formation, and the release of compounds such as organic acids, sugars, phenols, and amino acids.

Mulberries contain significant amounts of anthocyanins, including cyanidin-3-glycoside, cyanidin-3-rutinoside, pelargonidin-3-glycoside, and pelargonidin-3-rutinoside. These compounds have been shown to possess health-promoting effects, including the ability to inhibit the activity of cancer cells [14]. Cyanidin, a type of anthocyanin, is thought to form complexes with DNA, which may have chemical protective effects. This interaction can protect both cyanidin and DNA from oxidative damage caused by hydroxyl radicals [15].

This protective mechanism highlights the potential of anthocyanins to reduce oxidative stress–related damage. Mulberry fruits are rich in essential nutrients such as vitamins C and K, iron, potassium, and dietary fiber, contributing to overall health. They support immune function, digestion, and blood coagulation. Furthermore, due to their phenolic compounds, organic acids, and natural sugars, mulberries may help lower cholesterol, regulate blood sugar levels, and reduce cancer risk [16].

In Turkey, about 70% of mulberry fruits are used for molasses (syrup) production. Recently, industrial-scale mulberry juice production has increased, broadening its use in various food products. However, the commercial use of anthocyanins faces challenges, including low extraction yields and instability during processing due to sensitivity to heat, light, oxygen, and pH changes. Modern technologies in the food industry, such as the cold-press method, help preserve the nutritional value of fruits more effectively. This process does not use heat or chemicals, maintaining the fruit's natural nutrients. Cold-pressed juices are highly nutritious and increasingly popular due to their flavor and health benefits.

The bioactive compounds responsible for the therapeutic effects of mulberries include polyphenolic antioxidants, particularly flavonoids and resveratrol. The health-promoting properties of mulberries have been scientifically confirmed; experiments show that their consumption can improve cardiac muscle weakness (myocardial dystrophy). Patients who consumed mulberries experienced relief from shortness of breath and heart pain, normalized heartbeat, and reduction of edema caused by fluid retention [17,18,19,20].

Research methods. Determination of amino acid content. In laboratory conditions, the amino acid composition of mulberry fruits was studied according to GOST 34230-2017. Aqueous extracts of the fruits were prepared, and proteins and peptides were precipitated using centrifugation. For this purpose, 1 ml of the test sample was mixed with 1 ml of 20% TCA. After 10 minutes, the precipitate was separated by centrifugation at 8000 rpm for 15 minutes. Then, 0.1 ml of the supernatant was frozen and lyophilized. The prepared sample was analyzed using HPLC equipment.

The synthesis of free amino acid phenylthiocarbamyl (PTC) derivatives was carried out according to the Steven A. and Cohen Daniel method. Determination of PTC-amino acids was performed using an Agilent Technologies 1200 chromatograph with a 75 × 4.6 mm Discovery HS C18 column. Solvent A: 0.14 M CH_3COONa + 0.05% TEA, pH 6.4; solvent B: CH_3CN. Flow rate: 1.2 ml/min; detection wavelength: 269 nm. Gradient (%B/min): 1–6%/0–2.5 min; 6–30%/2.51–40 min; 30–60%/40.1–45 min; 60–60%/45.1–50 min; 60–0%/50.1–55 min.

Determination of flavonoid content. The analysis of flavonoids was carried out using HPLC in gradient elution mode with a diode-array detector (DAD). The mobile phase consisted of acetonitrile and a buffer solution. Spectral data were recorded in the range of 200–400 nm.

Chromatographic conditions: chromatograph – Agilent Technologies 1260; mobile phase (gradient mode) – acetonitrile–buffer solution, pH = 2.92 (4%:96%) 0–6 min;

(10%:90%) 6–9 min; (20%:80%) 9–15 min; (4%:96%) 15–20 min; injection volume – 10 μl; flow rate – 0.75 ml/min; column – Eclipse XDB–C18, 5.0 μm, 4.6 × 250 mm; detector – diode-array detector, wavelength 254 and 320 nm.

Main part. In laboratory conditions, the amino acid and flavonoid compositions of locally grown mulberry varieties – black mulberry (Morus nigra L.) and black pearl (Morus multicaulis Perr.) – from the Parkent district of Tashkent region were studied using the HPLC method. The results of the amino acid analysis are presented in the following table.

Table 1. Amino acid composition of mulberry varieties

Name of Amino Acids	Black Mulberry	Black Pearl
	Concentration, mg/g	
Aspartic acid	0,186579	0,215428
Glutamic acid	0,715006	0,187894
Serine	0,218112	0,878827
Glycine	0,563303	0,40376
Asparagine	1,122108	0,818123
Glutamine	0,471116	0,582337
Cysteine	10,02623	0,955191
Threonine	0,566634	0,177256
Arginine	0,081244	0,088191
Alanine	0,218318	0,198569
Proline	4,150485	0,808473
Tyrosine	0,706955	0,629347
Valine	0,869261	0,601399
Methionine	0,087693	0,075803
Histidine	0,771995	0,119579
Isoleucine	0,047803	0,161141
Leucine	0,095851	0,193953
Tryptophan	0,082213	0,094438
Phenylalanine	0,302994	0,034587
Lysine	0,108818	0,019639
Total	**21,39272**	**7,243935**

The black mulberry variety contained approximately three times more amino acids than the black pearl variety, with cysteine showing the highest concentration (10.02623 mg/g) among all amino acids. Proline content was also significantly higher compared to other amino acids. In the black pearl variety, cysteine (0.955191 mg/g), proline (0.808473 mg/g), and serine (0.878827 mg/g) were found in relatively higher amounts compared to the rest.

Thus, the nitrogen content in mulberry fruits is low, and their composition mainly consists of carbohydrates, vitamins, and polyphenolic compounds. The flavonoid composition of black pearl and black mulberry fruits was also analyzed.

In the Black Mulberry variety, the amount of amino acids is three times higher than in the Black Pearl variety, and among all amino acids, the cysteine content is the highest (10.02623 mg/g). The amount of proline is also significantly higher than other amino acids. In the Black Pearl variety, the contents of cysteine (0.955191 mg/g), proline (0.808473 mg/g), and serine (0.878827 mg/g) are relatively higher than others.

Thus, mulberry fruits contain a small amount of nitrogenous substances, and their composition mainly consists of carbohydrates, vitamins, and polyphenolic compounds.

The flavonoid composition of the Black Pearl and Black Mulberry fruits was studied.

Table 2. Phenolic compounds of mulberry varieties

Raw material	Content of phenolic compounds, mg/100 g				
	Apigenin	Robinin	Rutin	Hyperoside	Gallic acid
Black Pearl	22,098	1,546	20,900	0,021	0,017
Black Mulberry	19,211	2,262	27,659	0,044	0,043

It was found that the amount of apigenin in the Black Pearl variety is slightly higher, while the amounts of rutin, hyperoside, and robinin in the Black Mulberry fruits are higher. The apigenin content was higher in the Black Pearl variety, and the rutin and robinin contents were higher in Black Mulberry compared to Black Pearl.

Conclusion. When studying the amino acid content of the fruits of the Black Mulberry (Morus nigra L.) and Black Pearl (Morus multicaulis Perr.) varieties grown in the soils of Uzbekistan, it was found that the Black Mulberry variety contains three times more amino acids than the Black Pearl variety, and among all amino acids, the cysteine content is the highest (10.02623 mg/g). The proline content was also found to be significantly higher than other amino acids. It was also found that the apigenin content is higher in the Black Pearl variety, while the rutin and robinin contents are higher in the Black Mulberry variety.

References

1. Wei, H.; Zhu, J.J.; Liu, X.Q.; Feng, W.H.; Wang, Z.M.; Yan, L.H. Review of bioactive compounds from root barks of *Morus* plants (Sang-Bai-Pi) and their pharmacological effects. *Cogent. Chem.* 2016, *2*, 1212320. [Google Scholar] [CrossRef]

2. Yang, Y.; Tan, Y.X.; Chen, R.Y.; Kang, J. The latest review on the polyphenols and their bioactivities of Chinese *Morus* plants. *J. Asian Nat. Prod. Res.* 2014, *16*, 690–702. [Google Scholar] [CrossRef] [PubMed]

3. El-Mawla, A.M.; Mohamed, K.M.; Mostafa, A.M. Induction of Biologically Active Flavonoids in Cell Cultures of *Morus nigra* and Testing their Hypoglycemic Efficacy. *Sci. Pharm.* 2011, *79*, 951–961. [Google Scholar] [CrossRef] [PubMed]

4. Gerasopoulos, D., Stavroulakis, G. (1997). Quality characteristics of four mulberry (Morus sp) cultivars in the area of Chania, Greece. Journal of the Science of Food and Agriculture, 73(2), 261-264.

5. Kutlu, T., Durmaz, G., Ateş, B., Yilmaz, I., Çetin, M. Ş. (2011). Antioxidant properties of different extracts of black mulberry (Morus nigra L.). Turkish Journal of Biology, 35(1), 103-110.

6. Memete, A. R., Miere, F., Laslo, V., Purcarea, C., Vicas, L., Ganea, M., Vicas, S. I. (2023). An In Vitro Study of the Healing Potential of Black Mulberry (Morus nigra L.) Extract in a Liposomal Formulation. Applied Sciences, 13(2), 1041

7. Zhang, H., Ma, Z. F., Luo, X., Li, X. (2018). Effects of mulberry fruit (Morus alba L.) consumption on health outcomes: A minireview. Antioxidants, 7(5), 69.

8. Liu, S., Zhang, Y. (2024). Antioxidant properties and electrochemical activity of anthocyanins and anthocyanidins in mulberries. Journal of Food Measurement and Characterization, 1-8.

9. *Kutlu T., Durmaz G., Ateş B., Yilmaz I., Çetin M.Ş. Antioxidant properties of different extracts of black mulberry (Morus nigra L.) Turkish J. Biol. 2011;35:103–110.*

10. Suh, H. J., Noh, D. O., Kang, C. S., Kim, J. M., Lee, S. W. (2003). Thermal kinetics of color degradation of mulberry fruit extract. Food/Nahrung, 47(2), 132-135.

11. Sendri, N., Bhandari, P. (2024). Anthocyanins: a comprehensive review on biosynthesis, structural diversity, and industrial applications. Phytochemistry Reviews, 1-62.

12. Espín, J. C., Soler-Rivas, C., Wichers, H. J., García-Viguera, C. (2000). Anthocyanin-based natural colorants: a new source of antiradical activity for foodstuff. Journal of Agricultural and Food Chemistry, 48(5), 1588-1592.

13. Katsube, T., Imawaka, N., Kawano, Y., Yamazaki, Y., Shiwaku, K., Yamane, Y. (2006). Antioxidant flavonol glycosides in mulberry (Morus alba L.) leaves isolated based on LDL antioxidant activity. Food chemistry, 97(1), 25-31.

14. Chen, P. N., Chu, S. C., Chiou, H. L., Kuo, W. H., Chiang, C. L., Hsieh, Y. S. (2006). Mulberry anthocyanins, cyanidin 3-rutinoside and cyanidin 3-glucoside, exhibited an inhibitory effect on the migration and invasion of a human lung cancer cell line. Cancer letters, 235(2), 248-259.

15. Sarma, A. D., Sharma, R. (1999). Anthocyanin-DNA copigmentation complex: mutual protection against oxidative damage. Phytochemistry, 52(7), 1313-1318.

16. Naeem, M. Y. (2020). Medicinal potentials and health benefits of black mulberry. Eurasian Journal of Food Science and Technology, 4(1), 1-5.

17. Huang W.Y., Zhang H.C., Liu W.X., Li C.Y. Survey of antioxidant capacity and phenolic composition of blueberry, blackberry, and strawberry in Nanjing. J. Zhejiang Univ. Sci. B. 2012;13:94–102. doi: 10.1631/jzus.B1100137.

18. Ercişli S, Orhan E. Some physicochemical characteristics of black mulberry (Morus nigra L.) genotypes from Northeast Anatolia region of Turkey. Sci Hort 116: 41-46, 2008.

19. Chen P, Chu S, Chiou H et al. Mulberry anthocyanins, cyanidin 3-rutinoside and cyanidin 3-glucoside, exhibited an inhibitory effect on the migration and invasion of a human lung cancer cell line. Cancer Lett 235: 248-259, 2005.

20. Elmacı Y, Altuğ T. Flavour evaluation of three black mulberry (Morus nigra) cultivars using GC/MS, chemical and sensory data. J Sci Food and Agric 8: 632-635, 2002.

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