**Evaluation of chemical, bioactive compounds, and minerals of three mulberry species**

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**Novelty statement**

Given the impact of the growth under different ecological conditions on the fruits' chemical compounds and mineral content. However, to our knowledge, the chemical compounds and mineral element content of mulberry species fruits grown in Halabja, Iraq have not been studied. Our results confirm that the chemical compounds and mineral content vary in these species of mulberry fruit studied as compared to the grown in other places.

**Abstract**

This study was conducted to examine the chemical composition and mineral content of white mulberry, white shahtoot, and black mulberry fruits grown in Halabja City, Iraq. Berries were harvested manually in April 2021 at the commercially ripe stage, they were selected according to uniformity of shape and color. Fifty fruits from each species were used for analysis. The results indicated that the highest pH, total dry weight (TDW), total sugar, total soluble solids (TSS), reducing sugar, polyphenol oxidase activity, and peroxidase activity were observed in white shahtoot, which recorded the lowest moisture, and total acidity (TA) compared to other mulberry fruits species. While the lowest pH, TDW, Ash, and total sugar, reducing sugar, non-reducing sugar, TSS, polyphenol oxidase activity, and peroxidase activity were observed in black mulberry, which recorded the highest moisture, TA, niacin, riboflavin, and alkaloid compared to other mulberry fruits species. The highest minerals, including Ca, Na, Zn, K, Mn, and Fe, were observed in the black mulberry compared to other mulberry fruit species. The results concluded that the different species of mulberry could be exploited and made the best based on their nutritious value, which may provide useful amounts of minerals. Furthermore, white shahtoot and white mulberry fruits are advised for fresh fruit consumption, given their taller and more attractive fruits, and could be advised for processing, owing to raised TSS and lower acid contents.

**Keywords:** Mulberry, niacin, riboflavin, polyphenol oxidase, peroxidase.

1. **Introduction**

Mulberry yields fruit of high nutritive value and good quality and it is widely used in the human diet, industrial processing, and medicinal (Bošnjakovic et al. 2012). Mulberry fruits contain high amounts of polyphenols, flavonoids, anthocyanins, and minerals that are important for human health (Yaman, 2021). They also contain antioxidant, antiallergic, antimicrobial, and antihyperglycemic activities (Kostic et al. 2013; Liang et al. 2012).

The development of mulberry cultivation returned to the success of its farming owing to its fast-growing due to its ability to grow under different climatic states throughout the world. The increase in the demand of the mulberry fruits is due to their beneficial health properties, nutritional value, good taste, and biological activities (Arabshahi and Urooj, 2007; Ercisli and Orhan, 2007). Mulberry fruits can be consumed in different forms such as dried, juices, pulp, paste and in fresh. Due to their health advantages and high polyphenol contents, their consumption increased as fresh fruit (Brand et al. 2017; Khan et al. 2011). Mulberry fruits have been also utilized in folk medicine to treat arthritis, anemia, diabetes, and hypertension (Ozgen et al. 2009).

In Iraq, traditional products such as concentrated mulberry juice, and mulberry chocolate are made from fruits, the red, and black-colored fruits are consumed fresh while the white-colored fruits are consumed as dried, and as well as utilized in juices, and jam. White Shahtoot fruit could be used at the half-green stage when they are semi-sweet and crisp or used at full rip when they earn color to white and gain extreme sweetness. Their sweetness provides an ideal contrast to other foods in sambals and salads or on a cheese platter.

The impact of the growth under different ecological conditions on chemical compounds and minerals contents in the fruits. However, to our knowledge, the chemical compounds and minerals contents of mulberry species fruits grown in Halabja, Iraq have not been studied. Consequently, this study aimed to determine the chemical compounds and mineral elements contents of white mulberry, white shahtoot mulberry, and black mulberry species grown in Halabja city, Iraq.

**Materials and methods**

**Raw materials collection**

This study was conducted on three species of mulberry grown in Halabja city including Black mulberry (*Morus nigra* L.), White mulberry (*Morus alba* L.), and White Shahtoot (King White-a non-staining cultivar) (*Morus macroura* Miq). They were harvested manually in April 2021 at the commercially ripe stage, after that transported to the laboratory to prepare samples for analysis. Fifty berries from each species were selected for analysis according to uniformity of shape and color, then the samples were frozen until used.

**Determination of moisture, total dry weight, ash,** **total soluble solids, pH, and total acidity of mulberry fruit species**

The moisture (%) and the TDW (%) contents were determined according to the method reported by (Abdulrahman et al., 2021). The ash content was determined by weighing the burned remains obtained by Muffle Furnace at 550 °C until they reached constant weight (Liang et al., 2012). A hand Refractometer (Atago-Japan) was used to determine total soluble solids (%). A pH meter (Eu Tech-Singapore) was used to measure pH. Titratable acidity (%) of samples were titrated with 0.1N NaOH and phenolphthalein was used as an indicator (Taha and Aljabary 2022).

**Determination of Vitamins, alkaloids, reducing sugar, total sugar, and non-reducing sugar of mulberry fruit species**

Vitamins (Niacin and Riboflavin) were determined using the method reported by (Okwu, 2005). For niacin determination, briefly 5.0 g of the sample was treated with 50 mL of 1 N sulphuric acid and shacked for 30 min. and three drops of ammonia solution were added to the sample and filtered. And then, 10 mL of the filtrate sample was pipetted into a 50 mL volumetric flask and 5 mL of potassium cyanide was added. This was acidified with 5 mL of 0.02N H2SO4. The absorbance of the sample was measured using a spectrophotometer at 470 nm. Riboflavin determination, 5.0 g of the sample was extracted with 100 mL of 50% ethanol solution and shacked for 1 h. This was filtered into a 100 mL flask, and then 10 mL of the extract was pipetted into 50 mL volumetric flask, 10 mL of 5% potassium permanganate and 10 mL of 30% H₂O4 were added and allowed to stand over a hot water bath for about 30 min. Then 2 mL of 40% sodium sulfate was added. This was made up to 50 mL mark and the absorbance was measured at 510 nm by using a spectrophotometer. The alkaloid contents were determined gravimetrically. Horwitz (2010) method was used to determine reducing sugar and total sugar. While the non-reducing sugar is determined by subtracting reducing sugar from total sugar.

**Determination of polyphenol oxidase and peroxidase activity of mulberry fruit species**

Polyphenol oxidase (PPO) activity was estimated by using a buffer solution which was prepared by mixing of 0.2 M (K2HPO4), and (KH2PO4), the pH was adjusted to 7 and substrate solution (catechol). The absorption was recorded at 420 nm using a spectrophotometer. Then, the change in optical absorption was recorded within 5 minutes as the method reported by (Shi et al., 2002). The peroxidase activity in the fruits was evaluated by using a buffer solution (Potassium phosphate at pH 7), hydrogen peroxide and substrate solution (guaiacol). The absorption was recorded at 400 nm using a spectrophotometer as mentioned by (Aljabary, 2018).

**Estimation of mineral content**

According to (Gungor and Sengul, 2008). Mineral elements were estimated by using 0.50 g of fruits and digesting with 20 mL of concentrated sulfuric acid and 10 mL per-chloric acid, and heated gently on a hotplate. Then, the digested samples after cooling were transported to a 100 mL conical flask and completed with deionized water, and then filtered. An atomic absorption spectrophotometer (Analyst 700, Perkin Elmer, USA) was used for the mineral elements analyses.

**Statistical analysis**

One-way analysis of variance (ANOVA) was used. The collected data were analyzed by utilizing (SAS 9.1 software). For the means comparisons, Duncan's multiple range test (P<0.05) was used.

**Results**

The statistical differences were found in pH, moisture, TDW, ash, and total soluble solids in all the mulberry species (Table 1). The fruit juice pH for Black mulberry was acidic (3.47) but pH values for white mulberry and white shahtoot were higher and ranged between 6.70 and 6.79 respectively. The fruit's moisture content in this study varied from 71.07% in white shahtoot to 74.60% in white mulberry and the fruits of black mulberry 85.43% tend to have comparable higher moisture contents. The highest value of TDW in white shahtoot was recorded 28.93% and followed by white mulberry 25.40%. While the lowest values 14.57% of the black mulberry cultivar were recorded. The slight significant differences amongst the species in ash values, and the lowest percentages were found for black mulberry with the values of 0.987% and 0.989% in white shahtoot, they had lower ash content compared to white mulberry 0.990%. The values of TA ranged from 1.92% in white shahtoot to 2.27% in black mulberry. The TSS values ranged from 23.97 in white shahtoot to 20.50 in white mulberry to 11.00 in black mulberry.

Table (2) shows the contents of Niacin, Riboflavin, and Alkaloids of the mulberry fruit species studied. The chosen vitamins were nicotinic acid or niacin (vitamin B3) and riboflavin (vitamin B2) were estimated. A variable amount of niacin was found to range from 0.42 of *M. alba* to 2.93 mg/100g of *M. nigra*. An amount of riboflavin was recorded in the samples, which varied between 0.064 of *M. macroura* to 0.086 mg/100g of *M. nigra*. Furthermore, a higher amount of niacin and riboflavin were obtained in *M. nigra*. Significant differences were found among the mulberry species in alkaloid content in the range from 340.00 mg/100g of *M. macroura* to 628.00 mg/100g of *M. nigra*, while the *M. alba* content was 390.66 mg 100g-1.

The results in (Table 3) show that the white shahtoot was observed to have a higher total sugar content 12.06 g/100g but a lower value of 8.18 g/100g was observed for *M. nigra*. A similar result was recorded in reducing sugar. In the state of non-reducing sugar, a lowest amount of 1.77 g/100g was obtained for *M. nigra* and a highest amount of 1.96 g/100g was recorded in *M. alba*. Significant differences were found among the species in polyphenol oxidase activity, while white Shahtoot had higher polyphenol oxidase activity followed by white mulberry species with a high level of 236.0 and197.8 U/ml, respectively, and the lowest activity of the enzyme was reported in black mulberry with the value of 13.40 U/ml. Changes  in the activity of the peroxidase enzyme in white shahtoot, white mulberry, and black mulberry were studied in order to assess the role of the peroxidase enzyme in mulberry fruit which causes the browning. The higher activity was found in white shahtoot and black mulberry, which were 294.167 and 227.922 U/ml, respectively. Whereas the peroxidase was changed in the white mulberry which recorded the lowest activity at 124.133 U/ml.

The mulberry fruit species contents of the mineral elements are shown in (Table 4). The different mineral compositions were determined among the mulberry species. The P, Mn, Fe, and Zn values of mulberry species were ranged from 272.00 mg/100g (White Shahtoot) to 277.00 mg/100g (White Mulberry), 238.67 mg/100g (White Shahtoot) to 247.00 mg/100g (Black Mulberry), 3.01 mg/100g (White Shahtoot) to 4.58 mg/100g (Black Mulberry) and 50.37 mg/100g (White Shahtoot) to 54.18 mg/100g (Black Mulberry), respectively. While non-significant differences were found among mulberry species in Na, Ca, K, and Ni values.

**Discussion**

The results in (Table 1) show the differences in pH, moisture content, TDW, ash content, TA, and TSS in the mulberry fruit, these variations may be caused by a variety of species, cultivars, rootstocks, environmental factors, and orchard nutrition. Our results are in good harmony with the previous work by (Liang et al., 2012) who found that the pH of eight mulberry species from China ranged from 3.37 to 5.33. Ercisli and Orhan (2007) also found that the pH of white mulberry was 5.60 and black mulberry had the lowest pH, it was acidic at 3.52. They also indicated that the moisture content of black mulberry had higher (72.6%) moisture than white mulberry (71.5%), as well as found that the TDW of white mulberry was 29.5%, and black mulberry had the lowest TDW which was 27.4%. Furthermore, the same results were observed by (Hama et al.,2022)that the TDW in dried fruits of white shahtoot and white mulberry was higher than in black mulberry. The results of ash content found by (Imran et al., 2010) supported our results that the white mulberry had 0.57 g/100 g DW of ash which had the highest ash value compared to black mulberry which was 0.50 g/100 g DW. As for TA values, both the compositions and amounts of the organic acids were found to be variable in the different species, the main organic acids were citric, ascorbic, and tartaric (Özgen et al., 2009). Thus, these differences in the TA% in mulberry fruit species could be related to the variation in the amount of these acids, which makes fruits sour or sweet. Additionally, could be related to the variation of their pH as shown in the same table. Ercisli and Orhan (2007) reported that in *M. alba* TSS content is higher (20.4%) than in *M. nigra* 16.7%. Depending on our results, white shahtoot, and white mulberry fruits are advised for fresh fruit consumption, given their taller and more attractive fruits, and could be advised for processing, owing to raised TSS and lower acid contents.

Table (2) shows the contents of vitamins (Niacin and Riboflavin) and Alkaloids of the mulberry fruit species studied which were varied. Also, past studies indicated that the variation in concentrations of water-soluble vitamins was also mentioned for other plant species (Okwu, 2005; Imran et al. 2007; Hussain et al. 2008). Furthermore, the alkaloid recorded 0.28 mg/100g FW in *Aframomum melegueta* and 0.36 mg/100g FW in *Garcinia kola* Heckel (Okwu, 2005), which are lower than in our study. The overall result showed that the chosen *Morus* species, like *Morus alba*, *Morus macroura* and *Morus nigra*, might be the perfect sources of natural alkaloids and vitamins (Imran et al. 2010).

The results in (Table 3) show that the total sugar, reducing sugar, non-reducing sugar content, and the activity of polyphenol oxidase and peroxidase of the mulberry fruit species were varied. The results illustrated that in the *Morus* species of white shahtoot the sugar contents were lower than black mulberry 11.3%-16.2% (Elmaci and Altuğ, 2002). This could be related to the variation in geological and environmental conditions of growth. On the other hand, the high total sugar content in mulberry fruits could motivate their utilization as sugar sources in various food recipes (Imran et al. 2010). Significant differences were found among the species in polyphenol oxidase activity. Given this enzyme acts on the phenolic compounds in fruits which causes browning in fruit, the variation of phenolic compounds in the mulberry fruits depends on many factors such as genetic differences, and the degree of maturity at harvest (Liang et al. 2012). Changes in the activity of the peroxidase and polyphenol oxidase were measured in white shahtoot, white mulberry, and black mulberry were studied in order to assess the role of the peroxidase enzyme in mulberry fruit which causes the browning.

The difference in the mineral content is shown in (Table 4) for all the mulberry species. Besides varieties and species, the mineral contents depend on growing conditions such as geographical conditions, soil type, climatic, the amount of applied fertilizers, and agriculture management techniques (Ercisli, 2009). Due to the advantages of mulberry fruits for human health, knowledge of their trace element content is essential. The results indicated that mulberry was a better source for minerals, especially Na, P, Ca, K, Mn, Fe, Zn, and Ni, and could be recognized as a valuable horticultural product based on its rich and beneficial nutrient composition. The high level of Fe might be of nutritional importance, especially in those parts of the world where anemia and Fe deficiency are relatively widespread. The current study has presented a higher mineral content compared to the previous studies that reported by (Ercisli and Orhan, 2007; Okwu, 2005). Therefore, the existence of sufficient quantities of essential minerals in the studied fruits, which may act as better supplements of these elements through daily diet, allows one to easily meet a reasonable amount of the daily requirements (Imran et al. 2010). The nutritional value of mulberry fruit species is affected by specific agriculture management techniques and growing conditions (Ercisli and Orhan, 2007).

**Conclusion**

This results of this study that mulberry fruits are a highly valuable horticultural crop, according to their beneficial and rich nutrient ingredients. A significant difference in the chemical compounds, mineral content, and enzyme activity (polyphenol oxidase and peroxidase) among the mulberry species were found. The different species of mulberry could be exploited and made the best value based on their own nutritious value for various processes and purposes. Mulberry fruits are a perfect dietary source of some antioxidants, particularly polyphenols, and anthocyanin, which may provide useful amounts of minerals. According to the results, the highest amounts of some chemical compounds were observed in white shahtoot followed by black mulberry and white mulberry. While the highest moisture, TA, niacin, riboflavin, alkaloid, and mineral Ca, Na, Zn, Fe, K, Mn, and Ni contents were observed in the black mulberry compared to other mulberry fruit species.

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**Author Contributions**

All of them contributed to all parts of this article.

**Conflicts of Interest**

All authors declare no conflicts of interest.

**Data Availability**

Data presented in this study will be available on a fair request to the corresponding author.

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**Table 1.** The pH, moisture, TDW, Ash, TA, and TSS of mulberry fruits species.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Species | pH | Moisture (%) | TDW  (%) | Ash  (%) | TA  (%) | TSS  (%) |
| ﻿ White Shahtoot | 6.79 a | 71.07 c | 28.93 a | 0.989 ab | 1.92 b | 23.97 a |
| White Mulberry | 6.70 a | 74.60 b | 25.40 b | 0.990 a | 2.13 ab | 20.50 b |
| Black Mulberry | 3.47 b | 85.43 a | 14.57 c | 0.987 b | 2.27 a | 11.00 c |

TDW=Total dry weight, TA= Titratable acidity; TSS= Total soluble solid

Means with various letters in one column are statistically various (P<0.05) according to the Duncan test

**Table 2.** Niacin, Riboflavin and Alkaloid contents of mulberry fruits species

|  |  |  |  |
| --- | --- | --- | --- |
| Species | Niacin  (mg/100g) | Riboflavin  (mg/100g) | Alkaloids  (mg/100g) |
| White Shahtoot | 0.82 b | 0.064c | 340.00 c |
| White Mulberry | 0.42 c | 0.070b | 390.67 b |
| Black Mulberry | 2.93 a | 0.086a | 628.00 a |

Means with various letters in one column are statistically various (P<0.05) according to the Duncan test

Table 3. Total sugar, reducing sugar, non-reducing sugar, polyphenol oxidase activity, and Peroxidase activity in three fresh mulberry species

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Species | T. Sugar  (g/100g) | Reducing sugar  (g/100g) | Non-reducing sugar  (g/100g) | Polyphenol Oxidase activity  (units/ml) | Peroxidase activity  (units/ml) |
| ﻿White Shahtoot | 12.06 a | 10.25 a | 1.81 b | 236.00 a | 294.17 a |
| White Mulberry | 9.38 b | 7.42 b | 1.96 a | 197.80 a | 124.13 c |
| Black Mulberry | 8.18 c | 6.41 c | 1.77 b | 13.40 b | 227.92 b |

Means with various letters in one column are statistically various (P<0.05) according to the Duncan test

Table 4. Mineral contents of mulberry fruits species.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Minerals(mg/100g) | | | | | | | |
| Cultivars | Na | P | Ca | K | Mn | Fe | Zn | Ni |
| ﻿ White Shahtoot | 52.67 a | 272.00 b | 126.67 a | 1331.00 a | 2.39 b | 3.01 b | 2.37 b | 2.08 a |
| White Mulberry | 50.00 a | 277.00 a | 125.33 a | 1361.67 a | 2.39 b | 3.10 b | 5.91 a | 2.10 a |
| Black Mulberry | 53.67 a | 276.00 a | 126.67 a | 1412.67 a | 2.47 a | 4.58 a | 6.18 a | 2.09 a |

Means with various letters in one column are statistically various (P<0.05) according to the Duncan test