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Full Length Article

Evaluation of Chemical, Bioactive Compounds and Minerals of Three Mulberry Species

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Abstract

The study aims to examine the chemical composition and mineral content of while mulberry (*Morus alba* L.), White Shahtoot (*Morus macroura* Miq) and Black Mulberry (*Morus nigra* L.) fruits grown in Halabja province of Iraq. Fruits harvested manually in April 2021 at the commercially ripe stage were selected according to uniformity of shape and color. For each species, 50 fruits were taken to analyze the chemical constituents. Data revealed the highest pH, total dry weight (TDW), total sugar, total soluble solids (TSS), reducing sugar, polyphenol oxidase activity, and peroxidase activity in White Shahtoot, but the lowest moisture and total acidity (TA). However, Black Mulberry showed the lowest pH, TDW, ash, and total sugar, reducing sugar, non-reducing sugar, TSS, polyphenol oxidase activity, and peroxidase activity, but the highest moisture content, TA, niacin, riboflavin, and alkaloid. The highest minerals (Ca, Na, Zn, K, Mn and Fe) content was noted in Black Mulberry. Notably, all the mulberry species had great nutritious value in providing balanced amounts of minerals. 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; Nutrients

Introduction

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

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. An increase in the demand for mulberry fruits is due to their beneficial health properties, nutritional value, good taste, and biological activities (Arabshahi and Urooj 2007; Ercisli and Orhan 2007). It can be consumed in different forms such as dried, juices, pulp, paste and fresh. Due to their health advantages and high polyphenol contents, their consumption increased as fresh fruit (Khan *et al.* 2011; Brand *et al.* 2017). In addition, it has been utilized in folk medicine to treat arthritis, anemia, diabetes, and hypertension (Özgen *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, 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.

Different ecological conditions modify the plant phytochemical compounds and minerals contents of fruits, which have effect on growth. However, to our knowledge, the chemical compounds and mineral contents of mulberry species fruits grown in Halabja, Iraq have not been investigated. This study aimed to determine the chemical compounds and mineral elements contents of White Mulberry (*Morus alba*), and White Shahtoot (*M. macroura*) and Black Mulberry (*M. nigra*) species grown in Halabja City, Iraq.

Materials and Methods

Mulberries fruit samples harvesting and preparation

Three species of mulberry grown in Halabja city including Black Mulberry (*Morus nigra* L.), White Mulberry (*Morus*

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alba L.), and White Shahtoot; King White – a non-staining cultivar (*Morus macroura* Miq) were studied. The fruits were harvested manually in April 2021 at the commercially ripe stage, after that transported to the laboratory to prepare samples for analysis. Fifty fruits from each species were selected for analysis according to uniformity of shape and color, then the samples were frozen until used.

Mulberry biochemical attributes measurement

Moisture and TDW were determined according to the method of 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. The titratable acidity of samples was estimated with 0.1 N NaOH and phenolphthalein was used as an indicator (Taha and Aljabary 2022).

Vitamins (niacin and riboflavin) were determined using the method of Okwu (2005). For niacin determination, 5 g of the sample was treated with 50 mL of 1 N sulphuric acid and shaken for 30 min. Then three drops of ammonia solution were added to the sample and filtered. And then, 10 mL of the filtrate was pipette into a 50 mL volumetric flask, and 5 mL of potassium cyanide was added. This was acidified with 5 mL of 0.02 N H_2SO_4. The absorbance of the sample was measured using spectrophotometer at 470 nm. For riboflavin estimation, 5 g of the sample was extracted with 100 mL of 50% ethanol and shaken for 1 h, filtered into a 100 mL flask, and then 10 mL of the extract was pipetted into 50 mL volumetric flask. Then 10 mL of 5% potassium permanganate and 10 mL of 30% H_2O_4 were added and allowed to stand in a water bath for about 30 min. Then 2 mL of 40% sodium sulfate was added, and the volume was made up to 50 mL mark in a volumetric flask and the absorbance was measured at 510 nm by using a spectrophotometer. The alkaloid contents were determined gravimetrically (Adeniyi 2009). Horwitz (2010) method was used to determine reducing and total sugars. The non-reducing sugars were determined by subtracting reducing sugar from total sugar.

Determination of enzyme activities

Polyphenol oxidase (PPO) activity was estimated by using a buffer solution prepared by mixing 0.2 M (K_2HPO_4), and (KH_2PO_4), the pH was adjusted to 7. The absorption was recorded at 420 nm using a spectrophotometer (Spectrophotometer UV/Visible, Shimadzu, Japan). Then, the change in optical absorption was recorded within 5 min with the method of Shi *et al.* (2002). Fruit peroxidase activity 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 (Spectrophotometer UV/Visible, Shimadzu, Japan) (Aljabary 2018).

Estimation of mineral content

The method of Gungor and Sengul (2008) was used for these determinations. Mineral elements were estimated by digesting 0.5 g of fruits with 20 mL of concentrated sulfuric acid and 10 mL perchloric acid and heated gently on a hotplate. The digested cooled samples were diluted to 100 mL using a conical flask with deionized water and filtered. An atomic absorption spectrophotometer (Analyst 700, Perkin Elmer, USA) was used for mineral elements analyses.

Statistical analysis

The collected data were analyzed by utilizing (SAS 9.1 software). Randomized Complete Block Design (RCBD) was used with three replications and for the means comparisons, Duncan's multiple range test ($P<0.05$) was used.

Results

The significant 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 Black Mulberry tended to have comparable higher moisture contents (85.43%). The highest value of TDW in was recorded White Shahtoot (28.93%) followed by White Mulberry (25.40%), while the lowest value (14.57%) was noted in the Black Mulberry cultivar. Significant differences between the species in ash values. Lowest percentages were found for Black Mulberry (0.987%) and White Shahtoot (0.989%), while the highest ash content was noted in White Mulberry (0.990%). The value 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 1).

Table 2 shows the contents of Niacin, Riboflavin, and Alkaloids of the mulberry fruit. 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 White Mulberry to 2.93 mg/100 g of Black Mulberry. An amount of riboflavin was recorded in the samples, which varied between 0.064 of White Shahtoot to 0.086 mg/100 g of Black Mulberry. Furthermore, a higher amount of niacin and riboflavin were obtained in Black Mulberry. Significant differences were found among the mulberry species in alkaloid content in the range from 340.00 mg/100 g of

Table 1: The pH, moisture, TDW, Ash, TA, and TSS of mulberry fruit species

Species	pH	Moisture (%)	TDW (%)	Ash (%)	TA (%)	TSS (%)
White Shahtoot	6.79 ±0.09a	71.07 ± 0.75c	28.93±1.75a	0.989±0.0005ab	1.92±0.45b	23.97±0.057a
White Mulberry	6.70±0.27a	74.60± 0.9b	25.40±0.9b	0.990±0.001a	2.13±0.18ab	20.50±0.5b
Black Mulberry	3.47 ±0.02b	85.43±0.45a	14.57±0.45c	0.987±0.002b	2.27±0.075a	11.00±0.25c

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 fruit species

Species	Niacin (mg/100 g)	Riboflavin (mg/100 g)	Alkaloids (mg/100 g)
White Shahtoot	0.82±0.035b	0.064±0.001c	340.00±3c
White Mulberry	0.42±0.02c	0.070±0.0015b	390.67±1.5b
Black Mulberry	2.93±0.045a	0.086±0.001a	628.00±2a

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	Total sugars (g/100 g)	Reducing sugar (g/100 g)	Non-reducing sugar (g/100 g)	Polyphenol oxidase activity (units/mL)	Peroxidase activity (units/mL)
White Shahtoot	12.06±0.095a	10.25±0.075a	1.81±0.02b	236.00±55.80a	294.17±18.88a
White Mulberry	9.38±0.15 b	7.42±0.1b	1.96±0.05a	197.80±4.50a	124.13±9.76c
Black Mulberry	8.18±0.13 c	6.41±0.07c	1.77±0.06b	13.40±2.10b	227.92±43.41b

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

Table 4: Mineral contents of mulberry species fruits

Cultivars	Minerals (mg/100 g dry weight)							
	Na	P	Ca	K	Mn	Fe	Zn	Ni
White Shahtoot	52.67±5.50a	272.00±1.73b	126.67±1.52a	1331.00±10a	2.39±0.015b	3.01±1.44b	2.37±0.25b	2.08±0.05a
White Mulberry	50.00±4a	277.00±1a	125.33±5.50a	1361.67±11.59a	2.39±0.02b	3.10±0.47b	5.91±0.7a	2.10±0.01a
Black Mulberry	53.67±1.52a	276.00±2a	126.67±1.52a	1412.67± 2.51a	2.47±0.04a	4.58±0.43a	6.18±2.03a	2.09±0.01a

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

White Shahtoot to 628.00 mg/100 g of Black Mulberry, while the White Mulberry content was 390.66 mg/100 g.

Data showed that the White Shahtoot had a higher total sugar content 12.06 g/100 g but a lower value of 8.18 g/100 g was observed for Black Mulberry (Table 3). Similar results were recorded in the case of reducing sugar. In the state of non-reducing sugar, the lowest amount of 1.77 g/100 g was obtained for Black Mulberry, and the highest amount of (1.96 g/100 g) was recorded in White Mulberry. Significant differences were found among the species in polyphenol oxidase activity, while White Shahtoot had higher polyphenol oxidase activity followed by White Mulberry 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. A higher peroxidase activity was noted in White Shahtoot and Black Mulberry, which were 294.167 and 227.922 U/mL, respectively. However, peroxidase activity was reduced in White Mulberry (124.133 U/mL).

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

Discussion

The results indicated difference 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 (Table 1). Our results are in good harmony with the previous work by Liang *et al.* (2012) who reported that the pH of eight mulberry species from China ranged from 3.37 to 5.33. Ercisli and Orhan (2007) reported that the pH of White Mulberry was 5.60 and Black Mulberry had the lowest pH (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% (Table 1). In this context, Hama *et al.* (2022)

reported that the TDW in dried fruits of White Shahtoot and White Mulberry was higher than in Black Mulberry. In line with the findings of Imran *et al.* (2010). We found that 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, TA could be related to changes in their pH (Table 1). Ercisli and Orhan (2007) reported that in White Mulberry TSS content was higher (20.4%) than in Black Mulberry 16.7%. Based on our finding, 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 greater TSS and lower TA.

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

Total sugar, reducing sugar, non-reducing sugar content, and the activity of polyphenol oxidase and peroxidase of the mulberry fruit species were varied (Table 3). The results revealed that in the White Shahtoot species, 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, a higher 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 PPO 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 nutritional value of mulberry fruit species is affected by specific agriculture management techniques and growing conditions (Ercisli and Orhan 2007). Besides varieties and species, the mineral contents depend on growing conditions such as geographical conditions, soil types, 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 result 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 (Table 4). 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. This study has presented a higher fruit mineral content compared to the previous reports (Okwu, 2005; Ercisli and Orhan 2007). Therefore, sufficient quantities of essential minerals in the studied fruits, which may act as better supplements of these elements through daily diet, allow one to easily meet a reasonable amount of the daily requirements (Imran *et al.* 2010).

Conclusion

Significant differences in the chemical compounds, mineral content, and enzyme activity (polyphenol oxidase and peroxidase) among the mulberry species were found. The mulberry could be exploited and made the best value based on their own nutritious value for various processes and purposes. The highest amounts of some chemical compounds were observed in White Shahtoot followed by Black Mulberry and White Mulberry. However, 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.

Ethics Approval

Not applicable.

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