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Physico-chemical and anthocyanin profiling of *Morus* spp. cultivars grown in Tamil Nadu

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Abstract
The Physico-chemical and anthocyanin profile of different mulberry fruit cultivars, namely V, S13, MR2 and S36 grown in different regions of Tamil Nadu were investigated. The fruit properties of the cultivars varied based on soil type and cultivation location. The MR2 variety cultivated in Palladam area recorded the highest juice yield (532.6 g/1000 g fresh weight). The fruits of mulberry cultivars commonly grown in Tamil Nadu exhibited black to purple colour. The colour values of the fresh fruits measured in term of L*, a*, b* values indicated that the redness and blueness values between 14.73 to 24.26, 8.02 to 23.01 and-4.15 to-12.41, respectively. The average weight of single mulberry fruit ranged from 1.62 g to 1.84 g and the moisture content was between 75.50 to 88.00%. The compositions of the samples were 5.04 to 5.28 pH, total soluble solids (TSS) between 9.2ºBrix to 13.6 º Brix. Reducing sugars content was 7.02 to 10.07% per 100g fresh weigh. The total monomeric anthocyanin content, determined using the pH-differential method was in the range of 179.51 to 271.35 mg cyanidin-3-3-O-glucoside l^{-1} of fresh juice.

Keywords: Mulberry fruits, physico-chemical properties, anthocyanin profile, cultivars, *Morus* spp.

Introduction
In India, approximately 244 mulberry (*Morus* spp.) cultivars are maintained, and most of the states have adopted sericulture as an important agro-industry due to its high economic returns. Mulberry cultivation is practised under diverse agro-climatic conditions; with major sericulture zones located in state such Karnataka, Andhra Pradesh and Tamil Nadu. In India, 264 exotic and 836 indigenous mulberry accessions are conserved for mulberry fruit yield improvement at the bank of Central Sericultural Germplasm Research Centre, Hosur, Tamil Nadu (Kumaresan *et al.*, 2008) [11].

Mulberries ripen over an extended period, unlike many other fruits that mature simultaneously. In southern India, two fruiting seasons are observed annually: during October-November and March-May. Among various cultivars MR2 has been reported to yield about 550 kg of fruit per hectare (Singhal *et al.*, 2010) [16].

Mulberry fruits are potentially used in various valuable food products and are commercially processed into medicinal syrups, jam, marmalade, frozen desserts, juice, paste, ice cream, wine, distillates, natural food colorant and liquor (Singhal *et al.*, 2003) [17]. Mulberry juice is well recognized as one of the healthiest beverages, and due to its nutritional benefits, mulberry fruit juice can be used as an ideal medium for producing probiotics.

Mulberry fruits have been reported to exhibit a variety of biological activities, such as anti-thrombotic (Yamamoto *et al.*, 2006) [20], antioxidant (Kim *et al.*, 1999) [9], antimicrobial (Takasugi *et al.*, 1979), anti-inflammation (Kim and Park, 2006) [10] dietary modulator mechanisms for various diseases (Wrolstad, 2001) [19] and neuro-protective effects (Kang, 2006). These activities are mainly attributed to anthocyanin, which are a group of naturally occurring phenolic compounds for the responsible for the color of mulberries. Cyanidin-3-glucoside and Cyanidin-3-rutinoside are the major anthocyanins present in mulberry fruits (Liu *et al.*, 2004) [2].

Approximately 10.8 g of dried fruits accounts about 100 mg of anthocyanin content (Huo, 2003) [8]. The Antioxidants in mulberry fruits are 70% and 24% higher than blueberries and cranberries, respectively. Among various disciplines, there is a wider scope of scientific interest in using mulberry fruits due to their higher amounts of vitamin, fiber, resveratrol and

antioxidant properties have drawn significant attention in research studies.

Materials and Methods

Collection of samples

Fruits of four popular cultivars of mulberry (V, S13, MR2 and S36) were harvested during the fruit-bearing season i.e., September to November, from mulberry-growing districts of Tamil Nadu. Fruits were picked at fully ripened stage and collected in sterile zipped polythene bags. The fruits were stored at 4˚C until analysis.

Fruit yield and color characteristic of mulberry fruit

The fruits of mulberry cultivars commonly grown in Tamil Nadu were black in color. The color of mulberry fruits was measured using Hunter Color Lab (L*a*b*) (ColorFlex EZ). The equipment was calibrated using a white and black standard tile. Based on the spectra generated, the color parameters for the fruits were determined. The color coordinates of the samples followed for system defined by Commission International de l'Eclairage (CIE Lab), indicating lightness (L*), red-green chromaticity (a*), and yellow-blue chromaticity (b*). The L* value indicates lightness ranging from black (0) to white (C.I. =100), whereas a* value range from-60 (green) to + 60(yellow). The colour values were recorded in triplicates for each sample

Determination of fruit weight

Fresh weight of mulberry fruit was measured using a digital balance with a sensitivity of 0.1g. Fruit weight was expressed in grams per fruit.

Determination of moisture content

The moisture content was determined by drying the fruits at 60±1 °C until the sample reached constant weight (AOAC, 1984) [1]. The difference in initial and final weight was taken as moisture content and expressed as percentage.

Determination of pH

The mulberry fruit juice was extracted by squeezing the fruits and the pH of the juice was determined using a digital pH meter (ELICO DPH 500).

Determination of total soluble solids

Total soluble solids (TSS) content was determined by placing one drop of mulberry juice extract into a digital refractometer (ATAGO, Japan) calibrated in °Brix. Result was expressed percentage.

Determination of reducing sugar

The amount of reducing sugar in mulberry fruits was determined using Dinitrosalicylic acid (DNS) method (Miller, 1959) [13]. A standard graph was plotted using 200µg ml-1of glucose solution as the working standard. One ml of fruit extract from each cultivar of mulberry cultivar was mixed with 2 ml of DNS reagent in a tightly capped test tube. The mixture was heated at 90 °C for 15 minutes to develop a reddish-brown color.

Anthocyanin profile of mulberry fruit

Preparation of mulberry fruit extract

A quantity of 100 g of mulberry fruit was ground using a pestle and mortar. The mixture was separated by filtering through a nylon filter cloth. The filtrate was centrifuged at 5000 rpm for 15 minutes, and the supernatant was collected for further pigment analysis.

Determination of monomeric anthocyanin

The total monomeric anthocyanin content in the fruits extract was determined using the pH-differential method (Guisti and Wrolstad, 2003) [7]. anthocyanin shows a maximum of absorbance at 520 nm at pH 1.0. The colored oxonium form of anthocyanin predominates at pH 1.0, while the colorless hemiketal form predominates at pH 4.5. The pH-differential method is largely based on the reaction, producing oxonium forms, and allows accurate and rapid measurement of total monomeric anthocyanin content.

Total monomeric anthocyanin pigment was expressed as mg of cyanidin 3--3-O-glucoside, using a molar absorptivity (ὲ) of 26,900 L mol^{-1} cm^{-1} and a molecular weight of 449.2 g mol^{-1}. One ml of the different mulberry fruit extract was poured into two separate 10 ml volumetric flasks. One was filled upto the line with buffer solution of potassium chloride (pH 1.0) and the second with sodium acetate buffer (pH 4.5). The two diluted solutions were left to stand for 15 minutes at room temperature in a dark room. Finally, the absorbances of both the samples were measured at 520 nm and 700 nm. Absorbance (A) of the investigated extract was calculated as given below

A= (Aλ vis-max-A 700) pH 1.0-(Aλ vis-max-A 700) pH 4.5

Content of the monomeric anthocyanin pigment was calculated by

Monomeric anthocyanin pigment (mg/L) = (A x MV x DF x 1000)/(ὲ x l)

Were,
A= Absorbance
MV = Molecular weight (449.2 g mol-1)
DF = Dilution factor
l = Path length (1 cm)
ὲ = Molar absorptivity (26,900 dm3 mol-1 cm-1)

The results were expressed in terms of mg cyanidin 3--3-O-glucoside.

Statistical analysis

All multiple comparisons were subjected to Analysis of Variance (ANOVA). Duncan's multiple range test (DMRT) (Duncan, 1955) [4] was applied to the transformed values and were then transferred to the original means (Gomez and Gomez, 1984) [6].

Results

Fruit Yield and Color Characteristics

The study revealed significant variation in the fruit yield and color characteristics among different mulberry cultivars collected from major mulberry-growing regions of Tamil Nadu. The highest fruit yield per plant was observed in the Palladam region, indicating the suitability of red loamy soil for mulberry cultivation. Most of the cultivars produced black-colored fruits at maturity. The fruit yield and color characteristics of various mulberry cultivars collected from different mulberry-growing regions in Tamil Nadu are presented in Table 1.

Table 1: Fruit yield and color characteristics of mulberry cultivars in Tamil Nadu

Cultivars	Location	Fruit yield (g plant^{-1} Season^{-1})	Color of the fruit	Color value		
				L*	a*	b*
V1	Coimbatore	20.23	Black	16.32±1.43	8.02±0.44	-7.03±051
	Salem	23.63	Black	17.01±0.20	16.13±0.79	-9.10±0.00
	Avinashi	19.56	Black	23.72±0.76	23.01±1.83	-12.41±0.98
S13	Erikodi	22.26	Pink and black	19.29±0.65	15.09±0.10	-8.16±0.43
MR2	Dharmapuri	46.15	Purple and black	14.73±0.00	13.63±0.50	-4.15±0.07
	Palladam	55.24	Dark purple and black	24.26±0.96	19.38±0.54	-8.06±0.58
	Udumalpet	53.12	Purple and black	19.87±0.39	16.97±1.34	-9.23±0.26
S36	Coimbatore	18.12	Red and black	17.41±0.05	11.26±0.17	-12.10±0.86

Data mean±SD
L*= black (L*=0) to white (L*=100)
a*= 60 (Green) to + 60 (Red)
b*=-60 (Blue) to + 60(Yellow)

Among the regions, the highest fruit yield per plant was recorded in Palladam, which highlights the suitability of red loamy soil for mulberry cultivation.
Color values (L*, a*, b*) were measured using Hunter Lab Colorimeter. The L***** values indicated a dark coloration consistent with black mulberries, while *a****** and b***** values indicated the presence of red and blue pigments, typical of mature fruits. These color parameters reflect the transition of mulberries from green (immature) to purplish-black (mature) stages.

Physico-Chemical Properties
The physical and chemical characteristics of the fruits also varied among cultivars. Data on average fruit weight, moisture content, pH, total soluble solids (TSS), and reducing sugar are presented in Table 2.

Table 2: Physico-chemical characteristics of different cultivars of mulberry fruit

Cultivars	Location	Wet weight of fruit (g)	Moisture content (percent)	pH	Total soluble solids (per cent)	Reducing sugar (g^{-1} 100 g fresh weight)
V1	Coimbatore	1.19±1.02	78.80±3.69	5.17±0.21	13.09±0.80	4.06±1.48
	Salem	0.82±0.15	75.50±2.14	5.19±0.18	13.05±0.01	4.02±3.07
	Avinashi	1.20±0.80	81.50±6.04	5.28±0.31	14.66±1.15	4.46±0.30
S13	Erikodi	1.30±0.81	76.60±3.28	5.02±0.41	15.32±0.51	5.18±1.26
MR2	Dharmapuri	1.26±0.65	78.20±4.43	5.09±0.40	16.82±1.01	6.08±0.87
	Palladam	1.43±0.68	85.50±5.11	5.18±0.31	16.95±0.02	6.42±1.55
	Udumalpet	1.30±0.31	84.87±0.83	5.04±0.37	17.02±0.75	7.92±0.91
S36	Coimbatore	1.32±0.80	88.00±2.21	5.24±0.35	15.63±1.24	5.76±2.08

Data mean±SD

The fruit weight ranged from 0.82 to 1.43 g**,** with highest values observed in fruits from the S36 cultivar. Moisture content ranged from 75.50% (V1, Salem) to 88.00% (S36)**.** The pH of mulberry juice varied from 5.04 to 5.28, indicating mild acidity.
TSS values ranged from 13.05 to 17.02%, while reducing sugars varied from 4.02 to 7.92 g/100g fresh weight, suggesting regional and varietal variation in sugar accumulation.

3. Monomeric Anthocyanin Content
Regarding pigment analysis, the monomeric anthocyanin content of fresh mulberry juice was found to be between 179.51 and 271.35 mg/L, expressed as cyanidin-3-O-glucoside. These values represent the intensity of red to violet-black coloration and reflect the nutritional and functional richness of the studied cultivars (Table 3).

Table 3: Monomeric anthocyanin content of different cultivars of mulberry fruit

Cultivars	Location	Total anthocyanin (mg cyaniding-3-0-glucoside l^{-1} fresh juice)
V1	Coimbatore	204.56±08.89^{e}
	Salem	209.60±12.44^{de}
	Avinashi	200.38±16.82^{ef}
S13	Erikodi	192.03±15.84^{ef}
MR2	Dharmapuri	250.52±09.70^{bc}
	Palladam	271.35±13.68^{b}
	Udumalpet	229.60±01.60^{cd}
S36	Coimbatore	179.51±10.15^{f}

Values are means±SD, a-f values not sharing a common superscript latter within each column differs significantly at $p < 0.05$ (DMRT). These values are indicative of the pigment concentration contributing to fruit color and potential antioxidant capacity.

Discussion
Mulberry fruit yield and color parameters observed in this study correlate well with the environmental and soil conditions of Tamil Nadu. The superior yield in Palladam can be attributed to red loamy soil, which supports optimal plant growth. This agrees with findings by Singhal *et al.* (2010) [16], who reported mulberry yields of 40-50 g/plant in Tamil Nadu.
The black coloration of mature fruits aligns with typical South Indian cultivars. L*, a*, and b* values confirmed the

presence of deep purplish-black pigmentation. As noted by Datta (2002), mulberry fruit color varies among genotypes from white to deep red to black, with smoother peels that change color during ripening.

The observed fruit weight (0.82-1.43 g) was lower than values reported by Muhammad Iqbal *et al.* (2010), who recorded 2.54-3.02 g in Pakistan, possibly due to cultivar or environmental differences. Moisture content (75.5-88%) was higher than Turkish mulberries (71.5-74.6%; Ercisli and Orhan, 2007), suggesting better water retention or different harvest stages.

The pH range (5.04-5.28) was higher than the 3.02-4.78 reported in other *Morus* species (Muhammad Imran *et al.*, 2010), which might be due to genetic or agro-ecological differences. TSS (13.05-17.02%) was consistent with reports from Turkish cultivars (15.65-22.10%; Okatan *et al.*, 2010). Reducing sugar values (4.02-7.92%) were lower than the 12.11-19.56% range reported by Muhammad *et al.* (2010), which might be due to regional climatic conditions or genetic differences among cultivars.

Anthocyanin content (179.51-271.35 mg/L) showed notable variation among cultivars and was consistent with the ranges reported by Liu *et al.* (2004) [2], who observed 147.68 to 2725.46 mg/L across 31 cultivars. Anthocyanin levels are influenced by environmental conditions, ripening stage, and cultivar type (Danica *et al.*, 2013), which underscores the nutritional potential of mulberry fruits from Tamil Nadu for use as natural colorants and antioxidants in functional foods

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