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Comparison of Essential Amino Acids, Bioactive Compounds and Antioxidants on Fruit Ripening Stage of *Lepisanthes rubiginosa* (Roxb.) Leenh

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Abstract

Essential amino acids, bioactive compounds and antioxidants of *Lepisanthes rubiginosa* (Roxb.) Leenh were determined on the 3 stages of fruit ripening including unripe, half-ripe and ripe. Eight essential amino acids, identified by LCMS/MS, were found in most samples ranging from 5.63 ± 0.08 to 689.89 ± 25.12 µg/g DW. Glucose, fructose and caffeic acid contents ranged 49.86 ± 0.04 to 258.96 ± 0.86 mg/g, 61.41 ± 0.01 to 259.23 ± 2.71 mg/g and 114.67 ± 0.35 µg/g to 124.65 ± 0.33 µg/g, respectively. Total phenolic content (TPC) and total flavonoid content (TFC) ranged from 9.06 ± 0.22 to 124.65 ± 0.33 17 mg gallic acid equivalent (GAE)/100 g dry weight (DW) and 9.73 ± 0.02 to 105.83 ± 6.63 µg/g DW, respectively. These results showed the first to compare essential amino acids, bioactive compounds and antioxidants in the fruit ripening stages of *Lepisanthes rubiginosa*. The information of this study indicated promise for further development of functional ingredients in food or cosmetic products.

Keywords: Essential amino acids, Antioxidants, Bioactive compounds, *Lepisanthes rubiginosa*

Introduction

Ma huat (*Lepisanthes rubiginosa* (Roxb.) Leenh.) belongs to the family Sapindaceae that contains important forest species found in Northern and Northeastern Thailand with medicinal value and multiple uses. Plants from this family such as *Sapindus mukorossi*, *Dodonaea viscosa*, *Allophylus africanus* and *Sapindus trifoliatus* are traditionally used for oral and skin health care [1-5]. They comprise trees, shrubs and tendril-bearing vines with 140 to 150 genera and 1,400 to 2,000 species worldwide [6].

Sapindaceae are globally distributed in temperate and tropical regions. *L. rubiginosa* is an evergreen shrub, 2 - 3 m in height that can grow to 7 m, while *L. amoena* has been reported to possess antibacterial activity [7]. This plant is distributed in Thailand, Malaysia, Brunei, Myanmar Laos and South China. The fruits are edible but astringent and the wood is very hard [8]. *L. rubiginosa* have been traditionally used to cure antipruritic, headache, fever, decoction and antitussive disorders and as a tonic and food preservative. The stems are used for inducing sleep, while young leaves are scrubbed until foam appears and then pasted onto facial skin. Saburi *et al.* [9] investigated the methanolic fraction of *L. rubiginosa* bark. They isolated and characterized a new tetrasaccharide derivative of farnesol named rubiginoside along with known triterpenoid saponins. Adesanya *et al.* and Chuangbunyat *et al.* [10,11], found that an aqueous fruit extract of *L. rubiginosa* at doses of 20 and 100 mg/kg significantly ($p < 0.05$) decreased locomotion, and a dose of 100 mg/kg enhanced thiopental induced sleep, while Chabra *et al.* [2], (1991) determined that affinity to dopaminergic receptors and inhibition of apomorphine-induced climbing behavior in mice.

A comparative study by Chuangbunyat *et al.* [11], on the essential oil from flowers and fruits of *L. rubiginosa* showed anticancer activity against NCI-H187 (small cell lung cancer) and antioxidant activity in flower essential oil, while fruit essential oil did not show anticancer activity and possessed low antioxidant activity. However, both flower and fruit essential oils showed strong antimicrobial activity against Tricophytonmentagrophytes and moderate activity against *E. coli*, *S. aureus*, *Pseudomonas aeruginosa* and *Candida albicans*. Kuspradini *et al.* [7], reported that methanolic and ethanolic extracts of *L. amoena* leaves exhibited good inhibitory effects against *Candida albicans*, *Streptococcus mutans and*

Propionium acnes, while Hasan *et al.* [12], revealed that the ethanolic extract of *L. rubiginosa* leaves showed potential antioxidant, analgesic, antihyperglycemic, neuropharmacological and antidiarrheal activities. These findings suggested that the leaf extract of *L. rubiginosa* had bioactivity beneficial for human health.

Drugs currently used as antidiabetics have serious side effects and deleterious contraindications [13]. Hence, herbal medications with high therapeutic efficacy and minimal side effects have attracted increasing attention. Antidiabetic agents from traditionally acclaimed medicinal plants are now being investigated for their antidiabetic potential [14,15]. Fruit extracts of some plants were evaluated for alpha-amylase, alpha-glucosidase, total phenolic content (TPC) and total flavonoid content (TFC), with results showing significant antihyperglycemic and analgesic activities [16-18].

Variations in essential amino acids, antioxidant properties and bioactive compounds of *L. amoena* fruits at various stages of maturity have not previously been characterized. How maturation of fruits impacted sugar content, ascorbic acid, bioactive compounds, antioxidant and antiproliferative activities and total anthocyanins was reported in pomegranate [19], *Morinda citrifolia* L. [20], Mon Thong durian [21], *Manilkara hexandra* (Roxb.) [22]. and *Antidesma bunius* L. Spreng [23]. Characterization of amino acid compositions in fruits was reported in *Rosa roxburghii* [24], and amino acid, fatty acid and mineral contents of *Rubus amabil* were reported [25,26] and presented recent trends in the analysis of amino acids in fruits and derived foodstuffs. Fish [27], reported a reliable integrated extraction and analysis procedure for measuring complete physiological amino acid profiles of fruit and vegetables using HPLC instrumentation and Zeng *et al.* [28], identified 21 free amino acids in fruit juices by HPLC using a modification of the 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) method. Amino acid contents of fruits and fruit-derived foods were studied to determine their nutritional value, aroma, taste and health-promoting effects and possible uses as markers of origin and authenticity. However, no previous research has evaluated the amino acid contents of fruit extracts of *L. rubiginosa*.

This investigation was the first to compare essential amino acids, bioactive compounds and antioxidants in the fruit ripening stages of *Lepisanthes rubiginosa* (Roxb.) Leenh.

Materials and methods

Sample preparation

Fruits were harvested in the morning of March 2019 from the community forest “Na Si Naun”, Kantarawichai District, Maha Sarakham Province. The fruits were divided into 3 groups according to fruit ripening stage for instant unripe (green), half-ripe (pink and red) and ripe (dark purple or black) as shown in the **Figure 1**. Before performing the analyses, the samples were washed with running water and then with distilled water. Residual moisture was evaporated at room temperature. Methanolic extracts were obtained by homogenizing 2.5 g of dried tissue (pulp and peel) in 25 mL of deionized water until uniform consistency was achieved, using a homogenizer (IKA, Ultra Turray). The homogenates were filtered through Whatman no. 4 filter paper and then stored at −20 °C until analysis. Extraction was carried out according to Jelled *et al.* [29], with minor modifications. Briefly, samples (1.0 g) were extracted for 12 h with 10 mL of 80 % methanol at 37 °C on an incubator shaker set at 150 rpm. After that, the mixture was filtered through Whatman no. 1 filter paper, and the filtrate was collected. The pellet was re-extracted under identical conditions. The filtered samples were combined and used to analyze TPC, TFC and antioxidant activities.

Figure 1 Three fruit ripening stages of *L. rubiginosa* as unripe (green), half-ripe (pink and red) and ripe (dark purple or black).

Total phenolic content, total flavonoid content, antioxidation activities, sugar composition and amino acid content analysis

TPC was determined following Kubola *et al.* [30], using Folin-Ciocalteu's reagent. Briefly, 300 mL of the extract was combined with 2.25 mL of the Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water), and the combination was let to stand at room temperature for 5 min. Then, 2.25 mL of a sodium carbonate (60 g/l) solution was added to the mixture. Using a spectrophotometer, absorbance at 725 nm was measured after 90 min at room temperature. Results were given in mg gallic acid equivalents in 1 g of dried sample (mg GAE/g), while TFC was determined using the colorimetric method described by Kaisoon *et al.* [31]. Briefly, 0.5 mL of the extract was combined with 2.25 mL of distilled water before 0.15 mL of 5 % $NaNO_2$ solution was added. 0.3 mL of a 10 % $AlCl_3.6H_20$ solution was added after 6 min, and it was then given another 5 min to stand before 1.0 mL of 1 M NaOH was added. A vortex effectively blended the mixture. A spectrophotometer was used to detect the absorbance right away at 510 nm. The results were presented as mg rutin equivalents per 1 g of dried material (mg RE/g dry weight). DPPH free radical scavenging activity of the extracts was determined following the method of Kubola *et al.* [30], briefly, A 0.1 mL aqueous extract was added to 3 mL of a 0.001 M DPPH in methanol solution. After 30 min, the absorbance at 517 nm was measured, and the percent inhibition of activity was calculated as ((Ao - Ae)/Ao)/100 (Ao = absorbance without extract; Ae = absorbance with extract). Reducing power of the extracts was measured based on the FRAP assay following Siriamornpun *et al.* [32], An aliquot of 1.8 mL of FRAP reagent was briefly mixed with 180 µl of deionized water and 60 µl of extract. FRAP reagent was freshly prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, 20 mM $FeCl_36H_2O$ and distilled water in a 10:1:1:12 ratio at 37 °C. The mixture was shaken and incubated for 4 min at 37 °C in a water bath and then the absorbance was read at 593 nm. FRAP values were expressed as mmol $FeSO_4$ in 1.0 g of dried sample (mmol $FeSO_4$/g). Sugar compositions of the samples were determined using high-performance liquid chromatography (HPLC) apparatus equipped with refractive index detector as per the protocol of Asikin *et al.* [33], and Chumroenphat *et al.* [34]. Sugar samples (1 mg) were dissolved in 4 mL distilled water and then filtered through a 0.2 µm membrane filter. A Sugar-Pak I column (WAT085188, 6.5×30×10 mm^3µm, Waters Corporation, Milford, Massachusetts, USA) was used for HPLC separation. The column was maintained at a constant temperature of 80 °C was used as the mobile phase at a flow rate of 0.5 mL/min. The sugar samples and the standards (sucrose, glucose, and fructose) (10 µl) were injected using an autosampler. The concentrations of sucrose, glucose, and fructose were calculated using standard curves. The standard curves were obtained by plotting peak area against concentration for the respective sugar standards. Total sugar content was expressed as grams per g sugar. Amino acid extracts of *L. rubiginosa* were prepared according to Nimbalkar *et al.* [35]. Briefly, 1 mL of sample was centrifuged at 12,000 rpm for 5 min and then filtered through an Advantec 0.45 µm membrane filter and analyzed by LCMS/MS. Siriamornpun *et al.* [36], briefly, the pellet was re-extracted under identical conditions. The filtered samples were combined and used to analyze the total flavonoid and total phenolic contents and antioxidant activities.

Statistical analysis

SPSS version 29.0 software was used to analyze the data statistically. All data were analyzed by one-way analysis of variance (ANOVA), with results expressed as mean ± standard deviation (SD) of 3 replicates. The least significant difference (LSD) test was applied to determine significance relative to the control and Pearson's correlation test was used to assess correlations among the means. A p-value of < 0.05 was considered statistically significant.

Results and discussion

Antioxidant activities of *L. rubiginosa*

Antioxidant activities were expressed as DPPH radical scavenging Trolox equivalent concentration (TAC) and FRAP value (**Table 1**). DPPH radical scavenging activities were measured as mg Trolox equivalent, and results were converted to percentage inhibition. DPPH radical scavenging activities ranged 10.85 ± 0.02 in unripe fruits to 55.04 ± 0.06 mg Trolox/g in half-ripe fruits, with no statistically significant differences in ripe fruits at 54.27 ± 0.13 mg Trolox/g ($p < 0.05$). FRAP values of *L. rubiginosa* samples are presented in **Table 1**. Half-ripe fruits showed excellent reducing power (202.86 ± 10.20 mmol $FeSO_4$/g), followed by ripe fruits (52.76 ± 1.62 mmol $FeSO_4$/g) and unripe fruits (25.87 ± 2.83 mmol $FeSO_4$/g).

Total phenolic and flavonoid contents

TPC and TFC levels are indicative of the amounts of contained bioactive compounds which are beneficial to human health. Phenolic acids, which are readily assimilated through intestinal tract membranes, are advantageous to human health due to their antioxidant properties, which prevent cell injury caused by free-radical oxidation. Regular consumption of phenolic acids enhances the anti-inflammatory capacity of humans [37]. Flavonoids are widely used as anticancer, antimicrobial, antiviral, antiangiogenic, antimalarial, antioxidant, neuroprotective, antitumor, and anti-proliferative agents [38]. In this study as shown in **Table 1**, TPC was determined in the fruit ripening stages of *L. rubiginosa* as unripe, half-ripe and ripe, with results shown in TPC in the fruit ripening stages ranged from 5.00 ± 0.57 to 9.91 ± 0.51 mg GAE/g DW. Unripe fruits exhibited the highest TPC (9.91 ± 0.51 mg GAE/g DW) followed by half-ripe fruits (5.00 ± 0.03 mg GAE/g DW) and ripe fruits (5.00 ± 0.57 mg GAE/g DW). This consistency to Butkhup *et al.* [23], showed that the TPC in Maoluang fruits decreased from the immature to the over ripe stages, the highest content at the over ripe stage, with an average value of 141.94 mg/100 g FW and found TPC in mulberry fruit ranged from 104.78 to 213.53 mg GAE/100 g DW [39].

Flavonoids are phenolic compounds distributed in higher plants, with documented high antioxidant potential. Significant variations in TFC in the fruit ripening stages of *L. rubiginosa* fruits ranged from 6.63 ± 0.02 to fruits 46.82 ± 1.24 mg RE/g DW. The highest TFC was found in half-ripe fruits (46.82 ± 1.24 mg RE/g DW), followed by ripe (19.62 ± 0.94 mg RE/g DW) and unripe (6.63 ± 0.02 mg RE/g DW) as shown in **Table 1**. Consistent with our findings, the polyphenol content and antioxidant capacity of organically and conventionally grown tomato (*Solanum lycopersicum* L.) fruits both rise throughout ripening, with the former reaching a peak at the half-ripe stage. TPC and TFC levels fluctuated as fruits matured. To the best of our knowledge, however, there is a dearth of data on how different stages of fruit maturation affect the concentrations of antioxidants and phytochemicals in *L. rubiginosa* collected in Thailand in March 2019. Other fruits, like the date palm [40], tomato [41], and mango [42], have also been observed to experience a decrease in TPC as their fruits mature. The metabolism of phenolic compounds may be linked to the elevated quantities of these chemicals seen in the immature phases including the half-ripe stage. The decrease in TFC with increasing maturity suggests that the maturity stage has a major influence on the flavonoid content of the fruit [43].

Table 1 Antioxidant activities measured by means of DPPH radical scavenging and FRAP analyses, total phenolics (TPC) and total flavonoids (TFC) in *L. Rubiginosa*.

Sample	DPPH	FRAP	TPC	TFC
	mg Trolox/g DW	mg Fe^{2+}/g DW	mg GAE/g DW	mg RE/g DW
Unripe	10.85 ± 0.02[c]	25.87 ± 2.83[c]	9.91 ± 0.51[a]	6.63 ± 0.02[c]
Half-ripe	55.04 ± 0.06[a]	202.86 ± 10.20[a]	5.00 ± 0.57[b]	46.82 ± 1.24[a]
Ripe	54.27 ± 0.13[b]	52.76 ± 1.62[b]	5.00 ± 0.03[c]	19.62 ± 0.94[b]

Values are expressed as mean ± SD of triplicate measurements (n = 3).
Different letters within the same columns indicate statistically significant differences between ripening stages ($p < 0.05$).
FRAP: Ferric reducing antioxidant activities, DPPH: Radical scavenging activities.
TPC: Total phenolic contents, TFC: Total flavonoid contents.

Sugar composition

Concentrations of fructose and glucose throughout fruit maturation were higher than sucrose in all samples (**Table 2**). Fructose concentration in the maturity stages of *L. rubiginosa* fruits ranged from 61.41 ± 0.01 to 259.23 ± 2.71 mg/g. Highest fructose was found in ripe fruits (259.23 ± 2.71 mg/g), followed by half-ripe fruits (177.33 ± 0.28 mg/g) and unripe fruits (61.41 ± 0.01 mg/g). Glucose concentration in the fruit ripening stages of *L. rubiginosa* ranged from 49.86 ± 0.04 to 258.96 ± 0.86 mg/g. Highest glucose was found in ripe fruits (258.96 ± 0.86 mg/g), followed by half-ripe fruits (164.96 ± 0.16 mg/g) and unripe fruits (49.86 ± 0.04 mg/g). Besides sugars and organic acids, polyphenols as secondary metabolites, to a certain extent can also contribute to sweet, bitter or astringent flavours of fruit. Fruit sugar composition differs depending on its stage. These outcomes may be accounted for by the hydrolysis of sucrose into glucose and fructose by invertases or synthases during the stages of ripening [44]. Concentrations of fructose and glucose throughout fruit ripening stage were higher than sucrose in all samples. Highest fructose was found in ripe fruits. Contents of glucose and fructose increased considerably during fruit maturation, with relatively higher fructose content than glucose same as reported in mango [45]. The identical one as the report of Ivancic *et al.* [46], that showed skin and pulp sugar content, green olive skin was found to have the greatest concentration of glucose (28.89 mg/g FW). The amount of glucose in the skin considerably decreased to 18.87 mg/g FW. As soon as the skin turned from green to purple, there was a significant drop. The pulp's glucose level dropped linearly from 18.15 to 15.75 mg/g FW as the fruit ripened.

Table 2 Sugar composition analysis in *L. Rubiginosa.*

Sample	sugar content (mg/g)		
	sucrose	glucose	fructose
Unripe	0.61 ± 0.02[a]	49.86 ± 0.04[c]	61.41 ± 0.01[c]
Half-ripe	0.31 ± 0.02[b]	164.96 ± 0.16[b]	177.33 ± 0.28[b]
Ripe	0.05 ± 0.01[c]	258.96 ± 0.86[a]	259.23 ± 2.71[a]

Values are expressed as mean ± SD of triplicate measurements (n = 3).
Different letters within the same columns indicate statistically significant differences between ripening stages ($p < 0.05$).

Amino acid content

Both qualitative and quantitative LCMS/MS results of 10 amino acids were identified for the first time, including 9 essential amino acids in *L. rubiginosa*. Results showed arginine, isoleucine, leucine, lysine, methionine, phenylalanine and threonine in all the study samples, with histidine and valine absent in all samples (**Table 3**). Methionine had the highest value (689.89 ± 25.12 µg/100g DW). Investigation of the amino acids present in different kinds of plants has attracted research interest [47,48]. Numerous writers have reported on mulberry fruit, tomato and other fruits [44,49,50], which are consistent with our findings. Sorrequieta *et al.* [49], and Boggio *et al.* [50], discovered that the metabolizing enzymes that took place throughout the ripening process caused the free amino acid concentrations in tomato pericarp to grow significantly during the ripening stage. Here, both qualitative and quantitative results for 10 amino acids,

including 9 essential amino acids in *L. rubiginosa* were determined for the first time using LCMS/MS to provide useful information for further use of this plant. Results identified arginine, isoleucine, leucine, lysine, methionine, phenylalanine and threonine in all the study samples. Total levels of amino acids found in *L. rubiginosa* were previously reported to be lower than in other plants such as bean, potato, rice and mushroom. Amino acids are important nutrients for the human body [51].

Table 3 Contents of amino acid in *L. rubiginosa* analyzed by LCMS/MS.

Sample	Amino acid content (µg/100 g DW)									
	Arginine	**Histidine**	**Isoleucine**	**Leucine**	**Lysine**	**Methio -nine**	**Phenyla -lanine**	**Threo- nine**	**Trypto -phan**	**Valine**
Unripe	73.72 ± 5.14^{a}	nd	50.49 ± 2.69^{b}	101.04 ± 4.21^{a}	21.1 ± 1.98^{a}	601.8 ± 31.25^{b}	60.93 ± 3.37^{a}	89.11 ± 7.26^{a}	25.88 ± 1.13^{b}	nd
Half-ripe	69.27 ± 4.46^{a}	nd	58.05 ± 2.16^{a}	101.06 ± 3.49^{a}	17.13 ± 1.44^{b}	689.8 ± 25.12^{a}	50.94 ± 2.06^{b}	93.00 ± 1.45^{a}	65.57 ± 2.14^{a}	nd
Ripe	27.27 ± 1.11^{b}	nd	15.44 ± 0.37^{c}	6.71 ± 0.46^{b}	5.63 ± 0.08^{c}	194.0 ± 4.25^{c}	5.92 ± 0.40^{c}	29.11 ± 0.09^{c}	nd	nd

nd = not detected values are expressed as mean ± SD of triplicate measurements (n = 30).
Different letters within the same columns indicate statistically significant differences between ripening stages ($p < 0.05$).

Identification of phenolic and flavonoid compounds in *L. Rubiginosa*

Contents of *L. rubiginosa* extracts were quantified using HPLC-DAD. Distributions of phenolic acids and flavonoid compounds are presented in **Tables 4** and **5**. Among all samples of *L. rubiginosa*, caffeic acid was identified as the predominant phenolic acid, ranging from 114.67 ± 0.35 to 124.65 ± 0.33 µg/g. Highest caffeic acid was found in ripe fruits (124.65 ± 0.33 µg/g), followed by half-ripe fruits (123.10 ± 1.24 µg/g) and unripe fruits (114.67 ± 0.35 µg/g) as shown in **Table 4**.

Five flavonoids as quercetin, kaempferol, rutin, apigenin and myricetin were detected by RP-HPLC. Quantifications of the 5 flavonoids, based on calibration curves of authentic standards, are presented in **Table 5**. Flavonoids were detected in all samples, with significant differences ($p < 0.05$). Rutin was the major flavonoid, ranging from 38.39 ± 0.05 µg/g in ripe fruits to 105.83 ± 6.63 µg/g in unripe fruits, while Kaempferol was not detected in all samples as shown in **Table 5**. Phenolic and flavonoids content variation depending on the ripening stage of the fruit.

Table 4 Contents of phenolic acid in *L. rubiginosa* analyzed by LCMS/MS.

Sample	Phenolic acid content (µg/g)									
	GA	**PCCA**	**p-OHBA**	**VA**	**Ch A**	**CFA**	**SyA**	**p-CA**	**FA**	**SNA**
Unripe	10.61 ± 0.03^{b}	13.78 ± 1.04^{c}	nd	nd	Nd	114.67^{b} ± 0.35	14.36 ± 0.10^{c}	9.11 ± 0.01^{a}	25.74 ± 0.94^{a}	9.62 ± 0.01^{b}
Half-ripe	10.68 ± 0.09^{b}	17.00 ± 0.75^{b}	nd	10.00 ± 0.01^{b}	9.06 ± 0.22^{b}	123.10 ± 1.24^{a}	15.34 ± 0.09^{b}	9.52 ± 0.01^{b}	20.29 ± 0.19^{b}	9.67 ± 0.01^{a}
Ripe	13.82 ± 0.40^{a}	86.25 ± 3.53^{a}	nd	10.54 ± 0.01^{a}	25.09 ± 0.46^{a}	124.65 ± 0.33^{a}	15.75 ± 0.05^{a}	9.12 ± 0.01^{a}	14.88 0.37^{c}	9.56 ± 0.01^{c}

nd = not detected Values are expressed as mean ± SD of triplicate measurements (n = 3).
Different letters within the same columns indicate statistically significant differences between ripening stages ($p < 0.05$).
GA: Gallic acid; PCCA: Protocatechuic acid; *p*-OH: *p*-hydroxybenzoic; VA: Vanillic acid; Ch A: Chlorogenic acid; CFA: Caffeic acid; SyA: Syringic acid; *p*-CA: p-coumaric acid; FA: Ferulic acid; SNA: Sinapic acid

Table 5 Contents of flavonoid acids in *L. Rubiginosa.*

Sample	Flavonoid acids (µg/g)				
	Rutin	**Myricetin**	**Quercetin**	**Apigenin**	**Kaempferol**
Unripe	105.83 ± 6.63[a]	16.05 ± 0.05[b]	9.73 ± 0.02[a]	13.18 ± 0.05[b]	Nd
Half-ripe	56.63 ± 2.18[b]	16.03±0.06[b]	nd	60.89 ± 2.15[a]	Nd
Ripe	38.39 ± 0.05[c]	24.29±0.24[a]	nd	nd	Nd

Values are expressed as mean ± SD of triplicate measurements (n = 3).
Different letters within the same columns indicate statistically significant differences between ripening stages ($p < 0.05$).

Similar data were reported by Saensouk *et al.* [44]. The flavonoid compounds (rutin, catechin, quercetin, apigenin, myricetin and kaempferol) in mulberry fruit within the different ripening stages. The results show that the total flavonoid compounds were found in the range of 1.99 - 9.56 mg/g DW for M0 and M4. Zhang *et al.* [52], who found that TFC levels in the 2 plum cultivars decreased as the fruit developed and were higher in the peel than the pulp. comparable to the TPC outcomes at the fruit ripening stage. Other fruits, like the tomato [41], mango [42] and jujube [43], have also been observed to experience a decrease in TPC and TFC as their fruits mature.

Conclusions

Our results demonstrated that amino acid content and composition varied greatly among plant varieties; however, free amino acid variability may also be related to different environmental conditions using the discriminant and cluster analysis method [53,54]. Phenolic and flavonoids content variation depending on the ripening stage of the fruit. Therefore, further studies on this aspect are needed.

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