

Tree Age Affects the Physicochemical, Antioxidant, and Minerals Composition of Musang King Durian Fruits

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ABSTRACT

The quality of fruits, including durian, can be significantly affected by the age of the tree. The impact of tree age on the physicochemical, antioxidant, and mineral compositions of Musang King durian (MKD) fruit was investigated using physicochemical and biochemical approaches. The results revealed that firmness, pH, reducing sugar, polyphenol, and antioxidant activity were affected by tree age. Durian fruits from old trees exhibited higher firmness and reduced sugar compared to young and middle-aged trees. Fruits from old and middle-aged trees had higher polyphenol and antioxidant activity compared to young trees durian fruits. Calcium and nitrogen were significantly higher in durian fruits from young trees, while potassium and zinc were higher in fruits from old trees.

The finding demonstrated that tree age impacts the physicochemical, antioxidant content, and mineral composition of MKD fruits, and these changes may respond to the sensory perception of MKD fruits.

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INTRODUCTION

Malaysia is known for growing varieties of durian species and boasts a great variety

of durian cultivars (Siew et al., 2018). Among these, the Musang King durian (MKD), registered as D197, is well-known for its buttery, golden yellow flesh and intense flavor with a hint of bitterness aftertaste (Specialtyproduce, 2024). After Malaysian durians were introduced to China, demand increased tremendously, particularly for the MKD variety, resulting in a price premium over other varieties (MalaysiaSun, 2024). As a result, durians are now cultivated on a large scale in Malaysia. It was reported that the planting acreage and production of durian increased by 22.71% and 33.11%, respectively, between 2018 and 2022 (Department of Agriculture Malaysia, 2023).

Malaysian durians are categorized as harvested from young or old trees. Fruits from old trees are considered superior in flavor, and consumers are willing to pay more for them. MKD has been receiving positive reviews and now cultivated on a large scale. However, most of the MKD in the market comes from young trees, as durian begins to bear fruit from 4-5 years after grafting (Ketsa et al., 2020).

The eating quality of fruit is determined by sensory characteristics (e.g., flavor, aroma, firmness) and nutritional profile (e.g., antioxidant content) (Brasil & Siddiqui, 2018). Physicochemical properties, such as pH, titratable acidity (TA), and soluble solids content (SSC), define the fruit's eating quality (Athmaselvi et al., 2014). Antioxidants, such as phenolic compounds and ascorbic acid, enhance the nutritional and sensory appeal, influencing their acceptability and sensory perception of a fruit (Pott et al., 2019). These characteristics can be influenced by various preharvest factors, including tree age (Meena & Asrey, 2018), environmental conditions, and genetic variations (Brasil & Siddiqui, 2018).

Tree age was observed to significantly impact the quality characteristics of fruits, including the physicochemical and biochemical composition of fruits (Asrey et al., 2007; Khalid et al., 2012; Meena & Asrey, 2018a; Yaacob, 1983). The physiological changes that occur as a tree ages and grows significantly influence fruit composition and result in quality changes (Yaacob, 1983). These changes often involve differences in minerals allocation (Estravis-Barcala et al., 2020; Zhou & Melgar, 2020). For instance, compared to young trees, older trees allocate more dry matter and minerals to flowers and fruits (Karmacharya & Singh, 1992). Young guava trees produced fruits with higher iron content and low magnesium content (Asrey et al., 2007). Similarly, Kinnow mandarin harvested from young age trees (3 years old) contained higher Mn and Fe contents than old age trees (Khalid et al., 2012). Besides, the difference in tree ages has been associated with the change in the physicochemical and antioxidant characteristics of fruits. Meena and Asrey (2018a) revealed that Amrapali mango fruits from 18-year-old trees have the highest SSC and total sugars, while 30-year-old trees have lower TA. In addition, the antioxidant content of 6-year-old mango trees had higher total phenols, antioxidant capacity, and ascorbic acid than fruits from 18 and 30 years (Meena & Asrey, 2018b).

Despite the well-documented impacts of the development and ripening (Husin et al., 2022; Wiangsamut & Wiangsamut, 2023) and postharvest processing (Razali et al., 2022;

Tagubase & Ueno, 2016; Tan et al., 2020) on durian fruit quality, the influence of tree age on MKD fruit physicochemical properties, antioxidant content, and minerals composition remains uninvestigated. However, many durian lovers believe that older trees produce fruits with superior flavor. This claim is critical, but there is no scientific evidence to explain the influence of tree age on the eating quality of MKD fruit. Thus, this study aims to explore the impact of tree age on the eating quality attributes of MKD fruit.

MATERIALS AND METHODS

Plant Materials

Fresh and fully ripe MKD fruits from three tree age groups (5-9 years [young], 10-15 years [middle], and >16 years [old]) were harvested from commercial orchards in Raub (latitude 3° 03' N, longitude 101° 51' E), Pahang, Malaysia, during the peak season in November 2022. Fruits with similar size (1.3 ± 0.2 kg), well-formed shape, and no visual defects were selected for this study. Six fruits from each tree age group were used. After harvest, the pulp was removed from the locules for physicochemical analysis, while a separate set of pulp was extracted for mineral composition and antioxidant content analysis. The remaining pulp from each locule was lyophilized for 48 hours using a freeze drier (LaboGene Scanvac CoolSafe 4L, United Kingdom). The freeze-dried powder was then stored at -80 °C until the mineral's composition analysis.

Determination of Physicochemical Characteristics

Color

L^* , a^* , and b^* were measured using a chroma meter CR- 400 (Konica Minolta, Inc., Japan) at three different spots on the surface of each durian fruit pulp sample. The L^* (lightness), a^* ($+a^*$ = redness, $-a^*$ = greenness), b^* ($+b^*$ = yellowness, $-b^*$ = blueness), and total color change (ΔE^*). Given that MKD fruits are predominantly yellow, only the b^* value was reported. ΔE^* was determined using Equation [1];

$$\Delta E^* = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \quad [1]$$

Firmness

The firmness of the durian pulp was analyzed using an Instron texture analyzer (Model TA-XT2 Stable Micro Systems, UK). A 250-N load and a cylindrical probe (P/0.25) with a 5-mm diameter were used. The probe was pressed downwards to a depth of 3 mm at a speed of 20 mm/min, and the force (N) was recorded. Firmness measurements were taken at three different locations on the pulp (top, middle, and bottom) of each locule, and the

average values were calculated. The readings were recorded using the Instron Merlin Software version M12-13664-EN.

pH and titratable acidity (TA)

Durian pulp (7 g) was homogenized with 70 ml of distilled water, and the pH was measured using a pH meter (Professional Bench Top, Bp 3001, Singapore). The diluted durian homogenate was titrated against 0.1 N NaOH to pH 8.1 using phenolphthalein as an indicator according to Sospeter et al. (2025). TA (% citric acid) was calculated using Equation [2]:

$$\% \text{ citric acid} = \frac{\text{Volume of titre (ml)} \times 0.1 \times \text{volume made (ml)} \times \text{equivalent of citric acid} \times 100}{\text{Weight of the sample (g)} \times \text{volume of sample used for titration (ml)} \times 1000} \quad [2]$$

Equivalent of citric acid = 64.04g/equivalent

Soluble Solids Content

Soluble solid content (SSC) was measured using a method by Sospeter et al. (2025). 20 µl of the same homogenate of durian pulp used for pH analysis was applied onto the prism surface of the digital handheld refractometer (Atago Co., Tokyo, Japan) to obtain SSC readings. These readings were then multiplied by the dilution factor (10) to obtain the % SSC at 25 °C.

Determination of Antioxidant Content

Ascorbic acid content (AA)

The ascorbic acid content of the durian pulp in all treatment groups was estimated using the dye titration method (Nor et al., 2023). A total of 5 g of durian sample was blended with 20 ml of 3% metaphosphoric acid (HPO₃). Subsequently, 5 ml of the resulting extract was titrated with a standardized dye solution until the solution turned pink. The titration readings were recorded, and the ascorbic acid content was calculated using Equations [3] and [4].

$$\text{Ascorbic acid contents (mg/100 g)} = \frac{\text{Titre volume (ml)} \times \text{dye factor} \times \text{volume made up (ml)} \times 100}{\text{Weight of the sample (g)} \times \text{volume used for titration (ml)}} \quad [3]$$

$$\text{Dye factor} = \frac{0.5}{\text{Volume of the dye}} \quad [4]$$

Total Polyphenols (TP)

Extraction. Polyphenols were extracted according to the method described by Sospeter et al. (2025), with some modifications. One gram of freeze-dried powdered durian pulp samples was extracted using 15 ml of a binary mixture containing 0.2 M HCl and methanol (1:1, v/v). The sample mixture was then sonicated for 60 min at 50 °C and centrifuged for 7 min at 9000×g. This process was repeated three times, and thereafter, the extracts were concentrated at 40 °C by a centrifugal evaporator (Genevac EZ-2 Elite, Sysmex Belgium N.V.). Subsequently, the extract was examined for total polyphenol content.

Polyphenol Content Analysis. The Folin-Ciocalteu technique was used according to Singleton & Rossi (1965) with modification. A 100 µl aliquot of the extract was placed in a 15 ml test tube and diluted with Milli-Q water to achieve a total volume of 1 ml. Subsequently, 0.5 ml of Folin-Ciocalteu reagent and 2.5 ml of 20% (w/v) sodium carbonate solution were added to each test tube. The mixture was vortexed thoroughly and then left to react in the dark for 40 minutes. The absorbance measurements were taken at 765 nm using ultraviolet-visible spectroscopy (UV-VIS- Infinite M200) and gallic acid as the standard. The results were represented as milligrams of gallic acid equivalents (mg GAE) per 100 g of dry weight (DW) based on a calibration curve ($R^2 = 0.99$). Refer to the additional document (S1).

2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical-Scavenging Activity

DPPH radical-scavenging activity (AA) of durian pulp extract was assessed using the stable radical DPPH, as described by Saminathan & Doraiswamy (2000) with modification. 200 µg/ml of the diluted durian pulp extracts was adjusted to 1ml using methanol. The sample aliquots were well mixed with approximately 3 ml of a 0.1 mM DPPH solution. 1 ml of methanol was added to 3 ml of a 0.1 mM DPPH solution to generate a control sample. Then, the mixture was allowed to stand in the dark for 20 minutes at room temperature. The absorbances of the samples were measured at 517 nm using UV-VIS (Infinite M200) against the control. Next, the DPPH radical scavenging activity was calculated using Equation [5].

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{Absorbance (Control)} - \text{Absorbance (Sample)}}{\text{Absorbance (Control)}} \times 100$$

[5]

Determination of Macro and Microminerals

Sample Digestion

The sample was digested according to Uresti-Porras et al. (2021) with modification. A 0.25 g portion of the freeze-dried sample was mixed with 5 ml of concentrated sulfuric acid

in a test tube. The mixture was allowed to stand overnight. Following this, 2 ml of 50% hydrogen peroxide was added slowly to the mixture, and the test tube was then rotated gently to thoroughly mix the mixture. Subsequently, the test was placed in a digestion block operating at 285°C for 45 minutes until the solution became clear.

Atomic Absorption Spectroscopy (AAS) Measurements

The freeze-dried pulp samples were analyzed for the target elements using an Atomic Absorption Spectrometer (Shimadzu AA-670, Japan) equipped with appropriate hollow cathode lamps for each element. The concentrations of various elements were quantified by generating standard calibration curves using analytical reagent (AR) grade standard solutions of the respective elements. The elements analyzed included potassium (K), magnesium (Mg), calcium (Ca), nitrogen (N), iron (Fe), and zinc (Zn). The calibration and analysis followed standard AAS protocols to ensure accuracy and reliability.

Statistical Analysis

An experimental study was designed to investigate the null hypothesis that the tree age has no effects on the physicochemical quality parameters, antioxidant content and mineral composition of the whole MKD fruits. The collected physicochemical, antioxidant content and mineral composition data were analyzed using ANOVA with the JMP software version 17 pro (SAS Institute, Inc., Cary, NC, USA). Means were compared using Tukey's HSD ($p = 0.05$). In correlation analysis, Pearson correlation coefficients (r) as a measure for distance was used.

RESULTS

Physicochemical Characteristics of MKD Fruit

It was revealed that tree age significantly affected the firmness of MKD fruit; fruits from older trees exhibited higher firmness (1.35 N) compared to fruits from young (0.62 N) and middle-aged trees (0.81 N) ($p < 0.05$) (Figure 1a). While color parameters (b^* and E^* values), SSC and TA did not show any significant differences across the three tree age groups ($p < 0.05$) (Figures 1b, 1c and 1e). The pH of the MKD fruit from younger trees was higher (7.25) than that of durian from older trees (6.56). However, fruits from young- and middle-aged trees showed no significant differences. Furthermore, no significant differences were observed in the pH levels of fruit from middle- and old-aged trees ($p < 0.05$) (Figure 1d). Along with the aforementioned results, fruits from old trees exhibited significantly higher reducing sugars compared to those from young- and middle-aged trees (Figure 1f).

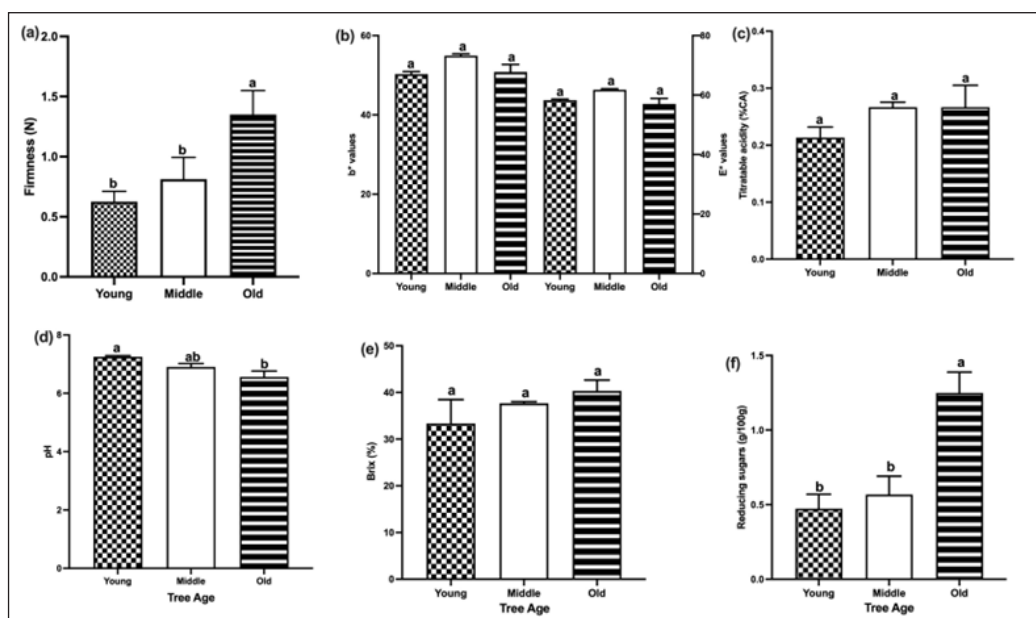


Figure 1. Means (n=6) of physicochemical parameters of Musang King durian fruits harvested from different tree ages (young, middle and old). Same letters indicate no significant differences between groups based on Tukey's HSD test ($p < 0.05$). Parameters include b^* = yellowness/ blueness, ΔE = change of color and CA = Citric acid. Error bars represent standard error

Antioxidant Content of MKD Fruit

The findings revealed that tree age did not affect the ascorbic acid content of MKD (Figure 2a). However, it affected the polyphenol content and antioxidant activity of MKD fruits ($p < 0.05$) (Figure 2b and 2c). Fruits from middle-aged trees displayed higher levels of polyphenol content and DPPH scavenging activity (antioxidant activity) as compared to those from young- and old-aged trees (Figure 2b and 2c).

Mineral Contents of MKD Fruit

The findings from the minerals analysis of MKD fruit reveal that an old-aged tree has significantly lower N than middle-aged and young trees (Figure 3a). Unlike N, K in old-aged trees was significantly higher than both young- and middle-aged trees (Figure 3b). For Ca, young-aged trees were significantly higher than middle- and old-aged trees (Figure 3c). However, there were no significant differences in Mg among the three tree ages (Figure 3d). For Zn content, just like the K, the old-aged tree has a significantly higher concentration of Zn than young- and middle-aged trees (Figure 3e). Unlike other minerals, the Fe concentration of middle-aged trees was significantly higher than young- and old-aged trees (Figure 3f). However, there was no significant difference between young- and old-aged trees.

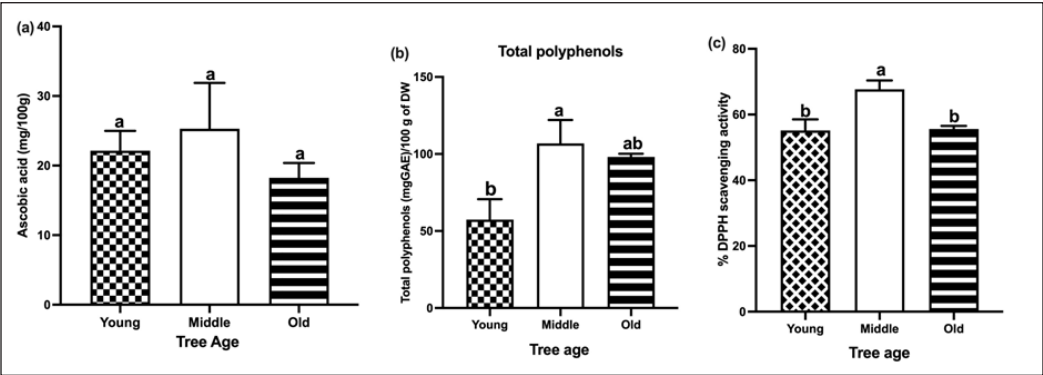


Figure 2. Means (n=6) of (a) ascorbic acid, (b) total polyphenol and % DPPH scavenging activity of Musang King durian fruits harvested from different tree ages (young, middle and old). Same letters indicate no significant differences between groups based on Tukey's HSD test ($p < 0.05$). DW = Dry weight and GAE = Gallic acid equivalent. Error bars represent standard error

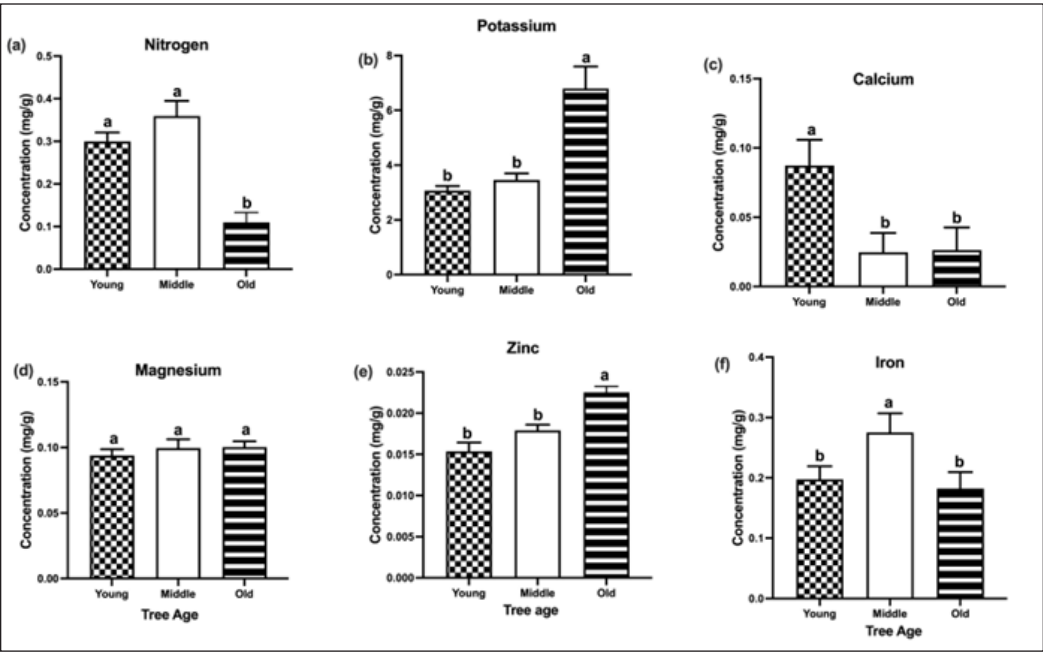


Figure 3. Means (n = 6) of macro- and microminerals concentrations in Musang King durian fruits harvested from trees of different age groups (young, middle, and old). The same letters indicate no significant differences between groups based on Tukey's HSD test ($p < 0.05$). Error bars represent standard error

Correlation between Minerals, Physicochemical, and Antioxidant Characteristics of Musang King Durian Fruit

In this study, Zn demonstrated significant positive correlations with the firmness and reducing sugar content of MK durian fruit ($r = 0.6, p < 0.05$) and negatively correlated with pH ($r = -0.7, p < 0.05$). Similarly, Fe showed a positive correlation with ascorbic acid content ($r = 0.6, p < 0.05$). K was positively associated with TA, firmness, and reducing sugar content ($r = 0.6, p < 0.05$). Additionally, N exhibited positive correlations with SSC, ΔE^* , and antioxidant activity. However, a negative correlation was observed between reducing sugar content and N content ($r = 0.5, p < 0.05$) (Figure 4: and Additional document (S2))

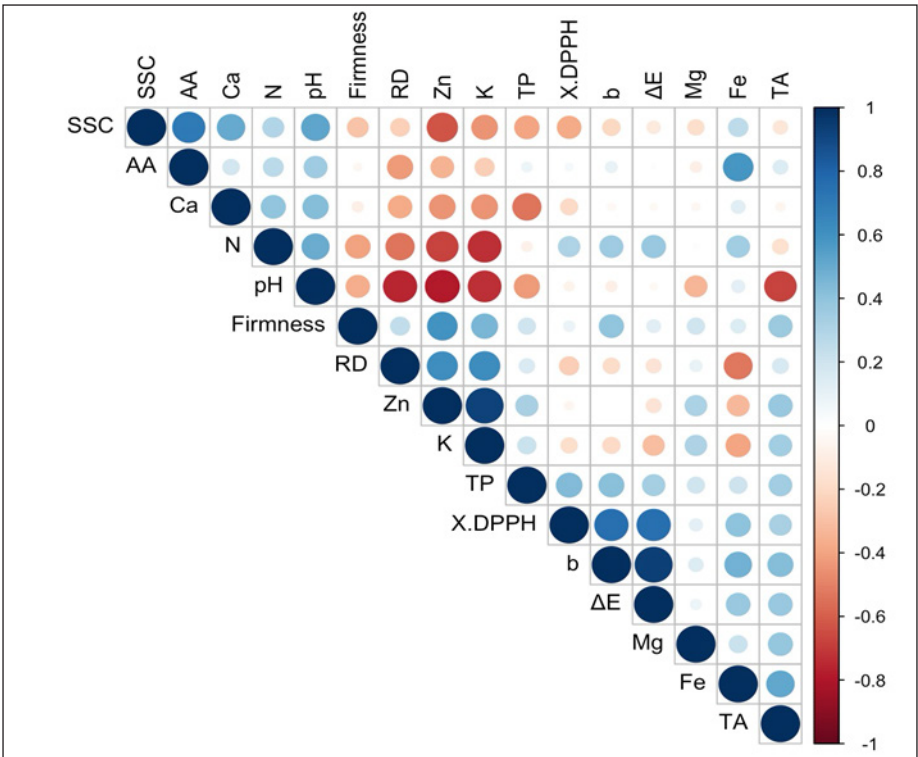


Figure 4. Correlation matrix between physico-chemical attributes, antioxidant content, and mineral content of Musang King durian across different tree ages. Correlation coefficients are represented by colors as indicated in the color key. Significant correlations are highlighted by .Abbreviations: TP = Total polyphenol; AA = Ascorbic acid; X.DPPH = 2,2-diphenyl-1-picrylhydrazyl; RD = Reducing sugar; SSC = Soluble solid content; TA = Titratable acidity; N = Nitrogen; K = Potassium; Mg = Magnesium; Fe = Iron; Ca = Calcium; Zn = Zinc

DISCUSSION

The study investigated the impact of tree age on physicochemical characteristics, antioxidant content, and mineral composition of MKD fruits. The findings reveal that tree age significantly affected the firmness of the MKD fruit. Fruit firmness is a complex

attribute perceived through the senses of touch, hearing, vision, and kinesthesia, which plays a crucial role in determining consumer acceptability (Feng et al., 2024). These results are inconsistent with the results obtained by Meena and Asrey (2018a), who observed that Amparali mango fruits from young-aged trees (6 years old) exhibit higher firmness. The authors suggested that firmness differences between fruits from different age groups could be attributed to the content of Ca and cell wall degrading enzymes. However, in our study the Ca content in old tree age fruits was lower compared to younger trees, suggesting a different mechanism influences durian fruit firmness. Furthermore, since cell wall-degrading enzyme activity, such as pectin methylesterase (PME) and polygalacturonase (PG) activities, was not measured in this study, we suspect that PG could contribute to firmness variations observed among tree ages. This enzyme has been shown to influence fruit softening (Lazan et al., 1995) and may exhibit species-specific activity patterns as observed in tomato and strawberry fruits (García-Gago et al., 2009). Additionally, firmness in this study was correlated with Zn content (Figure 4). Zn affects auxins, gibberellins, and abscisic acid hormones, which regulate cell wall enzymes and, hence, influence fruit firmness (Zhang et al., 2016). This may explain why durian from old trees has higher firmness compared to durian from younger trees.

Contrary to firmness, the current study also reveals that the pH value of MKD fruit was lower in fruits from old-aged trees, whereas reducing sugar content was higher in these fruits. The lower pH in durian from old-aged trees reflects their higher acidity. These results align with the study by Aregay et al. (2021) who reported that Gunda Gundo Orange (*Citrus* spp.) fruit from old-aged trees contained higher acidity than young-aged trees. These researchers reported that the high acidity was attributed by N content accumulation in the older trees. As it was hypothesized that N content is considered a phloem-mobile nutrient, and as trees mature, the transport of these mobile nutrients tends to be more pronounced (Khalid et al., 2018), N content influences the biosynthesis of organic acids, hence increasing fruit acidity (Zheng et al., 2023). However, our study observed lower N content in durian fruits from older trees, suggesting an alternative mechanism for the observed acidity. The lower pH and higher reducing sugar content in fruits from old-aged durian trees are speculated to result from the accumulation of K, as observed in the correlation analysis in this study. The high K content has been associated with increased acidity and sugar content in Cara Cara navel orange (*Citrus sinensis* L. Osbeck) (Wu et al., 2021). The K content enhanced sucrose metabolism and citrate synthesis in Cara Cara orange by increasing the activity of citrate-synthesizing enzymes, thereby contributing to higher acidity and reducing sugar levels (Wu et al., 2021). Further, K content was shown to up-regulate sugar and minerals transport genes, increasing sugar concentration in fruit and thereby increasing its quality (Shen et al., 2019). Additionally, Zn was also higher in durian from older trees. Zn content regulates enzymatic activities and the expression of

genes associated with sugar and acid metabolism, as observed in loquats (Ali et al., 2023). These mechanisms likely explain the observed low pH and increased reducing sugar content in MKD fruits in this study.

MKD fruits of middle-aged trees had higher polyphenols and DPPH antioxidant activity than fruits from young- and old-aged trees. This is inconsistent with Meena and Asrey (2018a), who found that phenolic content of Amrapali mangoes decreased with tree age. The inconsistencies in these findings may be attributed to variations between species. Different fruit species exhibit distinct gene expression profiles, which can influence the production of secondary metabolites, such as polyphenols (Xu et al., 2012). Additionally, the higher antioxidant content might be as a result of higher N content in fruits from middle-aged trees. N content was positively correlated with the antioxidant activity in this study (Figure 4). N content modulated the biosynthesis of carotenoids and phenolic acids hence improving the antioxidant content of the fruits (Narvekar & Tharayil, 2021; Zhang et al., 2024). A reduction in polyphenol content may indicate a loss of the nutritional value of durian fruit, as in apples, pears, durian, and peaches (Leontowicz et al., 2002, 2008).

Moreover, the MKD from young trees contained high concentrations of N and Ca. Previous studies showed that peach young trees allocated more of N, P, K, and Ca in fruits and pruned woods (Zhou & Melgar, 2020). The authors suggested a more efficient utilization of these nutrients for vegetative growth and fruit production (Zhou & Melgar, 2020). However, in our study, K content was higher in fruits from old trees. This could be due to the large size of the tree and reduced vegetative growth in old trees as compared with young trees (Khalid et al., 2018). This aligns with observations in forest canopy trees, where younger trees prioritize vegetative growth over older trees (Gavin & Peart, 1999).

Interestingly, the concentration of Zn and Fe was observed higher in fruit from old trees compared to young trees. This observation has not been reported previously in the literature. The higher content of these minerals could be due to age-related root patterns and the distribution of root hairs across soil strata (Mohsin et al., 2000), or efficiency in minerals uptake (Zhou & Melgar, 2020). Future research is needed to understand the mechanism behind this minerals variation across tree ages.

CONCLUSION

The impact of tree age on physicochemical characteristics, antioxidant content, and nutrient composition was investigated in this study. The findings reveal that MKD tree age significantly impacted fruit firmness and pH, reducing sugar content, antioxidant content, and mineral composition. Fruits from older trees exhibited greater firmness, higher reducing sugar levels, and a lower pH compared to those from middle-aged and young trees. In contrast, durians from middle-aged trees had the highest antioxidant content. Mineral composition analysis showed significantly higher concentrations of K,

Zn, and Fe in fruits from older trees, while fruits from younger trees had increased levels of N and Ca. These findings highlight tree age as a critical factor influencing both the physicochemical properties and nutritional value of MKD fruit. Moreover, Correlations between physicochemical, antioxidant, and mineral content were observed, suggesting associations. Future studies should focus on mechanistic research to confirm these links and better understand how tree age affects MKD fruit quality.

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