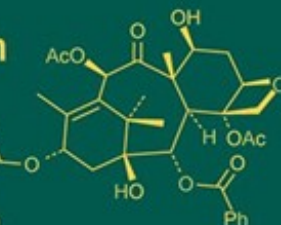
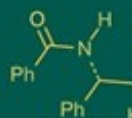
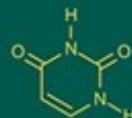
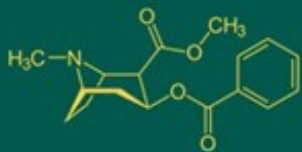


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Biochemical and biomass characteristics of naturally dropped immature mandarin (*Citrus Reticulata* Blanco) fruits at different developmental stages

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ABSTRACT

Naturally dropped immature citrus fruits are commonly discarded in orchards despite containing potentially valuable bioactive compounds. This study evaluated fresh dropped-fruit biomass, dry matter and selected biochemical attributes of mandarin (*Citrus reticulata* Blanco) fruits collected at five developmental sizes (9, 12, 15, 18 and 21 mm). Naturally dropped fruits were collected during 2023–24 and 2024–25, classified by diameter. To focus specifically on the quantity of dropped biomass, fresh and dry weights were expressed per plant rather than as the weight of 100 fruits. In pooled observations, fresh dropped-fruit weight increased from 202.5 g plant⁻¹ at 9 mm to 605.0 g plant⁻¹ at 21 mm, while dry weight increased from 52.85 to 171.20 g plant⁻¹. In contrast, antioxidant capacity was highest at 12 mm (13.21 mM L⁻¹ Trolox) and declined to 5.95 mM L⁻¹ Trolox at 21 mm. Total phenols reached 39.14 mg 100 g⁻¹ dry weight at 12 mm and declined to 27.75 mg 100 g⁻¹ dry weight at 21 mm. Total flavonoids were highest at 12 mm (26.70 mg 100 g⁻¹ dry weight) and declined progressively to 19.50 mg 100 g⁻¹ dry weight at 21 mm. Thus, fruit size influenced biomass accumulation and biochemical composition in opposite directions: larger dropped fruits contributed greater recoverable biomass, whereas smaller, particularly 9–12 mm, fruits retained higher concentrations of antioxidant-associated compounds. These results support size-specific utilization of mandarin dropped fruits, with early-stage fruits being of interest for bioactive-compound recovery and later-stage fruits providing greater dry biomass.

Keywords: Antioxidant capacity, *Citrus reticulata*, Dropped fruit, Flavonoids, Phenols, Waste valorization

1. Introduction

Mandarin (*Citrus reticulata* Blanco) is an important citrus fruit crop valued for its pleasant flavour, nutritional properties and commercial importance. In citrus production, a considerable proportion of young fruits may undergo natural physiological drop during early stages of fruit development. These dropped fruits are generally regarded as a loss of potential crop yield and are often left unutilized. However, immature citrus fruits may contain appreciable amounts of bioactive compounds, including phenolic compounds, flavonoids and other antioxidant constituents, indicating their potential for value addition and utilization [Ye *et al.*, 2011 [1]; Sun *et al.*, 2013 [2]; Zhang *et al.*, 2018 [3]; Kumar *et al.*, 2021 [4]].

Physiological fruit drop is a natural developmental process associated with competition for assimilates and changes occurring during fruit growth. The biochemical composition of dropped immature citrus fruits varies with fruit developmental stage and size. Studies have reported that young and physiologically dropped citrus fruits can possess substantial antioxidant activity and phenolic and flavonoid contents (Kumar *et al.*, 2022; Kumar *et al.*, 2021; Kumar *et al.*, 2023; Singh *et al.*, 2021; Luo *et al.*, 2023) [4, 5, 6, 7, 8]. reported significant bioactive composition and antioxidant activity in young mandarin fruits, while Sun *et al.*, 2013 [2] demonstrated that physiologically dropped citrus fruits contain phytochemicals with antioxidant potential. Similarly, Kumar *et al.*, 2021 [4] confirmed the presence of phenols, flavonoids and antioxidant activity in dropped *Citrus reticulata* fruits.

Phenolic compounds and flavonoids are important secondary metabolites of citrus fruits and contribute substantially to their antioxidant properties. Their concentration can change considerably during fruit development and maturation. (Bala *et al.*, 2025 [9]; García-Nicolás *et al.*, 2023 [10]; Fang *et al.*, 2022 [11]; Chen *et al.*, 2020 [12]; Luo *et al.*, 2023 [7]; Bentahar *et al.*, 2020 [13]; Zhang *et al.*, 2014). reported differences in phenolic and flavonoid contents among tissues of mandarin fruits and demonstrated their relationship with antioxidant capacity. (Kumar *et al.*, 2022; Kumar *et al.*, 2021; Heena *et al.*, 2022; Sun *et al.*, 2013; Kumar *et al.*

et al., 2023) [2, 4, 5, 6] Therefore, characterization of immature dropped fruits according to developmental stage may provide useful information regarding their biochemical value and possible utilization.

Although natural fruit drop represents a potential source of biomass, information relating fruit size with the fresh and dry biomass of naturally dropped mandarin fruits and their antioxidant-associated compounds is limited. Quantifying fresh dropped-fruit weight and corresponding dry weight per plant, together with antioxidant capacity, total phenols and total flavonoids, can help identify developmental stages having greater biomass and/or higher concentrations of valuable phytochemicals.

Therefore, the present study was undertaken to evaluate naturally dropped immature mandarin fruits (*Citrus reticulata* Blanco) at different fruit-size stages, with particular emphasis on fresh dropped-fruit weight, dry weight, antioxidant capacity, total phenolic content and total flavonoid content. The study aimed to determine the changes in these characteristics with fruit developmental stage and to assess the potential of naturally dropped mandarin fruits as a source of bioactive compounds.

2. Materials and Methods

2.1 Experimental material and design

The investigation was conducted during 2023–24 and 2024–25 in the Analytical and Post-Harvest Technology Laboratory, Department of Fruit Science, Faculty of Horticulture, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India. Naturally dropped mandarin fruits (*Citrus reticulata* Blanco) were collected from the experimental citrus material. Diseased, damaged and off-type fruits were excluded. Fruits were classified into five diameter classes—9, 12, 15, 18 and 21 mm—using a Vernier caliper. The dropped-fruit experiment comprised two citrus species and five fruit-size classes in the thesis design; the present manuscript reports only the mandarin treatment. The experiment used a factorial completely randomized design with three replications for the dropped-fruit experiment.

2.2 Fresh and dry dropped-fruit weight

Naturally dropped fruits were collected and separated into the five size classes. The total fresh weight of dropped fruits in each size class was determined using a digital balance, and fresh dropped-fruit weight was expressed on a per-plant basis. This approach represents the quantity of naturally dropped biomass and does not use the weight of 100 fruits. Representative samples from each size class were dried in a forced-air oven at 50 °C for 32 h, or until constant weight, and weighed using a precision balance. Dry weight per plant was calculated from the corresponding size-class biomass and expressed as g plant⁻¹.

2.3 Sample preparation

After drying, samples were ground to a fine homogeneous powder, passed through a 50-µm sieve and stored at -10 °C until biochemical analysis.

2.4 Antioxidant capacity

Antioxidant capacity was determined using the cupric ion reducing antioxidant capacity (CUPRAC) procedure of Apak *et al.*, 2008 [14]. Briefly, sample extract prepared in 80% ethanol was reacted with

copper(II) chloride, neocuproine and ammonium acetate buffer. Absorbance was measured at 450 nm after colour development. The thesis results are reported as mM L⁻¹ Trolox and this unit has been retained here to maintain consistency with the source dataset.

2.5 Total flavonoid content

Total flavonoids were determined by the aluminium chloride colorimetric method described by (Zhishen *et al.*) [15]. One millilitre of extract was mixed with distilled water, sodium nitrite and aluminium chloride, followed by sodium hydroxide. Absorbance was recorded at 510 nm. The results are presented according to the units used in the thesis results table, as mg 100 g⁻¹ dry weight.

2.6 Total phenolic content

Total phenolics were estimated using the Folin–Ciocalteu procedure based on Singleton and Rossi 1965 [16]. Dried fruit powder was extracted in 80% ethanol, centrifuged, and the supernatant was reacted with Folin–Ciocalteu reagent and sodium carbonate. Absorbance was recorded at 750 nm and gallic acid was used for calibration. Results are presented as mg 100 g⁻¹ dry weight, consistent with the thesis results table.

2.7 Statistical analysis

The observations were analyzed using the experimental statistical procedure described in the thesis. The factorial treatment effects of species and fruit size and their interaction were tested; the present paper extracts the mandarin treatment and reports the pooled means for the two experimental years. The thesis reports significant effects of fruit size and species × fruit-size interaction for the biomass and biochemical parameters. Pooled critical differences at 5% were 2.43 g plant⁻¹ for the fruit-size factor in fresh weight, 0.98 g plant⁻¹ for dry weight, 0.463 mM L⁻¹ Trolox for antioxidant capacity, 0.76 mg 100 g⁻¹ DW for phenols and 0.549 mg 100 g⁻¹ DW for flavonoids.

3. Results

3.1 Fresh dropped-fruit biomass

Fresh dropped-fruit weight increased consistently with fruit size (Fig. 1). The pooled mean across size classes was 402.0 g plant⁻¹. The increase from 9 to 21 mm represents nearly a threefold increase in fresh dropped biomass, indicating that larger fruit classes contribute substantially more material for physical recovery and subsequent processing.

3.2 Dry dropped-fruit biomass

Dry weight followed the same overall developmental pattern as fresh weight (Fig. 2). Pooled dry weights were 52.85, 94.95, 127.81, 134.65 and 171.20 g plant⁻¹ at 9, 12, 15, 18 and 21 mm, respectively, with a pooled mean of 116.29 g plant⁻¹. The increase indicates progressive dry-matter accumulation as fruit size advanced.

3.3 Antioxidant capacity

Antioxidant capacity showed a contrasting developmental response (Fig. 3). Pooled values increased from 12.51 mM L⁻¹ Trolox at 9 mm to a maximum of 13.21 mM L⁻¹ Trolox at 12 mm, then decreased to 10.10, 7.27 and 5.95 mM L⁻¹ Trolox at 15, 18 and 21 mm, respectively. The pooled mean was 9.81 mM L⁻¹ Trolox.

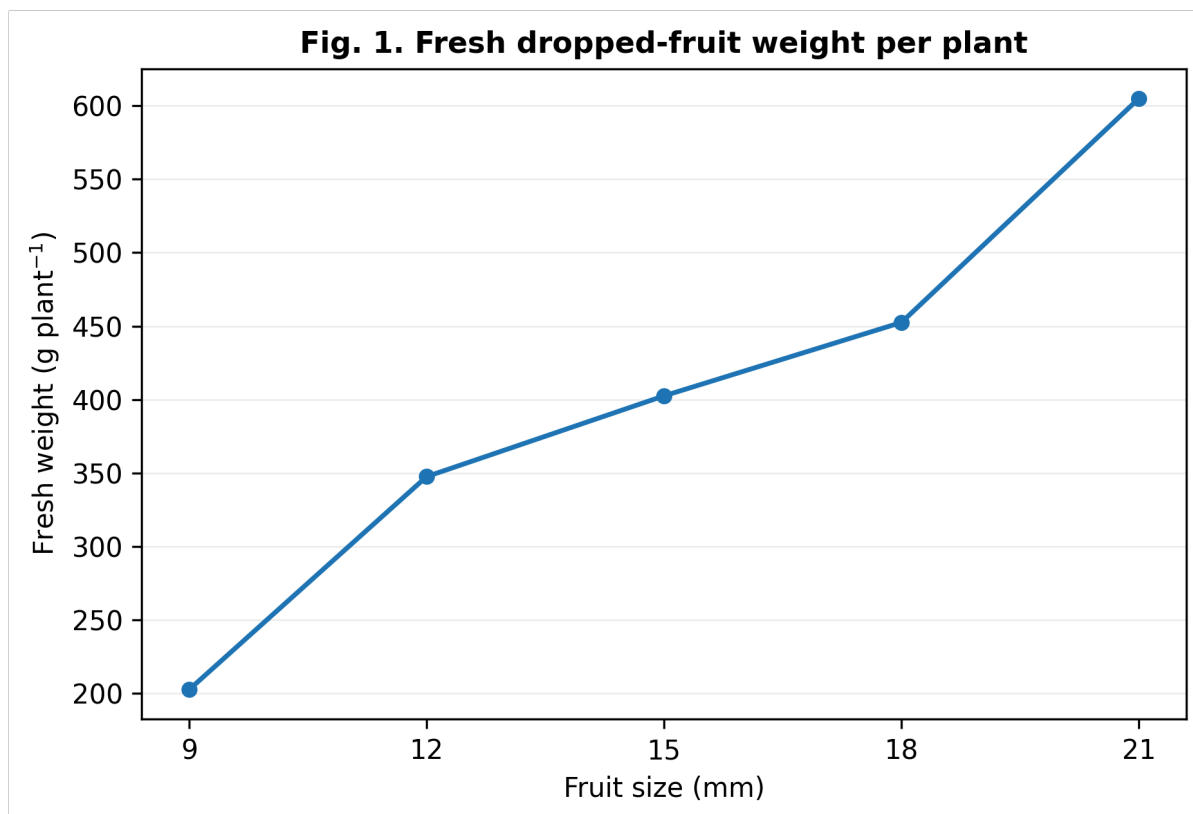


Figure 1. Fresh dropped-fruit weight per plant of mandarin at different fruit-size classes.

3.4 Total phenols and flavonoids

Total phenolic content was 33.26, 39.14, 35.30, 29.52 and 27.75 mg 100 g⁻¹ DW at 9, 12, 15, 18 and 21 mm, respectively. The highest value occurred at 12 mm, after which phenolic concentration de-

clined. Total flavonoid content showed a similar pattern, with pooled values of 26.15, 26.70, 22.95, 21.15 and 19.50 mg 100 g⁻¹ DW, respectively. The maximum flavonoid concentration was recorded at 12 mm (Fig. 4).

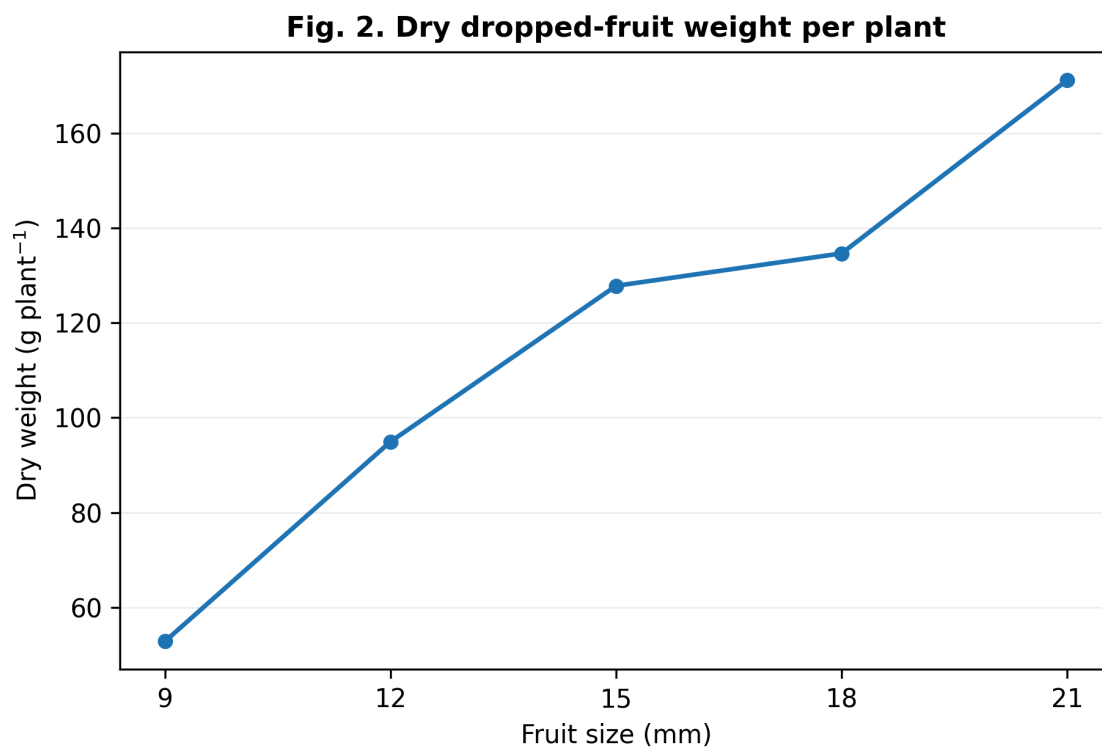


Figure 2. Dry dropped-fruit weight per plant of mandarin at different fruit-size classes.

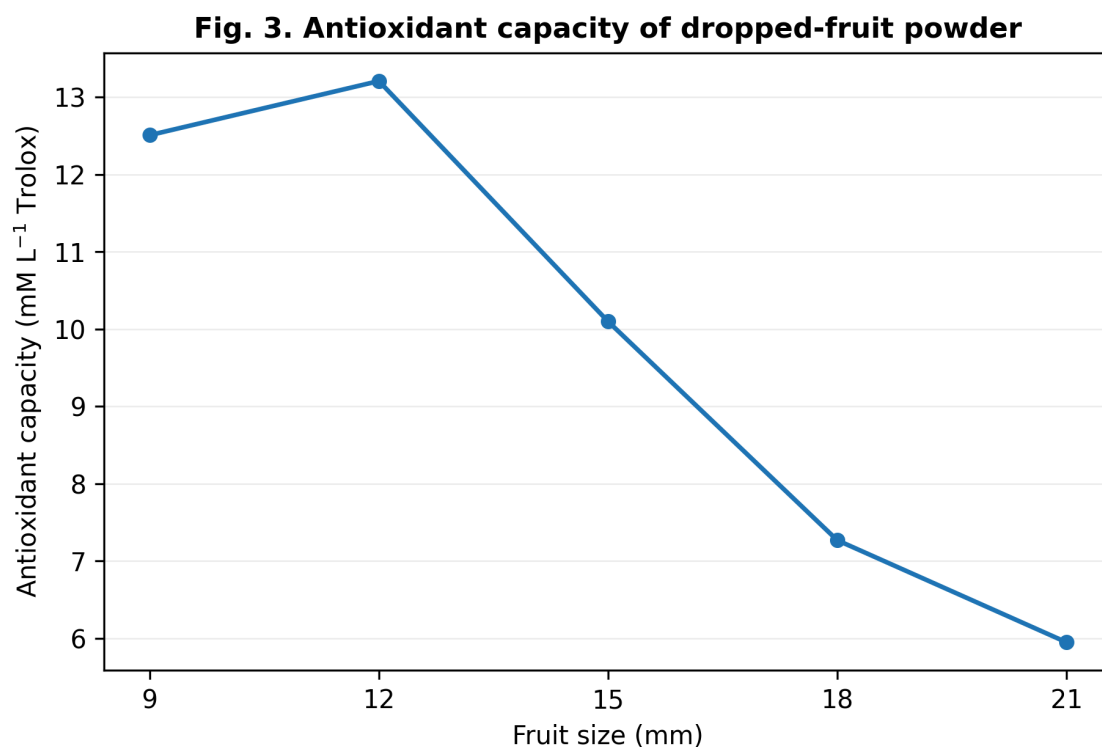


Figure 3. Antioxidant capacity of mandarin dropped-fruit powder at different fruit-size classes.

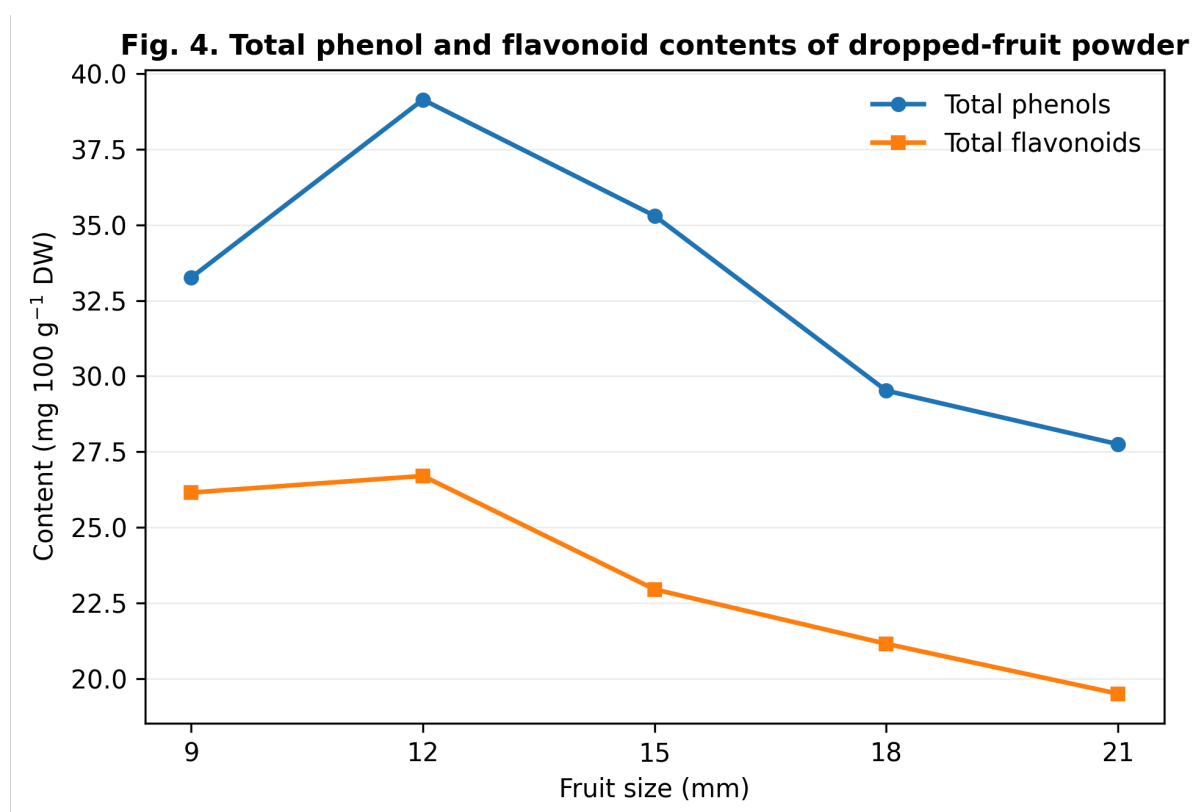


Figure 4. Total phenol and flavonoid contents of mandarin dropped-fruit powder at different fruit-size classes.

4. Discussion

The present results demonstrate a clear separation between biomass accumulation and bioactive-compound concentration during the development of naturally dropped mandarin fruits. Fresh and dry biomass increased markedly as fruit diameter increased, whereas antioxidant capacity, total phenols and total flavonoids were highest during the early developmental stages and declined with further enlargement. This inverse relationship is important from a utilization

perspective because the size class that supplies the greatest mass is not necessarily the size class with the greatest concentration of antioxidant-associated compounds.

The progressive increase in fresh weight from 202.5 g plant⁻¹ at 9 mm to 605.0 g plant⁻¹ at 21 mm is consistent with continued cell division, cell expansion and accumulation of water and structural dry matter during citrus fruit growth. The corresponding increase in dry weight from 52.85 to 171.20 g plant⁻¹ confirms that the increase was not simply a consequence of water accumulation. The magnitude of dry-matter accumulation indicates that larger dropped fruits can pro

vide a greater physical substrate for powder or other biomass-based value-added products (Bala *et al.*, 2025) [9].

In contrast, antioxidant capacity peaked at 12 mm and declined thereafter. The same general pattern was observed for phenols and flavonoids, suggesting that the early developmental phase contains a comparatively concentrated pool of antioxidant-associated secondary metabolites. Previous research on physiological citrus drop has demonstrated the presence of phenolic acids, flavonoids and other phytochemicals in young dropped fruits (Ye *et al.*, 2011; Sun *et al.*, 2013) [1, 2]. (Kumar *et al.*) [4] similarly reported that immature dropped *C. reticulata* fruits contained substantial flavonoids, phenolics and antioxidant potential and that fruit size and processing conditions influenced their retention. Their findings, together with the present results, support the concept that young dropped fruits can be considered a source of bioactive compounds rather than simply an orchard waste stream.

The maximum flavonoid concentration at 12 mm is also consistent with the broader observation that citrus flavonoid composition varies with developmental stage and genetic background. *Citrus* flavonoid profiles are strongly species- and genotype-dependent, and hesperidin and related flavanones are important constituents in mandarin and sweet orange (Zhao *et al.*, 2017; Sun *et al.*, 2015) [17, 18]. The decline in total flavonoids observed from 12 to 21 mm may reflect dilution associated with fruit expansion, developmental redistribution of secondary metabolites, or changes in biosynthetic activity. The present experiment did not measure individual flavonoids or transcript-level changes; therefore, these mechanisms should be considered as possible explanations rather than directly demonstrated processes.

Phenolic content followed the same general trend as flavonoids, reaching 39.14 mg 100 g⁻¹ DW at 12 mm before decreasing to 27.75 mg 100 g⁻¹ DW at 21 mm. Mandarin fruit tissues are known to differ in total phenolic content and antioxidant activity, and phenolics can contribute substantially to antioxidant responses (Zhang *et al.*, 2018) [3]. The parallel decline in phenols and antioxidant capacity in the present data is therefore biologically plausible, although the experiment did not establish causality or calculate a direct correlation coefficient between individual biochemical measurements.

From a utilization standpoint, the results suggest two distinct pathways. Fruits in the 9–15 mm range, particularly around 12 mm, are attractive when the objective is recovery of concentrated antioxidant-associated compounds. Fruits in the 18–21 mm range provide substantially greater fresh and dry biomass per plant and may therefore be more suitable where the primary objective is biomass recovery. This size-dependent approach is supported by earlier work showing that physiologically dropped citrus fruits can serve as raw materials for bioactive-compound recovery and other value-added applications (Ye *et al.*, 2011; Kumar *et al.*, 2021) [1, 4].

A limitation of the present analysis is that the biochemical measurements were made on dried powder, whereas the biomass measurements were expressed per plant. Consequently, concentration data should not be interpreted directly as total antioxidant or phenolic yield per plant. Future studies should combine concentration with total dry powder recovery and calculate the absolute mass of each target compound recovered per plant or hectare. Individual flavonoids such as hesperidin and narirutin should also be quantified to identify which compounds contribute most strongly to the observed antioxidant capacity.

5. Conclusion

Naturally dropped immature mandarin fruits showed pronounced developmental changes in both biomass and biochemical composition. Fresh and dry dropped-fruit weights increased progressively from 9 to 21 mm, reaching 605.0 and 171.20 g plant⁻¹, respectively, at 21 mm. In contrast, antioxidant capacity, total phenols and total flavonoids were highest at the early developmental stages, with maxima at 12 mm of 13.21 mM L⁻¹ Trolox, 39.14 mg 100 g⁻¹ DW and 26.70 mg 100 g⁻¹ DW, respectively. The results indicate that the uti-

lization strategy should be matched to fruit size: early-stage dropped fruits are particularly relevant for recovery of antioxidant-associated bioactives, whereas larger dropped fruits provide greater recoverable biomass. These findings strengthen the case for converting mandarin fruit drop into value-added raw material and provide a basis for future studies on compound-specific recovery and process economics.

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