



Comparative phytochemical analysis of the fruits of four Florida-grown finger lime (*Citrus australasica*) selections

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ABSTRACT

Finger lime (*Citrus australasica* F. Muell.), a citrus species native to Australia, is a distinct finger-shaped fruit with unique caviar-like pulp and skin color variations. In addition to visual and sensory characteristics, finger lime has recently gained attention from the American citrus industry due to its tolerance to a devastating citrus disease, Huanglongbing (HLB). Here, we characterized the phytochemical profiles (including acid and sugar) of four Florida-grown finger lime selections: two with red pulp, one with pale pink pulp, and one with white pulp. Two-three-fold higher levels of phenolic compounds and antioxidant capacity were observed in the peel compared to the pulp in the four selections. Selections with red pulp had more antioxidant capacity and higher phenolic compound content in both types of tissues than in the selections with white pulp. Citric acid was found abundant in all four selections making the selections a major source of organic acid. The overall results suggested that finger limes are rich in health-benefiting and flavor contributing compounds; more specifically, Florida-grown red selections of finger lime are richest in phytochemicals, vitamin C, citric acid, and sugars among evaluated genotypes, therefore, making them strong alternative for production in Florida and consumption.

1. Introduction

The Australian finger lime (*Citrus australasica* F. Muell.) is one of six different citrus species endemic to Australia (Delort & Jaquier, 2009; Hamilton, Ashmore, & Drew, 2005). Finger limes are known for their unique phenotype (Wang et al., 2019), growing either as thorny and vigorous shrubs or small trees up to 6 m tall in their natural habitat. The fruit is distinctively finger-shaped, can grow up to 12 cm in length, and are often slightly curved, narrowing at both the tip and base (Delort & Yuan, 2018). Commonly, the peel color is either green or red, whereas the pulp has a variety of colors ranging from green to yellow to various shades of red. The pulp is commonly called “lime caviar” as the juice vesicles are loosely adherent, reminiscent of caviar (Delort & Yuan, 2018).

In recent years, consumer preferences have leaned towards healthier lifestyles. There is an increasing interest in the health benefits of consuming different fruits and vegetables (Prior & Cao, 2000). Health-conscious consumers are currently inclined to consume fruits containing higher antioxidants and phytochemicals, leading to increased popularity and growing demand for such fruits (Gilbert et al., 2014). Several studies have shown that citrus fruit and juices are

healthy, as the health benefits imparted by their phytochemicals outweigh their sugar content (Liu, Heying, & Tanumihardjo, 2012). Citrus fruits are rich in flavonoids, anthocyanin, vitamin C, and other phenolic compounds. Composition, however, varies among cultivars and parts of citrus fruits; thus their human health benefits also differ owing to differences in biological functions, including antioxidant (Wang et al., 2017), antimutagenic (Liu et al., 2017), and anti-inflammatory activities (Cheng et al., 2017). Finger limes contain significant levels of phytochemical compounds such as total phenolics including anthocyanin, ascorbic acid, minerals (Netzel, Netzel, Tian, Schwartz, & Konczak, 2007; Sommano, Caffin, & Kerven, 2013), as well as organic acids and carbohydrate, thus making the nutrient and sensory profiles of finger lime fruits of interest to consumers.

Recent reports suggest that finger lime is tolerant to a devastating citrus disease, Huanglongbing (HLB), also known as citrus greening (Killiny et al., 2018). HLB has resulted in more than 70% decline in citrus production in Florida (USDA, 2019), and close to 90% infection spread in Florida. Sweet orange and mandarin varieties are highly susceptible to HLB and currently, there is no cure for HLB. Therefore, citrus growers are desperately looking for varieties that can endure HLB as well as be of interest to consumers. Finger lime is an excellent alternative

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crop for Florida under HLB prevalent conditions. Moreover, finger limes unique visual characteristics and high nutrient profile make it potentially desirable to consumers. However, the phytochemical profile of any fruit is also affected by the genotype-environment interaction. Currently, there is no information about finger limes grown in Florida, whose climatic conditions differ from those found in Australia. Thus, the objective of this study was to evaluate the phytochemical profile, sugar, acid, and antioxidant content of four distinct finger lime selections available in to our research program.

2. Materials and methods

2.1. Plant material

Finger lime fruit were harvested from 6-year-old trees growing at the Citrus Research and Education Center, Lake Alfred, Florida. The four finger lime selections evaluated in this study were: red pulp (cultivar *sanguinea* type) finger lime (FL-red), white pulp finger lime (FL-white), a low-seeded red pulp-large leaved finger lime hybrid (LL FL-red), and the commercially available *sanguinea* type Florida Division of Plant Industry 50–36 cultivar (DPI control) was used as control (Fig. 1). Fully mature fruit were harvested in November 2019; fruit maturity was determined on the basis of fruit firmness, size, color, and aroma. Eight fruits were collected from each selection (4 fruits per replicate; $n = 4$). Immediately after harvest, the peels were separated from the pulp. Peel and pulp were flash-frozen using liquid nitrogen, then ground finely, and stored at -80°C until further analysis. Peel and pulp tissue were finely ground using an analytical mill (Fex IKA A11; IKA-Werke, Staufen, Germany), each sample was milled for 1.5 min.

2.2. Reagents and standards

All chemicals used were analytical grade and purchased from Sigma-Aldrich (St. Louis, MO). The chemicals used were methanol (CH_3OH), Milli-Q® water, hydrochloric acid (HCl), Folin-Ciocalteu phenol reagent, sodium carbonate (Na_2CO_3), gallic acid ($\text{C}_7\text{H}_6\text{O}_5$), sodium nitrite (NaNO_2), aluminum chloride (AlCl_3), sodium hydroxide (NaOH), catechin hydrate ($\text{C}_{15}\text{H}_{14}\text{O}_6$), potassium chloride (KCl), sodium acetate

($\text{CH}_3\text{CO}_2\text{Na}\cdot 3\text{H}_2\text{O}$), 2,4,6-tris (2-pyridyl)-s-triazine (TPTZ), ferric chloride hexahydrate ($\text{FeCl}_3\cdot 6\text{H}_2\text{O}$), ferrous sulfate heptahydrate ($\text{FeSO}_4\cdot 7\text{H}_2\text{O}$), bromine water, metaphosphoric acid (HPO_3), acetic acid (CH_3COOH), thiourea ($\text{CH}_4\text{N}_2\text{S}$), 2,4-dinitrophenyl hydrazine (2,4-DNPH), and sulfuric acid (H_2SO_4).

2.3. Extraction of phytochemical compounds

Ground samples (5 g) were extracted with 15 mL of 80% aqueous methanol/0.5 mol equivalent/L HCl (mL:mL), mixed thoroughly, and incubated overnight at 4°C . Samples were then centrifuged for 20 min at $20,000 \times g$ at 4°C (Eppendorf AG, Hamburg, Germany). The recovered supernatant was then used for analysis of the contents of total phenolics, flavonoids, total anthocyanin, and antioxidant activity.

2.3.1. Total phenolic content (TPC)

Total phenolic content was determined using the Folin-Ciocalteu assay (Vashisth, Singh, & Pegg, 2011). First, 0.5 mL of supernatant was diluted with 8 mL of deionized water, after which 0.5 mL of Folin-Ciocalteu phenol reagent, a chemical that reacts with any reducing substance present, was added. Next, 1 mL of saturated Na_2CO_3 solution was added to the mixture, which was vortexed for 10 s and kept in the dark for 1 h at 25°C to allow for maximum color development. Afterward, 300 μL aliquots of the resultant mixture were measured at 750 nm using an Epoch 2 microplate spectrophotometer (BioTek Instruments, Inc., Winooski, VT). Results are expressed as gallic acid equivalents (mg GAE/100 g fresh weight).

2.3.2. Flavonoid content (FC)

Flavonoid content was measured using a colorimetric assay (Zhishen, Mengcheng, & Jianming, 1999). First, 1 mL of the supernatant was diluted with distilled water in a 1:2 ratio, followed by the addition of 0.3 mL of 5% NaNO_2 to create an alkaline medium. After 5 min, 0.3 mL of 10% AlCl_3 was added, resulting in a yellow complex formation. After 1 min, 2 mL of 1 mol equivalent/L NaOH was added, and the solution was vortexed briefly, causing the solution to turn into a reddish-brown color. Absorbance was measured at 510 nm using a spectrophotometer (BioTek Instruments, Inc., Winooski, VT). Results are expressed as catechin

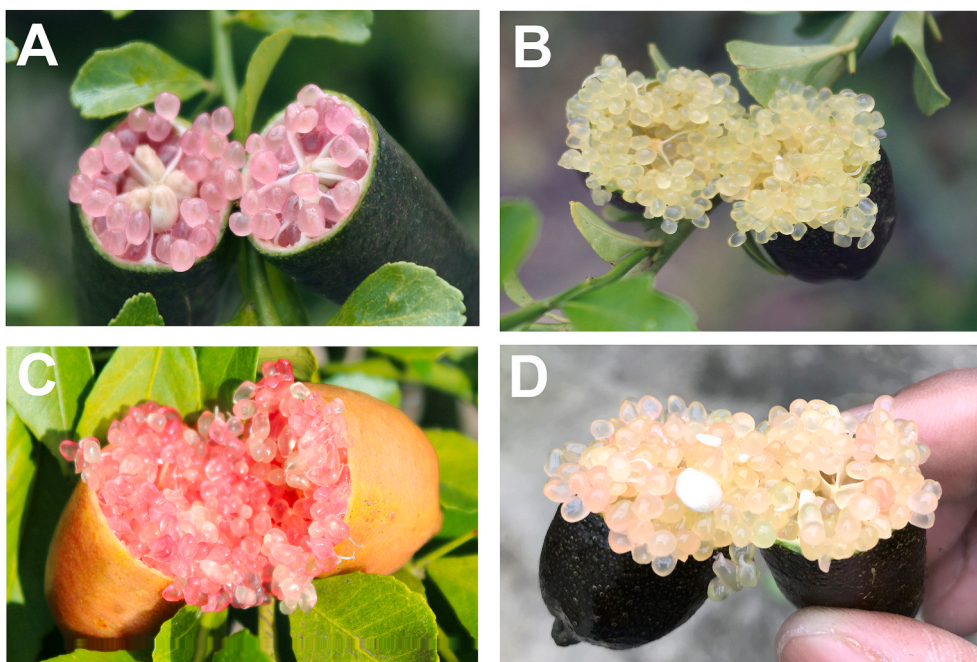


Fig. 1. Picture of caviar-like pulp with variable pulp and peel color of four different Florida grown finger-lime selections: FL-red (A), FL-white (B), LL FL-red (C), and DPI control (D).

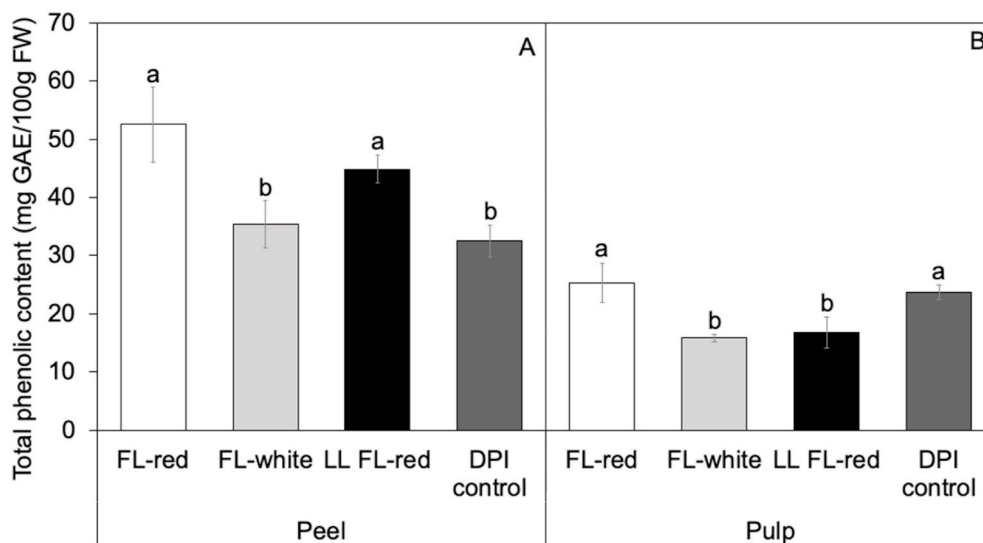


Fig. 2. Total phenolics content in peel (A) and pulp (B) (mean \pm standard deviation, $n = 4$) in four finger lime cultivars, red pulp finger lime (FL-red), white pulp finger lime (FL-white), red pulp-large leaved finger lime hybrid (LL FL-red), and the commercially available cultivar from Florida Division of Plant Industry 50–36 cultivar (DPI control). Bars marked with different lowercase letters indicate statistically significant difference using Tukey's honestly significant difference test at $\alpha = 0.05$.

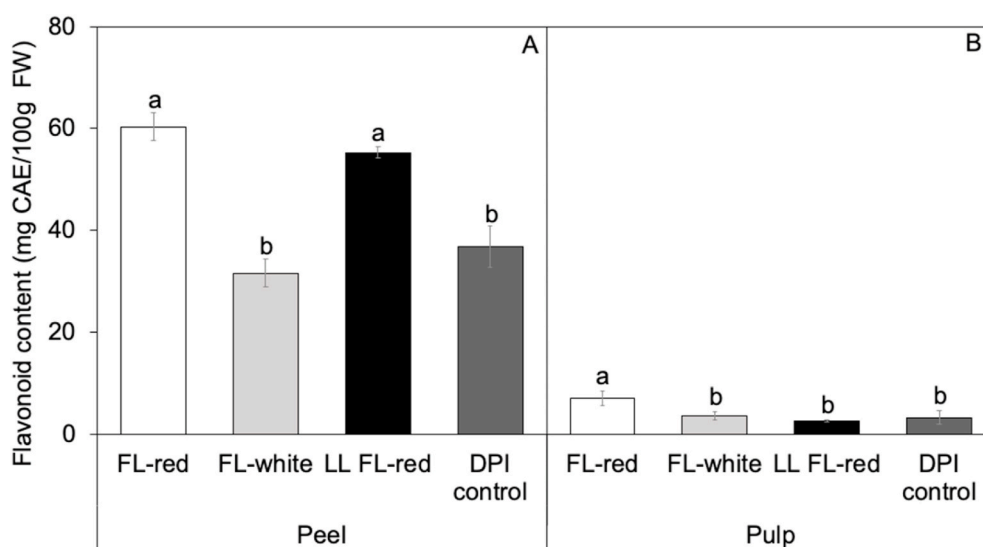


Fig. 3. Flavonoid content in peel (A) and pulp (B) (mean \pm standard deviation, $n = 4$) of four finger lime cultivars, red pulp finger lime (FL-red), white pulp finger lime (FL-white), red pulp-large leaved finger lime hybrid (LL FL-red), and the commercially available cultivar from Florida Division of Plant Industry 50–36 cultivar (DPI control). Bars marked with different lowercase letters indicate statistically significant difference using Tukey's honestly significant difference test at $\alpha = 0.05$.

equivalents (mg CAE/100 g FW).

2.3.3. Anthocyanin content (AC)

Anthocyanin content was measured using a pH differential assay (Lee, Durst, & Wrolstad, 2005). KCl (1.86 g) was mixed with 960 mL distilled water to prepare a pH 1.0 buffer (0.025 mol/L), and $\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$ (54.43 g) was mixed with 940 mL distilled water to prepare a pH 4.5 buffer (0.4 mol/L). The pH levels of both buffers were adjusted using HCl and the final volume was bulked up to 1 L by adding distilled water. The appropriate dilution factor was determined by diluting the test portion with the pH 1.0 buffer until the absorbance reading was within the linear range (0.2–1.4 AU). Finally, the absorbance of the dilutions using pH 1.0 and pH 4.5 buffers were measured at both 520 and 700 nm. Results are expressed as cyanidin-3-glucoside equivalents (mg C3GE/kg FW).

$$\text{Calculation} = \frac{A \times MW \times Df \times 10^3}{\epsilon \times l}$$

where, $A = (A_{520\text{nm}} - A_{700\text{nm}}) \text{ pH } 1.0 - (A_{520\text{nm}} - A_{700\text{nm}}) \text{ pH } 4.5$; MW = Molecular weight; 449.2 g/mol; Df = Dilution factor; l = Pathlength in cm; ϵ = Molar extinction coefficient: $26900 \text{ L mol}^{-1} \text{ cm}^{-1}$; and 10^3 = factor for conversion from g to mg.

2.3.4. Antioxidant activity

Ferric reducing antioxidant power (FRAP) assay was conducted to measure antioxidant activity with some modifications (Pulido, Bravo, & Saura, 2000). FRAP reagent was freshly prepared by combining of 0.01 mol/L TPTZ solution in 0.04 mol/L HCl, 0.02 mol/L $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (freshly prepared), and 0.3 mol/L of $\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$ at pH 3.6 (1:1:10 ratio

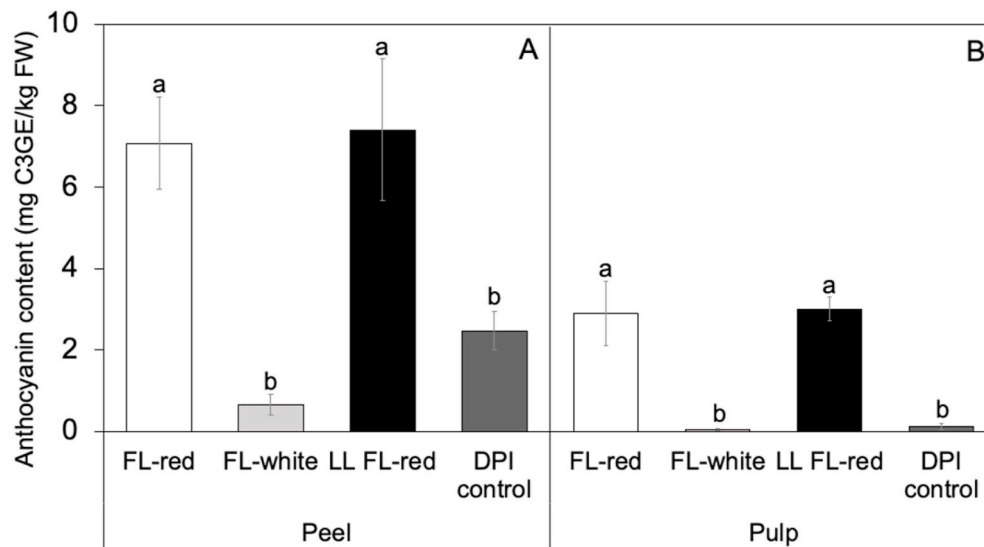


Fig. 4. Anthocyanin content in peel (A) and pulp (B) (mean \pm standard deviation, $n = 4$) of four finger lime cultivars, red pulp finger lime (FL-red), white pulp finger lime (FL-white), red pulp-large leaved finger lime hybrid (LL FL-red), and the commercially available cultivar from Florida Division of Plant Industry 50–36 cultivar (DPI control). Bars marked with different lowercase letters indicate statistically significant difference using Tukey's honestly significant difference test at $\alpha = 0.05$.

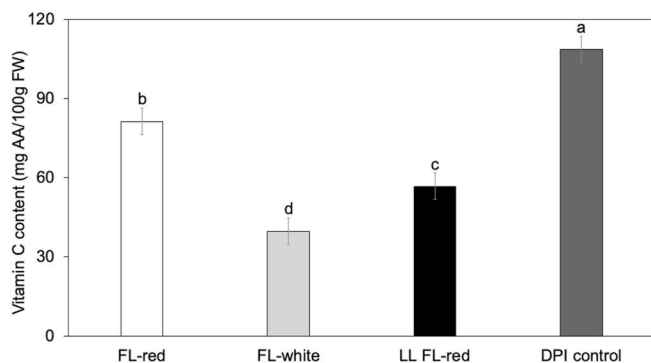


Fig. 5. Vitamin C content in pulp (mean \pm standard deviation, $n = 4$) of four finger lime cultivars, red pulp finger lime (FL-red), white pulp finger lime (FL-white), red pulp-large leaved finger lime hybrid (LL FL-red), and the commercially available cultivar from Florida Division of Plant Industry 50–36 cultivar (DPI control). Bars marked with different lowercase letters indicate statistically significant difference using Tukey's honestly significant difference test at $\alpha = 0.05$.

respectively). The FRAP reagent was warmed to 37 °C in a water bath, and 225 μ L of the reagent was mixed with 22.5 μ L deionized water and 7.5 μ L of test sample or blank (i.e., 1:1 aqueous methanol). The final solution was again incubated at 37 °C for 30 min, and reading was taken at 595 nm. Results are expressed as ferrous equivalents [Fe⁺²E (mmol/g FW)].

2.4. Vitamin C content

Vitamin C was measured using the assay described by [Rahman, Khan, and Hosain \(1970\)](#). Sample (10 g) was homogenized with 50 mL of 5% metaphosphoric acid-10% acetic acid (MA) solution. A flask was filled to the 100 mL mark using the MA solution and then filtered. Bromine water (3–4 drops) was added to the filtered solution, oxidizing the ascorbic acid into dehydroascorbic acid. Thiourea (3–4 drops) was then added to remove excess bromine. Finally, 1 mL of 2,4-DNPH was added. All of the samples, standards, and blank solutions were incubated in a water bath at 37 °C for 3 h, cooled in an ice bath for 10 min, and treated with 5 mL of 85% H₂SO₄ with constant stirring. The absorbance

reading was taken at 521 nm, and the results are expressed as ascorbic acid in mg/100 g FW.

2.5. Total soluble content (TSS) and titratable acidity (TA) of pulp

For TSS determination, 5 g of pulp was homogenized and centrifuged at 4,200 \times g at 4 °C for 10 min, after which 1 mL of supernatant was separated, poured, and measured in terms of Brix. For TA measurement, 49 mL of deionized water was mixed with 1 mL of juice supernatant. Both TSS and TA were determined using a handheld refractometer (Pocket PAL-BX1 ACID1; Atago USA, Bellevue, WA).

2.6. Malic acid and citric acid content of pulp

Malic and citric acid contents were quantified using an enzymatic UV-method citric acid and malic acid kit (citric acid: catalog no. 10139076035; malic acid: catalog no. 10139068035; R-Biopharm, Darmstadt, Germany). The frozen pulp was homogenized, and 1 g was mixed with 1 mL deionized water. All the reagents from the kit were prepared following the manufacturer's instructions. For both malic and citric acid, absorbance differences for both the blank and the samples were determined at 340 nm. Results are expressed as mg/g FW.

2.7. Carbohydrate analysis of pulp

The sucrose, fructose, and glucose levels in the pulp were quantified using ion chromatography (IC) with some modifications ([Cataldi et al., 2000](#)). Frozen pulp tissue was thawed, homogenized, and centrifuged at 20,000 \times g at 4 °C for 5 min. Ten μ L of the juice supernatant was diluted in 29.90 mL of deionized water, then 10 μ L of the diluted sample was filtered through a pre-filled chromatography column to aid in removing anion contaminants. Next, 250 μ L of the filtrate was re-filtered by transferring the liquid to a polytetrafluorethylene (PTFE) filter vial (0.45 μ m; Restek, Bellefonte, PA). Next, 25 μ L of eluted sample was injected in an IC (equipped with anion exchange and guard column (CarboPac PA200, Dionex, Sunnyvale, CA). A constant mobile phase with a flow rate of 0.4 mL/min was used, consisting of two solvents: 95% deionized water and 5% NaOH (1 mol equivalent/L). The standard curve obtained from this analysis was used for glucose, sucrose, and fructose quantification. Results are expressed as mg/L of finger lime pulp.

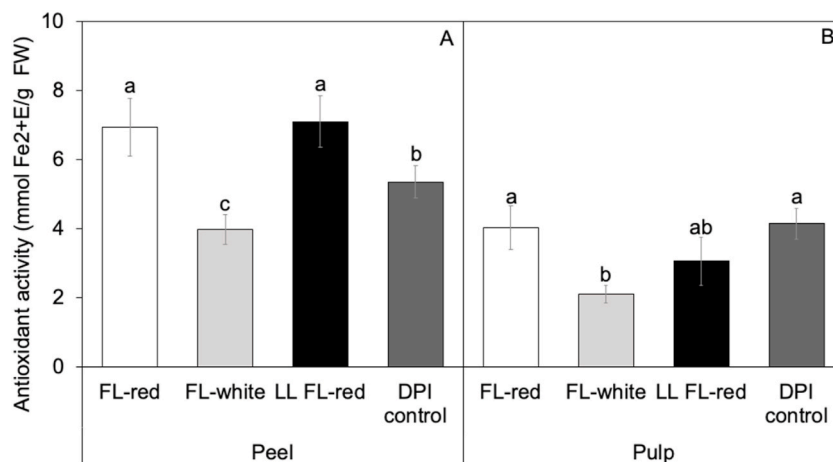


Fig. 6. Antioxidant activity in peel (A) and pulp (B) (mean \pm standard deviation, $n = 4$) of four finger lime cultivars, red pulp finger lime (FL-red), white pulp finger lime (FL-white), red pulp-large leaved finger lime hybrid (LL FL-red), and the commercially available cultivar from Florida Division of Plant Industry 50–36 cultivar (DPI control). Bars marked with different lowercase letters indicate statistically significant difference using Tukey's honestly significant difference test at $\alpha = 0.05$.

Table 1

Total soluble solids (TSS), titratable acidity (TA), and acid content (mean \pm standard deviation, $n = 4$) in four finger lime cultivars, red pulp finger lime (FL-red), white pulp finger lime (FL-white), red pulp-large leaved finger lime hybrid (LL FL-red), and the commercially available cultivar from Florida Division of Plant Industry 50–36 cultivar (DPI control).

	TSS (Brix)	TA (% citric acid)	TSS/TA ratio	Citric acid (mg/L)	Malic acid (mg/L)
FL-red	13.8 \pm 1.1 ^z a	2.8 \pm 0.3 c	4.9 \pm 0.5 a	36.2 \pm 3.6 a	5 \pm 1.1 c
FL-white	10.6 \pm 0.7 b	5.7 \pm 0.7 b	1.9 \pm 0.4 c	37.3 \pm 4.2 a	38 \pm 0.8 a
LL FL-red	8.5 \pm 0.3 c	8.0 \pm 1.2 a	1.1 \pm 0.1 d	14.6 \pm 1.9 b	10 \pm 3.8 bc
DPI control	13.7 \pm 0.6 a	3.6 \pm 0.2 c	3.8 \pm 0.2 b	38.8 \pm 3.3 a	15 \pm 3.2 b

^z Means followed by different letters are significantly different from each other ($P < 0.05$).

2.8. Statistical analysis

One-way analysis of variance (ANOVA) was used to analyze data with R software (version: February 1, 2019, RStudio, Inc., Vienna, Austria). Post-hoc comparisons were performed using Tukey's HSD ($\alpha \leq 0.05$). Pearson's correlation was calculated to study the relationships among phytochemicals and antioxidant capacity.

3. Results and discussion

Among the four finger lime selections, DPI 50–36 (DPI control) is commercially available in Florida and was used as a control for comparison. TPC was highest ($P < 0.001$) in the pulp of FL-red and DPI control, whereas for peel tissue, TPC was highest ($P < 0.001$) in both red selections, FL-red and LL FL-red, compared to DPI control and FL-white (Fig. 2). Sommano et al. (2013) reported a higher phenolic content of 457.5 mg GAE/100 g in finger limes, however our results are relatively low, approximately 10 times lower. Our results also indicate that the peel has 2–3 times higher TPC content (i.e., 35–55 mg GAE/100 g FW) compared to the pulp (15–25 mg GAE/100 g FW). Similarly, it has been reported that the peel of different citrus species such as lemons, oranges, and grapefruit contain higher levels of total phenolics, including flavonoids and anthocyanins, compared to the pulp (Goulas & Manganaris, 2012).

Flavonoid content (FC) was found to be significantly higher ($P < 0.001$) in the pulp of FL-red compared to DPI control, FL-white, and LL

FL-red (Fig. 3). Regarding the peel, both FL-red and LL FL-red had significantly higher FC ($P < 0.001$) than the DPI control and FL-white selection. FC was found to be significantly higher in the peel tissue (32–62 mg catechin/100 g FW) compared to the pulp (2.5–9 mg catechin/100 g FW), which suggests that the composition and content of flavonoids vary among the types of tissues in citrus. Flavonoids are major contributors of total phenolic content; however, it is worth noting that in the pulp of DPI control, TPC was similar to FL-red and higher than FL-white and LL FL-red. Nonetheless, the FC content of DPI control pulp was significantly lower than FL-red and similar to FL-white and LL FL-red. This suggests that in the DPI control, phenolic phytochemicals other than flavonoids contribute to TPC. Of the four selections, average AC was almost 3-fold lower in the pulp (1.5 mg C3GE/kg FW) compared to the peel (4.2 mg C3GE/kg FW; Fig. 4). In pulp tissue, the highest AC ($P < 0.001$) was found in both red selections, FL-red and LL-FL-red, compared to the DPI control whereas both FL-white and DPI control had very low to untraceable amounts of anthocyanin in pulp. Similarly, higher AC content ($P < 0.001$) was found in the peel of both red selections (FL-red and LL FL-red) compared to the DPI control. Cyanidin 3-glucoside are reported to be the major anthocyanin found in finger lime pulp (Netzel, Netzel, Tian, Schwartz, & Konczak, 2006), ranging from 0.13 to 0.21 mg C3GE/kg FW (Delort & Yuan, 2018). However, in the present study, the AC content was significantly higher compared to the previous literature. These results suggest that Florida red selections serve as a rich source of anthocyanin. Altogether, TPC, FC, and AC were highest in red selections. Fruit with dark red/purple color or high anthocyanin content are reported to have high TPC and antioxidant activity (Wang, Cao, & Prior, 1996). The results show that the peel is rich in AC as compared to the pulp (for all selections) therefore, it is likely that the high AC content of finger lime peels contribute to high TPC and FC content as compared to the pulp. In addition, high AC content in peel and pulp of red selections, respectively contribute to high TPC and FC content of red selection.

Citrus species, including the finger lime, are well known for their vitamin C content (Delort & Yuan, 2018). It has been reported that red finger limes contain more vitamin C (91 mg/100 g FW) than the green finger limes i.e., 26 mg/100 g FW (Konczak, Zabarar, Dunstan, & Aguas, 2010). In the present study, the vitamin C content of the three selections (FL-red, FL-white, and LL FL-red) were significantly lower ($P < 0.001$) than the DPI control (Fig. 5). Konczak et al. (2010) reported that the vitamin C content ranged from 20 to 40 mg/100 g FW in finger limes; however, in the present study, we obtained higher vitamin C content (35–115 mg AA/100 g FW). Interestingly, all finger lime selections in this study had a higher vitamin C content than other citrus species such

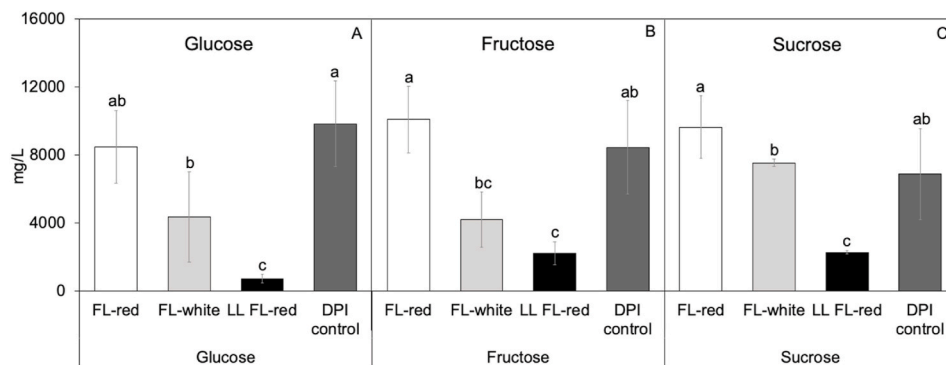


Fig. 7. Sugar content (glucose, fructose and sucrose) (mean ± standard deviation, n = 4) in four finger lime cultivars, red pulp finger lime (FL-red), white pulp finger lime (FL-white), red pulp-large leaved finger lime hybrid (LL FL-red), and the commercially available cultivar from Florida Division of Plant Industry 50–36 cultivar (DPI control). Bars marked with different lowercase letters indicate statistically significant difference using Tukey’s honestly significant difference test at $\alpha = 0.05$.

as orange, grapefruit, and lemon (Tareen et al., 2015). Higher AC and vitamin C content and lower TPC in Florida-grown finger limes compared to those in earlier studies can most likely be attributed to the intrinsic characteristics of the genotypes as well as environmental effects (Wang, Zheng, & Galletta, 2002). The genotype (G), environment (E), and their interaction (G x E) can play significant roles in phytochemical production. Generally, the genotype defines the potential of a crop to

produce phytochemicals; however, different abiotic factors like temperature and relative humidity in the growing environment can influence production (Jones & Hartley, 1999).

The finger lime with red pulp (FL-red) had significantly higher antioxidant capacity ($P < 0.001$) in both peel and pulp compared to finger limes with white pulp (FL-white and DPI control; Fig. 6). This can be due to high TPC, as a significant linear correlation between TPC and

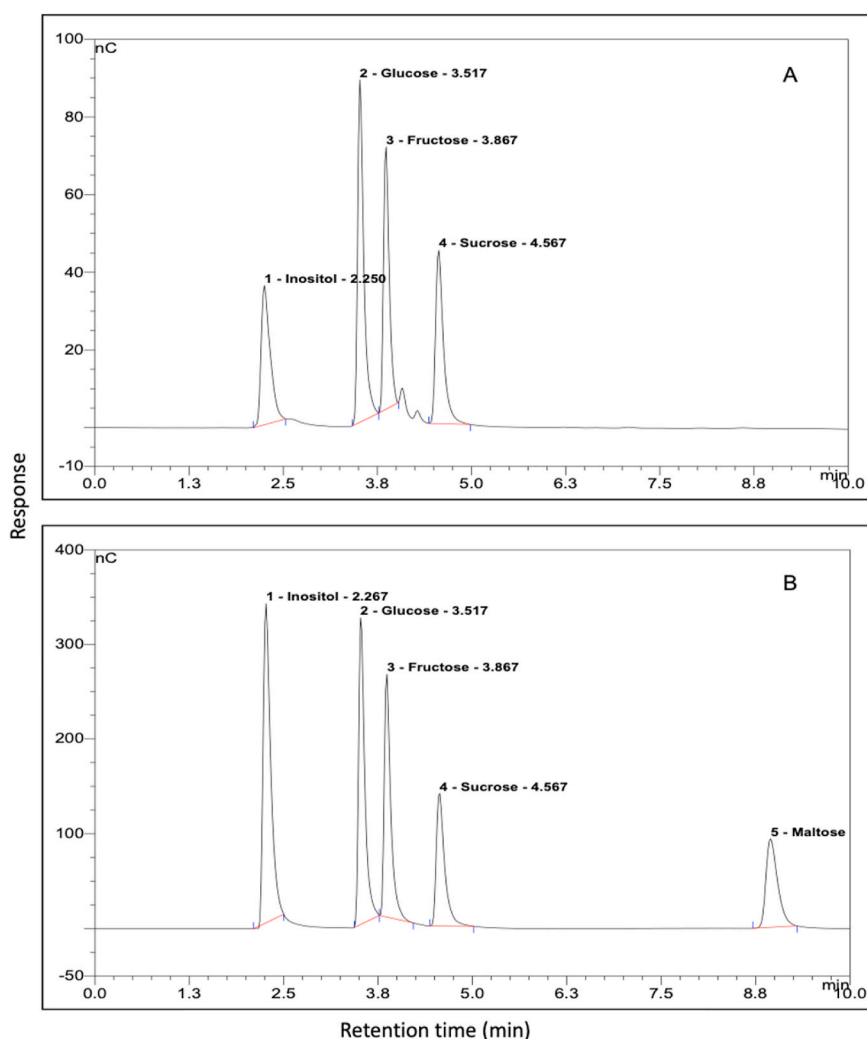


Fig. 8. Overlay of ion chromatogram separated from pulp sample (A) of the four Florida-grown finger lime selections; panel B shows the chromatogram of the standards run with each sample set.

antioxidant activity ($r = 0.77$, $P < 0.001$) was observed. It is well demonstrated that TPC is a major contributor to antioxidant activities in citrus, as well as in other fruit crops such as tomato and blackberry (Mertz et al., 2009). A positive correlation ($r = 0.84$, $P < 0.001$) between peel FC and antioxidant activity indicates that phenolic compounds, such as flavonoids, also play a major role in antioxidant activity. Similarly, a strong correlation between vitamin C and antioxidant activity ($r = 0.80$, $P < 0.001$) was observed. Overall, red selections of finger lime had higher antioxidant capacity. Therefore, when breeders are selecting for Florida finger lime selections, red colored peel and pulp can be used as an initial indicator for potentially high TPC, FC, AC, and antioxidant activity.

Citric and malic acid content, TSS, TA, and TSS/TA ratio were quantified in the pulp tissue of all four finger lime selections (Table 1). FL-red had the highest TSS ($P < 0.001$), followed by DPI control, FL-white, and LL FL-red. The TSS/TA ratio was highest in FL-red and lowest in LL FL-red ($P < 0.001$), suggesting that the FL-red pulp is rich in sugars; however, an inverse order for TA content was observed, wherein LL FL-red had the highest TA, and FL-red had the lowest ($P < 0.001$). Upon further evaluation, FL-white had significantly higher malic acid content, while FL-red has the lowest ($P < 0.001$). On the contrary, the citric acid content was significantly higher ($P < 0.001$) in FL-red followed by FL-white, DPI control, and finally lowest in LL-FL-red. Interestingly, there was a strong positive correlation between citric acid and TSS ($r = 0.77$, $P < 0.001$) and a negative correlation between citric acid and TA ($r = -0.75$, $P < 0.001$). Citric acid has been reported as the main organic acid and malic acid as a minor organic acid in finger limes (Konczak et al., 2010). The citric acid content in finger lime typically ranges from 1.1 to 67.8 mg/g FW, which is higher than any other citrus crops (Lin et al., 2015). In the present study, citric acid ranged from 14 to 36 mg/g FW in the red pulp finger lime and 34–42 mg/g FW in the white pulp finger lime. This supports the idea that citric acid is abundantly available in most finger lime selections, while malic acid is less abundant.

Glucose, sucrose, and fructose are the dominant sugar compounds found in citrus pulp (Wang et al., 2019). Glucose was significantly higher ($P < 0.001$) in the DPI control, followed by FL-red, FL-white, and the lowest in LL FL-red (Figs. 7 and 8). Similarly, fructose was significantly higher ($P < 0.001$) in FL-red followed by DPI control, FL-white, and the lowest in LL FL-red. Likewise, sucrose was greatest in FL-red followed by DPI-control, FL-white, and lowest in LL FL-red. It has been reported that finger lime has approximately 1200 mg/100 g of FW sugars (fructose and glucose) and 5000 mg/100 g FW carbohydrates, indicating that sugars contribute to less of the total carbohydrate content and that the remaining carbohydrates are possibly dietary fibers (Richmond, Bowyer, & Vuong, 2019). Moreover, the glucose, sucrose, and fructose content in six citrus cultivars range from 416 to 2600, 926–5092, and 372–1699 mg/100 g, respectively (Zhou et al., 2018), which are relatively higher than the sugar content present in the four finger lime selections. Overall, our results suggest that finger limes are not a significant source of fruit sugars and are a potentially good source of dietary fibers, in addition, finger limes are rich in organic acids that are of high nutritious value.

4. Conclusion

Among the four finger lime selections evaluated in this study, FL-red had the highest phenolics, flavonoid, and anthocyanin content with a high antioxidant capacity, followed by LL FL-red, DPI control, and FL-white. Vitamin C was highest in FL-red, followed by DPI control, LL FL-red, and FL-white. Similarly, finger lime peel had a significantly higher phytochemical content than pulp tissue in all four selections, indicating that the phytochemical content varies depending on the tissue type. In addition, Florida-grown red finger lime selections FL-red and LL FL-red (peel and pulp) have comparatively higher anthocyanin content than finger limes in previous studies. Moreover, vitamin C content was

high, while the sugar level was low compared to other citrus crops, making these four selections more desirable for diet-conscious consumers. Citric acid was abundant in each of the four finger lime selections, marking them as a potential source of organic acids. Low malic acid content was found in the red pulp selections compared to the DPI control, while FL-white had the highest malic acid content. Overall, the Florida-grown red selections are rich in phytochemicals, high antioxidant activity, and well-balanced composition of organic acid and sugars making them suitable candidates for growers as well as for consumers.

CRedit authorship contribution statement

Bikash Adhikari: Methodology, Formal analysis, Data analysis, Manuscript Writing. **Manjul Dutt:** Conceptualization, Writing - review & editing. **Tripti Vashisth:** Conceptualization, Writing - review & editing, Supervision.

References

- Cataldi, T. R. I., Margiotta, G., Iasi, L., Di Chio, B., Xiloyannis, C., & Bufo, S. A. (2000). Determination of sugar compounds in olive plant extracts by anion-exchange chromatography with pulsed amperometric detection. *Analytical Chemistry*, 72(16), 3902–3907. <https://doi.org/10.1021/ac000266o>.
- Cheng, L., Ren, Y., Lin, D., Peng, S., Zhong, B., & Ma, Z. (2017). The anti-inflammatory properties of citrus wilsonii tanaka extract in LPS-induced RAW 264.7 and primary mouse bone marrow-derived dendritic cells. *Molecules*, 22(7). <https://doi.org/10.3390/molecules22071213>.
- Delort, E., & Jaquier, A. (2009). Novel terphenyl esters from Australian finger lime (*Citrus australasica*) peel extract. *Flavour and Fragrance Journal*, 24(3), 123–132. <https://doi.org/10.1002/ffj.1922>.
- Delort, E., & Yuan, Y.-M. (2018). Finger lime/The Australian caviar—*Citrus australasica* exotic fruits. <https://doi.org/10.1016/b978-0-12-803138-4.00025-3>, 203–210.
- Gilbert, J. L., Olmstead, J. W., Colquhoun, T. A., Levin, L. A., Clark, D. G., & Moskowitz, H. R. (2014). Consumer-assisted selection of blueberry fruit quality traits. *HortScience*, 49(7), 864–873. <https://doi.org/10.21273/hortsci.49.7.864>.
- Goulas, V., & Manganaris, G. A. (2012). Exploring the phytochemical content and the antioxidant potential of citrus fruits grown in Cyprus. *Food Chemistry*, 131(1), 39–47. <https://doi.org/10.1016/j.foodchem.2011.08.007>.
- Hamilton, K. N., Ashmore, S. E., & Drew, R. A. (2005). Development of conservation strategies for citrus species of importance to Australia. *Acta Horticulturae*, 694, 111–115. <https://doi.org/10.17660/ActaHortic.2005.694.15>, 1999.
- Jones, C. G., & Hartley, S. E. (1999). A protein competition model of phenolic allocation. *Oikos*, 86(1), 27. <https://doi.org/10.2307/3546567>.
- Killiny, N., Jones, S. E., Nehela, Y., Hijaz, F., Dutt, M., Gmitter, F. G., et al. (2018). All roads lead to Rome: Towards understanding different avenues of tolerance to Huanglongbing in citrus cultivars. *Plant Physiology and Biochemistry*, 129, 1–10. <https://doi.org/10.1016/j.plaphy.2018.05.005>.
- Konczak, I., Zabarás, D., Dunstan, M., & Aguas, P. (2010). Antioxidant capacity and hydrophilic phytochemicals in commercially grown native Australian fruits. *Food Chemistry*, 123(4), 1048–1054. <https://doi.org/10.1016/j.foodchem.2010.05.060>.
- Lee, J., Durst, R. W., & Wrolstad, R. E. (2005). Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: Collaborative study. *J. AOAC International*, 88(5), 1269–1278.
- Lin, Q., Wang, C., Dong, W., Jiang, Q., Wang, D., Li, S., et al. (2015). Transcriptome and metabolome analyses of sugar and organic acid metabolism in Ponkan (*Citrus reticulata*) fruit during fruit maturation. *Gene*, 554(1), 64–74. <https://doi.org/10.1016/j.gene.2014.10.025>.
- Liu, Y., Heying, E., & Tanumihardjo, S. A. (2012). History, global distribution, and nutritional importance of citrus fruits. *Comprehensive Reviews in Food Science and Food Safety*, 11(6), 530–545. <https://doi.org/10.1111/j.1541-4337.2012.00201.x>.
- Liu, Y., Ren, C., Cao, Y., Wang, Y., Duan, W., Xie, L., et al. (2017). Characterization and purification of bergamottin from *Citrus grandis* (L.) Osbeck cv. Yongjiazaoxiangyou and its antiproliferative activity and effect on glucose consumption in HepG2 cells. *Molecules*, 22(7). <https://doi.org/10.3390/molecules22071227>.
- Mertz, C., Gancel, A. L., Gunata, Z., Alter, P., Dhuique-Mayer, C., Vaillant, F., et al. (2009). Phenolic compounds, carotenoids and antioxidant activity of three tropical fruits. *J. Food Composition and Analysis*. <https://doi.org/10.1016/j.jfca.2008.06.008>.
- Netzel, M., Netzel, G., Tian, Q., Schwartz, S., & Konczak, I. (2006). Sources of antioxidant activity in Australian native fruits. Identification and quantification of anthocyanins. *Journal of Agricultural and Food Chemistry*, 54(26), 9820–9826. <https://doi.org/10.1021/jf0622735>.
- Netzel, M., Netzel, G., Tian, Q., Schwartz, S., & Konczak, I. (2007). Native Australian fruits - a novel source of antioxidants for food. *Innovative Food Science & Emerging Technologies*, 8(3), 339–346. <https://doi.org/10.1016/j.ifset.2007.03.007>.
- Prior, R. L., & Cao, G. (2000). Antioxidant phytochemicals in fruits and vegetables: Diet and health implications. *HortScience*, 35(4), 588–592. <https://doi.org/10.21273/hortsci.35.4.588>.
- Pulido, R., Bravo, L., & Saura-Calixto, F. (2000). Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay.

- J. Agricultural and Food Chemistry*, 48(8), 3396–3402. <https://doi.org/10.1021/jf9913458>.
- Rahman, M. M., Khan, M. M. R., & Hosain, M. M. (1970). Analysis of vitamin C (ascorbic acid) contents in various fruits and vegetables by UV-spectrophotometry. *Bangladesh Journal of Scientific & Industrial Research*, 42(4), 417–424. <https://doi.org/10.3329/bjsir.v42i4.749>.
- Richmond, R., Bowyer, M., & Vuong, Q. (2019). Australian native fruits: Potential uses as functional food ingredients. *J. Functional Foods*, 62, 103547. <https://doi.org/10.1016/j.jff.2019.103547>.
- Sommano, S., Caffin, N., & Kerven, G. (2013). Screening for antioxidant activity, phenolic content, and flavonoids from Australian native food plants. *Inter. J. Food Properties*, 16(6), 1394–1406. <https://doi.org/10.1080/10942912.2011.580485>.
- Tareen, H., Mengal, F., Masood, Z., Mengal, R., Ahmed, S., Bibi, S., et al. (2015). Determination of vitamin C content in citrus fruits and in non-citrus fruits by titrimetric method, with special reference to their nutritional importance in Human diet. *Biological Forum - An Inter. J.*, 7(2), 367–369.
- Vashisth, T., Singh, R. K., & Pegg, R. B. (2011). Effects of drying on the phenolics content and antioxidant activity of muscadine pomace. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology*, 44(7), 1649–1657. <https://doi.org/10.1016/j.lwt.2011.02.011>.
- Wang, H., Cao, G., & Prior, R. L. (1996). Total antioxidant capacity of fruits. *J. Agricultural and Food Chemistry*, 44(3), 701–705.
- Wang, Y., Ji, S., Zang, W., Wang, N., Cao, J., Li, X., et al. (2019). Identification of phenolic compounds from a unique citrus species, finger lime (*Citrus australasica*) and their inhibition of LPS-induced NO-releasing in BV-2 cell line. *Food and Chemical Toxicology*, 129, 54–63. <https://doi.org/10.1016/j.fct.2019.04.006>.
- Wang, Y., Qian, J., Cao, J., Wang, D., Liu, C., Yang, R., et al. (2017). Antioxidant capacity, anticancer ability and flavonoids composition of 35 citrus (*Citrus reticulata* Blanco) varieties. *Molecules*, 22(7), 1–20. <https://doi.org/10.3390/molecules22071114>.
- Wang, S. Y., Zheng, W., & Galletta, G. J. (2002). Cultural system affects fruit quality and antioxidant capacity in strawberries. *J. Agricultural and Food Chemistry*, 50(22), 6534–6542. <https://doi.org/10.1021/jf020614i>.
- Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*, 64(4), 555–559. [https://doi.org/10.1016/S0308-8146\(98\)00102-2](https://doi.org/10.1016/S0308-8146(98)00102-2).
- Zhou, Y., He, W., Zheng, W., Tan, Q., Xie, Z., Zheng, C., et al. (2018). Fruit sugar and organic acid were significantly related to fruit Mg of six citrus cultivars. *Food Chemistry*, 259(1), 278–285. <https://doi.org/10.1016/j.foodchem.2018.03.102>.