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Total Phenolics as an Indicator of Quality in Florida-grown Tea

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Tea (*Camellia sinensis*) is emerging as a potential specialty crop for Florida, but quality evaluations of Florida-grown tea have not yet been reported. Phytochemicals contributing to tea quality include phenolics, such as catechins, tannins, and flavonoids, which contribute astringency and bitterness. We harvested two varieties of Florida-grown tea and processed them into green tea, then evaluated each variety of made-tea for total phenolics (TP): 'Fairhope', sourced from Alabama and 'Georgian' from Mississippi State University. Both have been growing in North-central Florida since 2016. TP levels were comparable to commercially-sourced green tea in both methanolic extracts and hot water tea infusions, measured as milligrams of catechin equivalent (CE) per gram dry weight of made-tea. In methanolic extracts, 'Fairhope' had 99.67 ± 5.77 mg·g^{-1} CE and 91.90 ± 5.54 mg·g^{-1} CE in July 2020 and May 2021 harvests, respectively. 'Georgian' extracts had 106.27 ± 5.96 mg·g^{-1} CE and 124.86 ± 6.46 mg·g^{-1} CE in July 2020 and May 2021 respectively. A commercially-sourced green tea showed TP levels of 81.58 ± 5.22 mg·g^{-1} CE. There were no significant differences ($P = 0.05$) between spring and summer harvests, or between the Florida-grown teas and the commercially-sourced tea, except for the May harvest of 'Georgian,' which had higher TP than both 'Fairhope' and the commercial standard. Hot water infusions of made-tea from the 2020 harvests of 'Fairhope' and 'Georgian' and the commercial control also did not differ significantly, with 25.95 ± 2.94 mg·g^{-1} CE, 30.55 ± 3.19 mg·g^{-1} CE and 24.19 ± 2.86 mg·g^{-1} CE, respectively. This work indicates that Florida-grown tea has phenolics levels of suitable quality for processing and marketing as green tea.

Tea (*Camellia sinensis*) is an important beverage worldwide, both culturally and for health-promoting qualities (Khalesi et al., 2014). A variety of compounds present in tea leaves contribute to the quality of prepared tea (Kottawa-Arachchi et al., 2019). One important class of compounds found in tea is phenolics; these include catechins, tannins, and flavonoids. Catechins are the dominant fraction of this class; the total dry weight of tea leaves can comprise up to 30% catechins (Han et al., 2016). Phenolics in tea leaves have been reported to change with processing method and season of harvest, contributing to changes in flavor and quality (Fang et al., 2017). This can have varying effects on quality of made-tea. Phenolics primarily contribute to the astringency, bitterness, and color aspects of tea quality (Zhang et al., 2020).

Tea is emerging as a specialty crop in the United States. Tea thrives in the subtropics, preferring sandy, acidic soils and humid conditions. Tea is of special interest for Florida growers, since it thrives in conditions similar to those preferred by citrus and blueberry and could be a potential economic buffer crop. Tea has marketing potential both as a finished horticultural product (made-tea) and as a nursery crop intended for home growers (Mavrakis, 2016). Establishment trials have identified several accessions of tea that perform well in North Florida field conditions, and some accessions that, despite showing lower vigor, may have high quality traits. The quality of teas made from mature (≥ 5-year-old) tea plants grown in field conditions has not yet been assessed. Given the large contribution of phenolics to tea quality, an assessment was made of the total phenolics (TP) of green tea made from two accessions growing in North Florida field conditions. One of these accessions, 'Fairhope', has shown good performance in the field regarding survivorship and vigor, and produces a mid-quality green tea. The other accession, 'Georgian', has proved to be less vigorous than 'Fairhope,' but produces a green tea with pleasant taste and aroma. Green tea made from these two accessions was compared to Maeda-en Sencha, a sencha-style green tea produced in Japan and commercially available in the US.

The objectives of this study were to determine the total levels of phenolics present in each accession and whether they differ between seasons or by accession, and compare the TP levels to a commercial standard to assess the quality of Florida-grown teas.

Materials and Methods

Tea plants were grown at the Plant Science Research and Education Unit in Citra, FL (29°24'27.6"N; 82°08'27.7"W). One-year-old plants were established in Spring 2016. Two accessions were used in this study: Fairhope, sourced from Fairhope Tea Plantation in Fairhope, AL, and Georgian, sourced from Mississippi State University.

The apical meristem and first two leaves of the two different tea accessions were harvested in July of 2020 and May of 2021. Harvested leaves were processed into green tea. This procedure includes withering (5 h at 20 °C.), steaming (5 min), rolling (10–20 min), and drying (2 h at 77 °C) (Fig. 1). Processed tea leaves were stored at 4 °C until analysis.

Three biological replicates were used for every sample in both methanol and hot water extractions.

Methanol Extraction. Dry processed tea leaves (50 mg) were frozen in liquid nitrogen, homogenized in 6 mL of 95% methanol for 30 s, inverted 3×, and incubated for 16 h at 22 °C. Samples were centrifuged at 200 g for 10 min in an IEC Clinical Centri-

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Fig. 1. Processing harvested tea leaves into green tea. Leaves were plucked at two-and-a-bud stage, then withered in paper bags for about 5h. The leaves were then steamed for 5 min, after which they were rolled in a stand mixer for 15 min. The processed leaves were dried at 77 °C until a stable weight was reached.

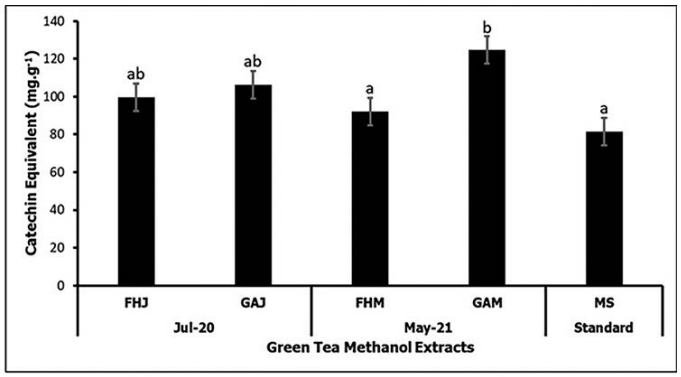

Fig. 2. Total phenolics (TP) of methanol extrctions of green tea, expressed as catechin equivalents (mg·g-1). FHJ = 'Fairhope' July 2020. FHM = 'Fairhope' May 2021. GAJ = 'Georgian' July 2020. GAM = 'Georgian' May 2021. MS = Maeda-en Sencha.

fuge (IEC, Irvine, CA) and the supernatant was collected (Yao et al., 2004).

Extraction efficiency was evaluated by three sequential extractions of two samples of Maeda-en Sencha and one sample of 'Fairhope.' Dry processed tea leaves (50 mg) were extracted according to the method described above. The tissue was re-extracted in a fresh 6 mL aliquot of 95% methanol for 2 h at 22°C, and this step was then repeated. First, second, and third extracts were then reacted in the Folin-Ciocalteu assay to determine TP levels in each successive extraction.

Hot Water Extraction. Dry processed tea leaves (500 mg) were placed in glass tubes with 29.5 mL of water and held at 80 °C in a hot water bath for 1 min. The tea leaves were removed and the extract was used in the Folin-Ciocalteu TP Assay.

Folin–Ciocalteu TP Assay. Extracts were reacted in a Folin-Ciocalteu assay modified after Ainsworth and Gillespie (2007). Diluted extract samples (10 μL of extract and 90 μL 95% MeOH) were placed into glass test tubes. Freshly prepared Folin–Ciocalteu reagent (10%, 200 μL) was added. Samples were vortexed and 800 μL of 700 mM sodium carbonate (Na_2CO_3) were added. The reaction was incubated at 22 °C for 2 h and the absorbance at 765 nm was read using a DU730 UV visible spectrophotometer (Beckman Coulter, Brea, CA). The standard used was ± catechin ($C_{15}H_{14}O_6$). Total phenolics levels are reported as catechin equivalents (CE).

Results and Discussion

Methanol Extraction. Total phenolics levels for green tea made from Florida-grown tea plants were found to be comparable to commercial standard green tea (Fig. 2). 'Fairhope' had 99.67 ± 5.77 mg·g-1 CE in leaves from the July 2020 harvest and 91.90 ± 5.54 mg·g-1 CE in leaves from May 2021. 'Georgian' extracts had 106.27± 5.96 mg·g-1 CE in July 2020 and 124.86 ± 6.46 mg·g-1 CE in May 2021. Maeda-en Sencha, the commercially-sourced green tea, showed TP levels of 81.58± 5.22 mg.g-1 CE. There was no difference between Maeda-en Sencha and the July 2020 harvests of 'Fairhope' and 'Georgian,' or the May 2021 harvest of 'Fairhope.'

There was no significant seasonal change in TP within accessions. Among accessions, the May 2021 harvest of 'Georgian,' showed TP levels significantly higher than the May 2021 harvest of 'Fairhope' ($P < 0.01$) and Maeda-en Sencha ($P < 0.001$). The higher levels of TP seen in the May 2021 harvest of 'Georgian' could be explained by the presence of lesions on the leaves of most 'Georgian' plants that season. A separate assay of only lesioned leaves collected in June 2021 showed TP levels higher than all samples except for the May 2021 'Georgian' harvest (data not shown).

The extraction efficiency for the methanolic extractions was > 97% recovery of TP in the first of three sequential extractions tested (data not shown).

Hot Water Extraction. The July 2020 harvests of 'Fairhope' and 'Georgian' used for the hot water extractions showed TP levels comparable to Maeda-en Sencha, with 'Fairhope' at 25.95 ± 2.94 mg·g-1 CE, 'Georgian' at 30.55 ± 3.19 mg·g-1 CE and Maeda-en Sencha at 24.19 ± 2.86 mg·g-1 CE (Fig. 3). These data indicate that total phenolics of Florida-grown tea extracted by hot water, as would be done to make tea for drinking, are of a suitable level to meet commercial quality standards. The hot water extracts showed much lower levels of TP in comparison with the methanolic extracts, with 26% of methanol-extracted TP present in 'Fairhope,' 29% in 'Georgian,' and 30% in Maeda-en Sencha. The hot water extracts were only incubated for 1 min, while the methanolic extractions were incubated for 16 h. However, the temperature of incubation was much higher for the hot water extraction (80 °C) compared to the methanolic extraction (22 °C). Previous studies have found that methanol results in higher recovery of phenolics from plant matter, compared to other solvents including hot water, ethanol, and chloroform (Yao

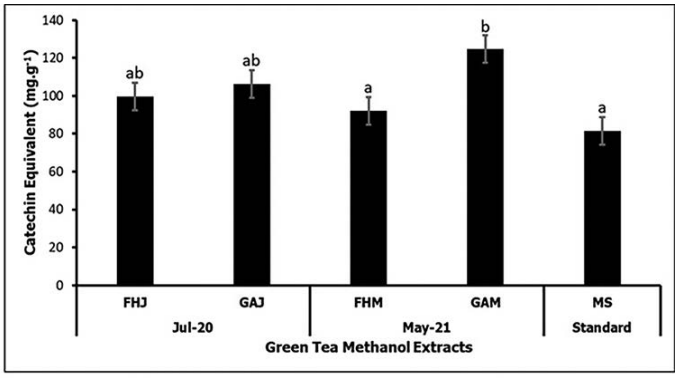

Fig. 3. Total phenolics (TP) of green tea extracted in hot water (80 °C) expressed as catechin equivalents (mg·g-1). FHT = 'Fairhope' tea. GAT = 'Georgian' tea. MST = Madea-en Sencha tea.

et al., 2004). This result can serve to inform an estimate of what proportion of the total plant phenolics are being consumed when drinking green tea.

These results indicate that the TP levels found in Florida-grown green teas are comparable to those found in commercially available green tea. Further analyses will investigate the profile of phenolics present in each accession by mass spectrometry, as well as other quality factors including L-theanine and caffeine.

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