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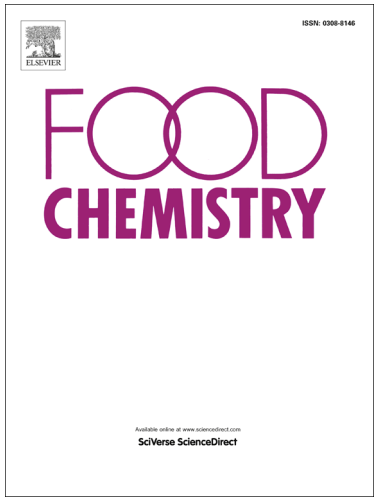

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Semkaleng Mpai, Rosemary du Preez, Yasmina Sultanbawa, Dharini Sivakumar

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Phytochemicals and nutritional composition in accessions of Kei-Apple (*Dovyalis caffra*): Southern African Indigenous fruit

Semkaleng Mpai[1], Rosemary du Preez[2], Yasmina Sultanbawa[3], Dharini Sivakumar[1]

[1]Phytochemical Food Network Research Group, Department of Crop Sciences, Tshwane University of Technology, Pretoria West. 0001, South Africa

[2]Institute for Tropical and Subtropical Crops Agricultural Research Council -Agricultural Research Council, Nelspruit, South Africa

[3]Queensland Alliance for Agriculture and Food Innovation, Center for Food Science and Nutrition, The University of Queensland, Australia

Abstract

Current study was initiated to identify the phytochemicals and the nutritional profile of eleven Kei-apple fruit accessions. Accession FH29 showed the highest level (492.45 mg 100 g^{-1} fresh weight) of total phenolic content, higher than the referral fruit, blueberry. Pyrogallol was identified as the predominant phenolic compound in all accessions. Accession FH 29 showed the highest (49.75 µmol TEAC g^{-1} fresh weight) antioxidant capacity. Catechin content was higher in accessions; FH151, FH15, FH14, FH29, FH243, FH 239 and FH 231. Accessions, FH14 and FH232 exhibited higher levels of β-carotene than the referral fruit apples (cv. Top red) and peaches (cv. Excellence). The total sugar (glucose and fructose) was highest (50 mg g^{-1} fresh weight) in accession FH240. Asparagine (3122.18 mg L^{-1}) and gamma-aminobutyric (1688.87 mg L^{-1}) were higher in accessions FH239 and FH243 respectively. Overall, the accession Kei-apple FH236 can be regarded as a good source of essential amino acids.

Keywords: Traditional fruits, Phenylalanine, Bioactive compounds, Pyrogallol, β-carotenes

1. Introduction

Phytochemicals are becoming popular due to their health benefits and fruits and vegetables are a rich source thereof. Fruits such as strawberries, (Diamanti et al., 2014) blueberries (Balducci et al., 2016) and raspberries (Bowen-Forbes, Zhang, & Nair, 2010) contain high levels of antioxidants. Recently, the research focus has fallen on exploring the phytochemicals in indigenous or underutilised fruits. Diet diversification with underutilised fruits and vegetables is a sustainable and affordable strategy to improve human nutrition and wellbeing (Fanzo, Hunter, Borelli, & Mattei, 2013). Low nutritional diets have negative effects on the population, for example, malnutrition, non-communicable diseases and obesity (Vorster, 2010).

Southern African indigenous Kei–apple fruit, belonging to the family Flacourtiaceae was reported to possess high levels of polyphenol compounds (Loots, van der Westhuizen, & Jerling, 2006). The juice obtained from the fruit contains the following major nonflavonoid phenolic compounds such as: p-coumaric acid (15.76 mg L^{-1}), p-hydroxyphenylacetic acid (10.62 mg L^{-1}), 3-methoxy-4-hydroxyphenylacetic acid (6.24 mg L^{-1}), m-hydroxybenzoic acid (4.27 mg L^{-1}), vanillic acid (3.64 mg L^{-1}) (Loots et al., 2006), chlorogenic acid and procatechuic acid (Minnaar, Jollya, Paulsen, Du Plessis, &Van Der Rijst, 2017). Kei–apple fruit juice also contains a higher concentration of ascorbic acid (669 mg L^{-1}) (Loots et al., 2006). The fruit provide opportunities to improve the rural economy by developing food products such as jam, jelly etc (Du Preez, 2003). Domestication programmes have been employed to integrate the traditional food crops into organised cultivation through the implementation of home gardens for the traditional fruit crops with minimal water use in order to supplement diets. (Du Preez, 2003). Eleven accessions of Kei-apple trees have been identified in the Southern African region (Du Preez, 2003). Kei-apple from Egypt was reported to contain 15 amino acids and glutamic acid was identified as abundant amino acids (Morton, 1987). Due to an increasing interest in the utilisation of Kei-apple as a source of

phytonutrients (Loots et al., 2006; Du Preez, 2003), it is important to determine the health beneficial phytochemicals and the nutritional compounds in the eleven accessions of Kei-apples.

The carbohydrate content of the fruit has been reported by Du Preez. (2003). However, detailed information on the concentration of glucose and sucrose need to be investigated. Since the use of Kei-apple is receiving more prominence to be included as a food ingredient, the aim of this study is to investigate the total phenolic compounds, β-carotene, anthocyanin content, antioxidant capacity, glucose, fructose and free amino acid composition in the eleven Kei-apple accessions in order to select the suitable accessions as functional food or diet diversification. To our best knowledge, there is very little information available on the phytonutrient properties of Kei-apple accessions.

2. Materials and methods

2.1 Plant material and sample preparation

Kei-apple fruits from eleven accessions (FH236, FH14, FH243, FH232, FH204, FH240, FH239, FH151, FH15, FH231, FH29) were harvested at the mature yellow stage (Brix 11-12°) (Du Preez, 2003) during summer (December to January in 2015 and 2016) from the Friedenheim Farm, Mpumalanga Province, South Africa. Disease-free with uniform shape and size and without any visible injuries or defects were harvested manually early in the morning. The commercial referral fruits, apples (*Malus Domestica* cv. Top red), peaches (*Prunus persica* cv. Excellence) and blueberries (*Vaccinium corymbosum* southern highbush var. Oz Julieta) were obtained from the commercial farms (Ceres, Goosen farms, Hargrove Heaven farm) in the Western Cape, South Africa. The fruits were de-seeded manually and a set of 20 replicate fruits per accession were frozen and stored at -20 °C until processing in order to compare the total phenolics, antioxidant capacity and the scavenging activity with the Kei-apple fruits and referral fruit (apple, peach and

blueberries). Fruit samples were homogenised directly using an Ultra Turrax (T25 digital ULTRA-TURRAX ®, Lab limited, Surry, UK) and the homogenised sample was used for different biochemical analysis (Colaric, Stampar, Solar, & Hudina (2006) Additionally, sugars (glucose and fructose), free amino acids and phenolic compounds were quantified from the edible portion (pulp) of the eleven Kei-apple accessions.

2.2 Reagents

Acetone, *n*-hexane, Folin-Ciocalteau reagent, sodium carbonate, gallic acid, methanol, Butylated hydroxytoluene (BHT), dichloromethane, ribitol, methoxyamine hydrochloride, pyridine, N-methyl-N-(trimethylsilyl) trifluoroacetamide, acetone, acetate buffer, 2,4,6-tripyridyl-*s*-triazine, HCl, ferric chloride ($FeCl_3.6H_2O$), ethanol, sodium acetate, potassium chloride, acetone, hexane, dichloromethane, sodium borate, methanol, , acetonitrile, vanillic acid (≥97.0), protocatechuic acid (≥97.0), syringic,(≥98.0%) p-coumaric acid (≥98.0%), ferulic acid (≥99.0%), caffeic (≥98.0%) and ellagic acid (≥95.0%), pyrogallol (≥98.0%), 2,2-diphenylpicrylhydrazyl (DPPH) solution, a AccQ-Tag Ultra amino acid kit, acetonitrile and *L*-Norvaline were purchased from Sigma Aldrich (Johannesburg, South Africa).

2.3 Phenolic content

Concentrations of phenolic acids such as. vanillic acid, protocatechuic acid, syringic, p-coumaric acid, ferulic acid, caffeic and ellagic acid were determined according to the HPLC analysis described by Colaric et al. (2006). using 1 g homogenised sample. The sample was extracted in 10mL of methanol containing 1% BHT in an in an ultrasonic bath for 45 min (Colaric et al. 2006). Thereafter, extracted samples were subject to centrifugation at 12 000 × *g*, 7min,10 ◦C.

The resulting supernatant was filtered via 35 µL filtered via a hydrophobic PTFE syringe filters (0.22 µm pore size) and 10 µL injected three times for high-performance liquid chromatography (HPLC) [with a photo diode array ultraviolet detector, C18 column (100 × 4.6 mm; 5 µm particle size), Model Flexar ™ 89173-556 PerkinElmer, Waltham, Massachusetts, USA and the mobile phase, flow rate and gradient elution programme were according to Colaric et al. (2006). Chromatogram was read at 272, 280, 310 and 320 nm. The phenolic acids and flavonols were identified and quantified using pure external standards and constructing the calibration curves according to Colaric et al. (2006). Results for phenolic acids and major flavonols (catechin or quercetin) are reported in mg per kg of FW.

Total phenolic content was quantified according to the Folin-Ciocalteau method (Singleton et al., 1999) using homogenised sample (0.2 g) extracted with 2 mL acetone: water (1:1 v/v) for 1 h at 25 °C in an ultrasonic bath. The total phenolic compounds were calculated according to Pereira, Knor, Vellosa, and Beltrame (2014) using a standard curve of pyrogallol and expressed as mg of pyrogallol equivalents per 100 g fresh weight (FW).

2.4 β-carotene

Estimation of β-carotene was performed using fresh pulp samples (5 g). β-carotene was extracted using 1.5 mL acetone-hexane mixture (4:6 v/v). The procedure adopted was similar to that described by Nagata and Yamashita (1992). The β-carotene was determined using the following calculation: β-carotene = 0.216 A663 – 0.304 A505 + 0.452 A453, and was expressed as mg β-carotene per 100 g FW. A663, A505, and A453 are the absorbance at 663, 505 and 453 nm.

2.5 Anthocyanins

Anthocyanins were determined using the pH differential method (Lee, 2005). A fresh pulp sample (10 g) was mixed with 50 mL of 70% methanol and homogenised for 60 s. Subsequently, the mixture incubated for 24 h at 25 °C. Thereafter, the mixture was centrifuged and filtered.

The filtrate was subjected to spectrophotometric analysis to quantify the anthocyanins using the two sets of buffer solutions [(pH 1 KCl 0.025 M), pH 4.5 buffer (sodium acetate)]. The anthocyanin content (cyanidin-3-glucoside equivalents) was determined using the equation where

$$\text{Anthocyanin content (cyanidin-3-glucoside equivalents, mg /L)} = \frac{A \times Mw \times DF \times 10^3}{\epsilon \times l}$$

[A (A_{520}-A_{700}) in (pH 1)- (A_{520}-A_{700}) in (pH 4.5)]

[M_W = molecular weight of cyanidin-3-glucoside (449 g mol^{-1}), DF = dilution factor (50 mL 10 g^{-1}), l = path length (1 cm) ε = molar extinction coefficient of cyanidin-3-glucoside (26,900 L mol^{-1} cm^{-1}), 10^3 is the factor for conversion from g to mg]. A_{520} and A_{700} are absorbances at 200 and 700 nm.

2.6 Antioxidant capacity

Ferric Reducing Antioxidant Potential (FRAP) assay was performed using a freshly prepared FRAP solution in 25 mL of 0.3 M acetate buffer (pH 3.6) and 2.5 mL of 10 mM 2,4,6-tripyridyl-*s*-triazine solution in 40 mM HCl and 2.5 mL of 20 mM ferric chloride ($FeCl_3.6H_2O$) according to the method describe by Llorach, Tomas-Barberan, and Ferreres (2004). A fresh sample pulp (5 g) was mixed with methanol:water (4:1 v/v). A FRAP solution (950 µL) at 37 °C was mixed with 50 µL of the sample mixture and the antioxidant capacity (FRAP) was expressed as µmol TEAC (The trolox equivalent antioxidant capacity) per g FW.

The DPPH assay was determined according to Tinyane, Sivakumar, and Soundy (2013) by extracting 2 g of the fruit pulp in methanol:water (3:2 v/v) and thereafter, the extract was diluted with an extraction solution to obtain different sample concentrations (0.09-100 mg mL^{-1}). The reaction mixture contained 250 µL DPPH (90 µM) solution and 28 µL of the sample in a 96-well microplate, shaken and kept in darkness for 60 min. Absorbance was read at 515 nm (Zenyth 200rt Microplate Reader). The results were expressed as the concentration of antioxidants required to decrease the initial DPPH absorbance by 50% (IC_{50}).

2.7 Glucose and fructose content

The glucose and fructose content were estimated following the method of Roessner, Wagner, Kopka, Trethewey, and Willimitzer (2000) using a fresh sample (100 mg) dissolved in 1.4 mL of 100% methanol and 50 µL of an internal standard [2 mg ribitol per L (w/v) in water]. The derivatised sugars were analysed using GC/MS with an Agilent J&W DB-17 (50 %-164 phenyl)-methyl-polysil-oxane column 30 m x 250 µm x 0.25 µm, with helium as a carrier gas at a flow rate of 1 mL min^{-1}. The GC conditions and run parameters were set up according to Roessner et al. (2000). The glucose and fructose content was identified and quantified by comparison of the peak areas with that of the known standard, expressed as mg per g of FW.

2.8. Amino acids

Amino acid analysis was performed according to Grobbelaar (2013) using a fresh sample (100 mg) which was vortexed with 6 N HCl 0.5 mL. The resulting mixture was then held in an oven at 110 °C for 18 h and after cooling, centrifuged and filtered. The resulting filtrate was dried using a speed vac and reconstituted in borate buffer (70 µL) for derivatisation. Samples were derivatised using the AccQ- Tag Ultra amino acid kit and the samples were analysed twice. The derivatisation kit contains five vials of each of the following: AccQ-Tag derivatising agent [6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC)], dry acetonitrile for preparing the AQC, and sodium borate buffer (0.2 M, pH 8.8) to be used in the derivatization reaction. Initially, the samples were undiluted and then 10 times diluted in order to quantify the amino acids that are present in higher concentrations. The derivatization process was performed by adding 10 µL aliquot of the prepared undiluted sample (that contained 20 µL *L*-Norvaline in 80 µL of the sample) to the 20 µL of AQC, vortexed and held in the oven at 55 °C for 10 min. Afterwards, once the vials were cooled, the samples were ready for the Ultra Performance Liquid Chromatograph (UPLC) analysis (Armenta et al. 2010)..

Amino acid separation and detection were performed using a Waters Acquity Ultra Performance Liquid Chromatograph (UPLC) fitted with a photodiode array (PDA) detector. An aliquot of 1 µL of sample was injected into the mobile phase which conveys the derivatized amino acids onto a Waters UltraTag C_{18} column (2.1 x 50 mm x 1.7 µm) held at 60 °C. The gradient was set up and commenced with 99.9% eluent A (water) and 1% eluent B (acetonitrile). The total run time was 9.5 min and the run flow rate was 0.7 mL min $^{-1}$. Chromatographic separation was done according to Armenta et al. (2010).

2.9. Statistical analysis

A completely randomised design was adopted in this study. Fifteen replicate fruits were used for the assessment of each biochemical parameter separately and repeated twice 2015 and 2016 seasons in order to confirm the observations. For each replicate fruit per parameter three sub samples (triplicate analysis) were used. Data were subjected to analysis of variance (ANOVA) using GenStat for the Windows (2004) statistical package (VSN) International, Hempstead, UK. Fisher's protected least significant difference at ($p<0.05$) level of significance was performed. Pearson's correlation coefficients were calculated to determine the strength of the linear relationships between antioxidant activity and the pyrogallol concentrations separately per Kei-apple accession.

3. Results and Discussion

Phenolic compounds: Pyrogallol, protocatechuic acid, ellargic acid, ferulic acid, syringic acid, *p* coumaric acid, vanillic acid, 3-methoxy-4-hydroxyphenyl propionic acid were identified and quantified in different Kei-apple accessions (Table 1). However, Caffeic acid (Loots et al., 2006) or chlorogenic acid (Minnaar, Jollya, Paulsen, Du Plessis, & Van Der Rijst, 2017) were not identified

in these accessions. Pyrogallol was identified as the predominant phenolic compound in Kei-apple and the concentration ranged between 2602.85 to 917.07 mg kg^{-1}. The pyrogallol concentration in Kei-apple accessions was significantly higher than the concentrations detected in the referral fruit, Top red apples (277.10 mg kg^{-1} FW). Accessions FH29 revealed the highest concentration of pyrogallol (Table 1) which is remarkably higher than the levels reported in avocados cv. Hass (453.1-475.9 mg kg^{-1} FW) at ready to eat stage ripeness (Glowacz, Bill, Tinyane, & Sivakumar, 2017) and in tomatoes (28 mg kg^{-1} FW) (Bao, Xiao, Zhu, Xin, & Zhang, 2015). Protocatechuic acid was not detected in FH151 and FH29. Similarly, ellagic acid was not present in FH204 and FH15 (Table 1). Accessions FH236 and FH232did not reveal the presence of syringic acid. Also, *p* coumaric acid was not found in the following accessions; FH236, FH232, FH204, and FH29. Vanilic acid was not detected in accessions, FH236, FH243, FH232, FH240, and FH239. Protocatechuic acid and pyrogallol are the predominant polyphenolic compounds in the Kei-apple accessions (Table 1, Fig 1). Kei-apple accessions, FH14, and FH243 showed higher protocatechuic acid and syringic acid respectively. Protocatechuic acid was reported in Kei-apple in the findings of Minnaar et al. (2017). Protocatechuic acid levels in FH14 is less than the levels observed in avocado cv. Hass (479.2 mg kg^{-1} FW) (Glowacz et al., 2017). However, Kei-apple accession FH14 showed higher concentrations of protocatechuic acid compared to the referral fruits [apples (cv. Top red), peaches (cv. Excellence), and blueberries (var. Oz Julieta)] in this study (Table 1). Although the syringic acid levels in FH243 almost falls within the levels reported in Williams' pears (*Pyrus communis* L.) (95.46–131.32 mg kg^{-1} FW) (Colaric et al., 2006), the levels noted in FH243 were higher than the levels found in the referral fruit peaches (cv. Excellence) (Table 1). The highest concentration of ellagic acid was obtained in Kei-apple accessions FH 151. Whilst . ellagic acid content in FH29 and FH239 were higher than the levels noted in other accessions. The concentration of ellagic acid in FH151 on fresh weight basis was similar to the levels noted in pears and tangerines on dry weight basis (Williner, Pirovani, & Güemes 2003) but lower than the levels reported in strawberry on fresh weight basis (122.5 mg kg^{-1} FW) (Milivojević et al., 2011). Ellagic

acid levels in FH 29 and FH 239 were higher than levels reported in bananas on dry weight basis (Williner et al., 2003). However, Kei-apple accessions FH240, FH239, FH151 and FH29 showed higher levels of ellagic acid compared to the referral fruits in this study [apples (cv. Top red), peaches (cv. Excellence), and blueberries (var. Oz Julieta)] in this study (Table 1). Gallic acid was not detected in all Kei-apple accessions in our study but it was detected at lower concentrations by Loots et al. (2006) in Kei-apple juice. Furthermore, pyrogallol, protocatechuic acid, ellagic acid, and syringic acid are of interest mainly for their nutritional and pharmacological potential as antioxidants, antibacterial, anticancer, antihyperlipidemic, antidiabetic, and anti-inflammatory agents. (Loots et al., 2006; de Beer, 2006; Bhattacherjee, & Datta, 2015). Results for the Kei-apple accession FH29 showed significantly higher levels ($p<0.05$) (492.45 mg 100 g^{-1} FW) of total phenolic content than the referral fruits blueberry (var. Oz Julieta) (293.38 mg 100 g^{-1}), apples cv. Top Red (130.18 mg 100 g^{-1}) peaches cv. Excellence (22.73 mg 100 g^{-1} FW) and the other Kei-apple accessions (Fig. 1). Kei-apple accessions FH240, FH232, FH151, FH15 and blueberries showed similar levels of total phenolic compounds but higher than that of the apples and peaches in this study (Fig. 1). The total phenolic content in Kei-apple was reported to be 225 mg 100 g^{-1} and 943 mg 100 g^{-1} on wet and dry weight basis respectively by de Beer (2006) using gallic acid as standard. In this study, the accession FH 29 revealed higher total phenolic content with pyrogallol as standard (Fig. 1). However, the total phenolic content of Kei-apple FH29 was higher than the reported blueberry cultivars Northblue, Duke, Bluetta, and Elliott which showed a total phenolic content of 400 - 401.6 mg 100 g^{-1} FW (Li et al., 2017; Gündüz, Serçe, & Hancock, 2015). The blueberry cultivar St. Cloud (Li et al., 2017) showed a lower concentration of total phenolic content (ca. 200 mg 100 g^{-1} FW), than all the Kei-apple accessions reported in this study where the total phenolic content ranged from 217.25 to 492.46 mg 100 g^{-1} FW. Blueberry cultivars Liberty (256.4 mg 100 g^{-1} FW) and Legacy (259.9 mg 100 g^{-1} FW) (Gündüz et al., 2015) exhibited more or less similar concentration of total phenolic content as the Kei-apple accession FH243 (258.05 mg 100 g^{-1} FW).

Overall all eleven accession of Kei-apple contained catechin concentration over 1000 mg kg^{-1} on fresh weight basis. Kei-apple accessions FH151, FH15, FH14, FH29, FH243, FH 239 and FH 231 revealed more or less 1750 mg of catechin kg^{-1} FW (Fig. 2A). But the referral fruit blue berry (var. Oz Julieta) showed five times higher concentrations of catechin compared to the above-mentioned accessions of Kei apples (Fig. 2A). Higher concentration of catechin in Kei-apple juice was reported in previous reports (Loots et al 2006). Quercetin is present in Kei-apple accessions; FH232, FH151, FH236, FH15, FH204, FH239, FH231 (Fig. 2B). The concentration of quercetin is lower than the concentration of catechin and it varied from 40 to 100 mg kg^{-1} FW. Accession FH151 showed the highest concentration of quercetin among the all the eleven accession of Kei apples in this study. Also, the quercetin concentration was significantly higher in FH151 than all the referral fruits (Fig. 2B).

β-carotene and anthocyanin: The β-carotene content of Kei-apple accessions FH14 (4.78 mg 100 g^{-1}) FH232 (4.51 mg 100 g^{-1} FW), FH239 (4.17 mg 100 g^{-1} FW) and FH236 (4.11 mg 100 g^{-1} FW), demonstrated higher levels than the other Kei-apple accessions and referral fruits, apples (cv. Top red), peaches (cv. Excellence) and blueberries (cv. Oz Julieta) (Fig. 3A). β- carotene levels in the Kei-apple accessions FH240 (3.78 mg 100 g^{-1} FW), FH204 (3.50 mg 100 g^{-1} FW), and FH231 (3.94 mg 100 g^{-1} FW), were similar to the levels obtained for cv. Top Red apples (Fig. 3A). The Kei-apple FH243 (1.41 mg 100 g^{-1} FW) showed the lowest level of β-carotene content among all the Kei-apple accessions investigated (Fig. 3A), which is similar to the levels reported in the yellow-fleshed peaches (cv. Excellence) and blueberries (cv. Oz Julieta). However, the β carotene content in peaches cv. Artic queen (10.4 mg 100 g^{-1} FW) and cv. Brite pearl (8 mg 100 g^{-1} FW) (Gill, Francisco, Tomas-Barberán, Hess-Piece, & Kader, 2002) was higher than the levels reported in all the Kei-apple accessions in this study. Apple cultivars Beni Shogun (0.32 mg 100 g^{-1}) and Gala (0.11 mg 100 g^{-1} FW) (Vieira et al., 2011) exhibited lower β carotene content than all the Kei-apple accessions. Blueberries were reported to contain < 0.1 mg kg^{-1} β carotene (Bouzari, Holstege, & Barrett, 2015).

The referral fruit blueberry was higher in anthocyanins, compared to all Kei-apple accessions including apple and peach fruit (Fig. 3B). According to the previous findings, Kei-apple juice was reported to contain 23.7 mg L^{-1} anthocyanin (Loots et al., 2006). The anthocyanin (cyanidin-3-glucoside) content was highest in FH204 (90.84 mg kg^{-1} FW) and FH29 (83.89 mg kg^{-1} FW) (Fig. 3B). However, the anthocyanin concentration in other Kei-apple accessions varied between 40.21 and 60.23 mg kg^{-1} FW) (Fig. 3B). Kei- apple accessions FH236, FH14, FH243, FH232, FH204, FH240, FH239, FH151, FH15, FH231 and FH29 showed similar concentration of anthocyanin as reported in the referral fruit (cv. Excellence) peaches in this study (Fig. 3B).

Antioxidant capacity and activity: Due to the complex nature of phytochemicals and involvement of multiple reaction mechanisms, performing different antioxidant assays can offer more accurate information on the antioxidant properties of fruits (Silva, & Sirasa, 2018). The total antioxidant capacities determined using FRAP and DPPH assays for different Kei-apple accessions are given in Fig. 3C and Fig. 3D. FRAP assay is common method performed to determine the antioxidant capacity of fruits and vegetables (Silva, & Sirasa, 2018). According to this method the Kei-apple accessions FH29, FH14, FH204, FH240, FH239, FH151, FH15, FH231 and FH243 showed higher antioxidant capacity than the referral fruits apple (cv. Top red), and peaches (cv. Excellence) (Fig. 3C). Based on the FRAP method Kei-apple accessions FH29, and FH236 showed the highest and the lowest antioxidant capacities respectively compared to the other accessions (Fig. 3C). However, the observed antioxidant capacity of FH29 was lower than the value noted in the referral fruit blueberry (var. Oz Julieta).

The DPPH radical scavenging assay is a popular method to demonstrate the ability of antioxidant to scavenge free radicals and the degree of discoloration demonstrates the scavenging potentials of the antioxidant extract (Sowndhararajan & Kang 2013). During this assay the lower value of IC 50 indicates the higher antioxidant activity (Sowndhararajan & Kang 2013). Therefore, Kei apple accessions, FH236, FH232, FH15 and FH243 showed higher antioxidant activity than the accessions, FH29, FH151, FH239, FH240, FH204, and FH14 (Fig. 3D). Also the Kei-apple

accession FH29 (0.62 IC 50 values μg mL^{-1}) by showing a lower IC_{50} value demonstrated higher antioxidant activity than the referral fruit blueberry (var. Oz Julieta). (Fig. 3D). A strong positive correlation ($r = 0.87$, $P < 0.01$) exists between the pyrogallol concentration and the antioxidant capacity (FRAP assay) in this study. This observation confirms the active contribution of the pyrogallol in increasing the antioxidant capacity in accession FH29. Preliminary studies indicated that the aantioxidant assays such as FRAP and ORAC (Oxygen Radical Absorbance Capacity) can be used as predictors to measure the antioxidant activity in Kei- apples (Loots et al., 2006). Generally, consumption of fruit is encouraged owing to the high antioxidant properties. Thus, the quantification and comparison of the antioxidant properties of indigenous fruit Kei-apple accessions with the commonly consumed commercial fruits indicate that it is important to include these fruits in the South African food composition database.

Glucose and fructose composition: The glucose and fructose content in the Kei-apple accessions are given in Fig. 4. Kei-apple selection FH236 (15.06 mg g^{-1} FW), FH240 (14.74 mg g^{-1} FW) and FH239 (13.21 mg g^{-1} FW) showed the higher concentration of glucose content whilst accessions FH14 (1.21 mg g^{-1} FW) and FH232 (2.69 mg g^{-1} FW) showed lower glucose concentrations (Fig. 4). Italian Apple cv. Braeburn showed similar levels of glucose content as the Kei-apple accessions, FH236 FH239, FH29, and FH240 with values between 11.8 mg g^{-1} FW to 15.6 mg g^{-1} FW (Baiamonte et al., 2016). Kei-apple s accessions FH236, FH240, FH239, FH29 and FH204 showed much higher glucose content than the non-melting peach (*Prunus persica* (L). Batsch) progeny which contains between 3.8 and 9.6 mg g^{-1} FW (Abidi et al., 2015). However, the glucose concentrations of accessions FH243 and FH15 falls within the range reported in the non-melting peach (Batsch) progeny (Abidi et al., 2015).

Fructose content was highest in the Kei-apple accession FH240 (35.28 mg g^{-1} FW) (Fig. 4). Accessions FH29 (28.32 mg g^{-1} FW), FH204 (26.60 mg g^{-1} FW), and FH243 (23.27 mg g^{-1} FW) showed a higher fructose concentration than FH231 (12.63 mg g^{-1} FW), FH15 (13.06 mg g^{-1} FW),

FH151 (11.12 mg g^{-1} FW), FH239 (12.47 mg g^{-1} FW), and FH14 (13.88 mg g^{-1} FW) (Fig. 4). Blueberry cultivars Bluecup and Reka (30 mg g^{-1} FW) showed more or less similar fructose concentration as accessions FH240 (Forney, Kalt, Jordan, & Vinqvist-Tymchuk 2012). Fructose concentration in Kei-apple accessions FH14 (13.88 mg g^{-1} FW), FH15 (13.06 mg g^{-1} FW), FH231 (12.62 mg g^{-1} FW), and FH151 (11.12 mg g^{-1} FW) (Fig. 4), were higher than the levels reported for the non-melting peach (*Prunus persica* (L). Batsch) progeny (4.5 and 10.8 mg g^{-1} FW) (Abidi et al., 2015). However, the fructose concentration in FH240 was lower than the levels reported in Apple cv. Braeburn which showed concentrations ranging from 53 to 58 mg g^{-1} FW (Abidi et al., 2015). The Kei-apple accession FH240 showed the highest total sugar content of 50.07 mg g^{-1} FW, followed by accessions FH29 (41.26 mg g^{-1} FW) and FH204 (37.31 mg g^{-1} FW) (Fig. 4). However, accession FH232 showed significantly lower total sugar content than all the other Kei-apple accessions included in this study (Fig. 4). The accession FH240 showed a moderately lower total phenolic content and antioxidant capacity than the FH29.

Amino acids; The total free amino acids in the eleven accessions of Kei-apple was identified and quantified using UPLC analysis. Twenty amino acids [Histidine (His), Serine (Ser), Arginine (Arg), Glycine (Gly), Aspartate (Asp), Glutamate (Glu), Threonine (Thr), Alanine (Ala), Proline (Pro), Cysteine (Cys), Lysine (Lys), Tyrosine (Tyr), Valine (Val) Isoleucine (Ile), Leucine (Leu), Phenylalanine (Phe), Asparagine (Asn), Glutamine (Gln), gamma-aminobutyric acid (GABA) and *Ornithine* (Orn) (non-proteinogenic amino acid)] were found in Kei-apple accessions FH236, FH15, FH243, FH232, FH204, FH239, FH151, FH15 and FH29 (Fig 5 & Fig 6A-C). Histidine was not detected in accessions FH239 and FH231. The concentration of phenylalanine in FH236 (27.79 mg L^{-1}) (Fig. 5) were higher than the concentrations reported in citrus Valencia (17.7 mg L^{-1}) [Valencia Late (*Citrus sinensis* (L.) Osb] (Cerdán-Calero, Sendra, & Sentandreu, 2012). Kei-apple accession FH 236 (23.17 mg L^{-1}) showed a higher concentration of leucine than the citrus Valencia Late (5.8 mg L^{-1}) (Cerdán-Calero et al., 2012) but slightly lower than the tomatoes (Cal Ace) (30 mg L^{-1}) (Fig. 5). Based on the amino acid analysis, it can be concluded that accession FH236 can be

regarded as a good source of essential amino acids (Fig. 5). All eleven Kei-apple accessions contained asparagine and gamma-aminobutyric acid (Fig. 6A). However, accession FH239 (3122. 18 mg L^{-1}) showed the highest concentration of gamma-aminobutyric acid followed by FH243 (654.65 mg L^{-1}) (Fig. 6A). All the Kei-apple accessions showed higher asparagine concentration than those reported for tomato (Cal Ace) which contained approximately 101.00 mg L^{-1} (Kader, Stevens, Albright, & Morris, 1978). Gamma-aminobutyric acid concentration in FH243 (654.65 mg L^{-1}) (Fig. 6A) is approximately closer to the levels present in tomatoes (710 mg L^{-1}) (Cal Ace) at the table ripe stage (Kader, 1978). Kei-apple accessions FH243, FH232, FH151, FH15 and FH29 showed higher concentrations of alanine ranging from 117 to 110 mg L^{-1} (Fig. 6B). The amount of alanine present in these accessions is similar to the levels found in tomatoes (Cal Ace) (118 mg L^{-1}) at the table ripe stage (Cerdán-Calero et al., 2012), but higher than the amount reported in citrus Valencia Late (87.3 mg L^{-1}). Although proline was not reported in tomato (Cal Ace) at table ripe stage (Kader, 1978), accessions FH 236 and FH 15 showed 120.69 mg L^{-1} and 139.87 mg L^{-1} of proline respectively at harvest maturity (Fig. 6B). Concentration of glutamine in FH243 (75.20 mg L^{-1}) and FH239 (68.29 mg L^{-1}) (Fig. 6B), are lower than the concentrations reported in tomato (Cal Ace) (624 mg L^{-1}) (Kader, 1978). Accession FH15 showed the highest concentration (159. 89 mg L^{-1}) of glutamate (Fig. 6B). However, the glutamate concentration in accession FH15 is much lower than the concentrations reported in tomatoes (Cal Ace) (Kader, 1978). Concentration of non-essential amino acid aspartate was highest in FH243 (125.74 mg L^{-1}) (Fig. 6B), although the amount presented here is lower than that which has been reported for tomatoes (434 mg L^{-1}) (Kader, 1978). Also, serine was highest in selection FH243 (76.79 mg L^{-1}) (Fig. 6B); this concentration was lower than the concentrations reported for tomatoes (434 mg L^{-1}) and citrus Valencia Late (104.6 mg L^{-1}). Furthermore, arginine (8.16 to 3-01 mg L^{-1}), glycine (12.67 to 3.47 mg L^{-1}) cysteine (0.77 to 0.24 mg L^{-1}), lysine (4.16 to 1.37 mg L^{-1}) and ornithine (0.6-0.22 mg L^{-1}) were also detected in all Kei-apple accessions (Fig. 6C). Kei-apple accessions, FH240 and FH232 showed higher concentrations of glycine and, arginine compared to the other accessions (Fig. 6C).

Although literature-based information on amino acids in commonly consumed fruits is available, it is difficult to compare the findings due to the differences in the analytical methods; the units and some of the results are presented on a dry rather than fresh weight basis.

Conclusion

To our knowledge, this is the first report on the phenolic compounds, antioxidant properties, sugar components and the amino acid profile of Kei-apple accessions from the Southern African region. Thus, the quantification and comparison of the antioxidant properties of indigenous fruit Kei- apple accessions with the commonly consumed commercial fruits indicate that it is important for food supplementation or diet diversification and is useful to promote the cultivation of suitable Kei-apple accessions as a source of phytochemicals and amino acids for supplement to diet and potential for added value. Based on this study daily consumption of Kei-apple accession FH236 needs to be encouraged to supplement the diet. Non-essential amino acids serine, aspartate, glutamate, alanine, proline, asparagine and glutamine as well as gamma-aminobutyric acid possess numerous health benefits and Kei-apple accession that are rich in these compounds can offer an ideal dietary supplementation for those who are vegetarian. Also due to the higher total sugar content and moderate levels of total phenols, accession FH240 can be well suited for consumption or for value-added product development.

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Fig. 1 Total phenol content in eleven Kei-apple accessions in comparison to the referral fruits.

Means values of each bar were calculated based on 15 samples per Kei-apple accession. Those followed by a different alphabet letter in a bar (biochemical parameter) were significantly different (at $p < 0.05$) according to the Fisher's protected least significant test

Fig. 2. (A) Catechin and (B) Quercetin content in eleven Kei-apple accessions in comparison to the referral fruits

Means values of each bar were calculated based on 15 samples per Kei-apple accession. Those followed by a different alphabet letter in a bar (biochemical parameter) were significantly different (at $p < 0.05$) according to the Fisher's protected least significant test. FW- Fresh weight

Fig. 3 (A) β-carotene, (B) anthocyanin content and (C) antioxidant capacity and (D) antioxidant activity in eleven Kei-apple accessions in comparison to the referral fruits

Means values of each bar were calculated based on 15 samples per Kei-apple accession. Those followed by a different alphabet letter in a bar (biochemical parameter) were significantly different (at $p < 0.05$) according to the Fisher's protected least significant test. FW-Fresh weight

Fig. 4 Glucose, fructose and total sugar composition in eleven Kei-apple accessions.

Means values of each bar were calculated based on 15 samples per Kei-apple accession. Those followed by a different alphabet letter in a bar (sugar component) were significantly different (at $p < 0.05$) according to the Fisher's protected least significant test. FW- Fresh weight

Fig. 5 Composition of essential amino acids in eleven Kei apples accessions

Means values of each bar were calculated based on 15 samples per Kei-apple accession. Those followed by a different alphabet letter in a bar (specific amino acid) were significantly different (at $p < 0.05$) according to the Fisher's protected least significant test

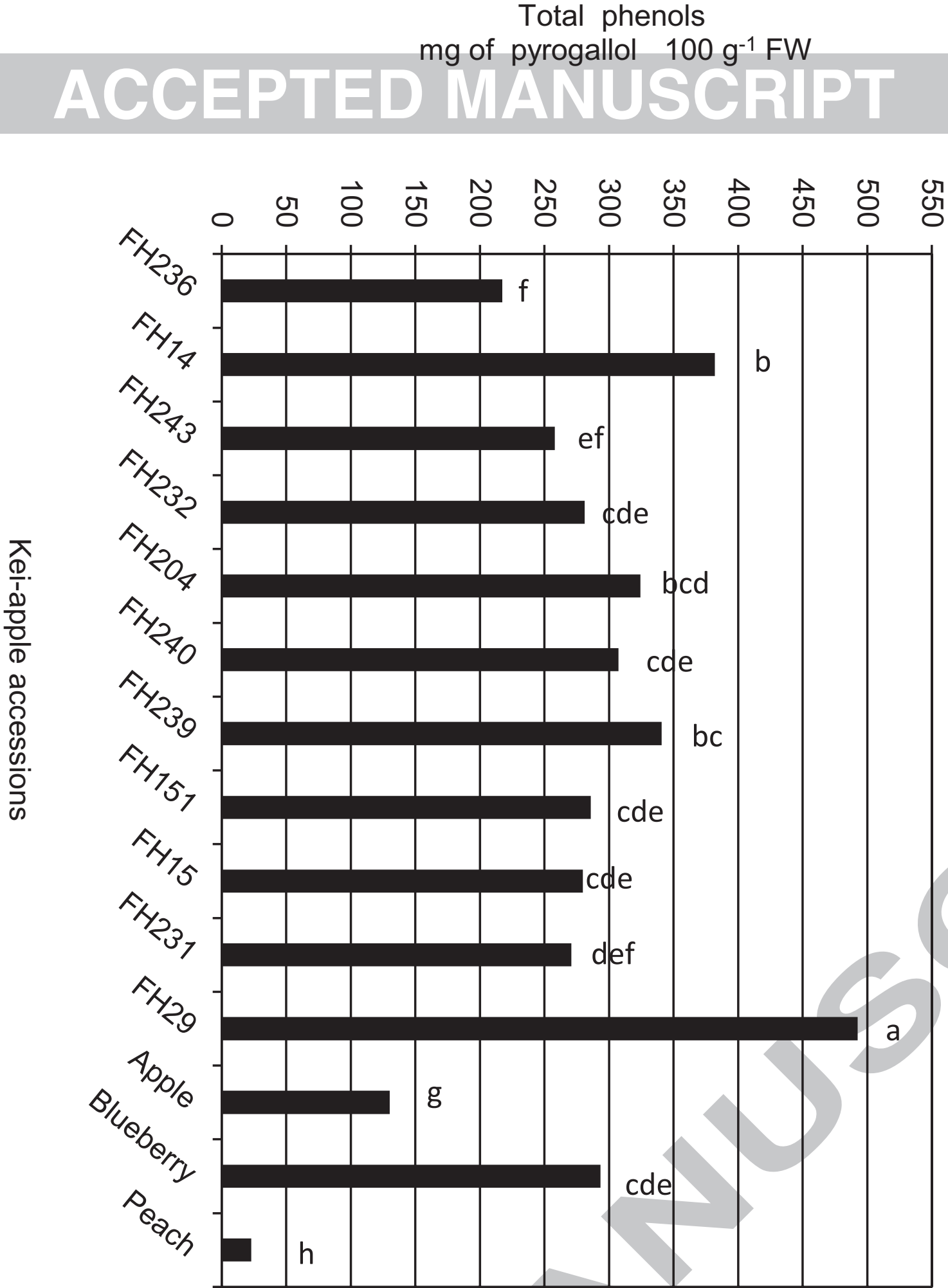
Fig 6 Non-essential amino acids in eleven Kei-apple accessions A). major non-essential amino acids B) moderately available non-essential amino acids C) non-essential amino acids that are present in lower concentrations

Means values of each bar were calculated based on 15 samples per Kei-apple accession. Those followed by a different alphabet letter in a bar (specific amino acid) were significantly different (at $p < 0.05$) according to the Fisher's protected least significant test

Table 1. Pyrogallol content and phenolic acid concentrations in Kei apple accessions

Kei apple accessions	Phenolic compounds (mg kg^{-1})								
	Pyrogallol	Protocatechuic acid	Ellagic acid	3-Methoxy-4-hydroxyphenylacetic acid	p-Coumaric acid	Syringic acid	Vanillic acid	Ferulic acid	p-Hydroxyphenylacetic acid
FH236	1659.55c	119.81b	6.68d	1.09cd	nd	nd	nd	5.54c	nd
FH14	1939.48b	227.58a	4.85d	1.39c	9.03c	72.28b	15.94b	nd	11.36b
FH243	1689.55c	97.66bc	4.43d	3.01b	2.23de	111.05a	nd	6.74c	7.34b
FH232	1257.52d	87.53c	3.90d	nd	nd	nd	nd	9.30b	6.38bc
FH204	1195.03d	32.83e	nd	nd	nd	69.70bc	11.91cd	12.28a	9.37b
FH240	917.07e	32.83e	28.44c	0.32d	1.34e	34.37e	nd	4.20d	4.66c
FH239	1755.91c	67.27d	32.52bc	2.13bc	2.43d	39.28de	nd	6.57c	12.18b
FH151	1817.03c	nd	42.71a	10.02a	3.61d	62.72c	6.69e	5.95c	17.31a
FH15	1991.93b	64.96d	nd	nd	11.62ab	68.25bc	9.93de	11.05a	10.42b
FH231	1199.29d	81.77c	4.56d	nd	8.31c	40.21d	12.2bc	8.34b	6.21c
FH29	2602.85a	nd	38.77b	nd	nd	47.58d	12.80bc	9.01b	9.53b
Referral fruits									
Apple	277.1f	36.71e	3.56d	nd	11.50b	nd	9.09de	1.89e	nd
Blueberry	nd	nd	6.71d	1.49c	12.42a	nd	32.89a	4.22d	nd
Peaches	nd	6.20f	5.04d	0.63d	3.34d	14.81f	nd	2.22e	0.075d

Means values of each column were calculated based on 15 samples per Kei apple accession on fresh weight basis. Those followed by a different alphabet letter in a column (chemical parameter) were significantly different (at $p < 0.05$) according to the Fisher's protected least significant test. nd-not detected



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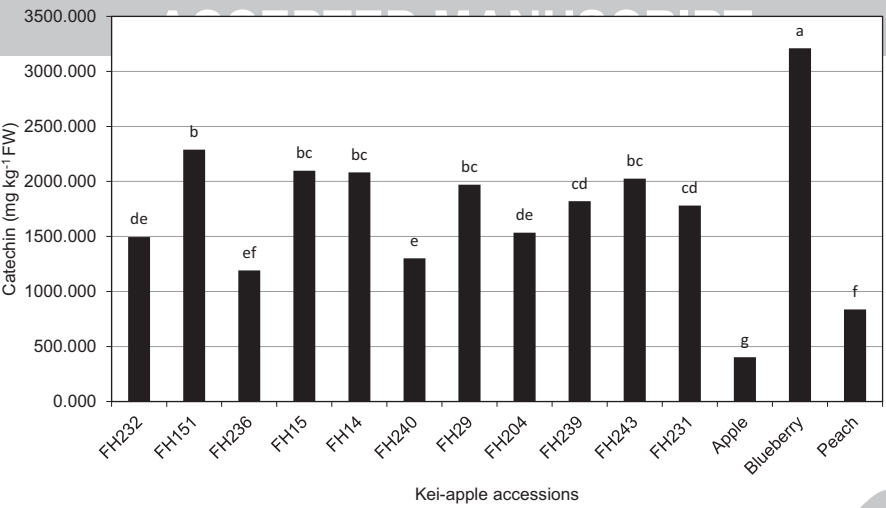

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