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Mango rejects and mango waste: Characterization and quantification of phenolic compounds and their antioxidant potential

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Abstract

The high demand and production rate of Mango (*Mangifera indica* L.) inevitably lead to a significant wastage of excess produce. The current research aims to screen and characterize phenolic compounds and estimate their antioxidant potential in rejected mangoes. It was found that Honey Gold variety possessed the highest Total Phenolic Content (TPC) (2.37 ± 0.06 mg GAE/g) and antioxidant capacity through 2,2′-diphenyl-2-picryl-hydrazyl (DPPH) (2.13 ± 0.09 mg AAE/g) assay. The LC-ESI-QTOF-MS/MS characterized a total of 86 phenolic compounds in different mango varieties including Kensington Pride (31), Keitt (30), Honey Gold (29), Calypso (28), and Palmer (14). In high-performance liquid chromatography-photo diode array (HPLC-PDA) quantification, the noteworthy levels of chlorogenic acid, caffeic acid, gallic acid, quercetin, and kaempferol were found in all five samples. The significant abundance of phenolics and its corresponding antioxidant capacity indicate the potential of rejected mango in food, pharmaceuticals, nutraceuticals, and feed industries.

Novelty impact statement: Liquid chromatography coupled mass spectrometry and high-performance liquid chromatography-photometric diode array analysis allows researchers to establish the various chemical profiles exhibited by different foods. Our present study focused on applying such techniques to tentatively identify, characterize, and quantify the phenolic compounds present in waste mango pulp. With the identification of such beneficial compounds, future studies can focus on developing innovative functional foods, food bioactives, pharmaceuticals, and nutraceuticals on a commercial scale. More importantly, such studies attempt to dissolve the growing concerns about food waste by effectively repurposing rejected foods.

1 | INTRODUCTION

Mango (*Mangifera indica* L.) belongs to the family of Anacardiaceae and is mainly grown in tropical regions but consumed abundantly worldwide. Although consumed fresh, approximately 20% of mangoes are chemically and physically preserved through innovative processing technologies such as high-pressure processing (HPP) and pulsed electric field processing, to manufacture mango products such as beverages, jams, jellies, and pickles (Peng et al., 2019). With worldwide production volumes reaching approximately 39.1 million tonnes in 2018, successive rises in demand for the fruit are expected (Altendorf, 2019). As a consequence of the high production rate of mango, a significant amount of wastage is inevitably produced along the food supply chain. In developed countries, food waste

is generally linked to the consumer's perception of product quality (Nicolae & Corina, 2011), one being the complete rejection of fruits that are perfectly nutritious but deemed esthetically unpleasing for retail. It has been well documented that mangoes provide an excellent source of carbohydrates, dietary fiber, proteins, and phytochemicals (Lauricella et al., 2017).

Phytochemicals including phenolic compounds of mangoes have been of particular interest to scientists mainly due to the recent surge in epidemiological literature, establishing a link between the consumption of a diet rich in natural antioxidants and decreased risk of oxidative stress associated diseases (Hatamnia et al., 2014). A significant amount of research has already proposed the high content of phenolic compounds present in mangoes and their health benefits, particularly in managing oxidative stress and degenerative diseases through the modulation of singling networks in the body (Arbizu-Berrocal et al., 2019; Saha et al., 2016). Phenolic compounds can be extracted and studied using a variety of organic solvents. Antioxidant capacity is frequently analyzed through spectrophotometric methods involving different chemical assays. Ferric reducing antioxidant power (FRAP), 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 2,2′-diphenyl-2-picryl-hydrazyl (DPPH), reducing power assay (RPA), hydroxyl radical scavenging activity ($^{\bullet}$OH-RSA), ferrous ion chelating activity (FICA), and total antioxidant capacity (TAC) methods are different assays which help in estimating the phenolic and antioxidant activity of a sample, which can be further evaluated to test its response against free radicals produced by certain diseases (Rubio et al., 2016). Moreover, recent technology such as liquid chromatography coupled with electrospray-ionization triple quadrupole time-of-flight mass spectrometry (LC-ESI-QTOF-MS) and high-performance liquid chromatography-photo diode array (HPLC-PDA) detector allow the structural characterization quantification of these phenolic compounds.

Phenolic compounds in mango by-products and mango flesh have been previously identified (Gu et al., 2019; Peng et al., 2019). However, information regarding the phenolic content in different varieties of mango flesh is still limited, especially in Australian grown varieties. Moreover, questions regarding the difference in phenolic content between imperfect or rejected and retail mango flesh are yet to be answered. Identification of health-promoting compounds from the imperfect and rejected flesh will provide further information in developing innovative functional foods, nutraceuticals, and pharmaceuticals on a commercial scale. The search for such methods aims to repurpose rejected foods in an attempt to dissolve the growing concerns presented by food waste while improving market development.

Therefore, this research study aims to determine the composition of phenolic compounds and the antioxidant capacity of rejected mango flesh. Moreover, the study will utilize five different varieties of mangoes including Calypso, Kensington Pride, Keitt, Palmer, and Honey Gold, which will be investigated through phenolic compound estimation—Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and Total Tannins Content (TTC) and various modified methods of antioxidant assays, including DPPH, FRAP, ABTS, RPA, $^{\bullet}$OH-RSA, FICA, and TAC. Furthermore, the samples will be subjected to characterization and quantification using LC-ESI-QTOF-MS/MS and HPLC-PDA, respectively. The purpose of the results is to provide sufficient evidence to facilitate further research on the potential application of mango phenolic compounds commercially.

2 | MATERIALS AND METHODS

2.1 | Chemical and reagents

The chemicals used for extraction and characterization were of analytical grade and were purchased from Thermo Fisher Scientific Inc. (Waltham, MA, USA), Chem-Supply Pty Ltd. (Adelaide, SA, Australia), RCI Labscan (Rongmuang, Thailand), Sigma-Aldrich (Castle Hill, NSW, Australia), Sigma-Aldrich (St. Louis, MO, USA). For the extraction of polyphenols, analytical grade hydrated sodium acetate, methanol, ethanol, hydrochloric acid, anhydrous sodium acetate, glacial acetic acid, and formic acid were purchased from Thermo Fisher Scientific Inc. (Waltham, MA, USA). Anhydrous sodium acetate was supplied by Chem-Supply Pty Ltd (Adelaide, SA, Australia). Furthermore, RCI Labscan (Rongmuang, Thailand) provided the 98% sulfuric acid. The chemicals required for the assays consisted of Folin and Ciocalteu's phenol reagent, gallic acid, quercetin, 2,4,6-tripyridyl-*s*-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH), L-ascorbic acid, vanillin, hexahydrate aluminum chloride, ferric chloride, 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS), ferric chloride ($Fe[III]Cl_3.6H_2O$), sodium carbonate anhydrous, sodium acetate hydrated, potassium ferricyanide $K_3[Fe(CN)_6]$, trichloroacetic acid (TCA), sodium phosphate buffer, hydrogen peroxide (H_2O_2), 3-hydroxybenzoic acid, ferrous chloride, ferrozine, and ethylenediaminetetraacetic acid (EDTA), which were all procured from Sigma-Aldrich (Castle Hill, NSW, Australia). Reference standards for HPLC were used to perform the chromatographic analysis. The chemicals included caffeic acid, chlorogenic acid, gallic acid, *p*-hydroxybenzoic acid, protocatechuic acid, syringic acid, catechin, epicatechin gallate, kaempferol, kaempferol-3-*O*-glucoside, quercetin, quercetin-3-*O*-galactoside, and quercetin-3-glucuronide, which were purchased from Sigma-Aldrich (St. Louis, MO, USA). To perform the antioxidant assays, 96 well plates were purchased from Thermo Fisher Scientific (VIC, Australia). Additionally, HPLC vials (1.5 ml) were purchased from Agilent technologies (VIC, Australia).

2.2 | Sample preparation

Five different varieties of "unwanted" mangoes—Calypso, Kensington Pride, Keitt, Honey Gold, and Palmer, were purchased from a local produce market in Melbourne, Australia (Figure 1). About 2–3 kg of each variety of rejected mangoes was purchased; peels and seeds were discarded. The mango flesh was cut into small pieces and blended with a 1.5-L blender (Russell Hobbs Classic, model DZ-1613, Melbourne, VIC, Australia). The pulp was

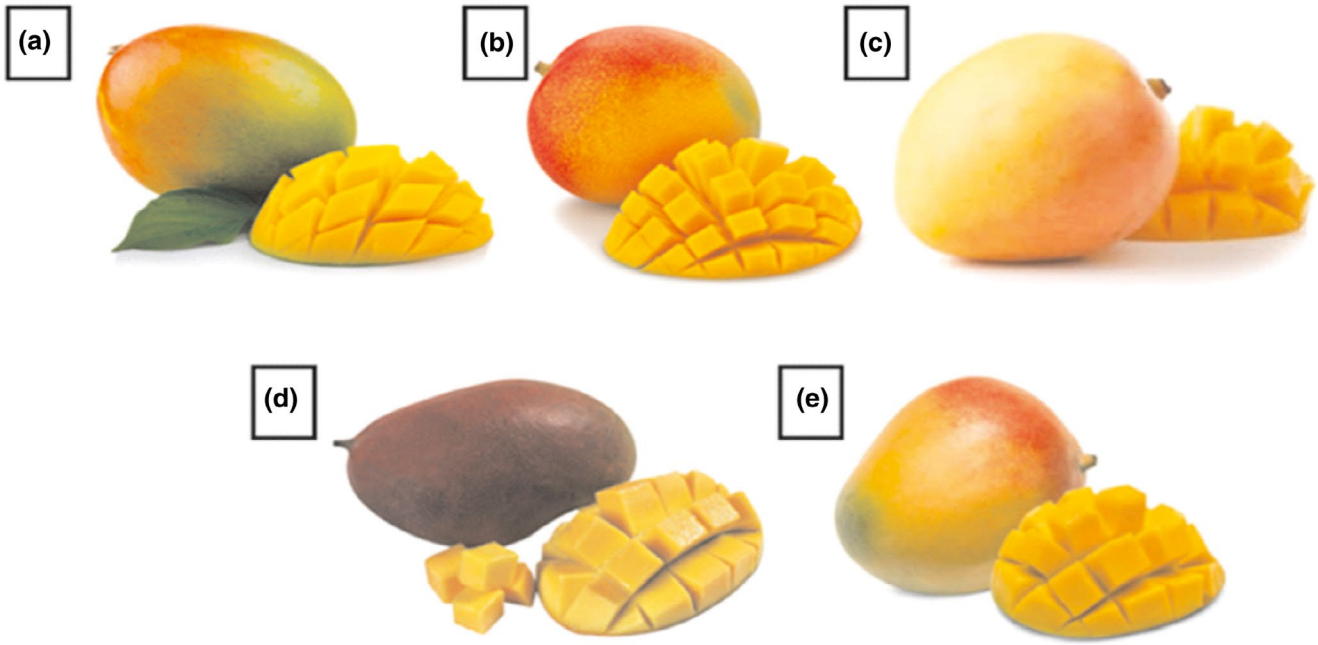

FIGURE 1 Five different varieties of Australian grown mango. (a)—Keitt; (b)—Kensington Pride; (c)—Honey; Gold; (d)—Palmer; (e)—Calypso

stored in a flat vacuum sealed aluminum foil Ziplock bag (Best supply, NSW, AU) and was kept at −20°C for 2–3 weeks for further analysis.

2.3 | Extraction of phenolic compounds

To extract the phenolic compounds, 5.0 ± 0.5 g of pulp from each mango was individually mixed with 20-ml 70% ethanol. The samples were homogenized using the IKA Ultra-Turrax T25 homogenizer (Rawang, Selangor, Malaysia). Upon homogenization, the mixture was subjected to partial concentration and incubation overnight using a shaking incubator (ZWYR-240, Labwit, Ashwood, VIC, Australia), which was set at 120 rpm 4°C. The mango pulp extracts were centrifuged for 15 min, 4°C at 24,400*g* using a benchtop centrifuge (Hettich Rotina 380R, Tuttlingen, Germany) (Subbiah et al., 2021). The filtrate was extracted and stored at −20°C. For the preparation of HPLC and LC-MS/MS analysis, the extracts were further subjected to another round of filtration using a micro-membrane (0.45 μm) syringe filter (Thermo Fisher Scientific Inc., Waltham, MA, USA).

2.4 | Total phenolic estimation and antioxidant potential

The total phenolic estimation and antioxidant assays were performed into 96-well plates based on the methods performed by Suleria et al. (2020) and Tang et al. (2020). The standard curves are created with $R^2 > 0.995$.

2.4.1 | Determination of TPC

The TPC of the extracts was measured adopting the Folin-Ciocalteu Method (Ali et al., 2021) with adjustments. To a 96-well plate (Thermo Fisher Scientific, VIC, Australia), 25 μl of sample extract is added, along with 25 μl of Folin–Ciocalteu's phenol reagent, which was diluted three times with water (1:3). Additionally, 200 μl of water was added, and the mixture was allowed to incubate for 5 min at room temperature. Upon incubation, 25 μl 10% (w:w) sodium carbonate was added, and the mixture was further incubated for 60 min at 25°C. The absorbance was measured at 765 nm using a microplate reader (Multiskan™ FC Microplate Photometer, Thermo Scientific, Waltham, Massachusetts, USA). A standard curve was constructed using concentrations of 0–200 μg/ml gallic acid, which was serially diluted with 70% ethanol. The results were expressed as mg gallic acid equivalents (GAE) per g of fresh sample weight (f.w) ± standard deviation (*SD*).

2.4.2 | Determination of TFC

Colourimetric-based methods were used to estimate the TFC, which were initially produced by Christ and Müller (1960). For our experiment, the TFC of the extracts was measured and modified to meet Horszwald et al. (2013) and Gu et al. (2019)'s specifications, which used sodium acetate as the medium and quercetin as the standard compound. Summarily, into a 96-well plate, 80 μl of sample extract was combined with 80 μl of 2% aluminum chloride (AlCl3) dissolved in ethanol and 50 g/L sodium acetate solution. The mixture was allowed to incubate at 25°C for 2.5 hr. Following incubation, the

absorbance was measured at 440 nm. The results were expressed as mg quercetin equivalents (QE) per g of fresh sample weight (f.w) ± standard deviation (*SD*).

2.4.3 | Determination of TTC

The total condensed tannin content of the extracts was measured adopting and modifying methods produced by Price et al. (1978). For this, 150-µl 4% vanillin solution and 25-µl sulfuric acid were diluted with ethanol and then added to 25 µl of sample extract. The mixture was allowed to incubate for 15 min at 25°C, followed by absorbance reading at 500 nm. A standard curve was constructed using concentrations of 0–1,000 µg/ml catechin, which was serially diluted with 70% ethanol. The results were expressed as mg catechin equivalents (CE) per g of fresh sample weight (f.w) ± standard deviation (*SD*).

2.4.4 | DPPH antioxidant assay

The radical scavenging ability of DPPH assay based on the method provided (Mensor et al., 2001) was adopted and modified to produce this experiment. Into a 96-well plate, 40-µl sample extract and 260 µl of 0.1 M DPPH radical methanol solution was added and incubated for 30 min at 25°C. The absorbance was measured at 517 nm using a microplate reader. A standard curve was generated using 0–50 µg/ml ascorbic acid aqueous solution. The results were expressed as mg ascorbic acid equivalents (AAE) per g of fresh sample weight (f.w) ± standard deviation (*SD*).

2.4.5 | FRAP assay

The FRAP assay was conducted to evaluate the reducing ability of the sample extracts based on the methods described by Benzie and Strain (1996). To prepare the FRAP reagent, a stock solution of 300 mmol/L sodium acetate buffer was added to 10 mmol/L 2,4,6, -tripytidyl-*s*-triazine (TPTZ) solution, and 20-mM ferric chloride in a ratio of 10:1:1 (v/v/v). The sample extracts (20 µl) and the freshly prepared FRAP reagent (280 µl) were mixed in a 96-well plate and allowed to incubate for 10 min at 37°C. Upon incubation, the absorbance was measured at 593 nm. A standard curve was achieved using concentrations of 0–50 µg/ml ascorbic acid. The results were expressed as mg AAE per g of fresh sample weight (f.w) ± standard deviation (*SD*).

2.4.6 | 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay

The ABTS antioxidant assay conducted acted in accordance with the procedure expressed by Re et al. (1999) with alterations, similar to the methods produced by Gu et al. (2019). First, a stock solution of $ABTS^{+}$ was prepared with 5 ml of 7 mmol/L of ABTS solution mixed with 88 µl of 140-mM potassium persulfate solution. It was important that this mixture was placed in the dark at room temperature for 16 hr. Ethanol (45 ml) was added to the $ABTS^{+}$ solution (0.5 ml) to dilute the solution to ensure a stable standard absorbance reading of 0.7. The absorbance was measured at 734 nm. The sample extracts (10 µl) and prepared ABTS+solution (290 µl) were allowed to incubate in a 96-well plate for 6 min at 25°C. Subsequently, the absorbance was analyzed at 734 nm. A standard curve was achieved using concentrations of 0–150 µg/ml ascorbic acid. The results were expressed as mg AAE per g of fresh sample weight (f.w) ± standard deviation (*SD*).

2.4.7 | Reducing power assay (RPA)

The reducing power activity was determined by modifying the method of Ferreira et al. (2007). About 10 µl of extract, 25 µl of 0.2-M sodium phosphate buffer (pH 6.6) and 25 µl of $K_3[Fe(CN)_6]$ were added sequentially followed by incubation at 25°C for 20 min. Then, 25°C of 10% TCA solution was added to stop the reaction, followed by the addition of 85 µl of water and 8.5 µl of $FeCl_3$. The solution was further incubated for 15 min at 25°C. Then the absorbance was measured at 750 nm. Ascorbic acid from 0 to 500 µg/ml was used to obtain a standard curve, and data were presented as mg AAE per g of fresh sample weight (f.w) ± standard deviation (*SD*).

2.4.8 | Hydroxyl radical scavenging activity ($^{\bullet}$OH-RSA)

The Fenton-type reaction method of Smirnoff and Cumbes (1989) was used to determine $^{\bullet}$OH-RSA with some modifications. About 50-µl extract was mixed with 50 µl of 6-mM $FeSO_4.7H_2O$, and 50 µl of 6-mM H_2O_2 (30%), followed by incubation at 25°C for 10 min. After incubation, 50 µl of 6-mM 3-hydroxybenzoic acid was added and absorbance was measured at a wavelength of 510 nm. Ascorbic acid from 0 to 300 µg/ml was used to obtain a standard curve, and data were expressed as AAE per g of fresh sample weight (f.w) ± standard deviation (*SD*).

2.4.9 | Ferrous ion chelating activity (FICA)

The Fe^{2+} chelating activity of the sample was measured according to Dinis et al. (1994), with modifications. About 15-µl extract was mixed with 85 µl of water, 50 µl of 2-mM ferrous chloride (with additional 1:15 dilution in water) and 50 µl of 5-mM ferrozine (with additional 1:6 dilution in water), followed by incubation at 25°C for 10 min. Then the absorbance was measured at a wavelength of 562 nm. Ethylenediaminetetraacetic acid (EDTA) from concentrations of 0 to 50 µg/ml was used to obtain a standard curve and data was presented as mg EDTA/g f.w.

2.4.10 | Determination of TAC

The TAC of the extracts were measured adopting the protocol of (Wang et al., 2021). Briefly, 40-µl extract was added to 260 µl of phosphomolybdate reagent (0.6 M H_2SO_4, 0.028 M sodium phosphate, 0.004 M ammonium molybdate). The mixture was allowed to incubate for 10 min at 95°C. The mixture was then cooled at room temperature. Upon cooling, the absorbance was measured at 695 nm. A standard curve was generated using concentrations of 0–200 µg/ml ascorbic acid. The results were expressed as mg AAE per g of fresh sample weight (f.w) ± standard deviation (*SD*).

2.5 | Characterization of phenolic compounds using LC-ESI-QTOF-MS/MS analysis

The phenolic compound characterization was performed on Agilent 1200 series HPLC (Agilent Technologies, Santa Clara, CA, USA) along with an Agilent 6520 Accurate- Mass Q-TOF LC/MS (Agilent Technologies, Santa Clara, CA, USA). The separation was conducted using a Synergi Hydro-RP 80A, LC column (250 mm × 4.6 internal diameter, 4-µm particle size) (Phenomenex, Lane Cove, NSW, Australia) at a column temperature of 25°C and sample temperature of 10°C. As previously described and demonstrated by Zhong et al. (2020). The mobile phase was compromised of mobile phases A and B, which are aqueous acetic acid (98:2, v/v) and acetonitrile/water/acetic acid (100:99:1, v/v/v), respectively. The gradient profile was accomplished over 85 min in a wide range of conditions (Time [min]: Mobile Phase [A][%]: Mobile Phase [B][%])—0 min: 90% A: 10% B; 20 min: 75% A: 25% B; 30 min: 65% A: 35% B; 40 min: 60% A: 40% B; 70 min: 45% A: 55% B; 75 min: 20% A: 80% B; 77–79 min: 0% A: 100% B; 82–84 min: 90% A: 10% B. The mobile phase flow rate was set at 0.8 ml/min with a sample injection volume of 6 µl. Peaks were identified in both positive and negative ion modes with the capillary and nozzle voltage set to 3.5 kV and 500 V, respectively. Additionally, following conditions were maintained: (i) nitrogen gas temperature at 300°C, (ii) sheath gas flow rate of 11 L/min at 250°C, and (iii) nitrogen gas nebulization at 45 psi. A complete mass scan ranging from *m/z* 50 to 1,300 was used; MS/MS analyses were carried out in automatic mode with collision energy (10, 15, and 30 eV) for fragmentation. Peak identification was performed in both positive and negative modes, while the instrument control, data acquisition, and processing were performed using MassHunter workstation software (Qualitative Analysis, version B.03.01) (Agilent Technologies, Santa Clara, CA, USA).

2.6 | Quantification of polyphenols through HPLC-PDA analysis

According to the modified method of Ma et al. (2019), the quantification of phenolic compounds present in the samples was carried out by HPLC (Waters Alliance 2690, Chromatograph Separation Module) along with a photodiode array (PDA) detector (Model 2998, Waters), which was set at λ 280, 320, and 370 nm with 1.25 scan/s (peak width = 0.2 min). Similar to LC-ESI-QTOF-MS/MS analysis, the column conditions remained the same; however, 20 µl of sample volume was used. Individual phenolic compounds were determined using calibration curves generated from standards that were produced from commonly found phenolic compounds present in mangoes. The results were expressed as µg/g of sample. All aspects of instrument control, data acquisition, and chromatography processing were conducted with Empower Software (2010).

2.7 | Statistical analysis

Each sample was tested in triplicates. The results obtained from the antioxidant assays were expressed as mean ± *SD* of three independent analysis ($n = 3$). Statistical analysis was conducted using Minitab Statistical Software Version 18.0 (Minitab Inc., USA) and Prism 7 Statistical Software. The difference in antioxidant activity was established through a statistical test- One-way analysis of variance (ANOVA). Furthermore, the significance was determined using Tukey's honestly significant differences (HSD) at $p < .05$.

3 | RESULTS AND DISCUSSION

3.1 | Polyphenol estimation

Five different varieties of Australian grown "rejected/unwanted" mangoes were subjected to polyphenol estimation, which were measured through TPC, TFC, and TTC methods (Table 1). Among the five different varieties, the "Honey Gold" variety displayed the significantly higher TPC, TFC, and TTC values than the rest of the varieties ($p \leq .05$), while the Kensington Pride variety possessed the least polyphenol content.

In terms of TPC values, all five varieties were significantly different from each other ($p \leq .05$), with Honey Gold (2.37 ± 0.06 mg GAE/g) having the highest phenolic content, followed by Palmer, Keitt, Calypso, and lastly, Kensington Pride. The current study takes into consideration the internal bruising and overall physical state of the "waste" mangoes used during the experiment, which were observed to be significantly wounded and ripe. Most mangoes that are unable to make it to retail tend to be neglected and generally left to over ripen and ultimately undergo senescence. In the event of over ripening of a fruit caused by bruise damage, chemical and physical changes tend to occur more rapidly, with drastic changes affecting the overall composition of the fruit. Our data suggest that rejected mango pulp contains considerable amounts of phenolic compounds, higher than what has been previously reported for retail mangoes (Manthey & Perkins-Veazie, 2009). Being aware that our samples are rejected mangoes that have suffered through mechanical, thermal, and other different unknown stressors; our data contradict previous studies which suggest that phenolic content decreases

TABLE 1 Polyphenol estimation and antioxidant potential of five different varieties of mango pulp

Antioxidant assays	Honey gold	Kensington pride	Calypso	Keitt	Palmer
TPC (mg GAE/g)	2.37 ± 0.06^{a}	1.08 ± 0.01^{e}	1.31 ± 0.02^{d}	1.71 ± 0.01^{c}	1.93 ± 0.01^{b}
TFC (mg QE/g)	0.37 ± 0.02^{a}	0.13 ± 0.05^{d}	0.24 ± 0.04^{b}	0.21 ± 0.01^{b}	0.18 ± 0.09^{c}
TTC (mg CE/g)	0.18 ± 0.04^{a}	0.09 ± 0.01^{c}	0.12 ± 0.01^{b}	0.03 ± 0.08^{d}	0.11 ± 0.07^{b}
DPPH (mg AAE/g)	2.13 ± 0.09^{a}	1.15 ± 0.04^{c}	1.13 ± 0.02^{c}	1.87 ± 0.04^{b}	1.93 ± 0.10^{a}
FRAP (mg AAE/g)	1.94 ± 0.01^{a}	1.07 ± 0.05^{e}	1.19 ± 0.09^{d}	1.32 ± 0.05^{c}	1.84 ± 0.04^{b}
ABTS (mg AAE/g)	1.93 ± 0.07^{a}	1.03 ± 0.09^{d}	1.01 ± 0.07^{d}	1.47 ± 0.09^{b}	1.39 ± 0.03^{c}
RPA (mg AAE/g)	1.14 ± 0.07^{a}	0.09 ± 0.01^{d}	1.03 ± 0.02^{b}	1.13 ± 0.04^{a}	0.97 ± 0.01^{c}
•OH-RSA (mg AAE/g)	0.79 ± 0.03^{a}	0.28 ± 0.07^{b}	0.07 ± 0.01^{d}	0.18 ± 0.05^{c}	0.27 ± 0.03^{b}
FICA (mg EDTA/g)	0.31 ± 0.02^{c}	0.74 ± 0.03^{a}	0.47 ± 0.02^{b}	0.21 ± 0.07^{d}	0.77 ± 0.09^{a}
TAC (mg AAE/g)	1.98 ± 0.03^{a}	0.93 ± 0.01^{d}	1.31 ± 0.03^{b}	0.95 ± 0.07^{d}	1.12 ± 0.02^{c}

Note: The results are illustrated in mean ± standard deviation of three independent measurements ($n = 3$). The total phenolic content (TPC) is expressed as mg of gallic acid equivalent GAE/g of fresh sample weight (f.w). The total flavonoid content (TFC) is expressed as mg of quercetin equivalent QE/g of fresh sample weight (f.w). The total tannins content (TTC) is expressed as mg of catechin equivalent QE/g of fresh sample weight (f.w). The antioxidant potential of the samples was measured through DPPH, FRAP, ABTS, RPA, •OH-RSA, and TAC, which are expressed as mg ascorbic acid equivalents (AAE) per g of fresh sample weight (f.w), while FICA are expressed as mg ethylenediaminetetraacetic acid (EDTA) per g of fresh sample weight (f.w). The different superscript letters $^{(a,b,c,d,e)}$ indicate significant differences at ($p \leq .05$).

Abbreviations: AAE, ascorbic acid equivalent; ABTS, 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid; DPPH, 2,2′-diphenyl-2-picryl-hydrazyl; FICA, ferrous ion chelating activity; FRAP, ferric reducing antioxidant power; OH-RSA, hydroxyl radical scavenging activity; RPA, reducing power assay; TAC, total antioxidant capacity.

with an increase in mechanical damage during fruit postharvest handling (Maia et al., 2014). Underhill and Critchley (1995) previously explained that when the cell membrane of a fruit encounters mechanical damage, polyphenol oxidase (PPO) is released and activated in the presence of oxygen from the cell membrane. PPO catalyzes a reaction that converts phenolic compounds into quinones, which further undergoes nonenzymatic processes to produce brown pigments that are responsible for the browning of fruits. Thus, with increase in damaged surface area caused by mechanical damage, temperature, and other stressors, it is expected that a greater amount of PPO is activated due to increased oxygen exposure to the cell membranes. Consequently, the level of polyphenols should decrease. Additionally, it should be noted that varying levels of total polyphenol may potentially be influenced by differences in cultivars, origins, and genetic variation (Huang et al., 2014). In comparison to our demonstrated data, other cultivars such as Lvsong and Xiao Tainang have exhibited a significantly lower concentration of total phenolics (Abbasi et al., 2015). Overall, our TPC results suggest that the pulp of the five varieties of Australian grown rejected mangoes is valuable as they possess a potential to create nutritional products.

Honey Gold variety contained the highest flavonoid content (0.37 ± 0.02 mg QE/g), whereas Kensington Pride held the lowest (0.13 ± 0.05 mg QE/g). There was no significant difference between Calypso and Keitt varieties ($p \leq .05$). Our TFC results for Australian grown Keitt variety were comparatively higher than previously recorded data for the same variety (Abbasi et al., 2015). However, it is possible to suggest that the conflicting data could be due to geographical or regional discrepancies. More particularly, factors such as the temperature and climate of the area play an integral role in the development of the plant—from seed to fruit (Hatfield & Prueger, 2015), such that excess heat may promote thermal stress, which tends to increase the levels of polyphenols in fruits. Rivero et al. (2001) previously investigated the effect of thermal stress on fruit plants during development and deduced that heat stress tends to increase soluble phenolic compounds in fruits by activating their biosynthesis and inhibiting their oxidation, further suggesting that the TPC in fruit is affected by the climate of the region. Although the environmental conditions have a significant influence on the growth of the plant, the ultimate usefulness of the fruit and its quality is dependent on how well the fruit is handled postharvest (Paull et al., 1997). Improper handling and storage conditions have suggested to hasten the ripening rate of fruits significantly, especially in conditions that promote water-deficit stress and ethylene production (Adato & Gazit, 1974). For this reason, it is observed that neglected fruits tend to overripen at a much quicker rate than those fruits that were handled properly.

Similar to the decreasing levels of TPC, it was observed that with increase in damage and over-ripening, flavonoid levels tend to also decrease (Maldonado-Celis et al., 2019). Palafox et al. (2012) concluded that with over ripening, expression of flavonol synthase drastically decreases, which reduces the flavonoid content of the fruit. Contrarily, green/underripe mangoes tend to have 45% more flavonoids than those in mature mangoes (Hu et al., 2018). Additionally, a higher TFC value may have been observed as a result of pulping during the sample preparation process. The pulp extraction process facilitates the release of polyphenol compounds caused by the

disruption of the cellular matrix (Arampath & Dekker, 2019). Honey Gold (0.18 ± 0.04 mg CE/g) and Keitt (0.03 ± 0.08 mg CE/g) varieties contained the highest and lowest tannins, respectively. Calypso (0.12 ± 0.01 mg CE/g) and Palmer (0.11 ± 0.07 mg QE/g) possessed similar tannins and were not significantly different. Previous studies have revealed that differences in TFC levels may occur among mango varieties, their growing conditions, and harvest periods (Kim et al., 2007). Additionally, in general, with increase in overripening, phenolic compounds are expected to decrease in climacteric fruits like mango (Haard & Chism, 1996). Moreover, it is essential to consider the effects of storage conditions on the quality parameters of mangoes. Rejected mangoes tend to be treated with poor storage conditions, which as a result have significant diminishing impacts on phenolic compounds (Sharma & Rao, 2017).

3.2 | Antioxidant potential (DPPH, FRAP, ABTS, RPA, •OH-RSA, FICA, and TAC)

DPPH, FRAP, ABTS, RPA, •OH-RSA, FICA, and TAC are among the most frequently conducted methods used to evaluate and deduce antioxidant activity in many foods and beverages (Shahidi & Zhong, 2015). Similar to the results obtained from polyphenol estimation, Honey Gold and Kensington Pride varieties contained the highest and lowest overall antioxidant potential, respectively. Table 1 suggests that the antioxidant potential of all five varieties is significantly different from each other ($p \leq .05$).

The DPPH assay is an antioxidant assay that allows antioxidants to be evaluated by spectrophotometry. It is based on reduction of DPPH radicals, which are scavenged by antioxidant compounds (Molyneux, 2004). In the DPPH assay, it was observed that Honey Gold (2.13 ± 0.09 mg AAE/g) and Palmer (1.93 ± 0.10 mg AAE/g) showed the greatest activity followed by Keitt (1.87 ± 0.04 mg AAE/g). Kensington Pride (1.15 ± 0.04 mg AAE/g) and Calypso (1.13 ± 0.02 mg AAE/g) had the lowest yet similar radical scavenging ability compared to the rest.

The ABTS assay utilizes preformed radical monocation of ABTS ($ABTS^{\bullet+}$), which is reduced in the presence of hydrogen-donating antioxidants (Re et al., 1999). The ABTS values suggested that Honey Gold (1.93 ± 0.07 mg AAE/g) showed the greatest activity, followed by Keitt (1.47 ± 0.09 mg AAE/g) and Palmer (1.39 ± 0.03 mg AAE/g). Similar to the values of the DPPH assay, Kensington Pride (1.03 ± 0.09 mg AAE/g) and Calypso (1.01 ± 0.07 mg AAE/g) varieties showed no significant differences in ABTS values between them.

The FRAP assay mechanism is based on a single electron transfer that measures the reducing potential of an antioxidant reacting with ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex. Accordingly, a colored ferrous ferrous tripyridyltrizine (Fe^{2+}-TPTZ) complex is produced with a high absorbance at 593 nm (Rajurkar & Hande, 2011). The FRAP assay results emphasized that the reducing ability of all varieties was significantly different from each other ($p \leq .05$). In terms of TAC, Honey Gold (1.98 ± 0.03 mg AAE/g) and Calypso (1.31 ± 0.03 mg AAE/g) showed the highest activity, followed by Palmer (1.12 ± 0.02 mg AAE/g), Keitt (0.95 ± 0.07 mg AAE/g) and lastly, Kensington Pride (0.93 ± 0.01 mg AAE/g).

Our results remained mainly consistent with previously recorded antioxidant potential studies conducted on mangoes (Gu et al., 2019), suggesting that the pulp of mango rejects are as valuable as retail mango pulp as they hold similar amounts of antioxidant potential. However, it is essential to note that the overripened nature of our samples could have potentially influenced the antioxidant potential values presented in Table 1.

Quiros-Sauceda et al. (2019) confirmed the effect of ripening and its impact on the accessibility of the phenolic compounds present in "Ataulfo" mango. The study suggests that phenolics are firmly bound to the fruit matrix of unripe mango, but the ripening process liberates the compounds as the major polysaccharides are hydrolyzed. As a result, covalent bonds between carbohydrate and phenolic complexes are broken, releasing the phenolics. Ibarra-Garza et al. (2015) further studied the influence of ripening stages in Keitt mangoes and concluded that antioxidant capacity varies and fluctuates at different ripening stages, with results demonstrating that the lowest antioxidant capacity was observed at the later or overripened stages of the fruit. Considering that our study was based on mango rejects, it was expected that our results showed lower antioxidant potential with over ripening and bruising, as these factors have been previously known to affect antioxidant potential in mangoes (Palafox et al., 2012).

In RPA, Honey gold and Keitt mangoes had higher antioxidant potential followed by Calypso, Palmer, and Kensington Pride varieties. Previously, Molla et al. (2020) study showed the reducing power activity in mangoes of different varieties of BARI mango and cultivar Langra that were in agreement with our study. However, •OH-RSA, Honey gold mangoes had higher antioxidant potential when compared to other varieties. Soh et al. (2017) study showed that aqueous extract has better inhibitory activity that hydroethanolic extract of African mango. In Jose et al. (2018) study, the mango leaves that have antioxidant potential (•OH-RSA) slightly higher than our results might be due to environmental factors, such as light, temperature, agronomic practices, and genetic variation. In FICA assay, Palmer and Kensington Pride varieties of our study had higher antioxidant potential followed by Calypso, Honey Gold, and Keitt varieties. To the best of our knowledge, the FICA assay was first time performed on mango fruit.

It is recommended that future studies focus on exploring the antioxidant potential of the five varieties above at different ripening stages to potentially produce the highest concentration of specific bioactive compounds. As a result, more top-quality nutraceutical, pharmaceutical, or cosmetic products can be produced. Additionally, such data would promote proper storage conditions for mango rejects as the additional insight would provide an incentive to preserve the quality of the mango. However, not all mango rejects are based on physical injuries but also on other factors such as size. Therefore, although our samples were of mainly overripe and bruised mango rejects, our results cannot be used to conclude the antioxidant potential for mango rejects based on other physical deformities such as size.

3.3 | Correlation between polyphenol and antioxidant potential

Numerous authors have previously investigated and demonstrated the positive correlation between total phenolic compounds and antioxidant activity (Babbar et al., 2011; Jayaprakasha et al., 2008). In the present study, the correlation between polyphenol and antioxidant potential was conducted using Pearson's correlation test (Table 2).

The obtained results indicated that TPC displayed a statistically significant ($p \leq .01$) positive correlation with DPPH ($r = 0.94$; $p \leq .01$), FRAP ($r = 0.94$; $p \leq .01$), and ABTS ($r = 0.95$; $p \leq .01$). The strong correlation between these variables may suggest that the phenolic compounds present in the mango samples are likely to contribute to radical scavenging activity. Additionally, the similar correlation coefficient between TPC and the antioxidant assays may be due to the similarity in the mechanism by which the assays undertake; that is, these spectrophotometric methods tend to be based on similar redox reactions (Huang et al., 2005). TFC and TAC had a correlation coefficient of 0.95 ($p \leq .01$), indicating a strong correlation between the two variables. Rumainum et al. (2018) have previously reported a strong correlation between TFC and TAC in fruits of six Thai mangoes and suggested that due to this high correlation, it may be assumed that flavonoids are primary contributors to the antioxidant activity of mango fruits. Although significant ($p \leq .05$), a relatively lower correlation was observed between the antioxidant assays in our experiment. Our results stated that DPPH showed significant yet a lower correlation coefficient with FRAP ($r = 0.87$; $p \leq .05$) and ABTS ($r = 0.93$; $p \leq .05$). In our result, ABTS has showed high significant correlation with •OH-RSA ($r = 0.84$, $p \leq .01$). In contrast to our results, previous studies have demonstrated a much stronger correlation ($r = 0.93$; $p < .001$) between DPPH and ABTS (Hoyos-Arbelaez et al., 2018). Additionally, Thaipong et al. (2006) have also previously reported the high correlation between ABTS and FRAP ($r = 0.97$; $p \leq .01$). All in all, our findings suggest that total phenolics and flavonoids are the major compounds that contribute to the antioxidant activity in our sample.

3.4 | LC-ESI-QTOF-MS/MS analysis of phenolic compounds

In recent times, LC-ESI-QTOF-MS/MS has proven to be a powerful and useful tool in efficiently identifying and characterizing phenolic compounds in plants. A qualitative analysis of the phenolic compounds from the extract of five different varieties of mangoes was conducted using LC-ESI-QTOF-MS/MS analysis in negative and positive ionization modes. Using the Agilent LC/MS MassHunter Qualitative Software, along with the Personal Compound Database and Library (PCDL), the present study was able to tentatively analyze and establish phenolic compounds based on their *m/z* value, retention time (min), and ionization mode (ESI^-/ESI^+) (Supplementary Data). It is worth mentioning that only compounds that exhibited a score greater than 80 (PCDL score) and mass error $< \pm 5$ ppm were selectively chosen for characterization.

A total of 86 different phenolic compounds were identified in five different mango pulp samples, including 24 phenolic acids, 45 flavonoids, 3 stilbenes, 5 lignans, and 9 other polyphenols (Table 3). It may be concluded that phenolic acids and flavonoids were the two major phenolic compound groups present in our samples. In particular, it was noticed that Kensington Pride was abundant in phenolic acids and flavonoids compared to the other samples. In contrast, Palmer lacked diversity in both those groups; however, it was interestingly rich in other polyphenols.

In brief, it was recorded that Kensington Pride possessed the most significant number of phenolic compounds (31), out of which 16 compounds were phenolic acids, 11 were flavonoids, a single

TABLE 2 Pearson's correlation coefficients (*r*) of phenolic contents (TPC, TFC, and TTC) and the antioxidant activities (DPPH, FRAP, ABTS, RPA, •OH-RSA, FICA, TAC)

Variables	TPC	TFC	TTC	DPPH	FRAP	ABTS	RPA	•OH-RSA	FICA
TFC	0.76								
TTC	0.49	0.69							
DPPH	0.94**	0.55	0.20						
FRAP	0.94**	0.62	0.59	0.87*					
ABTS	0.95**	0.78	0.42	0.93*	0.83				
RPA	0.70	0.67	0.16	0.63	0.56	0.58			
•OH-RSA	0.75	0.73	0.71	0.64	0.71	0.84**	0.16		
FICA	−0.40	−0.65	0.09	−0.39	−0.08	−0.53	−0.66	−0.23	
TAC	0.71	0.95**	0.88*	0.47	0.66	0.71	0.48	0.81	−0.38

Abbreviations: AAE, ascorbic acid equivalent; ABTS, 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid; DPPH, 2,2′-diphenyl-2-picryl-hydrazyl; FICA, ferrous ion chelating activity; FRAP, ferric reducing antioxidant power; OH-RSA, hydroxyl radical scavenging activity; TAC, total antioxidant capacity; TFC, total flavonoid content; TPC, total phenolic content; TTC, total tannins content.

*Significant correlation with $p \leq .05$; **Significant correlation with $p \leq .01$.

TABLE 3 Characterization of phenolic compounds present in five varieties of mango pulp extract using LC-ESI-QTOF-MS/MS

No.	Compound name	Molecular formula	RT (min)	Ionization mode	Molecular weight (*m/z*)	Theoretical weight (*m/z*)	Observed weight (*m/z*)	Error (ppm)	MS/MS product ions	Samples
Phenolic acids										
	Hydroxybenzoic acids									
1	Vanillic acid 4-sulfate	$C_8H_8O_7S$	5.068	$[M-H]^-$	247.9991	246.9918	246.9911	−2.8	167	CL
2	Gallic acid 4-*O*-glucoside	$C_{13}H_{16}O_{10}$	6.253	$[M-H]^-$	332.0743	331.0670	331.0678	2.4	169, 125	KP*, CL
3	Protocatechuic acid 4-*O*-glucoside	$C_{13}H_{16}O_9$	7.520	$[M-H]^-$	316.0794	315.0721	315.0719	−0.6	153	KP, *CL
4	2,3-Dihydroxybenzoic acid	$C_7H_6O_4$	9.135	$[M-H]^-$	154.0266	153.0193	153.0196	2.0	109	*KP, KE
5	4-Hydroxybenzoic acid 4-*O*-glucoside	$C_{13}H_{16}O_8$	10.924	$^{**}[M-H]^-$	300.0845	299.0772	299.0774	0.7	255, 137	HG, *KP, CL
6	2-Hydroxybenzoic acid	$C_7H_6O_3$	19.922	$^{**}[M-H]^-$	138.0317	137.0244	137.0250	4.4	93	HG, KP, *PL, CL
7	Gallic acid 3-*O*-gallate	$C_{14}H_{10}O_9$	20.499	$[M-H]^-$	322.0325	321.0252	321.0260	2.5	169	KP*, CL
8	Ellagic acid	$C_{14}H_6O_8$	50.283	$[M-H]^-$	302.0063	300.9990	300.9999	3.0	284, 229, 201	KP
	Hydroxycinnamic acids									
9	Sinapic acid	$C_{11}H_{12}O_5$	6.405	$^{**}[M-H]^-$	224.0685	223.0612	223.0614	0.9	205, 163	HG, KP, *PL, KE
10	Feruloyl tartaric acid	$C_{14}H_{14}O_9$	10.742	$[M-H]^-$	326.0638	325.0565	325.0570	1.5	193, 149	KP*, CL
11	*p*-Coumaroyl tartaric acid	$C_{13}H_{12}O_8$	13.345	$[M-H]^-$	296.0532	295.0459	295.0461	0.7	115	HG*, PL
12	Ferulic acid	$C_{10}H_{10}O_4$	18.100	$[M-H]^-$	194.0579	193.0506	193.0501	−2.6	178, 149, 134	KP, PL*, KE
13	*p*-Coumaric acid 4-*O*-glucoside	$C_{15}H_{18}O_8$	19.143	$[M-H]^-$	326.1002	325.0929	325.0923	−1.9	163	*HG, KP, CL
14	Ferulic acid 4-*O*-glucuronide	$C_{16}H_{18}O_{10}$	19.886	$^{**}[M-H]^-$	370.0900	369.0827	369.0845	4.9	193	*KP, CL
15	Isoferulic acid 3-sulfate	$C_{10}H_{10}O_7S$	21.327	$[M-H]^-$	274.0147	273.0074	273.0086	4.4	193, 178	KP
16	Ferulic acid 4-*O*-glucoside	$C_{16}H_{20}O_9$	23.216	$[M-H]^-$	356.1107	355.1034	355.1046	3.4	193, 178, 149, 134	*KP, PL, KE, CL
17	3-Caffeoylquinic acid	$C_{16}H_{18}O_9$	27.244	$[M-H]^-$	354.0951	353.0878	353.0886	2.3	253, 190, 144	*PL, KE
18	3-*p*-Coumaroylquinic acid	$C_{16}H_{18}O_8$	27.450	$^{**}[M-H]^-$	338.1002	337.0929	337.0943	4.2	265, 173, 162	HG, *KE
19	3-Feruloylquinic acid	$C_{17}H_{20}O_9$	30.100	$^{**}[M-H]^-$	368.1107	367.1034	367.1040	1.6	298, 288, 192, 191	HG, *KE, CL
20	Verbascoside	$C_{29}H_{36}O_{15}$	54.046	$[M-H]^-$	624.2054	623.1981	623.1984	0.5	477, 461, 315, 135	HG
21	1-Sinapoyl-2-feruloylgentiobiose	$C_{33}H_{40}O_{18}$	63.926	$[M-H]^-$	724.2215	723.2142	723.2165	3.2	529, 499	KE
	Hydroxyphenylacetic acids									
22	3,4-Dihydroxyphenylacetic acid	$C_8H_8O_4$	9.815	$^{**}[M-H]^-$	168.0423	167.0350	167.0357	4.2	149, 123	HG, *KP, CL
23	2-Hydroxy-2-phenylacetic acid	$C_8H_8O_3$	10.767	$^{**}[M-H]^-$	152.0473	151.0400	151.0396	−2.7	136, 92	HG, KP, KE, *CL
	Hydroxyphenylpropanoic acids									
24	3-Hydroxy-3-(3-hydroxyphenyl) propionic acid	$C_9H_{10}O_4$	33.927	$^{**}[M-H]^-$	182.0579	181.0506	181.0513	3.9	163, 135, 119	HG, PL, *KE, CL

(Continues)

TABLE 3 (Continued)

No.	Compound name	Molecular formula	RT (min)	Ionization mode	Molecular weight (*m/z*)	Theoretical weight (*m/z*)	Observed weight (*m/z*)	Error (ppm)	MS/MS product ions	Samples
Flavonoids										
	Anthocyanins									
25	Cyanidin 3-*O*-(2-*O*-(6-*O*-(E)-caffeoyl-D glucoside)-D-glucoside)-5-*O*-D-glucoside	$C_{43}H_{49}O_{24}$	5.532	$[M\text{-}H]^+$	949.2614	950.2687	950.2697	1.1	787, 463, 301	CL
26	Cyanidin 3-*O*-(6″-*p*-coumaroyl-glucoside)	$C_{30}H_{27}O_{13}$	14.547	$^{**}[M\text{-}H]^+$	595.1452	596.1525	596.1508	−2.6	287	*KP, CL
27	Petunidin 3-*O*-(6″-acetyl-glucoside)	$C_{24}H_{25}O_{13}$	27.285	$^{**}[M\text{-}H]^+$	521.1295	522.1368	522.1369	0.2	317	KE
28	Delphinidin 3-*O*-xyloside	$C_{20}H_{19}O_{11}$	43.392	$[M\text{-}H]^-$	435.0927	434.0854	434.0841	−3.0	303	KP
	Dihydrochalcones									
29	3-Hydroxyphloretin 2′-*O*-glucoside	$C_{21}H_{24}O_{11}$	10.792	$^{**}[M\text{-}H]^-$	452.1319	451.1246	451.1253	1.6	289, 273	HG, *KP, CL
30	3-Hydroxyphloretin 2′-*O*-xylosyl-glucoside	$C_{26}H_{32}O_{15}$	36.793	$[M\text{-}H]^-$	584.1741	583.1668	583.1678	1.7	289	CL
31	Phloridzin	$C_{21}H_{24}O_{10}$	50.567	$[M\text{-}H]^-$	436.1369	435.1296	435.1301	1.2	273	HG
	Dihydroflavonols									
32	Dihydroquercetin	$C_{15}H_{12}O_7$	11.661	$[M\text{-}H]^-$	304.0583	303.0510	303.0510	0.0	285, 275, 151	CL
33	Dihydromyricetin 3-*O*-rhamnoside	$C_{21}H_{22}O_{12}$	36.776	$^{**}[M\text{-}H]^-$	466.1111	465.1038	465.1046	1.7	301	KE
	Flavanols									
34	(+)-Gallocatechin	$C_{15}H_{14}O_7$	10.535	$^{**}[M\text{-}H]^-$	306.0740	305.0667	305.0679	3.9	261, 219	HG, *CL
35	Procyanidin dimer B1	$C_{30}H_{26}O_{12}$	14.900	$^{**}[M\text{-}H]^-$	578.1424	577.1351	577.1339	−2.0	451	HG, *KP, KE
36	Procyanidin trimer C1	$C_{45}H_{38}O_{18}$	19.067	$[M\text{-}H]^-$	866.2058	865.1985	865.1944	−4.7	739, 713, 695	CL
37	(+)-Catechin	$C_{15}H_{14}O_6$	19.091	$^{**}[M\text{-}H]^-$	290.0790	289.0717	289.0726	3.1	245, 205, 179	HG, *KP, CL
38	4′-*O*-Methyl-(-)-epigallocatechin 7-*O*-glucuronide	$C_{22}H_{24}O_{13}$	35.799	$[M\text{-}H]^-$	496.1217	495.1144	495.1138	−1.2	451, 313	KE
39	4′-*O*-Methylepigallocatechin	$C_{16}H_{16}O_7$	37.008	$[M\text{-}H]^+$	320.0896	321.0969	321.0965	−1.3	302	KE
40	(+)-Gallocatechin 3-*O*-gallate	$C_{22}H_{18}O_{11}$	49.606	$[M\text{-}H]^-$	458.0849	457.0776	457.0769	−1.5	305,169	HG
	Flavanones									
41	Hesperetin 3′-*O*-glucuronide	$C_{22}H_{22}O_{12}$	63.714	$^{**}[M\text{-}H]^-$	478.1111	477.1038	477.1026	−2.5	301, 175, 113, 85	HG, KP, PL, KE, *CL
	Flavones									
42	Apigenin 7-*O*-glucuronide	$C_{21}H_{18}O_{11}$	11.250	$[M\text{-}H]^+$	446.0849	447.0922	447.0908	−3.1	227, 199, 171	KP
43	Apigenin 7-*O*-apiosyl-glucoside	$C_{26}H_{28}O_{14}$	31.261	$^{**}[M\text{-}H]^+$	564.1479	565.1552	565.1532	−3.5	296	*KP, PL, KE, CL
44	Apigenin 6-C-glucoside	$C_{21}H_{20}O_{10}$	37.522	$^{**}[M\text{-}H]^-$	432.1056	431.0983	431.0983	0.0	413, 341, 311	HG, KP, *KE, CL
45	Apigenin 6,8-di-C-glucoside	$C_{27}H_{30}O_{15}$	42.815	$^{**}[M\text{-}H]^-$	594.1585	593.1512	593.1523	1.9	503, 473	*HG, KE
46	6-Hydroxyluteolin 7-*O*-rhamnoside	$C_{21}H_{20}O_{11}$	44.090	$^{**}[M\text{-}H]^-$	448.1006	447.0933	447.0941	1.8	301	*HG, KP, KE

(Continues)

TABLE 3 (Continued)

No.	Compound name	Molecular formula	RT (min)	Ionization mode	Molecular weight (*m/z*)	Theoretical weight (*m/z*)	Observed weight (*m/z*)	Error (ppm)	MS/MS product ions	Samples
47	Chrysoeriol 7-*O*-glucoside	$C_{22}H_{22}O_{11}$	48.770	$^{**}[M-H]^+$	462.1162	463.1235	463.1237	0.4	445, 427, 409, 381	HG, KP, KE*
48	Cirsilineol	$C_{18}H_{16}O_7$	80.994	$[M-H]^+$	344.0896	345.0969	345.0957	−3.5	330, 312, 297, 284	HG
	Flavonols									
49	Patuletin 3-*O*-glucosyl-(1->6)-[apiosyl(1->2)]-glucoside	$C_{33}H_{40}O_{22}$	28.535	$[M-H]^-$	788.2011	787.1938	787.1965	3.4	625, 463, 301, 271	HG
50	3-Methoxynobiletin	$C_{22}H_{24}O_9$	30.697	$[M-H]^+$	432.1420	433.1493	433.1482	−2.5	403, 385, 373, 345	KE
51	Myricetin 3-*O*-rutinoside	$C_{27}H_{30}O_{17}$	32.362	$[M-H]^-$	626.1483	625.1410	625.1437	4.3	301	*HG, KP, CL
52	Quercetin 3-*O*-(6″-malonyl-glucoside) 7-*O*-glucoside	$C_{30}H_{32}O_{20}$	33.728	$[M-H]^+$	712.1487	713.1560	713.1568	1.1	551, 303	KE
53	Kaempferol 3-*O*-(2″-rhamnosyl-galactoside) 7-*O*-rhamnoside	$C_{33}H_{40}O_{19}$	34.772	$^{**}[M-H]^-$	740.2164	739.2091	739.2073	−2.4	593, 447, 285	HG, KP, PL, *KE
54	Kaempferol 3,7-*O*-diglucoside	$C_{27}H_{30}O_{16}$	36.073	$^{**}[M-H]^-$	610.1534	609.1461	609.1484	3.8	447, 285	*HG, KP, PL, KE
55	Kaempferol 3-*O*-xylosyl-glucoside	$C_{26}H_{28}O_{15}$	39.096	$^{**}[M-H]^+$	580.1428	581.1501	581.1490	−1.9	419, 401, 383	HG, *KP
56	Myricetin 3-*O*-rhamnoside	$C_{21}H_{20}O_{12}$	39.402	$^{**}[M-H]^-$	464.0955	463.0882	463.0897	3.2	317	*HG, KE
57	Kaempferol 3-*O*-glucosyl-rhamnosyl-galactoside	$C_{33}H_{40}O_{20}$	39.469	$^{**}[M-H]^-$	756.2113	755.2040	755.2050	1.3	285	HG, KP, *PL, KE
58	Myricetin 3-*O*-galactoside	$C_{21}H_{20}O_{13}$	44.819	$[M-H]^-$	480.0904	479.0831	479.0837	1.3	317	HG
59	Quercetin 3-*O*-arabinoside	$C_{20}H_{18}O_{11}$	45.905	$^{**}[M-H]^-$	434.0849	433.0776	433.0778	0.5	301	HG, KP, *CL
60	Isorhamnetin	$C_{16}H_{12}O_7$	56.116	$^{**}[M-H]^-$	316.0583	315.0510	315.0520	3.2	300, 271	HG
	Isoflavonoids									
61	Sativanone	$C_{17}H_{16}O_5$	13.942	$[M-H]^-$	300.0998	299.0925	299.0931	2.0	284, 270, 253	HG
62	3′-Hydroxygenistein	$C_{15}H_{10}O_6$	16.866	$^{**}[M-H]^-$	286.0477	287.0550	287.0547	−1.1	269, 259	HG, *KP, PL, KE
63	3′-Hydroxydaidzein	$C_{15}H_{10}O_5$	26.561	$[M-H]^+$	270.0528	271.0601	271.0603	0.7	253, 241, 225	HG*, KP
64	6″-*O*-Acetyldaidzin	$C_{23}H_{22}O_{10}$	29.504	$^{**}[M-H]^-$	458.1213	457.1140	457.1121	−4.2	221	KP, *CL
65	Glycitin	$C_{22}H_{22}O_{10}$	38.881	$[M-H]^+$	446.1213	447.1286	447.1269	−3.8	285	KP
66	5,6,7,3′,4′-Pentahydroxyisoflavone	$C_{15}H_{10}O_7$	45.197	$^{**}[M-H]^-$	302.0427	303.0500	303.0488	−4.0	285, 257	*HG, KP, KE, CL
67	2′,7-Dihydroxy-4′,5′-dimethoxyisoflavone	$C_{17}H_{14}O_6$	61.298	$^{**}[M-H]^-$	314.0790	315.0863	315.0858	−1.6	300, 282	*HG, CL
68	2-Dehydro-*O*-desmethylangolensin	$C_{15}H_{12}O_4$	77.381	$[M-H]^-$	256.0736	255.0663	255.0656	−2.7	135, 119	CL
69	Dihydrobiochanin A	$C_{16}H_{14}O_5$	81.740	$[M-H]^+$	286.0841	287.0914	287.0918	1.4	269, 203, 201, 175	HG

(Continues)

TABLE 3 (Continued)

No.	Compound name	Molecular formula	RT (min)	Ionization mode	Molecular weight (*m/z*)	Theoretical weight (*m/z*)	Observed weight (*m/z*)	Error (ppm)	MS/MS product ions	Samples
Other polyphenols										
	Hydroxybenzoketones									
70	2,3-Dihydroxy-1-guaiacylpropanone	$C_{10}H_{12}O_5$	28.676	**[M-H]$^-$	212.0685	211.0612	211.0617	2.4	167, 123, 105, 93	HG, KP, PL, *KE, CL
	Hydroxycoumarins									
71	Scopoletin	$C_{10}H_8O_4$	79.689	**[M-H]$^-$	192.0423	191.0350	191.0344	−3.1	176	*PL, KE
	Other polyphenols									
72	Lithospermic acid	$C_{27}H_{22}O_{12}$	5.051	[M-H]$^-$	538.1111	537.1038	537.1048	1.9	493, 339, 295	CL
73	Arbutin	$C_{12}H_{16}O_7$	20.493	[M-H]$^-$	272.0896	271.0823	271.0830	2.6	109	HG, *KE
	Phenolic terpenes									
74	Carnosic acid	$C_{20}H_{28}O_4$	79.672	[M-H]$^-$	332.1988	331.1915	331.1915	0.0	287, 269	PL
75	Rosmanol	$C_{20}H_{26}O_5$	81.552	[M + H] $^+$	346.1780	347.1853	347.1864	3.2	301, 241, 231	KE
	Tyrosols									
76	Hydroxytyrosol 4-*O*-glucoside	$C_{14}H_{20}O_8$	9.739	[M-H]$^-$	316.1158	315.1085	315.1081	−1.3	153, 123	CL
77	3,4-DHPEA-AC	$C_{10}H_{12}O_4$	16.161	**[M-H]$^-$	196.0736	195.0663	195.0662	−0.5	135	*HG, KE, CL
78	Demethyloleuropein	$C_{24}H_{30}O_{13}$	19.375	[M-H]$^-$	526.1686	525.1613	525.1623	1.9	495	KP, *PL, KE
Stilbenes										
	Stilbenes									
79	3′-Hydroxy-3,4,5,4′-tetramethoxystilbene	$C_{17}H_{18}O_5$	10.356	[M-H]$^+$	302.1154	303.1227	303.1238	3.6	271, 241, 225	KP
80	Resveratrol 5-*O*-glucoside	$C_{20}H_{22}O_8$	39.161	[M-H]$^-$	390.1315	389.1242	389.1244	0.5	227	KE
81	Piceatannol 3-*O*-glucoside	$C_{20}H_{22}O_9$	49.838	[M-H]$^-$	406.1264	405.1191	405.1207	4.0	243	HG
Lignans										
	Lignans									
82	Sesamin	$C_{20}H_{18}O_6$	13.643	**[M-H]$^-$	354.1103	353.1030	353.1019	−3.1	338, 163	*HG, KE
83	7-Oxomatairesinol	$C_{20}H_{20}O_7$	30.283	[M-H]$^+$	372.1209	373.1282	373.1293	3.0	358, 343, 328, 325	KE
84	7-Hydroxymatairesinol	$C_{20}H_{22}O_7$	49.441	**[M-H]$^-$	374.1366	373.1293	373.1296	0.8	343, 313, 298, 285	HG
85	Conidendrin	$C_{20}H_{20}O_6$	78.509	[M-H]$^+$	356.1260	357.1333	357.1323	−2.8	339, 221, 206	HG
86	Schisandrol B	$C_{23}H_{28}O_7$	79.402	[M-H]$^+$	416.1835	417.1908	417.1897	−2.6	224, 193, 165	KP, *PL

**Indicates the detection of the compound in both ionisation modes (ESI$^-$/ESI$^+$). The following compounds were selected and discussed based on the negative (ESI$^-$) mode. Mango samples were indicated in abbreviations: CL, Calypso; HG, Honey Gold; KE, Keitt, KP, Kensington Pride; PL, Palmer.

stilbene and lignan, and 2 other polyphenols. Keitt also displayed a significant number of phenolic compounds (30), including 10 phenolic acids, 11 flavonoids, a single stilbene, 2 lignans and 6 other polyphenols. Similarly, Honey Gold (29) showed 11 phenolic acids, 11 flavonoids, a single stilbene, 3 lignans, and 3 other polyphenols. Not far off from Honey Gold and Keitt, a total of 28 phenolic compounds were recorded in Calypso, including 14 phenolic acids, 11 flavonoids, and 3 other polyphenols. Among the five samples, Palmer demonstrated the least number of phenolic compounds (17), possessing half of what was observed in the other samples.

3.4.1 | Phenolic acids

In the current study, hydroxybenzoic acids, hydroxycinnamic acids, hydroxyphenyl acetic acids, and hydroxyphenyl propanoic acids were the four subclasses of phenolic acids that were recorded in the samples of the five different mango varieties. Hydroxybenzoic acids and hydroxycinnamic acids were the two dominant subclasses demonstrated by the samples.

Hydroxybenzoic acids

A total of eight different hydroxybenzoic acids were identified in the mango pulp samples. Kensington Pride, in particular, was deduced to be potent in hydroxybenzoic acids, possessing seven out of eight different hydroxybenzoic acids (Compounds **2–8**). Likewise, six out of eight different hydroxybenzoic acids (Compounds **1**, **2**, **3**, **5**, **6**, **and 8**) were tentatively characterized in Calypso. Honey Gold, Palmer, and Keitt lacked diversity in hydroxybenzoic acids and were limited to Compounds (**4**, **5**, **and 6**).

Compound (**1**), possessing the molecular formula C_8 H_8 O_7 S, was detected in the negative mode $[M\text{-}H]^-$, at RT = 5.068, *m/z* 246.9911, and was tentatively characterized as Vanillic acid 4-sulfate and further confirmed by the MS/MS experiment which displayed a characteristic loss of SO_3 (80 Da) at *m/z* 167 (Yang et al., 2016). The observed compound was only identified in Calypso. Vanillic acids have been previously discovered in "Altaulfo" mango pulp as one of the major phenolic compounds (Palafox-Carlos et al., 2012).

Compound (**2**) possessing the molecular formula $C_{13}H_{16}O_{10}$ was tentatively identified in the negative mode $[M\text{-}H]^-$ at *m/z* 331.0678, which was then characterized as Gallic acid 4-*O*-glucoside. In the MS^2 spectra, the loss of the glucoside moiety [M-H-162] and consecutive loss of CO_2 (44 Da) from the precursor ion (*m/z* 169, gallic acid ion) were observed in gallic acid 4-*O*-glucoside (Rajauria et al., 2016). Compound (**3**) was characterized to be protocatechuic acid 4-*O*-glucoside, also identified in the negative ionization mode $[M\text{-}H]^-$ at *m/z* 315.0719. The compound was further identified based on the product ions at *m/z* 153 after the loss of glucoside (162 Da) in the MS^2 experiment (Sun et al., 2010). Similarly, Compound (**7**) was tentatively identified in the negative $[M\text{-}H]^-$ at *m/z* 321.0260, with retention time (RT) = 20.4999 min. The following compound was characterized as Gallic acid 3-*O*-gallate. The loss of galloyl moiety [M-H-152] was observed in gallic acid 3-*O*-gallate (Chen et al., 2015). Compounds (**2**), (**3**), and (**7**) were established in Kensington Pride and Calypso mango varieties; however, they were absent in the rest of the samples. Gallic acid and protocatechuic acid are common hydroxybenzoic acid derivatives that have been previously detected in mango pulp (Masibo & He, 2008; Palafox-Carlos et al., 2012). Multiple studies have crowned gallic acid and protocatechuic acid as primary phenolic acids present across a wide variety of mangoes (Corrales-Bernal et al., 2014; Kim et al., 2009).

Compounds (**4**) and **(6)** were tentatively identified to be 2,3-dihydroxybenzoic acid and 2-hydroxybenzoic acid, respectively. Compound **(4)** was identified in the negative ionization mode $[M\text{-}H]^-$ at *m/z* 153.0196, whereas Compound **(6)** was found to be present in both ionization modes but yielded $[M\text{-}H]^-$ *m/z* 137.0250. The following compounds further displayed product ions at *m/z* 109 and *m/z* 93, representing the loss of CO_2 from the precursor ions (Escobar-Avello et al., 2019; Wang et al., 2016). Our data suggest that these compounds were prominent in the Honey Gold, Kensington Pride, Palmer, and Calypso varieties. Previous studies have reported the presence of *p*-hydroxybenzoic acids in mango pulp (Masibo & He, 2008). Moreover, 2-hydroxybenzoic acid, more commonly known as salicylic acid, is a known phenolic phytohormone present in plants, responsible for the regulation of its growth, development, and defense.

Recorded in the negative mode $[M\text{-}H]^-$, with *m/z* 300.9999 and RT = 50.283, Ellagic acid (EA) (Compound **8**) was only found in Kensington Pride variety. El Ansari et al. (1971) previously reported the evidence of Ellagic acid in mango fruits. EAs have also been previously characterized in mango seed extract and was reported to contain 3–156 mg equivalents of gallic acid per 100 g, depending on the extraction method (Soong & Barlow, 2006). Moreover, EAs have also been tentatively identified by LC-MS in raw and ripe mango peels (Ajila et al., 2010). EAs are generated from the enzymatic hydrolyzation of ellagitannins, commonly present in mango pulp (Sepúlveda et al., 2011). In present times, EAs have received considerable attention due to its wide applications in enhancing human health (Landete, 2011). As a result, demand for natural EA has been increasing, especially in the functional food and pharmaceutical industries. However, currently, majority of EA has been chemically synthesized, involving harsh treatments and extra costs (Aguilera-Carbo et al., 2009). Hence, our study suggests that the pulp of Kensington Pride Australian variety is deemed suitable for the extraction and production of functional foods and medicines as it naturally contains EA.

Moreover, recent studies have revealed the efficiency of microbes (*Micrococcus luteus*) in the optimal production of ellagic acid from rejected mango pulp (Rubavathi et al., 2020). *Aspergillus niger* has also suggested being competent in the bioconversion of ellagitannin present in mango pulp industrial waste into EA (Murugan et al., 2020). Therefore, such processes provide the potential for rejected mangoes in the production of health benefiting compounds, including EA, while providing an alternative to the chemical extraction.

Hydroxycinnamic acids

Observed in both ionization modes, Sinapic acid (Compound **9**) was tentatively deduced from $[M-H]^-$ with *m/z* 223.0614 at RT = 6.405. In the MS^2 spectra, sinapic acid showed the fragments at *m/z* 205 and *m/z* 163, representing the loss of H_2O and 2CHO from precursor ion (Geng et al., 2014). The results suggest that sinapic acid existed in the majority of our mango pulp samples, including Honey Gold, Kensington Pride, Palmer, and Keitt Australian mango varieties. Sinapic acid is considered as one of the four most common hydroxycinnamic acids that dwell within the plant kingdom (Nićiforović & Abramovič, 2014). Sumitra et al. (2010) have previously extracted and characterized sinapic acid in mango fruit and was measured to contain 7.55 ± 0.3 μg/g dry weight (d.w.).

Ferulic acid (Compound **12**) was carefully identified in the negative ESI^- mode with *m/z* 193.0501 in three mango samples—Kensington Pride, Palmer, and Keitt. In an MS^2 experiment, ferulic acid displayed the product ions at *m/z* 178, *m/z* 149, and *m/z* 134, indicating the loss of CH_3, CO_2, and CH_3 with CO_2 from the precursor, respectively (Wang, Jia, et al., 2017). Abbasi et al. (2015) have previously quantified and documented the significant concentrations of ferulic acid in the pulp and peel of Da Tainang and Xiao Tainang mangoes of Jidan cultivars.

Five out of 13 hydroxycinnamic acid derivatives (Compounds **10**, **13**, **14**, **16**, **and 19**) were tentatively established in Calypso, including feruloyl tartaric acid, *p*-Coumaric acid 4-*O*-glucoside, ferulic acid 4-*O*-glucuronide, ferulic acid 4-*O*-glucoside, and 3-feruloylquinic acid, respectively. Accordingly, Compounds (**10**) and (**14**) were selected in the negative ESI^- mode, at *m/z* 325.0570 and *m/z* 369.0845. Feruloyl tartaric acid was identified by its product ions at *m/z* 193 and *m/z* 149, demonstrating the presence of ferulic acid and tartaric acid ions (L. Yang et al., 2017), while the fragment of *m/z* 193 [M-H – glucoside, loss of 162 Da], *m/z* 178 [M-H–$C_7H_{13}O_5$, loss of 177 Da], *m/z* 149 [M-H–$C_7H_{10}O_7$, loss of 206 Da] and *m/z* 134 [M-H–$C_8H_{13}O_7$, loss of 221 Da] allowed the identification of ferulic acid 4-*O*-glucoside (Wang, Liu, et al., 2017).

3-Caffeoylquinic acid (Compound **17**) and 3-*p*-Coumaroylquinic acid (Compound **18**) were detected in Keitt samples. Compound (**17**) was characterized in ESI^- at *m/z* 353.0886 and was further confirmed by the product ions of *m/z* 253 [M-H–HCOOH–$3H_2O$] (loss of 100 Da), *m/z* 190 [M-H- $C_6H_5O_2$-$3H_2O$] (loss of 163 Da) and *m/z* 144 [M-H–$C_7H_{11}O_6$–H_2O] (loss of 209 Da) from the parent ion (Lin et al., 2019). Furthermore, 3-*p*-coumaroylquinic acid (Compound **18**) displayed a precursor ion in both ionization modes. However, results were established based on the negative ESI^-; $[M-H]^-$ at *m/z* 337.0943. The product ions of Compound (**18**) were obtained at *m/z* 265 [M-H - 72], *m/z* 173 [M-H - 164] and *m/z* 162 [M-H - 175] were due to the loss of $4H_2O$, $C_9H_7O_3$, and $C_7H_{11}O_5$, respectively (Lin et al., 2019).

Compound (**15**) was tentatively assigned as Isoferulic acid 3-sulfate, after being identified in the negative mode $[M-H]^-$ with *m/z* 273.0086, with its product ions at *m/z* 193 (M-H- 80 Da) and *m/z* 178 (M-H - 95), representing the loss of SO_3 and further loss of CH_3 (Piazzon et al., 2012). Additionally, Verbascoside (Compound **20**) was determined in the negative mode $[M-H]^-$ with *m/z* 623.1984 and 1-Sinapoyl-2-feruloylgentiobiose (Compound **21**) at [M-H] -, *m/z* 723,2,165. According to Table 3, it was deduced that Isoferulic acid 3-sulfate was exclusively present in the Kensington Pride sample of our study. Similarly, Compounds (**20**) and (**21**) remained exclusive to the Honey Gold and Keitt, respectively.

Compounds (**20**) and (**21**) has been previously characterized in mango peels by (Peng et al., 2019). However, to the best of our knowledge, limited studies stand available to suggest the presence of Compounds (**20**) and (**21**) in mango pulp. Moreover, the detection of 1-Sinapoyl-2-feruloylgentiobiose in our Keitt sample introduces curiosity, as this compound is generally found in *brassicas* and broccoli (Plumb et al., 1997).

Multiple studies have acknowledged the presence of hydroxycinnamic acids in mango pulp and their by-products (Agatonovic-Kustrin et al., 2018; Gu et al., 2019). In regard to the current study, the majority of the detected hydroxycinnamic acids were identified in their conjugated forms. It should be noted that cinnamic acids rarely exist in uncombined forms (Campa et al., 2012). That is, cinnamic acid derivatives generally occur primarily in conjugated forms, including esters (Bolzani et al., 1991), glycosylated derivatives (Herrmann, 1989), and quincic acid (Schuster & Herrmann, 1985).

Hydroxyphenylacetic acids and hydroxyphenylpropanoic acids

A total of two hydroxyphenyl acetic acids derivatives (Compounds **22** and **23**) were established in our mango pulp samples. Compound (**22**) was tentatively characterized as 3,4-dihydroxyphenylacetic acid, and compound (**23**) as 2-hydroxy-2-phenylacetic acid. Compounds (**22** and **23**) were found in both ionization modes; however, the presented data are based on $[M-H]^-$ at *m/z* 167.0357 (RT = 9.815) and *m/z* 151.0396 (RT = 10.767), respectively. It was observed that the derivatives of hydroxyphenyl acetic acids were present in the majority of our rejected mango samples, except for Palmer.

Observed in both ionization modes, 3-hydroxy-3-(3-hydroxyphenyl) propionic acid, or Compound (**24**), was the only hydroxypehylpropanoic acid derivative that was tentatively detected in our samples (Honey Gold, Palmer, Keitt, Calypso). Compound (**24**) yielded a main product ion in the negative mode $[M-H]^-$ at *m/z* 181.0513, RT = 33.927.

A study presented by Hernández-Maldonado et al. (2019) suggested that quercetin, a common flavonoid present in mangoes, is susceptible to microbial biotransformation to dihydroxyphenylpropionic acids by a C-ring cleavage. The study continued to mention the formation of hydroxyphenylacetic acids from the dehydroxylation of dihydroxyphenylacetic acids. Additional studies have also implied the liberation of hydroxyphenylacetic acids, as catabolites of mango fermentation by microbes (Low et al., 2016). However, it is essential to note that the following results were based on in vitro colonic fermentation.

As far as we are aware, metabolites of quercetin have not been previously characterized in mango pulp; however, we acknowledge quercetin as a major polyphenol in mangoes (Masibo & He, 2008). The present study further considers the possibility of quercetin and its derivatives, established in our mango samples, to microbial transformation. This has been suggested as we take into consideration the initial physical state of our rejected mango samples, which were highly susceptible to microbial spoilage.

3.4.2 | Flavonoids

Flavonoids are considered a main polyphenol class and are the most abundant polyphenol in the human diet. They are divided into several classes, including, but not limited to, anthocyanins, flavonols, flavanols, flavanones, flavones, and isoflavones. Catechin, epicatechin, quercetin, isoquercetin, fisetin, and astragalin are previously identified flavonoids in mangoes (Masibo & He, 2008). The current study was able to tentatively establish 43 flavonoids among the five samples, which were further subdivided into classes of flavonols (12), isoflavonoids (9), flavones (7), flavanol (7), anthocyanins (4), dihydrochalcones (3), dihydroflavonols (2), and flavanones (1).

Anthocyanins are acknowledged due to their antioxidant potential and their pigmenting power, with respect to coloring of fruits (Einbond et al., 2004). Silva et al. (2014) previously quantified the amount of Anthocyanins in Brazillian mango pulp to be 7.85 ± 0.80 mg/100 g dry basis. However, some Chinese varieties have shown to have very low ranging contents of total Anthocyanins (0.0001–0.0005 mg/100 ml) (Abbasi et al., 2015). Ranganath et al. (2018) observed that mangoes' total anthocyanin content varies across cultivars, with red cultivars having the highest, followed by yellow, and then green.

Four anthocyanin derivatives (Compounds **25–28**) were observed among Kensington Pride, Keitt and Calypso mango varieties in the present study. Cyanidin 3-*O*-(2-*O*-(6-*O*-(E)-caffeoyl-D glucoside)-D-glucoside)-5-*O*-D-glucoside (Compound **25**), cyanidin 3-*O*-(6″-*p*-coumaroyl-glucoside) (Compound **26**), petunidin 3-*O*-(6″-acetyl-glucoside) (Compound **27)** were detected in both positive (ESI^+) and negative modes (ESI^-) with an observed $[M\text{-}H]^-$ *m/z* at 950.2697, *m/z* 596.1508, and *m/z* 522.1369, respectively. Although cyanidin derivates have been previously detected in mango peels, our study is the first to characterize and report the presence of such compounds in mango pulp.

Flavanols

A total of seven flavanols were detected within our mango pulp samples (Compounds **34–40**). Out of the seven compounds, four of them were tentatively characterized in the Honey Gold variety of mango pulp; these include—(+)-gallocatechin (Compound **34**), procyanidin dimer B1 (Compound **35**), (+)-catechin (Compound **37**), and (+)-gallocatechin 3-*O*-gallate (Compound **40**). The occurrence of (+)-catechin, (+)-gallocatechin and its derivatives in mango have been widely recorded over multiple studies (Masibo & He, 2008; Monribot-Villanueva et al., 2019). Such compounds represent most of the phenolic fraction within an extract of *Mangifera indica* (Scartezzini & Speroni, 2000). In recent times, these compounds have received significant recognition due to their health-enhancing properties (Zanwar et al., 2014).

According to Table 3, our data suggest that only Keitt samples possessed esters of epigallocatechin (Compounds **38** and **39**). 4′-*O*-Methyl-(-)-epigallocatechin 7-*O*-glucuronide (Compound 38) was observed in the negative ionization mode $[M\text{-}H]^-$, with *m/z* 495.1138. In contrast, 4′-*O*-Methylepigallocatechin (Compound **39**) was tentatively identified in the positive ionization mode, providing an observed $[M + H]^+$ *m/z* value of 321.0965.

The occurrence of epigallocatechin esters has been previously documented in mango peels of "Haden" and "Tommy Atkins" mango variety (Coelho et al., 2019). However, to the best of our knowledge, this may be the first time recorded in mango pulp, especially in the Australian variety of Keitt. Esters of epigallocatechin have demonstrated potent anti-inflammatory and antioxidant properties in physiological processes (Nagle et al., 2006). Our present study indicates the potential of rejected Keitt mangoes for the extraction of epigallocatechin esters and its use in the nutraceutical and pharmaceutical industries.

Flavones

In the present study, it was noticed that most of the flavones detected in the mango pulp samples were mainly glycosides and C-glycosides of apigenin. The detected flavone derivatives are commonly occurring compounds found in mango, with comparative analysis suggesting that it may be five times higher than other fruits such as durian and avocado (Sumitra et al., 2010). Four out of seven flavone derivatives were tentatively characterized in the mango pulp samples as apigenin glycosides, including apigenin 7-*O*-glucuronide (Compound **42**), apigenin 7-*O*-apiosyl-glucoside (Compound **43**), apigenin 6-C-glucoside (Compound **44**), and apigenin 6,8-di-C-glucoside (Compound **45**). Apart from the apigenins, our study was able to establish cirsilineol in Honey Gold mango pulp tentatively. With the molecular formula of $C_{18}H_{16}O_7$, the following compound was detected according to the precursor ions at $[M\text{-}H]^-$ *m/z* 345.0969. The major fragments at *m/z* 330 [M + H–CH_3], *m/z* 312 [M + H–CH_3–H_2O], *m/z* 297 [M + H–2CH_3–H_2O] and 284 [M + H–CH_3–H_2O–CO] further contributed to the identification of this particular compound (Pandey & Kumar, 2016). Cirsilineol has been previously characterized in different fruit peels, including Dragon fruit, Banana, Kiwifruit, and Lemon (Suleria et al., 2020). However, to the best of our knowledge, the presence of cirsilineol in mango pulp has not yet been recorded until now.

Flavonols

In fruits and vegetables, flavonols generally present themselves as glycosides based on aglycons, such as kaempferol, quercetin, myricetin, isorhamnetin, and rhamnetin (Terahara, 2015). Isorhamnetin, tamarixetin, and kaempferol are also commonly known metabolites of quercetin. Meneses et al. (2015) were able to recover several flavonols in industrial mango waste, through supercritical antisolvent extraction. The study identified the main compounds: quercetin 3-*O*-galactoside, quercetin 3-*O*-glucoside, quercetin 3-*O*-xyloside, and quercetin 3-*O*-arabinoside, quercetin, and kaempferol.

Quercetin 3-*O*-arabinoside (Compound **59**), observed in both ionization modes, with $[M\text{-}H]^-$ at *m/z* 433.0776, was tentatively characterized in Honey Gold, Kensington Pride, and Calypso mangoes. The identity of Compound (**59**) was proven by its MS^2 fragment at *m/z* 301, corresponding to the loss of arabinoside (132 Da) from the precursor (Xiao et al., 2018).

Quercetin 3-*O*-arabinoside was previously characterized by HPLC diode array and mass spectrometric detection and was considered one of the predominant flavonol glycosides, measuring up to 5 mg/kg of mango puree concentrate (Schieber et al., 2000).

Kaempferol 3,7-*O*-diglucoside (Compound **54**), kaempferol 3-*O*-(2″-rhamnosyl-galactoside) 7-*O*-rhamnoside (Compound **53**), and kaempferol 3-*O*-glucosyl-rhamnosyl-galactoside (Compound **57**) were identified in the majority of the mango pulp samples (Honey Gold, Kensington Pride, Palmer, and Keitt), except for Calypso. Evidence of kaempferol glycosides has been previously obtained; however, it was only preliminarily characterized (Schieber et al., 2000). We believe that our study may be the first to accurately identify, characterize, and report different kaempferol glycosides present in the pulp of Honey Gold, Kensington Pride, Palmer, and Keitt varieties of Australian mango.

Other polyphenols

In brief, a total of nine other polyphenolic compounds were recorded and grouped into hydroxybenzoketones (1), hydroxycoumarins (1), phenolic terpenes (2), tyrosols (3) and other polyphenols (2).

In particular, it was noticed that Compound (**70**) was proposed as 2,3-Dihydroxy-1-guaiacylpropanone based on the observed *m/z* 211.0617, RT = 28.676; it displayed its presence in all of the mango pulp samples. Carnosic acid (Compound **74**) was only exhibited by Palmer mango pulp, with the precursor ion $[M\text{-}H]^-$, at *m/z* 331.1915. The molecular ion of carnosic acid (*m/z* 331.1905) produced the major fragment ions at *m/z* 287 and *m/z* 269, representing the loss of CO_2 and further loss of H_2O from the parent ion (Pacifico et al., 2017). Similarly, Rosmanol (Compound **75**) was only detected in Keitt variety, displaying the product ions $[M + H]^+$ at *m/z* 347.1864. The following compound was further identified by the product ions at *m/z* 301 and *m/z* 231, resulting from the loss of a unit of H_2O and CO (46 Da) and cleavage of molecules pentene, water, and carbon monoxide (Jesionek et al., 2017). Carnosic acid (CA) is a phenolic diterpene specific to the Lamiaceae family and found to be abundant in rosemary (*Rosmarinus officinalis*) (Loussouarn et al., 2017). Hu et al. (2018) have previously reported rosmaniric acid, a compound also specific to rosemary, in mango fruits of Chinese origin. However, to the best of our knowledge, this study is the first to report CA's presence in mango pulp.

Current literature has stated CA's ability to fight against oxidative stress-related diseases due to its potent antioxidative properties (Etsassala et al., 2019). CA's health benefiting properties have resulted in an increase in demand for the compound within food, especially in the nutritional health and cosmetic industries (Birtić et al., 2015). Thus, our findings suggest that rejected Palmer mangoes pose a potential candidate for the extraction and production of functional foods containing CA, as it is naturally available within the pulp.

Stilbenes and lignans

Our study revealed three stilbenes (Compound **79–81**) in the samples of Kensington Pride, Keitt, and Honey Gold, respectively. Compound (**79**) was tentatively assigned as 3′-hydroxy-3,4,5,4′-tetramethoxystilbene, yielding a characteristic peak at *m/z* 303.1238, with the precursor ion $[M + H]^+$. Compounds (**80** and **81**) corresponded to resveratrol 5-*O*-glucoside and piceatannol 3-*O*-glucoside, which produced precursor ions $[M\text{-}H]^-$ at *m/z* 389.1244 and *m/z* 405.1207, respectively. The excepted loss of glucoside (162 Da) was observed in the MS^2 spectrum of resveratrol 5-*O*-glucoside, which allowed the identification of this compound (Reed, 2009). Additionally, the presence of product ions at *m/z* 243 indicated the loss of glucoside (162 Da) from Compound (**82**), which also corresponded to its identification (Fan, 2009).

A total of five lignans were tentatively characterized in our samples, out of which three (Compounds **83**, **85**, and **86**) were present in the pulp of Honey Gold. Compounds (**82** and **84**) were tentatively characterized as Sesamin and 7-hydroxymatairesinol, respectively. Although observed in both ionization modes, the compounds were characterized according to the product ion $[M\text{-}H]^-$ at *m/z* 353.1019 and *m/z* 373.1296. Furthermore, Compound (**85**) tentatively corresponded to Conidendrin, with a characteristic peak at *m/z* 357.1323; $[M + H]^+$. To the extent of our knowledge, this is the first time lignan derivatives have been characterized and reported in the pulp of mango.

3.5 | Quantitative determination of phenolic compounds by HPLC-PDA

HPLC-PDA is a technique used widely for the separation and quantification of phenolic compounds. In the current study, a total of 10 phenolic compounds were subjected to HPLC-PDA quantification, including 5 phenolic acids (gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, chlorogenic acid, and caffeic acid) and 5 flavonoids (catechin, epicatechin, epicatechin gallate, quercetin, and kaempferol) based on the LC-ESI-QTOF-MS/MS characterization and previously discussed antioxidant activities.

Figures 2 and 3 illustrate the targeted phenolic compounds' data in all mango pulp samples quantified using HPLC-PDA. In particular, the following data suggest that chlorogenic acid and caffeic acid were the two dominant phenolic acid derivatives present in all the mango pulp samples. In contrast, it was acknowledged that gallic acid levels were relatively low in the samples, with Calypso containing negligible amounts. Abbasi et al. (2015) have previously quantified gallic acid using HPLC techniques in multiple mango varieties and cultivars grown in China. The following study reported the gallic acid contents to be 1.79 mg/100 $g_{f.w.}$ in Keitt, 1.54 mg/100 $g_{f.w.}$ in Narcissus, and 0.93 mg/100 $g_{f.w.}$ in Thai mango, which is relatively lower than the quantity recorded in our current samples (Keitt: 3.69 ± 0.18 mg/$g_{f.w.}$, Honey Gold: 3.25 ± 0.16 mg/$g_{f.w.}$, Kensington Pride: 2.14 ± 0.11 mg/$g_{f.w.}$, and Palmer: 2.39 ± 0.12 mg/$g_{f.w.}$). The difference in gallic acid content may be associated with multiple factors such as the difference in cultivars, regional discrepancies, or the physical condition of fruit samples.

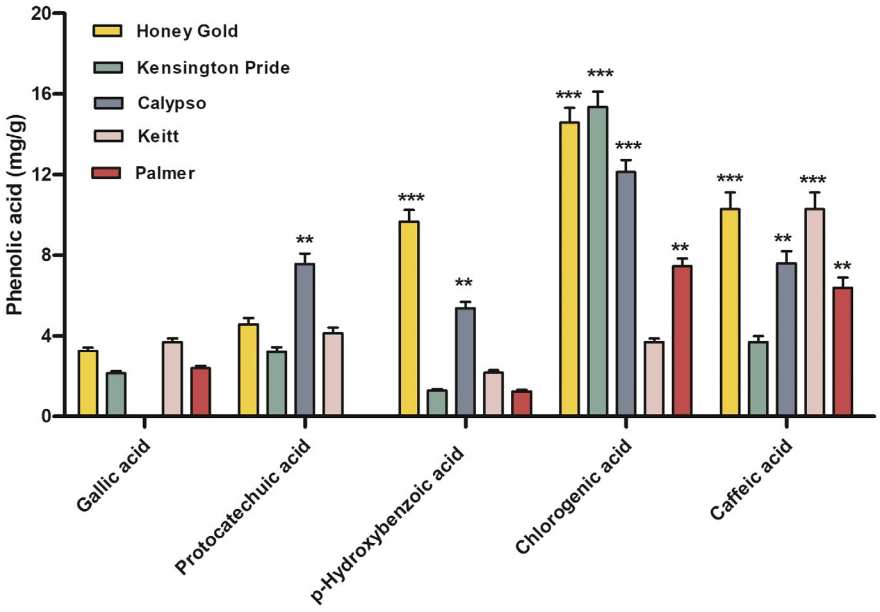

FIGURE 2 Quantification of subjected phenolic acids by high-performance liquid chromatography (HPLC) in mango pulp

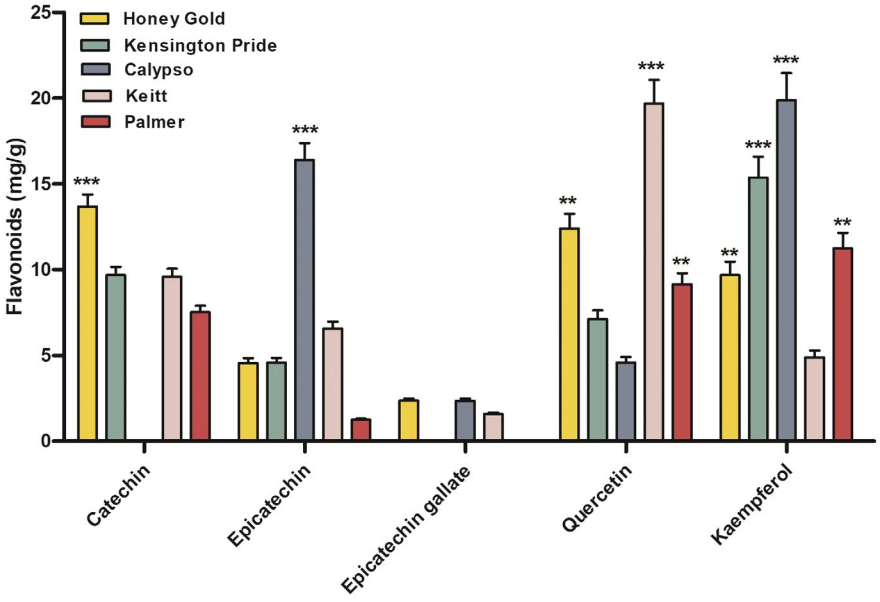

FIGURE 3 Quantification of subjected flavonoids by high-performance liquid chromatography (HPLC) in mango pulp

Quercetin and kaempferol were significant contributors to the flavonoid content in our samples. In particular, it was observed that Calypso was especially rich in flavonoids, mainly kaempferol and epicatechin. Additionally, Keitt displayed high levels of quercetin. Glycosides of quercetin have been previously quantified using HPLC diode array, measuring up to 5 mg/kg of mango puree concentrate (Schieber et al., 2000). From the following data, it may be assumed that kaempferol and epicatechin were the main contributors to Calypso's antioxidant capacity, whereas quercetin was for Keitt.

Briefly, through the characterization and quantification of phenolic compounds present in the pulp of five mango pulp samples, we can suggest the significance of rejected mango pulp phenolics. The high and robust presence of antioxidant compounds, particularly chlorogenic acid, caffeic acid, gallic acid, quercetin, and kaempferol, indicates that rejected mango pulp can be an excellent source of phenolic compounds with high antioxidant potential. Moreover, it suggests the true value and potential of rejected mangoes for the use in multiple industries.

4 | CONCLUSIONS

According to the current research, it was found that among the five varieties, the Honey Gold sample displayed higher level of phenolic compounds (TPC, TFC, and TTC) and higher antioxidant potential (DPPH, FRAP, RPA, •OH-RSA, FICA, and ABTS), whereas Kensington Pride displayed the least. With the successful application of LC-ESI-QTOF-MS/MS analysis, our study was able conclude a total of 86 different phenolic compounds within our five

samples, including 24 phenolic acids, 45 flavonoids, 3 stilbenes, 5 lignans, and 9 other polyphenols. Interestingly, it was recorded that Kensington Pride possessed the most significant number of phenolic compounds (31), followed by Keitt (30), Honey Gold (29) Calypso (28), and lastly, Palmer (14). HPLC-PDA quantification analysis suggested the significant levels of chlorogenic acid, caffeic acid, gallic acid, quercetin, and kaempferol in the five samples of rejected mango pulp.

A large body of evidence have indicated the various health benefits exerted by Mango and its by-products. With ongoing research in both animals and human cells in vitro, the body of evidence continues to evolve, supporting the contention that even rejected mango and mango by-products possess the potential to be created into a valued product by different industries. The present study suggests the significance of mango phenolics and its ability to enhance health by preventing different degenerative diseases. It further emphasizes on the utilization and incorporation of bioactive compounds present in rejected Mango and mango by-products for the development of nutritious and functional food products; primarily as an effort to prevent food waste and environmental pollution.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

AUTHOR CONTRIBUTIONS

Fung Ying Lee: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Writing-original draft. **Gia Toan Vo:** Formal analysis; Methodology; Visualization. **Colin Barrow:** Conceptualization; Funding acquisition; Investigation; Project administration; Resources; Supervision. **Frank R. Dunshea:** Conceptualization; Funding acquisition; Investigation; Project administration; Resources; Supervision. **Hafiz Ansar Rasul Suleria:** Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Writing-review & editing.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the supporting information of this article and will be provided on request.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.

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