

Article

The Effect of Juicing Methods on the Phytochemical and Antioxidant Characteristics of the Purple Prickly Pear (*Opuntia ficus indica*)—Preliminary Findings on Juice and Pomace

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Abstract: Prickly Pear (PP) is often overlooked due to its' short shelf-life. Juicing may improve marketability but often affects quality, thereby warranting investigation. Purple PP (whole (WF) and flesh (FF)) was juiced using blenders; stick (SB) and jug (JB); and juicers; commercial (CJ) and cold-pressed (CP). Juices and methanolic (70%) pomace extracts were analysed for; bioactives; Total Phenolic (TPC; $\mu\text{g}_{\text{GAE}}/\text{mL}$), Flavonoid (TFC; $\mu\text{g}_{\text{CE}}/\text{mL}$) and Betalain Content (TBC; $\text{mg}/100\text{ g}$; Betacyanin; BE; Betaxanthin; IE); and antioxidant characteristics; DPPH, FRAP (μM_{TE}) and vitamin C ($\text{mg}_{\text{AAE}}/\text{mL}$). Juicing techniques had effects on phytochemicals in; juice: TPC (WF/FF; $p = 0.022\text{--}0.025$), TFC (FF; $p = 0.034$), Betacyanin (WF/FF; $p = 0.029\text{--}0.026$), FRAP (WF/FF; $p = 0.016\text{--}0.024$) and Vitamin C (WF/FF; $p = 0.015\text{--}0.016$); and pomace: TPC (WF/FF; $p = 0.015$), TBC (FF; $p = 0.034$), Betacyanin (FF; $p = 0.047$), Betaxanthin (FF; $p = 0.017$), DPPH (WF/FF; $p = 0.016\text{--}0.024$), FRAP (WF/FF; $p = 0.015\text{--}0.023$) and Vitamin C (WF/FF; $p = 0.016\text{--}0.022$). Processing-style (blend/juice) affected; TPC, DPPH and FRAP in juice and pomace. Overall, fruit-preparation (WF/FF) had minimal effects. Additionally, correlations existed between; juice TFC and TBC ($p = 0.001$; $\tau = -0.044$); TBC and vitamin C ($p = 0.001$; $\tau = -0.637$); pomace TPC and DPPH ($p = 0.003$; $\tau = 0.440$), TPC and vitamin C ($p = 0.011$; $\tau = 0.440$); and TFC and FRAP ($p = 0.001$; $\tau = 0.519$). The best methods overall for juice were SB (FRAP), JB (TPC, TBC), CJ (TFC) and CP (DPPH, VitC); and for pomace extracts; SB(FRAP), JB (TPC, VitC), CJ(TFC), and CP (TBC, DPPH).

Keywords: juice; phytochemical; *Opuntia ficus indica*; Prickly Pear; cold pressed

1. Introduction

The Prickly Pear (PP) is a relatively short-seasoned fruit of the *Opuntia* spp. cacti family. The cactus is known for its' uses as; a source of food, traditional medicine and as a hedge or ornamental plant [1–4]. This cacti family is known globally, because of its' thriving nature, particularly in arid and semi-arid regions, which in Australia, has led to classification as a 'Weed of National Significance' (NWOS), apart from the *Opuntia ficus indica* species [4–6]. Although the species has been studied in other countries, the Australian PP has minimal research regarding the nutritional content [7].

As a source of food, the PP is eaten fresh or dried and exists in an extensive array of products [4–6], ranging from confectionary such as jams, jelly sweets, teas, tinctures and more recently, supplements, many with associated health claims [1–4]. Numerous consumption associated properties are reported including; anticarcinogenic [8–10], anti-arteriosclerotic [11,12], antiulcerogenic [13–17], hypoglycaemic [18–23] and hepatoprotective [24,25] properties.

The species of PP fruit is reportedly a good source of fibre [6], notably from the fruit seeds; numerous amino acids; as well as significant; lipid and phytosterol content [6,26–29]. From a micronutrient perspective; the fruit contains substantial mineral composition (particularly in calcium and potassium) [6,26–29], and an extensive profile of phytochemicals including bioactives; polyphenols and betalains; as well as numerous antioxidant characteristics [6,26–29]. The nutritional composition of the PP varies between fruit colour and component, as well as experimental processing methods [30].

The Australian PP as a food source, faces two major challenges; social misconception as a ‘NWOS’ and ‘farm- to- plate’ challenges. As a short seasoned, the fruit is associated with agricultural distribution challenges, primarily stemming from seasonal availability and short shelf life in a vast country. Therefore, there is an increasing need to modify the fruit during the harvesting season and extend the shelf life of the fruit via the development of products such as juice.

From a food preservation perspective, juicing is a fast and readily available processing method that requires minimal preparation. Although ideal for timely large-scale fresh-produce processing with minimal associated costs, juice processing is limited by short shelf-life, particularly for fresh fruit juices [31]. Additional challenged include; nutritional degradation associated with commercial processes, including the commonly known degradation of vitamin C (VitC) with heat processing such a pasteurisation [32]; and dependent on processing method, juice yield and waste output.

Agriculturally accessible ‘on-site’ juice processing technologies are extensive, ranging from; blender-style processing [33–38], commercial and cold-press juicing [35,39,40], as well as various forms of manual pressing [41]. The effect of juicing technologies on nutritional content, is studied [35] with regard to; apple, pear, persimmon and mandarin fruits, where the effect of processing methods on phytochemical content and was demonstrated to be statistically significant ($p < 0.05$) between juice-style and blender-style techniques.

Although the nutritional content of PP juice has been reported to be nutrient dense and have number of potentially health beneficial compounds, the comparison of various juicing techniques on the PP fruit juice composition has not been investigated. Therefore, the aim of this study is to determine the effect of several readily available juicing techniques on the physicochemical, phytochemical and bioactive composition of the Australian grown purple prickly pear (*Opuntia ficus indica*).

2. Materials and Methods

2.1. Study Design

The effect the various juicing and blending techniques on PP fruit juice and pomace (waste) was determined, in triplicate, by investigating the phytochemical content, specifically antioxidant characteristics (DPPH, FRAP and VitC) and bioactive content (Total Betalain; Phenolic and Flavonoid content). Physicochemical properties (pH, titratable acidity (TA), Total Soluble Sugars (TSS; Brix°) were also determined and compared.

Fresh purple PP fruits from the same location and harvest were prepared from fresh and randomly assigned to ‘pools’, representing a typical harvest and variations in maturity available to consumers. Once prepared, pooled fruits were juiced as; whole fruits (WF), and as peeled fruit flesh (FF), containing seeds. Purple type fruits were juiced using four juicing techniques; jug-blender, stick-blender, commercial-juicer and cold-press juicer, following a ‘homogenise and filter’ protocol [35]. Double-strained juice and waste were collected and stored separately in 50 mL light protected tubes at $-18\text{ }^{\circ}\text{C}$.

2.2. Chemicals

All chemicals, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Sodium nitrate, Sodium phosphate 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Copper(II) Chloride dehydrate, Ammonium Acetate, Ammonium molybdate, Neocuprine, Potassium persulfate, Sodium Acetate trihydrate, Iron (III) Chloride hexahydrate, Folin Ciocalteu reagent, Sodium Carbonate, Gallic Acid, Aluminium Chloride, sodium hydroxide, Catechin, L-Ascorbic Acid, Glacial acetic acid, 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), Methanol, Ethanol, Hydrochloric Acid and Sulfuric Acid were purchased from Sigma Aldrich (Castle Hill, Sydney, NSW, Australia). The De-ionised (DI) water was prepared on the day using the Millipore water purification system with resistivity greater than 18 M Ω cm (Millipore Australia, North Ryde, NSW, Australia).

2.3. Sample Preparation

Fresh purple PP fruits of the same morning harvest (February 2017) were donated from 'Chiron Health Products' (Victoria, Australia) farm (36°25'37.2 S; 143°37'55.2 E) and within 24 h of the morning harvest, transported on ice to the University of Canberra Food Science Laboratory. On receipt, fresh samples were washed under cold running water and gently rubbed with gloved hands to remove the prickles. Fruits mass of randomly pooled samples (~1 kg) was recorded and juiced either as: WF (randomly pooled); or appropriately peeled (randomly pooled). The prepared samples were stored separately, in darkness at -18 °C prior to juicing due to the fruit short harvest season and shelf life [33–38].

2.4. Juicing Methods and Pomace Separation

After thawing at 4 °C for 12 h, the WF (pooled) and FF (pooled) samples were homogenised individually (~1 kg) and juiced using four different commercially available juicer techniques; the stick-blender (Sunbeam Stickmaster SM7200, Florida, FL, USA); jug-blender (Kenwood Blend-X Classic BLP615WH, Sydney, NSW, Australia); commercial-juicer (Breville BJE200, Melbourne, Victoria, Australia) or a cold-press juicer (Oscar NeoDA100, Brisbane, Queensland, Australia) [35,39,40]. The individual fruit slurries were strained through wire mesh (0.8 mm) to separate the juice from pomace. The obtained juice and pomace were stored separately, in darkness at -18 °C prior to further analysis [33–38].

2.5. Titratable Acidity (TA), Total Soluble Solids (TSS) and pH

The juice and pomace of WF and FF samples were thawed at 4 °C prior to analysis. Samples that were exceeding the measurement (due to upper limits of instrument detection) were diluted in DI water (18 M Ω cm) prepared on the day, prior to analysis.

Titrate Acidity (TA) of diluted juices (10%; WF and FF), were determined by titration against 0.1M NaOH until pH of 8.1 was achieved, where values were expressed as a percentage [42,43]. NaOH was aliquoted until a pH of 8.1 was achieved, determined by a pH meter 'Butech Instruments pH 700' (Mettler Toledo, Port Melbourne, VIC, Australia).

The pH of the various juices was determined using a pH meter (Butech Instruments pH 700; Mettler Toledo, Victoria, Australia).

The Total Soluble Solids (TSS) were determined using handheld refractometer (OPTi Digital Hand Held Refractometer, Bellingham and Stanley, UK) following the procedures previously described and expressed as °Brix [42].

2.6. Extraction of Phytochemicals from Pomace

The phytochemical content of thawed pomace after juicing, were extracted according to Moussa-Ayoub et al., 2014 [44]. Briefly, 1 g of pomace was suspended in 10 mL of Methanol (70%

v/v) and sonicated (FXP4, Ultrasonics, Sydney, NSW, Australia) for 15 min at maximum power [44]. Samples were cooled on ice and stored in darkness at 4 °C until further analysis.

2.7. Bioactive Content (Total Betalain Content (TBC), Total Polyphenolic Content (TPC) and Total Flavonoid Content (TFC))

2.7.1. Total Betalain Content

The Total Betalain Content (TBC) was determined following the modified method by Parakash Maran et al., 2013 [45] and values were calculated using the formula proposed by Moßhammer et al., 2006 [46]. Briefly, the absorbance's of diluted fruit juices and methanolic pomace extracts (10% *v/v*) a were measured at 482, 532 and 600 nm (NovaspecPlus, GE Healthcare, Castle Hill, NSW, Australia).

The Betacyanin (BE) and Betaxanthin (IE) content was estimated relative to the maximum absorbance, molecular weight and coefficient extinction of betanin and indicaxanthin following the formula [45,46]:

$$etalain (mg/100g) = \frac{(A_{max} - A_{600nm}) \times Dilution Factor \times MW \times 100}{\epsilon \times 1} \quad (1)$$

Betacyanin; $A_{max} = 532$ nm, $MW = 550$ g/mol and $\epsilon = 60,000$ Lmol⁻¹cm⁻¹ (betanin equivalent (BE)); and betaxanthin; $A_{max} = 482$ nm, $MW = 308$ g/mol and $\epsilon = 48,000$ Lmol⁻¹cm⁻¹ (indicaxanthin equivalent (IE)) [45,46].

TBC was calculated as a sum of BE and IE values, expressed as mg/mL of total betalain content [45,46].

2.7.2. Total Phenolic (TPC) and Flavonoid (TFC) Content

The Total Phenolic (TPC) and Flavonoid (TFC) content of the thawed juice (WF and FF) and methanolic pomace extracts were determined spectrophotometrically (NovaspecPlus, GE Healthcare, Castle Hill, NSW, Australia). The TPC of the juice or pomace was determined as of the 'Folin Ciocalteu' method [47,48] and expressed as Gallic acid (GAE) equivalents ($\mu\text{g}_{\text{GAE}}/\text{g}$) [47]. The TFC, expressed as Catechin Equivalents (CE) ($\text{mg}_{\text{CE}}/\text{g}$), was determined following the aluminium chloride method [49].

2.7.3. Antioxidant Characteristics (DPPH and FRAP)

The antioxidant characteristics of thawed juices (WF and FF) and methanolic pomace extracts were measured spectrophotometrically (NovaspecPlus, GE Healthcare, Castle Hill, NSW, Australia) following the Free Radical Capacity (DPPH) and antioxidant capacity by ferric reducing antioxidant power (FRAP) [50,51] methods. All values were expressed in μM_{TE} Trolox (TE) equivalents/g.

2.7.4. Vitamin C Content

The VitC content of thawed juices (WF and FF) and methanolic pomace extracts were determined using the molybdite method [52–54]. Samples were combined with a working reagent (0.6 M Sulfuric Acid, Sodium phosphate and Ammonium molybdite). Absorbances were determined spectrophotometrically (NovaspecPlus, GE Healthcare, Castle Hill, NSW, Australia). Samples were expressed as L-Ascorbic Acid Equivalents ($\text{mg}_{\text{AAE}}/\text{g}$) [53].

2.8. Statistical Analysis

All samples were analysed in triplicates, where values were represented as 'mean \pm standard deviation' if the variance was small, or 'mean \pm standard deviation (median)' if not normally distributed. Values were reported as per 'Rule of Decimals' [55], exceptions to this include limitations of the equipment. Juicing methods were compared based on phytochemical content using a Kruskal-Wallis test, where statistical significance was defined as $\alpha < 0.05$. The differences between (i) style of juicing (blend vs. juice-type processing) and (ii) fruit preparation (WF vs. FF) were investigated

using Mann-Whitney U test. Correlations were determined to assess the strength and direction between two antioxidant or bioactive characteristics within PP fruit juice or pomace extracts using a Kendall-tau B (two-tailed; τ) between investigated compounds. Comparison of the four juicing methods based on phytochemical content were determined using the 'Statistical Processing Package for Social Sciences' (SPSSv23, IBM, Australia).

3. Results and Discussion

3.1. Physiochemical Characteristics

3.1.1. Juice Yield

Juice yield (%) (Table 1) of purple PP fruit, juiced as WF and FF, is reported to range between 65.83% to 85.32%. The highest juice yield was measured in stick-blended juice (FF; 85.32%) followed by cold-pressed (WF; 84.53%) and jug-blend (FF; 82.29%) juice. The preparation of the fruit; juiced (WF/FF; $p = 0.404$) and; type of processing method (blend vs. juice; $p = 0.703$) was not significant, when considering juice yield ($p = 0.404$).

Table 1. Juice yield, titratable acidity, total soluble solids and pH of purple Prickly Pear fruit juices.

| Fruit Component | Stick Blend | | Jug Blend | | Commercial Juice | | Cold Press Juicer | |
|-----------------|-------------|-------------|-------------|------------|------------------|-------------|-------------------|-------------|
| | WF | FF | WF | FF | WF | FF | WF | FF |
| Juice yield (%) | 69.72 | 85.32 | 68.01 | 82.29 | 65.83 | 67.13 | 84.53 | 75.76 |
| TA (%) | 0.19 ± 0.03 | 0.16 ± 0.04 | 0.18 ± 0.03 | 0.2 ± 0.05 | 0.18 ± 0.02 | 0.24 ± 0.09 | 0.23 ± 0.01 | 0.14 ± 0.01 |
| TSS (Brix°) | 13.8 | 13.0 | 11.0 | 10.7 | 10.3 | 10.8 | 10.8 | 10.4 |
| pH | 5.65 | 5.92 | 5.72 | 5.70 | 5.83 | 5.50 | 5.66 | 5.97 |
| pH Temp (°C) | 13.5 | 15.5 | 15.8 | 14.9 | 16.2 | 15.0 | 16.4 | 17.2 |

Note: Values represented as mean ± standard deviation; 'WF' Whole fruit; 'FF' Fruit Flesh; 'TA' Titratable Acidity; 'TSS' Total Soluble Solid; pH and temperature have a STD of 0.

3.1.2. Titratable Acidity

Titrateable Acidity (TA; %) is an indicator of total acid content and reported a better predictor of acid contents impact on flavor, than alternate measures including pH [56]. According to Pyo et al. (2014) the main acids in fruit juices to include citric, malic and ascorbic acids [35]. Titrateable acidity (TA; %; Table 1) in Australian grown purple PP fruit ranged between $0.14 \pm 0.01\%$ (FF; cold-press)- $0.24 \pm 0.09\%$ (FF; commercial-juice). The results reported in Table 1 were consistent with those of previous literature [37,57] with the highest content using the in commercial juicer (FF; $0.24 \pm 0.09\%$) followed by cold-pressed (WF; $0.23 \pm 0.01\%$), jug-blend (FF; $0.2 \pm 0.05\%$) and stick-blend (WF; $0.19 \pm 0.03\%$). Fruit components processed did not differ significantly (WF vs. FF; $p = 0.778$), nor did type of processing methods (blend vs. juice; $p = 0.664$). Although, Pyo et al. (2014) reports different associations when investigating the predominate acids of fruit juices; to be greater in blended kernel fruit juices for malic and citric acid contents, rather than juices, which contained greater amounts of ascorbic acids [35].

3.1.3. Total Soluble Solids (TSS)

Total Soluble Solids (TSS; °Brix), in addition to firmness, are measures of internal quality attributes in determining fruit maturity [58]. The TSS is reported to increase with ripeness and is used as an indicator of soluble simple sugars or acids, reported to predominantly include sugars; glucose, fructose and sucrose; or acids; citric, ascorbic and mallic acids [59]. TSS of purple PP fruit (Table 1) is reported to range between 10.3°Brix (WF; Jug-blend) to 13.8°Brix (WF; stick-blend). No significant differences were observed between the fruit components (WF vs. FF; $p = 0.426$) or between the different types of processing (blend vs. juice; $p = 0.147$).

3.1.4. pH

In addition to TA, pH can be used in sensory analysis to detect and establish the potential for bitterness and composition of different food products [56,60]. It is well established that pH varies depending on juice composition, particularly with relations to common fruit acids (malic, citric, lactic and ascorbic) [60]. The pH, (Table 1) ranged in purple PP juice between 5.5 (FF; commercial-juice)-5.97 (FF; cold-pressed). No significant differences were determined between fruit preparation methods (WF vs. FF; $p = 0.725$) or style of processing (blend vs. juice; $p = 0.964$).

3.2. The Phytochemical Content of Prickly Pear Fruit Juice and Methanolic Pomace Extracts

3.2.1. Total Polyphenol Content (TPC) of Prickly Pear Fruit Juice and Methanolic Pomace Extract

Total Phenolic Content (TPC) includes the cumulative content of compounds including subgroups of flavonols and betalains [61]. Phenols are a large group of bioactive-phytochemicals that exhibit numerous effects including antioxidant and anti-inflammatory effects [35]. A study by Pyo et al. (2014) has demonstrated TPC and TFC can be significantly affected by juicing methods (juice vs. blend; $p < 0.05$) in Korean kernel fruits [35] however, this was not investigated in the juice waste product commonly referred to as pomace.

The juice of the Australian-grown purple PP fruit contained TPC, ranging from $1516 \mu\text{g}_{\text{GAE}}/\text{mL}$ (FF; stick-blend)- $3031 \mu\text{g}_{\text{GAE}}/\text{mL}$ (WF; jug-blend) (Table 2). The highest TPC were in jug-blended juices, followed by commercially-juiced (FF; $2015 \pm 142 \mu\text{g}_{\text{GAE}}/\text{mL}$), cold-press ($1524 \pm 125 \mu\text{g}_{\text{GAE}}/\text{mL}$) and lastly, stick-blended juices (WF; cold-press ($2301 \pm 89.8 \mu\text{g}_{\text{GAE}}/\text{mL}$)). The findings of this study indicate that juicing method has a significant effect on purple PP fruit juices, processed as both; WF ($p = 0.022$) and FF ($p = 0.025$). Additionally, style of processing (blending vs. juicing) also had an effect ($p = 0.001$), although fruit preparation (whole fruit vs. fruit flesh) did not ($p = 0.100$).

Table 2. Total Polyphenolic, Flavonoid and betalain content, as well as individual Betacyanin and Betaxanthin of purple Prickly Pear fruit juices and corresponding methanolic (70% v/v) pomace extracts.

| | STICK BLEND | | Fruit Preparation | Jug Blend | | Fruit Preparation | Commercial Juicer | | Fruit Preparation | Cold Press | | Fruit Preparation | KRUSKAL-WALLIS | | Style of Processing |
|--|--------------------------|--------------------------|-------------------|---------------------------|--------------------------|-------------------|--------------------------|--------------------------|-------------------|--------------------------|--------------------------|-------------------|----------------|---------|---------------------|
| | WF | FF | p | WF | FF | p | WF | FF | p | WF | FF | p | WF | FF | p |
| Prickly Pear fruit Juice | | | | | | | | | | | | | | | |
| TPC (GAE) | 2301 ± 89.8 (2316) | 1516 ± 162 (1560) | 0.100 | 3031 ± 73.9 (3060) | 2432 ± 27.4 (2419) | 0.100 | 1048 ± 0.001 (1048) | 2015 ± 142 (1987) | 0.100 | 1001 ± 288 (1079) | 1524 ± 125 (1503) | 0.100 | 0.022 * | 0.025 * | 0.001 * |
| TFC (CE) | 396 ± 51.6 (409) | 346 ± 8.58 (347) | 0.400 | 396 ± 45.6 (422) | 273 ± 24.8 (269) | 0.100 | 428 ± 68.6 (409) | 266 ± 6.14 (264) | 0.100 | 291 ± 35.4 (277) | 382 ± 54.4 (370) | 0.100 | 0.091 | 0.038 * | 0.590 |
| TBC | 0.269 ± 0.107 (0.210) | 0.491 ± 0.013 (0.492) | 0.100 | 0.395 ± 0.087 (0.372) | 0.709 ± 0.105 (0.560) | 0.100 | 0.431 ± 0.017 (0.434) | 0.555 ± 0.85 (0.596) | 0.100 | 0.287 ± 0.038 (0.292) | 0.377 ± 0.064 (0.387) | 0.200 | 0.086 | 0.053 | 0.514 |
| Betacyanin (BE) | 0.086 ± 0.049 (0.081) | 0.172 ± 0.013 (0.165) | 0.100 | 0.199 ± 0.019 (0.205) | 0.563 ± 0.038 (0.236) | 0.100 | 0.212 ± 0.006 (0.215) | 0.273 ± 0.040 (0.293) | 0.100 | 0.126 ± 0.017 (0.132) | 0.182 ± 0.045 (0.196) | 0.200 | 0.029 * | 0.036 * | 0.551 |
| Betaxanthin (IE) | 0.182 ± 0.067 (0.169) | 0.319 ± 0.017 (0.313) | 0.100 | 0.196 ± 0.080 (0.194) | 0.309 ± 0.020 (0.314) | 0.100 | 0.219 ± 0.012 (0.219) | 0.282 ± 0.045 (0.303) | 0.200 | 0.161 ± 0.021 (0.160) | 0.195 ± 0.021 (0.191) | 0.200 | 0.459 | 0.053 | 0.178 |
| Methanolic (70%) Pomace extract | | | | | | | | | | | | | | | |
| TPC (GAE) | 44.5 ± 0.072 (44.4) | 25.8 ± 0.444 (25.7) | 0.100 | 23.0 ± 17.1 (32.7) | 28.3 ± 0.494 (28.5) | 0.700 | 41.96 ± 0.106 (42.0) | 37.2 ± 0.327 (37.3) | 0.100 | 41.6 ± 0.049 (41.6) | 40.2 ± 0.024 (40.2) | 0.100 | 0.015 * | 0.015 * | 0.039 * |
| TFC (CE) | 12.78 ± 1.34 (13.3) | 9.86 ± 0.048 (9.58) | 0.100 | 7.92 ± 0.417 (7.92) | 9.86 ± 0.636 (10.0) | 0.100 | 9.19 ± 0.868 (7.92) | 8.75 ± 1.10 (8.33) | 0.400 | 8.75 ± 1.10 (8.33) | 10.3 ± 2.30 (9.17) | 0.400 | 0.066 | 0.476 | 0.101 |
| TBC | 0.014 ± 0.001 (0.014) | 0.011 ± 0.001 (0.011) | 0.100 | 0.040 ± 0.049 (0.012) | 0.009 ± 0.003 (0.008) | 0.200 | 0.047 ± 0.064 (0.100) | 0.015 ± 0.005 (0.012) | 0.700 | 0.898 ± 2.29 (0.008) | 0.030 ± 0.006 (0.029) | 0.100 | 0.430 | 0.034 * | 0.551 |
| Betacyanin (BE) | 0.006 ± 0.001 (0.006) | 0.004 ± 0.001 (0.004) | 0.100 | 0.023 ± 0.0307 (0.008) | 0.003 ± 0.003 (0.002) | 0.200 | 0.026 ± 0.039 (0.003) | 0.007 ± 0.004 (0.005) | 0.700 | 0.029 ± 0.485 (0.004) | 0.013 ± 0.003 (0.012) | 0.100 | 0.730 | 0.047 * | 0.319 |
| Betaxanthin (IE) | 0.009 ± 0.002 (0.009) | 0.008 ± 0.001 (0.008) | 0.400 | 0.016 ± 0.018 (0.008) | 0.006 ± 0.001 (0.006) | 0.700 | 0.021 ± 0.025 (0.007) | 0.008 ± 0.001 (0.008) | 0.700 | 0.009 ± 0.004 (0.004) | 0.018 ± 0.006 (0.014) | 0.100 | 0.148 | 0.017 * | 0.630 |

Note: All values represented as 'Mean ± Standard Deviation (Median)' (no range reported owing to data being preliminary); **WF**—whole fruit; **FF**—Fruit flesh; **TPC**—Total Phenolic Content ($\mu\text{g}/\text{mL}_{\text{GAE}}$); **TFC**—Total Flavonoid Content ($\mu\text{g}/\text{mL}_{\text{CE}}$); **TBC**—Total Betalain Content ($\text{mg}/100 \text{ mL}$); **IE**—Total Betaxanthin Content ($\text{mg}_{\text{IE}}/100 \text{ mL}$); **BE**—Total Betaxanthin Content ($\text{mg}_{\text{BE}}/100 \text{ mL}$); * indicates a statistically significant difference (* $p < 0.05$).

The pomace generated during the juicing of purple PP was tested as a methanolic extract for the bioactive composition and antioxidant activity. The TPC of the purple PP pomace ranged between $23.0 \pm 17.1 \mu\text{g}_{\text{GAE}}/\text{mL}$ (WF; jug-blend) to $44.4 \mu\text{g}_{\text{GAE}}/\text{mL}$ (WF; stick-blend). The TPC was affected by juicing method, in juice derived from FF ($p = 0.015$) but not in juice derived from WF ($p = 0.015$). Additionally, style of processing (blend vs. juice) was also found to have an effect ($p = 0.039$) overall on TPC in pomace but fruit preparation did not ($p = 0.100\text{--}0.700$).

3.2.2. The Total Flavonoid Content (TFC) of Prickly Pear Fruit Juice and Methanolic Pomace Extracts

A study by Pyo et al. (2014) reported that the TFC of Korean kernel fruit juices vary ($p < 0.05$) between juice-type and blender-type processing [35]. Additionally, same study has identified that there were significant differences between methods of preparation in blended juices, in three of the four kernel fruit investigated [35]. The findings of our study (Table 2) indicate that juicing methods affects the TFC of purple PP fruit juices, that were processed as FF ($p = 0.038$), but not WF ($p = 0.091$). Pyo et al. (2014) observations in kernel fruit were not consistent with our findings in PP fruit juices, as no significant differences in TFC were drawn between blender-style and juice-style processing ($p = 0.590$), nor preparation of fruit (WF vs. FF; $p = 0.100\text{--}0.400$). The TFC composition of the purple PP ranged between commercially-juiced $266\text{--}428 \mu\text{g}_{\text{CE}}/\text{mL}$ (FF; WF). Conclusively, the highest TFC contents of the purple PP juices were reported to be using the commercial juicer ($428 \pm 68.6 \mu\text{g}_{\text{CE}}/\text{mL}$) followed by; jug-blender ($396 \pm 45.6 \mu\text{g}_{\text{CE}}/\text{mL}$), stick-blender ($396 \pm 51.6 \mu\text{g}_{\text{CE}}/\text{mL}$), and cold-pressed ($382 \pm 54.4 \mu\text{g}_{\text{CE}}/\text{mL}$).

Interestingly, corresponding PP pomace extracts did not share similar results (Table 2). Significant differences in TFC content were not observed between the different juicing methods in the pomace extracts, nor processing (blend vs. juice; $p = 0.101$) or fruit preparation ($p = 0.100\text{--}0.400$). In addition, there was no significant relationship between the TPC and TFC ($\tau = 0.658$). The TFC in pomace extracts ranged between $7.92 \mu\text{g}_{\text{CE}}/\text{mL}$ (WF; commercially-juiced) to $12.78 \mu\text{g}_{\text{CE}}/\text{mL}$ (WF; stick-blend). Resultantly, the highest contents were reported in pomace derived after stick-blending, followed by; cold-pressed (FF; $10.3 \pm 2.3 \mu\text{g}_{\text{CE}}/\text{mL}$), jug-blend (FF; $9.86 \pm 0.636 \mu\text{g}_{\text{CE}}/\text{mL}$) and commercially-juiced (WF; 9.19 ± 0.860).

3.2.3. Total Betalain, Betacyanin and Betaxanthin Content of Prickly Pear Fruit Juice and Methanolic Pomace Extracts

Total Betalain, Betacyanin and Betaxanthin Content of Prickly Pear Fruit Juice

The betalain composition is associated with yellow (betaxanthins) to red (betacyanin's) colored plant materials and it is widely studied in variety of different plants including; beetroot, pomegranate and amaranth [62]. The variation in composition of betalain subgroups (betaxanthin and betacyanin's) allow for the wide variation in colored plant materials today [62]. The betalains are a bioactive group of compounds [62] with in vitro and in vivo antioxidative [63–66] and antilipidemic [67–70] effects. The broader stability of betalains, when compared to other natural dyes, has allowed these compounds to be utilised as food dyes, and also promotes the use of purple PP juice as an alternative during the product development [71].

The findings of this study (Table 2; Figure 1) indicate that the juice derived from purple Australian PP contains the TBC with ranges from $0.269 \text{ mg}/100 \text{ mL}$ (WF; stick-blend) to $0.709 \text{ mg}/100 \text{ mL}$ (FF; jug-blend). The juices derived from different fruit components (WF vs. FF) did not demonstrate significant differences in TBC. Juice processing ($p = 0.053\text{--}0.086$) and style of processing ($p = 0.100$) did not affect the TBC significantly. The highest contents per method were observed in juices derived from fruit flesh for jug-blended ($0.560 \text{ mg}/100 \text{ mL}$) juices followed by; commercially juiced ($0.555 \text{ mg}/100 \text{ mL}$) stick-blend ($0.491 \text{ mg}/100 \text{ mL}$), and lastly, cold-pressed ($0.377 \text{ mg}/100 \text{ mL}$).

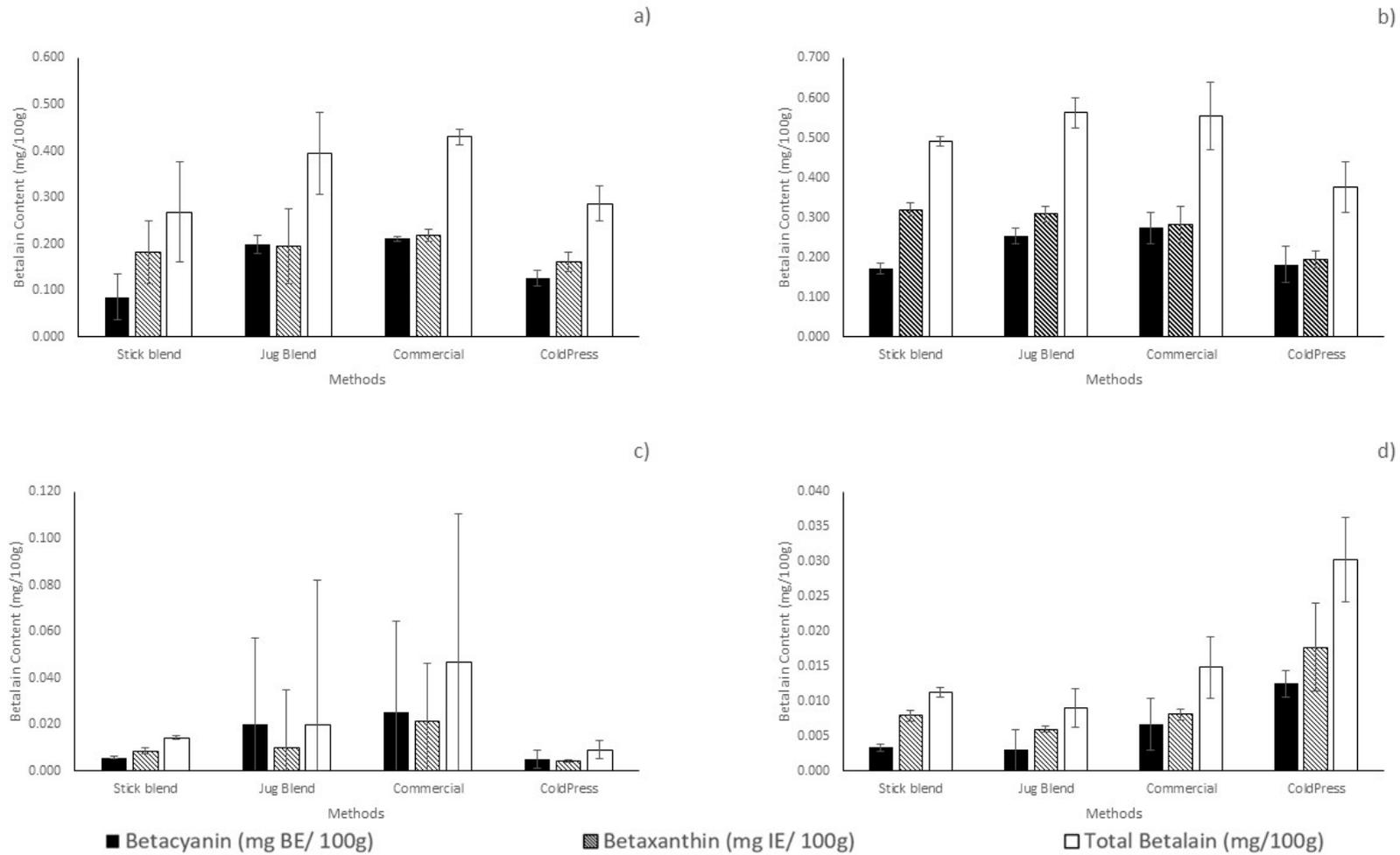


Figure 1. The Betalain Composition (mg/100 mL) of Prickly Pear fruit juice and methanolic pomace extracts: (a) Whole fruit (WF) juice (b) Fruit flesh (FF) juice (c) Whole Fruit (WF) pomace extract (d) Fruit Flesh (FF) pomace extract. Note: All values represented as Mean ± SD.

The juice derived from the purple PP juice (Table 2; Figure 1) contained 0.086–0.563 mg_{BE}/100 mL of betacyanin and 0.161–0.319 mg_{IE}/100 mL of betaxanthin. In the presented study, the juicing methods were shown to have a significant effect on betalain constituents, betacyanin, juiced as WF ($p = 0.029$) and FF ($p = 0.036$) but not betaxanthin. Style of juicing methods ($p = 0.178$ – 0.551) did not influence either sub-groups content, nor did fruit preparation ($p = 0.100$ – 0.200). The highest contents of betacyanin were measured in FF juiced using the jug-blender (0.563 ± 0.038 mg_{BE}/100 mL), followed by the juices obtained by; commercial juicer (0.273 ± 0.040 mg_{BE}/100 mL), cold-press (0.182 ± 0.045 mg_{BE}/100 mL), stick blend (0.172 ± 0.013 mg_{BE}/100 mL). The highest betaxanthin contents were reported in juices derived after the use of the stick-blender (0.319 ± 0.017 mg_{IE}/100 mL) followed by juices obtained after the use of; jug-blender (0.309 ± 0.020 mg_{IE}/100 mL), commercial-juicer (0.219 ± 0.012 mg_{IE}/100 mL) and cold-press juiced (0.182 ± 0.045 mg_{IE}/100 mL).

Total Betalain, Betacyanin and Betaxanthin Content of Prickly Pear Methanolic Pomace Extracts

The TBC was found to be in the range of 0.030–0.089 (FF; WF; cold-press). Like the values obtained for fruit juice, the TBC of PP pomace extracts had significant variations between juicing techniques in fruit juiced as FF ($p = 0.034$) but not as a WF ($p = 0.430$). The style of processing was also found to not affect the TBC in pomace extracts ($p > 0.05$). Method of fruit preparation ($p = 0.100$ – 0.700) and style of processing ($p = 0.551$) were found to not be significantly affected. The highest contents, by juicing method, were reported in cold-pressed juice extracts (WF; 0.898 ± 2.29 mg/100 mL) followed by; commercially-juiced (WF; 0.047 ± 0.064 mg/100 mL), jug-blended (WF; 0.040 ± 0.04 mg/100 mL) and stick-blended (FF; 0.014 ± 0.01 mg/100 mL).

The PP pomace extracts also contained varying levels (Figure 1) of betacyanin and betaxanthin constituents (Table 2). The betacyanin content of pomace extracts ranged between 0.003 mg_{BE}/100 mL (FF; jug-blended) to 0.029 mg_{BE}/100 mL (WF; cold-pressed), where betaxanthin is reported to range between 0.006 (FF; jug-blend) to 0.021 (WF; cold-pressed). The highest amounts of betacyanin was reported in the pomace of cold-pressed (WF; 0.029 ± 0.485 mg_{BE}/100 mL) followed by; commercially-juiced (WF; 0.026 ± 0.039 mg_{BE}/100 mL), jug-blend (WF; 0.023 ± 0.307 mg_{IE}/100 mL), and stick-blended (WF; 0.006 ± 0.001 mg_{BE}/100 mL) and products. Again, betaxanthin content was at its greatest in the pomace of commercially-juiced (WF; 0.021 ± 0.025 mg_{IE}/100 mL) followed by; cold-pressed (FF; 0.018 ± 0.006 mg_{IE}/100 mL); jug-blended (WF; 0.016 ± 0.006 mg_{IE}/100 mL) and lastly, stick-blended (WF; 0.009 ± 0.002 mg_{IE}/100 mL) juices. Both betacyanin ($p = 0.047$) and betaxanthin ($p = 0.017$) constituents had significant differences in content between juicing methods, in FF juices but not in WF. Such variation may be a result of differences in composition between products including PP fruit skins (WF) Additionally, fruit preparation ($p = 0.100$ – 0.700) and processing methods (blend vs. juice; $p = 0.630$) did not affect betacyanin and betaxanthin contents within purple PP juice pomace extracts.

3.3. Antioxidant Characteristics (DPPH, FRAP and Vitamin C)

Phytochemical-bioactive compounds exhibit antioxidant activities and have antioxidant effects [61]. Antioxidant characteristics; Free Radical Scavenging Activity (DPPH; μM_{TE}), Antioxidant Capacity by Ferric Reducing Antioxidant Power (FRAP; μM_{TE}) and Vitamin C (VitC; mg_{AA}/mL) provide an indication of potential antioxidant activity of the bioactive compounds within the PP fruit, including those investigated (Table 2). The study by Pyo et al. (2014) reported that in three of the four Korean kernel fruits investigated, juicer-processed juice had a higher antioxidant content than juices processed by blenders [35]. It was also reported fruits juiced as a WF to exhibit a higher antioxidant activity [35] probably due to the higher antioxidant content in skin. Our findings (Table 3) however, indicated that style of processing (blend vs. juice) only affected DPPH (juice $p = 0.039$; pomace; $p = 0.028$) and FRAP (pomace; $p = 0.024$). Similarly, the different juicing methods did have significant effects on DPPH (pomace; $p = 0.016$ – 0.024), FRAP (juice $p = 0.016$ – 0.024 ; pomace; $p = 0.015$ – 0.023) and VitC (juice $p = 0.015$ – 0.016 ; pomace $p = 0.016$ – 0.022) antioxidant characteristics. Additionally, preparation of the

fruit (WF vs. FF) predominately did not affect the measured characteristics. Supporting associations between bioactives content and antioxidant characteristics were also demonstrated within PP fruit juice and pomace extracts, when compared by Kendall's tau-B analysis of correlations. The PP juices were found to demonstrated significant inverse relationships between; TFC and TBC ($p = 0.001$; $\tau = -0.044$); and TBC and VitC ($p = 0.001$; $\tau = -0.637$). Interestingly, this was not observed in the PP fruit pomace although there were significant correlations in PP pomace extracts between; TPC and DPPH ($p = 0.003$; $\tau = 0.440$); TPC and VitC ($p = 0.011$; $\tau = 0.440$); and TFC and FRAP ($p = 0.001$; $\tau = 0.519$).

3.3.1. Free Radical Capacity (DPPH) of Prickly Pear Fruit Juice and Methanolic Pomace Extracts

The DPPH values in the juice of the Australian grown purple PP did not vary in content between the studied juice methods, nor were they affected by style of processing (Table 3). Preparation of fruit (WF vs. FF) did not have an effect ($p = 0.100$) on DPPH characteristics. The findings of our study disagreed with those of Pyo et al. (2014) who found significant variations in DPPH content between types of juicing methods, blend and juice, in three of the four fruits studied [35]. Variations between the two studies may include investigation into different fruits, protocol of processing methods or differences in analysis. Conclusively, Australian grown purple PP fruit juices varied in DPPH characteristics between $667 \mu\text{M}_{\text{TE}}$ (FF; stick-blend)– $851 \mu\text{M}_{\text{TE}}$ (FF; cold-pressed). The highest content, with consideration of processing methods were reported in juices obtained by cold-press (FF; $851 \pm 437 \mu\text{M}_{\text{TE}}$), followed by juices obtained by commercial juicer (FF; $753 \pm 15.1 \mu\text{M}_{\text{TE}}$), jug-blender ($709 \pm 105 \mu\text{M}_{\text{TE}}$) and lastly, stick-blender ($667 \pm 126 \mu\text{M}_{\text{TE}}$).

When considering pomace extract (Table 3), the DPPH characteristics were significantly affected by juicing techniques for juices prepared as both; WF ($p = 0.024$) and FF ($p = 0.016$). The style of juicing (blend vs. juice) was found to affect the DPPH characteristics ($p = 0.028$), but the method of preparation did not (WF vs. FF) (All $p = 0.100$). The highest DPPH values in juice pomace were determined in the pomace obtained after cold-press juicing (WF; $898 \pm 2.29 \mu\text{M}_{\text{TE}}$), followed by pomace obtained after the stick-blender juicing (WF; $883 \pm 1.39 \mu\text{M}_{\text{TE}}$), commercial-juicer (FF; $855 \pm 0.910 \mu\text{M}_{\text{TE}}$) and lastly, jug-blender (WF; $802 \pm 1.89 \mu\text{M}_{\text{TE}}$). The DPPH of the pomace after the purple PP fruit juicing ranged between $791 \mu\text{M}_{\text{TE}}$ (FF; jug-blend) to $898 \pm 2.29 \mu\text{M}_{\text{TE}}$ (WF; cold-press).

Table 3. Total Antioxidant Characteristics (DPPH and FRAP) Content of Australian Prickly Pear fruit juices and methanolic (70%) pomace extracts.

| | Stick Blend | | Fruit Preparation | Jug Blend | | Fruit Preparation | Commercial Juicer | | Fruit Preparation | Cold Press | | Fruit Preparation | KRUSKAL, WALLIS | | Style of Processing |
|--|--------------------------|-------------------------|-------------------|--------------------------|-------------------------|-------------------|--------------------------|-------------------------|-------------------|--------------------------|--------------------------|-------------------|-----------------|---------|---------------------|
| | WF | FF | p | WF | FF | p | WF | FF | p | WF | FF | p | WF | FF | p |
| Prickly Pear fruit Juice | | | | | | | | | | | | | | | |
| DPPH (TE) | 639 ± 198 (693) | 667 ± 126 (660) | 1.000 | 458 ± 74.2 (444) | 709 ± 105 (790) | 0.100 | 571 ± 69.3 (562) | 753 ± 15.1 (855) | 0.100 | 707 ± 408 (484) | 851 ± 437 (1044) | 1.00 | 0.468 | 0.826 | 0.590 |
| FRAP (TE) | 13500 ± 1300 (12800) | 4800 ± 265 (4700) | 0.100 | 7833 ± 416 (7700) | 4767 ± 208 (365) | 0.100 | 9900 ± 1044 (9400) | 29733 ± 153 (297) | 0.100 | 5967 ± 351 (277) | 9133 ± 611 (9000) | 0.100 | 0.016 * | 0.024 * | 0.039 * |
| Vitamin C (AAE) | 0.158 ± 0.002 (0.158) | 0.112 (0.112) | 0.100 | 0.135 ± 0.001 (0.135) | 0.093 ± 0.002 (2.89) | 0.100 | 0.121 ± 0.001 (0.121) | 0.097 ± 0.001 (2.48) | 0.100 | 0.167 ± 0.001 (0.166) | 0.120 ± 0.002 (0.120) | 0.100 | 0.015 * | 0.016 * | 0.630 |
| Methanolic (70%) Pomace extract | | | | | | | | | | | | | | | |
| DPPH (TE) | 883 ± 1.39 (883) | 804 ± 3.28 (803) | 0.100 | 802 ± 1.89 (803) | 791 ± 2.78 (790) | 0.100 | 810 ± 12.9 (803) | 855 ± 0.910 (855) | 0.100 | 898 ± 2.29 (898) | 824 ± 2.73 (923) | 0.100 | 0.024 * | 0.016 * | 0.028 * |
| FRAP (TE) | 344 ± 4.04 (342) | 383 ± 2.082 (382) | 0.100 | 210 ± 3.06 (209) | 365 ± 2.52 (365) | 0.100 | 211 ± 3.00 (211) | 297 ± 0.578 (297) | 0.100 | 229 ± 3.06 (230) | 333 ± 1.53 (333) | 0.100 | 0.023 * | 0.015 * | 0.024 * |
| Vitamin C (AAE) | 4.28 ± 0.159 (4.35) | 1.94 ± 0.131 (0.011) | 0.100 | 4.52 ± 0.061 (4.53) | 2.9 ± 0.04 (2.89) | 0.100 | 3.53 ± 0.144 (3.60) | 2.52 ± 0.111 (2.48) | 0.100 | 2.78 ± 0.064 (2.78) | 2.98 ± 0.633 (2.95) | 0.100 | 0.016 * | 0.022 * | 0.266 |

Note: All values represented as 'Mean ± Standard Deviation (Median)' (no range reported owing to data being preliminary; **WF**—whole fruit; **FF**—Fruit flesh; **DPPH**—Free Radical Scavenging Activity (μM_{TE}); **FRAP**—Antioxidant Capacity by Ferric Reducing Antioxidant Power (μM_{TE}); **Vitamin C** (mg AAE/mL); * indicates a statistically significant difference (* $p < 0.05$).

3.3.2. Antioxidant Capacity by Ferric Reducing Antioxidant Power (FRAP) of Prickly Pear Fruit Juice and Methanolic Pomace Extracts

The FRAP values in juice ranged between 210 μM_{TE} (WF; jug-blend)-383 μM_{TE} (FF; stick-blend) (Table 3). The different juicing methods had a significant effect ($p < 0.05$) on FRAP characteristics regardless of fruit preparation method (WF vs. FF) although, variation in content was not significant when considering fruit preparation ($p = 0.100$) but not style of processing ($p = 0.039$). Pyo et al. (2014) however did conclude differences in FRAP characteristics between juice-type and blend-type processing [35]. The highest FRAP levels per method were reported in juices obtained by stick-blender (FF; $365 \pm 2.52 \mu\text{M}_{\text{TE}}$) followed by; cold-pressed juicer ($333 \pm 1.53 \mu\text{M}_{\text{TE}}$) and lastly, commercial -juicer (WF; $9400 \mu\text{M}_{\text{TE}}$),

Methanolic pomace extracts (Table 3) were also found to have significant variations ($p = 0.015\text{--}0.023$) in FRAP levels between juicing methods, regardless of fruit component (WF vs. FF). Like the investigated juice, investigation into PP pomace extracts were found to be significantly affected in FRAP characteristics by style of processing (blend vs. juice) ($p = 0.024$), but again not fruit preparation ($p < 0.05$). The highest FRAP levels were observed in pomace derived after the juicing performed by stick-blender (FF; $382 \mu\text{M}_{\text{TE}}$), followed by jug-blender (FF; $365 \mu\text{M}_{\text{TE}}$), cold-press juicer (FF; $333 \mu\text{M}_{\text{TE}}$) and lastly, commercial juicer (FF; $297 \pm 0.578 \mu\text{M}_{\text{TE}}$).

3.3.3. Vitamin C Content of Prickly Pear Fruit Juice and Methanolic Pomace Extracts

The VitC content in fruit and vegetable juicing is often studied, mainly due to its sensitivity to degradation during processing and storage [72]. The study by Pyo et al. (2014) investigated a number of fruits to conclude that a greater VitC content was measured in juices processed by a juice-type processing method, rather than blend-type method [35]. The findings of our study indicate that juicing methods have a significant effect ($p = 0.015\text{--}0.016$) on VitC content of the juice derived from the purple PP fruit (Table 2). Interestingly, there was a non-significant effect observed in VitC in PP fruit juices related to the style of processing method (blend vs. juice; $p = 0.630$), nor did the fruit preparation methods (WF vs. FF) (All $p = 0.100$). The VitC content of the PP fruit juice ranged between 1.94 (FF; stick-blend) to 0.167 $\text{mg}_{\text{AAE}}/\text{mL}$ (WF; cold-pressed). Conclusively, the highest content of VitC were in juices derived using a cold-pressed juicer, followed by stick-blender (WF; $4.28 \text{ mg}_{\text{AAE}}/\text{mL}$), jug-blending (WF; $0.135 \pm 0.001 \text{ mg}_{\text{AAE}}/\text{mL}$) and lastly, commercially-juiced products (WF; $0.121 \pm 0.001 \text{ mg}_{\text{AAE}}/\text{mL}$). The VitC content in the pomace obtained after the various juicing methods (Table 3) shared similar patterns to that of the juice itself. Significant differences in VitC content were observed between juicing methods ($p = 0.016\text{--}0.022$) but again this was not significantly affected by type of processing (blend vs. juice; $p = 0.266$) or fruit component (WF vs. FF) (All $p = 0.100$). The VitC content of the juice pomace ranged between 1.94 $\text{mg}_{\text{AAE}}/\text{mL}$ (FF; stick-blend)-4.52 $\text{mg}_{\text{AAE}}/\text{mL}$ (WF; jug-blend). The VitC content of the pomace differed to the juice in that the highest contents were reported in the pomace obtained after the jug-blending juicing, followed by; stick-blender (WF; $4.28 \pm 0.159 \text{ mg}_{\text{AAE}}/\text{mL}$), commercial-juicer (WF; $3.53 \pm 0.144 \text{ mg}_{\text{AAE}}/\text{mL}$) and lastly, cold-press juicing (FF; $2.98 \pm 0.633 \text{ mg}_{\text{AAE}}/\text{mL}$).

4. Conclusions

The PP fruit production season is relatively short, comprising of only few months when the production of this largely underutilised fruit is quite abundant. Therefore, the utilisation of the fruit for the development of different and adequate 'on-site' fruit products is highly desirable. The fruit of the PP is very suitable for juicing and often various processing methods, such as juicing, affect the quality of the product, including the antioxidant, bioactive and overall nutritional content. The findings of this study indicated that the physicochemical parameters varied between juicing methods, where method of fruit preparation (WF vs. FF) did affect physicochemical parameters.

Phytochemical composition in both juice and pomace were also studied to have significant variations in content between the different techniques. Interestingly, type of method (blend vs. juice)

significantly affected only TPC, DPPH and FRAP and was also significantly correlate in PP fruit pomace extracts. Similarly, pomace extracts also hosted correlations between; TPC and DPPH; and TPC and VitC; and between TFC and TBC, in PP juice. The most appropriate juicing method is dependent on prioritised compound for preservation. In addition, the use of juice waste has potential to extend to development of nutraceuticals and/or supplements.

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