Nutrients, Antioxidants, and Bioactive Compounds on Passion Fruit (*Passiflora edulis*) Peel

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Key words: Passion fruit peel, Nutrients, Antioxidants, Total phenolic compounds, Anthocyanin

Summary

The aim of this study was to determine nutrient composition of passion fruit peel of three cultivars as 'Tainung No.1', 'Full-Star', and 'Golden'. Passion fruit peel cv. Golden significantly contained high values in both macronutrients and micronutrients. Antioxidants (POD, DPPH, FRAP, and APX) were also higher in cv. Golden, and SOD were higher in cv. Tainung No.1 and Full-Star. The highest of anthocyanin was obtained in cv. Tainung No.1. Total phenolics were higher in cv. Tainung No.1 and Full-star. The results were obtained passion fruit peel is a good source of several antioxidant compounds and nutrients, could be a combination with by-products into food products for add-values the fruit waste, reduced fruit waste, and support to a sustainable food system.

Introduction

Passion fruit is one of the Passifloraceae family, planted widely in the tropical and subtropical regions of the world. The passion fruit is a commercial of fruit. The two main commercial cultivars are purple fruit (*Passiflora edulis*) and yellow fruit (*P. edulis flavicarpa*). The passion fruit is native from Brazil and the largest manufacturer of passion fruit in the world. The mainly of *Passiflora* species are found in tropical regions in some despite records of species in China, India, Australia and the Pacific islands (Cerqueira-Silva *et al.*, 2014 and Cutri *et al.*, 2013). Brazil is the second world fruit producer, it's more than 58 million tons in 2016 (FAO,

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2016). Similarly, FAO (2011) reported world production of passion fruit increased from about 1.05 million MTs in 2005 year to 1.15 million MTs in 2009.

Passion fruit is a source of bioactive phytochemical compounds and therapeutic such as anticarcinogenic and antioxidant activities. In the same way, passion fruits not only are nutritious but also have health benefits, such as refreshing, solve thirst, help digestion, and eliminate fatigue and other effects. Passion fruit mostly consumed in fresh, juice, jam and other, which had many fruit wastes especially fruit processing industries are extremely high (Laufenberg *et al.*, 2003, Parfitt *et al.*, 2010) i.e. mango 30 – 50%, banana 20%, pomegranate 40 – 50% and passion fruit 45 - 50%. The waste (seeds and peels) could be used as a by-products for food ingredients and value-added materials, like a beverage (de Toledo *et al.*, 2018), bakery products (Martínez-Cervera *et al.*, 2011), meat products (Fernández-López *et al.*, 2008, Sánchez-Zapata *et al.*, 2010), and dairy products (Sendra *et al.*, 2008), these are contributing to the recovery of fruits and vegetable waste (Ayala-Zavala *et al.*, 2011).

Nowadays, passion fruit peel can use as an ingredient for the production pasta, bakery, flour, milk, yogurts, ice creams and others (Fioreze, 2004). In addition, passion fruit can be used in the composition of many process foods, for the enrichment of food products such as animal feed, fertilizer or raw material (material abundant in passion fruit waste) (Buckeridge and Tiné, 2011).

The objective of determining the passion fruit peel for three cultivars as 'Tainung No.1', 'Full-Star', and 'Golden' on nutrient composition, antioxidant capacity (according to POD, FRAP, DPPH, SOD, and APX), total phenolic compounds (TPC), and anthocyanin.

Materials and Methods

1. Plans materials

Passion fruit (*P. edulis*) at the ripening stage. The passion fruits were grown and harvested at Puli district, Nantou district, Taiwan and then transported to the Department of Horticulture, National Chung Hsing University. Passion fruits were sorting with no defect or spoilage external for use in the experiment.

2. Experiment

The passion fruits for 3 cultivars were 'Tainung No.1', 'Full-Star', 'Gloden'. After transportation to the laboratory, passion fruits were cleaned and separated the seed from the peel. Whereas, the fruits were determining the nutrient composition, antioxidant capacity (according to POD, FRAP, DPPH, SOD, and APX), total phenolic compounds (TPC), and anthocyanin.

3. Nutrients

The samples were washed with 1% HCl and then rinsed three times under distilled water. Samples were oven-dried at 100℃ for 1 hour and then oven at 70℃ for 48 hours. The passion fruit peels were milled with 150 G high speed grinder (RT-02A) and stored in paper bag. For analysis, sample powder 2 g taken into a cup glass and oven at 500℃ for 7 hours. After heating, cooled down and added 5 ml of 2 N HCl for AA. Used Whatman #42 to be filtered and diluted to 25 ml by distilled water. The supernatant was used for element analysis.

3.1 Total nitrogen (Micro-Kjeldahl) analysis

The sample powder 0.2 g packed in filter paper (Whatman 01) and then placed in digestion tubes. Added 1 g of Se mixture powder (Merck 8030) with H_2SO_4 for 4.5 ml into the tubes. The digestion tubes heat at 410℃ for 3 hours until solution turn to light blue and cooled down. Added 15 of distilled water with 12N NaOH•H_2O for 20 ml and the solution placed into a Micro-Kjeldahl apparatus. The analysis measured into a 20 ml of 2% Boric acid (19 μM Bromocresol and 25 μM Methyl red indicator solution), when total solution volume increase to 25 ml, then titration the solution with 1/14 H_2SO_4 and recorded volumes. The total nitrogen (%) was calculated as: (Conc. – BK)/2 × F (0.97804).

3.2 Phosphorus (P) analysis

The supernatant sample 1 ml mixed with 3 ml of distilled water and 1 ml of reagent [$(NH_4)_6Mo_7O_{24}$•4H2O, Ammonium Vanadate, and HNO_3] and vortex. Placed the mixture solution at room temperature for 30 min and using an Elisa Reader (BMG LABTECH, FLUO star Omega Ω, Germany) and evaluate absorbance at 470 nm. The phosphorus concentration (%) was calculated as: Conc. × 5 × 25/0.5 × 10^{-4}.

3.3 Potassium (K) and Magnesium (Mg) analysis

The supernatant solution 0.1 ml mixed with 3.9 ml of distilled water and vortex. Dilution the mixing solution (400x) 1 ml, added 4.5 ml of distilled water and vortex. The analysis was measured by using Polar Zeeman Atomic Absorption Spectrophotometer (Z-2300) and recorded volumes. The analysis concentration (%) were calculated as: (Conc. – BK) × dilution factor × 0.001 × 25/0.5.

3.4 Calcium (Ca), Iron (Fe), Manganese (Mn) and Zinc (Zn) analysis

Used the supernatant sample to be measured into a Polar Zeeman Atomic Absorption Spectrophotometer (Z-2300) and recorded volumes. The analysis concentration (ppm) was calculated as: (Conc. – BK) × 25/0.5.

3.5 Boron (B)

Transferred supernatant sample 0.2 ml into centrifuge tube, then added 0.4 ml buffer-masking and 0.2 ml azomethine-H boron reagent after added reagent vortex the mixing supernatant. After the mixing supernatant color develop for 1 hour, using Elisa Reader (BMG

LABTECH, FLUO star Omega Ω, Germany) and read ABS at 420 nm.

4. Antioxidant capacity

4.1 Peroxidase (POD)

According Johnson and Cunningham (1972). Sample (fresh passion fruit peel) 2 g were homogenized with 0.1 M phosphate buffer (pH 7.0) [1% PVP, 0.25% triton x-100] and centrifuged at 20,000×g at 4℃ for 20 min. Then extract solution was filtered to through miracloth and supernatant liquid used to analysis. The absorbance was read at 470 nm by using Elisa Reader (BMG LABTECH, FLUO star Omega, Germany). After extraction, the mixture contained 200 µL 3.6×10^{-3} M guiacol (100 ml 0.1 M phosphate buffer pH 6.0 mix with 0.04 ml guaicol), deionized water for 40 µL, enzymatic extract for 10 µL, and 0.0135 M hydrogen perioxide for 20 µL, in a total volume of 270 µL. Then taken sample solution to an Elisa Reader (BMG LABTECH, FLUO star Omega Ω, Germany) and evaluate absorbance at 470 nm. POD activity calculated as: conc × 0.1 (ml)/sample (0.01 ml) × (5 ml buffer + FW)/FW and expressed unit as △A•min/g•FW.

4.2 Ferric reducing ability of plasma (FRAP)

According Benzie and Strain, (1996). Using 0.5 gram of sample (fresh passion fruit peel) was homogenized with 30 mM acetate buffer (pH 3.6) for 5 ml and then centrifuged at 20000×g at 4℃ for 10 min. The extract solution was filtered through the miraclot and after centrifuged used the supernatant liquid to analysis.Using supernatant for 50 µL mixed with working reagent [20 µL $FeCl_3$•$6H_2O$, 10 mM TPT (2,4,6-tripyridyl-s-triazine), and 30 mM acetate buffer pH 3.6, three solutions were mixed in ratio 1:1:10 (v:v:v)], then vortex and incubated the supernatant at 37℃ for 10 min. FRAP assays using an Elisa Reader (BMG LABTECH, FLUO star Omega Ω, Germany) and evaluate absorbance at 593 nm. The FRAP was calculated as: Conc (µM) × 5 (mL)/1000/g (FW) and expressed as $\mu mol \cdot g^{-1} \cdot FW$.

4.3 2,2-diphenyl-1-picrylhydrazyl (DPPH)

The passion fruit peel for 2 grams were homogenized with 50% alcohol buffer for 10 ml and then centrifuge at 13000×g for 10 min at 4℃. After centrifuge filter by used miracloth and used the supernatant for analysis. Preparation extraction for analysis, the supernatant divided into 2 group: the supernatant 1 ml added 0.2 ml DPPH + methanol solution (DPPH) and only methanol solution (Methanol). Distilled water 1 ml mixed with 0.2 ml methanol solution and the sample supernatant 1 ml mixed with 0.2 ml DPPH + methanol solution as a blank (BK) and control (CK), respectively. The supernatants were put at the dark for 30 minutes and then determined by using UV - 2000 machine absorption at 517 nm. The DPPH (%) was calculated as: [CK - (Methanol - DPPH)]/CK × 100.

4.4 Superoxide dismutase (SOD)

SOD activity was evaluated according Wang *et al.*, (2008). Using 2 grams of sample (fresh

passion peel fruit) were homogenized on ice-cold with 0.1 M phosphate buffer (pH 7.8) (1 mM PEG 6000, 0.1 mM EDTA), 0.1 g PVPP and then centrifuged at 12000×g at 4℃ for 12 min. The extract solution was filtered through the miraclot and the supernatant used to analysis according Beauchamp and fridovich, (1971). The mixture used supernatant for 10 µL with 50 mM sodium carbonate-sodium bicarbonate buffer (pH 10.1) for 250 µL, 300mM L-methionine for 10 µL, 3mM nitro blue tetrazolium blue chloride (NBT) for 10 µL, 3 mM EDTA for 10 µL, and 60 mM riboflavin for 10 µL. After mixed, kept the solution at the dark for 5 min and light at 2500 Lux for 5 min. And then evaluated by using Elisa Reader (BMG LABTECH, FLUO star Omega, Germany) and read absorbanc e at 560 nm. Blank was used 0.1 M phosphate buffer (pH 7.8) [1 mM PEG 6000, 0.1 mM EDTA] for 10 mL. SOD activity was calculated as: (blank $\triangle A_{560}$/sample $\triangle A_{560}$)/0.5 blank $\triangle A_{560}$ × (5/0.01) (dilution factor)/g (fresh weight)/5 (min) and expressed unit as µg•g^{-1}•FW. Specific activity (unit•g^{-1}•protein) = CAT (unit•g^{-1}•FW)/total soluble protein content (mg•g^{-1}).

4.5 Ascorbate peroxidase (APX)

APX activity was evaluated according Wang *et al.*, (2008). Using 2 grams of sample (fresh passion peel fruit) were homogenized with 0.1 M phosphate buffer (pH 7.8) [1 mM PEG 6000, 0.1 mM EDTA], 0.1 g PVPP and then centrifuged at 12000×g at 4℃ for 20 min. The extracted solution was filtered through the miraclot and the supernatant used to analysis according Nakano and Asada (1981). The mixture used supernatant for 10 µL with 150 mM phosphate buffer (pH 7.0) for 100 µL, 1.5 mM ascorbate for 100 µL, 0.75 mM EDTA for 40 µL, and 6 mM H_2O_2 for 50 µL. The activity was read absorbance at 290 nm by using Elisa Reader (BMG LABTECH, FLUO star Omega, Germany). The blank was used 10 µL of 0.1 M phosphate buffer (pH 7.8) [1 mM PEG 6000, 0.1 mM EDTA]. APX activity was calculated as: $\triangle A_{290}$/g (fresh weight)/1 (min) and expressed unit as unit•g^{-1}•FW.

5. Total phenolic compound (TPC)

Preparation the extract, using 1 gram of passion peel fruit were homogenized with 5 mL phosphate buffer (pH 7.0) by pestle and mortar. The solution was centrifuged at 13,000×g at 4℃ for 20 min. After centrifuged, solution was filtered to through miracloth and supernatant liquid was collected to analysis. The extract solution 0.1 mL mixed with 0.9 mL distilled water and 0.1 ml of folin-ciocalteus reagent. Immediately added 0.2 mL of 20 % Na_2CO_3 and 8.7 mL of distilled water. The mixture solution was cubated in a water bath at 100℃ for 3 min and then immediately cooled. The evaluate absorbance at 660 nm by using Elisa Reader (BMG LABTECH, FLUO star Omega, Germany). The total phenolic compounds were calculated as: Conc (ppm) × (5 + FW)/FW × 10 (dilution factor) and expressed unit as µg•g^{-1}•FW.

6. Anthocyanin

The fresh sample 1 g (without white sponge under passion fruit peel) extracted with 5 ml of 0.025 M potassium chloride buffer (pH 1.0) for 1 tube, and the new tube also used the sample 1 g (without white sponge under passion fruit peel) extracted with 5 ml of 0.4 M sodium acetate buffer (pH 4.5). These extracted solutions were kept at 4℃ under dark overnight (24 hours). Then, vortex the solution and dilute with distilled water. The analysis of anthocyanin was determined by using Elisa Reader (BMG LABTECH, FLUO star Omega, Germany) at an absorbance of 370, 520 and 530 nm. The anthocyanin was calculated as: [(Conc. pH 1.0 – Conc. pH 4.5) × MW (449.2) × dilution factor × 1000]/Ɛ (26,900) × 1 and expressed as mg/L.

7. Statistical analysis

The data was carried out statistical analysis by using SAS 9.0 (Institute Inc., 2000) and determined one-way analysis of variance (ANOVA) for a completely randomized design (CRD) statistical model. Means values among treatments were compared by t-Tested (LSD) and least significant difference at the 5% ($p \leq 0.05$) level of significance.

Results

The macronutrient and micronutrient elements of different cultivars of passion fruit peel as showed in Table 1 and 2. The nutrient values showed significant differences in all cultivars. The highest of all macronutrient values were shown in passion fruit peel cv. Golden than other cultivars contained, and potassium (K) values higher than other macronutrient elements, respectively. The micronutrient elements of the manganese (Mn) and boron (B) were not significantly different in all cultivars while the iron (Fe) and zinc (Zn) were significant differences in all cultivars. The peel of passion fruit cv. Golden also showed the highest micronutrient values followed by cv. Tainung No.1 and cv. Golden, respectively. However, passion fruit peel contains nutrient and nutrient values difference depends on cultivars.

The results of the antioxidant capacity on passion fruit peel were shown in Table 3. The FRAP activity was not significantly different among all cultivars while POD, DPPH, APX, and SOD were significant differences between cultivars. The POD and APX activity were higher in passion fruit peel cv. Golden than other cultivars and the SOD activity of passion fruit peel cv. Golden had a significantly lower than the other cultivars. Finally, passion fruit peel cv. Golden presented high levels of antioxidant capacity in 2,2-diphenyl-1-picrylhydrazyl (DPPH), the ferric reducing ability of plasma (FRAP), and ascorbate peroxidase (APX) activity, followed by cv. Tainung No. 1 and cv. Full Star, respectively.

Table 1. The macronutrient elements of different cultivars on passion fruit peel.

Cultivars	N (%)	P (%)	K (%)	Mg (%)	Ca (%)
Tainung No. 1	0.86 a*	0.04 b	12.71 a	0.12 b	0.79 b
Full-Star	0.54 a	0.05 b	8.51 b	0.06 c	1.06 a
Golden	0.81 a	0.18 a	13.44 a	0.22 a	1.06 a

*Values followed the same letter (a-c) within the same column differ from each other according LSD ($p \leq 0.05$).

Table 2. The micronutrient elements of different cultivars on passionfruit peel.

Cultivars	Fe (ppm)	Mn (ppm)	Zn (ppm)	N (ppm)	P (ppm)	B (ppm)
Tainung No. 1	180.9 a[Z]	119.6 a	67.2 a	0.0042 a	0.043729 b	1.26 a
Full-Star	89.7 b	117.7 a	47.4 b	0.0026 a	0.05131 b	1.41 a
Golden	175.0 a	117.5 a	53.2 ab	0.0040 a	0.17675 a	1.21 a

[Z] Values followed the same letter (a-c) within the same column differ from each other according LSD ($p \leq 0.05$).

Table 3. The antioxidant capacity of different cultivars on passion fruit peel.

Cultivars	POD[Z] ($\triangle A_{470}$/g•FW/min)	DPPH (%)	FRAP (μmol/g•FW)	SOD (unit/g•FW)	APX (unit/g•FW)
Tainung No 1	6.5 b[Y]	93.7 a	1.3 a	25.8 a	66.4 b
Full star	8.7 b	86.1 b	2.4 a	23.3 a	81.5 b
Golden	15.5 a	89.6 ab	1.6 ab	17.0 b	114.4 a

[Z]POD = Peroxidase activity, DPPH = 2,2-diphenyl-1-picrylhydrazyl, FRAP = Ferric reducing ability of plasma, SOD = Superoxide dismutase, APX = Ascorbate peroxidase.

[Y]Values followed the same letter (a - c) within the same column differ from each other according LSD ($p \leq 0.05$).

The results shown on Table 4. Anthocyanin contents had significant different among of cultivars. The highest of anthocyanin contents were observed in peel of passion fruit cv. Tainung No. 1 followed by cv. Full Star, and cv. Golden, respectively. In contrast, the peel of passion fruit

cv. Golden had significantly lower total phenolic compounds. The peel of passion fruit cv. Tainung No. 1 presented the best results of anthocyanins, total phenolic.

Table 4. The anthocyanin and total phenolic compounds of different cultivars on passion fruit peel.

Cultivars	Anthocyanin (mg/100g)	TPC[Z] (μg/g•FW)
Tainung No 1	81.7 a[Y]	1731.0 a
Full star	27.6 b	1787.4 a
Golden	1.5 c	1018.1 b

[Z] TPC = Total phenolic compounds, SP = soluble protein.

[Y]Values followed the same letter (a-c) within the same column differ from each other according LSD ($p \leq 0.05$).

Discussion

Wijeratnam, (2016) reported passion fruit had high mineral content, which rich source of iron, copper, magnesium, and phosphorus. Our experiment of the study did not find copper in passion fruit peel, may these nutrients could be found in other parts. Liu *et al*., (2008) reported that passion fruit seeds contained sodium, magnesium, potassium, and calcium values approximately 2.980 ± 0.002 mg/g, 1.540 ± 0.001 mg/g, 0.850 ± 0.001 mg/g, and 0.540 ± 0.002 mg/g, respectively. The highest nutrient of our study was potassium which has many benefits for health. Nowadays, several authors indicated that passion fruit peel powder has a beneficial effect in reducing blood glucose, total cholesterol and low-density lipoproteins (LDL), and also increasing the high-density lipoproteins (HDL) of diabetic group people (Medeiros *et al*., 2009; Janebro *et al*., 2008; Ramos *et al*., 2007)

Halliwell (2012) presented that passion fruit rich in antioxidants and reduced the oxidative and therapeutic. Recently, several studies have reported the functional properties of P. edulis peel powder (PEPF), particularly its dietary fiber content and antioxidant capacity. Likewise, Sihombing *et al*, (2015) presented that the highest of antioxidant activity from twenty-two peel fruits was *Cytrus hystrix, Ipomoea batatas* L., *Dimocarpus longan*, *Solanum betaceum, Passiflora edulis*, *Sechium edule*, *Annona squamosa* L., *Archidendron pauciflorum*, and *Parkia speciosa*. Passion fruit peel is one of rich antioxidant. Likewise, our study also presented high levels of antioxidants and passion fruit cv. Golden higher in all antioxidant except superoxide dismutase

(SOD). The antioxidant capacity of some *Passiflora* species range between 28% to 95% of DPPH values. Our results obtained passion fruit peel contained high DPPH values (86.1-93.7%). FRAP assays, the passion fruit peel extraction had 30.94 μg TE/g (Wong *et al.*, 2014), 4.47 ± 0.36 μmol (Lima *et al*., 2018). And our results FRAP compounds were range from 6.5 to 15.5 (△A470/g•FW/min). P. edulis was reduced the SOD activity, thus indicate excess peroxide of these tissues, and represented that there was no increase in CAT and APX activity. Like our results because passion fruit peels cv. Golden was higher APX compounds while cv. Golden was lower than other passion fruit peel cultivars.

The high level of anthocyanin extensively found in berries fruit such as blueberries and blackcurrants (Kahkonen *et al*., 2001) due to anthocyanin mostly contained in pink, red, blue or purple color of fruit and our study passion fruit peel cv. Tainung No.1 and cv. Full-star is a purple and purple-red color of peel fruit. These cultivars showed a high of anthocyanin compounds than cv. Golden (Yellow). Da Silva *et al*., (2014) presented the by-products higher levels of total anthocyanins than pulps on papaya 1.87and 22.43 mg/100g dry basis, mango 7.85 and 2.29 mg/100g dry basis, passion fruit 3.48 and 3.70 mg/100g dry basis, and surinam cherry fruit 226.90 and 1021.22 mg/100g dry basis, respectively. In addition, Da Silva *et al.*, (2014) presented the by-products higher levels of total anthocyanins than pulps on papaya 1.87and 22.43 mg/100g dry basis, mango 7.85 and 2.29 mg/100g dry basis, passion fruit 3.48 and 3.70 mg/100g dry basis, and surinam cherry fruit 226.90 and 1021.22 mg/100g dry basis, respectively. The residue part of passion fruit had high amounts of total phenolic compounds than pulp of fruit, 103 ± 10.4 and 20 ± 2.6 mg GAE 100 g^{-1}, respectively (de Oliveira *et al.*, 2009). Our results of total phenolic compounds had high content 1787.4 μg/g•FW. Finally, this best results of this study passion fruit peel cv. Golden was high levels on nutrients and antioxidants, and passion fruit peel cv. Tainung No. 1 was a high level of anthocyanin and total phenolic compounds.

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百香果果殼之營養成分、抗氧化和生物活性物質

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關鍵字：百香果果殼、營養成分、抗氧化劑、總酚含量、花青素

摘要：本研究分析'台農一號'、'黃金'、'滿天星'三個品種的百香果殼中，營養元素、抗氧化能力 (POD、FRAP、DPPH、SOD 及 APX)、總酚類化合物 (TPC)和花青素含量。黃金百香果的果殼中含有大量元素和微量元素，並顯著高於其他兩個品種。在抗氧化能力方面，'黃金'百香果相較其他品種含有較高的 2,2-二苯基-1-苦基肼 (DPPH)、鐵還原能力 (FRAP)及過氧化酶 (POD)、抗壞血酸過氧化物酶 (APX)之活性。'台農一號'百香果殼中具有較高的花青素'台農一號'、'滿天星'百香果殼中具有較高的總酚類化合物。試驗結果顯示百香果果殼是幾種抗氧化劑和營養元素的良好來源，是為果實副產品循環再利用，增加附加價值、減少農業廢棄物。

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