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Comparison of Total Phenolic Contents (TPC) and Antioxidant Activities of Fresh Fruit Juices, Commercial 100% Fruit Juices and Fruit Drinks

(Perbandingan Jumlah Kandungan Fenolik (TPC) dan Aktiviti Antioksidan Jus Buah-buahan Segar, 100% Jus Buah-buahan Komersial dan Minuman Buah-buahan)

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ABSTRACT

There is an increasing trend of fruit juice consumption due to increasing reported health benefits of antioxidant content present in fruit juices. The aim of this study was to compare the total phenolic contents (TPC) and antioxidant activities of fresh fruit juices, commercial 100% fruit juices and fruit drinks. Seven types of freshly blended fruit juices and their commercial counterparts were selected. Folin-Ciocalteu method was used to determine the total phenolic content, whilst ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays were performed to evaluate the antioxidant activities of fruit juices. The TPC contents of fresh fruit juices, commercial 100% fruit juices and fruit drinks were at the ranges of 13.38-80.40, 21.65-130.39 and 3.32-45.10 mg GAE/100 mL, respectively. Both fresh guava juice and commercial guava drinks have exhibited the highest antioxidant activities in DPPH assay (205.71-770.12 µmol TE/100 mL) and FRAP assay (320.80-843.13 µmol TE/100 mL). Pomegranate juices demonstrated the highest antioxidant activities among commercial 100% fruit juices with DPPH and FRAP values of 2705.01 and 2953.85 µmol TE/100 mL, respectively. Fruits drinks group had the lowest TPC and antioxidant activities for all types of fruits. TPC was significantly correlated (p<0.05) to FRAP (r=0.954) and DPPH (r=0.908) assays. In conclusion, the TPC and antioxidant activities of commercial 100% fruit juices and fresh juices were comparable as no significantly difference (p>0.05) was found between these two groups. Commercial fruit drinks in this study were not good source of antioxidants. These findings provide some useful information especially for ageing population in choosing healthy fruit juice or drinks for their health maintenance purposes.

Keywords: Antioxidant activity; fresh fruit juice; fruit drink; total phenolic content; 100% commercial fruit juice

ABSTRAK

Penggunaan jus buah-buahan semakin meningkat kerana kandungan antioksidan dalam jus buah-buahan dilaporkan boleh memberi manfaat kepada kesihatan. Tujuan kajian ini adalah untuk membandingkan jumlah kandungan fenolik (TPC) dan aktiviti antioksidan antara jus buah-buahan segar, 100% jus buah-buahan komersial dan minuman buah-buahan. Tujuh jenis jus buah-buahan segar serta jus komersial bagi buah-buahan tersebut telah dipilih. Kaedah Folin-Ciocalteu telah digunakan untuk menentukan jumlah kandungan fenolik, manakala asai FRAP dan DPPH telah dijalankan untuk menilai aktiviti antioksidan jus buah-buahan. Kandungan TPC jus buah-buahan segar, 100% jus buah-buahan komersial dan minuman buah-buahan masing-masing berada dalam julat 13.38-80.40, 21.65-130.39 dan 3.32-45.10 mg GAE/100 mL. Kedua-dua jus jambu batu segar dan minuman jambu batu komersial telah menunjukkan aktiviti antioksidan yang tertinggi dalam asai DPPH (205.71-770.12 µmol TE/100 mL) dan asai FRAP (320.80-843.13 µmol TE/100 mL). Jus delima menunjukkan aktiviti antioksidan yang paling tinggi antara 100% jus buah-buahan komersial dengan nilai DPPH, 2705.01 µmol TE/100 mL dan nilai FRAP, 2953.85 µmol TE/100 mL. Sampel minuman buah-buahan pelbagai jenis mempunyai TPC dan aktiviti antioksidan yang paling rendah bagi semua jenis buah-buahan. TPC berkait secara signifikan (p<0.05) dengan asai FRAP (r=0.954) dan DPPH (r=0.908). Kesimpulannya, 100% jus buah-buahan komersial adalah setanding dengan jus buah-buahan segar kerana TPC dan aktiviti antioksidan antara kedua-dua kumpulan tidak berbeza secara signifikan (p>0.05). Minuman buah-buahan komersial dalam kajian ini didapati bukan sumber antioksidan yang baik. Kajian ini menyediakan maklumat yang berguna terutamanya kepada populasi penuaan dalam pemilihan jus atau minuman buah- buahan yang sihat.

Kata kunci: Aktiviti antioksidan; jumlah kandungan fenolik; jus buah-buahan segar; minuman buah-buahan; 100% jus buah-buahan komersial

INTRODUCTION

World Health Organization (2003) has recommended the intake of five servings or equivalent to 400 g of fruits and vegetables in daily diet. Malaysia Dietary Guidelines (2010) also encourage the public to consume various fruit daily, ranging from fresh, canned, dried or 100% fruit juice.

For ageing people, as with younger adults, the diet should follow the principles of a healthy balanced diet which include fruit juices. Previous studies reported that oxidative stress in human body resulted from excessive free radicals which was associated with high risk of non-communicable diseases (NCD) (Alfadda & Sallam 2012; Durackova 2010; Gupta et al. 2014). According to National Health Morbidity Survey, NHMS (2011), 92.5% adults do not consume enough fruits and vegetables per day while the non-communicable diseases like type II diabetes, hypercholesterolemia and hypertension are on the rise.

Consumption of fruits rich in antioxidant substances such as phenolic compounds and vitamin C is inversely associated with risks of non-communicable diseases. Phenolic compounds have a wide spectrum of health benefits such as anti-bacterial, anti-mutagenic and anti-inflammatory, antioxidant activity and minimize oxidative stress (Celep & Rastmanesh 2013). Epidemiological studies and meta-analyses proved that frequent and adequate intake of fruits could help to prevent cardiovascular diseases (Rautiainen et al. 2012), neurodegenerative diseases (Albarracin et al. 2012), cancer (Wang et al. 2014), diabetes (Hegde et al. 2013) and osteoporosis (Shen et al. 2014).

Besides, fruit juice could be a great alternative for whole fruits, which might be less palatable with its tangy, sour taste or course in texture. Fruit juices could promote better ingestion, especially for elderly and children (Wootton-Beard & Ryan 2011). Scientific evidence suggested that fruit juices may be as effective as whole fruits in prevention of chronic diseases (Abirami et al. 2014; Ruxton et al. 2006). Ready-to-drink fruit juices are growing fast in the market as consumers are looking for convenient fruit products with high sensory and nutritional qualities (Lau et al. 2012).

Previous local studies have evaluated antioxidant properties of fresh fruits (Addai 2013; Ibrahim et al. 2013; Ikram et al. 2009; Tan et al. 2012), but limited studies were conducted to compare the antioxidant capacity of different categories of fruit juices. Lek et al. (2012) and Mahdavi et al. (2010) showed that the antioxidant activities of commercial juices were relatively low as compared to their fresh juice counterparts. Minimal research has been conducted to study the differences in antioxidant properties between fresh fruit juices and commercial counterparts, either commercial 100% juices or drinks. According to Food Act 1983 and Food Regulation 1985 Malaysia, commercial fruit juice is the reconstituted product of concentrated juice while fruit drink contain not less than 5% (w/v) of fruit juice. The present study was important to provide an insight to consumers for a better understanding on fresh and commercial juices or drinks for various fruits, with regard to both phenolic compound content and their antioxidant properties.

The antioxidant properties of fresh fruit juices would be expected to be different from the commercial counterparts due to several factors. Fruit mashing, clarification, filtration and heat pasteurization during juice production could alter antioxidant properties of commercial fruit juices or drinks (Savatović et al. 2009). Thus, this study was aimed to determine the differences of total phenolic contents (TPC) and antioxidant activities in freshly blended fruit juices, commercial 100% fruit juices and fruit drinks. The relationship between total phenolic content and antioxidant activity of all the fruit juices was also determined in the present study.

Materials & Methods

Sample Collection

Seven types of fresh fruits, namely apple, grape, guava, mango, pineapple, pomegranate and orange along with commercial 100% fruit juices and fruit drinks were purchased from local markets or hypermarkets in Kuala Lumpur, Malaysia. Two brands were selected for each commercial juice or drink. Only five types of commercial 100% fruit juices and six types of fruit drinks were included in the present study as commercial 100% guava, mango juices and pomegranate fruit drink were not available in the market.

Chemicals & Reagents

Folin-Ciocalteu reagent, gallic acid, sodium carbonate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-Hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox), 2,4,6-tri-(2-pyridyl)-s-triozine (TPTZ) and ferric chloride hexahydrate (FeCl3. 6H2O) (Sigma-Aldrich, USA), sodium acetate trihydrate, glacial acetic acid, hydrochloric acid (sp.gr.1.18, England), methanol (Systerm ChemAR). All chemicals and reagents used in this study were of analytical grade (99%).

Sample Preparation

All fresh fruits were thoroughly washed under running tap water and the juices were collected using electric juice extractor (Breville JE98XL, USA). The fruit juices were filtered through muslin cloth and kept under -20°C in freezer (Sanyo, Japan) for less than four weeks. Juices were centrifuged at 3000 rpm (1556 × g) for 10 min according to the method used by Aa et al. (2011) using centrifuge (Gyrozen 406, Korea) and supernatant was used for the analysis. Each juice was collected from two locations. Each sample was analyzed twice and the average reading was taken as the final results. Juice was diluted at factor of 10 to 60 prior to analysis (Pisoschi et al. 2009).

Total Phenolic Content (TPC)

Total phenolic content of juices was determined spectrophotometrically according to Folin-Ciocalteu method with slight modification by Mahdavi et al. (2010) and Singleton & Rossi (1965). An amount of 0.4 mL sample or standard solution was added into 10 mL volumetric flask, containing 3.6 mL of distilled water. Folin-Ciocalteu reagent (0.4 mL) was added into the mixture. About 4 mL of 7% sodium carbonate was also added following 5 min. The solution was made up to 10 mL with distilled water, mixed thoroughly and allowed to stand at room temperature for 90 min. The absorbance was measured at 765 nm using UV-visual spectrophotometer (Secomam Prim, France) against distilled water as blank. Calibration curve was plotted using gallic acid standard solution of 0 - 250 mg/L. The result was expressed as gallic acid equivalent (mg GAE /100 mL).

DPPH FREE RADICAL SCAVENGING ASSAY

This assay was carried out based on the method of Costa et al. (2012) and Plank et al. (2012) with slight modification. 0.1 mM of methanolic DPPH stock solution was prepared freshly using 10 mg DPPH dissolved in 125 mL methanol in a 250 mL volumetric flask. A 0.4 mL diluted sample or standard solution was added into test tube containing 5.6 mL methanolic DPPH. Test tubes sealed with parafilm were incubated in water bath (Memmert, Germany) at 37°C for 30 min. The absorbance was measured against methanol (blank) at 517 nm using UV-visual spectrophotometer (Secomam Prim, France). Trolox calibration solutions of 50-500 μM concentration were used to generate the standard curve. The results were expressed as μmol TE/100 mL.

FRAP FERRIC REDUCING ANTIOXIDANT POWER

The FRAP assay was performed according to previous studies (Álvarez et al. 2014; Wootton-Beard et al. 2011) with slight modifications. The FRAP reagent was made up of 300 mM acetate buffer (3.1 g $C_2H_3NaO_2{\cdot}3H_2O$ and 16 mL $C_2H_4O_2$), 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution dissolved in 40 mM HCl and 20 mM $FeCl_3{\cdot}6H_2O$ solution. The fresh working solution was prepared by mixing 25 mL acetate buffer at pH3.6, 2.5 mL TPTZ solution and 2.5 mL $FeCl_3{\cdot}6H_2O$ solution. The mixture was warmed at 37°C prior to analysis. 2 mL of warmed distilled water at 37°C was added to 50 μL of sample and 2 mL of reagent in test tube. The mixture was incubated at 37°C for 4 min in the dark condition and monitored until 8 min. Absorbance was taken against methanol as blank at 593 nm using UV-visual spectrophotometer (Secomam Prim, France). The standard curve was linear between 100 and 1000 μM Trolox. The results were expressed in μmol TE/100 mL.

STATISTICAL ANALYSIS

Descriptive data were reported as mean ± standard deviation. Statistical analyses were performed using statistical package SPSS v 22.0. Differences at $p<0.05$ (95% confidence level) were considered to be significant. Analysis of variance (One-way ANOVA) was used to compare different group of samples while post-hoc test was carried out for paired comparisons. Independent t-test was used when comparing two groups of samples. Pearson's correlation coefficient (r) was used to determine the relationship between total phenolic content and antioxidant activities.

RESULTS & DISCUSSION

TOTAL PHENOLIC CONTENT (TPC)

TPC of juice samples was quantified by Folin-Ciocalteu assay which depends on the reduction of Folin-Ciocalteu reagent by phenolic compounds under alkaline condition. Absorbance is directly proportional to the concentration of phenolic compounds, which represented by the intensity of blue color produced in each solution (Huang et al. 2005). Linear standard curve of gallic acid with $R^2 = 0.9994$ was used to determine TPC. TPC among fresh juices, commercial 100% fruit juices and fruit drinks were at the range of 13.38 - 80.40 mg, 21.65 - 130.39 mg and 3.32 - 45.10 mg GAE/100 mL, respectively (Table 1).

The alphabets A, B and C represent categories of fresh fruit juices, commercial 100% juices and fruit drinks, respectively. The TPC content was outlined as B4 > B2 > A4 > B5 > A3 > A7 > B3 > A5 > A2 > C3 > A1 > B1 > A6 > C6 > C4 > C5 > C2 > C1. The highest TPC value was detected for mango (80.40 ± 0.36 mg GAE/100 mL) among all fresh juices, pomegranate among commercial 100% juices (130.39 ± 25.39 mg GAE/ 100 mL) and guava among fruit drinks (45.10 ± 7.75 mg GAE/100 mL). Fresh pomegranate juice had the lowest TPC (13.38 ± 0.42 mg GAE/100 mL) which contradicted with values reported in previous studies. Mena et al. (2013) reported the values between 300 - 407 mg GAE/100 mL for cultivars in Spain while Li et al. (2015) reported values 315 - 743 mg GAE/100 mL for cultivars in China. Pomegranate used in the present study was grown in China, which contains lower TPC as compared to cultivars from Turkey, Spain and California (Hmid et al. 2013). Besides, different method of sample preparation might cause the variations of TPC.

Previous studies have prepared the samples by pressing the arils, whereas blending was performed in the present study. Blending might not be able to extract the phenolic compounds thoroughly, thus resulting in the deviation of TPC. The results for other fresh fruit juices were in agreement with previous studies (Aa et al. 2011; Fu et al. 2011; Keskin-Šašić et al. 2012; Mahdavi et al. 2010). Previous reported values were 17.50 - 21.54 mg GAE/100 mL for grape juice; 45.38 mg GAE/100 mL for apple juice; 24.64 mg GAE/100 mL for guava juice; 56.72 mg GAE/100 mL for mango juice; 35.74 mg GAE/100 mL for pineapple juice and 54.28 mg GAE/100 mL for orange juice. According to Fu et al. (2011), phenolic compounds that was commonly found in the juice sample included in the present study were quercetin, chlorogenic acid, kaempferol, luteolin, gallic acid and caffeic acid.

DPPH FREE RADICAL SCAVENGING ASSAY

Standard curve of Trolox with $R^2 = 0.9998$ was used to determine the antioxidant co-centration in each sample. Radical scavenging activities among fresh juices ranged from 267.78 - 770.12 μmol TE/100 mL, commercial 100% fruit juices ranged from 109.43 - 2705.01 μmol TE/100 mL

TABLE 1. Antioxidant activities and TPC of different categories of fruit juices and drinks

Code	Fruits	DPPH µmol TE/100 mL	FRAP µmol TE/100 mL	TPC mg GAE/mL
	Fresh juices			
A1	Apple	329.32 ± 13.70[c]	321.87 ± 26.98[b]	44.82 ± 15.94[a]
A2	Grape	708.52 ± 21.40[a]	592.13 ± 26.63[a]	49.80 ± 1.33[a]
A3	Guava	770.12 ± 5.01[a]	843.13 ± 44.47[c]	62.94 ± 4.95[ac]
A4	Mango	514.36 ± 15.77[d]	288.47 ± 41.30[b]	80.40 ± 0.36[c]
A5	Pineapple	275.00± 120.69[bc]	307.15 ± 88.60[b]	57.88 ± 16.70[ac]
A6	Pomegranate	267.78 ± 5.15[b]	292.13 ± 27.23[b]	13.38 ± 0.42[b]
A7	Orange	302.74 ± 71.53[bc]	410.45 ± 80.73[b]	60.02 ± 12.07[ac]
	Commercial 100% fruit juices			
B1	Apple	109.43 ± 37.20[c]	95.50 ± 25.81[c]	21.65 ± 7.02[c]
B2	Grape	849.26 ± 367.08[a]	758.00 ± 296.60[a]	102.41 ± 39.79[ab]
B3	Pineapple	315.91 ± 173.55[cd]	350.90 ± 201.50[d]	59.60 ± 23.81[a]
B4	Pomegranate	2705.01 ± 853.61[b]	2953.85 ± 1182.07[b]	130.39 ± 25.39[b]
B5	Orange	304.13 ± 39.43[d]	393.15 ± 62.01[ad]	76.84 ± 12.15[a]
	Fruit drinks			
C1	Apple	31.02 ± 22.16[a]	19.77 ± 13.55[a]	3.32 ± 2.24[a]
C2	Grape	13.32 ± 8.08[a]	13.75 ± 8.21[a]	3.52 ± 0.89[a]
C3	Guava	205.71 ± 5.01[b]	320.80 ± 51.30[b]	45.10 ± 7.75[b]
C4	Mango	58.69 ± 53.17[a]	65.17 ± 62.77[a]	6.79 ± 5.82[a]
C5	Pineapple	33.70 ± 36.25[a]	35.73 ± 32.42[a]	4.14 ± 2.28[a]
C6	Orange	66.96 ± 63.87[a]	77.65 ± 71.57[a]	11.31 ± 9.48[a]

Values were expressed as mean ± SD
Different letters in the same column represent significant difference (p<0.05) within one category of fruit juice or drink

and fruit drinks ranged from 13.32 - 205.71 µmol TE/100 mL. The free radical scavenging activity measured using DPPH assay in the present study was in the order of B4 > B2 > A3 > A2 > A4 > A1 > B3 > B5 > A7 > A5 > A6 > C3 > B1 > C6 > C4 > C5 > C1 > C2. The highest radical scavenging activity was observed for commercial 100% pomegranate juice (2705.01 ± 853.61 µmol TE/100 mL), commercial 100% grape juice (849.26 ± 367.08 µmol TE/100 mL) and fresh guava juice (770.12 ± 5.01 µmol TE/100 mL). Based on literatures, 251-1105 µmol TE/100 mL was reported for grape juice (Burin et al. 2010); 48.76-122.92 µmol TE/100 mL for apple juice (Pyo et al. 2014); 1518±81 µmol TE/100 mL for guava (Fu et al. 2011); 310.00 µmol TE/100 mL for mango juice (Vasco et al. 2008); 152.93 µmol TE/100 mL for pineapple juice (Kongsuwan et al. 2009) and 156.44 -860.00 µmol TE/100 mL for orange juice (Pyo et al. 2014). The findings of all fresh fruit juices in the present study were consistent with values reported by published studies, except for fresh pomegranate juice, which was greatly different from that of reported in the previous studies (Li et al. 2015; Mena et al. 2013) with values of 1479.00 -2486.00 µmol TE/100 mL.

FRAP FERRIC REDUCING ANTIOXIDANT POWER ASSAY

FRAP assay measures the ability of antioxidant compounds to reduce Fe (III) to Fe (II) under acidic condition (pH 3.6) (Carlsen et al. 2010). The results was calculated from calibration curve of Trolox, with R^2 = 0.9996. Reducing ability among fresh juices ranged from 288.47 - 843.13 µmol TE/100 mL, commercial 100% fruit juices ranged from 95.50 - 2953.85 µmol TE/100 mL and fruit drinks ranged from 13.75 - 320.80 µmol TE/100 mL (Table 1). A similar trend as DPPH assay was observed for all fruit juices and drinks demonstrated in FRAP assay. The reducing power for the tested samples was in the order of B4 > A3 > B2 > A2 > A7 > B5 > B3 > A1 > C3 > A5 > A6 > A4 > B1 > C6 > C4 > C5 > C1 > C2.

The highest reducing ability was observed for commercial 100% pomegranate juice (2953.85 ± 1182.07 µmol TE/100 mL), fresh guava juice (843.13 ± 44.47 µmol TE/100 mL) and commercial 100% grape juice (758.00 ± 296.60 µmol TE/100 mL). Similar to the findings reported in DPPH assays, all values obtained were in fair agreement with previous studies, except pomegranate juice. High antioxidant activity in guava juice is mainly attributed to its high content of vitamin C (Thaipong et al. 2006). Anthocyanin, resveratrol and hydroxycinnamate are reported as main constituents in grape juice (Mullen et al. 2007). A great variation in antioxidant activities for each type of fruit juices as compared to current literatures is mainly ascribed to the maturity index of fruits selected, technique used in juice extraction and parts of fruits used (peel, flesh, seed) (Burin et al. 2010; Gull et al. 2012; Sreekumar et al. 2014). Additionally, exposure to oxygen or light during sample handling and laboratory analysis might also influence the results (Wang & Xu 2007).

COMPARISON OF TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITIES AMONG DIFFERENT CATEGORIES OF JUICES OR DRINKS FOR EACH TYPE OF FRUIT

TPC of fresh, commercial 100% and fruit drinks were compared within types of fruits (Figure 1). Different categories of juices or drinks of the same fruit were compared in term of the radical scavenging (DPPH) (Figure 2) and reducing ability (FRAP) (Figure 3). Both assays demonstrated identical trends in the comparison of the antioxidant activities. All fruit drinks showed the lowest TPC and antioxidant activity as compared to their 100% fruit juice and fresh juice ($p<0.05$).

Overall, there was no significant difference ($p>0.05$) observed between TPC of fresh juices and commercial 100% juices (apple, pineapple and orange), although commercial 100% pineapple and orange juice were slightly higher than their fresh counterparts while fresh apple was higher than the commercial juice counterparts. Both 100% grape and pomegranate juice were significantly ($p<0.05$) higher TPC than their fresh counterparts.

The results were correspond to the ingredients list labelled on the packaging of the commercial juices and drinks. The ingredients listed in descending order based on the quantity per serving of fruit drinks are water,

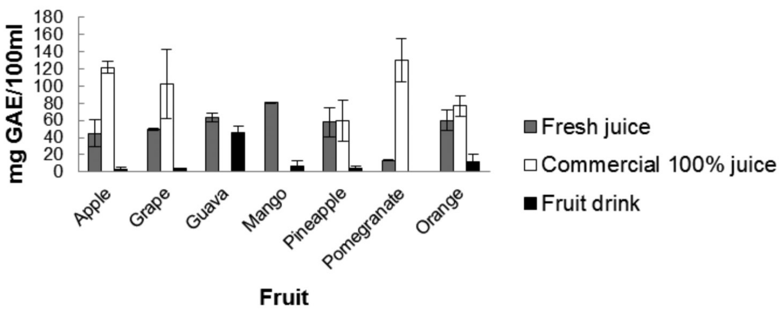

FIGURE 1. TPC (mg GAE/100 mL) of different categories of juices or drinks for each type of fruit

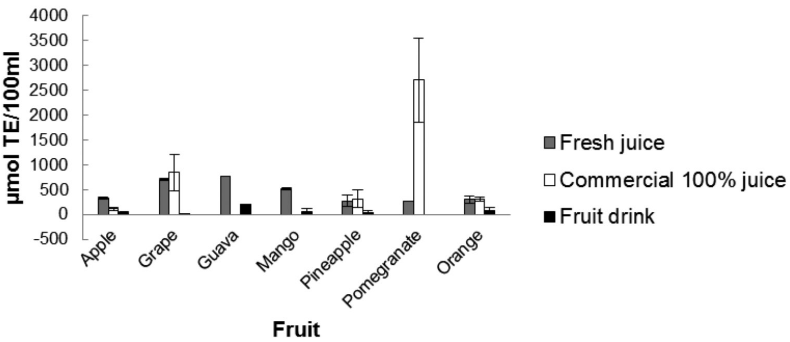

FIGURE 2. DPPH radical scavenging activities (µmol TE/100 mL) of different categories of juices or drinks for each type of fruit

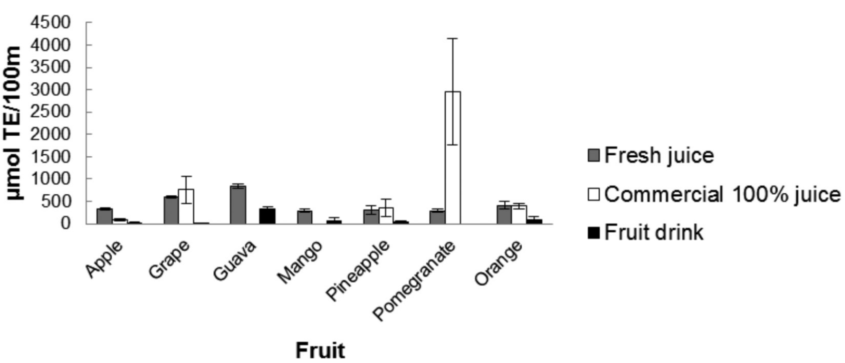

FIGURE 3. FRAP values (µmol TE/100 mL) of different categories of juices or drinks for each type of fruit

sugar, fruit juices and various kinds of food additives and fortified nutrients. Low proportion of pure fruit juice results in lower concentration of phenolic contents and hence less potent antioxidant activity in fruit drinks. On the other hand, the ingredients listed for commercial 100% juices are reconstituted form of fruit juice or juice concentrate and its corresponding amount of water, without added sugar. There was no significant difference (p>0.05) observed between commercial 100% juices and fresh juices for grape, pineapple and orange. In DPPH and FRAP assays, commercial 100% pomegranate juice was significantly higher (p<0.05) in antioxidant activity than its fresh counterpart whereas fresh apple juice showed higher antioxidant activity than its commercial 100% juice significantly (p<0.05).

Fröhling et al. (2012) studied on TPC and antioxidant capacities of commercial nectars, suggesting that TPC in fruit juice are influenced by several factors such as selection of fruit variety to yield juice, processing methods and storage conditions. Commercial fruit juices processing such as clarification, filtration and pasteurization would strongly affect the phenolic contents of the juices. Clarification and filtration, which aim to yield clear fruit juice, might remove part of the phenolic compounds that bound to the fiber and pectin (Candrawinata et al. 2012). Heat treatment could degenerate anthocyanins found abundantly in grape (Kechinski et al. 2010). Storage temperature at 4°C or lower over short period is optimum to preserve the antioxidants (Mgaya-Kilima et al. 2014).

Lower TPC and antioxidant activity in fresh pomegranate juice as compared to commercial 100% counterpart can be explained in several ways. First is the selection of cultivars used to yield juice. There was more than 1000 cultivars of pomegranate grown in Middle East, Mediterranean, China, India, California, South-West America and Mexico (Çam et al. 2009). Differences in geographical condition, climate and postharvest condition are the main parameters affecting the composition of phenolic compounds (Zarei et al. 2010). Besides, the mechanical pressing during juice production of pomegranate has significant impacts on the phenolic content. High level of punicalagins that present in rind or husk migrate to juices (Tezcan et al. 2009). Besides, pasteurization (high temperature/short time) can enhance the level of punicalagins but reduces ellagic acid and anthocyanins (Mena et al. 2012). The loss of ellagic acid and anthocyanins could be negligible as punicalagins is the main phytochemical in pomegranate (Nuncio-Jáuregui et al. 2015). Apparently, juices obtained in laboratory through blending the arils using juice extrator is relatively low in TPC and antioxidant activity.

CORRELATIONS BETWEEN TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITIES

The results showed that total phenolic content was positively and strongly correlated with both antioxidant activity assays DPPH (r = 0.908) and FRAP (r = 0.954) (p<0.01) (Table 2). In a similar comparative study of homemade and commercial grape juice by Burin et al. (2010), the correlation coefficient reported between TPC and DPPH was 0.957 which was higher than the values obtained in the present study. Likewise, high correlation was found between TPC and FRAP (r = 0. 904) reported by Xu et al. (2008) who studied on antioxidant of citrus fruit juices. This indicated that phenolic compounds were the main contributor to antioxidant activity in terms of radical scavenging and ion reducing ability. However, the antioxidant activities of fruit juices cannot be entirely predicted on the basis of their phenolic contents, as vitamin C and carotenoids in the juices also partially contribute to antioxidant functions (Almeida et al. 2011), which were not quantified in the present study. The samples with low phenolic content might show high antioxidant activity because other methanol-soluble compounds such as methylxanthine or certain pigment from fruits can also react with DPPH radicals (Belščak et al. 2009). Besides, overestimation of phenolic content by Folin-Ciocalteu assay could occur, by taking into account other non-phenolic reducing agents such as organic acid, sugar and ascorbic acid. Therefore, characterization of individual phenolic compounds using accurate analytical platform are required to provide more reliable quantification of phenolic compounds in future study.

High positive correlation was also observed between DPPH radical scavenging assay and FRAP ferric reducing assay with r = 0.959. This result was in agreement with previous study (Pyo et al. 2014) that determined the antioxidant capacity of fruit juices (r = 0.922). Both antioxidant assays rely on the principle of reduction using the mechanism of electron transfer.

CONCLUSION

The findings from the present study suggested that consumption of fresh fruit juices or commercial 100% fruit juices are equally good since there was no significant difference between TPC and antioxidant activities of these

TABLE 2. Associations between TPC and antioxidant activities

	TPC	DPPH	FRAP
TPC	1.000	0.908**	0.954**
DPPH	0.908**	1.000	0.959**
FRAP	0.954**	0.959**	1.000

**Correlation is significant at p<0.01 (2-tailed) two categories of food juices. These findings provide some useful information especially for ageing population in choosing healthy fruit juice or drinks for health maintenance purposes. The findings will also increase alertness among consumers and regulatory bodies on misleading claims by food manufacturers on food label of fruit juices or drinks. Future studies should quantify polyphenolic profiles of fruit juices or drinks using analytical platforms such as HPLC.

REFERENCES

Aa, Z., Kong, K. & Ismail, A. 2011. Antioxidant properties of tropical juices and their effects on *in vitro* hemoglobin and low density lipoprotein (LDL) oxidations. *International Food Research Journal* 18(2): 549-556.

Abirami, A., Nagarani, G. & Siddhuraju, P. 2014. *In vitro* antioxidant, anti-diabetic, cholinesterase and tyrosinase inhibitory potential of fresh juice from *Citrus hystrix* and *C. maxima* fruits. *Food Science and Human Wellness* 3(1): 16-25.

Addai, Z.R., Abdullah, A., Mutalib, S.A., Musa, K.H. & Douqan, E. 2013. Antioxidant activity and physicochemical properties of mature papaya fruit (*Carica papaya* L. Cv. eksotika). *Advance Journal of Food Science and Technology* 5: 859-865.

Albarracin, S.L., Stab, B., Casas, Z., Sutachan, J.J., Samudio, I., Gonzalez, J., Gonzalo, L., Capani, F., Morales, L. & Barreto, G.E. 2012. Effects of natural antioxidants in neurodegenerative disease. *Nutritional Neuroscience* 15(1): 1-9.

Alfadda, A.A. & Sallam, R.M. 2012. Reactive oxygen species in health and disease. *Journal of Biomedicine and Biotechnology* 2012: Article ID. 936486.

Almeida, M.M.B., de Sousa, P.H.M., Arriaga, Â.M.C., Do Prado, G.M., de Calvalho Magalhães, C.E., Maia, G.A. & de Lemos, T.L.G. 2011. Bioactive compounds and antioxidant activity of fresh exotic fruits from Northeastern Brazil. *Food Research International* 44(7): 2155-2159.

Álvarez, J., Pastoriza, S., Alonso-Olalla, R., Delgado-Andrade, C. & Rufián-Henares, J. 2014. Nutritional and physicochemical characteristic of commercial Spanish citrus juices. *Food Chemistry* 164: 396-405.

Belščak, A., Komes, D., Horžić, D., Ganić, K.K. & Karlović, D. 2009. Comparative study of commercially available cocoa products in terms of their bioactive composition. *Food Research International* 42(5): 707-716.

Bunea, A., Rugina, O.D., Pintea, A.M., Sconţa, Z., Bunea, C.I. & Socaciu, C. 2011. Comparative polyphenolic content and antioxidant activities of some wild and cultivated blueberries from Romania. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 39(2): 70-76.

Burin, V.M., Falcão, L.D., Gonzaga, L.V., Fett, R., Rosier, J.P. & Bordignon-Luiz, M.T. 2010. Colour, phenolic content and antioxidant activity of grape juice. *Ciênc. Tecnol. Aliment., Campinas* 30(4): 1027-1032.

Çam, M., Hışıl, Y. & Durmaz, G. 2009. Classification of eight pomegranate juices based on antioxidant capacity measured by four methods. *Food Chemistry* 112(3): 721-726.

Candrawinata, V.I., Blades, B., Golding, J., Stathopoulos, C. & Roach, P. 2012. Effect of clarification on the polyphenolic compound content and antioxidant activity of commercial apple juices. *International Food Research Journal* 19(3): 1055-1061.

Carlsen, M., Halvorsen, B., Holte, K., Bohn, S., Dragland, S., Sampson, L., Willey, C., Senoo, H., Umezono, Y., Sanada, C., Barikmo, I., Berhe, N., Willett, W., Phillips, K., Jacobs, D. & Blomhoff, R. 2010. The total antioxidant content of more than 3100 foods, beverages, spices, herbs and supplements used worldwide. *Nutrition Journal* 9(1): 3.

Celep, G.S. & Rastmanesh, R. 2013. Polyphenol consumption and metabolic diseases. *Journal of Nutrition Disorders & Therapy* 3(e106).

Costa, A.S.G., Nunes, M.A., Almeida, I.M.C., Carvalho, M.R., Barroso, M.F., Alves, R.C. & Oliveira, M.B.P.P. 2012. Teas, dietary supplements and fruit juices: a comparative study regarding antioxidant activity and bioactive compounds. *LWT - Food Science and Technology* 49(2): 324-328.

Durackova, Z. 2010. Some current insights into oxidative stress. *Physiology Research* 59(4): 459-469.

Fröhling, B., Patz, C., Dietrich, H. & Will, F. 2012. Anthocyanins, total phenolics and antioxidant capacities of commercial red grape juices, black currant and sour cherry nectars. *Fruit Process* 3: 100-104.

Fu, L., Xu, B.T., Xu, X.R., Gan, R.Y., Zhang, Y., Xia, E.Q. & Li, H.B. 2011. Antioxidant capacities and total phenolic contents of 62 fruits. *Food Chemistry* 129(2): 345-350.

Gull, J., Sultana, B., Anwar, F., Naseer, R., Ashraf, M. & Ashrafuzzaman, M. 2012. Variation in antioxidant attributes at three ripening stages of guava (*Psidium guajava* L.) fruit from different geographical regions of Pakistan. *Molecules* 17(3): 3165-3180.

Gupta, R.K., Patel, A.K., Shah, N., Chaudhary, A.K., Jha, U.K., Yadav, U.C., Gupta, P.K. & Pakuwal, U. 2014. Oxidative stress and antioxidants in disease and cancer: a review. *Asian Pacific Journal of Cancer Prevention* 15(11): 4405-4409.

Hegde, S.V., Adhikari, P., M. Nandini & D'souza, V. 2013. Effect of daily supplementation of fruits on oxidative stress indices and glycaemic status in Type 2 Diabetes Mellitus. *Complementary Therapies in Clinical Practice* 19(2): 97-100.

Hmid, I., Elothmani, D., Hanine, H., Oukabli, A. & Mehinagic, E. 2013. Comparative study of phenolic compounds and their antioxidant attributes of eighteen pomegranate (*Punica granatum* L.) cultivars grown in Morocco. *Arabian Journal of Chemistry* doi.org/10.1016/j.arabjc.2013.10.011

Huang, D., Ou, B. & Prior, R.L. 2005. The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry* 53(6): 1841-1856.

Ibrahim, D., Hazali, N., Jauhari, N., Omar, M.N., Yahya, M.N.A., Ahmed, I.A., Mikail, M.A. & Ibrahim, M. 2013. Physicochemical and antioxidant characteristics of *Baccaurea angulata* fruit juice extract. *African Journal of Biotechnology* 12(34): 5333-5338.

Ikram, E.H.K., Eng, K.H., Jalil, A.M.M., Ismail, A., Idris, S., Azlan, A., Nazri, H.S.M., Diton, N.A.M. & Mokhtar, R.A.M. 2009. Antioxidant capacity and total phenolic content of Malaysian underutilized fruits. *Journal of Food Composition and Analysis* 22(5): 388-393.

Kechinski, C.P., Guimaraes, P.V., Norena, C.P., Tessaro, I.C. & Marczak, L.D. 2010. Degradation kinetics of anthocyanin in blueberry juice during thermal treatment. *Journal of Food Science* 75(2): C173-176.

Keskin-Šašić, I., Tahirović, I., Topčagić, A., Klepo, L., Salihović, M., Ibragić, S., Toromanović, J., Ajanović, A. & Velispahić, E. 2012. Total phenolic content and antioxidant capacity of fruit juices. *Glas. Hem. Tehnol. Bosne Herceg* 39: 25-28.

Kongsuwan, A., Suthiluk, P., Theppakorn, T., Srilaong, V. & Setha, S. 2009. Bioactive compounds and antioxidant capacities of phulae and nanglae pineapple. *Asian Journal of Food and Agro-Industry* 2: 44-50.

Lau, T.C., Chan, M.W., Tan, H.P. & Kwek, C.L. 2012. Functional food: a growing trend among the health conscious. *Asian Social Science* 9(1): 198.

Lek, K.B., Zuraini, Z., Boon, K.B., Wan, Y.H., Swee, K.Y. & Noorjahan, B.M.A. 2012. Comparison of total phenolic content and antioxidant activities of freeze-dried commercial and fresh fruit juices. *Journal of Medicinal Plants Research* 6(48): 5857-5862.

Li, X., Wasila, H., Liu, L., Yuan, T., Gao, Z., Zhao, B. & Ahmad, I. 2015. Physicochemical characteristics, polyphenol compositions and antioxidant potential of pomegranate juices from 10 Chinese cultivars and the environmental factors analysis. *Food Chemistry* 175: 575-584.

Mahdavi, R., Nikniaz, Z., Rafraf, M. & Jouyban, A. 2010. Determination and comparison of total polyphenol and vitamin C contents of natural fresh and commercial fruit juices. *Pakistan Journal of Nutrition* 9(10): 968-972.

Mena, P., Vegara, S., Marti, N., Garcia-Viguera, C., Saura, D. & Valero, M. 2013. Changes on indigenous microbiota, colour, bioactive compounds and antioxidant activity of pasteurised pomegranate juice. *Food Chemistry* 141(3): 2122-2129.

Mena, P., Martí, N., Saura, D., Valero, M. & García-Viguera, C. 2012. Combinatory effect of thermal treatment and blending on the quality of pomegranate juices. *Food and Bioprocess Technology* 6(11): 3186-3199.

Mgaya-Kilima, B., Remberg, S.F., Chove, B.E. & Wicklund, T. 2014. Influence of storage temperature and time on the physicochemical and bioactive properties of roselle-fruit juice blends in plastic bottle. *Food Science & Nutrition* 2(2): 181-191.

Ministry of Health Malaysia. 1985. Food Act 1983. (Act 281).

Ministry of Health Malaysia. 2010. Malaysian Dietary Guidelines. National Coordinating Committee on Food and Nutrition: Technical Working Group on Nutritional Guidelines.

Ministry of Health Malaysia. 2011. National Health Morbidity Survey Malaysia. Fact Sheet.

Mullen, W., Marks, S.C. & Crozier, A. 2007. Evaluation of phenolic compounds in commercial fruit juices and fruit drinks. *Journal of Agriculture and Food Chemistry* 55(8): 3148-3157.

Nuncio-Jáuregui, N., Calín-Sánchez, Á., Vázquez-Araújo, L., Pérez-López, A.J., Frutos-Fernández, M.J. & Carbonell-Barrachina, Á.A. 2015. Chapter 76 - Processing pomegranates for juice and impact on bioactive components. In *Processing and Impact on Active Components in Food*, edited by Preedy, V. San Diego: Academic Press. pp. 629-636.

Pisoschi, A.M., Cheregi, M.C. & Danet, A.F. 2009. Total antioxidant capacity of some commercial fruit juices: electrochemical and spectrophotometrical approaches. *Molecules* 14(1): 480-493.

Plank, D.W., Szpylka, J., Sapirstein, H., Woollard, D., Zapf, C.M., Lee, V., Chen, C.Y., Liu, R.H., Tsao, R., Dusterloh, A. & Baugh, S. 2012. Determination of antioxidant activity in foods and beverages by reaction with 2,2'-diphenyl-1-picrylhydrazyl (DPPH): Collaborative Study First Action 2012.04. *Journal of AOAC International* 95(6): 1562-1569.

Pyo, Y.H., Jin, Y.J. & Hwang, J.Y. 2014. Comparison of the effects of blending and juicing on the phytochemicals contents and antioxidant capacity of typical Korean kernel fruit juices. *Preventive Nutrition and Food Science* 19(2): 108-114.

Rautiainen, S., Larsson, S., Virtamo, J. & Wolk, A. 2012. Total antioxidant capacity of diet and risk of stroke a population-based prospective cohort of women. *Stroke* 43(2): 335-340.

Ruxton, C.H., Gardner, E.J. & Walker, D. 2006. Can pure fruit and vegetable juices protect against cancer and cardiovascular disease too? a review of the evidence. *International Journal of Food Science and Nutrition* 57(3-4): 249-272.

Savatović, S.M., Tepić, A.N., Šumić, Z.M. & Nikolić, M.S. 2009. Antioxidant activity of polyphenol-enriched apple juice. *Acta Periodica Technologica* 40: 95-102.

Shen, C.L., Mo, H., Smith, B.J., Chen, C.H., Chen, L., Chyu, M.C. & Kwun, I.S. 2014. Chapter 52 - Green Tea and Other Fruit Polyphenols Attenuate Deterioration of Bone Microarchitecture. In *Polyphenols in Human Health and Disease,* edited by Watson, R.R., Preedy, V.R. & Zibadi, S. San Diego: Academic Press. pp. 681-693.

Singleton, V. & Rossi, J.A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture* 16(3): 144-158.

Siow, L.F. & Hui, Y.W. 2013. Comparison on the antioxidant properties of fresh and convection oven-dried guava (*Psidium guajava* L.). *International Food Research Journal* 20(2): 639-644.

Sreekumar, S., Sithul, H., Muraleedharan, P., Azeez, J.M. & Sreeharshan, S. 2014. Pomegranate fruit as a rich source of biologically active compounds. *Biomed Research International* 2014: Article ID. 686921.

Tan, E.S., Abdullah, A., Musa, K.H., Ghani, M.A. & Maskat, M.Y. 2012. Antioxidant properties of three banana cultivars (*Musa acuminate* 'Berangan','Mas' and 'Raja') extracts. *Sains Malaysiana* 41(3): 319-324.

Tezcan, F., Gültekin-Özgüven, M., Diken, T., Özçelik, B. & Erim, F.B. 2009. Antioxidant activity and total phenolic, organic acid and sugar content in commercial pomegranate juices. *Food Chemistry* 115(3): 873-877.

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L. & Hawkins Byrne, D. 2006. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis* 19(6-7): 669-675.

Vasco, C., Ruales, J. & Kamal-Eldin, A. 2008. Total phenolic compounds and antioxidant capacities of major fruits from Ecuador. *Food Chemistry* 111(4): 816-823.

Wang, W.D. & Xu, S.Y. 2007. Degradation kinetics of anthocyanins in blackberry juice and concentrate. *Journal of Food Engineering* 82(3): 271-275.

Wang, X., Ouyang, Y., Liu, J., Zhu, M., Zhao, G., Bao, W. & Hu, F.B. 2014. Fruit and vegetable consumption and mortality from all causes, cardiovascular disease, and cancer: systematic review and dose-response meta-analysis of prospective cohort studies. *British Medical Journal* 349: 5472.

WHO/FAO. 2003. *Diet, Nutrition and the Prevention of Chronic Diseases*. WHO Technical Report Series 916. Geneva: World Health Organisation.

Wootton-Beard, P.C., Moran, A. & Ryan, L. 2011. Stability of the total antioxidant capacity and total polyphenol content of 23 commercially available vegetable juices before and after *in vitro* digestion measured by FRAP, DPPH, ABTS and Folin-Ciocalteu methods. *Food Research International* 44(1): 217-224.

Wootton-Beard, P.C. & Ryan, L. 2011. Improving public health?: the role of antioxidant-rich fruit and vegetable beverages. *Food Research International* 44(10): 3135-3148.

Xu, G., Liu, D., Chen, J., Ye, X., Ma, Y. & Shi, J. 2008. Juice components and antioxidant capacity of citrus varieties cultivated in China. *Food Chemistry* 106(2): 545-551.
Zarei, M., Azizi, M. & Bashiri-Sadr, Z. 2010. Studies on physico-chemical properties and bioactive compounds of six pomegranate cultivars grown in Iran. *Journal of Food Technology* 8(3): 112-117.

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