

## Artikel Asli/Original Articles

# Evaluation of Total Phenolic Content, Antioxidant Activities and Sugar Content of Fresh Mixed Fruit and Vegetables Juices (Penilaian Jumlah Kandungan Fenolik, Aktiviti Antioksidan dan Gula dalam Jus Campuran Buah dan Sayur Segar yang Terpilih)

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### ABSTRACT

The notion of fruit and vegetables (FV) in preventing chronic diseases has long been discerned. To meet the recommended FV intake, FV juices have emerged as a convenient and healthy choice. Mixed fruit and vegetable juices (MFVJ) cater the blend of desirable flavor and taste of consumers. This study was carried out to determine the total phenolic content (TPC), antioxidant activities and sugar content of selected MFVJ. Folin-Ciocalteu assay was employed to quantify TPC, whereas DPPH and FRAP assays were used to evaluate the antioxidant activities of MFVJ. The sugar content was determined using phenol-sulfuric acid method. MFVJ extracted from bitter melon, green apple and orange (BGO) had the highest TPC content ( $76.4 \pm 4.2$  mg GAE/100 ml). Celery and green apple (CG) juice showed the highest DPPH value ( $522.3 \pm 7.6$  mg TE/100 ml) whereas carrot and starfruit (AS) juice have the highest FRAP value ( $419.6 \pm 21.6$  mg TE/100 ml). The sugar content of MFVJ was within the range of 5.7-13.3 g/100 ml. MFVJ can be considered as healthy beverages with considerable amounts of phenolic compounds and low sugar content. This study provides some useful reference for consumers who consume juices with combinations of FV. Future studies need to discover more combinations of FV juices, providing more data pertaining to MFVJ. Identification of individual phenolic compounds should also be part of future research using various instrumental analyses.

**Keywords:** Total phenolic content; antioxidant activities; sugar content; fresh; mixed fruit and vegetable juices

### ABSTRAK

Tanggapan mengenai sayur-sayuran dan buah-buahan (FV) adalah baik dalam pencegahan penyakit kronik telah lama dikenal pasti. Jus FV merupakan minuman yang mudah dan sihat bagi memenuhi pengambilan FV yang disyorkan. Jus campuran buah dan sayur (MFVJ) dapat memenuhi perisa dan rasa yang disukai oleh pengguna. Kajian ini dijalankan untuk menentukan jumlah kandungan fenolik (TPC), aktiviti antioksidan dan kandungan gula bagi MFVJ yang terpilih. Asai Folin-Ciocalteu telah dijalankan bagi menentukan TPC, manakala asai DPPH and FRAP telah digunakan untuk menilai aktiviti antioksidan MFVJ. Kandungan gula pula ditentukan dengan menggunakan kaedah fenol-asid sulfurik. MFVJ yang diekstrak daripada peria, epal hijau dan oren (BGO) mengandungi TPC yang tertinggi ( $76.4 \pm 4.2$  mg GAE/100 ml). Jus saderi dan epal hijau (CG) menunjukkan nilai DPPH yang tertinggi ( $522.3 \pm 7.6$  mg TE/100 ml) manakala jus lobak merah dan belimbing mempunyai nilai FRAP yang tertinggi ( $419.6 \pm 21.6$  mg TE/100 ml). Kandungan gula MFVJ berada dalam julat 5.7-13.3 g/100 ml. MFVJ boleh dianggap sebagai minuman sihat kerana mengandungi sebatian fenolik yang agak tinggi dan mempunyai kandungan gula yang rendah. Kajian ini menyediakan beberapa kombinasi FV sebagai rujukan yang berguna kepada pengguna untuk menyediakan MFVJ. Kajian masa depan patut menggunakan lebih banyak kombinasi FV untuk jus MFVJ, supaya dapat memberi lebih banyak maklumat berkaitan dengan kebaikan MFVJ. Pengenalpastian sebatian fenolik juga harus menjadi sebahagian daripada kajian pada masa akan datang dengan menggunakan pelbagai analisis berinstrumen.

**Kata kunci:** Jumlah kandungan fenolik; aktiviti antioksidan; kandungan gula; segar; jus campuran buah dan sayur

### INTRODUCTION

Epidemiological studies reported that diet rich in fruits and vegetables (FV) is important in the prevention of a series of pathological conditions such as heart diseases, cancer, diabetes mellitus, and other diseases related to oxidative stress (Bhupathiraju et al. 2013; Bradbury

et al. 2014; Mursu et al. 2014). The protective effects of FV intake are related to their polyphenolic antioxidants content (Eastwood 1999).

Mixed fruit and vegetable juices (MFVJ) contain a combination of two or more fruits and vegetables. MFVJ may increase the acceptance of consumers as it can offset certain flavor or taste of fruits or vegetables that is not well-

accepted by consumers, thus improving the palatability of the juices. Besides, MFVJ ensure the consumption of wide variety of fruit and vegetables as compared to single fruit or vegetable juices. MFVJ serve as a healthy option for consumers, in order to meet the recommended intake of fruits and vegetables. To date, limited studies have been conducted to evaluate the antioxidant activities of MFVJ.

Sugar is another concern when choosing healthy beverages, as high sugar intake was associated with an array of chronic diseases. Consumption of sugar-sweetened beverages was associated with the increase of adiposity, long-term weight gain and risk of diabetes (de Ruyter et al. 2012; Ebbeling et al. 2012; Malik et al. 2010; Qi et al. 2012). Fresh FV juices without added sugar are preferable over other sugar-added beverages. To the best of our knowledge, paltry research has worked to determine the sugar content of fresh juices including MFVJ. Thus, this study was carried out to determine TPC, antioxidant activities and sugar content in juice combinations of FV which are locally available.

## EXPERIMENTAL METHODS

### SAMPLE COLLECTION

Eleven types of fresh mixed fruit and vegetable juices (MFVJ) were selected, namely carrot, celery and green apple (ACG), carrot and green apple (AG), carrot and starfruit (AS), carrot, tomato and orange (ATO), carrot, cucumber and green apple (AUG), bitter gourd, carrot and orange (BAO), bitter gourd, celery and green apple (BCG), bitter gourd, green apple and orange (BGO), bitter gourd and starfruit (BS), celery and green apple (CG) and celery, cucumber and green apple (CUG). The fruits were blended based on each whole fruit. For example, AS juice made from combination of one carrot and one star fruit. All selected juices were purchased from fruit juice stalls and the juices were extracted using electric juice extractor (Breville JE98XL, USA) without addition of water and additives. The juice was stored in the freezer prior to the analyses carried out the next day.

### CHEMICALS & REAGENTS

1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma-Aldrich), gallic acid (Sigma-Aldrich), Folin-Ciocalteu's reagent (Sigma-Aldrich), sodium carbonate (SystemChemAR), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) (Sigma-Aldrich), 2,4,6-tri-(2-pyridyl)-s-triazine (TPTZ) and ferric chloride hexahydrate ( $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ) (Sigma-Aldrich, USA), sodium acetate trihydrate, glacial acetic acid, hydrochloric acid (sp.gr. 1.18, England), phenol (R&M Chemicals), sulphuric acid (R&M Chemicals), glucose (HmbG Chemicals) and methanol (SystemChemAR). All chemicals and reagents used in this study were of analytical grade.

### SAMPLE PREPARATION

Prior to analysis, centrifugation was performed based on the method of Aa et al. (2011) where the juices were centrifuged at 3000 rpm ( $1556 \times g$ ) for duration of 10 minutes using centrifuge (Gyrozen 406, Korea) and supernatant was collected for the analysis. All the following analyses were performed in four replicates and the final results were expressed as mean  $\pm$  standard deviation.

### DETERMINATION OF TOTAL PHENOLIC CONTENT (TPC)

The total phenolic content was determined spectrophotometrically using Folin-Ciocalteu reagent according to the method described by Mahdavin et al. (2010) with minor modification. A total of 3.6 ml of distilled water was mixed with a 0.4 ml of juice sample and 0.4 ml of Folin-Ciocalteu's reagent solution in a 10 ml volumetric flask. The mixture was homogenized using a vortex (Scientific Industries, USA) and incubated at room temperature for 5 minutes. Then, a total of 4.0 ml of sodium carbonate solution (7%, w/v) was added to the mixture. The mixture was made up to 10 ml with distilled water and was shaken thoroughly. The mixture was incubated at room temperature for 90 minutes. This was followed by measurement of the sample absorbance at 765 nm using a spectrophotometer (Secomam CE, Fance) against distilled water as a blank. The calibration curve from gallic acid standard (0 to 250 mg/ml) was obtained and the total phenolic content of the samples was expressed as mg GAE (gallic acid equivalent)/100 ml of samples.

### DETERMINATION OF DPPH RADICAL SCAVENGING ACTIVITY

DPPH radical scavenging activity was determined using a slight modification method proposed by Costa et al. (2012) and Plank et al. (2012). A total of 0.4 ml of juice sample was mixed with 5.6 ml of freshly prepared 0.1 mM methanolic DPPH solution in a test tube. The sample mixture was homogenized using a vortex (Scientific Industries, USA) and was incubated in water bath at 37°C for 30 minutes. Then the absorbance of the sample mixture was measured at 517 nm using spectrophotometer (Secomam CE, France). Standard curve was plotted using different concentrations of Trolox solution (0 to 500 mg/ml). The results were expressed as mg TE/100 ml of samples.

### DETERMINATION OF FRAP FERRIC REDUCING ANTIOXIDANT POWER

The FRAP assay was performed in accordance to the method of Álvarez et al. (2014) and Wootton-Beard et al. (2011) with slight modification. The FRAP reagent was prepared from 25 mL of 300 mM acetate buffer (3.1 g  $\text{C}_2\text{H}_3\text{NaO}_2 \cdot 3\text{H}_2\text{O}$  and 16 mL  $\text{C}_2\text{H}_4\text{O}_2$ ), 2.5 mL of 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution dissolved in 40 mM HCl and 2.5 mL of 20 mM  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  solution. The

mixture was warmed at 37°C prior to analysis. A volume of 2 mL of warmed distilled water at 37°C was added to 50 µL of sample, followed by addition of 2 mL of FRAP reagent in test tube. The mixture was incubated at 37°C for 4 min in the dark condition. Absorbance was read against methanol as blank at 593 nm using UV-visual spectrophotometer (Secomam CE, France). The standard curve was produced from 0-1000 mg/mL of Trolox solutions. The results were expressed in mg TE/100 ml.

#### DETERMINATION OF SUGAR CONTENT

Phenol-sulfuric acid method was applied to determine the total sugar content of juices (Dubois et al. 1956). A total of 1.0 ml of sample was mixed with 1.0 ml of 5% aqueous phenol solution in test tube. The mixture was homogenized using vortex (Scientific Industries, USA). Then, a total of 5.0 ml of concentrated sulphuric acid was added and the mixture was shaken to ensure the solution is mixed well. After allowing the test tube to stand for 15-30 minutes at room temperature, the absorbance was measured at 490 nm using spectrophotometer (Secomam CE, France). Standard calibration curve was generated using different concentrations (0-1000 µl/ml) of standard glucose solution. The total sugar content of sample was expressed as gram sugar per 100 ml of samples.

## RESULTS AND DISCUSSION

#### TOTAL PHENOLIC CONTENT (TPC) OF SELECTED FRESH MIXED FRUIT AND VEGETABLE JUICES (MFVJ)

The TPC of the MFVJ samples was in the range of 49.6-76.4 mg GAE/100 mL (Table 1). Out of all samples, bitter gourd, orange and green apple (BGO) contained the highest TPC with the value of  $76.4 \pm 4.2$  mg GAE/100 ml. Bitter gourd, celery and green apple (BCG) contained similar fruits or vegetables as BGO except orange was replaced with celery, but the TPC value of BCG were considerably lower ( $55.3 \pm 1.3$  mg GAE/100 ml). Previous study also reported celery has lower phenolic content as compared to other vegetables such as broccoli, spinach, carrot, lettuce and some other vegetables with TPC value of  $14.95 \pm 0.51$  mg GAE/100 g fresh weight (Chu et al. 2002). This value was apparently lower than TPC of orange ( $77.23 \pm 0.08$  mg GAE/100 g) reported in another study (Fu et al. 2011). Similarly, bitter gourd, carrot and orange (BAO) with substitution of green apple with carrot has demonstrated a lower TPC value ( $59.9 \pm 1.0$  mg GAE/100 ml). These scenarios depicted the importance of fruit and vegetable selection as the ingredients of MFVJ.

TABLE 1. Total phenolic content and sugar content of selected mixed fruit and vegetable juices (MFVJ)

| Mixed fruit and vegetable juice      | Abbreviation of juice | Total phenolic content (mg GAE/100 ml) | Sugar content (g/100 ml) |
|--------------------------------------|-----------------------|--|--------------------------|
| Carrot, celery and green apple       | ACG                   | $55.8 \pm 1.7$                         | $6.1 \pm 0.4$            |
| Carrot and green apple               | AG                    | $60.2 \pm 0.5$                         | $9.3 \pm 0.6$            |
| Carrot and starfruit                 | AS                    | $68.8 \pm 3.0$                         | $13.3 \pm 0.9$           |
| Carrot, tomato and orange            | ATO                   | $69.9 \pm 4.5$                         | $7.4 \pm 0.4$            |
| Carrot, cucumber and green apple     | AUG                   | $49.6 \pm 0.4$                         | $11.3 \pm 1.5$           |
| Bitter gourd, carrot and orange      | BAO                   | $59.9 \pm 1.0$                         | $9.3 \pm 0.1$            |
| Bitter gourd, celery and green apple | BCG                   | $55.3 \pm 1.3$                         | $5.7 \pm 1.0$            |
| Bitter gourd, green apple and orange | BGO                   | $76.4 \pm 4.2$                         | $10.3 \pm 0.7$           |
| Bitter gourd and starfruit           | BS                    | $61.1 \pm 1.5$                         | $6.8 \pm 0.8$            |
| Celery and green apple               | CG                    | $65.8 \pm 1.1$                         | $9.5 \pm 1.3$            |
| Celery, cucumber and green apple     | CUG                   | $55.0 \pm 1.4$                         | $9.0 \pm 0.4$            |

Four of the selected samples (carrot and green apple (AG), carrot and starfruit (AS), bitter gourd and starfruit (BS), celery and green apple (CG)) were made up from a combination of two fruits or vegetables. AS has emerged as the juice with the highest TPC ( $68.8 \pm 3.0$  mg GAE/100 ml) when compared with BS, CG and AG with TPC values of  $65.8 \pm 1.1$ ,  $61.1 \pm 1.5$  and  $60.2 \pm 0.5$  mg GAE/100 ml, respectively. In juice extraction, ripe starfruit is preferred over the unripe ones. Previous study showed ripe starfruit contained higher TPC than the green unripe fruits with peel having higher TPC than that of pulp. The TPC for ripe starfruit peel and pulp were  $98.19 \pm 13.83$  and  $39.89 \pm$

$5.29$  mg TAE/100 g fresh weight, respectively (Lim & Lee 2013). The high TPC of starfruit might be the contributing factor of high TPC in AS juice. Surprisingly, these juices exhibited higher TPC values as compared with most of the juices with three fruits or vegetables. Combination of carrot, cucumber and green apple (AUG) was the MFVJ with the lowest TPC ( $49.6 \pm 0.4$  mg GAE/100 ml), ranking after cucumber and green apple (CUG), bitter gourd, celery and green apple (BCG) and carrot, celery and green apple (ACG) exhibited slightly higher TPC values of  $55.0 \pm 1.4$  mg GAE/100 ml,  $55.3 \pm 1.3$  mg GAE/100 ml and  $55.8 \pm 1.7$  mg GAE/100 ml, respectively.

#### ANTIOXIDANT ACTIVITIES OF SELECTED FRESH MIXED FRUIT AND VEGETABLE JUICES (MFVJ)

Phenolic compounds have important roles to play in antioxidant activities of MFVJ. Despite this, other antioxidant constituents such as vitamins and minerals also contribute to the antioxidant capacity of MFVJ. Thus, determination of antioxidant activities using antioxidant assays such as DPPH and FRAP are important in evaluating the synergistic effects of all antioxidant compounds.

Celery and green apple (CG) juices demonstrated the highest DPPH value ( $522.3 \pm 7.6$  mg TE/100 ml) whereas carrot and starfruit (AS) exhibited the highest FRAP value ( $419.6 \pm 21.6$  mg TE/100 ml) in all MFVJ samples (Figure 1). Both celery and green apple in CG contain substantial amounts of phenolic compounds, ascorbic acid (AA) and  $\alpha$ -tocopherol, which may act synergistically to exert the strong antioxidant effects. The TPC, AA and  $\alpha$ -tocopherol of green apple were 1.87 mg GAE/g fresh weight, 20.6  $\mu$ g

AA/g fresh weight and 3.97  $\mu$ g/g fresh weight, respectively (Isabelle et al. 2010a). Whilst, celery has a TPC of 0.31 mg GAE/G fresh weight, 26.4  $\mu$ g AA/g fresh weight and contained 7.05  $\mu$ g/g fresh weight of  $\alpha$ -tocopherol (Isabelle et al. 2010b). Another study used 2',7'-dichlorofluoresceindiacetate (DCFH-DA) cell based to determine the antioxidant properties of carrot juice, revealing carrot juice has a low  $IC_{50}$  value of  $57 \pm 37$   $\mu$ g/ml. This value was close to that of  $\alpha$ -tocopherol ( $49.6 \pm 15.0$   $\mu$ g/ml) and showed that the antioxidant activity of carrot juice was superior to those of blueberry, peach and kiwi juices (Girard-Lalancette et al. 2009). This phenomenon was further supported by evidence from a human study which indicated drinking carrot juice increased the plasma total antioxidant capacity and decreased the plasma malondialdehyde (MDA) production (Potter et al. 2011). This might be able to elucidate the high FRAP value of juice combination of carrot and starfruit (AS).

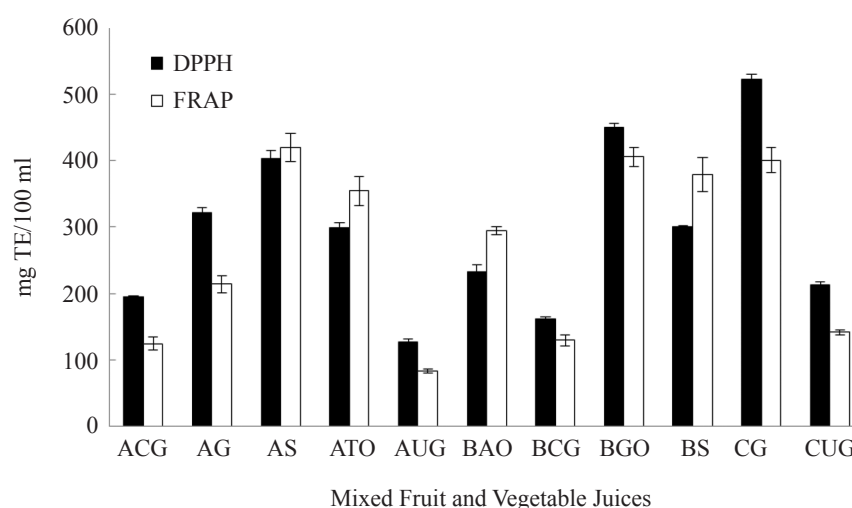


FIGURE 1. Antioxidant activities of selected fresh mixed fruit and vegetable juices (MFVJ)

Consistent to the results of TPC, carrot, cucumber and green apple (AUG) was the MFVJ sample with the lowest values in both DPPH and FRAP assays, confirming the roles of phenolic compounds in antioxidant activities are somehow important. Both bitter gourd, green apple and orange (BGO) and celery and green apple (CG) juices also possessed high FRAP values ( $>400$  mg TE/100 ml). AS and BGO exhibited high radical scavenging ability with DPPH values of  $402.3 \pm 12.5$  and  $449.4 \pm 7.1$  mg TE/100 ml, respectively, ranking after CG.

#### SUGAR CONTENT OF SELECTED FRESH MIXED FRUIT AND VEGETABLE JUICES (MFVJ)

The sugar content of MFVJ was in the range of 5.7-11.3 g/100 mL (Table 1). Generally the binary MFVJ (combinations of 2 fruits or vegetables) had relatively high sugar content

as compared to those of ternary MFVJ (combination of 3 fruits or vegetables) except for bitter gourd and starfruit (BS) that have sugar content of  $6.8 \pm 0.8$  g/100 mL. Carrot and starfruit (AS) has the highest sugar content ( $13.3 \pm 0.9$  g/100 ml) while bitter gourd, celery and green apple (BCG) contained the least sugar content ( $5.7 \pm 1.0$  g/100 ml). The sugar content was hardly predictable since the change of one ingredient can affect the sugar content of MFVJ as depicted in ACG ( $6.1 \pm 0.4$  g/100 ml) and CUG ( $9.0 \pm 0.4$  g/100 ml). Despite this, the sugar content in the selected MFVJ was considered low, and the difference among sugar content of juices was small. Low sugar content of MFVJ can serve as the suitable beverages for diabetic individuals in order to control glycemic response. The limitation of the study would be samples could not be collected from various stalls due to cost limitation.



## CONCLUSION

MFVJ contains considerable high amount of phenolic and low content of sugar, catering a healthy choice for consumers. With its high antioxidant activities, MFVJ will be able to confer a wide spectrum of health benefits. Celery and green apple (CG) juice contained high amount of antioxidants and moderate amount of sugar would be the best MFVJ in this study. Future studies should continue to explore combinations of FV for fresh MFVJ preparation. Identification of phenolic compounds using different analytical platforms as well as quantification of other antioxidant nutrients such as vitamins C and E need to be analysed for future research.

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