

Artikel Asli/Original Articles

Phenolic Content and Antioxidant Activities of Commercial Pomegranate and Date Concentrates

(Kandungan Fenolik dan Aktiviti Antioksidan Pati Delima dan Kurma Komersial)

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ABSTRACT

The aim of this study was to determine the polyphenol content and antioxidant activities in commercialized date and pomegranate concentrates that are available in local market. A total of seven samples comprised of two brands of date concentrates (D1 and D2) and five brands of pomegranate concentrates (P1, P2, P3, P4, and P5) have been analyzed for their polyphenol content and antioxidant activity. The Folin-Ciocalteu assay was used to determine the polyphenol content (TPC) of the samples while 2,2-diphenyl-1-picrylhydrazyl-hydrate (DPPH) and Ferric Reducing Antioxidant Power (FRAP) assays were used to determine the antioxidant activity. The results showed the commercialized date concentrate D2 contained the highest TPC (1243.00 ± 67.00 mg GAE/100 ml) and antioxidant activity using FRAP assay (2.67 ± 0.01 mmol TE/100 ml). Date concentrate D1 showed the highest antioxidant activity using DPPH assay (0.39 ± 0.02 mmol TE/100 ml). The pomegranate concentrate P5 showed the highest reading for TPC (3260.10 ± 428.70 mg GAE/100 ml), DPPH (1.61 ± 0.05 mmol TE/100 ml) and FRAP assays (20.98 ± 0.42 mmol TE/100 ml). As a conclusion, the studied commercial date and pomegranate concentrates available in the local market contained high polyphenol and antioxidant contents although the content levels were different from one brand to another. Further study is needed to identify the causes of this distinction. The results of this study could help the consumers to make a better selection of commercialized date and pomegranate concentrates that are available in local market in term of polyphenol content and antioxidant activity.

Keywords: Pomegranate; date; commercial juice; concentrate; polyphenol; antioxidant

ABSTRAK

Tujuan kajian ini adalah bagi menentukan kandungan polifenol dan antioksidan aktiviti dalam pati kurma dan delima komersial yang terdapat di pasaran tempatan. Sejumlah tujuh sampel yang terdiri dari dua jenama pati kurma (D1 dan D2) dan lima jenama pati delima (P1, P2, P3, P4, dan P5) telah dianalisa kandungan polifenol dan aktiviti antioksidan. Asai Folin-Ciocalteu telah digunakan untuk menentukan kandungan polifenol (TPC) sampel manakala asai DPPH dan FRAP telah digunakan untuk menentukan aktiviti antioksidan. Keputusan kajian menunjukkan pati kurma komersial D2 mengandungi TPC yang paling tinggi (1243.00 ± 67.00 mg GAE/100 ml) dan aktiviti antioksidan menggunakan asai FRAP (2.67 ± 0.01 mmol TE/100 ml). Pati kurma D1 menunjukkan aktiviti antioksidan paling tinggi menggunakan asai DPPH (0.39 ± 0.02 mmol TE/100 ml). Pati delima P5 menunjukkan bacaan tertinggi bagi TPC (3260.10 ± 428.70 mg GAE/100 ml), asai DPPH (1.61 ± 0.05 mmol TE/100 ml) dan FRAP (20.98 ± 0.42 mmol TE/100 ml). Kesimpulannya, pati kurma dan delima komersial yang dikaji dalam kajian ini mengandungi polifenol dan antioksidan yang tinggi walaupun aras kandungannya adalah berbeza dari satu jenama ke jenama lain. Kajian selanjutnya diperlukan bagi mengenal pasti penyebab kepada wujudnya perbezaan ini. Hasil kajian ini dapat membantu pengguna untuk membuat pilihan yang lebih baik bagi pati kurma dan delima komersial yang terdapat dipasaran tempatan dari segi kandungan polifenol dan aktiviti antioksidan.

Kata kunci: Delima; kurma; jus komersial; pati; polifenol; antioksidan

INTRODUCTION

According to Centers for Disease Control and Prevention (CDC) only one in ten adults consume enough fruits and vegetables as recommended which is at least 1 ½ to 2 cups per day of fruits and 2 to 3 cups per day of vegetables or 400 g per day of fruits and vegetables as recommended by WHO (Lee-Kwan et al. 2017). Fruits and vegetables intake could

reduce the risk of non-communicable diseases such as cardiovascular disease and cancer (Wang et al. 2014). Fruits and vegetables are the source of phytochemicals and major contributors of dietary polyphenols that provide protective mechanism such as antioxidant and anti-inflammatory for human (Hervert-Hernández et al. 2011; Slavin & Lloyd 2012). Polyphenol consumption also shows beneficial effects on cognitive and mental health status especially among elderly (Rosli et al. 2014).

Pomegranate and date are among the fruits mentioned in the Holy Quran and they are beneficial to human being (Al-Quran, Al-An'am 6:141). Pomegranate and dates are high in polyphenol content which act as antioxidant and provide protection for liver, gastrointestinal and neuronal function (Viuda-Martos et al. 2010; Asgary et al. 2013; Rahmani et al. 2014; Essa et al. 2015). A study by Rosenblat et al. (2015) found that the combination of date and pomegranate can protect against atherosclerosis which is the thickening and hardening of arterial wall due to cholesterol oxidation and lipid accumulation. This could lead to strokes or heart attacks. Polyphenol compounds in date and pomegranate act as anti-atherosclerotic agent by reducing the oxidation process (Rosenblat et al. 2015).

Besides consuming as a whole fruit, pomegranate and date were also made into juice or concentrate. Some people consume pomegranate or date concentrate daily as dietary supplement for their antioxidant benefit. Most of commercially available pomegranate or date concentrates have claimed to contain high antioxidant. Thus, this study was carried out to determine and compare the polyphenol content and antioxidant activity in commercialized pomegranate and date concentrates that are available in the local market that might help consumers to make a better brand choice.

EXPERIMENTAL METHODS

CHEMICALS AND MATERIALS

Folin-Ciocalteu reagent, sodium carbonate, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Trolox, 2,4,5-Tripyridyls-triazine (TPTZ), iron (III) chloride, hydrochloric acid, sodium acetate, methanol and acetic acid were obtained from Sigma-Aldrich, USA.

Pomegranate and date concentrates (100% pure) were purchased from suppliers of health food products located in three different locations which is in Kajang, Ampang and Kampung Baru. Five brands of pomegranate concentrates (P1, P2, P3, P4 and P5) and two brands of date concentrates (D1 and D2) were chosen based on their availability in the local market. All the date and pomegranate concentrates were imported from Turkey as stated in the products label but the type or cultivar of date or pomegranate used was not mentioned specifically. The samples were diluted with distilled water before they were analyzed. Each sample was analyzed in triplicate.

TOTAL POLYPHENOL CONTENT

The polyphenol content of the juice was determined using Folin-Ciocalteu assay based on the method described by Zhang et al. (2006) with a few modifications. About 20 µl sample and 100 µl Folin-Ciocalteu agents were mixed into 96-wellplate flat bottom. Samples were left for 5 minutes before adding 80 µl of 7.5% sodium carbonate and kept in the dark at room temperature. Following two

hours, the absorbance was measured at wavelength 750 nm by using microplate reader (iMark, Bio-Rad, Canada). The results were expressed as mg Gallic acid equivalent (GAE)/100 ml.

DPPH RADICAL SCAVENGING ACTIVITY

DPPH assay was carried out according to the method of Wang et al. (2012) with a few modifications. About 100 µl of sample and 100 µl 0.3 mM DPPH were mixed in 96-wellplate flat bottom and left for 30 minutes in the dark at room temperature before the absorbance was measured by using microplate reader (iMark, Bio-Rad, Canada) at wavelength of 490 nm. The results were expressed as mmol Trolox equivalent (TE)/100 ml.

FERRIC REDUCING ANTIOXIDANT POWER (FRAP)

FRAP assay was carried out according to the method of Benzie and Strain (1996) with a few modifications. About 50 µl of product sample and 175 µl of warm FRAP reagent were mixed in 96-wellplate flat bottom and incubated in the dark at room temperature for 5 minutes before the absorbance was measured at wavelength 595 nm using microplate reader (iMark, Bio-Rad, Canada). The results were expressed as mmol Trolox equivalent (TE)/100 ml.

STATISTICAL ANALYSIS

Data were analysed by using SPSS version 20 (IBM Corporation, Armonk, New York, USA). All the data were presented in mean ± standard deviation from triplicate analysis of samples. One-way ANOVA and Independent t-test were used to analyse the mean difference of polyphenol content and antioxidant activity in the pomegranate and date concentrates. The significant difference is at ($p < 0.05$).

RESULT AND DISCUSSION

POLYPHENOL AND ANTIOXIDANT IN POMEGRANATE

Table 1 showed the total polyphenol content of pomegranate concentrates. The result showed P5 brand has significantly ($p < 0.05$) highest polyphenol content (3260.10 ± 428.70 mg GAE/100 ml) and antioxidant activities based on DPPH (1.61 ± 0.05 mmol TE/100 ml) and FRAP (20.98 ± 0.42 mmol TE/100 ml) assays. Meanwhile, P1 brand showed the lowest value for all the assays followed by P2 brand. Overall, the total polyphenol content were between 18.30 to 3260 mg GAE/100 ml.

Previous studies (Tezcan et al. 2009; Karaca 2011; Wern et al. 2016; Zografou et al. 2017) reported the total polyphenol content in commercial pomegranate juice were between 14.40 to 13039.00 mg GAE/100 ml which is parallel with current study. The total polyphenol content in commercial pomegranate concentrate (515.85 mg GAE/100

ml) was higher compared to pomegranate juice (72.06 mg GAE/100 ml) (Zografou et al. 2017). Meanwhile Wern et al. (2016) reported that commercial 100% pomegranate juice was significantly higher in total polyphenol content (13039 mg GAE/100 ml) as compared to fresh squeezed pomegranate juice (1338 mg GAE/100 ml).

TABLE 1. Total polyphenol content and antioxidant activity in pomegranate concentrates

| Brand of pomegranate concentrate | Parameters | | |
|----------------------------------|-------------------------------|----------------------------|---------------------------|
| | TPC mg GAE/ 100 ml | DPPH mmol TE/ 100 ml | TPC mmol TE/ 100 ml |
| P1 | 18.30 ± 3.00 ^a | 0.13 ± 0.00 ^a | 0.74 ± 0.02 ^a |
| P2 | 22.30 ± 4.73 ^a | 0.13 ± 0.00 ^a | 0.81 ± 0.01 ^a |
| P3 | 441.00 ± 59.20 ^a | 0.37 ± 0.01 ^b | 2.09 ± 0.00 ^b |
| P4 | 1288.70 ± 182.00 ^b | 0.68 ± 0.09 ^c | 6.76 ± 0.21 ^c |
| P5 | 3260.10 ± 428.70 ^c | 1.61 ± 0.05 ^d | 20.98 ± 0.42 ^d |

Note. Data were expressed as mean ± standard deviation from nine replicate of samples. The mean that has different letter in the same column were significantly different ($p < 0.05$) using one-way ANOVA and post-hoc Tukey HSD.

The antioxidant activities in pomegranate concentrates using DPPH and FRAP assays for current study were ranged between 0.13 to 1.61 mmol TE/100 ml and 0.74 to 20.98 mmol TE/100 ml respectively. Mena et al. (2013) reported almost the same result in fresh pomegranate juice which was 1.48 mmol TE/100 ml using DPPH assay. However, Wern et al. (2016) reported higher values for antioxidant activity (26.78 mmol TE/100 ml) in fresh pomegranate juice and 270.50 mmol TE/100 ml in commercial pomegranate juice using the same assay. The same study also reported higher values compared to this study using FRAP assay in fresh pomegranate juice and commercial pomegranate juice which were 29.21 mmol TE/100 ml and 295.39 mmol TE/100 ml respectively (Wern et al. 2016). Meanwhile Mena et al. (2013) reported the FRAP value in fresh juice was 1.79 mmol TE/100 ml which is higher than FRAP value for P1 and P2 but lower compared to P3, P4 and P5.

The antioxidant potential in pomegranate juices depend on polyphenol content of the pomegranate. There are multiple factors that could affect the polyphenol content level in pomegranate juice such as cultivar, origin of the fruit, maturity index, extraction method, part of fruit used and storage condition (Fawole & Opara 2013). Pomegranate juice pressed from the whole fruits including their arils and peels contained higher polyphenol and antioxidant activity compared to juice produced from arils only (Anahita et al. 2015). Commercial pomegranate juice also showed higher antioxidant compared to fresh juice (Zografou et al. 2017). Technique of juice extraction is important as it will affect the polyphenol in the juice. According to Pyo et al. (2014), juice produced by blending technique contains higher polyphenol compound and antioxidant compared to juicing. Juicing technique involved extraction of juice

by squeezing the edible part of a fruit (pulp and flesh) while blending technique involved crushing the whole fruit including the peel to produce juice. The removal of peels in juicing technique may result in loss of certain polyphenol compounds that are valuable for the human health (Pyo et al. 2014).

POLYPHENOL AND ANTIOXIDANT IN DATE

Based on the result shown in Table 2, date concentrate D2 (1243.00 ± 67.00 mg GAE/100 ml) contained significantly ($p < 0.05$) higher polyphenol content compared to D1 (727.00 ± 21.00 mg GAE/100 ml). Date concentrate D1 showed the highest antioxidant activity ($p < 0.05$) using DPPH assay (0.39 ± 0.02 mmol TE/100 ml) while date concentrate D2 showed the highest antioxidant activity ($p < 0.05$) using FRAP assay (2.67 ± 0.01 mmol TE/100 ml).

Most previous studies were focussing on polyphenol content and antioxidant activity that involved fresh date. There is a paucity in study on commercialized date concentrates or juices. This is maybe due to the nature of date which commonly consumed as a whole fruits, not in the form of juice or concentrate. Previous studies on the polyphenol content of fresh date were reported from various countries such as Tunisia, America, Saudi Arabia and Algeria and the results ranged between 28.96 to 709.00 mg GAE/100 g (Al-Turki et al. 2009; Saafi et al. 2009; Al-Humaid et al. 2010; Mrabet el al. 2012; Benmeddour et al. 2013). The polyphenol content in date concentrates D1 and D2 were higher compared to the fresh date used in the previous studies possibly due to the extraction technique involved.

The antioxidant activity using DPPH and FRAP assays of dates from previous studies ranged between 0.10 µmol to 4.94 mmol TE/100 g and 21.21 µmol to 27.5 mmol TE/100 g respectively (Abbas et al. 2008; Bilgari et al. 2008; Al-Turki et al. 2009; Al-Humaid et al. 2010; Mrabet el al. 2012; Mohamed et al. 2014). The dates used in these studies originated from various countries such as Tunisia, America, Saudi Arabia and Sudan.

The difference in polyphenol content and antioxidant activity in dates possibly due to numerous factors such as

TABLE 2. Total polyphenol content and antioxidant activity in date concentrates

| Brand of date concentrate | Parameters | | |
|---------------------------|--------------------------|----------------------------|----------------------------|
| | TPC mg GAE/ 100 ml | DPPH mmol TE/ 100 ml | FRAP mmol TE/ 100 ml |
| D1 | 727.00 ± 21.00 | 0.39 ± 0.02* | 1.35 ± 0.02 |
| D2 | 1243.00 ± 67.00* | 0.34 ± 0.02 | 2.67 ± 0.01* |

Note. Data were expressed as mean ± standard deviation from nine replicate of samples. The mean that has (*) in the same column were significantly different ($p < 0.05$) using Independent t-test.

cultivar, geographical condition, soil, climate, agricultural practices, amount of sunlight received, fertilizer used, exposure to diseases and pesticide, processing technique, storage condition and handling method (Al-Turki et al. 2010; Samad et al. 2016). Furthermore, date fruit maturity during harvest will influence the polyphenol content and antioxidant activity since different stages of maturity produce different polyphenol content (Awad et al. 2011; Shahdadi et al. 2015). Fruiting stage of date palm after pollination consisted of five phases which include *hanbauk*, *kimri*, *khalal*, *rutab* and *tamr* (Ghnimi et al. 2017). Date fruit in *khalal* phase showed higher polyphenol level compared to *tamr* phase (Amira et al. 2012, Eid et al. 2013). The extraction and processing method of date juice or concentrate will affect the phytochemical exist in the fruits thus, could influence the polyphenol content and antioxidant activity in the juice that will be produced (Abbes et al. 2013; Pyo et al. 2014; El-Sohaimy et al. 2015).

CONCLUSION

As a conclusion, pomegranate concentrate P5 and date concentrate D2 showed the highest polyphenol content and antioxidant activity. The variation in polyphenol content and antioxidant activity of each brand could be due to the origin or cultivar of the fruits used and the extraction method involved. But further study is needed to identify the exact causes of this distinction. There is no doubt that date and pomegranate concentrates contain high polyphenol and antioxidant. This study could help the consumers to make a better brand selection of commercialized date and pomegranate concentrates that are available in local market as an antioxidant supplement to maximize the intake of polyphenol.

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