Chemical Characteristics, Antioxidant Activity, Total Phenol, and Caffeine Contents in Coffee of Date Seeds (*Phoenix dactylifera* L.) of Red Sayer Variety

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ABSTRACT

Red Sayer is one of the date varieties from the United Arab Emirates. This variety is one type of dates that is imported into Indonesia and used as processed date palm juice producing waste, namely date seeds. Date seeds can be made into some coffee that is rich in antioxidants and does not contain caffeine. The purpose of this study is to determine the chemical characteristics, antioxidant activity, total phenol, and caffeine contents in coffee of the Red Sayer variety date seeds. Coffee of date seeds is made into powder and then analyzed for its contents of water, ash, protein, fat, carbohydrates and total sugar, besides its antioxidant activity, total phenol, and caffeine levels. The results of the analysis obtained are, as follows: 4.42±0.01% water, 1.17±0.04% ash, 8.55±0.64% protein, 7.34±0.07% fat, 78.52±0.76% carbohydrate, 16.39±0.01% total sugar, 23.81±0.01 µg/mL antioxidant activity (IC50) and 340.65±1.53 mg GAE / 100g total phenol, while caffeine is not detected. Red Sayer date seed coffee, therefore, can be a source of carbohydrates and natural antioxidants that does not contain caffeine.

Keywords: Antioxidants, Date Seeds, Caffeine, Coffee, Red Sayer

INTRODUCTION

Dates (*Phoenix dactylifera*) are plants found in many Middle Eastern countries, such as the United Arab Emirates. One of the date varieties from the United Arab Emirates is Red Sayer. This variety is a type of dates that is imported to Indonesia. In Indonesia, dates besides being ready-to-eat food are also used as raw materials in the production of date palm juice. In the production of date palm juice there is some waste generated in the form of date seeds. According to data from the Central Statistics Agency, import of dates to Indonesia in April 2017 was recorded at 8.6 million kg [1]. While dates contain about 11-18% of the weight of dates comes from the seeds [2].

Many studies have been conducted on the compositions of date seeds from various parts of the world. Date seeds of Khaliti variety contain 6.88% water, 8.12% total sugar, 6.63% reducing sugar, 1.49% sucrose, 5.31% protein and 8.33% fat [3]. While date seeds of Deglet...
Nour and Allig cultivars, respectively contain 5.56 and 5.17% protein, 1.19 and 12.67% fat, 1.15 and 1.12% ash and 83.1 and 81.0% total carbohydrates [4]. The dates of Kabkab and Shahani varieties from Bushehr, Iran consist of 10.50% water, 5.56% protein, 12.59% fat, 62.18% acid soluble fiber and 1.35% ash [5]. Date seeds consist of 3.10-7.10% water, 2.30-6.40% protein, 5-13.20% fat, 0.9-1.80% ash and 22.50-80.20% dietary fiber [6]. In addition, date palm seeds reported contain many minerals such as sodium, potassium, magnesium, calcium, phosphorus, iron, manganese, zinc, copper, nickel, cobalt, chromium, lead and cadmium [2,7].

The waste of date palm seeds in Indonesia is quite abundant, but until now the date palm seeds are still considered as waste from processing date palm-based products. Although if handled further, dates can be a material that has benefits, one of which is as a raw material in making coffee. Date seed coffee of Khalas, Khunaizy, and Fard varieties contain steroids, tannins, high phenolic compounds, but no caffeine is found there [8]. The purpose of this study is to determine the chemical characteristics, antioxidant activity, total phenol, and caffeine contents in date seed coffee of the Red Sayer variety.

EXPERIMENT

Chemicals and instrumentation

Chemicals used for research are DPPH (Sigma), methanol (Merck), gallic acid standard (Sigma), Folin-Ciocalteu reagent (Merck), magnesium oxide (Sigma), and caffeine standard (Sigma). Samples of research are date seeds from the Red Sayer variety obtained from a date palm juice factory CV. Sehat Prima Lestari, Bogor, West Java, Indonesia.

Instruments applied for analysis are spectrophotometer UV-Vis (Shimadzu) and HPLC (Agilent).

Procedure

The making of date seed coffee

Date seeds are washed to be removed from the date flesh which is still attached and baked at 125 °C for 30 minutes until the color changes to light brown. Seeds are smoothed and sifted to get a fine powder. Then, it is sifted once more to get a fine seed powder (60 mesh) [9].

Chemical characterization of date seed coffee

Determination of water and ash content refer to previously report from [10]. Meanwhile determination of protein, fat, and carbohydrate content, and also total sugar following reference [11].

Antioxidant activity determination of date seed coffee

A 13.5 g of sample in a 100-mL of volumetric flask. It is squeezed with methanol, and then filtered off. Separately, a solution of 0.004% of DPPH (2,2-diphenyl-1-picrylhydrazyl) is prepared. In the reaction tube, a 1 mL of sample or blank is pipetted into a 15 mL tube, and a 5 mL of 0.004% DPPH solution is added. This mixture is stirred for 30 seconds, and incubated for 20 minutes in the dark condition. The mixture is taken for further analysis using UV-VIS spectrophotometer at a wavelength of 517 nm. The absorbance values are recorded for each sample. Repetition of sample is prepared and further analysis using similar procedure. The antioxidant activity of the sample is expressed in IC50 [12].
Total phenol determination of date seed coffee

The sample is weighed as much as 10 grams, and put into a 100-mL of volumetric flask and squeezed with distilled water. A 1.0 mL of sample is pipetted to a 15 mL test tube. It is further added with 5 mL of 10% a Folin-Ciocalteu reagent, and leave for 3-8 minutes. Then, this mixture is added with 4 mL of 7.5% Na$_2$CO$_3$, stirred and leave at room temperature for 2 hours (protect from light). Then, the mixture is analyzed using a UV-VIS spectrophotometer at a wavelength of 768 nm [13]. Separate procedure analysis is also undertaken using standard solution as sample and a blank solution. The procedure and analysis is undertaken triple.

Several gallic acid solutions are prepared separately as standard samples with different concentrations, i.e. 10 ppm, 25 ppm, 50 ppm, 75 ppm, and 100 ppm, respectively. Analysis are performed using UV-VIS spectrometer at a wavelength of 768 nm. The absorbance recorded are plotted toward the concentration of gallic acid. The phenol content of the sample from date seed coffee is determined based on its absorbance value to the gallic acid equivalent concentration.

Caffeine content determination of date seed coffee

The determination of caffeine contents in the sample is carried out using high performance liquid chromatography (HPLC) methods. Caffeine in the sample is extracted with water at 90 °C in the presence of magnesium oxide. The sample is weighed as much as 0.25 gram, and also 2.5 grams of magnesium oxide. Then, both are poured into a 100 mL Erlenmeyer, added with hot distilled water, and further heated at 90 °C under stirring for 20 minutes. It is cooled at room temperature (under running water), then the sample solution is transferred to a 100 mL volumetric flask and then squeezed and being homogeneous. Next, it is filtered with Whatman filter paper No. 41, then filtered again with a 0.45 µm pore sized syringe filter. The sample is ready to be injected into HPLC [10].

RESULT AND DISCUSSION

Date seeds, waste from date juice production, are processed into coffee and their chemical contents are analyzed including water, ash, protein, fat, total sugar, carbohydrate, caffeine, total phenol, and antioxidant activity. The characteristics of the coffee powder of date palm seeds are presented in Table 1.

| No. | Parameter                   | Results       |
|-----|-----------------------------|---------------|
| 1   | Water (%)                   | 4.42 ± 0.01   |
| 2   | Ash (%)                     | 1.17 ± 0.04   |
| 3   | Protein (%)                 | 8.55 ± 0.64   |
| 4   | Fat (%)                     | 7.34 ± 0.07   |
| 5   | Sugar (%)                   | 16.39 ± 0.01  |
| 6   | Carbohydrate (%)            | 78.52 ± 0.76  |
| 7   | Caffeine (%)                | ND            |
| 8   | Total phenol (mg GAE/100 g) | 340.65 ± 1.53 |
| 9   | Antioxidant activity (IC$_{50}$) (µg/mL) | 23.81 ± 0.22 |

Note: ND is not detected

Based on the results of previous studies, roasted dates contain 7.1% protein, 8.1% fat, 1% ash, and 62.3% carbohydrates [5,14]. If the results are compared with fresh date seeds, the various
varieties shown in Table 2 have differences, especially in their water content, this is due to the roasting process which can eliminate the water content in dates. In addition, differences can be caused by differences in cultivars studied and also climatic conditions [3]. The fat content in date seeds also depends on the variety, origin, time of harvest and fertilizer [2]. The determination of sugar content in the coffee of date palm seeds has not been done in previous studies, but the date palm seeds of Khalti variety contain 8.12% total sugar, 6.63% reducing sugar, and 1.49% sucrose [3].

| No | Date varieties                  | Chemical composition (%) | References |
|----|---------------------------------|--------------------------|------------|
| 1  | Fard (UAE)                      | 10.3 9.9 5.7 1.4 -       | [15]       |
| 2  | Khalas (UAE)                    | 7.1 13.2 6.0 1.8 -       | [15]       |
| 3  | Lulu (UAE)                      | 9.9 10.5 5.2 1.0 -       | [15]       |
| 4  | Phoenix canariensis seeds (Saudi Arabia) | 10.2 10.36 5.67 1.18 72.59 | [16] |
| 5  | Allig (Tunisia)                 | - 12.67 5.17 1.12 81.0   | [4]        |
| 6  | Deglet Nour (Tunisia)           | - 10.19 5.56 1.15 83.1   | [4]        |
| 7  | Khalti (Tunisia)                | 6.86 8.33 5.31 -         | [3]        |
| 8  | Date seed powder (Oman)         | - 8.08 7.08 0.98 62.31   | [14]       |
| 9  | Kabkab & Shahani (Iran)         | 10.5 12.59 5.56 1.35 80.65 | [5] |
| 10 | Rajshahi (Bangladesh)           | 5-10 7-10 - 1-2 55-65    | [17]       |
| 11 | Mabseeli (Oman)                 | 3.14 5.05 3.92 1.03 86.89 | [18]       |
| 12 | Um-sellah (Oman)                | 4.4 5.9 5.4 1.16 83.14   | [18]       |
| 13 | Shahal (Oman)                   | 5.19 5.09 2.29 0.89 86.54 | [18]       |

The total phenol of coffee powder from date seeds of Red Sayer variety is 340.65 mg GAE (gallic acid equivalent)/100 g, when compared to previous studies total phenol in coffee of date seeds from the United Arab Emirates reachest 500 mg TAE (tannic acid equivalent)/100 g [8]. High levels of phenolic compounds in date palm seeds are also previously reported, that is 3100-4400 mg GAE/100 g [19]. This difference is due to differences in varieties, roasting processes, extraction processes, and others. Phenolic compounds in date palm seeds are primarily proanthocyanidin, which are classified as condensed tannins [20].

It can also be seen that the higher the total phenol, the stronger the antioxidant activity is, this is consistent with previous studies that high total phenol in date seed coffee is proportional to% inhibition of DPPH radicals, the total phenol of date seed coffee from United Arab Emirates of 500 mg TAE/100 g has a % inhibition of 64.9% [8]. The antioxidant activity of date seed coffee can also be expressed as an IC50 value. IC50 is defined as the concentration of material needed to inhibit 50% DPPH free radicals, meaning that the smaller the IC50 value, the higher the antioxidant activity of the material. A material is said to have very strong antioxidant activity if it has an IC50 value of less than 50 µg/ml, strong if 50-100 µg/ml, medium if 100-150 µg/ml, and weak if 150-200 µg/ml [21]. The IC50 value of date seed coffee is 23.81 µg/ml, meaning that date seed coffee has very strong antioxidant activity.

The results show that date seed coffee from Red Sayer variety does not contain caffeine, this is consistent with previous studies that date seed coffee of Khalas, Khunaizy, and Fard
varieties does not contain caffeine either [8]. Compared with normal coffee beans which have caffeine content of 20-40%, the caffeine content in date seed coffee is 0% [9]. The absence of caffeine content in date seed coffee can be one of the benefits for consumers who are sensitive and pay attention to the caffeine contents in the products they consume. This is because, although caffeine is a compound that is commonly used or consumed daily, caffeine is still an addictive compound. Caffeine is a psychoactive substance that is often associated with negative health effects such as pregnant women who consume caffeine will be at high risk for depression and anxiety symptoms. Babies born to mothers who consume more caffeine during pregnancy also experience problems such as low birth weight [22]. In addition, other studies have also proven the existence of violent behavior in adolescents who consume caffeine [23].

CONCLUSION
Red Sayer date seed coffee contains high carbohydrates and sugar as well as total phenol so it is included in a very strong class of antioxidants, but date seed coffee of Red Sayer variety does not contain caffeine.

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CONFLICT OF INTEREST
Authors declare no competing interest.

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