

Physicochemical properties, total phenolic content, and antioxidant capacity of homemade and commercial date (*Phoenix dactylifera* L.) vinegar

*Hafzan, Y., Saw, J.W. and Fadzilah, I.

School of Health Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia

Article history

Received: 4 July 2016

Received in revised form:

23 October 2016

Accepted: 25 October 2016

Abstract

Previous studies proved the antioxidant properties of dates. However, studies on date byproducts especially date vinegar are still lacking. Hence, it is the aim of the present study to compare the physicochemical properties, total phenolic content, and antioxidant capacity between homemade and commercial date vinegar. Physicochemical properties such as total sugar content, pH, and total titratable acidity of homemade and commercial date vinegar were studied. Both homemade and commercial date vinegar showed significant difference in physicochemical properties including pH, sugar content and total titratable acidity ($p < 0.05$). Total phenolic content ranged from 281.17 mg GAE/L to 641.17 mg GAE/L with significant differences ($p < 0.05$) noted between homemade and commercial date vinegar. Homemade semisoft date vinegar possessed significantly higher ($p < 0.05$) hydrogen peroxide inhibition capacity (310.20 $\mu\text{g AAeq/mL}$) as compared to soft date vinegar (200.06 $\mu\text{g AAeq/mL}$) and commercial date vinegar (190.81 $\mu\text{g AAeq/mL}$). Pearson's correlation statistical test showed no significant correlation ($p > 0.05$) between hydrogen peroxide scavenging activity and total phenolic content of date vinegar. Only the homemade date vinegar showed metal chelating property with values of 0.34 ± 0.10 and 2.90 ± 0.03 for semisoft and soft date cultivars respectively. Pearson's correlation statistical test showed significant correlation between metal chelating rate and total phenolic content ($r = 0.50$; $p < 0.01$) of the two samples. Homemade date vinegar showed generally higher antioxidant activity than commercial date vinegar and both samples were significantly different in terms of their physicochemical properties.

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Keywords

Physicochemical

Antioxidant

Phenolic

Date vinegar

Introduction

Consumption of dates has been ascribed to various medicinal uses, such as remedy for intestinal problems, sore throat, colds, and bronchial cough, relief of fever, liver and abdominal aches, cystitis, gonorrhoea, and oedema (Al-Yahyai and Manickavasagan, 2012). Research revealed that dates are rich in phenolic compounds. Among the phenolic compounds found in dates are hydroxycinnamates, flavonols, flavan-3-ols, flavan-3,4-diols, proanthocyanidins, and tannins (Mansouri *et al.*, 2005). These phenolic compounds are believed to exhibit various significant health benefits to the body due to their ability to prevent oxidation-linked chronic or degenerative illnesses such as cancer and cardiovascular disease, which are the major chronic diseases worldwide (Silva *et al.*, 2007). The antioxidant metabolites in dates are believed to be able to maintain a specific cellular homeostasis, thereby giving beneficial effects across diverse biological systems and cell types (Valko *et al.*, 2007).

Date byproducts have been widely used mainly by the Arab communities in the form of jam, jelly,

juice, syrup, fermented beverages, and vinegar. Content of bioactive compounds in these byproducts has increased the industrial value of dates. In recent years, there is an increase in the consumption of commercial date vinegar observed among Malaysian population despite the dearth of scientific data to support the benefit of its consumption. Thus, this study was carried out to evaluate physicochemical properties, antioxidant capacity, as well as the total phenolic content of commercial date vinegar, as compared to the homemade date vinegar.

Materials and Methods

Sample

Commercial date vinegar was purchased from traditional medicine shops in Kota Bharu, Kelantan, Malaysia. Homemade date vinegars were self-prepared from semisoft (variety Fardh, originated from Iran) and soft dates (variety Honey dates, originated from Iran). Confirmation of the type of dates (either soft or semisoft) during selection of dates was based on the date varieties labelled on the products' packaging (Adel and Awad, 2009; Singh *et*

al., 2012). The date vinegar sample used for analysis was also from similar varieties as the dates samples used to make homemade vinegar (based on the package labeling).

Preparation of homemade date vinegar

Homemade date vinegar was prepared based on method by Mohammed and Mohammed (2013) and a website Tibb-e-Nabawi (2014) with slight modification. The samples were pitted, crushed, and blended with distilled water (2:5 w/w sample: distilled water) to form a suspension. The suspension was stored in a glass jar, covered with muslin cloth and tightened with a rubber band and placed in a dark cupboard at room temperature (28°C). The juice was then let to spontaneous (without artificial inoculation) and simultaneous fermentation for 40 to 60 days. For the first 3 weeks, every third day, the jars were opened and the juices were stirred properly to avoid any yeast accumulation at the top. After 30 days, primary fermentation was completed and the fermented juices were filtered. The empty jars were washed with distilled water and the same process was repeated for further fermentation (at least 1 month). After 40-60 days, the juices were again filtered, bottled and refrigerated. Confirmation on the production of vinegar was done via total titratable acidity test. The parameters modified were the temperature in which the date suspensions were fermented and the absence of starter culture for speeding up the fermentation. Date vinegar was then centrifuged at 5000rpm for 10 minutes. The supernatant was collected for further analysis.

Preparation of potassium hydrogen phthalate standard

0.2 g potassium hydrogen phthalate ($\text{KHC}_8\text{H}_4\text{O}_4$) was dissolved in 25 mL water in a 50 mL volumetric flask. The volume was made up to 50 mL with distilled water and the flask was capped and mixed thoroughly. This solution was used to standardize sodium hydroxide solution used in the experiment. The whole process was repeated to prepare another potassium hydrogen phthalate solution.

Preparation of sodium hydroxide solution

Two grams of sodium hydroxide pellets were weighed precisely into 500 mL volumetric flask. Before the weighed pellets were added into the volumetric flask, small amount of distilled water was added into the volumetric flask. The volumetric flask was then shaken in a water bath to dissolve the pellets. Distilled water was then added slowly into the volumetric flask to further dissolve the pellets.

The volume was made up to a point slightly below the mark and the content of the flask was then mixed thoroughly by using a magnetic stirrer. Distilled water was then further added to make up final volume of 500 mL. The stock solution was further diluted with 500 mL distilled water in a 1000 mL volumetric flask to produce 0.05 M NaOH solution.

Standardization of the sodium hydroxide solution

A clean 50 mL burette was selected, rinsed with a small portion of the prepared sodium hydroxide solution and filled in to just below the zero mark. The initial volume to the nearest 0.01 mL was read and recorded. A 250 mL Erlenmeyer flask was then rinsed with distilled water to make sure it was clean. 50 mL of the prepared potassium hydrogen phthalate solution was transferred into the flask. Three drops of phenolphthalein indicator were added and titrated with the sodium hydroxide solution until a permanent color change was detected. The palest pink color denoted the end point of the neutralization reaction. The color should persist throughout the entire solution when swirled for at least 10 seconds. The color faded slowly on standing. The titration was repeated on 50 mL aliquot of the primary standard solution. The titration was repeated if the titration volume did not agree within ± 0.1 mL and the best two titrations were reported.

Determination of acetic acid in vinegar

Five milliliters of the homemade date vinegar were centrifuged, pipetted into a 250 mL Erlenmeyer flask, and 75 mL of distilled water were then added. Three drops of phenolphthalein indicator were then added. The acetic acid was then titrated with the standard base to a pale pink equivalence point. The burette readings were then recorded. The titration was repeated at least once more using a fresh aliquot of vinegar. The best two titration results that agreed within ± 0.2 mL were reported.

Determination of total phenolic content using Folin-Ciocalteu method

Total phenolic content of vinegars were determined by the Folin-Ciocalteu method with slight modification (Singleton and Rossi, 1965). In a solution of 3.9 mL water and 0.1 mL sample, 0.5 mL diluted Folin-Ciocalteu reagent (1:4 reagent: water) was added. The light of fume hood and lab were switched off while mixing the sample with Folin-Ciocalteu reagent as this reagent is very sensitive to light. After 5 minutes, 0.5 mL saturated sodium carbonate (20% w/v) was added. After vigorous vortexing, the final solution was left to stand at room temperature for

30 minutes. The reading was performed at $\lambda_{\max} = 725$ nm (UV-Vis Spectrophotometer, Carry 100). The results were expressed as milligram gallic acid equivalents per litre of sample (mg GAE/L), using a gallic acid standard curve. The formula used to calculate the total phenolic content was: $y = ax + b$, whereby “y” represents the absorbance of sample and “x” represents the total phenolic content of sample.

Hydrogen peroxide assay

The hydrogen peroxide assay was carried out with slight modification (Yen and Chen, 1995). An aliquot of vinegar sample (1.0 mL) was pipetted into a screw-cap test tube and deionized distilled water was added to make the total volume of 4.0 mL. Then, 0.6 mL of 4.0 mM H₂O₂ solution in 0.1 M phosphate buffer pH 7.4 was added. The reaction mixture was shaken and left to stand at room temperature for 10 minutes. The absorbance for the sample (A_{sample}) was measured against a buffer blank at $\lambda_{\max} = 230$ nm. The control reaction (A_{control}) reading was taken after adding 0.6 mL of H₂O₂ solution to 4.0 mL of deionized distilled water. The percent inhibition of H₂O₂ of the sample was calculated according to the equation: Percent inhibition of H₂O₂ = $[1 - (A_{\text{sample}} / A_{\text{control}})] \times 100$. Every sample was analyzed in triplicate and the H₂O₂ scavenging activity was expressed as micrograms of ascorbic acid equivalent per millilitre of sample ($\mu\text{g AAeq/mL}$) using ascorbic acid standard curve (100 – 500 $\mu\text{g/mL}$).

Metal chelating assay

With slight modification on the method of metal chelating assay (Dinis *et al.*, 1994), 0.5 mL of sample, 1.6 mL of deionized water and 0.05 mL of FeCl₂ (2 mM) was added, followed by the addition of 0.1 mL of ferrozine (5 mM) after 15 minutes. Then, the mixture was left to stand for 10 minutes at room temperature in a dark room. The absorbance of the Fe²⁺-ferrozine complexes red or violet color was measured at $\lambda_{\max} = 562$ nm. One milliliter of distilled water was used as a control. The chelating antioxidant activity for Fe²⁺ was calculated according to the following formula: Chelating rate (%) = $(A_c - A_s) / A_c \times 100$, where, A_c was the absorbance of the control reaction and A_s was the absorbance of the sample extract. EDTA (100 $\mu\text{g/mL}$) was used as a positive control. Every sample was analyzed in triplicate.

Data analysis

Comparisons between samples were performed by using one-way ANOVA followed by Tukey HSD post hoc test. Correlation between antioxidant capacity and total phenolic content of each sample

Table 1. Physicochemical properties of dates vinegar

Mean \pm SD	Dates Vinegar		
	Homemade		Commercial
	Soft	Semisoft	
pH	2.73 \pm 0.00*	2.77 \pm 0.01*	2.70 \pm 0.00*
Sugar content Brix (%)	14.35 \pm 0.07*	7.80 \pm 0.00*	8.40 \pm 0.00*
Total titratable acidity (%)	1.89 \pm 0.02*	1.19 \pm 0.02*	5.66 \pm 0.06*

*The mean difference is significant at the 0.05 level ($p < 0.05$).

used was done by using Pearson’s correlation. All values presented are the means of three independent replications. Data were presented as mean \pm standard deviation (Mean \pm SD).

Results and Discussion

Physicochemical properties : pH, total sugar content, and titratable acidity

The pH value, sugar content, and total titratable acidity of date vinegar was shown in Table 1. There was significant difference in pH values of homemade and commercial date vinegar ($p < 0.05$). There was also significant difference between semisoft and soft date vinegar for the homemade date vinegar ($p < 0.05$). The pH of date vinegar ranged from 2.70 to 2.77. The soft date vinegar showed higher total sugar content than semisoft and commercial date vinegar ($p < 0.05$). However, commercial date vinegar had significantly higher total sugar content than semisoft date vinegar ($p < 0.05$). Soft cultivars were shown to have conversion of sucrose to fructose and glucose that caused increase in total sugar of the fruit (Adel and Awad, 2009). Hence, this could be explained that soft date vinegar produced from soft date cultivars had higher total sugar content than semisoft date vinegar.

The profile of kinetic curve depicted in Figure 1 showed that the maximum concentration of acetic acid produced by semisoft date vinegar (1.47%) and soft date vinegar (3.01%) was reached at about 20 days of fermentation. However, the production of vinegar started to decrease generally after this time for both types of date vinegar. At 35 days of fermentation, the production of vinegar slightly increased again for both types of date vinegar. Production started to decrease probably due to the oxidation of acetic acid by oxygen present in the fermentation medium (Mohammed and Mohammed, 2013). In general, the spontaneous fermentation of

Table 2. Correlation between total phenolic content and antioxidant properties of dates' vinegar.

Mean \pm S.D	Dates Vinegar			Correlation coefficient, (r)
	Soft	Homemade Semisoft	Commercial	
Total phenolic content (mg GAE/L)	570.74 \pm 0.17	641.17 \pm 0.03	281.17 \pm 0.05	
Antioxidant properties				
H ₂ O ₂ scavenging AAeq (μ g/ml)	200.06 \pm 21.8	310.20 \pm 38.5	190.81 \pm 11.4	0.074**
Metal chelating rate (%)	2.90 \pm 0.03	0.34 \pm 0.10	- 35.95 \pm 0.05	-0.999**

** Significant correlation at the 0.01 level (2-tailed).

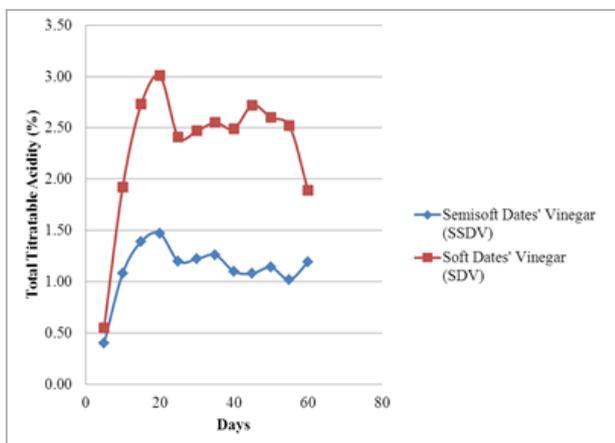


Figure 1. Total titra acidity of homemade vinegar along 60 days of fermentation period

vinegar by the simple batch process is generally slow and requires 4-5 weeks for complete fermentation. According to Mohammed and Mohammed (2013), they showed that the maximum concentration of acetic acid produced by low-cost "Khastawi" dates was 4.02% (w/v) at about 33 days of fermentation. The difference in acetic acid production at different time of fermentation could be probably due to the different temperature the date juice being fermented. In the present study, the fermentation temperature was set at 28°C while the temperature in the study by Mohammed and Mohammed (2013) was set at 30°C. Commercial date vinegar (5.66%) was shown to have significantly higher acetic acid than homemade date vinegar (1.89% for soft date vinegar and 1.19% for semisoft date vinegar) ($p < 0.05$). This was due to additional acetic acid being added into commercial vinegar.

Total phenolic content

Total phenolic content of date vinegars ranged from 281.17 mg GAE/L to 641.17 mg GAE/L. Table 2 showed that homemade date vinegar significantly has

higher total phenolic content (semisoft = 641.17 mg GAE/L; soft = 570.74 mg GAE/L) than commercial date vinegar ($p < 0.05$). Homemade semisoft date vinegar significantly showed a higher total phenolic content than the soft counterpart ($p < 0.05$). Dry date varieties had been reported to exhibit higher total phenolic content than soft date varieties (Biglari *et al.*, 2009). Study showed that Kharak dates (dry dates) had the highest total phenolic content and Jiroft dates (soft dates) had the lowest total phenolic content (Biglari *et al.*, 2008). The study showed that dry dates had the highest total phenolic content followed by semisoft dates, and soft dates (Biglari *et al.*, 2008). Although there is not much study carried out on date vinegars, there are numerous studies carried out on date fruits. From the result of total phenolic content of dry, semisoft, and soft dates, the soft date vinegar had the least total phenolic content in comparison with dry and semisoft date vinegars.

Hydrogen peroxide inhibition

Hydrogen peroxide inhibition of date vinegar ranged from 190.81 μ g AAeq/mL to 310.20 μ g AAeq/mL (Table 2). Semisoft date vinegar showed the highest hydrogen peroxide inhibition (310.20 μ g AAeq/mL), while soft date vinegar showed slightly higher (200.06 μ g AAeq/mL) hydrogen peroxide inhibition than commercial date vinegar (190.81 μ g AAeq/mL). Generally, homemade date vinegar had higher hydrogen peroxide inhibition than commercial date vinegar. However, statistical analysis did not show significant difference among homemade date vinegar and commercial date vinegar ($p > 0.05$). Hydrogen peroxide (H₂O₂) forms hydroxyl radical, which is deleterious to the mammalian and bacterial cell (Wissal *et al.*, 2014). The quenching of hydroxyl radical by different date vinegars extracts may be related to the prevention of lipid peroxidation propagation process and the reduction

of chain reaction due to the presence of phenolic compounds (Wissal *et al.*, 2014).

Metal chelating property

From the experiment, metal chelating property of commercial date vinegar was undetectable while homemade date vinegar showed low metal chelating rate (Table 2). EDTA (100 µg/mL), which was the positive control for this test significantly showed a very high metal chelating rate of 98.3% while homemade date vinegar only showed significantly very low metal chelating rate of 0.34% to 2.91% ($p < 0.05$). Hence, date vinegar can be considered as a weak metal chelator. A study showed that Fardh date extract at tamr (dried) stage had high metal chelating activity of 80% (Singh *et al.*, 2012). However, present study that used Fardh dates variety as semisoft dates to produce the semisoft date vinegar showed very low metal chelating activity. This might be due to the fermentation process during vinegar production, which affected the metal chelating property of the dates.

Correlation between TPC and antioxidant properties

Pearson correlation coefficient was computed to assess the relationship between total phenolic content and antioxidant properties of date vinegar (Table 2). Negative correlation was depicted between TPC and metal chelating property ($r = -0.999$, $n = 6$, $p = 0.000$). Overall, there is a strong, negative correlation between total phenolic content and metal chelating rate of date vinegar. Decrease in total phenolic content of date vinegar correlated with the increase in metal chelating rate of date vinegar. This negative correlation between total phenolic content of date vinegar and metal chelating rate might be due to the presence of non-phenolic compounds in date vinegar that contribute to metal chelating property of date vinegar (Stanisavljević *et al.*, 2015). This current study also evidenced no significant correlation ($r = 0.074$, $p > 0.05$) between total phenolic content and hydrogen peroxide scavenging activity of both homemade and commercial date vinegar. Correlation analyses by Wissal *et al.* (2014) indicated a positive linear relationship between antioxidant activities and total phenolic content, but the study was done on the date flesh extract not on the vinegar.

Conclusion

Homemade date vinegar had significantly higher ($p < 0.05$) total phenolic content and hydrogen peroxide scavenging activity in comparison with commercial date vinegar. Present study also evidenced very low

metal chelating property of home made date vinegar. The physicochemical properties (pH, sugar content and total titratable acidity) of homemade date vinegar were significantly different from the commercial date vinegar. The findings of this present study would suggest further study to be done using other antioxidant assays to confirm the potential of date's vinegar and to characterize the phenolic compounds exist or remain after the completion of fermentation process of vinegar.

Acknowledgements

Authors would like to thank Universiti Sains Malaysia for the research grant (304/PPSK/61312092) and Science Laboratory Management Unit for the facilities provided. In addition, sincere appreciations are extended to all the staffs and seniors in nutrition laboratory and food preparation laboratory of School of Health Sciences, Health Campus, USM for their assistance and guidance towards the success of this research.

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