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Effect of date palm cultivars on chemical and phytochemical properties of date vinegars

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ABSTRACT

Introduction: *Phoenix dactylifera*, commonly known as date or date palm, is a flowering plant species in the palm family Arecaceae, cultivated for its edible sweet fruit called dates. Dates are high in dietary fibre and antioxidant compounds, known for preventing cancer and cardiovascular diseases. This research studied the chemical properties, antioxidant activities, and total phenolic content of fermented date vinegars from *Phoenix dactylifera* L. fruits, which had three cultivars - Barhi, Siam S1, and KL1. **Methods:** The first step was making date wines by using *Saccharomyces cerevisiae* (0.75% v/v of date juice content) to produce alcohol; the second step was making date vinegars by using *Acetobacter pasteurianus* (10% v/v of inoculum). Thereafter, the wines and vinegars were analysed for their chemical properties [high performance liquid chromatography (HPLC)], antioxidant activities [2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay], and total phenolic content (folin ciocalteu method). **Results:** Results showed that the highest alcohol content was 9.35% (v/v) in Siam S1 wine. The highest acetic acid was 7% (v/v) in Siam S1 vinegar. From the phytochemical analysis of vinegars, the highest antioxidant activity was found to be 24.96 mg/mL in Siam S1 vinegar, while the highest total phenolic content was found to be 208.35 mg GAE/L in KL1 vinegar. **Conclusion:** This novel research showed that the Siam S1 date had the highest acetic acid and antioxidant activity in vinegar. Thus, this cultivar could be processed to make new, healthy products that can further lead to income generation for the people in Thailand.

Keywords: antioxidant activity, chemical properties, date fruits, vinegar, wine

INTRODUCTION

Vinegar, a kind of acidic condiment, has been in use for more than 3000 years (Solieri & Giudici, 2009). Both solid-state and liquid-state fermentation methods are being used in the production of vinegar (Xia *et al.*, 2020). Vinegar is made from sources that contain sugar such as grains, fruits, and honey. This leads to the different types of vinegars available in the market. Different raw materials support the different physiochemical properties of vinegar products. Natural vinegar is a superior food additive as it contains nutrients such as carbohydrates, amino acids and peptides, vitamins and minerals, and antioxidants such as carotenoids and phenolic compounds. From the early days of agriculture until today, mankind has always used vinegar for various purposes, including as a condiment, pickling or preserving

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agent, disinfectant, cleaning agent, and beverage (Matloob, 2014). Vinegar has been proven useful for the prevention and treatment of diabetes because it decreases the presence of glucose and insulin in the blood (Brahim *et al.*, 2014).

Date vinegar has beneficial effects similar to those of other types of vinegar. It is made from date palm (*Phoenix dactylifera* L.), that is grown in the hot and dry regions of North African countries and the Middle East. Dates are important for agriculture and economy in the Arab Gulf area. They are predominantly grown in Egypt (1,352,950 metric tons), Saudi Arabia (1,078,300 metric tons), Iran (1,023,130 metric tons), the United Arab Emirates (775,000 metric tons), and Algeria (710,000 metric tons) (Chandrasekaran & Bahkali, 2013). Date fruit is divided into various development and ripening stages (Hababouk, Kimri, Bisr or Khalal, Rutab, and Tamer). Generally, the date fruit is harvested and brought to market at three stages, namely Bisr, Rutab, and Tamer—depending on the cultivar (Ahmed *et al.*, 2021). The fruit is high in dietary fibre, proteins, carbohydrates, minerals, vitamins, tannins, phenolic and antioxidant compounds. These phytochemical compounds can prevent the oxidation of other molecules such as proteins and lipids, thus providing a protective role in the body against cancer, cardiovascular diseases, and other degenerative conditions (Matloob & Balakit, 2016). The tannin contained in date palm fruits is used medicinally as a defensive astringent for intestinal troubles. It is commonly administered as a treatment for colds, sore throat and bronchial catarrh, as well as to relieve cystitis, fever, oedema, gonorrhoea, liver and abdominal troubles. It is also said to counteract alcohol intoxication (El-Sohaimy & Hafez, 2010).

The overall objective of this research was to study the effect of date fruit varieties on the chemical and phytochemical properties of date vinegar. This research aimed 1) to produce fermented vinegar from date fruits which had three cultivars – Barhi, Siam S1, and KL1, and 2) to study the chemical properties, antioxidant activity, and total phenolic content of date vinegars.

MATERIALS AND METHODS

Chemicals and reagents

All reagents and solvents used during the experiment were of analytical grades and purchased from various suppliers. 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) was bought from Sigma–Aldrich (Steinheim, Germany). Gallic acid standard was supplied by Fluka (Buchs, Switzerland). Folin-ciocalteau reagent was from Merck (Darmstadt, Germany) and sodium carbonate (anhydrous) from Univar (Downers Grove, IL, USA).

Raw materials

Phoenix dactylifera L. fresh date cultivars, namely Barhi (yellow as fresh), Siam S1 (purple as fresh), and KL1 (yellow as fresh) (Khalal developmental stages) were harvested in June 2021 in the Mahasarakham province.

Date vinegar production

The vinegar fermentation process started with date fruits being crushed and mixed with water at a ratio of 1:4 (w/w) to prepare the juice. After adjustment of total soluble solid content up to 20ºBrix by cane sugar, the date juice was pasteurised for 30 minutes (min) at 65ºC. Alcoholic fermentation was conducted for three days at room temperature in plastic vessels containing 2 L of the date juice inoculated with wine yeast, *S. cerevisiae* (LALVIN K1-V1116) at a ratio of 0.75% (v/v). At the end of the fermentation process, the wine was separated from the sediment by allowing it to settle in glass bottles, followed by

pasteurisation for 30 min at 65°C. Then, vinegar production began from the alcohol content of the obtained wine, adjusted to 6% (v/v) and inoculated with *A. pasteurianus* TISTR 521 (Thailand institute scientific and technological research), which was grown in glucose yeast broth at a ratio of 10% (v/v). A glass flask containing 135 mL of the date wine was shaken for 15 days at 30°C on a shaker (150 rpm). After fermenting vinegar for 15 days, the fermentation process was stopped by pasteurisation of vinegar at 65°C for 30 min (Figure 1).

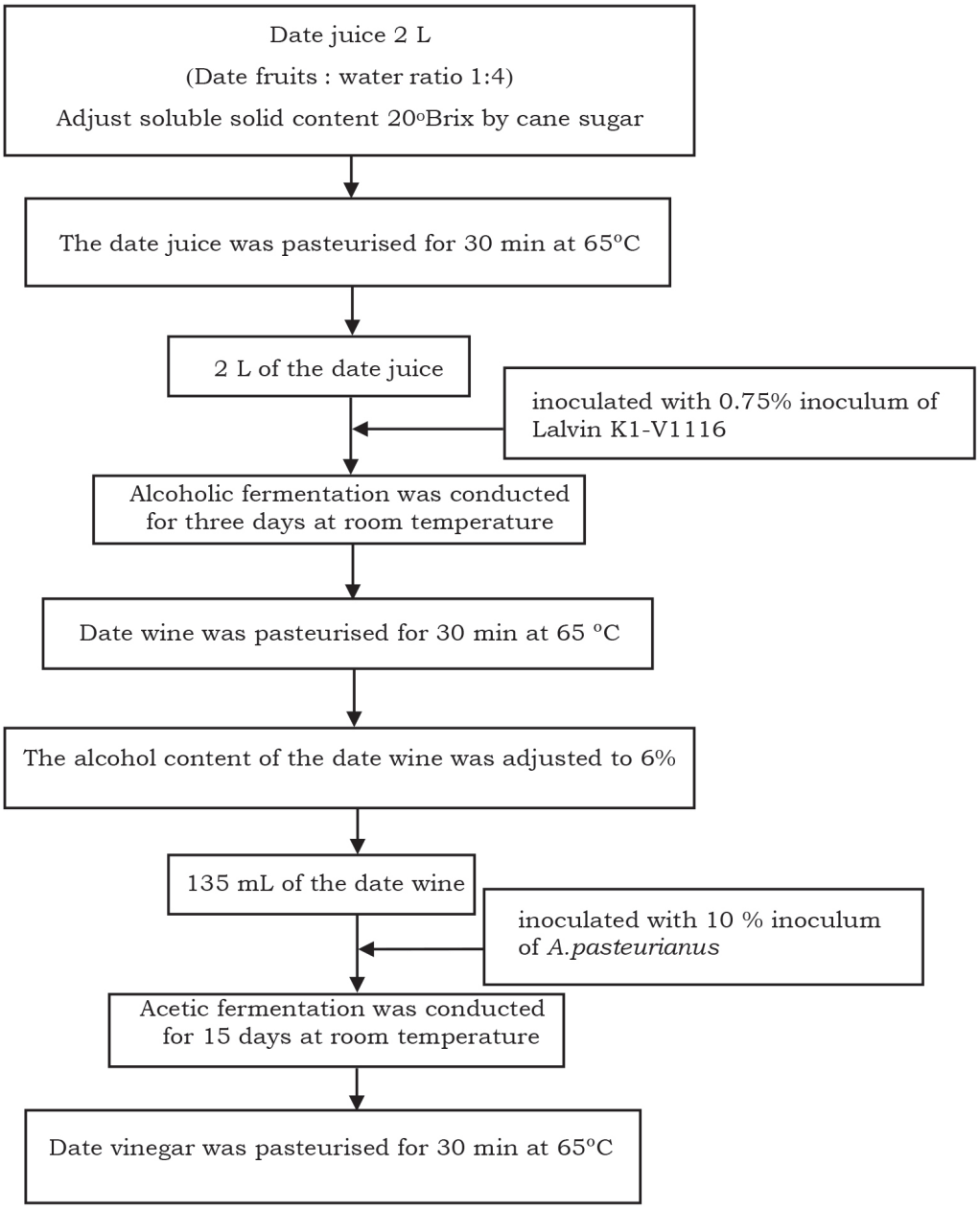

Figure 1. Diagram of date vinegar production

Chemical analysis

The wine and vinegar samples were centrifuged and filtered through a 0.45 µm filter before injection into the HPLC system. The analysis was performed on a Shimadzu HPLC-RID system (Shimadzu, Japan) consisting of Shimadzu LC-20AD pumps and RID-10A refractive index detector. The analytical column was Aminex HPX-87H column (300 mm × 7.8 mm i.d., 9 µm, Bio-Raand Laboratories, Inc., USA) coupled to a cationic exchange pre-column (Bio-Rad Laboratories, Inc., USA). H_2SO_4 (5 mM) was conducted as the mobile phase. The injection volume was 20 mL with a flow rate of 0.6 mL/min. The column temperature was set at 45°C (Aguiar *et al.* 2005). A series of standard solution [ranging from 0-16% of fructose (w/v), glucose (w/v), absolute ethanol (v/v), and acetic acid glacial (v/v)] were prepared. A standard curve with R^2 greater than 0.99 was plotted, and then the concentrations of fructose, glucose, absolute ethanol, and acetic acid glacial in wine and vinegar were quantified accordingly.

Antioxidant activity

The antioxidant activities of the samples were determined by DPPH radical assay (Brand-Williams, Cuvelier & Berset, 1995) during which DPPH radical was used as a stable radical. In brief, 5 mL of every sample was added to 5 mL of 0.1 mM DPPH radical solution prepared in ethanol, and the mixture was incubated for 20 min at room temperature in the dark. After incubation, absorbance was measured at 517 nm using the Shimadzu UV-1800 spectrophotometer (Shimadzu, Japan), and the DPPH radical scavenging activities were expressed as mg ascorbic acid equivalents in 1 mL of sample (mg/mL).

Total phenolic content analysis

The Folin-Ciocalteu method was utilised for the determination of total phenolic content in date vinegars (Singleton, Orthofer & Lamuela-Raventós, 1999). Briefly, 1 mL of each sample was diluted with 9.5 mL of distilled water and then mixed with 0.5 mL of Folin-Ciocalteu reagent and 2 mL of 10% Na_2CO_3 solution. After an incubation time of 30 min at room temperature, absorbance was measured at 765 nm using the Shimadzu UV-1800 spectrophotometer (Shimadzu, Japan). Results were expressed as mg gallic acid equivalents in 1 mL of the sample (mg GAE/L). A standard gallic acid curve was constructed by preparing the dilutions of 0, 20, 40, 60, 80, and 100 µg/10 ml in ethanol.

Statistical analysis

The vinegars were produced with 450 ml of each cultivar in a 250 mL three-glass flask. The trials were repeated three times from the same batch of vinegar. Results were presented as mean±standard deviation (*SD*). The obtained data were analysed by one-way analysis of variance (ANOVA) with Duncan multiple range tests (DMRT) to determine the significance between samples, using SPSS software version 22.0 (SPSS-IBM Chicago, IL, USA). In all cases, $p<0.05$ was regarded as statistically significant.

RESULTS

Chemical properties of date wines and vinegars

The date wines produced via a three-day alcoholic fermentation process using *S. cerevisiae* as an inoculant were analysed for their chemical compositions, and the results are presented in Figure 2. It was observed that the Siam S1 date wine contained the highest alcohol content of 9.35% (v/v). The date vinegars produced via a 15-day acetous fermentation process using *A. pasteurianus* TISTR 521 as an inoculant were analysed for their chemical compositions, and the results are presented in Figure 3. All date

vinegars showed a significant decrease in alcohol content as it was converted to acetic acid by acetic acid bacteria, which was consistent with the increased acetic acid content. However, the alcohols were not completely depleted, with vinegar produced from the Siam S1 cultivar containing the highest acetic acid content of 7.00% (v/v).

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Total phenolic content and antioxidant activities

Bioactive properties, namely DPPH radical scavenging activities and total phenolic content of the wine and vinegar samples, are shown in Figures 4 and 5, respectively. Results showed that the date wine derived from Siam S1 exhibited the highest antioxidant activity of 73.79±0.77 mg/mL. The vinegar produced from Barhi was observed to exhibit the highest antioxidant activity of 24.96±0.29 mg/mL. The levels of total phenolic content detected in the date wines and vinegars produced from different cultivars via a two-stage fermentation process are given in Figure 5. It was noted that the total phenolic content of date wine derived from Siam S1 contained the highest level (343.42±1.43 mg/L). The KL1 vinegar measured at the end of acetous fermentation exhibited the highest total phenolic content of 208.35±0.96 mg/L.

DISCUSSION

Chemical properties of date wines and vinegars

From Figure 2, It was observed that on day 0 of fermentation, the KL1 cultivar had the highest glucose content and the Barhi cultivar had the highest fructose content. On day 3, the Siam S1 date wine contained the highest alcohol content of 9.35% (v/v), which was similar to that detected in Zahdi date wine at 5.61%(v/v), which was produced for a period of four days at a yeast ratio of 10 g/L (Matloob, 2014) and unripe banana, Musa (ABB) 'Kluai Namwa' wine by mixed strains of *S. cerevisiae* for ten days [13.2±0.07% (w/v)] (Thongpoem, *et al.*, 2021). As given in Figure 2, on day 3 of fermentation, glucose and fructose in most wine samples were not completely depleted, except for the glucose in Barhi and Siam S1 date wines. Glucose and fructose were least depleted in KL1 wine. Usually, a typical wine fermentation process comprises a lag phase, which lasts for several hours, a short growth phase of 24–36 hours, followed by a stationary phase, during which most of the sugar (between 50 and 80%) is fermented. During this phase, yeast activity continually decreases, although the viability level remains high, generally over 90%, until the sugar is exhausted (Marsit & Dequin, 2015). According to Jakabová *et al.* (2021), who studied the chemical composition of white wines produced from different grape varieties, glucose and fructose are the main fermentable sugars in wine. In alcoholic fermentation, yeast converts most of the glucose and fructose contents into alcohol and CO_2. Grape must contain equal amounts of glucose and fructose, and during fermentation, glucose is consumed at a higher rate than fructose, which leads to an increased proportion of fructose as fermentation progresses.

In the final process of acetous fermentation, the vinegar produced from the Barhi cultivar contained the highest alcohol content of 0.09% (v/v), while that produced from Siam S1 and KL1 cultivars were completely depleted. According to a previous study by Matloob (2014), homemade date vinegar has an alcohol content of 0.22% (w/v), while commercial date vinegar has an alcohol content of between 0.01-2.53% (w/v). Similarly, Boonsupa & Kerdchan (2021) revealed that at the end of acetic fermentation in prunus vinegar, the alcohol content ranged between 1.14 –

3.35% (v/v). At the end of an acetous fermentation process, acetic acid content was found to range from 2.97-7.00% (v/v), with the highest value of 7.00% (v/v) observed in the date vinegar produced from the Siam S1 cultivar, similar to that detected in homemade date vinegar produced from the Zahdi cultivar [7.24% (w/v)] (Matloob, 2014) and close to the acetic acid content determined in Chinese pomegranate vinegar (7.50±0.21%) (Boonsupa *et al.*, 2021), as well as the lowest of 2.97% (v/v) produced from the Barhi cultivar.

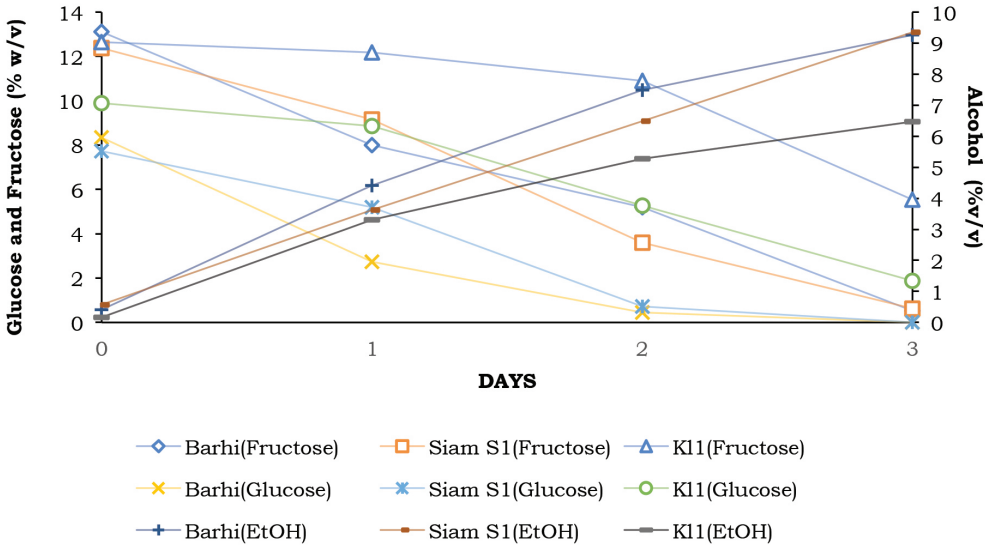

Figure 2. Physicochemical properties of date wines during the 3-day fermentation process

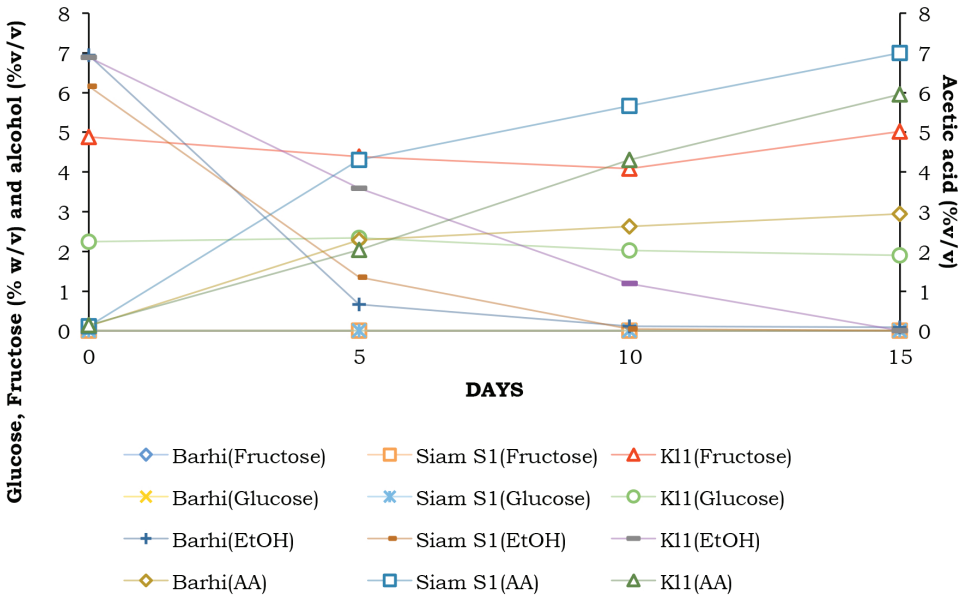

Figure 3. Physicochemical properties of date vinegar during a 15-day fermentation process

Total phenolic content and antioxidant activities

Organic acids and polyphenols, mainly acetic acid, plays a significant role in the beneficial properties provided by fruit vinegar. Recently, studies are being carried out to determine and identify the phenolic composition of vinegar such as palm vinegar having gallic acid (14.14±0.07 µg/mL), catechin (8.61±0.32 µg/mL), rutin (6.67±0.03 µg/mL), isoquercetin (11.27±0.12 µg/mL), and quercetin (10.33±0.16 µg/mL) (Chatatikun & Kwanhian, 2020). The type and quantity of bioactive compounds in vinegar are closely related to the raw matter used to produce vinegar, including the cultivar of the fruits.

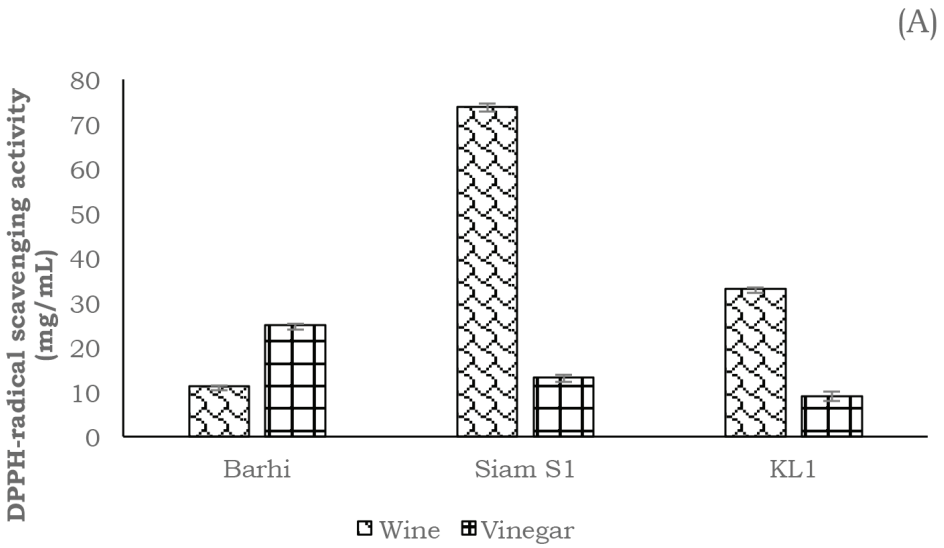

Figure 4. Antioxidant activities of the three date wines and vinegars produced via a two-stage fermentation process.

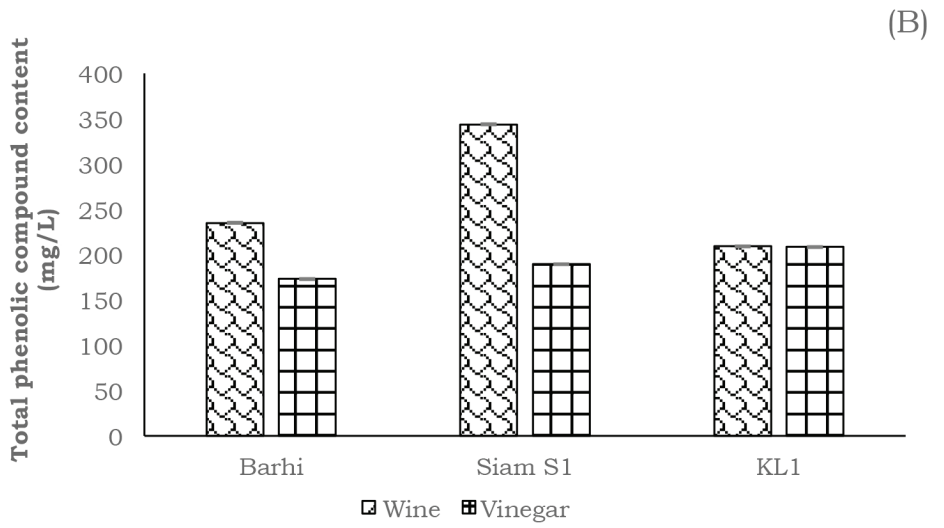

Figure 5. Total phenolic content of the three date wines and vinegars produced via a two-stage fermentation process.

According to Boonsupa (2021), research on the different cultivars of fruits affects the content of phytochemical substances contained in the vinegar. For example mango vinegar, which is produced from the mahacharnok cultivar, has the highest antioxidant activity because of the nature of microorganisms to produce vinegar and the technique selected in the fermentation process. Several studies revealed that bioactive compounds such as polyphenols in vinegar may have protective effects such as anti-hyperglycaemic effect, anti-hyperlipidaemic effect, antimicrobial effect, antioxidant effect, and anti-inflammatory effect (Ousaaid *et al.*, 2022; Chen *et al.*, 2016). Results showed that the date wine derived from Siam S1 exhibited the highest antioxidant activity of 73.79±0.77 mg/mL, which was greater than that produced from black rose hip wine (27.84 mg/mL), a wine with a fermentation period of 45 days (Pashazadeh *et al.*, 2021). The vinegar produced from Barhi was observed to exhibit the highest antioxidant activity of 24.96±0.29 mg/mL, which was greater than that of commercial red date vinegar (1.16±0.16 mg/mL) (Ali *et al.*, 2019). It was noted that the total phenolic content of date wine derived from Siam S1 was highest (343.42±1.43 mg GAE/L), but was much lesser than that of Khistawi date wine (1,179.8±111 mg GAE /L), which is characterised by its high sugar content (Matloob & Balakit, 2016). The KL1 vinegar measured at the end of acetous fermentation exhibited the highest total phenolic content of 208.35±0.96 mg GAE/L, but was much lesser than that detected in Khistawi date vinegar (1,453.4±220 mg GAE /L) (Matloob & Balakit, 2016) and cider vinegar (289±13 mg GAE/L) (Benedek *et al.*,2022). According to Andlauer, Stumpf & Furst (2000), the acetification process is accompanied by a decrease in total phenolic content in cider white and red vinegar.

Nowadays, reports show that date composes intrinsic nutritional and functional properties. They are a good source of simple carbohydrates, mainly in the form of glucose and fructose. Dates are abundant in dietary non-starch polysaccharides (NSPs) and some minerals, such as magnesium and potassium. Bioactive constituents that have been detected in *P. dactylifera* fruits include phenolic acids, carotenoids, and flavonoids. Bioactive compounds of *P. dactylifera* are of two kinds, namely non-nutritive or nutritive molecules. Examples of non-nutritive bioactive molecules detected in *P. dactylifera* are phenolic acids, flavonoids, carotenoids, phytosterols, and polyphenols. The other group can be considered as nutritive bioactive compounds such as soluble and insoluble NSPs, α-tocopherols, β-carotene, ascorbic acid, and selenium (Al-Mssallem, Alqurashi & Al-Khayri, 2019).

CONCLUSIONS

This study was conducted to compare the levels of acetic acid, total phenolic and antioxidant contents of the date vinegars produced from three cultivars of dates via a two-stage fermentation process. The results showed that the vinegar produced from Siam S1 exhibited the highest level of acetic acid (7.00%), while those produced from Barhi displayed the highest antioxidant activities (24.96 mg/mL) measured through DPPH radical assay. Meanwhile, the vinegar produced from KL1 was observed to have the highest total phenolic content (208.35 mg/L). Currently, farmers in Thailand grow a large number of dates, resulting in a large amount of yields. Therefore, research into dates processing will encourage value added to agriculture

and interested entrepreneurs to invest in the production of vinegar from date palms that can be further developed into industrial production in future.

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Authors' contributions

Boonsupa W, principal investigator, conceptualised and designed the study, prepared the draft of the manuscript and reviewed the manuscript; Thammajit C, Sittisumran T, Thiansai O, and Kaewsura T conducted the study, data analysis and interpretation, assisted in drafting of the manuscript and reviewed the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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