



PHENOLIC CONTENT OF VARIOUS DATE PALMS FRUITS AND VINEGARS FROM IRAQ

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ABSTRACT

Vegetables and fruits and all the drinks derived from them, are rich in phenolic compounds, which have been confirmed to exhibit positive effects on certain types of inflammatory disorders, coronary disease and cancers. In this study, the total phenolic contents of selected Iraqi date's and vinegars were evaluated and compared with other fermented fruits. The production of date vinegar was carried out by anaerobic conversion of date juice to ethanol using bakery yeast and aerobic oxidation of ethanol to acetic acid at 30°C by old vinegar. Total phenolics were determined colorimetrically by Folin-Ciocalteau reagent. The levels of sugars, ethanol and acetic acid in homemade and 21 commercially available vinegars were determined by refractometry, gas chromatography and titrimetry. The results indicated that: (i) Khistawi variety gave the highest phenolic content (475.5 mg GAE/100 g), followed by Khadrawi (411.6 mg GAE/100 g), Sultani (371.6 mg GAE/100 g), Zahdi (335.6 mg GAE/100 g), Maktoom (331.6 mg GAE/100 g) and Ashursi (315.6 mg GAE/100 g) and (ii) the total phenol content of khistawi date's vinegar (1453 mg GAE/L) was very high compared with other fermented fruits. Accordingly, Iraqi date palm and its vinegar could be considered as a potential source of antioxidant. These natural compounds can be used in food industry as a source of bioactive human health promoters.

Key words: Iraqi dates, Khistawi, Fermentation, Polyphenols, Vinegars.

INTRODUCTION

Dates are an important crop in the hot desert regions of the world and are marketed internationally as a high-value fruit. Industrially they are utilized to produce several products such as syrup, jam, date-jelly, date butter, vinegar and wine. Date fruits have high carbohydrates content, making them a good source of energy. Moreover, dates contain fat, protein, fiber, minerals and some vitamins¹. Besides nutritional compounds, date is rich in

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polyphenols. These biologically active compounds can prevent the oxidation of other molecules such as proteins and lipids providing a protective role in the body against cardiovascular disease, cancer, and other degenerative conditions²⁻⁴. Polyphenols are regular constituents of human food, with varied consumption depending on the diet. The average consumption of polyphenols is about 1 g per day⁵.

Over the past few years there has been increasing interest from Tunisia, Spain, Oman, Algeria, Kuwait, Iran, Bahrain and the USA in determining the relevant dietary sources of antioxidant phenolic compounds of their date fruits⁶. However, little information has been published about the total phenolic content of Iraqi dates. Thus, further studies were necessary to characterize the contents of phenolic compounds. Such detailed information is essential in order to promote the use of these fruits.

Moreover, the latest strategic report from Iraq indicated that about 36% of the total date production is sold as animal feed and 14% of the crops are wasted⁷. This is an intolerable great loss for developing countries like Iraq. A feasible solution to avoid this loss could be through the bio-conversion of low quality date into natural vinegar. The health benefits of natural vinegar are well known and include an immune modulation effect, resistance to cardiovascular disease, appetite suppression, improved digestion, decreased serum cholesterol and blood pressure, and reduced fasting blood glucose⁸⁻¹⁰.

The aim of this study was to determine the total phenolic content in the 10 most frequently consumed dates' cultivars in Iraq and to compare the phenolic levels of Iraqi date vinegar with other fermented fruits. Date vinegar was produced by a two stage fermentation process; the first one being the conversion of fermentable sugars to ethanol by commercial baker yeast and the second the oxidation of ethanol by old vinegar¹¹. Total phenolics were determined colorimetrically using Folin-Ciocalteu reagent. The levels of ethanol, acetic acid and sugars in homemade and twenty one commercially available vinegars were monitored by gas chromatography, titrimetry, refractometry and UV-vis spectroscopy.

EXPERIMENTAL

Materials and methods

Extraction of antioxidant compounds

Ten date cultivars namely Khistawi, Shukari, Hamrawi, Khadrawi, Maktoom, Sultani, Ashursi, Degla, Zahdi and Barban were collected at "tamr stage" (full ripeness) from Babylon province (Iraq). Hundred grams from each variety were directly stored at -18°C prior to

analysis. After washing with water and removing the seeds, the edible part of date was dried at room temperature before extraction. Two grams of date were mixed for 2 h with 20 mL of H₂O at 70°C with continuous agitation. The mixture was centrifuged at 4000 g for 15 min, and the supernatant was decanted. The extraction process was repeated several times with new distilled water until no significant amount of phenols was left in the extract. The supernatants were finally combined and analyzed using Folin-Ciocalteu reagent.

Fermentation processes

Khistawi dates at the ripening stage (which is characterized by a high sugar content) were selected to follow the steps of the fermentation process. After cleaning properly, the date fruits were extracted trice with distilled water (2:1) at 70°C, filtered and concentrated to the required percentage using rotary evaporator. Approximately 4.60 L of juice containing 15.65% (w/v) total soluble sugars were obtained from 1 kg of khistawi dates. The clarified juice was inoculated at 30°C through the addition of 0.5% (w/v) Chinese bakery yeast. The fermented juices reached maximum ethanol concentrations of 6.62% (w/v) ethanol in 40 to 80 hours. The date wine formed was centrifuged and mixed with old vinegar in accordance with the surface culture method^{11,12}. In 12 to 18 days of acetic fermentation about 4.25 L of natural vinegar was produced from three replicates of the experiment, containing 6.84% (w/v) of acetic acid. The homemade vinegar was compared with twenty one commercial vinegars currently available in Iraqi markets.

Physiochemical analysis

Polyphenols in dates and vinegars were determined using Folin-Ciocalteu reagent to form a blue phosphotungstic-phosphomolybdenum complex. To 100 µL of the sample extract, 2 mL of distilled water and 1.0 mL of Folin–Ciocalteu reagent (diluted at 1/10) were added. The mixture was allowed to stand for 5 min and then 0.75 mL of Na₂CO₃ solution (60 g/L) was added. The mixture was allowed to stand for 90 min and absorption was measured at 765 nm by a Shimadzu 1700 double beam UV-visible spectrophotometer against water as a blank. The total phenol concentration was expressed as mg of gallic acid equivalent (GAE) per 100 g of fresh sample for three replicates (Fig. 1).

Ethanol determinations were carried out by Shimadzu-2010 gas chromatograph^{11,13}. The percentage (w/v) of the total soluble sugars was analyzed by refractometry and the phenol-sulphuric acid method¹⁴. Total acidity was evaluated by titration with standardized solution of 0.1M sodium hydroxide and the pH of the samples was measured using a pH-meter (Model 240, WTW).

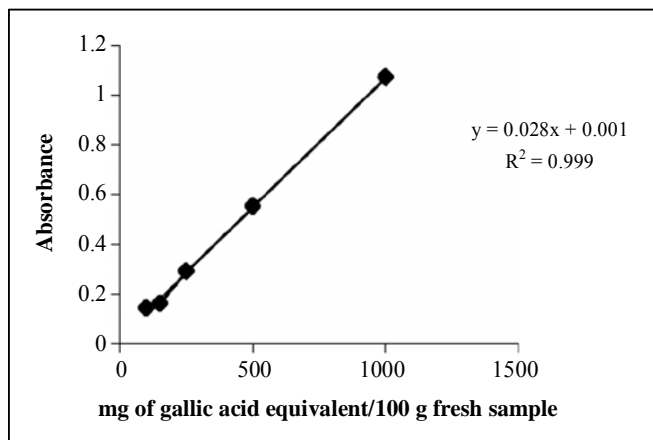


Fig. 1: Calibration curve of gallic acid as a reference material for the determination of polyphenols in dates extract and vinegars by Folin-Ciocalteu reaction after 90 min

RESULTS AND DISCUSSION

Effect of solvent system

The recovery of phenolic contents in different samples is influenced by the polarity of extracting solvents and the solubility of these compounds in the solvent used for the extraction process¹⁵. In this study, different solvents such as methanol/H₂O, ethanol/H₂O and acetone/H₂O mixtures and distilled H₂O have been tried for the extraction of phenolic compounds from different date's varieties. Among all the solvents used, water at 70°C was found to be the most efficient solvent for extracting phenolic compounds probably due to its high polarity.

Extraction temperature

Fig. 2 shows the influence of temperature on extraction efficiency of total phenolics from date fruits. Water showed a steady increase in the phenolics content with temperature increases, from 167.6 at 30°C to 335.6 mg/100 g at 70°C probably due to the enhancement in both the solubility of solute and the diffusion coefficient. Heating also might soften the plant tissue and weaken the phenol–protein and phenol–polysaccharide interactions in date tissues. However, despite the positive effects of higher temperatures on the phenolics extraction, this cannot be increased indefinitely because many phenolic compounds are easily hydrolyzed and oxidized which decrease the yield of phenolics in the extracts¹⁶. Therefore, 70°C was selected as optimum temperature to extract phenolics from date fruits.

The increased extraction time and sustained exposure to high temperature increases

the loss of solvent by vaporization, which affects the solvent to solid ratio, and increases the loss of phenolics by oxidation. Therefore, 2 hours extraction time was selected as optimum time for phenolic extraction from date fruits.

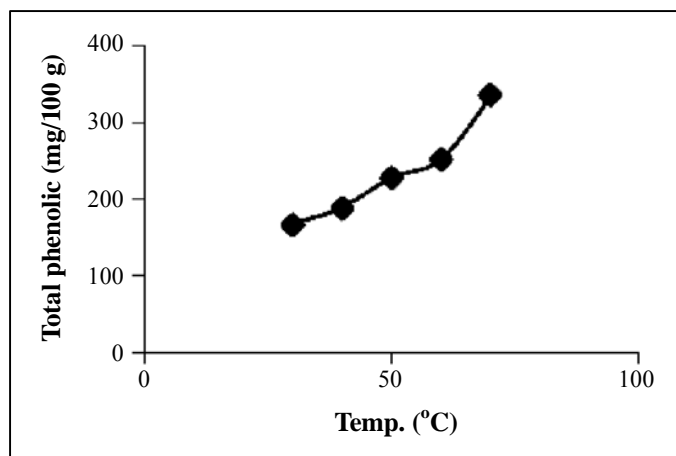


Fig. 2: Influence of extraction temperature on extraction efficiency of total phenolics from date fruits

Total phenolic contents in date extracts

The content of total phenolics in fresh date's weight (FW) is shown in Table 1. Significant difference ($p < 0.05$) in total phenolic contents was observed. Khistawi variety gave the highest phenolic content (475.5 mg of GAE/100 g) followed by Khadrawi (411.6 mg of GAE/100 g), Sultani (371.6 mg of GAE/100 g), Zahdi (335.6 mg of GAE/100 g), Maktoom (331.6 mg of GAE/100 g) and Ashursi (315.6 mg of GAE/100 g), while it ranged from 147.6 to 283.4 mg of GAE/100 g for the varieties of Barban, Degla, Hamrawi and Shukari, respectively. The darkest variety of date like Khistawi was found to have the highest concentration of polyphenols, whereas the lightest variety (Barban) contained the lowest (Table 1). This finding agreed well with the study of Beretta et al.¹⁷ who found a high correlation ($r = 0.93$) between polyphenols concentration and the color of honey.

Compared to other countries the total phenolic content of Iraqi dates is within the range reported by Benmeddour et al.¹⁸ for Algerian dates (167-709 mg GAE/100 g FW), Besbes et al.¹⁹, Saafi et al.²⁰ and Kchaou et al.⁶ for Tunisian dates (199.43-681.8 mg GAE/100 g FW), Singh et al.²¹ for Omani dates (81-235 mg GAE/100 g FW), Saleh et al.²² for Saudi dates (238.54-455.88 mg GAE/100 g FW) and Anjum et al.²³ for Pakistani dates (486.6-564.8 mg GAE/100 g DW). Surprisingly, the Iraqi phenolic level was much higher

than those reported by Mansouri et al.²⁴ and Ghiaba et al.²⁵ for Algerian dates (3.49-23.05 mg GAE/100 g FW) and Biglari et al.²⁶ for Iranian dates (2.89-141.35 mg GAE/100 g DW). The observed differences may be attributed to the cultivars and extraction conditions such as solvent and ratio material/solvent.

Table 1: Concentration of polyphenols expressed as mg of Gallic acid equivalent (GAE) in 100 g of fresh dates

| No. | Type of date | (mg GAE/100 g FW) |
|-----|--------------|-------------------|
| 1 | Khistawi | 475.5 |
| 2 | Khadrawi | 411.6 |
| 3 | Sultani | 371.6 |
| 4 | Zahdi | 335.6 |
| 5 | Maktoom | 331.6 |
| 6 | Ashursi | 315.6 |
| 7 | Shukari | 283.4 |
| 8 | Hamrawi | 275.6 |
| 9 | Degla | 247.6 |
| 10 | Barban | 147.6 |

In comparison with other fresh fruits²⁷⁻²⁹, our results indicated that Iraqi date fruits might be considered as a remarkable source of total phenolics (Table 2). However, dried fruits, such as prune (760 mg GAE/100 g DW), apricots (630 mg GAE/100 g DW) and figs (520 mg GAE/100 g DW) may have more polyphenols compared to fresh dates³⁰.

Table 2: Concentration of polyphenols in fresh fruits from literature data expressed as mg of gallic acid equivalent (GAE) in 100 g of fresh weight

| No | Type of fruit | Dragovic-Uzelac ²⁷ | Fue et al. ²⁸ | Faller and Fialho ²⁹ |
|----|---------------|-------------------------------|--------------------------|---------------------------------|
| 1 | Apple | 56.7 | 74.0 | - |
| 2 | Apricot | 50.6 | - | - |
| 3 | Blueberry | 219.6 | - | - |
| 4 | Mandarin | 116.1 | - | 13.7 |

Cont...

| No | Type of fruit | Dragovic-Uzelac ²⁷ | Fue et al. ²⁸ | Faller and Fialho ²⁹ |
|----|---------------|-------------------------------|--------------------------|---------------------------------|
| 5 | Orange | 127.8 | 77.0 | 114.6 |
| 6 | Sour cherry | 265.0 | - | - |
| 7 | Strawberry | 112.7 | - | - |
| 8 | Peach | 40.7 | - | - |
| 9 | Pomegranate | - | 147.0 | - |
| 10 | Red grape | - | 80.0 | - |
| 11 | Green citrus | - | 54 | - |
| 12 | Tomato | - | 30 | - |
| 13 | Pineapple | - | - | 85.1 |
| 14 | Banana | - | - | 215.7 |
| 15 | Papaya | - | - | 15.3 |
| 16 | Mango | - | - | 110.5 |

Antioxidant capacities

Fruits contain different antioxidant compounds. Therefore, measuring the antioxidant capacity of each compound individually becomes very difficult. Instead several methods have been developed to estimate the antioxidant potential by measuring the ability of antioxidants to scavenge specific radicals, inhibit lipid peroxidation or chelate metal ions³¹. The outcome of these measurements confirms the presence of a significant correlation between the total phenolic content and the antioxidant capacities of date fruits⁶. So this study implies that date palm fruit (especially Khistawi) have good antioxidant potential and can be used to produce novel natural antioxidants.

Vinegar analysis

Generally, commercial vinegar can be divided into three groups; wine vinegar or brewed vinegar, distilled vinegar and artificial vinegar. All these products are solutions containing mainly acetic acid, which has been reported to possess physiological effects. In contrast to distilled and artificial vinegars, wine vinegar may contain important materials such as amino acids, carbohydrates, vitamins, minerals, carotenoids and phenolic compounds⁸. The concentration of polyphenols in wine vinegars can vary due to the different sources of raw materials³²⁻³⁴.

The mean values of total phenol content of Khistawi date juice, wine and vinegar are

shown in Table 3. The mean value of total phenol content in fresh juice (15.65% w/v) was 1211.8 mg GAE/L which decreased slightly (-2.6%) during wine formation. Because little is known about the ability of *Saccharomyces cerevisiae* strains to affect the phenolic content of wines during the fermentation process and aging, some authors attributed the decrease in phenolic level to the adsorption of phenolic compounds on the yeast cell walls³⁵.

Table 3: Total phenol content of Khistawi date juice, wine and vinegar

| Sample | Total phenol (mg GAE/L) |
|---------|-------------------------|
| Juice | 1211.3 ± 76 |
| Wine | 1179.8 ± 111 |
| Vinegar | 1453.4 ± 220 |

Values are mean ± SD of 3 observations

On the other hand, the total phenolic content of Khistawi date wine increased significantly (+20.1%) during the acetification process and reached a peak value of 1453.4 mg GAE/L within 14 days. This increase could be attributed to the presence of glycosidic bonds between date polyphenols and sugar, protein, cellulose and starch. These glucoside bonds are degraded by the acids produced during natural fermentation leading to the liberation or synthesis of new antioxidative phenolics³⁶. The present study verified that fermentation is a good technology with great potential for the production of antioxidant active compounds from natural sources.

However, the current results contradict those of Andlauer et al.³⁷ and Vithlani and Patel³⁸ who found a remarkable decrease in total phenol content due to the acetification process of other fruit juices. Conflicting results between the positive and negative effects of fermentation may arise from uncertainty in the production or degradation of antioxidant activity compounds. The production yields of antioxidant activity compounds can be improved with a suitable choice of microorganism or substrate.

Table 4 shows the physiochemical parameters of 21 commercial vinegars currently consumed in Iraq. The pH of the samples presented values between 2.40 and 3.26, which are similar to those reported by other workers³⁹. Excluding three brands; all analyzed vinegars presented minimum acidity of 4.00%, value that is demanded by the North American legislations⁴⁰. On the other hand, 76.2% of the commercial vinegars had alcohol contents between trace and 0.50% (w/v), whereas 23.8% contained significantly higher ethanol concentration of 0.50 to 2.53% (w/v). The highest concentration detected are much higher than the maximum ethanol content (0.5-1.0% v/v) set by the Codex Alimentarius commission for wine vinegars⁴¹.

The quality of 21 samples of vinegar commercially available in Iraq was evaluated according to their total polyphenol content (Table 4). Based on the total polyphenol level, the commercial vinegar samples in this study can be divided into three groups. Group 1 included 9 vinegars (mainly distilled or artificial) in which the content of total polyphenols was between trace and 70 mg GAE/L. Group 2 consisted of 5 vinegars with total polyphenol content ranging from 100-352 mg GAE/L. Group 3 (consisted of 7 brands) contained total polyphenols higher than 352 mg GAE/L which included mainly date vinegars. Among all wine vinegars investigated, Khistawi date vinegar was the darkest in color and acquired the highest polyphenol content (Table 4).

Table 4: pH, ethanol, total titratable acidity, polyphenols and total soluble sugar in homemade Khistawi vinegar and 21 commercial vinegars currently consumed in Iraq¹

| Vinegar label | Type | Source | pH | Ethanol (% w/v ¹¹) | Acidity (% w/v) | Polyphenol (mg/L) | Total sugar (% w/v) |
|-------------------|------------|---------|------|-----------------------------------|--------------------|----------------------|------------------------|
| Homemade | Date | Iraq | 3.07 | 0.22 | 6.84 | 1453 | 0.66 |
| Commercial | | | | | | | |
| Al-Wafe | Artificial | Iraq | 2.67 | < 0.01 | 4.08 | nd | nd |
| Al Arabi | Date | Iraq | 3.03 | 0.25 | 3.18 | 392.6 | 0.19 |
| Ibin Hayan | Date | Iraq | 3.02 | 1.44 | 5.28 | 421.6 | 1.17 |
| Hello | Artificial | Iraq | 2.65 | 0.03 | 4.86 | nd | nd |
| Albadawi | Artificial | Iraq | 2.57 | < 0.01 | 4.56 | nd | 2.47 |
| Canz Alnumaniah | Date | Iraq | 3.26 | 0.56 | 5.04 | 798.9 | 0.75 |
| Sham gardens | Date | Syria | 2.99 | 2.53 | 5.26 | 577.9 | 0.58 |
| Durra | Date | Syria | 3.23 | 1.07 | 4.22 | 752.9 | 1.73 |
| Hamada | Garlic | Syria | 2.84 | 0.31 | 3.18 | 539.9 | 0.03 |
| Sham gardens | Grape | Syria | 2.95 | 0.50 | 4.63 | 352.9 | 0.46 |
| Al Walaem | Garlic | Syria | 3.12 | < 0.01 | 4.98 | 196.7 | 0.55 |
| Alwadi alakhdar | Grape | Lebanon | 2.86 | 0.18 | 5.64 | 346.9 | 0.21 |
| Hamra (White) | Grape | Lebanon | 2.49 | < 0.01 | 6.18 | nd | nd |
| Hamra (Red) | Grape | Lebanon | 2.55 | < 0.01 | 5.76 | 17.73 | nd |

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| Vinegar label | Type | Source | pH | Ethanol (% w/v ^{!!}) | Acidity (% w/v) | Polyphenol (mg/L) | Total sugar (% w/v) |
|-----------------|------------|---------|------|-----------------------------------|--------------------|----------------------|------------------------|
| Teeba Al Jabal | Artificial | Lebanon | 2.52 | < 0.01 | 5.34 | nd | nd |
| Zer | Apple | Turkey | 2.71 | 0.09 | 5.17 | 63.10 | 1.48 |
| Kemal Kukrer | Grape | Turkey | 2.99 | < 0.01 | 5.11 | 294.9 | 0.42 |
| American garden | Vegetable | U.S.A | 2.40 | 0.16 | 6.12 | nd | 2.47 |
| American garden | Grape | U.S.A | 2.53 | 0.18 | 5.40 | 128.9 | 0.22 |
| Baider | Sugarcane | K.S.A. | 2.43 | < 0.01 | 6.36 | nd | 2.40 |
| Taksan | Apple | Iran | 2.96 | 0.42 | 2.85 | 137.9 | 0.29 |

!All averages derived from three readings with a maximum standard deviation of 5%

!!% (w/v): (g/100 mL), nd: not detected

According to Alonso et al.⁴², Davalos et al.⁴³, Mariana-Atena et al.⁴⁴ and Shahidi et al.⁴⁵ the antioxidant capacity of different wine vinegars is strongly correlated to their polyphenolic content. These directives clearly imply that dates vinegar offer a very competitive price to all imported brands. Moreover, the great advantage of vinegar as a matrix for polyphenols in the diet is that, in vinegars, they are present in the soluble state and hence are more biologically available, whereas in fruits and vegetables, they are strongly bonded and hence less easily absorbed.

CONCLUSION

- (i) The results showed that Iraqi dates were rich in phenolics that are capable of scavenging free radicals involved in human diseases.
- (ii) Low cost date could be used in the manufacture of value added products such as vinegar with commercial value. The beneficial health effects of fruit vinegars may in part be related to the structural breakdown of plant cell walls during bacterial fermentation, leading to the liberation or synthesis of new antioxidative phenolics.
- (iii) Modification of fermentation process could be customized so as to increase the bioaccessibility of bioactive compounds. Therefore, in the future, it can be anticipated that fermentation could be used to design food with health effects.

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