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Original Article

Determination and evaluation of some basic phytochemical properties of date vinegar

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Abstract:

Date vinegar is a traditional functional food that, thanks to the rich nutritional content of dates and the bioactive compounds formed through natural fermentation, exhibits beneficial effects, particularly on the digestive system, metabolism, and metabolic health. Traditional vinegar production stands out as an alternative source, as the fruits used in the process have a high natural sugar content and rich nutritional profile, making it a natural and economical health-supporting product. In this study, the production method and phytochemical profile of date vinegar were thoroughly examined, including total phenolic content, total flavonoid level, DPPH antioxidant capacity, and organic acid composition through HPLC analysis. The results revealed that date vinegar contains a high amount of antioxidant compounds (total phenolic content of 429.00 mg GAE L^{-1}, total flavonoid content of 302.00 µg QE mL^{-1}), and exhibits a strong DPPH free radical scavenging activity of 89%. In terms of organic acid composition, tartaric acid (556.33 mg 100 g^{-1}) and oxalic acid (354.33 mg 100 g^{-1}) were detected at the highest concentrations. The presence of these organic acids contributes not only to the taste of the vinegar but also to its positive biological effects on health. The findings indicate that date vinegar has a complex and diverse phytochemical structure and possesses significant potential as a functional food due to its strong antioxidant capacity. Moreover, these results are in parallel with similar studies reported in the literature, supporting the use of date vinegar as a traditional product with health-promoting biological activities. In conclusion, the functional properties and rich organic acid content of date vinegar make it a valuable natural health-supporting and functional food product.

Key words:
Phoenix dactylifera L., date vinegar, functional foods, fermentation, antioxidant, health effects

Apstrakt:

Određivanje i procena nekih osnovnih fitohemijskih svojstava urminog sirćeta

Urmino sirće je tradicionalna funkcionalna hrana koja, zahvaljujući bogatom nutritivnom sastavu urmi i bioaktivnim jedinjenjima nastalim prirodnom fermentacijom, pokazuje povoljne efekte, naročito na digestivni sistem, metabolizam i metaboličko zdravlje. Tradicionalna proizvodnja sirćeta ističe se kao alternativa, jer plodovi korišćeni u procesu imaju visok prirodan sadržaj šećera i bogat nutritivni profil, čineći ga prirodnim i ekonomičnim proizvodom poziitivnim po zdravlje. U ovoj studiji detaljno su ispitani način proizvodnje i fitohemijski profil urminog sirćeta, uključujući ukupni sadržaj fenola, ukupan nivo flavonoida, DPPH antioksidativni kapacitet i sastav organskih kiselina putem HPLC analize. Rezultati su pokazali da urmino sirće sadrži visoku količinu antioksidativnih jedinjenja (ukupni sadržaj fenola 429,00 mg GAE L^{-1}, ukupni sadržaj flavonoida 302,00 µg QE mL^{-1}) i pokazuje snažnu sposobnost hvatanja slobodnih radikala od 89%. Što se tiče sastava organskih kiselina, najviše su prisutne vinska kiselina (556,33 mg na 100 g) i oksalna kiselina (354,33 mg na 100 g). Prisutnost ovih organskih kiselina doprinosi ne samo ukusu sirćeta, već i njegovim pozitivnim biološkim efektima na zdravlje. Nalazi ukazuju da urmino sirće ima složenu i raznovrsnu fitohemijsku strukturu i poseduje značajan potencijal kao funkcionalna hrana zbog snažnog antioksidativnog kapaciteta. Štaviše, ovi rezultati su u skladu sa rezultatima sličnih publikovanih literaturnih podataka, podržavajući upotrebu urminog sirćeta kao tradicionalnog proizvoda sa zdravstveno-promotivnim biološkim aktivnostima. U zaključku, funkcionalna svojstva i bogat sadržaj organskih kiselina čine urmino sirće vrednim prirodnim proizvodom koji podržava zdravlje i funkcionalnom hranom.

Ključne reči:
Phoenix dactylifera L., urmino sirće, funkcionalna hrana, fermentacija, antioksidans, zdravstveni efekti

Introduction

Date palm (*Phoenix dactylifera* L.) has been cultivated in hot climates and arid and semiarid regions, especially in the Middle East and North Africa, for 5,000 years. It has always played an important role in these regions' economic and social life and is considered one of the most important fruits of the Arabian Peninsula. Date palm (*Phoenix dactylifera* L.), from the Arecaceae (Palmae) family, is a dioecious fruit-bearing tree. The Arecaceae family contains more than 200 genera and 2500 species. It is a functional fruit species that attracts attention with its rich carbohydrate content, vitamins (especially B group vitamins), minerals (potassium, magnesium, iron), and high phenolic compound content (Al-Bulushi et al., 2017; Hamden et al., 2022; Barakat & Alfheeaid, 2023; Pepe et al., 2024; Çolak & Alan, 2025). While a low-quality date with dark fruit color, small, rotten, and undesirable taste cannot be marketed, its high sugar content strengthens its use as a raw material in the production of various value-added products such as date syrup, date paste, liquid sugar, and vinegar (Mohamed & Fennir, 2017).

Food safety, healthy nutrition, and sustainable food production are among the issues that are becoming increasingly important today. Therefore, functional foods obtained by traditional fermentation techniques have become the focus of attention again due to their contributions to health and natural production methods. Vinegar is an important fermented product used as both a food additive and a natural treatment tool for centuries. While fruits and vegetables with high sugar or starch content are generally used in vinegar production, in recent years, tropical fruits with high nutritional value, such as dates, have also begun to be evaluated in this field (Al-Farsi & Lee, 2008). It is a liquid product containing alcohols, acids, esters, aldehydes, and ketones, the main volatile component of which is acetic acid, which gives vinegar its sour taste and defines it as vinegar (Ho et al., 2017). Dates constitute a suitable carbon source substrate for alcohol fermentation in vinegar production since they contain 70-80% natural sugar (glucose, fructose, and sucrose) (Sahari et al., 2014). Date vinegar, which is obtained by subjecting date juice to alcoholic fermentation with yeast species such as *Saccharomyces cerevisiae* and then to a second fermentation with acetic acid bacteria such as *Acetobacter aceti*, attracts attention due to both its sensory and functional properties (Ali et al., 2018).

Historically, numerous plant species have been studied and used in treating or preventing various diseases. Factors such as the increase in population, insufficient drug supply, side effects of various synthetic drugs, and the emergence of new diseases due to resistance to currently used drugs have led to an increasing emphasis on the use of plant materials as a source of medicine for a wide variety of human ailments (Siddiqui, 2011). Thus, palm vinegar, which can exhibit antimicrobial, antioxidant, hypoglycemic, and cholesterol-lowering effects thanks to the organic acids (especially acetic acid), flavonoids, phenolic compounds, and volatile compounds it contains, is also reported to have positive effects on the digestive system, balancing stomach acidity, and supporting intestinal microbiota (Al-Hooti et al., 2002). Palm vinegar, which has a wide range of uses in traditional medicine from stomach disorders to detoxification, is currently being studied, with scientific studies supporting this traditional knowledge, and it especially reveals its potential as a functional food and natural preservative due to its high antioxidant capacity (Al-Hilfy et al., 2020).

This article compiles the production process, chemical components, and potential effects of date vinegar on health in accordance with the existing literature and evaluates its potential for use as a functional food.

Materials and Methods

Materials

In this study, a sample of date (*Phoenix dactylifera* L.) vinegar, which was fermented at home using traditional methods, was used. This vinegar is not widely available commercially but is available through online sales platforms. Vinegar samples were stored in the refrigerator at +4 °C until the analysis day. All analyses were carried out using standardized methods in a laboratory environment. In the study, the phytochemical properties of date vinegar produced from tropical fruit species were investigated. The applied analysis methods are explained in detail below.

Method

Vinegar installation

The production of date vinegar used in this study used the traditional method (Akarca et al., 2020; Öztürk, 2022). After the supplied fruits were washed, the date fruits were dried without any pretreatment, and their seeds were removed. The fruits prepared for vinegar production were added to 5 L glass jars at a rate of one-third of the jar volume. In addition, 0.5% honey was added to each sample to assist the fermentation process. Following this process, the jars were filled using spring water taken from its natural source, and the jars were closed with a cheesecloth. The prepared samples were left to ferment at room temperature and in a dark environment by utilizing the natural microbiota of the fruits, and the samples were mixed every day during the fermentation period. The fermentation process was carried out for 60 days, during which a sufficiently large vinegar mother was formed on the surfaces of the jars (approximately 0.5 cm). Following fermentation, the vinegar samples were filtered and transferred to new glass jars to separate the fruit pulp. Following this process, the jars were closed tightly, and the vinegar samples were stored in a dark environment at 25 °C until analysis was performed.

Determination of total phenolic content

This study used the Folin-Ciocalteu method to determine the total phenol content (TPC) (Kähkönen et al., 1999). A 200 µL volume of vinegar samples, obtained by the conventional method, and 500 µL of Folin-Ciocalteu reagent (diluted 10-fold with water) were added to a 10 mL test tube. The solution was left in the dark for 5 min before adding 1000 µL of sodium carbonate solution (7.5% w/v in water). The tubes were closed, shaken, and then left in the dark for 1 h again. UV spectrophotometric analyses for all absorbance measurements were carried out in a double-beam spectrophotometer at 765 nm using 1.0 cm quartz cells. They were compared with the gallic acid calibration curve. The results were expressed as mg GAE L^{-1}. Each experiment was carried out in triplicate.

Determination of total flavonoid content

The total flavonoid content of vinegars was determined by the aluminum chloride colorimetric method (Chang et al., 2002). Briefly, a 50 µL vinegar sample was placed in a 10 mL test tube. It was mixed with 950 µL of methanol and 4 mL of distilled water, then with 300 µL of sodium nitrite solution (5% in water). After incubation, 300 µL of aluminum chloride solution (10% in water) was added, and the mixture was left to stand for 6 min. Then, 2 mL of sodium hydroxide solution (1 M, in water) was added, and the mixture was adjusted to a final volume of 10 mL with distilled water. The mixture was left to stand for 15 min. UV spectrophotometric analyses were carried out on a double-beam spectrophotometer at 510 nm using 1.0 cm quartz cells for all absorbance measurements. Total flavonoid content was calculated from the quercetin calibration curve, and the result was expressed as mg quercetin equivalent per liter.

DPPH (2,2-diphenyl-1-picrylhydrazyl) method

The DPPH (2,2-diphenyl-1-picrylhydrazyl) experiment was performed by Thaipong et al. (2006) with minor modifications. DPPH Stock solution: 24 mg DPPH was weighed and dissolved in methanol. It was transferred to a 100 mL flask, and the volume was completed with 100 mL of methanol. It was stored at -18 °C until use. The working solution was obtained by mixing 20 mL of the stock solution with 90 mL of methanol. An absorbance value of 1.1 +/- 0.02 was obtained at 515 nm using a spectrophotometer. Three hundred µL of the vinegar sample was filtered and taken into a tube, and then 5700 µL of the DPPH working solution was added and mixed. It was allowed to react for one hour in a dark place. Then, the absorbance of this solution was measured at 515 nm on a spectrophotometer. Antioxidant activity was calculated as the decrease in absorbance value using the following formula:

$$\textbf{Antioxidant activity (\%)} = (A_0 - A1)/A \times 100$$

Where A_0 is the absorbance value of the control solution without a sample.
A_1: Absorbance of the mixture containing the sample.

Organic acid analysis

Vinegar samples were homogenized with Heidolph Silent Crusher M (Heidolph, Germany) and mixed on a shaker (Heidolph Unimax 1010, Germany) for 1 h, and then centrifuged at 14,000 rpm for 15 min. The supernatant was filtered through a 0.45 µm membrane filter (Millipore Millex-HV Hydrophilic PVDF; Millipore, USA). This separation was carried out using a reverse-phase ACE-C18 column (4 mm x 150 mm, 5 µm). The mobile phase was 10 mM potassium dihydrogen phosphate aqueous solution adjusted with ortho-phosphoric acid. The mobile phase was vacuum filtered through a 0.45 µm nylon filter and degassed online with a micro vacuum degasser. Chromatographic separation of these compounds was carried out at room temperature. The analysis was carried out at a flow rate of 1 mL/min; the run time was 10 min. The detector for ascorbic acid was set at λ=245 nm and for other organic acids, the detector was set at λ= 210 nm. The injection volume was 10 µL (Büyüktuncel et al., 2017). Each experiment was performed three times, and the results were expressed in g L^{-1} fresh weight.

Quantification of organic acids and phenolic compounds by HPLC-UV

Vinegar samples were first shaken for 1 h and then centrifuged at 14,000 rpm for 15 min. Then, the supernatant was filtered using a 0.45 µm membrane filter. The filtered vinegar samples were analyzed with an Agilent 1260 HPLC instrument (Agilent Technologies, CA, USA) equipped with Chemstation software, a quaternary pump, an autosampler, and a UV detector.

Organic acids were determined using an ACE-C18 column (4 mm × 150 mm, 5 µm; Hichrom Ltd., Theale, United Kingdom). The mobile phase consisted of a 10 mM potassium phosphate aqueous solution (pH 2.2, with ortho-phosphoric acid) with a 1 mL/min flow rate. The injection volume was 20 µL, and the detector was set at 245 nm for ascorbic acid and 210 nm for all other organic acids (Fu et al., 2015). An ACE-C18 (4.6 mm × 150 mm, 5 µm; Hichrom Ltd., Theale, United Kingdom) column was used for the chromatographic separation of phenolic compounds. Details of the chromatographic conditions are given in **Tab. 1**.

The mobile phase flow rate was kept at 1.0 mL min-1. Mobile phase A was ultrapure water, while mobile phase B was acetonitrile; both contained 0.1% acetic acid. Gradient conditions were 0–3.25 min, 8–10% B; 3.25–8 min, 10–12% B; 8–15 min, 12–25% B; 15–15.8 min, 25–30% B; 15.8–25 min, 30–90% B; 25–25.4 min, 90–100% B; 25.4–30 min, 100% B. The injection volume was 10 µL and the column temperature was kept at 25 °C. Detection wavelengths were selected according to the wavelengths where the phenolic compounds to be analyzed had maximum absorption. Syringic acid, protocatechuic acid, ferulic acid, ellagic acid, quercetin, and gallic acid were detected at 280 nm. Caffeic acid was detected at 330 nm and *p*-coumaric acid was detected at 305 nm (Wen et al., 2005).

Statistical analysis

The data obtained were analysed using the Minitab software package. Basic descriptive statistics were calculated using the 'Basic Statistics' command.

Results and discussion

This study summarizes the quantitative analysis results of various phytochemical components in date vinegar. These analyses were conducted to reveal the chemical composition of date vinegar and to characterize the product phytochemically. The study determined total phenolic and flavonoid contents, along with DPPH activity reflecting antioxidant capacity; in addition, levels of different phenolic acids (protocatechuic, gentisic, caffeic, *p*-coumaric, and ellagic acids) and organic acids (oxalic, tartaric, acetic, and citric acids) were analyzed. The data indicate that date vinegar possesses a complex and rich phytochemical composition (**Tab. 2**).

The results showed that the total phenolic content was 429.00 mg GAE L^{-1}, the total flavonoid content

Table 1. Chromatographic conditions of HPLC method

Parameters	Conditions	
Mobile phase	A: Ultrapure water containing 0.1% acetic acid.	
	B: Acetonitrile containing 0.1% acetic acid.	
Mobile phase flow rate	1.0 mL min^{-1}	
Column	ACE-C18 (4.6 mm × 150 mm, 5 µm)	
Column temperature	25 °C	
Injection volume	10 µL	
Run time	30 min.	
Detection wavelengths	280 nm for syringic acid, protocatechic acid, and gallic acid.	
	225 nm for vanillic acid.	
	305 nm for p-coumaric acid.	
	330 nm for caffeic acid and chlorogenic acid.	
Elution	Gradient Time min.	B% (Volume)
	0.00	8
	3.25	10
	8.00	12
	15.00	25
	15.8	30
	25	90
	25.40	100
	30.00	100

Table 2. Phytochemical analysis results of date vinegar

Variable	Mean±StDev	SE Mean±StDev	CoefVar	Minimum	Maximum
Total Phenolic (mg GAE L-1)	429.00±1.00	0.58±1.00	0.23	428.00	430.00
Total Flavonoids (μg QE mL^{-1})	302.00±1.00	0.58±1.00	0.33	301.00	303.00
DPPH (%)	89.00±1.00	0.58±1.00	1.12	88.00	90.00
Protocatechic Acid (mg 100g^{-1})	6.10±0.08	0.05±0.08	1.31	6.02	6.18
Gentisic Acid (mg 100g^{-1})	56.20±0.14	0.08±0.14	0.25	56.06	56.34
Cafeic Acid (mg 100g^{-1})	1.50±0.22	0.13±0.22	14.67	1.28	1.72
Coumaric Acid (mg 100g^{-1})	1.82±0.16	0.09±0.16	8.84	1.67	1.99
Ellagic Acid (mg 100g^{-1})	9.20±0.15	0.09±0.15	1.63	9.05	9.35
Oxalic Acid (mg 100g^{-1})	354.33±14.98	8.65±14.98	4.23	342.00	371.00
Tartaric Acid (mg 100g^{-1})	556.33±14.64	8.45±14.64	2.63	543.00	572.00
Acetic Acid (mg 100g^{-1})	167.67±7.37	4.26±7.37	4.40	162.00	176.00
Citric Acid (mg 100g^{-1})	1.13±0.23	0.13±0.23	20.38	1.00	1.40

was 302.00 μg QE mL^{-1}, and the DPPH activity was 89.00%. Among the organic acids, the highest values were detected in tartaric acid (556.33 mg 100 g^{-1}) and oxalic acid (354.33 mg 100 g^{-1}), indicating that date vinegar possesses a rich and diverse phytochemical profile and suggesting that these components may have significant biological activities in addition to contributing to the product's potential antioxidant capacity. The health benefits of the organic acids identified in this study were discussed in light of the literature and related to our own findings.

Hegazy et al. (2024) aimed in their study to determine the total phenolic content (TPC) and total flavonoid content (TFC) of four different commercial date-based vinegars marketed in Saudi Arabia (date, date and garlic, date and pomegranate, date and turmeric), reported that TPC values varied from 240.81 ± 34.71 to 2228.79 ± 81.24 mg GAE L^{-1} in different vinegar samples. It was noted that these high phenolic and flavonoid contents increased the antioxidant capacity of the vinegars. Additionally, tests conducted on eight different bacterial strains emphasized the high antimicrobial potential of the vinegar samples. Similarly, in our study, the presence of high phenolic and flavonoid contents and 89% DPPH activity supports the fact that date vinegar possesses potent antioxidant and potential health-protective properties.

Akarca et al. (2020) determined the total phenolic content of vinegar produced by traditional methods from Iranian Mazafati dates as 231.37±44.44 mg GAE L^{-1}. They also reported that the vinegar contains beneficial components for human health such as carotenoids, phytosterols, B-group vitamins, and phosphorus, which may contribute to the prevention of various chronic diseases. The phenolic and flavonoid levels detected in our study are higher compared to those in this study, which may be due to differences in date variety, production method, and geographical factors. This finding provides an important basis for considering date vinegar as a functional food.

Tang et al. (2024) reported that date vinegar has high total acidity and phenolic contents, with acetic, L-malic, and oxoglutaric acids being dominant. Our data found tartaric and oxalic acids at the highest concentrations. This diversity of organic acids contributes both to the taste and aroma characteristics of the vinegar and its health benefits. For example, the antioxidant and antimicrobial effects of tartaric acid have been supported in the literature (Kumar et al., 2018). In this context, our findings support the positive health effects of organic acids in date vinegar.

Boonsupa et al. (2023) reported that the antioxidant activity of vinegar produced from different date varieties in Thailand was at the level of 24.96 mg mL^{-1}, emphasizing that these values correlated with the phenolic content in the vinegar. The 89% DPPH activity observed in our study indicates that date vinegar possesses strong antioxidant activity. Measuring antioxidant activity is critically important for determining the product's capacity to neutralize free radicals and thus evaluating its potential health benefits. Considering that oxidative stress plays a significant role in the development of chronic diseases, the antioxidant capacity of date vinegar offers functional properties that may support the prevention of such diseases (Lobo et al., 2010).

Nosratabadi et al. (2024) investigated the changes in microflora during fermentation and demonstrated that phenolic compounds increased while the pH value decreased as fermentation progressed. This supports the fact that date vinegar's chemical and biological properties are closely related to the fermentation process. The high phenolic contents obtained in our study indicate that appropriate fermentation conditions enhance the beneficial components in the vinegar.

Benhammadi et al. (2025) detailed the acidic pH, dry matter, and sugar contents of date vinegars; our findings are consistent with these data and provide information about the chemical stability and flavor profile of date vinegar. Habiba et al. (2024) reported that vinegar obtained from wild date palm fruit exhibited high nutritional value, acidity, and phenolic content; additionally, it demonstrated strong free radical scavenging capacity. This confirms that date vinegar is rich in health-beneficial compounds. Al-Malki et al. (2023) stated that traditional date vinegars are safe regarding microbial contamination. Similarly, our study suggests that healthy and hygienic production conditions contribute to preserving phenolic and antioxidant components.

In conclusion, the phenolic compounds, flavonoids, and antioxidant values obtained in our study are consistent with many studies in the literature, with some being higher or at moderate levels than others. The main reasons for these differences include the type of date and its growing region, fermentation duration and conditions, vinegar production method, analytical techniques, and additives.

Conclusion

Date vinegar stands out as a functional food obtained through traditional production methods, with its rich chemical content and positive effects on health. Date vinegar, prepared from fermented dried date fruit, is widely used due to its nutritional and health benefits, ability to act as an antimicrobial agent, and ability to add flavor to foods. Although dates are produced in many countries and date vinegar has been consumed since ancient times, scientific studies on date vinegar have increased in recent years.

In particular, the production techniques of date vinegar produced by natural fermentation methods should be standardized. Additionally, its usability in industrial production, microbiological quality, shelf life, microbiological reliability, and bioavailability should be increased. In this direction, contributing to the academic literature by examining the production process, chemical composition and health effects of date vinegar in detail and developing both traditional and alternative functional food sources can make significant contributions to the functional food sector.

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