



## Physicochemical, Antimicrobial and Bioactive Properties of Date Vinegar

Ahmed G. Hegazi<sup>1</sup>, Mohammad Melebari<sup>2</sup>, Fayez M. Al Guthami<sup>3</sup>, Mohamed F. A. Ramadan<sup>3,4</sup>, Ahmed F. M. Al Gethami<sup>3</sup>, Khaled S. Gazi<sup>2</sup>, Mohammed A. Thabet<sup>2</sup>, Tariq Alpakistany<sup>5</sup>, Ali A. Hroobi<sup>2</sup>, Abdulaziz F. Al Guthami<sup>3</sup>, Taher M. Taha<sup>6</sup> and Salud J. Serrano<sup>7</sup>.



<sup>1</sup> National Research Centre, Dokki, Giza 12622, Egypt.

<sup>2</sup> Department of Biology, Faculty of Science, Al-Baha University, Al-Baha 65799, Saudi Arabia.

<sup>3</sup> Al Guthami Company, Makkah 24211, Saudi Arabia.

<sup>4</sup> Pesticide Analysis Research Department, Central Agricultural Pesticides Laboratory, Agricultural Research Centre.

<sup>5</sup> Ministry of Health, King Faisal Medical Complex, Taif, Saudi Arabia.

<sup>6</sup> Department of Botany and Microbiology, Faculty of Science, Al-Azhar University, Assiut, Egypt.

<sup>7</sup> University of Córdoba, Spain.

### Abstract

VINEGAR has been proven to be an effective antibacterial against different types of pathogenic bacteria, making it useful for various applications as a food preservative and cosmetic ingredient as well as some medical applications including reducing cholesterol, weight management, and controlling blood sugar.

This study investigates the physicochemical properties and antibacterial activities of four types of commercial date-based vinegar marketed in Saudi Arabia (date, date & garlic, date & pomegranate, and date & turmeric). Determinations of pH, acetic acid content, conductivity, dry matter, total soluble solids (brix values), and alcohol and mineral contents were carried out. The total phenolic compounds (TPC) and the total flavonoid compounds (TFC) were also measured. The antimicrobial activity was studied against eight pathogen and one non-pathogenic bacteria using the disk diffusion method. The physicochemical properties of the vinegar samples (n=264) showed high variability in the values, indicating remarkable differences in the studied vinegar qualities. The results showed a large diversity of vinegar products intended for direct use by the consumer. The values of phytochemical indicated that the different vinegars had a high value for TPC and TFC. The eight tested bacterial strains showed variable sensitivities to the different samples studied with high inhibition zones. It was obvious that the application of Saudi vinegar must take into account its phytochemical characteristics.

**Keywords:** Vinegar; physicochemical; bioactive, antimicrobial activity, mineral.

### Introduction

Vinegar is produced through the fermentation of starch and sugars [1]. Depending on the manufacturing method Vinegar is largely classified into synthetic vinegar, fermented vinegar, and others [2]. So, Vinegar is a liquid suitable for human consumption [3]. It is produced from the appropriate raw materials of agricultural origin [4]. Vinegar is an important ingredient in many food products [5].

The primary acid in vinegar is acetic acid which is well known for cooking and other household uses. Acetic acid is not harmful to human health at low levels [6] usually around 3 present concentrations

[7]. Many researchers have found that vinegar has an antibacterial effect on different pathogenic bacteria. It was documented to have a therapeutic effect on burns [6] and also, inhibit the growth of spoilage bacteria in meat such as beef and poultry [8]. Many factors affect the antimicrobial activity of organic acid including the concentration of acid, ionic strength, pH, and temperature. The majority of the organic acid is found in fruit and fermented food including [9].

There is an increasing interest in applying natural antimicrobial compounds in the food industry. These natural alternatives are needed to achieve a high level of safety against foodborne pathogenic

\*Corresponding authors: Ahmed G. Hegazi, E-mail: [ahmedhegazi128@gmail.com](mailto:ahmedhegazi128@gmail.com), Tel.: +201001440063

(Received 29 June 2024, accepted 16 November 2024)

DOI: 10.21608/EJVS.2024.299982.2208

©National Information and Documentation Center (NIDOC)

microorganisms [10]. The salad dressings provide a harsh environment for foodborne pathogens such as *Salmonella* and *E. coli* to survive because of the acetic or citric acids [9]. To the best of our knowledge, studies have been mostly interested in the inhibitory effect of vinegar on foodborne pathogens. So, this study contributes to investigating the physicochemical properties and antibacterial activities of four types of commercial date-based kinds of vinegar marketed in Saudi Arabia (date, date & garlic, date & pomegranate, and date & turmeric).

## **Material and Methods**

### *Vinegar samples*

Four different types of date-based vinegars were used. A total of 264 vinegar samples were kindly provided by Alnahl Aljwal industry (Makkah, Saudi Arabia): Date vinegar (55 samples), date & pomegranate vinegar (62 samples), date & turmeric vinegar (78 samples) and date & garlic vinegar (69 samples). All samples were centrifuged to reduce the turbidity and were stored at 4 °C for later use.

### *Physicochemical properties*

The pH was measured by using a pH meter (InoLab 720, WTW GmbH, Weilheim, Germany). The total acidity content is expressed in grams of acetic acid per L. The percentage of acetic acid in the samples was calculated using NaOH (0.1 mol/L) for the determination of pH values [11, 12]. Total acidity was expressed as acetic acid equivalent [11, 13, 14].

The Brix values and residual alcohol content are the percentage by volume of ethanol still contained in the vinegar after acetic fermentation was done as [11, 14, 15] using a refractometer. The total dry extract refers to all the substances which, under the conditions described as [15,16]. The total dry matter (%) was carried out according to the Association of Official Analytical Chemists methods [12]. The total soluble solids were measured by using a refractometer (ATOGO, Fujian, China) following [17].

Vinegar ash refers to all the incineration products of the evaporation residue of a known volume of vinegar [15, 16]. The vinegar turbidatable acidity was calculated as the percentage (%) of acetic acid [12]. A turbidimeter was used to detect the turbidity values of the different vinegars [18]. The values were expressed as Nephelometric Turbidity Unit (NTU) [19], mineral analysis was performed according to the method described by [15, 20].

### *Bioactive value*

The total phenolic content (TPC) was determined according to the Folin-Ciocalteu method [21]. The absorbance of the mixture was measured using a spectrophotometer [22]. The results were expressed as  $\mu\text{g GAE/mL}$  of vinegar sample. The total flavonoid content (TFC) was determined as described earlier [23]. The absorbance of the mixture

was measured at 510 nm using a spectrophotometer. TFC was expressed as Quercetin Equivalent per mL of vinegar ( $\mu\text{g QE/mL}$ )

### *Antimicrobial Analysis*

#### *Microbial Strains*

For studying the antibacterial activity of the different vinegar samples, Eight bacterial strains were used including three Gram-positive strains namely: *Staphylococcus aureus* (ATCC 25923), *Listeria monocytogenes* (ATCC 19115), and *Bacillus cereus* (ATCC 11778) and five Gram-negative strains namely: *Escherichia coli* Spp. (ATCC 25922), *Escherichia coli* O157 (ATCC 43888), *Salmonella typhimurium* (ATCC 14028), *Pseudomonas aeruginosa* (NCTC 10662), and *Vibrio* spp. (ATCC 17802). All microbial strains were provided by the Microbiology Laboratory, Faculty of Biology, Al-Baha University, Saudi Arabia. The bacterial cultures were stored in tryptic soy broth (SB) containing 20% glycerol at -80 °C till used. The different microbial strains were standardized and inoculated following the method described by [24].

#### *Disk Diffusion Assay*

Vinegar samples were purified from their microbial load by using membrane filters (0.22  $\mu\text{m}$ ) before the antibacterial activity test. The antibacterial activity was tested using the disc diffusion Kirby-Bauer method [25,26]. Briefly, the bacterial isolates were cultured in tryptic soy broth and incubated at 37 °C for 18 h. The standardized suspension ( $1-5 \times 10^8$  CFU/mL) of the previously prepared isolates was inoculated onto Mueller–Hinton agar (MHA). The diluted bacteria suspensions were spread over Muller-Hinton Agar plates. In parallel, vinegar-loaded discs were prepared as follows: Whatmann No. 1 filter papers were folded three times and punched with a paper puncher to make a six-layer disc. These discs were then autoclaved and completely dried in an oven at 70 °C. Vinegar samples were loaded (50  $\mu\text{L/disc}$ ) on the sterilized, dried discs, aseptically. Then the loaded discs were dried at 60 °C for 2 h. The dried loaded discs were applied on inoculated Muller-Hinton agar plates and incubated at 37 °C [27]. The clear zones were measured after 6, 12, 18, and 24 h. After incubation, the diameters of the inhibition zones were measured in mm. Fluconazole, ampicillin, and streptomycin were used as positive controls.

#### *Statistical Analysis*

All collected data were expressed as mean and standard deviation (SD). A one-way analysis of variance was used to analyse data, with  $p < 0.05$  representing a significant difference between means, as estimated with a multiple-range test using the least significant difference (LSD) or Duncan's test at  $\alpha < 0.05$ . The homogeneous subgroups were determined

to use multiple correspondence analyses. The determinations were conducted in triplicate.

## Results

The physicochemical properties of different kinds of vinegar (264 samples) are shown in Table 1. It was observed variability in the values, indicating remarkable differences among vinegar qualities. pH levels of the vinegars varied from  $3.10 \pm 0.01$  to  $3.90 \pm 0.02$ . In general, the vinegar of date & pomegranate had the lowest pH value ( $3.10 \pm 0.01$ ) compared to date & turmeric, date & garlic, and date vinegar ( $3.40 \pm 0.01$ ,  $3.72 \pm 0.01$  and  $3.90 \pm 0.02$ ), respectively.

It was observed that the total acidity levels of the vinegar samples were generally correlated with their pH values (Table 1). Total acidity was expressed as acetic acid equivalent. Date & garlic vinegar showed the highest total acidity ( $1.42 \pm 0.07$  g/L.) and the lowest acidity  $5.72 \pm 0.25$  was recorded with date & pomegranate vinegar.

The °Brix (Table 1) indicates the percentage of total soluble solids including sugar, salts, and proteins in an aqueous sample. In this study, the brix values of the vinegars varied in a wide range (from  $3.95 \pm 0.23$  to  $20.88 \pm 0.03$  g/cm<sup>3</sup>). The turbidity of vinegar is a result of the presence of suspended solids in the liquid medium. As it is seen in Table 1, turbidity levels of the different vinegars were variable between  $19.6 (\pm 0.57)$  and  $310.0 (\pm 26.8)$  NTU. The date & pomegranate vinegar had the highest turbidity ( $310.0 \pm 26.8$ ), while the date vinegar showed the lowest value ( $19.6 \pm 0.57$ ).

Total phenolic and flavonoid contents (TPCs and TFCs) of the tested vinegar samples are summarized in Table 1. The bioactive properties of the samples varied in a wide range and were not correlated with each other. Among the tested vinegar samples, the highest TPC and TFC levels were obtained in the date & pomegranate vinegar. The date vinegar had the lowest levels in terms of all the measured parameters.

Mineral contents are shown in Table 2. Na, K, Ca, Mg, Fe, and Cr were the most abundant minerals present in the vinegars. Interestingly, date vinegar was the richest in Na, K, Ca, Cu, Mg, Co, Cr, and Ni. Date & pomegranate vinegar was the richest in Mn, Fe, and Zn, while date & garlic is the richest in Se.

The antibacterial activity was tested using the disc diffusion Kirby-Bauer method against eight bacterial strains. Three Gram-positive strains (*S. aureus*, *L. monocytogenes* and *B. cereus*) and five Gram-negative strains (*E. coli*, *E. coli* O157, *S. typhi*, *P. aeruginosa* and *Vibrio* spp.) were tested. The antibacterial activity was measured after 6, 12, 18, and 24 hours. Figures 1 and 2 show the antibacterial activity of the four different vinegar types against the selected bacterial strains. All types of vinegar had excellent antibacterial activity against Gram-positive

and Gram-negative bacteria, mostly pathogens, used in this study. All the bacterial strains were sensitive to all vinegars in the first 6 and 12 hours, except for *E. coli* and *L. monocytogenes* which showed resistance to vinegar samples after 12 hours. The strains of *Vibrio* spp., and *S. aureus*, were sensitive to all vinegars for over 24 hours. Date and date & pomegranate vinegars samples showed nearly similar activities against all bacteria. However, date & pomegranate vinegar had less effect on *E. coli* spp. and *E. coli* O157 was compared to only Date-made vinegar. On the other hand, the date and turmeric vinegar was the most powerful against the bacterial strains used in this study. The Gram-positive bacteria (Fig. 1), *B. cereus* and *S. aureus* were sensitive even after 24 hours, while, *L. Monocytogenes* become resistant after 12 h which similar to other gram-negative bacterial strains. On the other hand, the gram-negative bacteria (Fig. 2) used in this study started showing resistant activity after 12 hours, except for *vibrio* spp. strain.

## Discussion

In general, vinegars were above a pH of 3.00 in accordance with the previous studies of [14] [14,20,28,29,30] stated that the total acidity levels were generally correlated with their pH values as accordance to the Codex Alimentarius Commission.

As a result of the study, it was determined that the mean density of date, date and pomegranate, date and turmeric and date and garlic vinegars were  $1.015 \pm 0.01$  g/cm<sup>3</sup>,  $1.003 \pm 0.001$  g/cm<sup>3</sup>,  $1.005 \pm 0.001$  g/cm<sup>3</sup> and  $1.011 \pm 0.003$  g/cm<sup>3</sup>, respectively (Table 1). [28] found that the density values of the vinegars were close and ranged between 0.962 and 1.018 g/cm<sup>3</sup>. Similarly to our results, [35] determined that the density of apple cider vinegar was  $1.08 \pm 0.05$  g/cm<sup>3</sup>. The Brix° ranged from  $3.95 \pm 0.23$  to  $8.18 \pm 0.03$  in date and turmeric and date vinegars, respectively (Table 1). [20] found that Brix (%) between 1.22 and 20.80 for grape vinegar, between 1.02 and 12.90 for apple vinegar, and a value of 1.26 for hawthorn vinegar. Where a value of 5.45% for *C. tanacetifolia* vinegar. Compared with traditional vinegar (Özdemir et al. 2021) Also, Karadag et al (2020) detected a rosehip vinegar value of 4.01%. [29,31] identified values between 8.6 and 13.4% in the case of apple vinegar.

In this study, the acidity of vinegars had values between  $3.0 \pm 0.03$  and  $3.6 \pm 0.03$  g acetic acid/100 mL. [20] found that commercial vinegars had higher values between 4.14 and 9.63 g acetic acid/100 mL. The total titratable acidity of the vinegar in this study ranged between  $19.6 \pm 0.57$  g/L (date vinegar) and  $57.2 \pm 2.9$  g/L (date and turmeric vinegar). [30] identified a total titratable acidity for traditionally obtained red wine vinegar with a value of 85.15 g/L and a value of 122.97 g/L in the case of industrial red wine vinegar. [32] found that differences in this

parameter depend on the method of production and the raw material used.

The dry matter values (Table 1) varied in the range of  $2.07 \pm 0.04$  % (date vinegar) to  $2.68 \pm 0.03$  % (date and garlic vinegar). Total soluble solids ranged between  $1.0 \pm 0.02$  (date and pomegranate vinegar) and  $1.5 \pm 0.03$  (date and turmeric). Bakir et al. (2016) found that dry matter values of grape and apple cider vinegar were determined to be  $3.8 \pm 0.30$ , and  $4.3 \pm 0.40$  g/L, respectively. The difference between that study and the results of our study can be attributed to the fact that water-insoluble dry matter (starch, cellulose, etc.) was less in date vinegar.

The mean conductivity values of vinegar samples (Table 1) were determined to range from  $260 \pm 0.03$  mS/cm (date and pomegranate. vinegar) to  $274 \pm 0.02$  mS/cm (date vinegar). In the study carried out by [33], it was determined that the conductivity value of date vinegar was  $3.10 \pm 0.15$   $\mu$ S/cm, which was lower than our results. This difference between the studies is attributed to be due to the types of dates used in the studies and, production and post-production storage times [34].

The range of ash was  $2.06 \pm 0.18$  g/L (date and turmeric vinegar) to  $2.62 \pm 0.12$  g/L (date and garlic vinegar). In the study of [35], the ash content in apple cider vinegar was determined to be  $3.25 \pm 1.25$  g/L. This difference between the studies is considered to be due to the higher mineral matter content of apple cider vinegar compared to date vinegar.

The alcohol content for all vinegar types was zero after six months of storage (Table 1). [36,37,38] stated that the alcohol content in the vinegar samples ranged from  $0.03 \pm 0.02$  to  $1.00 \pm 0.00$ % for V2 and V3, respectively. In another study carried out by Bayram et al. (2018), it was determined that the alcohol values of apple cider vinegar were below 0.5%. This difference between the studies may be due to the difference in time storage after production.

The mineral material values in examined vinegar were varied depending on the vinegar type. In general, Na, K, Ca and Fe were the most abundant minerals present in the vinegars. These results conformed with the maximum limit, which was 10 mg/L, approved by the Turkish Food Codex [20,35]. 2015).

Concerning the phytochemicals content of vinegar, many researchers have showed the profile of the bioactive compounds [14,37,38]. The means for total phenolic content were determined to be ranged in date vinegar ( $240.81 \pm 34.71$   $\mu$ g GAE/mL) to date and pomegranate vinegar ( $2228.79 \pm 81.24$   $\mu$ g GAE/mL). The results in the literature highlight a great variability regarding the presence of phenolic compounds [30,34]. To explain the differences between the values of total phenolic compounds as a result obtained of our study and that results obtained in other previous studies may be because date

vinegar is rich in carotenoids, phytosterols and bioactive components and total phenolic values are higher [21]. Phenolic compounds play a role in health outcomes due to their antioxidant activity reported that TPC of Algerian and Iranian date palm fruit respectively, from 2.49 to 8.36 mg GAE/100 g of fresh and from 2.89 to 6.64 mg GAE/100 g of dry weight. The importance of ingestion of foods rich in flavonoids plays a good role in defiance against oxidative stress- related human ailments [21]. The TFC of date vinegar ranged from ( $144.49 \pm 0.76$   $\mu$ g QE/mL) to ( $349.05 \pm 2.87$   $\mu$ g QE/mL) for date and date and pomegranate vinegar respectively (Table 1). In general, higher flavonoid values were related to the rutab stage which specifies that the drying process may have a caustic effect on these compounds. Vinegars bioactive properties can vary in a wide range depending on the type of raw material [20,34].

The antibacterial activity was measured after 6,12,18 and 24 hours of incubation. Figures 1 and 2 show the antibacterial activity of the vinegar samples against Gram-positive and Gram-negative selected bacteria. The sensitivity of the examined bacteria to the vinegars was highly variable. However, bacterial strains were sensitive to all four types of vinegar in the first 6 and 12 hours (except for *E. coli* spp. over countered vinegar of date and pomegranate after 12 hours). On the other hand, the date and turmeric vinegar was the most powerful among the four vinegars against the bacterial strains used in this study. Gram-positive bacteria (Fig. 1) *Bacillus cereus* and *Staphylococcus aureus* were sensitive even after 24 hours, except for the strain *Listeria Monocytogenes* which showed activity similar to other gram-negative bacterial strains. On the other hand, the gram-negative bacteria (Fig. 2) used in this study started showing resistant activity after 12 hours, except for vibrio spp. strain. [39] stated that weak acids including acetic acid show their antimicrobial activity by traversing the microbial membrane to an undissociated form dissociating by the intracellular pH and liberating a proton in the cytoplasm. Vinegars, containing considerable amounts of acetic acid, have been known to have strong antimicrobial activity against bacteria [40,41,42]. The variation of the antimicrobial activity is related indeed to, the qualitative and quantitative difference of organic acids, primary metabolites and polyphenols contained in each kind of vinegar [14,27]. The presence of bioactive compounds, such as gallic acid, caffeic acid, catechins, amino acids and acetic acid, in the vinegar, can inhibit the bacteria strains at low concentrations such as *S. aureus*, *S. mutans*, *E. coli* O157:H7 and *P. aeruginosa* [43,44].

Searching for new antibiotic agents, as alternative for traditional antibiotics, is one of the priorities of researchers worldwide due to increasing antibiotic

resistant bacteria [45]. All vinegar samples showed very strong antibacterial activity within the first 12 h of incubation against all the tested bacteria strains. The maximum inhibition zone (34 mm) was recorded with date & turmeric vinegar against *Vibrio* spp. Some bacterial strains (*E. coli* and *L. monocytogenes*) overcome the antibacterial activity of vinegar after 12 h. After 24 h of incubation, the most sensitive gram-positive bacterial strain was *S. aureus* with inhibition zones diameters of 23, 23, 28, and 23 mm with date, date & pomegranate, date & turmeric, and date & garlic respectively, while the most sensitive gram-negative bacterial strain was *V. spp.* with inhibition zone diameters of 19.5, 18, 22.5, and 20 mm with date, date & pomegranate, date & turmeric, and date & garlic, respectively. Studies of the antimicrobial activities of date vinegar are limited. For example, [46] reported inhibition zone diameters of 49 and 33 mm against *S. aureus* with two different date vinegar types namely: Deglet-Nour and Temjouhart, respectively using well diffusion method. In the same study the inhibition zone diameters were 20 and 12 mm Deglet-Nour and Temjouhart vinegars, respectively using disc diffusion method [46]. Comparing to [46] results, our results is higher (23 mm with date vinegar) when we use the same antibacterial assay method (disc diffusion method). In another study, natural date vinegar showed inhibition zone diameters of 19 against *E. coli* and 9 mm against *S. typhi* that isolated from minced meat and chicken meat, respectively. Comparing to Hussein results, the *E. coli* strain used in our study resist all types of date-based vinegars and *S. typhi* strain showed comparable results (8 mm) with that obtained by Hussein. The variation in antibacterial results may be due to the differences in antibacterial assay and/or bacterial strains used. Some parameters that can affect antibacterial properties of vinegar may be related to the qualitative and quantitative differences of organic acids, primary metabolites, and polyphenols contained in each kind of vinegar [14,27].

Acetic acid, a weak organic acid, is the major component of vinegar. It has been known to have strong antibacterial activity against a wide spectrum of bacterial species [40,41,42]. The bactericidal effect of weak organic acids may be due to one of two mechanisms: Firstly, the undissociated form of acetic acid is liposoluble and can diffuse through the bacterial plasma membrane. Inside the bacteria cell, the acetic acid dissociates into proton (decrease the pH) and acetate anion. The accumulation of proton and acetate can destroy the bacteria cell [39]. Secondly, the solubilisation of the undissociated acetic acid in cell membrane alters the structure and function of the membrane that can affect the cell permeability [47]. Other studies suggest that the presence of organic acids and polyphenolic

compounds may play a crucial role in antimicrobial properties of vinegar [27,48,49].

The presence of bioactive compounds, such as gallic acid, caffeic acid, catechins, amino acids and acetic acid, in the vinegar, can inhibit the bacteria strains at low concentrations such as *S. aureus*, *S. mutans*, *E. coli* O157:H7 and *P. aeruginosa* [43,44].

## Conclusions

It could be concluded that this study contributes to evaluate the physicochemical, biochemical properties, mineral, and antimicrobial activity of four different types of vinegar from Saudi Arabia. The results showed a large diversity of vinegar products intended for direct use by the consumer. The values of phytochemical indicated that the different vinegars had a high value for TPC and TFC. The eight tested bacterial strains showed variable sensitivities to the different samples studied with high inhibition zones. It was obvious that the application of Saudi vinegar must take into account its phytochemical characteristics.

## Author Contributions

Conceptualization, AGH, MM and AFM G; methodology, MFAR, KSG, MAT, TA, and AAH; data curation, AF G and TMT; writing—original draft preparation, AGH, KSG and SSJ.; writing-review and editing, SSJ and AGH supervision, FMG. All authors have read and agreed to the published version of the manuscript.

## Funding statement

The paper is funded by the National Research Centre, Egypt and Al Guthami Company, Saudi Arabia.

## Institutional Review Board Statement

Not applicable.

## Informed Consent Statement

Not applicable.

## Data Availability Statement

Not applicable.

## Acknowledgements

The authors extend their appreciation to The National Research Centre, Egypt and Al Guthami Company.

## Conflicts of Interest

Data are available upon request.

## Sample Availability

Samples are available from the authors upon reasonable request.

TABLE 1. Physicochemical characteristics and bioactivity of different date vinegar varieties

Parameters	Vinegar			
	Date (n= 55)	Date & pomegranate (n= 62)	Date & turmeric (n= 78)	Date & garlic (n= 69)
pH	3.90 ± 0.02	3.10 ± 0.01	3.40 ± 0.01	3.72 ± 0.01
Total acidity	1.74 ± 0.06	5.72 ± 0.25	2.94 ± 0.06	1.42 ± 0.07
°Brix	8.18 ± 0.03	4.62 ± 0.09	3.95 ± 0.23	4.03 ± 0.03
Turbidity	19.6 ± 0.57	310.0 ± 26.8	57.2 ± 2.9	32.5 ± 0.5
Acetic Acid (%)	3.0 ± 0.03	3.1 ± 0.01	3.6 ± 0.03	3.5 ± 0.02
Density (g/cm <sup>3</sup> )	1.015 ± 0.01	1.003 ± 0.001	1.005 ± 0.001	1.011 ± 0.003
Alcohol (%)	0	0	0	0
Total Dry (%)	2.45 ± 0.03	2.22 ± 0.03	2.07 ± 0.04	2.68 ± 0.03
Total soluble solids	1.2 ± 0.01	1.0 ± 0.02	1.5 ± 0.03	1.1 ± 0.02
Conductivity (mS/cm)	274 ± 0.02	260 ± 0.03	266 ± 0.02	264 ± 0.01
Ash (g/L)	2.41 ± 0.11	2.21 ± 0.15	2.06 ± 0.18	2.62 ± 0.12
TPC (GAE)/L	240.81 ± 34.71	2228.79 ± 81.24	1439.52 ± 24.29	253.52 ± 9.49
TFC (µg QE/mL)	144.49 ± 0.76	349.05 ± 2.87	280.45 ± 5.56	207.33 ± 3.69

TABLE 2. Mineral content of date vinegars

Sample Element (ppm)	Vinegar			
	Date	Date & pomegranate	Date & turmeric	Date & garlic
Sodium (Na)	6551.00 ± 271.50	33.30 ± 1.40	89.60 ± 2.20	49.10 ± 1.20
Potassium (K)	4098.20 ± 131.20	3832.10 ± 74.42	1484.88 ± 56.30	2176.20 ± 35.30
Calcium (Ca)	937.90 ± 38.10	351.00 ± 14.30	179.30 ± 9.70	158.30 ± 2.40
Copper (Cu)	0.32 ± 0.01	0.17 ± 0.01	0.02 ± 0.01	0.04 ± 0.01
Magnesium (Mg)	243.60 ± 15.50	198.94 ± 7.10	99.20 ± 4.10	145.60 ± 3.20
Manganese (Mn)	2.13 ± 0.14	2.29 ± 0.08	1.31 ± 0.02	0.77 ± 0.01
Iron (Fe)	6521.01 ± 0.01	7525 ± 0.02	6012.01 ± 0.01	6368 ± 0.03
Cobalt (Co)	0.14 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.01
Zinc (Zn)	0.56 ± 0.04	0.78 ± 0.03	0.33 ± 0.01	0.65 ± 0.02
Selenium (Se)	0.14 ± 0.01	0.04 ± 0.01	0.12 ± 0.01	0.18 ± 0.02
Chromium (Cr)	0.70 ± 0.06	0.19 ± 0.03	0.15 ± 0.01	0.16 ± 0.02
Nickel (Ni)	0.20 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.05 ± 0.01

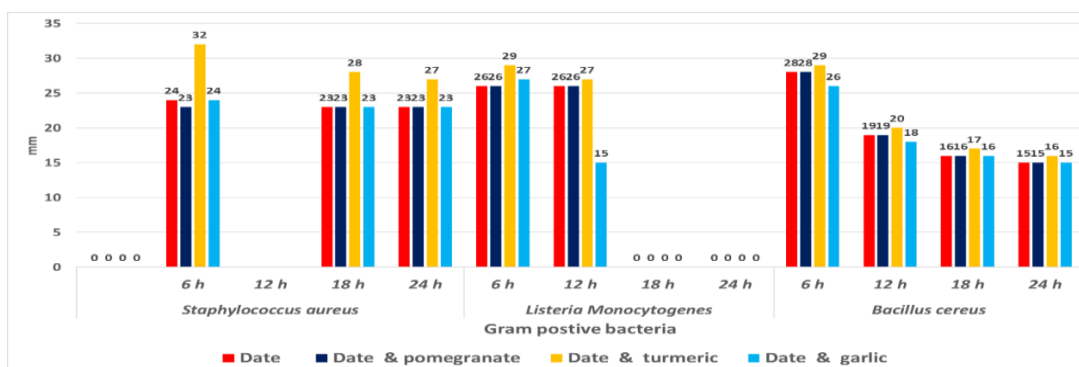


Fig. 1. Antibacterial activity of date vinegars on Gram positive bacteria at different intervals

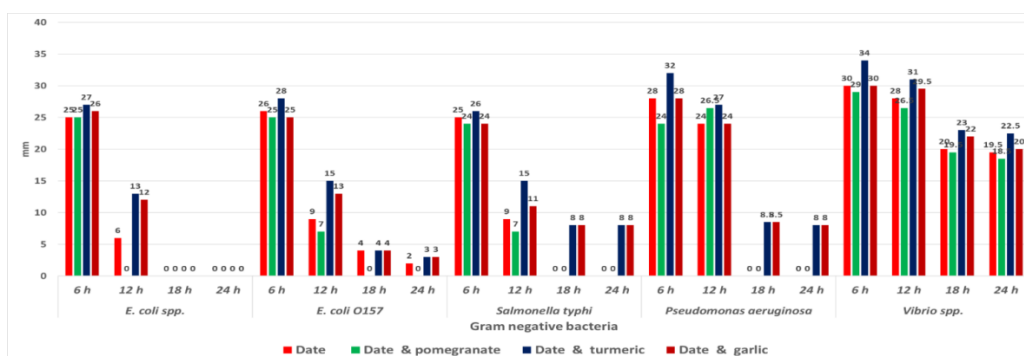


Fig. 2. Antibacterial activity of date vinegar on Gram negative bacteria at different intervals

## References

- Osuala OJ, Ezemba AS, Ajeh JE, Chude CO, and Ezemba CC. Evaluation of the Proximate and Elemental Composition of Traditional and Industrial Produced Vinegar. *International Journal of Innovative Research and Development*, **10**(5), 187-195 (2021a).
- Kim S.H. , Seo J.H. and Jeong Y.J. Quality comparison of non-thermal sterilized raw apple vinegar and commercial apple vinegar products. *Food Sci. Preserv.*, **31**(2), 235-244(2024).
- Osuala, O.J., Ezemba, C.C., Chude, C.O., Ezemba, A.S. and Anaukwu, C. Antimicrobial Analysis of Traditional and Industrial Produced Vinegar. *International Journal of BioSciences and Technology*, **14**(3), 28 – 43(2021b).
- Pooja S. and Soumitra. B. Optimization of Process Parameters for Vinegar Production using Banana Fermentation. *International Journal of Research in Engineering and Technology*, 501 – 515((2021).
- Ezemba, C.C., Osuala, O. and Ezemba, A. Review on Production and Functionality of Vinegar. *Academia Letters*, Article, 4160(2013). <https://doi.org/10.20935/AL4160>.
- Budak, N.H., Aykin, E., Seydim, A.C., Greene, A.K. and Seydim, Z.B. Functional properties of vinegar. *Journal of Food Science*, **79**(5), R757-R764(2014).
- CAC. Codex Alimentarius Commission. Proposed draft revised regional standard for vinegar Retrieved August 2014, from [ftp://193.43.36.92/codex/Meetings/CCEURO/cceuro22/CL00.\(2000\)](ftp://193.43.36.92/codex/Meetings/CCEURO/cceuro22/CL00.(2000)).
- Lingham, T., Besong18e, S., Ozbay, G. and Lee, J.L. Antimicrobial Activity of Vinegar on Bacterial Species Isolated from Retail and Local Channel Catfish (*Ictalurus punctatus*). *J. Food Process Technol.*, S11-001(2012). doi:10.4172/2157-7110.S11-001
- Miskiyah Juniawati, and Hidayati, I. Antimicrobial Activity of Coconut Water Vinegar Powder to *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. The 3<sup>rd</sup> International Conference on Agricultural Postharvest Handling and Processing IOP Conf. Series: Earth and Environmental Science 1024.012069 IOP Publishing (2022). doi:10.1 088/1755 -1315/1024/1/012069
- Rauha, P., Remes, S., Heinonen, M., Hopia, A., Kahkonen, M., Kujala, T., Pihlaja, K., Vuorela, H. and Vuorela, P. Antimicrobial effect of Finnish plant extracts containing flavonoids and other phenolic compounds. *Int. J. Food Microbiol.*, **56**, 3-12 (2000).
- Anonymous, A. Métodos Oficiales de Análisis, Tomo II, Ministerio de Agricultura, Pesca y Alimentación, Madrid, Sipán (1993).
- Nielsen, S.S. Standard Solutions and Titratable Acidity. In *Food Analysis Laboratory Manual*; Springer: Cham, Switzerland, 95–102 (2010).
- AOAC Official Methods of the Ass. Office. Agric. Chem., 14th edit., Arlington USA (1984) .
- Kara, M., Assouguem, A., Fadili, M.E., Benmessaoud, S., Alshawwa, S.Z., Kamaly, O.A., Saghrouchni, H., Zerhouni, A.R. and Bahhou, J. Contribution to the Evaluation of Physicochemical Properties, Total Phenolic Content, Antioxidant Potential, and Antimicrobial Activity of Vinegar Commercialized in Morocco. *Molecules*, **27** (3), 770-777.
- Curvelo-Garcia AS Wine vinegars. Methods of Analysis (Part Two). *Green Sheet of OIV* No. 1033 (1996) .
- CPIV Methods of Analysis, Annex 4 to the minutes of CPIV Technical Committee's Meeting, Stuttgart, Germany(1995) .
- Albornoz CEH. Microbiological Analysis and Control of the Fruit Vinegar Production Process. *PhD Thesis, Universitat Rovira i Virgili, Catalonia, Spain* (2012). Available online: <http://purl.org/dc/dcmitype/Text>.
- López, F., Pescador, P., Güell, C., Morales, M.L., García-Parrilla, M.C. and Troncoso, A.M. Industrial vinegar clarification by cross-flow microfiltration: effect on colour and polyphenol content. *Journal of Food Engineering* 68(1): 133-136(2005).
- Llaguno, C. and Polo, M.C. (1991) El Vinagre de Vino (The Wine Vinegar) Consejo Superior de Investigaciones Científicas (High Council of Scientific Research) Madrid, Spain.



20. Ozturk, I., Caliskan OZNUR, Tornuk, F., Ozcan, N., Yalcin, H., Baslar, M. and Sagdic, O. Antioxidant, antimicrobial, mineral, volatile, physicochemical and microbiological characteristics of traditional home-made Turkish vinegars. *LWT-Food Science and Technology*, **63** (1), 144–151(2015).
21. Ali, Z., Ma, H., Rashid, M.T., Wali, A. and Younas, S. Preliminary study to evaluate the phytochemicals and physicochemical properties in red and black date's vinegar. *Food Science & Nutrition*, **7**(6),1976-1985 (2019). doi: 10.1002/fsn3.1009
22. Lucas B.N., Nora FMD., Boeirani CP., Verruck S. and da Rosa CS. Determination of total phenolic compounds in plant extracts via Folin-Ciocalteu's method adapted to the usage of digital images. *Food Sci. Technol., Campinas*, **42**, e35122, (2022).
23. Saeed N, Khan MR. and Shabbir M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. *BMC Complement Altern Med.* **16**;12:221. (2012). doi: 10.1186/1472-6882-12-221
24. CLSI Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. Clinical and Laboratory Standards Institute; Wayne, PA, USA. (2012).
25. Bauer AW. Antibiotic susceptibility testing by a standardized single disc method. *American Journal of Clinical Pathology* **45**: 149-158, (1966).
26. Furtado GL and Medeiros AA. Single-disk diffusion testing (Kirby-Bauer) of susceptibility of *Proteus mirabilis* to chloramphenicol: significance of the intermediate category. *Journal of Clinical Microbiology* **12** (4): 550–553 (2022).
27. Quinto EJ, Caro I, Villalobos-Delgado LH, Mateo J, De-Mateo-Silleras B, and Redondo-Del-Río MP. Food Safety through Natural Antimicrobials. *Antibiotics* **8** (4): 208 (2019).
28. Kara M, Assouguem A, Kamaly OMA, Benmessaoud S, Imtara H, Mechchate H, Hano C, Zerhouni AR. and Bahhou J. The Impact of Apple Variety and the Production Methods on the Antibacterial Activity of Vinegar Samples. *Molecules* **26** (18): 5437 (2021).
29. El Abdali Y, Saghrouchni H, Kara M, Mssillou I, Allali A, Jordan YAB, Kafkas NE, El-Assri EM, Nafidi HA, Bourhia M, Almaary KS, Eloutassi N, and Bouia A. Exploring the Bioactive Compounds in some Apple Vinegar Samples and their Biological Activities. *Plants* **12**(22):3850 (2023). doi: 10.3390/plants12223850
30. Pashazadeh H, Özdemir N, Zannou O. and Koca I. Antioxidant capacity, phytochemical compounds, and volatile compounds related to aromatic property of vinegar produced from black rosehip (*Rosa pimpinellifolia* L.) juice. *Food Bioscience* **44**: 101318 (2021).
31. Cosmulescu, S.; Stoenescu, A.-M.; Trandafir, I. and Tut, ulescu, F. Comparison of Chemical Properties between Traditional and Commercial Vinegar. *Horticulturae*, **8**, 225, (2022).
32. Sung NH, Woo SM, Kwon JH, Yeo SH. and Jeong YJ. Quality characteristics of high acidity apple vinegar manufactured using two stage fermentation. *Journal of the Korean Society of Food Science and Nutrition* **43** (6): 877–883 (2014).
33. Chochevska, M.; Seniceva, E.J.; Veličkovska, S.K.; Naumova-Le, tia, G.; Mirčeski, V.; Rocha, J.M.F. and Esatbeyoglu, T. Electrochemical determination of antioxidant capacity of traditional homemade fruit vinegars produced with double spontaneous fermentation. *Microorganisms*, **9**, 1946 (2021).
34. Siddeeg A, Zeng XA, Rahaman A, Manzoor MF, Ahmed Z. and Ammar AF. Quality characteristics of the processed dates vinegar under influence of ultrasound and pulsed electric field treatments. *Journal of Food Science and Technology* **56**: 4380-4389 (2019).
35. Akarca G, Tomar O, Çağlar A. and İstek Ö Physicochemical and Sensory Quality Properties of Vinegar Produced by Traditional Method from Persian Mazafati Date (*Phoenix dactylifera* L.). *European Journal of Science and Technology* **19**: 429-434 (2020).
36. Dabija A, and Hatnean CA. Study concerning the quality of apple vinegar obtained through classical method. *Journal of Agroalimentary Processes and Technologies* **20**(4): 304-310 (2014).
37. ONSSA. Royaume du Maroc. Décret N°2-10-385 Du 23 Joumada II 1432 (27 Mai (2011) Portant Réglementation de La Fabrication et Du Commerce Des Vinaigres (2011).
38. Bakir S, Toydemir G, Boyacioglu D, Beekwilder J. and Capanoglu E. Fruit Antioxidants during Vinegar Processing: Changes in Content and in Vitro Bio-Accessibility. *International Journal of Molecular Sciences* **17**(10):1658 (2016).
39. Ousaaaid D, Imtara H, Laaroussi H, Lyoussi B. and Elarabi I. An investigation of Moroccan vinegars: Their physicochemical properties and antioxidant and antibacterial activities. *Journal of Food Quality* **66**18444 (2021).
40. Salmond CV, Kroll RG. and Booth IR. The effect of food preservatives on pH homeostasis in *Escherichia coli*. *Microbiology* **130**: 2845-2850 (1984).
41. Karapinar M. and Gonül, SA. Effects of sodium bicarbonate, vinegar, acetic and citric acids on growth and survival of *Yersinia enterocolitica*. *International Journal of Food Microbiology* **16**(4): 343-347 (1992).
42. Medina E, Romero C, Brenes M. and de Castro A. Antimicrobial activity of olive oil, vinegar, and various beverages against foodborne pathogens. *Journal of Food Protection* **70**(5): 1194-1199 (2007).



43. Hindi NK. In Vitro Antibacterial Activity of Aquatic Garlic Extract, Apple Vinegar and Apple Vinegar—Garlic Extract Combination. *American Journal of Phytomedicine and Clinical Therapeutics* 1 (1): 42–51 (2013).
44. Gálvez MC, Barroso C. and Pérez-Bustamante JA. Analysis of Polyphenolic Compounds of Different Vinegar Samples. *Zeitschrift für Lebensmittel-Untersuchung und-Forschung* 199 (1): 29–31 (1994).
45. Kelebek H, Kadiroglu, P, Demircan NB. and Selli S. Screening of bioactive components in grape and apple vinegars: Antioxidant and antimicrobial potential. *Journal of the Institute of Brewing* 123 (3): 407–416 (2017).
46. Muteeb G, Rehman MT, Shahwan M. and Aatif M. Origin of Antibiotics and Antibiotic Resistance, and Their Impacts on Drug Development: A Narrative Review. *Pharmaceuticals (Basel)*.16(11):1615 (2023).
47. Cherif, B.; Bouras, N.; Oumouna, M.; Ould el hadj, M. D.; Holtz, M. D. and Sabaou, N. Ethnopharmacological use and antimicrobial activity of traditional date vinegar of Ghardaia. *Algerian Journal of Arid Environment*, v. 4, n. 1, p. 83-93, (2014).
48. Henriques, M., Quintas, C. and Loureiro-Dias, M.C. Extrusion of benzoic acid in *Saccharomyces cerevisiae* by an energy-dependent mechanism. *Microbiology* 143, 1877–1883 (1997).
49. Kadiroğlu P. FTIR spectroscopy for prediction of quality parameters and antimicrobial activity of commercial vinegars with chemometrics. *J Sci Food Agric*. 98:4121–4127 (2018). doi: 10.1002/jsfa.8929.
50. Fernandes ACF, de Souza AC, Ramos CL, Pereira AA, Schwan RF. and Dias DR. Sensorial, antioxidant and antimicrobial evaluation of vinegars from surpluses of physalis (*Physalis pubescens L.*) and red pitahaya (*Hylocereus monacanthus*) *J Sci Food Agric*. 99:2267–2274 (2019).

### الخصائص الفيزيائية والكيميائية والمضادة للميكروبات والنشطة الحيوية لخل التمر

أحمد جعفر حجازي<sup>1</sup>، محمد مليباري<sup>2</sup>، فايز القثامي<sup>3</sup>، محمد رمضان<sup>3</sup>، أحمد القثامي<sup>3</sup>، خالد غازي<sup>2</sup>، محمد ثابت<sup>2</sup>، طارق عبد المطلب الباكستاني<sup>4</sup>، علي الحروبي<sup>2</sup>، عبد العزيز القثامي<sup>3</sup>، طاهر طه<sup>5</sup> و سالود سيرانو<sup>6</sup>

<sup>1</sup> المركز القومي للبحوث، الدقي، الجيزة 12622، مصر.

<sup>2</sup> قسم الأحياء، كلية العلوم، جامعة الباحة، الباحة، 65431، المملكة العربية السعودية.

<sup>3</sup> شركة القثامي، مكة المكرمة 24211، المملكة العربية السعودية.

<sup>4</sup> وزارة الصحة، مجمع الملك فيصل الطبي، الطائف، المملكة العربية السعودية.

<sup>5</sup> قسم النبات والأحياء الدقيقة، كلية العلوم، جامعة الأزهر، أسبوط، مصر.

<sup>6</sup> جامعة قرطبة، إسبانيا.

### الملخص

قد ثبت أن الخل مركب فعال مضاد للبكتيريا خاصة ضد أنواع مختلفة من البكتيريا المسببة للأمراض، مما يجعله مفيداً في تطبيقات مختلفة كمواد حافظة للأطعمة ومكونات تجميلية بالإضافة إلى بعض التطبيقات الطبية بما في ذلك خفض نسبة الكوليسترول وتحسين الوزن والتحكم في نسبة السكر في الدم.

تبحث الدراسة التأثيرات الخواص الفيزيائية والكيميائية والأنشطة المضادة للبكتيريا لأربعة أنواع من خل التمر التجاري المسوق في المملكة العربية السعودية (التمر، والتمر والثوم، والتمر والرمان، والتمر والكرم). وذلك لتحديد درجة الحموضة، ومحتوى حمض الأسيتك، والخاصية التوصيلية، والمادة الجافة، والمواد الصلبة الذائبة الكلية (قيم بريكس)، ومحتوى الكحول والمعادن. كما تم قياس المركبات الفينولية الكلية والمركبات الفلافونويدية الكلية. وقد تم دراسة النشاط المضاد للميكروبات ضد ثماني مسببات للأمراض وواحدة غير مسببة للأمراض باستخدام طريقة انتشار القرص. كما أظهرت الخصائص الفيزيائية والكيميائية لعينات الخل (ن = 264) تبايناً كبيراً في القيم، مما يشير إلى اختلافات ملحوظة في صفات الخل المدروسة. وأيضاً أظهرت النتائج تنوعاً كبيراً في منتجات الخل المخصصة للاستخدام المباشر من قبل المستهلك.

وقد أشارت قيم المواد الكيميائية النباتية إلى أن أنواع الخل المختلفة لها قيمة عالية للمركبات الفينولية الكلية والمركبات الفلافونويدية الكلية. كما أظهرت السلالات البكتيرية الثمانية المختبرة حساسية متفاوتة للعينات المختلفة المدروسة ذات مناطق التثبيط العالية. ونستخلص من هذه النتائج أن تطبيق الخل السعودي يجب أن يأخذ في الاعتبار خصائصه الكيميائية النباتية.

**الكلمات الدالة:** الخل، فيزيائية، نشطة بيولوجياً، نشاط مضاد للميكروبات، المعادن.