Sigma J Eng & Nat Sci 38 (3), 2020, 1643-1652

Sigma Journal of Engineering and Natural Sciences
Sigma Mühendislik ve Fen Bilimleri Dergisi

Research Article

BIOACTIVITY, PHYSICOCHEMICAL AND ANTIMICROBIAL PROPERTIES OF VINEGAR MADE FROM PERSIMMON (*DIOSPYROS KAKI)* PEELS

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Received: 14.03.2020 Revised: 09.08.2020 Accepted: 20.08.2020

ABSTRACT

Persimmon (*Diospyros kaki)* wastes are defined as a new alternative source for vinegar production. This approach enables the utilization of the persimmon peels which are generally thrown during the consumption or processing of the fruit. The present study investigated total phenolic compound (TPC), total flavonoid compound (TFC), antioxidant capacities (DPPH) radical scavenging activity and the copper reducing antioxidant capacity (CUPRAC)) of persimmon peel vinegar. Additionally, the antimicrobial and some physicochemical properties of the persimmon peels vinegar were evaluated. TPC, TFC were 292.28 mg gallic acid equivalent (GAE)/L and 17.36 mg catechin equivalent (CAE)/L, for persimmon vinegar, respectively. In terms of antioxidant activity, persimmon peels vinegar extracts had 225.44 mg Trolox equivalent (TE)/L for DPPH assay, and 589.07 mg TE/L for CUPRAC assay. Titratable acidity, pH and Brix values of vinegar samples were found as 4.5%, 3.64%, and 1.2%, respectively. The vinegar showed a well antimicrobial effect. Generally, upon increasing the extract concentration, the inhibition zone was also increased, and the highest inhibition zone was observed at direct (100%) extract concentration applied against *Escherichia coli* for antibacterial activity and the highest inhibition zone was observed at direct (100%) extract concentration applied against *Aspergillus niger* for antifungal activity. This study suggested that especially in vinegar production, food waste might be evaluated and also, this vinegar has health-promoting qualities and might be a competitive product in the commercial market.
Keywords: *Diospyros kaki* Peels, vinegar, waste, antioxidant, antimicrobial.

1. INTRODUCTION

Waste valorization processes are critically significant for the food industry [1]. In food factories, large amounts of food waste are produced as a result of the process and most of them are either disposed of (environmental pollution) or used for the production of low-value products (animal feed, fertilizer, etc.) using lower technologies. The effective use of these wastes is important not only for the prevention of environmental pollution but also for the creation of added value and diversification of products. However, in the food industry, the use of product-specific wastes, which comprise of organic residues of processed raw materials, is very limited.

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Fruit and vege table by-products in food and agricultural products processing have proven to contain phenolic antioxidants, dietary fiber, pectin, essential fatty acids, and vitamins that are important for nutrition [2, 3]. The edible fleshy parts of fruit have been reported to contain a lower amount of the phenolic compound than the peels of fruit [4]. The persimmon (*Diospyros kaki*), the perennial plant, belonging to the *Ebenaceae* family, is widely grown in Asian countries, also spreads to other parts of the world, particularly to Europe due to favorable climatic conditions [5]. Persimmon fruit is used in various food industries as it is consumed fresh and dried. It can also be used in vinegar production. Traditional vinegar is produced in two stages (alcohol and acetic acid fermentation) of fermentation spontaneously from the sugar-containing raw material [6, 7]. Traditionally, the vinegar process is generally initiated by the "vinegar mother" from obtained the previous vinegar [8]. The vinegar mother which comprises acetic acid bacteria and yeast on the surface performs the conversion of ethyl alcohol to acetic acid. Vinegar is produced very slowly in this method, but the quality of the produced vinegar is quite high. The fermentation process usually increases the bioactive compounds of vegetables and fruits [9].

By-products can be used as natural antioxidant sources, which are quite high in the number of phenolic substances, can be produced during the processing of agricultural and food products [2]. Phytochemicals such as phenolics and flavonoids found in fruits and vegetables are the main bioactive components that make positive contributions to health [3]. Wastes generated during the processing of fruit and vegetables can be used in the production of vegetable flavoring substances, natural colorants, and bioactive additives for functional foods, which are classified as food additives. Furthermore, these wastes can be converted into valuable products by fermentation. These products are lactic acid, methane, ethanol, food colorant, citric acid, surfactant, enzymes, other food components, and especially aroma compounds [10].

Persimmon involves several types of phytochemicals such as ascorbic acid, carotenoids, polyphenols, pectin, and fibers. Recent researches have demonstrated that fresh persimmon can decrease blood pressure and cholesterol, strengthens the immune system, cure as a resort for preventing cancer and diseases of the digestive system [11]. Besides, the bioactive compounds of persimmon vinegar might be different from that of persimmon fruit. According to recent research, there have been studies on bioactive compounds and other quality parameters of persimmon fruit, leaves. But vinegar production, bioactive compounds, and antimicrobial effect of the persimmon peel vinegar were not studied. This study aims to produce vinegar from persimmon peels and to investigate their potential for use as a bioactive food additive by determining the physicochemical and phytochemical properties of the vinegar obtained.

2. MATERIAL AND METHODS

2.1. Chemicals

Folin–Ciocalteu's phenol reagent, copper (II) chloride, sodium carbonate, aluminum trichloride, ammonium acetate, sodium nitrite, sodium bicarbonate, sodium hydroxide, ethanol, hydrochloric acid (37%), nutrient broth, nutrient agar, potato dextrose agar (PDA) purchased from Merck (Germany) and 2,2-Diphenyl-1-picrylhydrazyl (DPPH), gallic acid, -catechin hydrate standard, -6- Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), neocuproine, obtained from Sigma-Aldrich (Germany).

2.2. Plant material and vinegar production

Persimmon (*Diospyros kaki*) was obtained from Denizli (Turkey) and stored at 4 °C until the drying process. The drying process of the persimmon is performed after the raw fruit were washed and peeled. The vinegar, which was produced at 25-28 ^{o}C temperature for 8-10 weeks by a traditional method, was obtained from the waste persimmon peels (Figure 1).

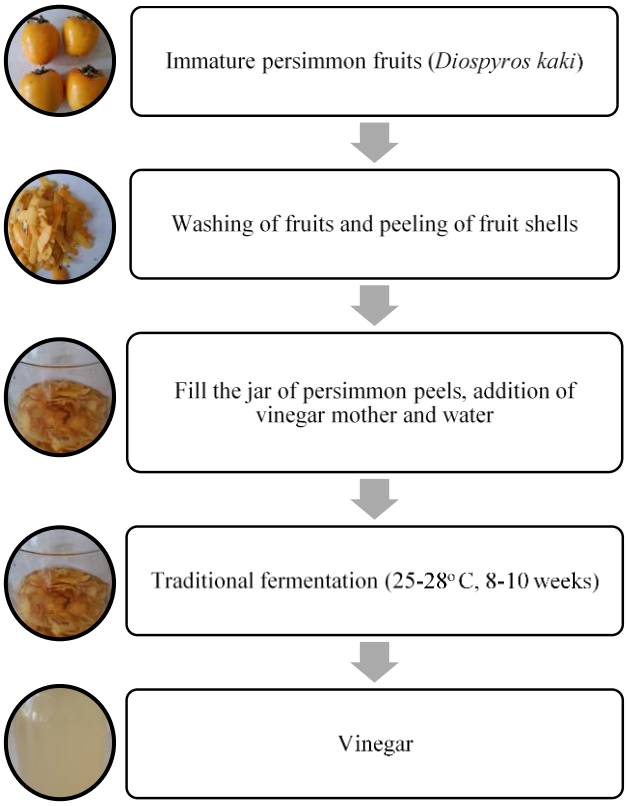

Figure 1. Production of traditional vinegar from persimmon peels

2.3. Physicochemical analysis

°Brix values of the persimmon peel vinegar were detected using a portable refractometer (Atago PAL-1 Pocket Refractometer, Japan) calibrated with pure water. The results were stated as °Brix [12]. 2 mL of persimmon peel vinegar was titrated up to pH 8.0 with using 0.1 N NaOH. The results were stated as acetic acid equivalent and also pH of the persimmon peel vinegar was detected with a pH meter (Ohaus Starter 3100, USA) calibrated with buffer solution.

2.4. Preparation of ethanolic extracts

10 mL of vinegar was mixed with 100 mL 80% ethanol for 2.5 hours on a magnetic stirrer at 25 °C and the vinegar extract store at –20 °C until analysis. The solution was centrifuged at 4100 rpm for 15 minutes at 4 °C (Hettich 320R, Germany) and then, the combined supernatants were filtered (Whatman No 1). The extracts were stored at -18 °C for farther analysis.

2.5. Assay of phenolics, flavonoids

The total phenolic content (TPC) analysis of persimmon peel vinegar extract was conducted with a spectrophotometer (Shimadzu UV-1800 spectrophotometer, Japan) at 760 nm by applying the Folin–Ciocalteu method [12] with modifications and using gallic acid as a standard. 0.5 mL

extract (1 mL of vinegar was dissolved in 10 mL ethanol), 2.5 mL of 0.2 N Folin–Ciocalteu reagent, and 2 mL of sodium carbonate solution (7.5% (w/v)) were mixed in that order. The absorbance of the mixture was read after 30 min incubation in a dark at 25 °C. The TPC was expressed with a linear range of 0.01–0.11 mg/mL (r^2=0.991) as mg gallic acid equivalents (GAE)/L in a fresh sample.

The total flavonoid content (TFC) of persimmon peel vinegar extract was analyzed by applying the aluminum trichloride ($AlCl_3$) method [13] at 510 nm with a spectrophotometer. 1 ml sample (1 mL of vinegar was dissolved in 10 mL ethanol) was mixed with 4 mL of pure water and 0.3 mL of sodium nitrite (5%). The reaction proceeded for 5 min. and then 0.3 mL of aluminum trichloride (10%) was added that the solution was left for 6 minutes. Later 2 mL of 1 M sodium hydroxide solution was supplemented and the mixture was adjusted to 10 mL with pure water. The TFC was expressed with a linear range of 0.01-0.3 mg/mL (r^2=0.996) as mg catechin equivalents (CAE)/L in fresh sample.

2.6. DPPH (radical scavenging activity) Assay

The scavenging activity was assayed using the method developed by Singh et al. [14] with some modification at 517 nm by a spectrophotometer. 0.1 mL sample was mixed with 4.9 mL of the DPPH solution (0.1 mM). Then the mixture was kept at 27 °C temperature for 25 min. The results were expressed as Trolox equivalents (TE) in mg/L fresh sample with a linear range of 0.025-0.6 mg/mL (r^2=0,996).

2.7. CUPRAC (Determination of copper reducing antioxidant capacity) Assay

The copper reducing capacity was determined using the method proposed by Apak et al. [15]. 1 milliliter of neocuproine (7.5 mM), $CuCI_2$ (0.01 M), and 1 M ammonium acetate buffer (pH 7.0) solutions were added to a test tube. Then 0.1 mL vinegar extract and 1 mL pure water were added into the test tube. The sample solutions were mixed and the reaction proceeded for one hour in a dark place at 25 °C. The absorbance of the resulting solution was detected at 450 nm by spectrophotometer (Shimadzu UV-1800 spectrophotometer, Japan). The results were expressed as mg Trolox equivalents (TE)/L in fresh sample with a linear range of 0.025-0.8 mg/mL (r^2=0,991).

2.8. Sensory evaluation

Persimmon peels vinegar was submitted to descriptive analysis. The panel for sensory analysis characterization consisted of seven assessors between the ages of 25 and 50. General parameters were chosen such as floral, acidity, fruity, sweetness, pungent and general impression, and were analyzed using a designed from 0 (absence) to 5 (very intense) six-level scale [16, 17]. All study procedures were in accord with the Yildiz Technical University Ethics Board for Human Subjects and met the Ethical Review Committee standards.

2.9. Antimicrobial activity assays

Antimicrobial activity of persimmon vinegar was described with agar diffusion method against 5 bacteria (*Escherichia coli* O157: H7 ATCC 33150, *Bacillus cereus* FMC19, *Listeria monocytogenes* ATCC 19118, *Staphylococcus aureus* ATCC 25923, *Salmonella* Typhimurium ATCC 14028) and 3 molds (*Aspergillus flavus, Penicillium carneum, Aspergillus niger*). The microorganisms from stock cultures were subjected to 2-step activation treatment at 18 and 24 h (bacteria at 36-37 ºC, Nutrient Broth, molds at 26-27 ºC on PDA solid medium). 100 µL of each microorganism stock culture was pipetted, sowed by spread plate method, and left for 20 min. Then, 4 equidistant wells (5 mm in diameter) were cut (using cork borer) from the agar.

Persimmon vinegar sample was dissolved in the distilled water as a final concentration of 10% (v: v) and 20 µL of extracts (100% and 10%) and blank (water) solutions were applied to the wells. The petri dishes were incubated at 27 ºC for 5 days for the molds, and at 37 ºC for 18-24 h for the bacteria. Zones around the wells were measured in mm.

2.10. Statistical analysis

The experiments were applied in triplicate. The results were expressed as mean ± standard deviation. The significant differences between samples were determined using Duncan multiple post hoc tests ($p < 0.05$) when the one-way analysis of variance (ANOVA) was significant.

3. RESULTS AND DISCUSSION

3.1. Physicochemical analysis of persimmon peels vinegar

The fermentation process of the persimmon peels vinegar was carried out at 25-28 ^{o}C for 8-10 weeks to produce vinegar. As a result of the study, titratable acidity, pH, and ^{o}Brix values of vinegar samples were found as 4.5% (45 g/L), 3.64, and 1.2%, respectively. The most significant quality criterion of vinegar is acetic acid content. According to the TS 1880 vinegar standard, the total acid content (TCA) of the wine vinegar must be at least 40 g/L in terms of acetic acid [18]. In a study where the physicochemical properties of persimmon vinegar were determined during fermentation, TCA increased from 0.83 to 32.5 g/L, while ^{o}Brix values decreased from 11,36 to 3,18 [19]. A similar trend was demonstrated by Roda et al. [20] who showed the pineapple wastes vinegar attained approximately 5% (w/v) acidity and at pH 3, at the end of the acidification process at 30 days. In another study, the organic acid content, the proximate composition of industrial Zhenjiang aromatic vinegar, and traditional Zhenjiang aromatic vinegar were detected pH value in all Zhenjiang aromatic vinegar varied 3.38 - 3.62, 5.80% - 6.69% respectively [21].

3.2. Total phenolic, flavonoid contents and antioxidant capacity of persimmon peel vinegar

Phenolic compounds have been shown to significantly influence the quality of vinegar and ıt has among at most identified components [22]. The result of the study TPC and TFC and antioxidant capacity of persimmon peels vinegar were given in Table 1. As shown in Table 1, the TPC and TFC of the vinegar were found as 292.28 mg GAE/L and 17.36 mg CAE/L vinegar respectively. The total antioxidant activity of persimmon peels vinegar sample was determined according to CUPRAC and DPPH methods. Antioxidant capacities evaluated with the Copper Reducing Antioxidant Capacity method were lower than the 2,2-diphenyl-1-picrylhydrazyl radical method. The antioxidant capacities of the sample were measured to be 225.44 mg Trolox equivalent (TE)/L (with using the DPPH method) and 589.07 mg TEAC/L vinegar (with using the CUPRAC method), respectively. Thus, in our study, the CUPRAC value of persimmon vinegar extract was higher than the value obtained by the DPPH method. Cu^{2+} ion takes part in the formation of free radicals and the reduction of cupric ion shows another mechanism reflecting the antioxidant capacity.

Table 1. Total phenolic, total flavonoid contents and antioxidant activities of ethanolic extract of vinegar obtained from persimmon peels

Bioactive characteristics	**Unit**	**Ethanolic extract**
TPC	mg GAE/L	292.28±3.47
TFC	mg CAE /L	17.36±0.34
DPPH	mg TE /L	225.44±3.44
CUPRAC	mg TE/L	589.07±4.56

The results were given as mean ± standard deviation of triplicate measurements, *TPC* total phenolic content, *TFC* total flavonoid content, *DPPH* 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity, *CUPRAC* Copper reducing antioxidant capacity; GAE gallic acid equivalent, CE catechin equivalent, TE Trolox equivalent.

Zou et al. [19] reported the changes in TPC of persimmon during fermentation. TPC of persimmon was found in the range of 300-350 mg GAE/L during the fermentation process. This study compared with our study, TPC of fruit vinegar was found similar. The amount of phenolic substance of peels vinegar is close to the values of the fruit vinegar. They reported that acetic and alcoholic fermentation increased the bioactive properties of *Diospyros kaki* and its tannin concentration. Gallic acid, which different phenolic compounds present in *Diospyros kaki*, represented the essential component, and its content enhanced during the alcoholic fermentation while decreased during acetic fermentation. Also, this study reported that during fermentation, the metabolic activity of acetic acid bacteria and yeast modifies the structure of the persimmon puree and can, as a result, affect the chemical content of persimmon.

Ubeda et al. [23], total phenols index (TPI) values were determined as 277-424, 268-397 mg gallic acid/kg in persimmon puree and vinegar and it was found that total phenols index values did not change significantly during the vinegar production process and also this study was reported that it obtained higher values than other commercial vinegar like red and white wine vinegar. TPI values of vinegar are very similar to our study. There is no difference between the vinegar made from persimmon fruit and the vinegar made from the peel in terms of total phenolic compounds.

Giuffrè et al. [24], which produces vinegar from waste products similar to our study, found the total amount of phenolic substance and antioxidant activity (DPPH % inhibition) of vinegar they obtained from *Citrus bergamia* by-products as 241-1953 (mg/L) and 9.87-21.80 % inhibition respectively. While our study results were consistent with the amount of phenolic substance of this study, DPPH (% inhibition) was higher than the values of the study. Zhao et al. [21] showed that the bioactive compounds of industrial and traditional Zhenjiang aromatic vinegar (ZAV) samples during the fermentation process. Consequently, TPC, TFC and antioxidant activities of ZAV were found as 2.07-6.45 mg GAE/mL, 1.21-4.15 mg RE/mL and 17.82- 54.72 mmol TEAC/L respectively. In another study, Ordoudi et al. [25] found the antioxidant activity value described by the CUPRAC method of pomegranate syrup as 7.8 mM trolox, and the DPPH value as 5 mM trolox. The differences in the results may be evaluated with differences in the extraction conditions, type of extraction solvent, environmental conditions, use a different part of the plant, plant varieties, and production of different vinegar.

3.3. Sensorial characteristics

The sensory data are shown in Fig. 2 and also the descriptors pungent, floral, fruity, acidity, sweetness, and general impression in the persimmon peels vinegar was graded as 3±0.71, 4±0,71, 2.4±0.55, 4±0.71, 1.6±0.55 and 4.2±0.84, respectively. 82% of panelists liked this vinegar in terms of a general impression. The aroma of this vinegar was deemed acceptable.

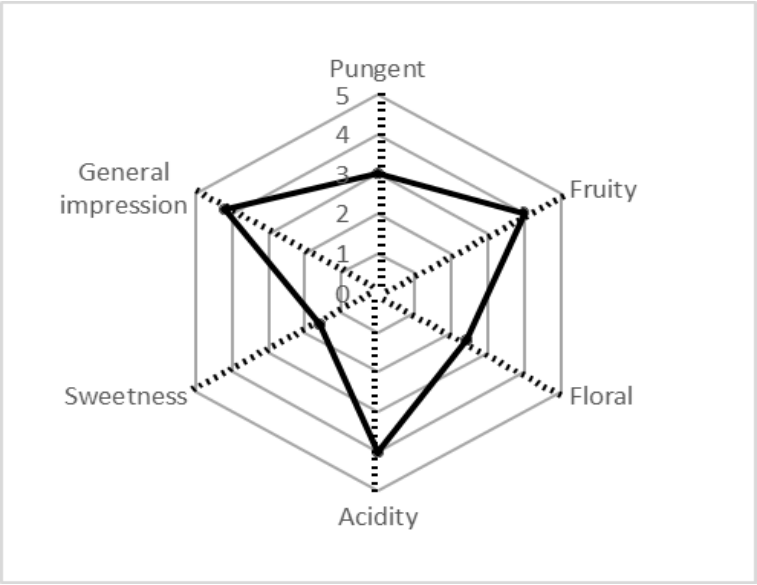

Figure 2. Mean values obtained from descriptive sensory analysis for vinegar from persimmon peels

Chen et al. [26] reported that the sensorial properties of citrus vinegar were usually dominated by sour, then by sweet, umami taste which can be a consequence of the balance and interaction among the varied flavor compounds. Similarly, our study was shown that high acidity and pungency, low sweetness characterizes for the persimmon peels vinegar.

3.4. Antimicrobial activity of persimmon peels vinegar

Table 2 shows the antimicrobial activity of the persimmon peels vinegar against selected mold and bacteria. The vinegar extract showed good antimicrobial effects. Between 10% and 100% of vinegar concentration, the differences in the inhibition against *Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella* Typhimurium, and *Escherichia coli* was significant ($p<0.05$). Generally, upon increasing the extract concentration, the inhibition zone was also increased, and the highest inhibition zone was observed at direct (100%) extract concentration applied against *Salmonella* Typhimurium and *Escherichia coli*. In terms of antifungal properties, the inhibition zone was highest against *Aspergillus niger*, and it was increased with the increase in extract concentration from 10% to 100%. Against *Aspergillus flavus*, *Penicillium carneum*, at 10% extract concentration no inhibition was observed and inhibition was increased with higher concentrations applied.

Table 2. Antimicrobial effect of vinegar obtained from persimmon peels

Microorganisms	**negative control (water)**	Extract concentration (%)	
		10	**100**
Bacteria	-		
Bacillus cereus	-	5.83±0.29[a]	7.00±1.00[b]
Staphylococcus aureus	-	-	9.33±0.58[a]
Listeria monocytogenes	-	-	9.67±0.58[a]
Salmonella Typhimurium	-	5.83±0.29[a]	10.33±0.57[b]
Escherichia coli	-	6.67±0.58[a]	10.67±0.58[b]
Molds			
Penicillium carneum	-	-	5.83±0.29[a]
Aspergillus flavus	-	-	5.67±0.28[a]
Aspergillus niger	-	6.83±0.28[a]	7.83±0.29[b]

The results were given as mean ± standard deviation of triplicate measurements.
Means with different letters in the same row are significantly different ($p < 0.05$).
- : no inhibition

In a study by Yagnik et al. [27] the effect of antimicrobial activity of apple cider vinegar against *S. aureus, E. coli* was studied. These bacteria were directly cultured with different concentrations of apple cider vinegar and also the zone of inhibition was measured. Apple cider vinegar had an antibacterial effect against *S. aureus* (22.5 mm) and *E. coli* (27.5 mm). These results were compared with our study and the antibacterial potency of persimmon peels vinegar for *S. aureus, E. coli* could be arranged as follows: *E. coli* (10.67 mm)> *S. aureus* (9.33 mm). Ozturk et al. [28] determined industrial and home-made traditional vinegar produced in Turkey concerning the antimicrobial effect. The sensitivity of the bacteria to the vinegar samples was notably variable. *B. cereus* was detected as the most sensitive strain, in which 90% and 100% of the industrial and traditional vinegar samples demonstrated antimicrobial activity at variable levels. Different studies have demonstrated that the acetic acid in vinegar was the most lethal acid to *E. coli* O157: H7 followed by citric, lactic, and malic acids [28].

4. CONCLUSION

Vinegar is a cheap product due to producing inexpensively from raw material. Our study has demonstrated that persimmon processing by-products might serve as a new alternative source for vinegar production and also may be evaluated that the new developments in process engineering such as the by-products. To reduce waste, it is very important to use fruit or vegetable peels as raw materials for converting into products such as vinegar. The results of our study show that vinegar produced from persimmon peels, which is waste, has phenolic content and antioxidant activity at least as much as vinegar produced from its fruit. This is the first study that emphasizes the importance of reusing waste materials. Besides, it is predicted that this study can contribute to the food industry by turning alternative persimmon wastes into efficient resources.

Acknowledgment

The authors will like to thank *Pamukkale University* (Project No: 2017KKP062) for the funding of this study and also thanks to the *Yildiz Technical University*, Department of Food Engineering for the analysis of this study.

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