



## Fig Vinegar as an Antioxidant and Antimicrobial Agent

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### ABSTRACT

Fig vinegar is a special product which has a long history and is handed down from generation to generation. In the current study, characteristics of homemade fig vinegars supplied from different cities of Turkey were compared in terms of their physicochemical, antioxidant and antimicrobial properties. Physicochemical properties of the samples were very diverse. Total phenolic and flavonoid content, and ABTS<sup>+</sup> and DPPH radical scavenging activities of the samples were ranged from 313.5 to 594.25 mg GAE/L and 42.52-132.42 mg catechin/L, and 0.44-3.98 µg TE/mL and 1.25-7.51 µg TE/mL, respectively. The antioxidant activity of vinegars is highly correlated with their phenolic and flavonoid contents. Furthermore, vinegar samples exhibited inhibitive effect on all test microorganisms and the minimum inhibition concentration values were ranged between 0.39 and 25% (v/v). These results indicated the distinct properties of homemade fig vinegars and the potential of them as antimicrobial and antioxidant substances.

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## Introduction

Vinegar is an acidic liquid product obtained from various sources including fermentable carbohydrates by the activity of *Saccharomyces cerevisiae* and acetic acid bacteria (AAB). It is also possible to produce vinegar from liquid containing ethanol such as wine (Sengun, 2015). Acetic acid produced by AAB during vinegar production is responsible for characteristic aroma of vinegar. There are very types of vinegar produced worldwide such as cereal vinegar, wine vinegar, fruit vinegar, traditional balsamic vinegar and cider vinegar. It is provided by firms that large scale production and/or produced in small scales traditionally, which have changed from region to region (Giudici et al., 2017).

Recently, the popularity of vinegars prepared at homes from a variety of substrates has been increased. Although the substrates and the final products in homemade vinegar productions have some variations, the process always includes alcoholic and acetous fermentation, which are the main steps of vinegar production (Rosma et al., 2016). Unlike commercial vinegars, they are produced under uncontrolled conditions and consumed without pasteurization. Hence, it may provide a suitable environment for the growth of undesirable

microorganisms. It is noted that the presence of sufficient amount of acid is essential to obtain high quality vinegars (Giudici et al., 2017).

Different plant species and products are used in the treatment of diseases in many parts of the world (Sevindik et al., 2017; Mohammed et al., 2019; Pehlivan et al., 2018; Mohammed et al., 2020). Vinegar has long been used in both natural folk medicine and traditional medicine for treating a variety of diseases. It is provided beneficial health effects including anticarcinogenic, antidiabetic, antiglycemic, antihypertensive, antimicrobial, antioxidant and lipid-lowering when consumed regularly due to including variety of organic acids, phenolic compounds, amino acids, vitamins, melanoidins (Karabiyikli and Sengun, 2017). Several researchers demonstrated that different kinds of vinegars effectively inhibit the growth of foodborne pathogens including *Bacillus cereus*, *Aeromonas hydrophila*, *Vibrio parahaemolyticus*, *Escherichia coli* O157:H7, *Salmonella enteritidis*, *S. typhimurium*, *Staphylococcus aureus*, and could be used for disinfection of food, food preparation surfaces and equipment (Karabiyikli and Sengun, 2017).

There is a limited knowledge on traditional homemade vinegars produced from different kinds of raw materials. Fig (*Ficus carica* L.) is grown in large areas in Turkey and used as raw material of fig vinegar (Harzallah et al., 2016). The quality properties of fig vinegar, which is produced by fresh and/or dried figs show differences depending on the production techniques and raw material used. There are several studies investigating the antioxidant activities and phenolic contents of vinegars produced from various fruits, mainly grape and apple. However, except our previous studies (Sengun et al., 2020; Şengün and Kılıç, 2020a, b), there are no studies determining the properties of fig vinegar including total phenolic content, total flavonoid content, antioxidant and antimicrobial activities.

Therefore, the purpose of the current study was to examine some properties of traditional fig vinegar supplied from different cities of Turkey in terms of their physicochemical, antioxidant and antimicrobial properties.

## Materials and Methods

### Materials

In the study, traditionally produced homemade fig vinegars were collected from various cities (Sample A from Aydın, Sample B from Gaziantep, Sample C and D from Izmir) in Turkey. Vinegar production was performed using two different varieties of figs grown in Aydın (Morguz-dark color), Gaziantep (Sari Lop-light color) and Izmir (Sari Lop and Sari Zeybek-light color) cities. The production steps include mixing fresh fruits and water (1:1), first fermentation (at room temperature for 2 weeks), filtration, second fermentation (at room temperature for 10-12 weeks) and bottling. Vinegar samples were stored at 4°C before used in the experiments.

### Methods

#### Physicochemical Analysis

The pH values of vinegars were determined by using pH meter (NEL Mod 821) (AOAC, 2007).

Acidity values of vinegars such as total, volatile and non-volatile were examined by titrimetric method. The results of these analyses were given as g acetic acid/100 mL of sample (AOAC, 2007).

Residual alcohol and specific weight of vinegars were examined by picnometric method. The results of these analyses were given as % (AOAC, 2007).

Brix values of vinegar samples were determined by refractometer (Hanna HI 96801), values were expressed as Brix (AOAC, 2007).

Ash contents of vinegar samples were detected by ashing the samples at 525°C to constant weight and the results of this analysis were given as g/L (AOAC, 2007).

Color values of the samples were determined using a HunterLab Colorflex (Management Company, USA) calibrated with standard calibration scale and the color properties of the samples were given as  $L^*$ ,  $a^*$ , and  $b^*$  (Rommel et al., 1990).

#### Total Phenolic Content

The amounts of total phenolic contents of vinegars were detected by the colorimetric Folin-Ciocalteu method. 1 mL of filtered (0.2 µm, Sartorius Stedim) vinegar, 5 mL of 10% Folin-Ciocalteu's phenol reagent (Sigma-Aldrich) and 75 mL of distilled water were mixed. After the mixture

was held at room temperature for 3 min, 10 mL saturated  $\text{Na}_2\text{CO}_3$  (Merck) was transferred to the mixture. The obtained mixture was complemented to 100 mL with distilled water and kept for 90 min in the dark. Then the absorbance value of this mixture was determined by a spectrophotometer (Cary60 UV-Visible, Agilent Technologies) at 720 nm (Cemeroglu, 2013). Total phenolic content of vinegars was given as mg of gallic acid equivalents/L (mg GAE/L).

#### Total Flavonoid Content

The amounts of total flavonoid contents of vinegars were detected with spectrophotometric method (Zhishen et al., 1999). 4 mL of distilled water and 1 mL of filtered vinegar (0.2 µm, Sartorius Stedim) was mixed. Then 0.3 mL of 10%  $\text{AlCl}_3$  (Merck), 0.3 mL of 5%  $\text{NaNO}_2$  (Merck) and 2 mL of 1 M NaOH (Merck) were transferred to the mixture. The obtained mixture was completed to 10 mL with distilled water, and then the absorbance value of the mixture was detected using a spectrophotometer (Cary60 UV-Visible, Agilent Technologies) at 510 nm. The amount of total flavonoid contents of vinegars were given as catechin equivalents (mg catechin/L).

#### Antioxidant Activity

The antioxidant activities of vinegars were analyzed according to the ABTS and DPPH methods.

ABTS radical scavenging activities vinegars were detected as described by Re et al. (1999). After dissolving 37.5 mg of  $\text{K}_2\text{S}_2\text{O}_8$  (Sigma-Aldrich) and 10 mg of  $\text{ABTS}^+$  [2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (Sigma-Aldrich)] in 1 and 2.57 mL of distilled water, respectively, and both solutions were mixed and stored in the dark for 12-16 hour to obtain ABTS radical cation ( $\text{ABTS}^+$ ) solution. After that, to obtain absorbance value of  $0.70 \pm 0.02$  at 734 nm, 1 mL of  $\text{ABTS}^+$  solution was diluted with ethanol. Furthermore,  $\text{ABTS}^+$  solution (3 mL) and filtered (0.2 µm, Sartorius Stedim) vinegars (300 µL) with concentrations in the range of 100-500 µg/mL, were mixed and stored for 6 min in the dark. Then the absorbance of this mixture was detected using a spectrophotometer (Cary60 UV-Visible, Agilent Technologies) at 734 nm. The percent inhibition of  $\text{ABTS}^+$  was measured as given below:

$$\% \text{ABTS}^+ = (A_c - A_s) \times 100 / A_c$$

Where;

$A_s$  = is absorbance of sample

$A_c$  = is absorbance of  $\text{ABTS}^+$  solution.

Calibration curve was prepared using Trolox (50-500 µg/mL) and the antioxidant capacity was given as µg of Trolox equivalents/mL (µg TE/mL).

DPPH radical scavenging capacity of vinegars was also examined by the modified method of Cemeroglu (2013). Filtered the sample (0.2 µm, Sartorius Stedim) at various concentrations (20-100 µL) was transferred to 300 µL of 0.1 mM 2,2-Diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich). After methanol was added to the obtained mixture until 3 mL was reached, it was held in the dark for 15 min at room temperature. After that, the absorbance value of this mixture was determined by a spectrophotometer (Cary60 UV-Visible, Agilent Technologies) at 517 nm.

The percentage inhibition was expressed as given below:

$$\text{Abs (\%)} = (A_c - A_s) \times 100 / A_c$$

Where;

$A_s$  is absorbance value of sample

$A_c$  is absorbance value of control (methanol).

The results were given as  $\mu\text{g}$  Trolox equivalents/mL ( $\mu\text{g TE/mL}$ ).

#### Antimicrobial Activity

Antimicrobial properties of vinegars were detected by determining minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC). *Bacillus subtilis* ATCC 6037, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 1103, *E. coli* O157:H7 ATCC 43895, *Listeria monocytogenes* Scott A, *Salmonella typhimurium* NRRL-B-4420, *Staphylococcus aureus* 6538P, and *Pediococcus acidilactici* ATCC 8042 were utilized as test microorganisms. This study is mainly focused on the pathogens (*E. coli*, *E. coli* O157:H7, *L. monocytogenes*, *S. typhimurium*, *S. aureus*) associated with food-borne diseases, also representative species for LAB (*E. faecalis* and *P. acidilactici*) and food spoilage bacteria (*B. subtilis*) have been contained. All test microorganisms were supplied from Laboratory of Food Microbiology, Department of Food Engineering, University of Ege, Turkey. The test microorganisms stored at  $-20^\circ\text{C}$  were reactivated in Mueller Hilton Broth (MHB, pH  $7.3 \pm 0.2$ , Oxoid) at  $37^\circ\text{C}$  for 18-24 h. The test microorganisms having the turbidity standard of 0.5 McFarland (DEN-1 McFarland Densitometer, Grant-bio) were utilized in the analyses.

For detecting MIC values of the samples, microdilution method was performed using standard 96-well microtiter plates (Deng et al., 2014). Dilutions of vinegars prepared in MHB (a total volume of 200  $\mu\text{L}$ ) and distributed in wells of the plate. The final concentrations of the samples in the wells of the prepared microplate were in the range of 50% - 0.10% (v/v). After that, 10  $\mu\text{L}$  of the test microorganism was inoculated in each well. The wells including only the test cultures and MHB were used as positive and negative controls, respectively. The analyze was separately repeated for each test microorganism and for each vinegar sample. After incubation (at  $37^\circ\text{C}$  for 18 h), 20  $\mu\text{L}$  of 2,3,5-triphenyl tetrazolium chloride (0.5%, w/v, Merck) aqueous solution was mixed in the wells of the microplate and incubated again for 30 min at  $37^\circ\text{C}$ , and the change of colors was observed. The lowest concentration of the sample necessary to inhibit observable growth of the test microorganisms was determined as the MIC value.

After determining the MIC values, to detect MBCs of vinegars (Tomas-Menor et al., 2013), inoculum was transferred from the first wells, where no color change was seen and on Mueller Hilton Agar (MHA, pH  $7.3 \pm 0.2$ , Oxoid) by streaking, and incubated at  $37^\circ\text{C}$  for 24 h were checked for colony formation.

#### Statistical Analysis

Analyses were conducted in three replicates and two parallels. Data were examined by one-way ANOVA using the SPSS software version 20. Differences among the means were evaluated by using Duncan's Multiple Range

test at a significance level of  $P < 0.05$ . In tables and figures, the values were given in terms of mean values and standard deviation.

## Results and Discussions

### Physicochemical Properties

Total acidity and pH values of fig vinegars were ranged from 1.93 to 4.47 g acetic acid/100 mL and  $3.51 \pm 0.02$  to  $3.75 \pm 0.21$ , respectively (Table 1). Except sample A, our results were not in conformity with FDA (United States Food and Drug Administration) and Turkish Standards, who declare that total acidity value of vinegar should be at least 4% acidity. The acidity is one of the main parameters of vinegars. In the previous study, the pH value and total acidity of fig vinegar were determined as 3.22 and 4.73 g acetic acid/100 mL (Sengun et al., 2020). Volatile and non-volatile acidity of vinegars were determined as in the range of 1.25 and 3.67 g acetic acid/100 mL, and 0.24 and 1.20 g acetic acid/100 mL, respectively (Table 1). Figs used as raw material in the study were belong to different varieties and grown in different locations. Hence, it could be reason of the variation of acidic values of vinegars. Giudici et al. (2017) also stated that the concentration of acetic acid, which is the main parameters of vinegars, is related with the raw material, the microorganisms involved during vinegar production and the technology employed.

Residual alcohol content is other important quality criteria for vinegars. In an ideal vinegar production, all alcohol in the medium is expected to be converted to acetic acid, and this case represents efficiency of vinegar (Giudici et al., 2017). It is stated that residual alcohol content of vinegar should be at most 0.5% (v/v) except wine vinegars (Anonymous, 2016). However, this value was ranged between 2.04 and 3.09% in this study (Table 1).

Brix and specific weights of fig vinegar samples varied in a wide range from 1.30 to 21.2 and 0.9955 to 0.9970, respectively (Table 1). In another studies, brix values of various vinegars were reported as ranging between 3.30 and 3.40 for alcohol vinegar, 3.80-5.00 for wine vinegar (Saiz-Abajo et al., 2004), 1.00-20.80 for apple, apple-lemon, apricot, artichoke, blackberry, fig, grape, hawthorne, mandarin, mulberry, lemon, persimmon, plum, pomegranate, rosehip, sour cherry vinegar (Budak, 2015; Ozturk et al., 2015; Sengun et al., 2020) and above 55 for traditional balsamic vinegar (Masino et al., 2008). As it can be seen from the studies, brix value shows characteristic properties of vinegar and may change depending on the type of vinegar and the raw material used during production (Saiz-Abajo et al., 2004; Masino et al., 2008).

The amount of ash was expressed as unburned inorganic materials found in samples. In the study, ash contents of fig vinegars were found between 2.6 g/L and 11.5 g/L ( $P < 0.05$ ) (Table 1). Other researchers stated that ash contents of grape and wine vinegars ranged between 0.74-3.56 g/L and 2.03-2.61 g/L, respectively (Gerbi et al., 1998; Akbas and Cabaroglu, 2010). Hence, the ash content of fig vinegars is much higher compared to the other vinegars. These results can be related to the fact that the fig fruit is a rich source in terms of mineral content (Vinson, 1999).

Table 1. Physicochemical properties of fig vinegars

| Analysis                         | A                       | B                       | C                       | D                       |
|----------------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| pH                               | 3.75±0.21 <sup>a</sup>  | 3.57±0.03 <sup>a</sup>  | 3.51±0.02 <sup>a</sup>  | 3.67±0.01 <sup>a</sup>  |
| Total acidity (g/100 mL)*        | 4.87±0.33 <sup>c</sup>  | 3.17±0.14 <sup>b</sup>  | 2.16±0.09 <sup>a</sup>  | 1.93±0.13 <sup>a</sup>  |
| Volatile acidity (g/100 mL)*     | 3.67±0.33 <sup>d</sup>  | 2.69±0.14 <sup>c</sup>  | 1.92±0.09 <sup>b</sup>  | 1.25±0.13 <sup>a</sup>  |
| Non-volatile acidity (g/100 mL)* | 1.20±0.00 <sup>d</sup>  | 0.48±0.00 <sup>b</sup>  | 0.24±0.00 <sup>a</sup>  | 0.68±0.00 <sup>c</sup>  |
| Brix <sup>o</sup>                | 21.2±0.00 <sup>d</sup>  | 1.80±0.00 <sup>b</sup>  | 1.30±0.00 <sup>a</sup>  | 2.30±0.00 <sup>c</sup>  |
| Residual alcohol (%; 20°C)       | 2.04±0.00 <sup>a</sup>  | 2.52±0.00 <sup>b</sup>  | 2.59±0.00 <sup>b</sup>  | 3.09±0.00 <sup>c</sup>  |
| Specific weight                  | 0.9970±0.0 <sup>c</sup> | 0.9963±0.0 <sup>b</sup> | 0.9962±0.0 <sup>b</sup> | 0.9955±0.0 <sup>a</sup> |
| Ash (g/L)                        | 11.5±0.00 <sup>d</sup>  | 3.6±0.00 <sup>c</sup>   | 3.2±0.00 <sup>b</sup>   | 2.6±0.00 <sup>a</sup>   |

\*: as acetic acid. Standard deviation of means is shown as ± SD. Values in the same row with different superscripts are significantly different (P<0.05).

Table 2. Antioxidant, total phenolic and total flavonoid contents of fig vinegars

| Analysis                                   | A                        | B                       | C                        | D                       |
|--|--------------------------|-------------------------|--------------------------|-------------------------|
| Total phenolic content (mg GAE/L)          | 594.25±6.01 <sup>d</sup> | 313.5±8.48 <sup>a</sup> | 444.25±8.13 <sup>b</sup> | 383±6.36 <sup>c</sup>   |
| Total flavonoid content (mg catechin/L)    | 132.42±0.53 <sup>d</sup> | 48.52±0.26 <sup>a</sup> | 110.11±0.16 <sup>c</sup> | 69.14±0.47 <sup>b</sup> |
| DPPH radical scavenging activity (µg/mL)*  | 7.51±0.69 <sup>b</sup>   | 1.49±0.01 <sup>a</sup>  | 2.09±0.05 <sup>a</sup>   | 1.25±0.007 <sup>a</sup> |
| ABTS+ radical scavenging activity (µg/mL)* | 3.98±0.001 <sup>d</sup>  | 0.44±0.003 <sup>a</sup> | 0.73±0.00 <sup>b</sup>   | 0.97±0.002 <sup>c</sup> |

\*: as TE. Standard deviation of means is shown as ± SD. Values in the same row with different superscripts are significantly different (P<0.05).

Table 3. The minimum inhibition concentration (MIC) values of fig vinegars (v/v, %)

| Microorganisms                  | A         | B         | C         | D         |
|---------------------------------|-----------|-----------|-----------|-----------|
| <i>Bacillus subtilis</i>        | 0.39±0.00 | 12.5±0.00 | 12.5±0.00 | 9.38±4.42 |
| <i>Enterococcus faecalis</i>    | 1.56±0.00 | 12.5±0.00 | 9.38±4.42 | 12.5±0.00 |
| <i>Escherichia coli</i>         | 3.12±0.00 | 6.25±0.00 | 12.5±0.00 | 9.38±4.42 |
| <i>Escherichia coli</i> O157:H7 | 1.56±0.00 | 6.25±0.00 | 12.5±0.00 | 6.25±0.00 |
| <i>Listeria monocytogenes</i>   | 1.56±0.00 | 9.38±4.42 | 12.5±0.00 | 9.38±4.42 |
| <i>Salmonella typhimurium</i>   | 1.56±0.00 | 12.5±0.00 | 25±0.00   | 9.38±4.42 |
| <i>Staphylococcus aureus</i>    | 6.25±0.00 | 12.5±0.00 | 12.5±0.00 | 12.5±0.00 |
| <i>Pediococcus acidilactici</i> | 12.5±0.00 | 4.69±2.21 | 9.38±4.42 | 4.69±2.21 |

\*Standard deviation of means is shown as ± SD.

Table 4. Minimum bactericidal concentration (MBC) values of fig vinegars (v/v, %)

| Microorganisms                  | A         | B   | C   | D   |
|---------------------------------|-----------|-----|-----|-----|
| <i>Bacillus subtilis</i>        | 3.12±0.00 | >50 | >50 | >50 |
| <i>Enterococcus faecalis</i>    | 12.5±0.00 | >50 | >50 | >50 |
| <i>Escherichia coli</i>         | 50±0.00   | >50 | >50 | >50 |
| <i>Escherichia coli</i> O157:H7 | >50       | >50 | >50 | >50 |
| <i>Listeria monocytogenes</i>   | 12.5±0.00 | >50 | >50 | >50 |
| <i>Salmonella typhimurium</i>   | 25±0.00   | >50 | >50 | >50 |
| <i>Staphylococcus aureus</i>    | 50±0.00   | >50 | >50 | >50 |
| <i>Pediococcus acidilactici</i> | >50       | >50 | >50 | >50 |

\*Standard deviation of means is shown as ± SD.

### Color Properties

Color properties of fig vinegar samples are shown in Figure 1 and  $L^*$ ,  $a^*$ ,  $b^*$  values of the samples were determined as ranging between 5.36-15.42, -1.65-8.51 and 3.96-8.29, respectively (P<0.05). Among the samples, the highest  $L^*$  and  $a^*$  values were determined in sample B and C, respectively. Meanwhile, Sample A as the only sample produced from dark-colored figs (Morguz) has the highest  $b^*$  value. Many researchers reported that the color properties of various vinegars show significant differences, which mainly change depending on the color of raw material used (Palacios et al., 2002; Lopez et al., 2005; Giudici et al., 2017). Besides, the technology employed during vinegar production could also affect the color of the product. For example, if the production steps include heat processing, the color of vinegar mainly comes to darker like in balsamic vinegar.

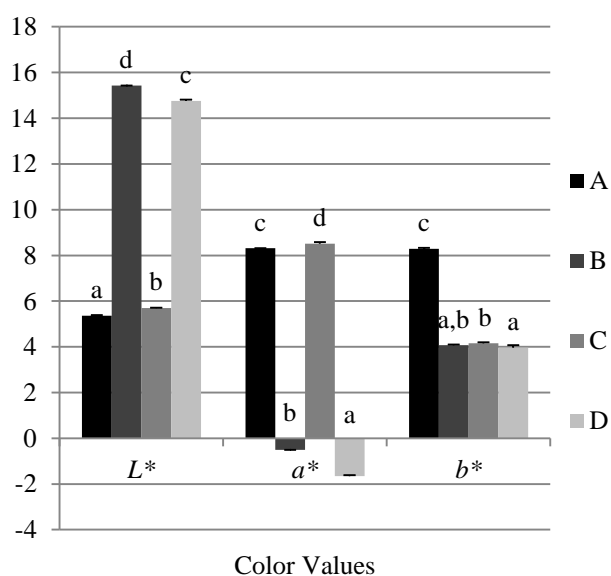
### Antioxidant Activities

The total phenolic contents of the samples ranged between 313.5 mg GAE/L and 594.25 mg GAE/L (P<0.05) (Table 2). Many researchers reported different total phenolic contents for various vinegars, such as 4684.50 mg GAE/L for apple vinegar, 32761.33 mg GAE/L for pomegranate vinegar, 2000-3000 mg GAE/L for grape vinegars, 200-1000 mg GAE/L for Spanish wine vinegar, 3092 mg GAE/100 g for pepper leave vinegar, 823.07 mg GAE/L for pomegranate vinegar, 481.40 mg GAE/L for young Sherry vinegar, 563.73 mg GAE/L for Reserva Sherry vinegar, 1051-4086 mg GAE/L for Shanxi aged vinegar (Alonso et al., 2004; Budak and Guzel-Seydim, 2010; Song et al., 2014; Aykin et al., 2015; Kharchoufi et al., 2018; Xia et al., 2018).

There is a positive correlation between the amount of total phenolic and flavonoid content. The total flavonoids

of fig vinegars were in the range of 48.52-132.42 mg catechin/L (Table 2). The results showed that the highest total flavonoid and also phenolic contents were obtained in Sample A, which is the only sample produced from dark-colored figs. Hence, it could be concluded that there is positive correlation between the darkness of figs and the total flavonoid and phenolic contents of vinegar. Although the color values ( $b^*$ ) of some samples close to each other ( $P>0.05$ ), such as in Sample B and C (samples grown in different cities, but belong to same variety-Sari Lop) or in Sample B (Sari Lop) and D (Sari Zeybek) (samples belong to different varieties and grown in different cities) (Figure 1), total phenolic and flavonoid contents of these samples were statistically different from each other ( $P<0.05$ ). In the previous study, the total phenolic and flavonoid contents of fig vinegar were determined as 935.5 mg GAE/L and 178.45 mg catechin/L, respectively (Sengun et al., 2020), which were higher than the results of the current study. In the literature, wide variety of total flavonoid contents were also reported for different vinegars, such as 14.43-349.05 mg catechin/L for homemade grape vinegars, 10.89-188.43 mg catechin/L for homemade apple vinegars (Ozturk et al., 2015) and 1655-3999 mg Rutin equivalent/L for nine different Shanxi aged vinegars (Xia et al., 2018). All these results confirm that the amount of total phenolic and flavonoid contents of vinegars may change depending on the variety and the growth area of fig fruit and the technology used during vinegar production.

There are numerous methods developed for the assessment of antioxidant activities of various materials (Sevindik et al., 2017; Mohammed et al., 2019). The outcomes of the methods based on the deactivation of radicals, such as DPPH and ABTS, differ inside reactions appearance and reaction kinetics (Layina-Pathirana et al., 2006; Mareček et al., 2017). As it can be seen from Table 2, DPPH and ABTS<sup>+</sup> radical scavenging activities of fig vinegars varied in the range of 1.25-7.51  $\mu\text{g TE/mL}$  and 0.44-3.98  $\mu\text{g TE/mL}$ , respectively ( $P<0.05$ ).



**Figure 1.** Color properties of fig vinegars (Different letters on data bars indicate a significant difference ( $P<0.05$ ). Statistical analysis was applied separately for  $L^*$ ,  $a^*$ ,  $b^*$  values).

As in parallel with the amount of total phenolic and flavonoid contents, the highest antioxidant activity was determined in Sample A. In the previous studies, wide range of antioxidant activities were reported for fruit vinegars, mainly for pomegranate, grape and apple vinegars (Budak et al., 2011; Bertelli et al., 2015; Ozturk et al., 2015; Bakir et al., 2017; Kharchoufi et al., 2018). However, there is no data about the antioxidant activity of fig vinegar. Hence, it is the first study to report antioxidant activity of fig vinegar, which is highly correlated with its total phenolic and flavonoid contents.

#### The MIC and MBC Values

The MICs of vinegar samples were determined against eight microorganisms using a 96-well microtiter plate method, containing the final concentrations of vinegar in the range of 0.10%-50% (v/v). Vinegar samples were found inhibitive for all microorganisms tested at concentration 12.5% (MIC, v/v). Sample A, which has the highest acidic, phenolic and flavonoid contents showed stronger antimicrobial activities than other samples against test microorganisms, except *P. acidilactici*. The most sensitive bacteria to sample A were found as *B. subtilis* with MIC of 0.39%. *P. acidilactici* and *S. typhimurium* was the most resistant bacteria against sample A and C, respectively (Table 3). Moreover, bactericidal effect was determined only in sample A for all test microorganisms, except *E. coli* O157:H7 and *P. acidilactici*, and also the highest bactericidal effect was observed to *B. subtilis* with MBC of 3.12% (Table 4). In our previous study, the MIC value of fig vinegar on *L. monocytogenes*, *E. faecalis*, *B. subtilis*, *S. aureus*, *E. coli* O157:H7, *S. Typhimurium*, *E. coli*, *B. cereus* and *P. acidilactici* was determined in the range of 3.12-6.25%, while the MBC value of the vinegar was 50% for all test cultures (Sengun et al., 2020). In a study carried out by Ozturk et al. (2015), *B. cereus* was determined as the most sensitive strain against traditionally produced fruit vinegars. In another study (Bakir et al., 2017), balsamic vinegar was shown the highest antimicrobial activity against *S. typhimurium*. All the results demonstrated that the antimicrobial activity of vinegars may change depending on the test culture and the amounts of acidity, total phenolic and flavonoid contents of vinegars.

#### Conclusion

The results of the study demonstrated that the properties of traditional fig vinegar vary in a wide range. Since the production of fig vinegar took place under uncontrolled conditions, some vinegar samples were not in conformity with the Standards, in terms of acidity and residual alcohol content. However, high levels of total phenolic and flavonoid contents, especially in the sample produced from dark colored figs (Sample A), were observed in vinegars, which lead to indicate high antioxidant activities. Besides, fig vinegars exhibited antimicrobial activity to all test bacteria in various sensitivities. Further analysis is needed to determine the phenolic profile of fig vinegar.

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