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Microbiological and chemical properties of fig vinegar produced in Turkey

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Fig vinegar is a traditional fermented product produced mainly from fresh or dried fig in Turkey. The aim of the present study was to investigate the microbiological and chemical properties of traditional fig vinegars. Vinegar samples produced with different receipts by using different types of raw materials and the fermentation conditions, were collected from eight different regions of Turkey and analysis results were compared to understand the factors affecting the properties of vinegars. Total mesophilic aerobic bacteria (TMAB), yeast, lactic acid bacteria (LAB) and acetic acid bacteria (AAB) ranged from 2.26 to 7.29 log cfu/ml, 0.00 to 6.49 log cfu/ml, 0.81 to 8.20 log cfu/ml and 2.68 to 8.23 log cfu/ml, respectively. Samples were found negative for mould, *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella* spp., *Escherichia coli* and *Bacillus cereus*. Chemical properties including pH value, total acidity, non-volatile acidity, volatile acidity, ash, specific gravity and alcohol content of vinegar samples were determined as 3.05 to 3.73, 2.10 to 6.97 g/100 ml, 0.07 to 0.53 g/100 ml, 1.97 to 6.46 g/100 ml, 1.11 to 5.60 g/l, 1.0002 to 1.1448 and <0.5%, respectively. The results presented showed that the microbiological and chemical properties of fig vinegar changed depending on the raw materials, the fermentation time and techniques used in its production.

Key words: Fig, vinegar, quality, microbiology, chemical.

INTRODUCTION

Several types of vinegar are used in foods as flavouring and acidifying agent, especially for salad vegetables. Fig (*Ficus carica*) fruit is native in Anatolia and Mediterranean Region, and Turkey has an important genetic source for the agricultural crops with widespread varieties (Şimşek, 2010). Turkey is one of the major fig producers with 210152 tons/year and exportation ratio of dry and fresh fig is increasing day by day with the increased possi-bilities and developments in packaging industry for table fruits (Şimşek, 2010). Although fig fruit is commonly consumed as fresh or dried fruit, it is also processed to vinegar for obtaining very special taste for flavouring.

In the classical world, Hippocrates of Kos was the first to prescribe vinegar as the main remedy against a variety of diseases, such as common cold and cough. It is reported that vinegar helps lower cholesterol; cures eye infections; fights age and liver spots; relieves night time leg cramps; soothes sprained muscles; forestalls osteo-porosis; helps fade away headaches; relieves calluses, skin rashes, athlete's foot, insect bites; is a good remedy for urinary problems, for coughs, colds; relieves heart and circulatory problems, lowers high blood pressure and destroys bacteria in foods (Orey, 2009). There are limited studies on the microbiological proper-ties of vinegar, which mainly focused on industrial vine-gars such as persimmon vinegar, rice vinegar, wine vinegar and traditional balsamic vinegar (De Vero et al., 2006; Giudici et al., 2009; Mamlouk et al., 2011; Hidalgo et al.,

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2013). On the other hand, there has been no study done regarding the properties of fig vinegar, according to the best of the author's knowledge.

Fig vinegar has a great importance in the historical development of vinegar production. There are several different receipts and production techniques for homemade fig vinegar in different regions of Turkey (Figure 1). It is mainly prepared from fresh or dried fig. On the other hand, fresh grape fruit can be added to the mix, which affects the colour and taste of the end product, depending on the region of the production. The fermentation process is obtained by two-stage: In the first step, fermentable sugars are converted into ethanol by yeasts and secondly, acetic acid bacteria (AAB) oxidize the ethanol to acetic acid. This spontaneous fermentation occurs for 8 to 14 weeks, at room temperature, until desired acidity (at least 4%, w/v) and flavour is obtained. It is commonly known that the production of fig vinegar is not an easy work. Low acidic value of fig fruit (0.18 to 0.48%, w/v) provides a suitable condition for uncontrolled microbial growth during fermentation period (Küden et al., 2008). If the production occurs under poor hygienic conditions and acidification starts too late, the product can be easily spoiled before the vinegar is produced (Sokollek et al., 1998). Moreover, there are some difficulties, which limit the production of fig vinegar in the industrial scale, such as filtration problems (cause of the fibrous structure of the fruit), lots of waste and high cost of refining.

The objective of this study was to investigate the microbiological and chemical properties of fig vinegar, produced traditionally by using different recipes.

MATERIALS AND METHODS

Sample collection

Homemade fig vinegar samples produced traditionally with different receipts were collected from eight different cities of the Aegean region in Turkey (Table 1). The samples, which completed the fermentation periods and ready for consumption, were stored at 4°C before used in the experiments.

Microbiological analyses

25 ml sample was taken under aseptic conditions, and transferred into 225 ml 0.1% peptone water (PW, pH 6.3 ± 0.2, Oxoid, Basignstoke, Hampshire, England) to determine the microbiological quality of fig vinegar. Appropriate ten-fold dilutions of the samples were prepared in PW and plated on growth media in duplicate to estimate microbial counts.

Total mesophilic aerobic bacteria (TMAB) count was determined by using pour plate method on Plate Count Agar (PCA, pH 7.1 ± 0.2, Oxoid) and the plates were incubated at 30°C for 24 to 48 h (FDA-BAM, 2001a). The counts of yeast and mould were determined on acidified Potato Dextrose Agar (PDA, pH 5.6 ± 0.2, Oxoid) with 10% of tartaric acid (Merck) by using pour plate method and plates were incubated at 25°C for 3 to 5 days (FDA-BAM, 2001b). Double plated Man Rogosa and Sharp Agar (MRS, pH 6.2±0.2, Oxoid) and M17 Agar (pH 6.9 ± 0.2, Oxoid) plates were used to count lactic acid bacteria (LAB) and these plates were incubated at 30°C for 3 to 5 days (Sharpe et al., 1966; Kandler and Weiss, 1986). Glucose Yeast Extract Calcium Carbonate Agar (GYC, 10% glucose, 1% yeast extract, 2% calcium carbonate, 1.5% agar, pH 6.8 ± 0.2) and Yeast Extract Peptone Mannitol Agar (YPM, 0.5% yeast extract, 0.3% peptone, 2.5% mannitol, 1.2% agar, pH 7.0±0.2) were used by surface plate method to count acetic acid bacteria (AAB) and the plates were incubated at 30°C for 5 to 10 days (De Vero et al., 2006).

Staphylococcus aureus was determined by surface plating on Baird-Parker Agar (BPA, pH 6.8 ± 0.2, Oxoid) and the plates were incubated at 37°C for 48 h (FDA-BAM, 2001c). After primary enrichment step, Oxford Agar (pH 7.0 ± 0.2, Oxoid) and Palcam Agar (pH 7.2 ± 0.2, Oxoid) were used for the isolation of *L. monocytogenes* and plates were incubated at 35°C for 48 h (FDA-BAM, 2011). *Salmonella* detection was applied after pre-enrichment and enrichment steps by using streak plate method on Xylose Lysine Desoxycholate Agar (XLD, pH 7.4 ± 0.2, Oxoid) and Brillant Green Agar (BGA, pH 6.9 ± 0.2, Oxoid) and incubating the plates at 37°C for 24 h (FDA-BAM, 2007). Enumeration of *Escherichia coli* was performed by most probable number technique. Lauryl Sulphite Tryptose Broth (LSTB, pH 6.8 ± 0.2, Oxoid) tubes were incubated at 37°C for 24 to 48 h and after incubation period, the tubes that produced gas, were inoculated to *E. coli* Broths (EC, pH 6.9 ± 0.2, Difco Laboratories, Detroit MI, 48232-7058 USA) and incubated at 44.5°C for 24 to 48 h (FDA-BAM, 2002). *B. cereus* counts were determined by using surface plating on Mannitol Egg Yolk Polimixin Agar (MYP, pH 7.2 ± 0.2, Oxoid) and plates were incubated at 30°C for 24 h (FDA-BAM, 2001d).

Chemical analysis

The pH value, total acidity, volatile and non-volatile acidity (AOAC, 1995), ash (Anonymous, 1983), specific gravity and alcohol (Anonymous, 1976) contents of the vinegar samples were determined. Experiments were conducted in three replicates. The pH value of fig vinegar samples were detected by using previously calibrated pH meter (Nel Mod 821). Total acidity, volatile and non-volatile acidity of the samples was determined by titrimetric method and the results were expressed as the acetic acid percentage. Ash content was determined by ashing the sample at 525°C to constant weight. Alcohol contents of the samples were expressed as % (v/v) by densimetry measure using a hydrostatic balance after distillation.

Statistical analysis

Results were expressed as means ± standard deviation of three determinations and one-way analysis of variance (ANOVA). A Tukey test was carried out to assess for any significant differences between the means (MINITAB 15 Statistical Package Program). A significance level of $P<0.05$ was used for all evaluations.

RESULTS AND DISCUSSION

Microbiological properties of fig vinegar samples

Microbiological results of fig vinegar samples are represented in Table 2. TMAB counts of the samples ranged from 2.26 to 7.29 log cfu/ml ($P<0.05$) (Table 2). The highest TMAB count was obtained from Sample F, which was the unique sample produced only from fresh fig. Sokollek et al. (1998) reported that TMAB counts of grape vinegar ranged between 6.5 and 10.6 log cfu/ml.

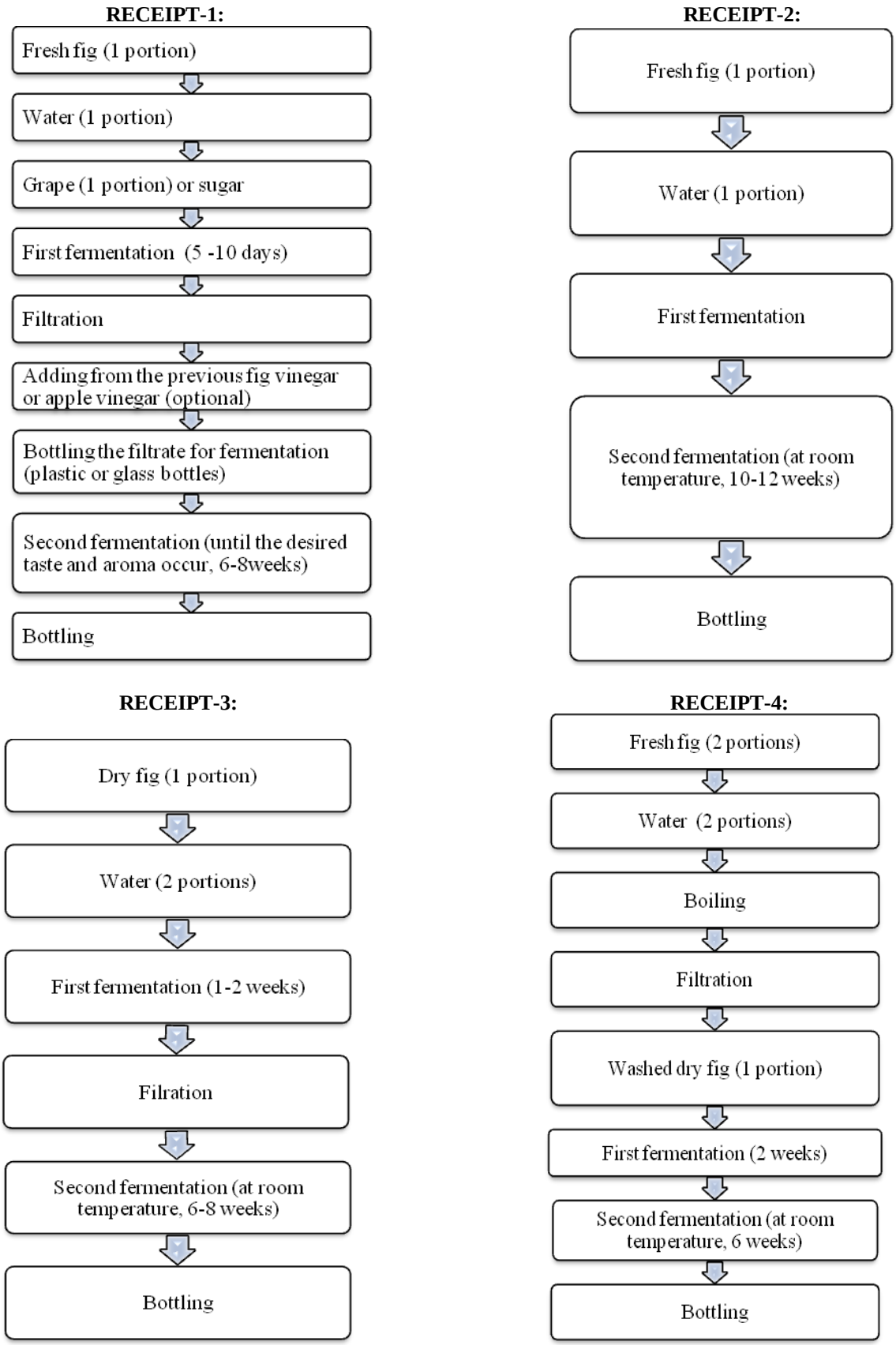

Figure 1. Different receipts used in the production of fig vinegar.

Table 1. Sampling sites, ingredients and production steps of fig vinegar samples.

Code	Sampling region/site	Ingredient	Production steps[a]
A	Aydin/Ortaklar	Dry fig, water	Receipt 3
B	Aydin/Ortaklar	Dry fig, water	Receipt 3
C	Izmir /City Center	Fresh fig , water, apple vinegar, sugar	Receipt 1
D	Izmir /City Center	Fresh fig, grape, water	Receipt 1
E	Izmir /Birgi	Fresh fig, dry fig, water	Receipt 4
F	Izmir/Hatay	Fresh fig, water	Receipt 2
G	Aydın/City Center	Dry fig, water	Receipt 3
H	Izmir /Odemiş	Dry fig, water	Receipt 3

[a] Receipts are given in Figure 1.

Table 2. Microbiological results of fig vinegar samples.

Samples[a]	Lactic acid bacteria (log-cfu/ml)[c]		Acetic acid bacteria (log-cfu/ml)[c]		Total mesophilic aerobic bacteria (log-cfu/ml)	Mould and yeast (log-cfu/ml)
	MRS	M17	GYC	YPM		
A	0.81±0.133**b**[a]	3.11±0.208[a]	3.09±0.075[a]	3.25±0.189[a]	3.25±0.211[a]	0.00±0.000[a]
B	2.52±0.297[b]	2.10±0.126[b]	2.68±0.140[b]	2.73±0.170[b]	2.26±0.360[b]	2.49±0.133[b]
C	6.88±0.125[c]	6.63±0.141[c]	5.85±0.419[c]	7.01±0.180[c]	6.90±0.615[cd]	6.49±0.162[g]
D	3.40±0.185[d]	2.99±0.132[a]	3.67±0.132[d]	4.84±0.114[d]	4.90±0.272[e]	4.85±0.164[c]
E	4.09±0.091[e]	4.01±0.081[d]	3.65±0.195[d]	4.33±0.108[e]	4.13±0.120[f]	3.82±0.115[d]
F	8.20±0.345[f]	7.59±0.280[e]	5.27±0.109[e]	8.23±0.150[f]	7.29±0.202[c]	0.00±0.000[a]
G	3.90±0.143[e]	3.89±0.169[d]	3.21±0.091[a]	5.18±0.125[g]	4.90±0.091[e]	4.78±0.111[e]
H	6.59±0.103[c]	6.59±0.179[c]	6.51±0.128[f]	6.71±0.209[h]	6.69±0.198[d]	4.28±0.059[f]

[a]For explanation see Table 1. **b**Values represent the mean of three determinations ± SD. Values in the same column with the different letter are statistically different (P < 0.05). [c]Two different media were used for enumeration of lactic acid bacteria (MRS and M17) and acetic acid bacteria (GYC and YPM).

These differences could be explained by the types of samples analysed, which were produced from different raw material, fermentation time, fermentation temperature, etc.

Yeast counts of the samples ranged between 0.00 to 6.49 log cfu/ml, while no mould growth was observed for eight vinegar samples (Table 2). Sample A and F were found negative for yeast, while other sample's yeast counts were significantly different from each other (P<0.05). During alcoholic fermentation, which is usually completed in a few weeks, the numbers of yeasts increase rapidly ranging between 10^2 and 10^6 cfu/g (Solieri et al., 2006). In this step, yeasts are able to metabolize carbohydrates to ethanol, carbon dioxide and lots of secondary products. Although, yeasts have an important role in the alcoholic fermentation, the mould growth is not wanted for a healthy vinegar fermentation process. As an alternative, the back-slopping practice is usually applied to speed up the fermentation and to avoid the growing of moulds (Solieri et al., 2006).

Lactic acid bacteria of samples were enumerated on two different media for the detection of *Lactobacillus* spp. (on MRS) and *Streptococcus* and *Lactococcus* spp. (on M17), separately. LAB counts on MRS and M17 ranged from 0.81 to 8.20 and 2.10 to 7.59 log cfu/ml, respectively (Table 2). The results showed that except sample A, no significant differences were detected between the counts on MRS and M17 plates (P>0.05). The highest LAB number was noted from sample F (Table 2), which also have the highest acidic value (Table 3). LAB population of vinegar plays a strong role in the microbial consortium of the alcohol fermentation step and also improves the taste. However, there are only a few studies which searches LAB in vinegar (Haruta et al., 2006; Wu et al., 2012).

It is known that all media do not support AAB growth equally and they are selective just for one strain or another (Gullo et al., 2006). Thus, AAB in fig vinegar samples were enumerated on two different media (Table 2). Gullo et al. (2006) reported that GYC was proposed as a medium that enable most strains to be recovered in traditional vinegars. However, in this study, the recovery of GYC and YPM plates were found significantly different in all vinegar samples (P<0.05) and lower counts were

Table 3. Chemical composition of fig vinegar samples.

Code[a]	pH	Total acidity[c] (g/100 ml)	Non-volatile acidity (g/100 ml)	Volatile acidity (g/100 ml)	Ash (g/l)	Specific gravity	Alcohol (%-v/v, 20°C)
A	3.53±0.035**b**[a]	4.72±0.106[a]	0.36±0.034[a]	4.36±0.147[a]	4.75±0.358[a]	1.0018±0[a]	<0.5
B	3.20±0.064[b]	5.51±0.053[b]	0.34±0.000[a]	5.18±0.053[b]	3.31±2.315[a]	1.0360±0[a]	<0.5
C	3.42±0.050[a]	2.10±0.000[c]	0.13±0.017[b]	1.97±0.017[c]	2.38±1.688[a]	1.0070±0[a]	<0.5
D	3.56±0.021[ac]	3.22±0.106[d]	0.17±0.034[c]	3.06±0.140[d]	1.11±0.066[a]	1.0088±0[a]	<0.5
E	3.50±0.085[a]	2.85±0.212[d]	0.07±0.034[cd]	2.78±0.243[d]	1.86±0.189[a]	1.0110±0[a]	<0.5
F	3.05±0.000[b]	6.97±0.106[e]	0.53±0.000[bce]	6.46±0.106[e]	1.62±0.486[a]	1.0341±0[a]	<0.5
G	3.71±0.014[ac]	4.57±0.106[a]	0.11±0.017[de]	4.47±0.123[a]	1.58±0.792[a]	1.0002±0[a]	<0.5
H	3.73±0.021[c]	4.65±0.000[a]	0.19±0.000[e]	4.46±0.000[a]	5.60±5.440[a]	1.1448±0[a]	<0.5

[a]For explanation see Table 1. **b**Values represent the mean of three determinations ± SD. Values in the same column with the different letter are statistically different ($P < 0.05$). [c]Acetic acid.

obtained by GYC plates. The counts obtained by GYC and YPM plates ranged from 2.68 to 6.51 and 2.73 to 8.23 log cfu/ml, respectively (Table 2). It is reported that the AAB population always have very high numbers in vinegar samples, such as 10^8 cells/ml (Torija et al., 2010). When this range is compared with the results of this study, the AAB counts are lower for the most of the fig vinegar samples. The extreme media of the fig vinegar and a significant amount of microbiota, which cannot be cultivated on standard laboratory media, may be the cause of decrease in the numbers of AAB (Sokollek et al., 1998). In recent years, interest on the microbiological aspects and function of AAB responsible for acetification processes has arisen (Mamlouk et al., 2011). A number of studies dealing with species diversity by culture and no-culture methods as well as others on the functionality of AAB and their mechanisms of resistance to vinegar environment have been published (De Vero et al., 2006; Gullo et al., 2006; Torija et al., 2010; Mamlouk et al., 2011; Wu et al., 2012; Hidalgo et al., 2013). Isolation, cultivation and preservation difficulties of AAB restrict the usage of this group as a starter culture in vinegar production. On the other hand, using AAB as a starter culture in the production of vinegar may lead to an improved fermentation process and an enhanced product quality. Thus, particular attention has been devoted to the understanding of AAB, especially viable but non-culturable state of AAB.

All samples were found negative for mould, *S. aureus*, *Listeria monocytogenes*, *Salmonella* spp., *E. coli* and *Bacillus cere*us, which means that analysed vinegars were produced under hygienic conditions with appropriate fermentation conditions. On the other hand, the chemical composition of the final product such as high acidic value can also affect the microbiological quality of the samples and show bacteriostatic effect against the pathogenic and spoilage microorganisms.

Chemical properties of fig vinegar samples

General composition of fig vinegar samples are given in Table 3. There are a few studies considering these parameters for different kinds of vinegars, but none of them is on the fig vinegar. As it can be seen from Table 3, pH values of the samples ranged from 3.05 to 3.73 ($P<0.05$). Some researchers reported that the pH values of different kind of vinegars ranged between 2.63 and 3.27 (Akbaş and Cabaroğlu, 2010) and 2.36 and 3.0 (Gerbi et al., 1998). The United States Food and Drug Administration (FDA) require that vinegar should contain at least 4% acidity, which is similarly given in Turkish Standards (Anonymous, 2004). In the present study, total acidity, non-volatile and volatile acidity of the samples were found ranging between 2.10 and 6.97 g/100 ml, 0.07 and 0.53 g/100 ml, and 1.97 and 6.46 g/100 ml, respectively (Table 3). The acidity of the samples was found meeting the criteria given in the standard (Anonymous, 2004). By the way, total acidic values of the samples, produced not only from fresh fig, but also containing some other ingredients such as apple vinegar and sugar (Sample C), grape (Sample D) or dried fig (Sample E), were found under the criteria given in the standard (Anonymous, 2004). Sample F, which had the longest fermentation time, has the highest acidity (Table 3). In the other researches, total acidity, non-volatile and volatile acidity of different kinds of vinegars were reported as between 5.0 and 8.0, 0.01 and 0.45, and 0.99 and 11.64 g/100 ml, respectively (Gerbi et al., 1998; Akbaş and Cabaroğlu, 2010). Şahin et al. (1977) showed that, addition of adjunct materials during grape vinegar production, especially wort of malt rootlets and wort of malt and yeast water, reduced the acetic acid fermentation time. The vinegar quality depends on process conditions including acidification speed. It was reported that acid production of vinegar samples were also affected by the temperature (Şahin et al., 1977). It can be concluded that AAB counts and acidity of fig vinegar samples depend on the availability of the fermentative substrates, the fermentation time and temperature of the production.

Ash determination is important to support vinegar characterization and quality evaluation (Masino et al.,

2008). Ash contents of vinegar samples ranged between 1.11 and 5.60 g/l (Table 3). Minimum limit of ash is 0.8 g/l for vinegars produced in Turkey (Anonymous, 2004). It means that all the samples met the standard for ash content. Different researchers reported that ash contents of vinegars ranged between 1.63 and 4.19 g/l (Şahin et al., 1977; Gerbi et al., 1998; Akbaş and Cabaroğlu, 2010). Specific gravity of the samples ranged between 1.0002 and 1.1448 (P>0.05) (Table 3). These results were found similar with the other researcher's findings, except sample H. Mu et al. (2003) reported the specific gravity of bamboo vinegar (Moso bamboo and Madake bamboo) as 1.0246to 1.0257 for the original vinegar, 1.0130 to 1.0095 for distilled vinegar and 1.0031 to 0.9666 for ether-extracted vinegar. In another study, it was reported that the specific gravity of grape vinegars ranged between 1.0100 and 1.0119 (Şahin et al., 1977). Some researchers investigated density, instead of specific gravity of the product. It is reported that the quality of traditional balsamic vinegar relies mainly on sugar content, density, Brix and dry residue and secondly on acidity (Masino et al., 2008). Density of grape vinegar was reported to range between 1.0016 and 1.0139 g/ cm^3 (Akbaş and Cabaroğlu, 2010).

In vinegar fermentation, alcohol is oxidized to acetic acid and residual alcohol content is used as an important parameter which represents the quality and efficiency of the product. It is reported that alcohol content of vinegars, except wine vinegars, should be under 0.5% (v/v) (Anonymous, 2004). In this study, alcohol contents of the samples were found to met the criteria given in the standard (Table 3). Similar results were obtained by Akbaş and Cabaroğlu (2010), who investigated the vinegars produced commercially in Turkey.

Conclusion

The present study is the first one which represents the chemical and microbiological properties of fig vinegar, produced traditionally in Turkey. The results of the study indicate that sample F, which is the unique sample produced only from fresh fig and has the longest fermentation time, has the highest number of TMAB, LAB and AAB. This result also explains why sample F has the highest total acidic value. Moreover, total acidic values of the samples produced by receipt 1 and 4 were less than 4% (w/v). On the other hand, it remains to be investigated in laboratory conditions how factors, such as raw materials, fermentation time, fermentation temperature, etc., affect the microbiological and chemical properties of vinegar. Although, uncontrolled fermentations have high pathogen growth risk as a result of contamination from raw material or during processing, this study showed that home-made vinegars have no risk in that point of view.

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