BURSA ULUDAĞ ÜNİVERSİTESİ ZİRAAT FAKÜLTESİ DERGİSİ
Journal of Agricultural Faculty of Bursa Uludag University
e-ISSN 2651-4044
https://dergipark.org.tr/tr/pub/bursauludagziraat
http://www.uludag.edu.tr/ziraatdergi
Haziran/2023, 37(1), s. 79-99

ARAŞTIRMA MAKALESİ RESEARCH ARTICLE

Geliş Tarihi (Received): 27.05.2022
Kabul Tarihi (Accepted): 26.12.2022

Determination of Antimicrobial Properties of Endemic Black Sakı Apple Vinegar Produced by Traditional Method Using Different Yeast Raw Materials[A]

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Abstract: In this study, it was aimed to determine the antibiotic effect of Black Sakı cider vinegar (homemade) produced with different yeasts against different pathogenic bacterial species (*E. faecalis* ATCC 29212, *S. aureus* ATCC 29213, *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *E. coli* ATCC 8739, *E. coli* (colistin R) ATCC 19846, *Klebsiella pneumoniae* ATCC 700603, *Salmonella enterica* subsp. *enterica* serovar Enteritidis ATCC 13076 and *Pseudomonas aeruginosa* ATCC 27853), with clinical antibiotic resistance by using disc diffusion and microdilution methods. In general, it had been determined that all vinegar samples had antibacterial effect, and the most antibacterial effect against all standard strains was commercial vinegar sample (No. 7 vinegar). It was determined that vinegar sample number 1 (vinegar containing 0.3% *Saccharomyces cerevisiae*) was the weakest effective vinegar sample against all other standard strains except for *Enterococcus faecalis ATCC 29212* strain. In addition, in *Escherichia coli ATCC 8739* strain, the sample number 6 was organic household vinegar, in which MIC values were obtained at 1/32 dilution, unlike the others. In conclusion, the antimicrobial effect of Black Sakı apple vinegar obtained from different yeast raw materials on various microorganisms was determined in detail. These results will form the basis of new studies and will enable studies to be conducted to investigate

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[A] This study does not require ethics committee approval. The article has been prepared in accordance with research and publication ethics.

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***Atıf/Citation:** Yangılar F., Gülhan, B., Kılıçgün H. 2023. Determination of Antimicrobial Properties of Endemic Black Sakı Apple Vinegar Produced by Traditional Method Using Different Yeast Raw Materials. *Bursa Uludağ Üniv. Ziraat Fak. Derg.,* 37(1), 79-99. https://doi.org/10.20479/bursauludagziraat.1122279

more bacterial species and their effects on human health by producing Black Sakı vinegar at different doses and techniques.

Keywords: Black sakı apple, antimicrobial, vinegar, homemade, commercial vinegar.

Farklı Maya Hammaddeleri Kullanılarak Geleneksel Yöntemle Üretilen Endemik Kara Sakı Elma Sirkelerinin Antimikrobiyal Özelliklerinin Tespiti

Öz: Bu çalışmada farklı mayalarla üretilen elma sirkelerinin (ev yapımı), kilinik olarak antibiyotik direnci olan farklı patojen bakteri türlerine karşı (*E. faecalis* 29212, *S. aureus* 29213, *S. aureus* 25923, *E. coli* 25922, *E. coli* 8739, *E. coli* (colistin R) 19846, *Klebsiella pneumoniae* 700603, *Salmonella enterica* ve *Pseudomonas aeruginosa* 27853), disk difüzyon ve mikrodilusyon metodları kullanılarak antibiyotik etkisinin belirlenmesi amaçlandı. Genel olarak bütün sirke örneklerinin antibakteriyel etkisinin olduğu, bütün standart suşlara karşı en fazla antibakteriyel etkinin ticari sirke örneği (7 numaralı sirke) olduğu tespit edilmiştir. 1 numaralı sirke örneğinin ise (%0.3 *Saccharomyces cerevisiae* içeren sirke*) Enterococcus faecalis ATCC 29212* suşu hariç bütün diğer standart suşlara karşı en zayıf etkili sirke örneği olduğu tespit edilmiştir. Ayrıca *Escherichia coli ATCC 8739* suşunda diğerlerinden farklı olarak 1/32 dilusyonda MIC değerlerinin elde edildiği örnek 6 numaralı organik ev sirkesi olmuştur. Sonuç olarak, bu çalışmada farklı maya hammaddelerinden elde edilen elma sirkelerin çeşitli mikroorganizmalar üzerindeki antimikrobiyel etkisi detaylı olarak belirlenmiştir. Bu sonuçlar yeni çalışmalara temel oluşturacak niteliktedir ve farklı dozlarda ve tekniklerde sakı elma sirkesi üretilerek daha fazla bakteri türüne ve insan sağlığına etkilerinin de araştırıldığı çalışmaların yapılmasına olanak sağlayacaktır.

Anahtar Kelimeler: Kara sakı elma, antimikrobiyal, sirke, ev yapımı, ticari sirke.

Introduction

Vinegar is a sour-tasting fermented product obtained by the anaerobic conversion of sugars to ethanol by yeasts and aerobic oxidation of ethanol to acetic acid by bacteria. Those obtained from rice and wheat are classified as “grain vinegar”, and vinegar obtained from grape, apple and coconut is classified as “fruit” vinegars (Chen et al., 2016). Vinegar, which has a history of about 3000 years, plays an important role in our daily life (Tesfaye et al., 2002; Chen et al., 2017; Jiang et al., 2019). In addition, scientists accept vinegar as a "superfood" that is claimed to be good for weight loss, digestion, and skin health. There are even vinegar diets available. In the oldest sources, there is information that Hippocrates (approximately 420 BC) used vinegar for the treatment of wounds 2300 years ago (Johnston and Gaas, 2006; Santos et al., 2019). Various vinegars are produced from different raw materials such as rice, onion, tomato, apple, cider, pineapple and honey (Solieri and Giudici, 2009). The role of

biologically active ingredients, which have many physiological effects on human metabolism, is important (Taş and Güneşer, 2021). Especially, vinegaris also a rich in nutritional and bioactive compounds such as amino acids, sugars, organic acids, polyphenols, melanoidins and tetramethyl pyrazine (Ho et al., 2017; Xia et al., 2018). These organic acids in vinegar are not only nutrients, but also bioactive compounds with antimicrobial and anti-inflammatory effects, suppressing fat accumulation and hyperlipidemia, regulating insulin resistance and metabolism, contributing to weight loss, antihypertensive and reducing fatigue (Hindi, 2013; Petsiou et al., 2014). In addition, some studies have suggested that it has an antitumor effect (Baba et al., 2013; Xia et al., 2020). Due to the use of a wide variety of fruits and vegetables in vinegar, which are well known to be important sources of phenolic compounds and organic acids, the final product has strong antioxidant potential and antimicrobial activity (Charles et al., 2000; Chang et al., 2005; Bakır et al., 2017). Fermented foods have been reported to exert anti-obesity effects by altering the composition of the gut microbiota and the expression of genes related to the metabolic syndrome (Han et al., 2015). Among these fermented foods, vinegar, which is an acidic food flavor, has become a product that has received a lot of attention recently, as it exhibits multiple bioactivities such as anti-hypercholesterolemic, anti-hyperglycemic, anti-hypertension, anti-microbial, anti-cancer, anti-thrombotic (Mohamad et al., 2015; Beh et al., 2017).

There are some reasons that give vinegar antimicrobial properties. Vinegar has been used among the people for the treatment of nail fungus, head lice, warts, ear cleaning and outer ear infections. Consumers generally prefer natural preservative methods to prevent the development of foodborne pathogenic microorganisms. Organic acids and mainly acetic acid in vinegar cause bacterial cell death by acting on the cell membranes of microorganisms. Vinegar, also known as acetic acid among natural products, contains disinfecting properties (Nascimento et al., 2003; Saqib, 2017).

Recently, various vinegars have been prepared by using different yeasts at home due to its contribution to the development of the immune system along with its use for seasoning purposes. Although the substrates and final products show some differences in homemade vinegar production, the process always includes alcohol and acetic acid fermentation, which are the main stages of vinegar production (Rosma et al., 2016; Kılıç and Şengün, 2021). There is limited information on traditional homemade vinegars produced from different types of raw materials. Apple species such as Fuji, Catarina, Golden Delicious, and Ida Red show differences in terms of chemical composition and phenolic content. In addition, the polyphenolic content is higher in the peel compared to apple pomace, as it is mostly found in the peel (Tsao et al., 2005; Vieira et al., 2009; Du et al., 2020). In addition, the production methods and raw materials used in vinegar production can also cause differences in the phenolic composition of vinegar (Budak and Güzel-Seydim, 2010; Bakır et al., 2016; Anonymous, 2022). Many studies have shown that the presence of phenolic compounds in vinegar supports antibacterial activity (Kara et al., 2021; Ousaaid et al., 2022). Some researchers report that different types of vinegar effectively inhibit the growth of foodborne pathogens, including *Bacillus cereus, Aeromonas hydrophila, Vibrio parahaemolyticus, Escherichia coli* O157:H7, *Salmonella enteritidis, S. typhimurium* and *Staphylococcus aureus* were used for disinfection of food preparation surfaces and equipment (Karabıyıklı and Şengün, 2017). In this study, based on the a fore mentioned promising health and food safety properties of vinegar products, it is from the Black Sakı

apple, an endemic apple variety that grows in eastern Turkey and has two types as Black Sakı and White Sakı (Gökşen and Keleş, 2020). It was aimed to elucidate the antimicrobial activities and mechanisms of action of 6 different Kara Sakı vinegar samples were produced using different yeasts by comparing them with commercial apple cider vinegar samples. In order to determine the antimicrobial activity, gram positive and gram negative bacteria were tried to be selected, which cause the most infections in humans. Standard ATCC strains (ATCC®, American Type Culture Collection) whose resistance profiles are known all over the world were used for standardization of results and comparisons.

Material and Method

Within the scope of the study, Black Sakı Apple grown under organic conditions for vinegar production was selected from Elmaköy Village of Erzincan province in September 2021 and stored until the production time. Yeast substances in vinegar production; for vinegar production, lyophilized yeast of *Saccharomyces cerevisiae,* which was purchased from Pak Maya used with an initial count of 10^7 cfu/mL as the first yeast Organic Kavılca wheat was obtained from a local production in Hacıpiri village of Akyaka district of Kars. Chickpea, barley, organic honey, molasses, and ready-made vinegar were obtained from local companies, while apple cider vinegar, traditionally produced from the same type of apple, was procured from the manufacturer and used in production. In addition, the whey obtained by producing cheese was used in the production of vinegar.

Vinegar Production

Traditionally produced from apples, Black Sakı apple cider vinegar was produced as two samples for each fermentation culture. The vinegar production flow chart was given in Figure 1. Briefly, in the production of vinegar by the slow method, some modifications were made to the method reported by Aktan and Yıldırım (2011). To produce vinegar, 350 grams of sliced apple after stem and seed separation. The mixture was then weighed into 1-liter glass jar for each trial. In another sterile beaker, 0.3 g of Pak Maya brand *Saccharomyces cerevisiae*, 2.5% chickpea, 2.5% barley, 2.5% buckwheat, 2.5% whey, natural vinegar and commercial vinegar were taken. Then except for natural vinegar and commercial vinegar they were added to the jar and mixed (2.5% honey + 2.5% molasses). In the next process, 15 ml vinegar and drinking water up to the neck level of the jar was added to the jar and mixed with the help of a spoon to ensure homogenization. The mouth of each jar was wrapped with cheese cloth and covered in an oxygen-proof way with the help of parafilm, this stage was and left for ethyl alcohol fermentation at 25˚C. Then, mixing process was applied every day until the shells collapsed to the bottom. After the shells settled to the bottom, vinegar (15 mL) was added and mixed again and left to acetic acid fermentation (25˚C) with only cheese cloth at the mouth. The step up to the ethyl alcohol production was the oxygen-free step, and the acetic acid production step was the oxygen step after observing the formation of mother of vinegar in the jar during fermentation and the collapse of this mother of vinegar, the vinegar produced

was filtered with the help of cheese cloth. Vinegar production was carried out in two parallels in all apple varieties and the pictures of the production stage were given in Figure 2. Vinegar samples were prepared for analysis by filtering through a 0.45 µm membrane filter before had used in the tests.

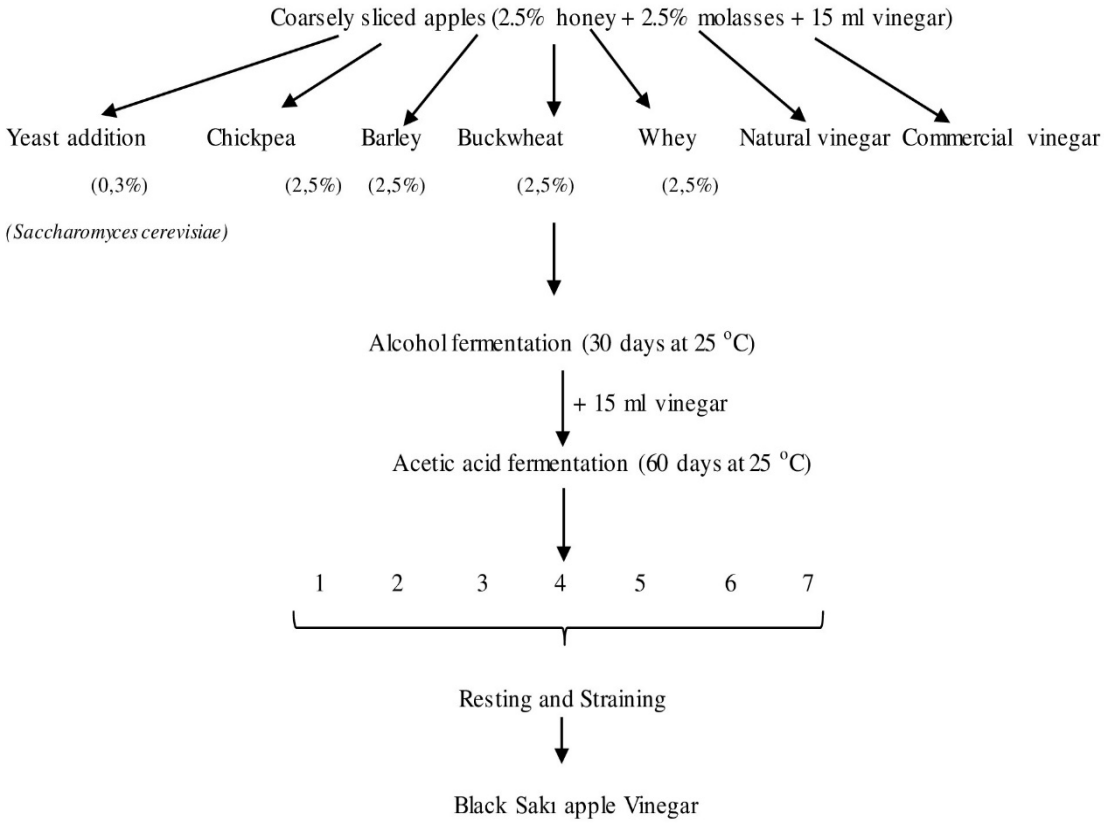

Figure 1. *Flow chart of Kara Sakı vinegar samples*

Figure 2. *Vinegar production stages*

Antimicrobial Analysis

The vinegar samples produced in the study and the test microorganisms was given in Table 1. For the evaluation of antimicrobial activity, *E. faecalis* ATCC 29212, *S. aureus* ATCC 29213, *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *E. coli* ATCC 8739, *E. coli* (colistin R) ATCC 19846, *Klebsiella pneumoniae* ATCC 700603, *Salmonella enterica subsp. enterica* serovar Enteritidis ATCC 13076 and *Pseudomonas aeruginosa* ATCC 27853 standard strains were used.

Clinical isolates of microorganisms were obtained from Erzincan Binali Yıldırım University Faculty of Medicine Microbiology laboratory. Standard strains were first transferred to Brain heart infusion broth (bio Merieux, France) medium and incubated for 1 night, then passaged into media with 5% sheep blood (bioMerieux, France), and then inoculated from fresh passages into brain heart infusion broth (bioMerieux, France) media. Standard strains inoculated into Brain heart infusion broth (bioMerieux, Fransa) media were prepared using DensiCHEK™ Plus densitometer device (bioMerieux, Fransa) at 0.5 McFarland turbidity standard ($1.5x10^8$ microorganisms per ml). These prepared bacterial suspensions were used in liquid microdilution method and disk diffusion method to investigate the antimicrobial effect of vinegar samples.

Table 1. Vinegar samples by groups, test microorganisms and antibiotics used

Vinegar code	**Different yeasts used in vinegar production**	
Vng 1	0.3% *Saccharomyces cerevisiae*	
Vng 2	Chickpea	
Vng 3	Barley	
Vng 4	Buckwheat	
Vng 5	Whey	
Vng 6	Organic household vinegar	
Vng 7	Commercial vinegar	
Test microorganisms		**Antibiotics**
P_1	*E. faecalis* ATTC 29212	Ampicillin
P_2	*S. aureus* ATTC 29213	Vancomycin
P_3	*S. aureus* ATTC 25923	Vancomycin
P4	*E. coli* ATTC 25922	Ertapenem
P5	*E. coli* ATTC 8739	Ertapenem
P6	*E. coli* (colistin R) ATTC 19846	Ertapenem
P7	*Klebsiella pneumoniae* ATTC 700603	Ertapenem
P8	*Salmonella enterica* subsp. *enterica* serovar Enteritidis ATCC 13076	Ampicillin
P9	*Pseudomonas aeruginosa* ATTC 27853	Meropenem

Liquid Microdilution Method, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Detection (this section was re named)

For antimicrobial tests, sterile microdilution plates with 96-well U-bottom wells were used. Serial dilutions of vinegars whose antimicrobial activity was investigated were done with brain heart infusion broth (bio Merieux, France). 200 microliters of 7 different vinegar samples were added to the first wells and 100 microliters of Brain heart infusion broth (bioMerieux, France) was added to the next wells. Then, 100 microliters were taken from the first wells and placed in the second wells and pipetted, and the vinegar sample was diluted in half. This process was continued by reducing the vinegar concentration by half. Then, standard bacterial suspensions prepared in 0.5 McFarland turbidity standard were added to these wells. In addition, the last three wells were designed as follows: only vinegar was placed in the first well, broth, which was a positive control with pathogen added to the second well, and brain heart infusion broth, which was used as a negative control, was placed in the last well. Following this procedure, the plates were incubated for 24 hours at 150 rpm at 35 degrees using a heidolph brand shaking incubator device (Germany), (Figure 3). After the MIC values were determined, 10-microliter passages were made on 5% sheep blood agar medium and incubated for another 24 hours, then the last well without growth was detected and MBC values were determined.

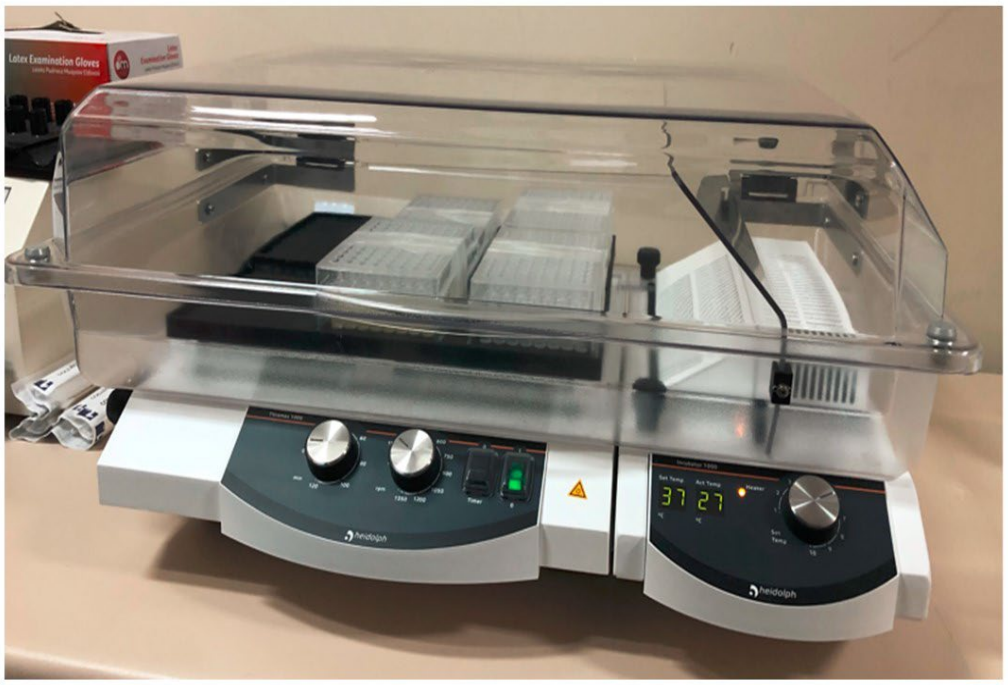

Figure 3. *Incubation of apple vinegar samples in the shaking incubator*

Preparation of Discs and Disc Diffusion Method

Vinegar samples, after passing through a 0.2 µm filter (Minisart Syringe Filter, Cellulose Acetate, Sartorious Stedim Biotech), were absorbed into 6 mm diameter sterile empty discs (Oxoid) as 20 µL. Ampicillin (AMP, 10 µg/disc, Oxoid) and gentamicin (CN, 10 µg/disc, Oxoid) discs were used as positive control, and sterile water-impregnated discs were used as negative control. Mueller Hinton Agar media (Biomerioux), vinegar-impregnated discs (Oxoid), and standard bacterial suspensions prepared in 0.5 McFarland turbidity standard were used for the disc diffusion method (Figure 4). The prepared bacterial suspensions were spread on the surface of the medium with sterile cotton swabs, and discs impregnated with vinegar were placed within 15 minutes. Vinegar-impregnated discs were placed on the media inoculated with the test microorganisms, with a minimum distance of 24 mm from each other and 18 mm from the petri dish. After this procedure, the petri dishes were incubated at 37 degrees. After 24 and 48 hours, the zone diameters were measured, and the results were evaluated.

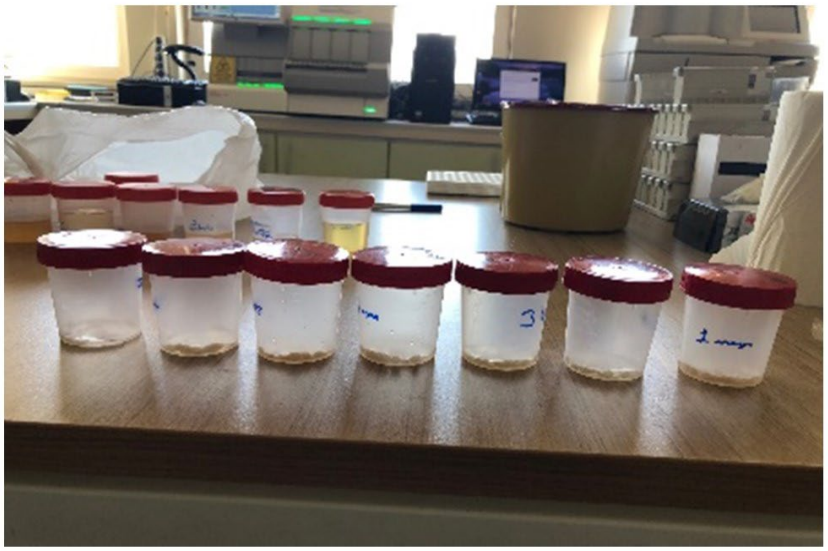

Figure 4. *Disk diffusion process*

Results and Discussion

Different microorganism's standard strains, which were *E. faecalis* ATCC 29212, *S. aureus* ATCC 29213, *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *E. coli* ATCC 8739, *E. coli* (colistin R) ATCC 19846, *Klebsiella pneumoniae* ATCC 700603, *Salmonella enterica subsp. enterica* serovar Enteritidis ATCC 13076 and *Pseudomonas aeruginosa* ATCC 27853 were used to observe the antimicrobial effect of Black Sakı apple vinegar. MIC and MBC values of vinegar samples prepared from different raw materials were given on the plates presented in Figure 5.

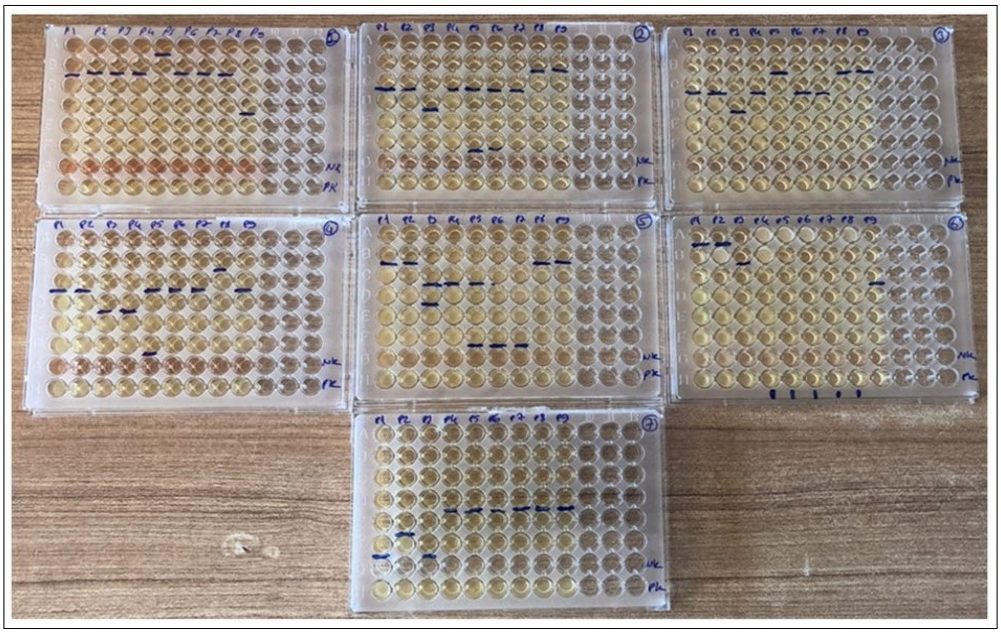

Figure 5. *Plates were used in MIC and MBC evaluation*

MIC and MBC values obtained with vinegars against standard strains were investigated. Considering the *Pseudomonas aeruginosa* ATCC 278536 strain, the most effective vinegar was vinegar number 7 with MIC values at 1/32 dilution and MBC at ¼ dilution. This was followed by MIC at ¼ dilution and MBC in the first well without dilution, followed by vinegar samples 2, 3, 4 and 6. The MIC value in vinegars 1 and 5 was obtained at ½ dilution, and the MBC value in the first well without dilution was found in the 5th vinegar, while the MBC value could not be determined in the vinegar number 1, Table 2.

Table 2. MIC and MBC breakpoint values obtained against standard strains

Pathogenic bacteria	Vinegar	MIC value dilution	MBC value dilution	Disc Diffusion
Pseudomonas aeruginosa ATCC 278536	Vng 1	1/2	-	-
	Vng 2	1/4	1	-
	Vng 3	1/4	1	-
	Vng 4	1/4	1	-
	Vng 5	1/2	1	-
	Vng 6	1/4	1	-
	Vng 7	1/32	1/4	8
Klebsiella pneumoniae ATCC 70063	Vng 1	1/2	-	-
	Vng 2	1/4	1	-
	Vng 3	1/4	1/2	-
	Vng 4	1/4	1	-
	Vng 5	1/2	1	-
	Vng 6	1/4	-	-
	Vng 7	1/16	1/2	8

Tablo 2. Devamı

Escherichia coli ATCC 25922	Vng 1	1/2	-	-
	Vng 2	1/8	1	-
	Vng 3	1/8	1	-
	Vng 4	1/8	1	-
	Vng 5	1/8	1	-
	Vng 6	1/8	1	-
	Vng 7	1/32	1/4	10
Escherichia coli (colistin R) ATCC 19846	Vng 1	1/2	-	-
	Vng 2	1/4	1	-
	Vng 3	1/4	1	-
	Vng 4	1/8	1	-
	Vng 5	1/4	1	-
	Vng 6	1/8	-	-
	Vng 7	1/8	1/4	10
Salmonella enterica subsp. *enterica* serovar Enteritidis ATCC 13076	Vng 1	1	-	-
	Vng 2	1/4	1	-
	Vng 3	1/2	1	-
	Vng 4	1/4	1	-
	Vng 5	1/4	1	-
	Vng 6	1/8	-	-
	Vng 7	1/8	1/4	-
Escherichia coli ATCC 8739	Vng 1	1/2	-	-
	Vng 2	1/4	1	-
	Vng 3	1/4	1	-
	Vng 4	1/4	1	-
	Vng 5	1/32	1	-
	Vng 6	1/32	-	
	Vng 7	1/8	1/4	12
Staphylococcus aureus ATCC 25923	Vng 1	1/2	-	-
	Vng 2	1/4	1	-
	Vng 3	1/4	1	-
	Vng 4	1/4	-	-
	Vng 5	1/4	1	-
	Vng 6	1/4	-	-
	Vng 7	1/8	1/4	12
Staphylococcus aureus ATCC 29213	Vng 1	1/2	-	-
	Vng 2	1/2	1	-
	Vng 3	1/2	1	-
	Vng 4	1/2	-	-
	Vng 5	1/2	1	-
	Vng 6	1/2	-	-
	Vng 7	1/8	1/4	10
Enterococcus faecalis ATCC 29212	Vng 1	1/8	-	-
	Vng 2	1/2	-	-
	Vng 3	1/2	-	-
	Vng 4	1/4	-	-
	Vng 5	1/2	-	-
	Vng 6	1/4	-	-
	Vng 7	1/8	1/2	-

When looked at *Klebsiella pneumoniae* ATCC 70063 strain, vinegar number 7 was found to be the most effective vinegar with MIC value at 1/16 dilution and MBC value at ½ dilution. This was followed by the example of vinegar 3 with MIC at ¼ dilution and MBC at ½ dilution. This was followed by vinegars 2 and 4 with ¼ MIC and MBC values in the first well without dilution. Then, while the MIC value was determined in ¼ dilution in vinegar number 6, the MBC value could not be determined. In addition, the MIC value was determined in vinegar number 5 at ½ dilution, and the MBC value was found in the first well without dilution, Table 2.

When MIC and MBC values were examined against *Escherichia coli* ATCC 25922 strain, it was observed that the most effective vinegar was 7 number sample with MIC value in 1/32 dilution and MBC value in ¼ dilution. Vinegars numbered 2, 3, 4, 5, 6 showed equal effects with MIC and 1 MBC values at 1/8 dilution. The least effective vinegar, on the other hand, was the vinegar sample number 1 with the MIC value at ½ dilution without the MBC value, Table 2.

When we loked at the ATCC 19846 of *Escherichia coli* (colistin R), which was a more resistant strain, the most effective vinegar was vinegar number 7 with MIC values at 1/8 dilution and MBC at ¼ dilution.

Considering only the MIC values, it was observed that the MIC value in 1/8 dilution and the vinegar number 7 had the same MIC value as the vinegars numbered 4 and 6. Considering the MBC values, vinegar sample number 4 with MIC values in 1/8 dilution and MBC values in the first well without dilution was followed by vinegar number 6 with MIC value at 1/8 dilution and no MBC value. This was followed by vinegars 2, 3 and 5 with MIC values in ¼ dilution and MBC in the first well without dilution. The least effective vinegar, on the other hand, was vinegar number 1, which had an MIC value at ½ dilution without MBC value (Table 2).

Considering the *Salmonella enterica* subsp. *enterica* serovar Enteritidis ATCC 13076 strain, vinegar number 7 was found to be the most effective with MIC value at 1/8 dilution and MBC value at ¼ dilution, followed by vinegar number 6 with MIC value at 1/8 dilution without MBC value. In vinegar samples (2, 4 and 5) MIC values were determined at ¼ dilution and MBC values in the first well without dilution.

In the vinegar sample number 3, MIC values were found at 1/2 dilution and MBC values were found in the first well without dilution. On the other hand, the least effective vinegar sample was the sample number 1 with the MIC value in the first well without the dilution without the MBC value, Table 2.

In *Escherichia coli* ATCC 8739 strain, MIC values were determined at 1/32 dilution in vinegars 5 and 6, unlike the others. However, when the MBC values were examined, MBC value was determined in the first well without dilution in vinegar number 5, but MBC value could not be determined in vinegar number 6. This was followed by the vinegar number 7. However, vinegar number 7 was found to be more effective than other vinegars with its MIC value in 1/8 dilution and MBC value in ¼ dilution MIC values at ¼ dilution and MBC values in the first well without dilution were determined in vinegars 2, 3 and 4. The vinegar sample that showed the least effect was vinegar number 1 with its MIC value at ½ dilution without determining the MBC value, Table 2.

In *Staphylococcus aureus* ATCC 25523 strain, number 7 was found to be the most effective sample with MIC values at 1/8 dilution and MBC at ¼ dilution. This was followed by vinegar samples 2, 3 and 5, with MIC at ¼ dilution and MBC values in the first well without dilution. In vinegar samples 4 and 6, MIC values were found at ¼ dilution without MBC values. In addition, the least effective vinegar was the number 1 vinegar, and the MIC value was determined at ½ dilution without the MBC value, Table 2.

When looking at *Staphylococcus aureus* ATCC 29213 strain, the most effective vinegar was found to be 7th vinegar with MIC values at 1/8 dilution and MBC at ¼ dilution, followed by vinegars numbered 2, 3 and 5 with MIC values at ½ dilution and MBC in the first well without dilution. This was followed by vinegars 1, 4 and 6, whose MIC value were determined only at ½ dilution without MBC value, Table 2.

When the *Enterococcus faecalis* ATCC 29212 strain was examined, vinegars 1 and 7 were found to be the most effective vinegars with a MIC value at 1/8 dilution, unlike the others. However, vinegar number 7 was found to be more effective than vinegar number 1 with its MBC value in ½ dilution, since vinegar number 1 did not have an MBC value. This was followed by vinegars 4 and 6 with MIC values at ¼ dilution, and vinegars numbered 2, 3 and 5 with MIC values at ½ dilution, Table 2.

When we look at the results of the disk diffusion test, zone diameters could be obtained with vinegar number 7 in all standard strains except *Enterococcus faecalis* ATCC 29212 and *Salmonella enterica* subsp. *enterica* serovar Enteritidis ATCC 13076. Discs prepared with other vinegars did not form zone diameters. Zone diameter values were not obtained in any of the vinegar samples in *Enterococcus faecalis* ATCC 29212 and *Salmonella enterica* subsp. *enterica* serovar Enteritidis ATCC 13076 strains. For vinegar sample number 7, zone diameters in order from largest to smallest were *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 25523, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 19846, *Pseudomon asaeruginosa* ATCC 278536, *Klebsiella pneumoniae* ATCC 70063 (Figure 6). It was determined as 12, 12, 10, 10, 10, 8, 8 mm for the standard strains, respectively. While negative control discs did not create inhibition zone diameters, antibiotic discs used as positive control were found to be effective when evaluated according to CLSI criteria (CLSI, 2020). Zones of microbial growth inhibition was indicated by clear zones and vary with vinegar dilutions for each microbe.

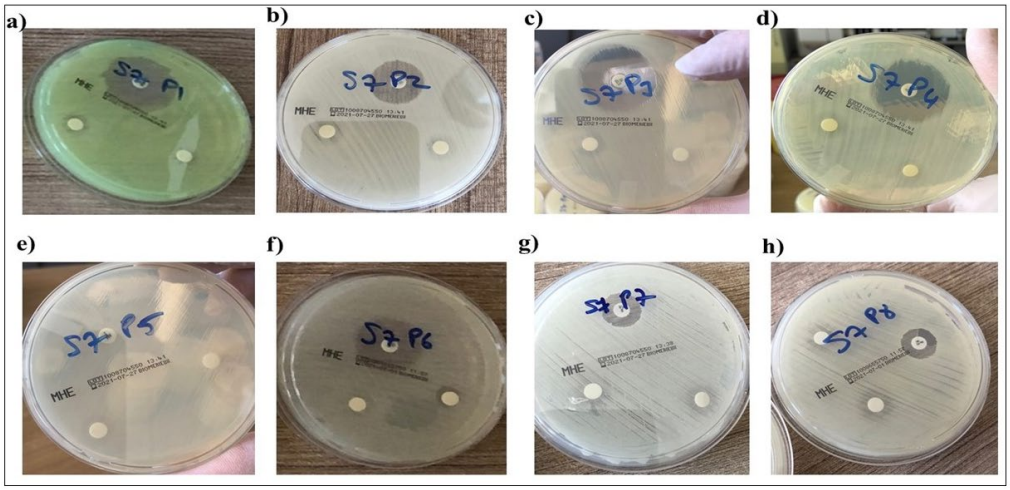

Figure 6. *Effect of varying concentrations of vinegar 7 on microbial growth after incubation at 37 °C for 24 h. (a) E. faecalis* ATCC 29212*; (b) S. aureus* ATCC 29213*; (c) S. aureus* ATCC 25923*; (d) E. coli* ATCC 25922*; (e) E. coli* ATCC 8739*; (f) E. coli (colistin R)* ATCC 19846*; (g) Klebsiella pneumoniae* ATCC 70063*; (h) Salmonella enterica* subsp. *enterica* serovar Enteritidis ATCC 13076.

Numerous studies had shown that homemade vinegar provides broad antibacterial activity on food pathogens (Janchovska et al., 2015; Öztürk et al., 2015; Gökırmaklı et al., 2019; Kalaba et al., 2019; Şengün and Kılıç, 2020a). Vinegar was rich in polyphenols such as gallic, protocatechuic, chlorogenic, caffeic acids and organic acids such as citric, malic, tartaric, lactic, acetic and succinic acids, which show antimicrobial activity (Yagnik et al., 2018; Liu et al., 2019). Organic acids act by destroying the outer membrane of bacteria, inhibiting the synthesis of macromolecules, and increasing the intracellular osmotic pressure (Chen et al., 2016). Polyphenols acted by changing the permeability of the bacterial cell wall (Bouarab-Chibane et al., 2019). Anonymous (2022) had been suggested that the raw materials in vinegars were responsible for the changes in antimicrobial activities.

Yagnik et al. (2018) investigated the antimicrobial effect of commercial apple cider vinegar on *E. coli, S. aureus* and *C. albicans*, and determined the minimum inhibitory concentrations of apple cider vinegar as 250 µg/ml for *C. albicans*, 62 µg/ml for *E. coli* and 125 µg/ml for *S. aureus*. Researchers had reported that apple cider vinegar had a direct antimicrobial effect against pathogenic microorganisms. When Table 1 was examined, there were 6 different apple cider vinegars in which different yeast raw materials were used and 1 commercial apple cider vinegar was available. When the MIC and MBC values obtained from vinegars against the standard strains in our study were examined, it was seen that the most effective vinegar for *Pseudomonas aeruginosa* ATCC 278536 was vinegar number 7 with MIC values at 1/32 dilution and MBC at ¼ dilution. Then, it was seen that MIC in ¼ dilution and MBC in the first well without dilution followed by vinegar samples 2, 3, 4 and 6. The MIC value in vinegars 1 and 5 was obtained at ½ dilution, and the MBC value in the first well without dilution was found in the 5th vinegar, while the MBC value could not be determined in the vinegar number 1, Table 2. In addition, for the vinegar sample number 7, the zone diameters from largest to smallest were

Escherichia coli ATCC 8739*, Staphylococcus aureus* ATCC 25523, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 19846, *Pseudomonas aeruginosa* ATCC 278536, *Klebsiella pneumoniae* ATCC 70063. These zone diameters were determined as 12, 12, 10, 10, 10, 8, 8 mm, respectively. The high antibacterial activity of vinegar number 7 suggests that it is due to the high amount of acetic acid found in industrial vinegar samples. Indeed, diluted organic acids and highly acidic liquids such as vinegar could inhibit microbial growth or survival, depending on their acidity levels. Weak acids, including acetic acid, exert their antimicrobial activity by switching the microbial membrane to its undissociated form and dissociating according to intracellular pH and releasing a proton in the cytoplasm (Salmond et al., 1984). It had been reported that vinegars containing a significant amount of acetic acid had strong antimicrobial activity against bacteria and fungi (Karapınar and Gönül, 1992; Wen-qiao et al., 2005; Öztürk et al., 2005; Medina et al., 2007; Pinto et al., 2008).

When viewed at *Klebsiella pneumoniae* ATCC 70063, one of our current strains, vinegar number 7 was found to be the most effective vinegar. This was followed by vinegar samples 3, 2 and 4. While the MIC value was determined in ¼ dilution in vinegar number 6, the MBC value could not be determined. In addition, the MIC value was determined in vinegar number 5 at ½ dilution and the MBC value in the first well without dilution, Tablo 2. All results were showed that the antimicrobial activity of vinegar might vary depending on the test culture, total phenolic content and acidity amounts of vinegar. Actually, Bakır et al. (2017) reported that balsamic vinegar showed the highest antimicrobial activity (16 mm) against *S. typhimurium*, while pomegranate vinegar showed the highest activity on *S. aureus* (13 mm) and *E. coli* (14 mm). In another study, it was found that mulberry vinegar showed the highest antimicrobial activity in the disc diffusion and microdilution test against various microorganisms such as *Candida albicans*, *Bacillus cereus*, *B. subtilis*, *Enterococcus faecalis*, *Erwinia carotovora*, *E. coli*, *Klebsiella oxytoca*, *S. aureus* and *Streptococcus pyogenes*. Among these strains the highest antimicrobial activity was seen againts on *S. aureus* (inhibition zone: 28mm) (Karaağaç et al., 2016).

When MIC and MBC values were examined against *Escherichia coli* ATCC 25922 strain, which was our other bacterial species in our study, it was observed that the most effective vinegar was vinegar number 7 with MIC value in 1/32 dilution and MBC value in ¼ dilution. Vinegars numbered 2, 3, 4, 5, 6 showed equal effects with MIC and 1 MBC values at 1/8 dilution. On the other hand, the least effective vinegar was the vinegar sample number 1 with the MIC value at ½ dilution without the MBC value, Table 2. When the ATCC 19846 of *Escherichia coli* (colistin R), which was a more resistant strain, was examined, the most effective vinegar was vinegar number 7 with MIC values at 1/8 dilution and MBC at ¼ dilution. Considering only the MIC values, it was observed that the MIC value in 1/8 dilution and the vinegar number 7 had the same MIC value as the vinegars numbered 4 and 6. Considering the MBC values, vinegar sample number 4 with MIC values in 1/8 dilution and MBC values in the first well without dilution was followed by vinegar number 6 with MIC value at 1/8 dilution and no MBC value Vinegar number 6 was followed by vinegars numbered 2, 3 and 5 with MIC values in ¼ dilution and MBC values in the first well without dilution. The least effective vinegar, on the other hand, was vinegar number 1, with an MIC value at ½ dilution without MBC value, as in *Escherichia coli* ATCC 25922, Table 2.

In *Escherichia coli* ATCC 8739 strain, MIC values were determined at 1/32 dilution in vinegars 5 and 6, unlike the others. However, when the MBC values were examined, MBC value was determined in the first well without dilution in vinegar number 5, but MBC value could not be determined in vinegar number 6. This was followed by the circus number 7. However, vinegar number 7 was found to be more effective than other vinegars with its MIC value in 1/8 dilution and MBC value in ¼ dilution. MIC values at ¼ dilution and MBC values in the first well without dilution were determined in vinegars 2, 3 and 4. The vinegar sample that showed the least effect was vinegar number 1 with its MIC value at ½ dilution without determining the MBC value, Table 2. Indeed, Hammouda et al. (2021) in their study using the well diffusion experiment was found that it had antibacterial activity of four vinegar samples such as grape, fig, prickly pear and date vinegar from various regions of Tunisia against three-gram positive *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 25912) and *Listeria monocytogenes* (ATCC 15313) and one gram negative (*Escherichia coli* ATCC 25922) strains. Date vinegar showed the most effective inhibition of *S. aureus* with a zone diameter of 24 ± 1.42 mm, and Grape vinegar showed the smallest inhibition zone (10 ± 1.42 mm). In addition, grape vinegar was only able to inhibit the growth of *S. aureus*, unlike other vinegar samples that inhibited the growth of all pathogenic bacteria tested. In the study, *E. coli* was observed as the most sensitive strain to all vinegar samples. This activity was mainly due to the acidic origin of the samples (pH values between 3.61 and 4.08) and this was confirmed by testing the antibacterial activity of neutralized vinegars at pH 6.5 where no activity was detected. In addition, it was known that acetic acid, the main compound in vinegar samples, had strong antimicrobial activity against bacteria (Medina et al., 2007). In our study, it was thought that there might be several reasons for the different antibacterial effect of apple cider vinegar made with different yeast raw materials on *E. coli* strains. One of them was thought to be the acetic acid content, which is the most important quality criterion of vinegars. Acetic acid usually lowers the pH of the medium. It had also been found that acetic acid passes through the cell wall and penetrates the cell and denatures the plasma. Since the antimicrobial effect of acetic acid occurs with its undissociated molecules, the effect of acetic acid increases as the pH of the environment decreases. Acetic acid has a greater antimicrobial effect against bacteria. The second one might be due to the phenolic content of Kara Sakı apple. Thus, it had been reported in studies that vinegar varieties are rich in phenolic compounds that indicate antimicrobial and antioxidant activity (Karabıyıklı and Şengün, 2017). These results were agreed with our study.

Considering the *Salmonella enterica* subsp. *enterica* serovar Enteritidis ATCC 13076 strain, vinegar number 7 was found to be the most effective with MIC value at 1/8 dilution and MBC value at ¼ dilution, followed by vinegar number 6 with MIC value at 1/8 dilution without MBC value. In vinegar samples 2, 4 and 5, MIC values were determined at ¼ dilution and MBC values in the first well without dilution. In the 3rd vinegar sample, MIC values were found in 1/2 dilution and MBC values in the first well without dilution. On the other hand, the least effective vinegar sample was sample number 1 with MIC value in the first well without dilution without MBC value, Table 2. In the study conducted by Hindi (2013), antibacterial effects of the garlic-apple cider vinegar mixture and apple cider vinegar alone were investigated on *S. aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Enterococcus feacalis*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*,

Pseudomonas fluorescence, *Enterobacter aerugenes*, *Klebsiella pneumoniae*, *E. coli*, *Salmonella typhi*, *Proteus mirabilis*, *Proteus vulgaris* and *Acinetobacter*. It was observed that the product obtained with the mixture of apple cider vinegar and garlic on all microorganisms had higher antimicrobial activity than only apple cider vinegar. While the inhibition zone of the mixture varied between 25-50 mm, it was determined that apple cider vinegar alone had an inhibition zone between 6-15 mm. Actually, since different yeast raw materials were used in our study, different antibacterial effects depending on the content of yeast raw materials were the expected possible results of our study and these results support the results we obtained from our study. In both *Staphylococcus aureus* ATCC 25523 and *Staphylococcus aureus* ATCC 29213*,* vinegar number 7 was found to be the most effective with MIC values at 1/8 dilution and MBC at ¼ dilution, followed by *Staphylococcus aureus* ATCC 25523 with MIC values at ¼ dilution in 2 wells and without MBC in 3 wells without dilution and vinegar examples number 5 followed. In *Staphylococcus aureus* ATCC 29213 strains vinegars numbered 2, 3 and 5 were followed by MIC values at ½ dilution and MBC values in the first well without dilution. MIC values of *Staphylococcus aureus* ATCC 25523 strain at ¼ dilution were found in vinegar samples 4 and 6 without MBC values. In addition, the least effective vinegar was the number 1 vinegar, and the MIC value was determined at ½ dilution without the MBC value. In *Staphylococcus aureus* ATCC 29213, vinegars numbered 1, 4 and 6, whose MIC value was determined only in ½ dilution without MBC value, Table 2. In fact, Şengün and Kılıç (2018) examined the antimicrobial effects of homemade mulberry and fig vinegars against the pathogens *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Staphylococcus aureus* and *Salmonella typhimurium.* While fig and mulberry vinegars were showed a similar effect on *E. coli* O157:H7 and *S. aureus*. They showed different effects on *L. monocytogenes* and *S. typhimurium*. These results support our study.

When the *Enterococcus faecalis* ATCC 29212 strain was examined, vinegars 1 and 7 were found to be the most effective vinegars with a MIC value at 1/8 dilution, unlike the others. However, vinegar number 7 was found to be more effective than vinegar number 1 with its MBC value in ½ dilution, since vinegar number 1 did not have an MBC value. This was followed by vinegars 4 and 6 with MIC values at ¼ dilution, and vinegars numbered 2, 3 and 5 with MIC values at ½ dilution, Table 2. As a matter of fact, like our study, Şengün and Kılıç (2018) examined the antimicrobial properties of mulberry vinegars (homemade and commercial). In their study, they worked with different microorganisms such as *E. coli* O157:H7 ATCC 43895, *L. monocytogenes* Scott A, *S. typhimurium* NRRLB 4420 and *S. aureus* 6538 P, *Bacillus subtilis* ATCC 6037, *E. coli* ATCC 1103, *Enterococcus faecalis* ATCC 29212 *and Pediococcus acidilactici* ATCC. Commercial vinegar sample showed antimicrobial effect on all the tested microorganisms, but they found that the antimicrobial effect of household vinegar was limited. When evaluated in general, vinegar number 7 was determined as the most effective vinegar sample against all standard strains. Vinegar number 1 was the weakest vinegar sample against all standard strains except for *Enterococcus faecalis* ATCC 29212. On the other hand, vinegar number 1 was also one of the 2 most effective vinegars against *Enterococcus faecalis* ATCC 29212 strain. It was thought that this effect might be due to the use of *Saccharomyces cerevisiae,* which was used as a yeast raw material in vinegar production, Table 2. In addition, while vinegar samples had MIC values, the absence of MBC values means that they only provide a

bacteriostatic effect. It was determined that vinegar samples 1 and 6 generally showed a bacteriostatic effect, Table 2.

When we look at the results of the disk diffusion test, zone diameters were obtained from industrial vinegar number 7 for all strains except for *Enterococcus faecalis* ATCC 29212 and *Salmonella enterica* subsp. *enterica* serovar Enteritidis ATCC 13076. Discs were prepared with other Kara Sakı apple vinegars did not form zone diameters. It was thought that this result was due to the amount of acetic acid in the industrial vinegar. In fact, Şengün and Kılıç (2020b) also found that the antimicrobial effect of traditional household vinegar was lower than that of industrial vinegar. Similarly, Chang and Fang (2007) applied commercial rice vinegar containing 5% acetic acid (pH 3.0) within 5 minutes in their study in which they contaminated the pathogens *E. coli* O157:H7 and *Salmonella enterica* (10^7 cfu/g) with lettuce samples. They determined that it caused a decrease of 3 log units in the *E. coli* O157:H7 population. They reported that commercial rice vinegars containing lower acetic acid (0.05%; pH 4.09 and 0.5%; pH 3.26) did not have an inhibitory effect on *E. coli* O157:H7. These results agreed with our study result.

Conclusion

As a result, Kara Sakı apple vinegars included in the study differed in terms of antimicrobial activity. We could also say that these differences were due to the difference in the raw material used in the preparation of vinegar and the acidity regulator and additives used in its production. Commercial vinegar sample showed antimicrobial effect on all the tested microorganisms, but the antimicrobial effect of Kara Sakı apple vinegar samples obtained from different yeast samples was limited. In addition, in our study, it was thought that chickpea, buckwheat and barley raw materials with high phenolic content also affect on MIC and MBC values. The obtained results show that the Kara Sakı apple vinegar had a significant potential in terms of antimicrobial activity.

Acknowledgment

No ethics commission permission is required in this manuscript. The manuscript has been prepared in accordance with publication and research ethics. The authors declare that there is no conflict of interest regarding the publication of this article. FY and HK; production of kinds of vinegar and article language. FY and BG; supervision, planning, and resources scanning. FY and BG; methodology, laboratory studies, and writing-original draft preparation, data analysis. BG and HK; article revision.

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