

Journal of the Hellenic Veterinary Medical Society

Vol 72, No 1 (2021)



Antimicrobial effects of fruit sauces on some pathogenic bacteria in vitro and on chicken breast meat

I VAR, S UZUNLU, I DEĞİRMENCI

doi: [10.12681/jhvms.26755](https://doi.org/10.12681/jhvms.26755)

Copyright © 2021, I VAR, S UZUNLU, I DEĞİRMENCI



This work is licensed under a [Creative Commons Attribution-NonCommercial 4.0](https://creativecommons.org/licenses/by-nc/4.0/).

To cite this article:

VAR, I., UZUNLU, S., & DEĞİRMENCI, I. (2021). Antimicrobial effects of fruit sauces on some pathogenic bacteria in vitro and on chicken breast meat. *Journal of the Hellenic Veterinary Medical Society*, 72(1), 2703–2712. <https://doi.org/10.12681/jhvms.26755>

Antimicrobial effects of fruit sauces on some pathogenic bacteria *in vitro* and on chicken breast meat

I. Var¹ , S. Uzunlu² , I. Değirmenci¹ 

¹Çukurova University Agricultural Faculty Food Engineering Department, Adana, Turkey

²Alanya Alaaddin Keykubat University RafetKayış Engineering Faculty Food Engineering Department, Alanya, Turkey

ABSTRACT: The use of natural food additives is currently a rising trend. In the present study, the aim was to determine the antimicrobial effects of plum, pomegranate, Seville orange and sumac sauces on *E. coli* O157:H7, *E. coli* type I, *Listeria monocytogenes*, *Listeria ivanovii*, *Salmonella* Typhimurium and *Staphylococcus aureus*. Different concentrations (1%, 10%, 100%, v/v) of the sauces were tested on the studied bacteria *in vitro* using the agar diffusion and minimal inhibition concentration (MIC) methods. The results showed that the sumac sauce had the highest antimicrobial activity. The Seville orange, plum and pomegranate sauces also exerted antimicrobial activity in descending order. The antimicrobial activity of the fruit sauces was more effective at a concentration of 100% than at 10% and 1%, v/v. The most inhibitory effect was recorded for sumac sauce at a concentration of 100% (v/v) on *L. monocytogenes* and *E. coli* O157:H7. The findings of the MIC method aligned with the agar diffusion method. In addition, the *in situ* (food method) antimicrobial effect of the sauces on the indigenous microflora of chicken breast samples sold in stores was determined. Chicken samples hosting aerobic mesophilic bacteria, coliforms and *E. coli* were treated for two hours at 4 °C with plum, pomegranate, Seville orange and sumac sauces and were then monitored. The findings revealed that the Seville orange and sumac sauces were the most effective in reducing the indigenous microbial growth on the chicken samples. The plum sauce showed higher antimicrobial activity than pomegranate sauce. The phenolic content and acidity of the samples significantly ($P < 0.05$) affected the antimicrobial activity both *in vitro* (agar diffusion and MIC) and *in situ* (chilled chicken breast). In conclusion, the sumac and Seville orange sauces were found to be the most promising natural antibacterial agents, and their use could be recommended, for example, in catering services to reduce the risk of foodborne illness.

Keywords: Antimicrobial effect, Chicken, Pomegranate, Seville orange, Sumac

Corresponding Author:
Sinan Uzunlu, Alanya Alaaddin Keykubat University RafetKayış Engineering
Faculty Food Engineering Department, Alanya, Turkey
E-mail address: sinan.uzunlu@alanya.edu.tr

Date of initial submission: 16-03-2020
Date of revised submission: 09-12-2020
Date of acceptance: 14-12-2020

INTRODUCTION

Economic losses resulting from food spoilage and foodborne illnesses are a global problem. To decrease foodborne illnesses, the growth of microbial organisms should be prevented during the food production chain. The Centers for Disease Control and Prevention (CDC) estimates that, each year, roughly 48 million people become ill, 128.000 are hospitalised and 3.000 die from foodborne diseases in the United States. Unspecified agents contribute to 80% of the total number of illnesses, and the remaining 20% are caused by 31 known pathogens. *Salmonella* species and *Staphylococcus aureus* are among the top five pathogens contributing to domestically acquired foodborne illnesses. In the United States, *Escherichia coli* (STEC) O157:H7 is among the top pathogenic bacteria resulting in hospitalisation and *Listeria monocytogenes* in death (Anonymous, 2018). Catering services are one of the sources of foodborne outbreaks. A recent survey reported that, among 28 European Union member states, catering services followed household environments as the primary source of foodborne salmonellosis. Various facilities such as hospital restaurants, takeaway restaurants, ethnic restaurants, hotels, and in-flight catering services were among the sources of infections (Osimani et al., 2016).

Chicken meat is one of the major sources of food poisoning cases because of the frequent presence of *Salmonella* spp., *E. coli* and *Campylobacter* spp. (Kim et al., 2019; Li et al., 2019; Vetchapitak and Misawa, 2019; Dantas et al., 2020; Saad et al., 2020; Shen et al., 2020). For instance, several virulence genes of *Salmonella* spp. were detected in chicken samples from retail markets in South Korea (Dantas et al., 2020).

To minimise the prevalence of foodborne diseases, some slaughter establishments have begun to add antimicrobial chemicals (e.g. acidified sodium chloride, chlorine, sodium hypochlorite) to rinse solution for chicken carcasses (Ebel et al., 2019). However, because of consumer concerns over synthetic additives in food production, recent research has focused on the use of natural antimicrobial agents. Concentrated fruit sauces, which have mostly been used in salad dressings, have been widely studied because of their antimicrobial activity. For example, pomegranate sauces were previously found to inhibit the growth of *S. aureus* and *E. coli* O157:H7 (Karabiyikli and Kislak, 2012). Plum sauce displayed a strong antimicrobial effect on the growth of coliform bacteria and *E. coli* on

ground beef and minced beef samples (Yapar, 2006). Sour orange juice completely inactivated *Salmonella* Typhimurium and *L. monocytogenes* at the end of the seventh day of incubation at 37 °C (Karabiyikli et al., 2014). Sumac sauce inhibited the growth of *E. coli* O157:H7 and *L. monocytogenes* in vitro conditions (Kunduhoglu and Pilatin, 2004). Hence, the use of fruit sauces could be a practical way of decreasing the microbial load and maintaining food safety in households and catering services. The aim of the present study was to determine the antimicrobial effects of fruit sauces on some pathogenic bacteria both *in vitro* (minimal inhibition concentration [MIC] and agar diffusion tests) and *in situ* (by placing chicken breasts in each fruit sauce at 4 °C for 2 h).

MATERIALS AND METHODS

Materials

Nutrient agar (NA), nutrient broth (NB), peptone water (0.1%), Mueller-Hinton broth (MB), Violet Red Bile agar (VRB), Brilliant Green Bile Broth (BGLB), Fluorocult Lauryl Sulphate Broth (Merck), KOVACS's indole reagent and Fluorocult *E. coli* O157:H7 agar were purchased from Merck (Darmstadt, Germany). Chemicals used in physicochemical analyses were purchased from Sigma-Aldrich (United Kingdom). Fresh pomegranates (*Punica granatum*) were purchased from local markets in the Antakya province of Turkey. Sumac (*Rhus coriaria* L.) fruits were purchased in the Gaziantep province. Plums (*Prunus domestica* cv. French) and Seville oranges (*Citrus aurantium*) were purchased in the Mersin province. Chicken samples were purchased in their original packages from two different suppliers in the Adana province and were immediately transferred to the laboratory and kept under refrigeration (4°C).

Preparation of fruit sauces

The pomegranate, plum and Seville orange samples were first cleaned and washed under tap water. Then, the fruits were pressed using a lab-type juice press (Waring, US). The obtained fruit juices were filtered through filter paper (Isolab, Germany). The filtrates were gently boiled in a kitchen saucepan, filtered, boiled again for 1 h and left to reach ambient temperature. However, the sumac fruits were not subjected to a heat treatment and were left at room temperature until sediments were observed by the naked eye. The filtrate was then sun dried until reaching a dark colour, after which it was filtered and bottled. All sauce types were bottled in sterile glass jars and

stored at 4 °C until further use.

Test microorganisms

E. coli O157:H7, *E. coli* type I, *L. monocytogenes*, *Listeria ivanovii*, *S. Typhimurium* and *S. aureus* were acquired from former foodborne isolates in our laboratory (Çukurova University Agricultural Faculty Food Engineering Department, Turkey) collection. Reference strains were purchased from local companies to control the studied bacterial types.

Antibacterial assay (*in vitro*) using the agar-well diffusion method

The agar-well diffusion method was used following the modifications of Ahmad and Beg (2001) to determine the antimicrobial activity of the fruit sauces. Freshly grown cultures were first propagated in 5 mL of NB in tubes and incubated at 30 °C for 24 h. Following incubation, tubes were centrifuged at 3,000 g for 10 min at 4 °C and washed twice in NB. The supernatant was discarded. Bacterial pellets of the test microorganisms were diluted in 3 mL of sterile distilled water and used as stock cultures. Appropriate concentrations (0.5 McFarland units, corresponding with approximately 1.5×10^8 CFU/mL) of the bacteria were determined with a densitometer and further diluted to 10^5 CFU/mL. One millilitre of prepared cells was spread onto the surface of nutrient agar plates and left to dry at ambient conditions for 30 min. A sterilised stainless steel borer was used to punch wells in the agar medium 6 mm in diameter. Each well was then filled with 0.5 mL of fruit sauce diluted in sterilised distilled water to achieve different final concentrations (1%, 10%, 100% v/v, water basis). The plates were left at ambient conditions to allow the diffusion of the sauces in the agar plates. The plates were then incubated at 37 °C for 24-48 h until visible growth of microorganisms was evident in the control plates. The diameter of the inhibition zone was measured using callipers and expressed in millimetres. The values were taken as the average of five repetitions. The sensitivities of the bacteria were classified by the diameter of the inhibition zone, as described by Ponce et al. (2003): not sensitive (-) for diameters less than 8 mm, sensitive (+) for diameters 9-14 mm, very sensitive (++) for diameters 15-19 mm and extremely sensitive (+++) for diameters larger than 20 mm.

Minimal inhibitory concentration test

The minimum inhibitory concentrations (MICs) were evaluated by the broth microdilution method

(M26-A) in accordance with the guidelines of the National Committee for Clinical Laboratory Standards (2003). Each fruit sauce was serially diluted two-fold in Mueller Hinton Broth (Merck, Darmstadt/Germany) to achieve increasingly more diluted concentrations (1:0.125, 1:0.25, 1:0.5, 1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256 µg/mL). Each dilution and a control tube (containing no fruit sauce) was inoculated with 5.0 log CFU/mL of the test microorganisms. Tubes were then incubated at 37 °C for 24-48 h. The MIC was assigned to the lowest concentration of each antimicrobial fruit sauce that prevented bacterial growth. The test was conducted five times per fruit sauce.

Antimicrobial activity (*in situ*) of fruit sauces on chicken samples

To determine the antimicrobial effect of the fruit sauces on the naturally existing bacterial microflora of the chicken samples, the following procedures were applied. Twenty grams of chicken breast samples were treated with 15 mL of each fruit sauce separately and mixed well in Petri plates with their lids. Samples were left to rest at 4 °C for 2 h to ensure that each sauce had coated the surface of the chicken samples. Then, 10 g of each chicken sample was diluted in 90 mL of peptone water, homogenized in a stomacher (BagMixer 400 P, Interscience, France) and serial decimal dilutions were made. Dilutions were surface plated on VRB agar to monitor coliform bacteria and incubated at 35 °C for 18-24 h according to the solid medium method (SMM).

To confirm the coliforms, at least 10 colonies were selected and incubated at 35 °C in BGLB broth, and Gram stains were made (Anonymous, 2002). The most probable number (MPN) method was used with Fluorocult Lauryl Sulphate Broth to identify the amount of bacteria. After incubation at 35 °C for 48 h, the tubes were checked for gas formation, which confirms the presence of coliform bacteria. The gas-positive tubes were checked for light blue fluorescence under UV light (366 nm). Afterwards, the tubes showing both positive and negative fluorescence were subjected to an indole test using KOVACS's indole reagent. Positive indole and fluorescence samples were marked as *E. coli* type I (BGA, 1992). Meanwhile, negative fluorescence and positive indole samples were marked as *E. coli* O157:H7. Fluorocult *E. coli* O157:H7 agar was used to confirm *E. coli* O157:H7, and the plates were incubated at 35 °C for 18-24 h (Szabo et al., 1986). NA (Nutrient Agar) was surface plated to carry

out the total aerobic mesophilic bacteria counts, and plates were incubated at 30 °C for 24-48 h (Beuchat et al., 1991).

Physicochemical analyses

The pH and total acidity of the fruit samples were measured in duplicate. A Mettler Toledo Seven Compact pH Meter (Port Melbourne, Victoria, Australia) was used for pH determination. The titrimetric method with NaOH (0.1 N) was used to measure the total acidity of the samples. The results were expressed as the citric acid percent (%) of the samples taken as the total acidity (Ting and Rouseff, 1986). To determine the total phenolic compounds, 2 mL of each fruit sauce was mixed with 8 mL of ethanol (80%, v/v) and then centrifuged at 4,000 rpm for 20 min. Fifty µL of supernatant phase was mixed with 100 µL of Folin-Ciocalteu solution and 1,500 µL of distilled water for 10 min. Then, 50 µL Na₂CO₃ (20%, v/v) was added, and the mixture was left in the dark for 2 h. The optical density of samples was then measured against the blank at a wavelength of 765 nm using a spectrophotometer (Shimadzu UV-1700 Pharmaspec, Japan). A standard curve was created using gallic acid to measure the total phenolic content of samples in mg gallic acid/L (Abdullakassim et al., 2007).

Statistical analyses

Data were analysed by ANOVA (one-way analysis) using Statistical Package for the Social Sciences (SPSS) software, version 21 (IBM, USA)). Duncan's post-hoc test was applied at a significance level of $P < 0.05$.

RESULTS

Physicochemical analyses

The pH of the sumac sauce was the lowest among the sauces and that of pomegranate was the highest. These findings were accompanied with the titration acidity of the fruit sauces (Table 1). The total phenolic content of the pomegranate sauce was the lowest and that of the sumac sauce was the highest.

Microbial inhibition analyses

The agar diffusion test results showed that all bacteria were extremely sensitive to plum, Seville orange and sumac sauces at a concentration of 100%, v/v (Table 2). *Escherichia coli* type I, *S. Typhimurium* and *E. coli* O157:H7 were also extremely sensitive to pomegranate sauce at a concentration of 100%, v/v. Sumac sauce at 10% (v/v) concentration showed high antimicrobial activity against test microorgan-

isms. The sensitivity of the bacteria to the remaining sauces at a concentration of 10% (v/v) varied, but in general, the studied bacteria were not sensitive at this concentration. The lowest concentration (1%, v/v) of the sauces was ineffective in inactivating microbial growth. Only *L. monocytogenes* was sensitive to sumac sauce at a concentration of 1%, v/v (Table 2).

The findings of the MIC method (Table 3) in general aligned with the those of the agar diffusion method, as shown in Table 2. The sumac sauce had a significantly ($P < 0.05$) higher antimicrobial activity than the remaining sauces on all of the studied bacteria except for *L. ivanovii*. The MIC value of sumac sauce and Seville orange sauce was the same for this latter bacterium (Table 3).

The pomegranate sauce had a significantly ($P < 0.05$) higher antimicrobial activity than the Seville orange and plum sauces against *S. Typhimurium*. The same sauce had significantly ($P < 0.05$) less activity than the other sauces (plum and Seville orange) against *L. ivanovii* and *S. aureus* and no activity against *L. monocytogenes*. The plum, pomegranate, and Seville orange sauces showed no differences in their antimicrobial activity against *E. coli* type I and *E. coli* O157:H7. The Seville orange sauce had a significantly ($P < 0.05$) higher antimicrobial activity against *L. ivanovii* and *S. aureus* compared to the plum and pomegranate sauces (Table 3).

According to the *in situ* (food method) test results, the Seville orange and sumac sauces both completely inactivated the indigenous microbial (total aerobic mesophilic bacteria, coliform bacteria and *E. coli* type I) growth in the chicken breast samples. The amount of coliform bacteria inactivated by the plum and pomegranate sauces was higher than the total aerobic mesophilic bacterial counts (Table 4).

DISCUSSION

Previous studies have shown that the antimicrobial activity of pomegranates (González et al., 2002; Duman et al., 2009; Tađi, 2010), plums (Shyamala Gowri and Vasantha, 2010; Mehta et al., 2014), Seville oranges (Değirmenci and Erkurt, 2020), and sumac (Ali-Shtayeh et al., 2013) depends on the high acidity and rich phenolic content of the fruits. Similarly, we found that the fruits with higher phenolic content and lower pH (Table 1) displayed significantly ($P < 0.05$) higher antimicrobial activity, as shown in Tables 2, 3, and 4.

Table 1 Physicochemical analyses

| | Plum | Pomegranate | Seville orange | Sumac |
|---|--------------------------|-------------------------|--------------------------|---------------------------|
| pH | 3.36±0.01 ^a | 5.12±0.02 ^b | 2.47±0.01 ^c | 2.13±0.00 ^d |
| Titration Acidity (% citric acid) | 3.268±0.17 ^a | 0.098±0.00 ^b | 16.195±0.00 ^c | 36.84±1.12 ^d |
| Total phenolic content (mg gallic acid/L) | 260.32±3.85 ^a | 74.30±0.94 ^b | 153.99±1.37 ^c | 2637.59±9.95 ^d |

Results are presented as means of two observations ± standard deviation.

Data in the same row bearing different superscript letters are significantly different ($P < 0.05$).

Table 2 Agar diffusion test results (mm)

| | <i>E.coli</i> Type I | <i>S.Typhimurium</i> | <i>E.coli</i> O157:H7 | <i>L.monocytogenes</i> | <i>L.ivanovii</i> | <i>S.aureus</i> |
|----------------|----------------------|----------------------|-----------------------|------------------------|--------------------|---------------------|
| Plum | 100% | 28 ^{ax} | 24 ^{abx} | 29 ^{axy} | 25 ^{abx} | 24.2 ^{abx} |
| | 10% | 1.0 | 1.0 | 2.0 | 2.0 | 1.0 |
| | 1% | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Pomegranate | 100% | 32 ^{ax} | 36.4 ^{ay} | 26 ^{bx} | 1.0 ^{dy} | 9.0 ^{ey} |
| | 10% | 17 | 3.0 | 3.0 | 1.0 | 6.0 |
| | 1% | 5.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Seville orange | 100% | 33.8 ^{ax} | 37 ^{ay} | 33.8 ^{ay} | 24 ^{bx} | 25 ^{bx} |
| | 10% | 2.0 | 7.0 | 2.0 | 3.0 | 22 |
| | 1% | 6.0 | 2.0 | 6.0 | 1.0 | 2.0 |
| Sumac | 100% | 35.2 ^{abx} | 38.2 ^{aby} | 40.2 ^{bz} | 40.7 ^{bz} | 32.5 ^{az} |
| | 10% | 22.5 | 21.5 | 20 | 15 | 21.2 |
| | 1% | 1.0 | 5.0 | 1.0 | 9.0 | 3.7 |

Results are presented as means of five observations.

Different superscript letters (a, b, c) within the same row indicate significant ($p < 0.05$) differences among the bacterial types as affected by each fruit sauces at 100% concentration.

Different superscript letters (x, y, z) within the same column indicate significant ($p < 0.05$) differences among the fruit sauces at 100% concentration against each bacterial types.

Table 3 MIC test results (µg/mL)

| | <i>E.coli</i> Type I | <i>S.Typhimurium</i> | <i>E.coli</i> O157:H7 | <i>L.monocytogenes</i> | <i>L.ivanovii</i> | <i>S.aureus</i> |
|----------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
| Plum | 1:32 (0.031) ^a | 1:32 (0.031) ^a | 1:32 (0.031) ^a | 1:32 (0.031) ^a | 1:16 (0.062) ^a | 1:32 (0.031) ^a |
| Pomegranate | 1:28 (0.035) ^a | 1:64 (0.015) ^b | 1:32 (0.031) ^a | <1:2 (0.5) ^b | 1:8 (0.125) ^a | 1:8 (0.125) ^b |
| Seville orange | 1:32 (0.031) ^a | 1:32 (0.031) ^a | 1:32 (0.031) ^a | 1:32 (0.031) ^a | 1:256 (0.003) ^b | 1:128(0.007) ^c |
| Sumac | 1:256 (0.003) ^b | 1:256 (0.003) ^c | 1:256 (0.003) ^b | 1:256 (0.003) ^c | 1:256 (0.003) ^b | 1:256 (0.003) ^d |

Results are presented as means of five observations.

Different superscript letters within the same column indicate significant ($p < 0.05$) differences.

Table 4 Antimicrobial activity of fruit sauces on chicken breast meat samples

| | TVC | Coliform (SMM) | Coliform (MPN) | <i>E.coli</i> |
|-------------------|-------------------------|-------------------------|----------------|---------------|
| Supplier 1 | | | | |
| Control | 5.49±0.05 ^d | 3.73±0.08 ^c | > 1100 | > 1100 |
| Plum | 3.04±0.04 ^c | 2.84±0.06 ^b | 26.7 | 26.7 |
| Pomegranate | 4.59±0.01 ^b | 2.84±0.00 ^b | 35 | 35 |
| Seville orange | < 0.1±0.00 ^a | < 0.1±0.00 ^a | < 3.0 | < 3.0 |
| Sumac | < 0.1±0.00 ^a | < 0.1±0.00 ^a | < 3.0 | < 3.0 |
| Supplier 2 | | | | |
| Control | 5.64±1.04 ^g | 5.25±0.35 ^h | > 1100 | > 1100 |
| Plum | 3.62±0.32 ^f | 2.72±0.12 ^g | 15 | 15 |
| Pomegranate | 4.87±0.02 ^{fg} | 3.65±0.18 ^f | 46 | 46 |
| Seville orange | < 0.1±0.00 ^e | < 0.1±0.00 ^e | < 3.0 | < 3.0 |
| Sumac | < 0.1±0.00 ^e | < 0.1±0.00 ^e | < 3.0 | < 3.0 |

Results are presented as means of two observations.

Different superscript letters within each supplier sample columns indicate significant ($p < 0.05$) differences for TVC and Coliform (SMM), and shown in log CFU/g.

No statistical analyses were performed on Coliform (MPN) and *E.coli*.

The phenolic compounds in plants are present in the form of monophenols, diphenols or triphenols, which are called simple phenolic compounds. In addition, phenolic acids such as gallic acid, caffeic acid, ferulic acid and furocoumarins are among the antimicrobial compounds (Daglia, 2012; Quinto et al., 2019).

Polyphenols are generally divided into two classes as flavonoids and nonflavonoids, which are important for their potential antimicrobial activity (Daglia, 2012). The freeze-dried arils of pomegranate fruits (FDAPs) contain both flavonoids (proanthocyanidin trimers and degradation of procyanidin dimers of flavan-3-ols) and nonflavonoids (caffeic acid and ferulic acid) in addition to palmitic and stearic acids, as determined by Uzunlu and Niranjana (2017). That study found that polycaprolactone (PCL)-incorporated pomegranate methanolic extract films had higher antimicrobial activity against *E. coli* and *S. aureus* than PCL-FDAP active films (Uzunlu and Niranjana, 2017).

Naz et al. (2007) directly isolated the bioactive phenolic compounds of pomegranate fruits and determined their antimicrobial activity. Of the identified phenolic compounds, gallic acid showed the highest antibacterial activity against tested Gram-positive and Gram-negative bacteria (Naz et al., 2007).

Duman et al. (2009) found that both *E. coli* and *S. Typhimurium* cells were highly affected by pomegranate sauce at a concentration of 100% (v/v), and

S. aureus was less affected. Var et al. (2016) and Kunduhoğlu and Pilatin (2004) also reported that *E. coli* and *Salmonella* cells were highly inactivated by pomegranate sauce at a concentration of 100%, v/v. Another study examined the antimicrobial effect of traditional and commercial pomegranate sauces in Turkey and found traditional sauces to be more effective than commercially produced sauces (Karabiyikli and Kislal, 2012).

An extensive review reported that commercial pomegranate juices from the whole fruit have higher antioxidant activity than from the arils only (Kalaycıoğlu and Erim, 2017). In addition, the use of different solvents (water, ethanol, petroleum ether, chloroform, acetone, methanol) to extract bioactive compounds from different parts (aril, peel, seed) of the pomegranate fruit resulted in differences in antimicrobial activity (Tanveer et al., 2015). For instance, the aqueous extract of pomegranate displayed lower (0.20 mg/mL) MIC value than the ethanolic extract against *E. coli* (Voravuthikunchai et al., 2004). González et al. (2002) documented a high content of polyphenolic compounds in pomegranate peel and seeds with a high antimicrobial activity.

Our pomegranate sauce had a lower total phenolic content (74.3 mg GAE/L) and higher pH than earlier reported studies (see Kalaycıoğlu and Erim, 2017). One study reported a similar phenolic content (88.5 mg GAE/kg) for a pomegranate fruit clone '351' grown in south-eastern Spain. The MIC values of the

present pomegranate sauce were found to be in the range of 0.015-0.5 µg/mL (Table 3), with inhibition zones of 9.0-36.4 mm against the tested microorganisms (Table 2).

Prashanth et al. (2001) found that the aqueous extract of pomegranate rind had a MIC value against *Salmonella* Typhi of 0.025 µg/mL. This was similar to our findings for *S. Typhimurium*, for which we found a MIC value of 0.015 µg/mL. We also determined, interestingly, that pomegranate sauce had higher antimicrobial activity against *S. Typhimurium* than the other pathogens in both the agar diffusion and MIC assays (Tables 2 and 3).

Pradeep et al. (2008) also found that methanolic extracts of pomegranate pericarp showed the highest antimicrobial activity against *S. Typhimurium* and *Shigella dysenteriae* serotype 2, with an inhibition zone of 25 mm (for each) among other *Salmonella* types and *E. coli*. In addition, Pérez and Anesini (1994) determined that the pericarp extract of pomegranate showed strong antimicrobial activity against the multidrug-resistant typhoid fever-causing *S. Typhi*. An *in vivo* study to treat salmonellosis successfully found that pomegranate peel extract effectively inhibited the growth of *S. Typhimurium* and significantly reduced mouse mortality (Choi et al., 2011).

However, the comparison of the phenolic contents and antimicrobial activity of fruits and their cultivars is not always possible because the variety, climate, growing and processing conditions of the studied fruits and methods used to extract the bioactive compounds from fruits can result in differences in the data (Weerakkody et al., 2010; Değirmenci and Erkurt, 2020).

For instance, Orak (2009) found a total phenol content of 9,870 µg/mL in a sour concentrate of pomegranate, which was about one-third lower than that of the fruit's juice. Conventional evaporation for nearly 8 h to produce sour concentrate mainly resulted in differences in the phenolic content of the fruit. Orak (2009) also stated that the difference in phenolic composition depends on the studied material and determination methods.

For instance, Shyamala Gowri and Vasantha (2010) found that the aqueous extracts of black plum (*Syzygium cumini* L.) leaves contained higher phenols than the methanolic extracts. Leaves of black plum (*S. cumini* L.) extracted in both methanol and water have been found to inhibit *Bacillus subtilis*, *S. Typhi*, *Pseudomonas aeruginosa*, *E. coli* and *Proteus vulgaris* (Shya-

mala Gowri and Vasantha, 2010). The authors referred to the antibacterial effect of tannins and other phenolic constituents present in black plum (*S. cumini*) leaves.

The present MIC values of plum sauce were the same (0.031 µg/mL) for the tested bacteria except for *L. ivanovii*, which had a value of 0.062 µg/mL, showing its resistance (Table 3). The inhibition zones were aligned with the MIC values, showing similar sensitivity of the bacteria. In the agar diffusion tests (Table 2), *L. ivanovii* was more resistant than the remaining bacteria, similar to the MIC test (Table 3).

Our research group previously documented that plum sauces have a high antibacterial effect on *Salmonella enteritidis*, *S. Typhimurium*, *E. coli*, *S. aureus* and *Bacillus* spp. at a concentration of 100%, v/v (Var et al., 2016). Fung and Thompson (2001) have reported that dried plum diluted at various concentrations inhibited the growth of *E. coli* O157:H7, *S. Typhimurium* and *S. aureus* on raw and cooked pork meat. In addition, Mehta et al. (2014) previously assessed extracts of dried plum samples against four bacterial pathogens, namely *S. aureus*, *Staphylococcus epidermidis*, *B. subtilis* and *Proteus mirabilis*. These researchers determined that dried plum extracts exercised an inhibitory effect against most of the tested bacteria, although no inhibition was detected against *B. subtilis*. Furfural and eugenol were determined as the antibacterial agents (Mehta et al., 2014).

Citrus aurantium (Seville orange) essential oil contains oxygenated monoterpenes, aliphatic hydrocarbons, monoterpene hydrocarbons and esters. Of a total of 77 different compounds, the main compound and dominant chemical class was linalool. Several chemical compounds present in fruits show antimicrobial activity against microorganisms (Değirmenci and Erkurt, 2020). For example, Karabıyıklı et al. (2014) examined the antimicrobial activity of sour orange juice against *L. monocytogenes* at different concentrations. At a concentration of 100% (v/v), *L. monocytogenes* was completely inactivated following incubation at 37 °C for 3 h, and a concentration of 10% (v/v) had a similar effect after 3 days of incubation and, at a concentration of 1% (v/v), after 1 day of incubation at 37 °C. *Salmonella* Typhimurium cells were totally inactivated at a concentration of 100% (v/v) after incubation at 37 °C for 3 h, and a concentration of 10% (v/v) also inactivated this bacterium after 2 days of incubation at 37 °C. However, at a concentration of 1% (v/v), this bacterium was inactivated after 7 days of incubation (Karabıyıklı et al., 2014).

The *in vitro* data revealed that both *L. monocytogenes* and *L. ivanovii* were extremely sensitive to Seville orange sauce at a concentration of 100%, v/v. *Listeria ivanovii* was also extremely sensitive to a concentration of 10% (v/v), although *L. monocytogenes* was not. There was no antimicrobial effect at a concentration of 1%, v/v. *Salmonella* Typhimurium cells were highly affected at a concentration of 100% (v/v), but were not affected at concentrations of 10% (v/v) and 1%, v/v (Table 2). Al-Oqaili et al. (2014) also reported that bitter orange juice at a concentration of 100% (v/v) showed very high antimicrobial activity against *S. aureus* using the agar diffusion method.

Sumac is used as astringent agent and for indigestion, anorexia, diarrhoea, haemorrhagia and hyperglycaemia in traditional folk medicine (Nasar-Abbas and Halkman, 2004; Fazeli et al., 2007). The bioactivity of sumac was previously documented and found to contain 211 phytochemicals. In particular, sumac is an abundant source of phenolic components, mainly hydrolysable tannins (Abu-Reidah et al., 2015).

The present findings for sumac sauce showed that the inhibition zones for *L. monocytogenes* and *E. coli* O157:H7 were 40.7 mm and 40.2 mm, respectively, at a concentration of 100% (v/v). Kunduhoğlu and Pilatin (2004) reported inhibition zones of 32 and 28 mm for *L. monocytogenes* and *E. coli* O157:H7, respectively, at a concentration of 75%, v/v. Another study found that the water extracts of sumac were effective against 12 bacterial strains, including *L. monocytogenes*, *S. aureus*, *E. coli* type I, *E. coli* O157:H7 and *S. enteritidis* (Nasar-Abbas and Halkman, 2004). The authors also studied a neutralised extract (pH 7.2) of sumac to exclude the inhibitory effect of the high citric and malic acid content, and the same inhibition patterns were found against all tested bacterial strains except *P. vulgaris* (Nasar-Abbas and Halkman, 2004). Another study (Digrak et al., 2001) extensively investigated the antibacterial and antifungal activities of medicinal plants, including sumac. The antimicrobial effect of the sumac samples was in the range of 35-51 mm based on the disc diffusion method (Digrak et al., 2001). Overall, our findings are in consistent with the literature.

Antimicrobial activity (*in situ*) of fruit sauces on chicken samples

The *in situ* antimicrobial effect of sauces on the indigenous microflora of the chicken samples showed that the Seville orange and sumac sauces completely inactivated the growth of total aerobic mesophilic bacteria, coliform

bacteria and *E. coli* type I after 2 h at 4 °C. Meanwhile, the plum and pomegranate sauces decreased the growth of all tested bacteria by one to two log units (Table 4). Similarly, Yapar (2006) found that plum and pomegranate sauces at a concentration of 100% (v/v) completely inactivated coliform bacteria and *E. coli* growths on minced beef meat and ground beef meat. Total aerobic mesophilic bacteria decreased by 3 logs on minced beef meat dipped in plum and pomegranate sauces after 2 h at 4 °C; a 2-log decrease was found for ground beef samples. Coagulase-negative *Staphylococci* decreased by one log unit on minced beef samples dipped in plum sauce. Finally, a very slight (~0.5 logs) decrease was reported for both ground and minced beef samples dipped in pomegranate sauce (Yapar, 2006).

Consistent with our data, Bazargani-Gilani et al. (2015) reported the initial total viable count (TVC) of chicken breast meat samples as 4.85 log CFU/g. These authors stated that pomegranate juice retarded the growth of TVC and extended the shelf life of chicken up to 15 days at 4 °C storage. Lytou et al. (2016) studied the effects of marination (with pomegranate juice, olive oil, dried thyme and honey) and chilling at 4 °C on chicken breast fillets. The initial population of TVC of the samples was 5.1 log CFU/g, which decreased by ca. 1.0 log units on the first day. Taking into account that the upper acceptability limit of the microbial load for fresh meat is 7 logs (Senter et al., 2000), these authors reported that the growth of TVC and *Pseudomonas* spp. on chicken breast fillets marinated with pomegranate juice and stored at 4 °C was controlled, extending the time required to reach this upper limit by up to 8 days and 4 days, respectively (Lytou et al., 2016). Notably, *Pseudomonas* spp. is one the dominant genera of bacteria in chicken meat in addition to *Moraxella*, *Brochotrix* and *Carnobacterium* species and plays a significant role in meat spoilage (Kim et al., 2019).

CONCLUSIONS

In summary, all of the studied fruit sauces showed antimicrobial activity against the test microorganisms. Sumac sauce exhibited the highest antimicrobial activity both *in vitro* and *in situ* (on chilled chicken breasts). We propose the use of fruit sauces (4 °C for 2 h) as a practical way for catering services and households to decrease the existing microbial population of chicken breast meat.

ACKNOWLEDGMENTS

This work was financially supported by the Scientific Research Projects Coordinatorship of Çukurova University under project number FYL-2014-3284.

REFERENCES

- Abdullakasm P, Songchitsomboon S, Techagumpuch M, Balee N, Swatsitang P, Sungpuang N (2007) Antioxidant capacity, total phenolics and sugar content of selected Thai health beverages. *Int J Food Sci Nutr* 58:77-85.
- Abu-Reidah IM, Ali-Shtayeh MS, Jamous RM, Arráez-Román D, Segura-Carretero A (2015) HPLC-DAD-ESI-MS/MS screening of bioactive components from *Rhus coriaria* L. (Sumac) fruits. *Food Chem* 166:179-191.
- Ahmad I, Beg AZ (2001) Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. *J Ethnopharmacol* 74:113-123.
- Ali-Shtayeh MS, Al-Assali AA, Jamous RM (2013) Antimicrobial activity of Palestinian medicinal plants against acne-inducing bacteria. *African J Microbiol Res* 7:2560-2573.
- Al-Oqailli RMS, Al-Alak SK, Mohammed BB (2014) Antibacterial activity of citrus juices against methicillin resistant *Staphylococcus aureus*. *J Biol Agric Healthcare* 4:7-10.
- Anonymous (2002) Official Methods of Analysis, 17th ed., Bacteriological Analytical Manual. Chapter 4: Enumeration of *Escherichia coli* and the Coliform Bacteria. Feng, P. Weagant, S. D. Grant, M. A. and W. Burkhardt. Retrieved on July 18, 2019 from: <https://www.fda.gov/food/laboratory-methods-food/bam-4-enumeration-escherichia-coli-and-coliform-bacteria>.
- Anonymous (2018) Burden of foodborne illness: findings. National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of Foodborne, Waterborne, and Environmental Diseases (DF-WED).
- Bazargani-Gilani B, Aliakbarlu J, Tajik H (2015) Effect of pomegranate juice dipping and chitosan coating enriched with *Zataria multiflora* Boiss essential oil on the shelf-life of chicken meat during refrigerated storage. *Innov Food Sci Emerg Technol* 29:280-287.
- Beuchat LR, Nail BV, Brackett RE, Fox TL (1991) Comparison of the Petrifilm™ yeast and mold culture film method to conventional methods for enumerating yeasts and molds in foods. *J Food Protect* 54:443-447.
- BGA (1992). Bundesgesundheitsamt: Amtliche Sammlung von Untersuchungsverfahren nach § 35 LMBG - Loseblattsammlung. Beuth Verlag Berlin, Köln.
- Choi J-G, Kang O-H, Lee Y-S, Chae H-S, Oh Y-C, Brice O-O, Kim M-S, Sohn D-H, Kim H-S, Park H, Shin D-W, Rho J-R, Kwon D-Y (2011) In vitro and in vivo antibacterial activity of *Punicagranatum* peel ethanol extract against *Salmonella*. *Evidence-Based Complementary and Alternative Medicine* 690518:1-8.
- Daglia M (2012) Polyphenols as antimicrobial agents. *Curr Opin Biotech* 23:174-181.
- Dantas STA, Camargo CH, Tiba-Casas MR, Vivian RC, Pinto JPAN, Pantoja JCF, Hernandez RT, Júnior AF, Rall VLM (2020) Environmental persistence and virulence of *Salmonella* spp. isolated from a poultry slaughterhouse. *Food Res Int* 129:108835.
- Değirmenci H, Erkurt, H (2020) Relationship between volatile components, antimicrobial and antioxidant properties of the essential oil, hydrosol and extracts of *Citrus aurantium* L. flowers. *J Infect Public Heal* 13:58-67.
- Digrak M, Hakkı Alma M, İlçim A (2001) Antibacterial and antifungal activities of Turkish medicinal plants. *Pharm Biol* 39:346-350.
- Duman AD, Ozgen M, Dayisoylu KS, Erbil N, Durgac C (2009) Antimicrobial activity of six pomegranate (*Punica granatum* L.) varieties and their relation to some of their morphological and phytonutrient characteristics. *Molecules* 14:1808-1817.
- Ebel ED, Williams MS, Tameru B (2019) Relatedness of *Salmonella* contamination frequency on chicken carcasses and parts when processed in the same establishment. *Food Control* 100:198-203.
- Fazeli MR, Amin G, Attari MMA, Ashtiani H, Jamalifar H, Samadi N (2007) Antimicrobial activities of Iranian sumac and avishan-e shirazi (*Zataria multiflora*) against some food-borne bacteria. *Food Control* 18:646-649.
- Fung DYC, Thompson L (2001) Effects of dried plums on suppression of growth of foodborne pathogens in liquid medium and ground meat. Report to the California Dried Plum Board, Sacramento, CA, 27 pp.
- González CN, Sánchez F, Quintero A, Usabillaga A (2002) Chemotaxonomic value of essential oil compounds in *Citrus* species. *Acta Hort* 576:49-51.
- Kalaycıoğlu Z, Erim FB (2017) Total phenolic contents, antioxidant activities, and bioactive ingredients of juices from pomegranate cultivars worldwide. *Food Chem* 221:496-507.
- Karabiyikli S, Kisla D (2012) Inhibitory effect of sour pomegranate sauces on some green vegetables and kisir. *Int J Food Microbiol* 155:211-216.
- Karabiyikli Ş, Değirmenci H, Karapınar M (2014) Inhibitory effect of sour orange (*Citrus aurantium*) juice on *Salmonella* Typhimurium and *Listeria monocytogenes*. *LWT-Food Sci Technol* 55:421-425.
- Kim H-E, Lee J-J, Lee M-J, Kim B-S (2019) Analysis of microbiome in raw chicken meat from butcher shops and packaged products in South Korea to detect the potential risk of foodborne illness. *Food Res Int* 122:517-527.
- Kunduhoğlu B, Pilatin S (2004) Nar ekşisivesumakekşisi'nin *Escherichia coli* O157:H7 ve *Listeria monocytogenes* üzerine antimikrobiyal etkisinin belirlenmesi (In Turkish). In: Proceedings of the 1. Geleneksel Gıdalar Sempozyumu, Van, Turkey: pp 90-92.
- Li Y, Pei X, Zhang X, Wu L, Liu Y, Zhou H, Ma G, Chen Q, Liang H, Yang D (2019) A surveillance of microbiological contamination on raw poultry meat at retail markets in China. *Food Control* 104:99-104.
- Lytou A, Panagou EZ, Nychas G-JE (2016) Development of a predictive model for the growth kinetics of aerobic microbial population on pomegranate marinated chicken breast fillets under isothermal and dynamic temperature conditions. *Food Microbiol* 55:25-31.
- Mehta S, Soni N, Satpathy G, Gupta RK (2014) Evaluation of nutritional, phytochemical, antioxidant and antibacterial activity of dried plum (*Prunus domestica*). *J Pharma Phytochem* 3:166-171.
- Nasar-Abbas SM, Halkman AK (2004) Antimicrobial effect of water extract of sumac (*Rhus coriaria* L.) on the growth of some food borne bacteria including pathogens. *Int J Food Microbiol* 97:63-69.
- National Committee for Clinical Laboratory Standards (2003) Clinical Laboratory Standards MIC testing. Performance standards for antimicrobial susceptibility testing (M100-S13), NCCLS, Wayne, PA (2003).
- Naz S, Siddiqi R, Ahmad S, Rasool SA, Sayeed SA (2007) Antibacterial activity directed isolation of compounds from *Punica granatum*. *J Food Sci* 72:M341-M345.
- Orak HH (2009) Evaluation of antioxidant activity, colour and some nutritional characteristics of pomegranate (*Punica granatum* L.) juice and its sour concentrate processed by conventional evaporation. *Int J Food Sci Nutr* 60:1-11.
- Osimani A, Aquilanti L, Clementi F (2016) Salmonellosis associated with mass catering: a survey of European Union cases over a 15-year period. *Epidemiol Infect* 144:3000-3012.
- Pérez C, Anesini C (1994) In vitro antibacterial activity of Argentine folk medicinal plants against *Salmonella typhi*. *J Ethnopharmacol* 44:41-46.
- Ponce AG, Fritz R, del Valle C, Roura SI (2003) Antimicrobial activity of essential oils on the native microflora of organic Swiss chard. *LWT-Food Sci Technol* 36:679-684.
- Pradeep BV, Manojbabu MK, Palaniswamy M (2008) Antibacterial activity of *Punica granatum* L. against gastro intestinal tract infection causing organisms. *Ethnobotanical Leaflets* 12:1085-1089.
- Prashanth D, Asha MK, Amit AA (2001) Antibacterial activity of *Punica granatum*. *Fitoterapia* 72:171-173.
- Quinto EJ, Caro I, Villalobos-Delgado, LH, Mateo J, De-Mateo-Silleras, Redondo-Del-Río MP (2019) Food safety through natural antimicrobials. *Antibiotics* 8:1-30.
- Saad SM, Ibrahim HM, Hassan MA, Eldin SNS (2020) Molecular detection of virulence factors in some food poisoning bacteria isolated

- from chicken meat and giblet. *BVMJ* 38:106-111.
- Senter SD, Arnold JW, Chew V (2000) APC values and volatile compounds formed in commercially processed, raw chicken parts during storage at 4 and 13 °C and under simulated temperature abuse conditions. *J Sci Food Agric* 80:1559-1564.
- Shen H, Chen H, Ou Y, Huang T, Chen S, Zhou L, Zhang J, Hu Q, Zhou Y, Ma W (2020) Prevalence, serotypes, and antimicrobial resistance of *Salmonella* isolates from patients with diarrhea in Shenzhen, China. *BMC Microbiol* 20:197.
- Shyamala Gowri S, Vasantha K (2010) Phytochemical screening and antibacterial activity of *Syzygiumcumini* (L.) (Myrtaceae) leaves extracts. *Int J PharmTech Res* 2:1569-1573.
- Szabo RA, Todd ECD, Jean A (1986) Method to isolate *Escherichia coli* O157:H7 from food. *J Food Protect* 49:768-772.
- Tağı Ş (2010) Nar Suyu Üretim Aşamalarında Antimikrobiyel Aktivite Fenolik Madde Miktarındaki Değişimler (In Turkish). Ankara Üniversitesi Bilimsel Araştırma Projesi Kesin Raporu. Ankara, Turkey, Proje Numarası: 08B4343002:37pp.
- Tanveer A, Farooq U, Akram K, Hayat Z, Shafi A, Nazar H, Ahmad Z (2015) Pomegranate extracts: A natural preventive measure against spoilage and pathogenic microorganisms. *Food Rev Int* 31:29-51.
- Ting SV, Rouseff RL (1986) Citrus fruits and their products. Analysis-technology. Marcel Dekker, New York, 293 pp.
- Uzunlu S, Niranjana K (2017) Laboratory antimicrobial activity of cinnamaldehyde and pomegranate-based polycaprolactone films. *J Appl Polym Sci* 134:45347.
- Var I, Sağlam S, Kamberoğlu MA (2016) Geleneksel Meyve Ekşilerinin Antimikrobiyal Özellikleri (In Turkish). In: Proceedings of the Gıda, Metabolizma & Sağlık: Biyoaktif Bileşenlerle Doğal Katkılar Kongresi, Istanbul, Turkey: pp 41-45.
- Vetchapitak T, Misawa N (2019) Current status of *Campylobacter* food poisoning in Japan. *Food Safety* 7:61-73.
- Voravuthikunchai S, Lortheeranuwat A, Jeeju W, Srirak T, Phongpaichit S, Supawita T (2004) Effective medicinal plants against enterohaemorrhagic *Escherichia coli* O157:H7. *J Ethnopharmacol* 94:49-54.
- Weerakkody NS, Caffin N, Turner MS, Dykes GA (2010) *In vitro* antimicrobial activity of less-utilized spice and herb extracts against selected food-borne bacteria. *Food Control* 21:1408-1414.
- Yapar F (2006) The antibacterial effects of plum sauce, pomegranate sauce and citric acid to chopped and minced meat (In Turkish). ÇukurovaÜniv Fen BilEnstMsC Thesis, Turkey, 49 pp.