

# Comparison of Biochemical, Microbiological, and Toxicological Properties of Wild and Cultivated Sour Cherry Genotypes (*Prunus cerasus* L.)

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### ABSTRACT

The investigation of two sour cherry genotypes such as the SC genotype, a small-fruited wild sour cherry, and the LC genotype, a large-fruited cultivated sour cherry, for antimicrobial, mutagen, antimutagen, and antioxidant activities, and bioactive compounds was aimed. Total phenolic, flavonoid, and ascorbic acid contents, antioxidant activity (DPPH, ABTS, FRAP), phenolic, sorbic, benzoic acids, sugar contents, and antibacterial activities (agar well diffusion, MIC, MBC, and MTC) were studied. Additionally, Salmonella typhimurium revision tests were made. The SC genotype had higher values for total phenolic, flavonoid, and ascorbic acid contents than the LC genotype. Similarly, antioxidant activity was found to be higher in the SC genotype. The dominant phenolic acids in both sour cherry genotypes were succinic acid and rutin trihydrate among the phenolic acid contents. While benzoic acid, sorbic acid, and sucrose could not be detected in both genotypes, glucose and fructose contents were higher in the LC genotype. Bacillus spizizenii ATCC 6633 was the most sensitive bacteria to both sour cherry extracts, and a weaker antibacterial activity was observed in the other test bacteria. In addition, no mutagenic and antimutagenic activities were found in both sour cherry genotypes. The SC genotype, a wild sour cherry, contains more bioactive components and exhibits higher antioxidant activity than the LC genotype, a cultivated variety. Consequently, because of its contents and biological activities, sour cherry has the potential to play a supportive role in human health.

# Yabani ve Kültüre Alınmış Vişne Genotiplerinin Biyokimyasal, Mikrobiyolojik ve Toksikolojik Ozelliklerinin Karşılaştırılması

### ÖZET

Bu çalışmada küçük meyveli yabani bir vişne olan SC genotipi ve büyük meyveli bir kültür vişnesi olan LC genotipi olmak üzere iki vişne genotipinin antimikrobiyal, mutajen, antimutajen ve antioksidan aktivite ve biyoaktif bileşenler yönünden araştırılması amaçlanmıştır. Vişne genotipleri toplam fenolik, flavonoid ve askorbik asit içerikleri, antioksidan aktivite (DPPH, ABTS ve FRAP), fenolik asit, sorbik asit, benzoik asit, şeker içerikleri ve antibakteriyel aktiviteler (agar kuyu difüzyon, MIC, MBC ve MTC) yönünden incelenmiştir. Ayrıca, Salmonella typhimurium revizyon testleri yapılmıştır. Sonuçlara göre SC genotipinin toplam fenolik, toplam flavonoid ve askorbik asit içerikleri açısından LC genotipine göre daha yüksek değerlere sahip olduğu görülmüştür. Benzer şekilde SC genotipinde antioksidan aktivitenin daha yüksek olduğu belirlenmiştir. Her iki vişne genotipinde baskın fenolik asitlerin süksinik asit ve rutin trihidrat olduğu belirlenmiştir. Benzoik asit, sorbik asit ve sakkaroz her iki genotipte de tespit edilememişken; LC genotipinde glikoz ve fruktoz içerikleri daha yüksek bulunmuştur. Bacillus spizizenii ATCC 6633'nin her iki vişne ekstraktına karşı en duyarlı bakteri olduğu tespit edilmiş ve diğer test bakterilerinde daha zayıf bir antibakteriyel aktivite gözlenmiştir. Ayrıca **Food Science** 

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### Anahtar Kelimeler

Fenolik asit,	
Minimum	inhibisyon
konsantrasyonu,	
Minimum	bakterisidal
konsantrasyon,	
Mutajen/antimuta	jen,
Antioksidan	

her iki vişne genotipinde de mutajenik ve antimutajenik aktiviteye rastlanmamıştır. Yabani vişne olan SC genotipinin, kültüre alınan bir çeşit olan LC genotipine göre daha yüksek miktarda biyoaktif bileşen içerdiği ve daha yüksek antioksidan aktivite gösterdiği görülmüştür. Sonuç olarak vişne, sahip olduğu içerik ve biyolojik aktiviteleri nedeniyle insan sağlığını destekleyici rol oynayabilecek potansiyele sahiptir.

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# INTRODUCTION

Sour cherry (Prunus cerasus L.) is a sour, stone fruit from the Rosaceae family (Ferretti et al., 2010). It took its name (cerasus) from "Kerasus" which is the old name of the Giresun Province of The Black Sea Region of Türkiye and its homeland is the North Anatolian mountains (Anonymous, 2008). It also grows in many parts of the world such as Europe, North America, and Asia (Wojdylo et al., 2014). Due to its acid/sugar ratio, sour cherry has a characteristic sour taste, which limits its consumption as fresh fruit (Yılmaz et al., 2019). So, sour cherry is generally consumed as jam, marmalade, and fruit juice and has recently been used in the production of vinegar and alcoholic beverages. Sour cherry is one of the fruits that are rich in phenolic substances, especially anthocyanins, and its unique color is due to the anthocyanins it contains (Chandra et al., 1992). Biochemical contents such as total phenolic substance and total anthocyanin content (Kim et al., 2005) differ according to the cherry variety.

In studies on the effects of sour cherry and cherry juice on human health, it has been reported that the consumption of sour cherry juice may be beneficial in regulating sleep quality in adults with insomnia (Pigeon et al., 2010) and it may reduce the average serum triglyceride amount, the very low-density lipoprotein (VLDL) amount and triglyceride to highdensity lipoprotein cholesterol (TG/HDL) ratio, and the amount of serum uric acid, which is a risk indicator of inflammation and cardiovascular diseases (Martin et al., 2011). Similarly, the consumption of sour cherry juice can significantly reduce the loss of strength and pain caused by exercise (Connolly et al., 2006) and post-race pain in runners (Kuehl et al., 2010).

In this study, the antibacterial, antioxidant, mutagen, and antimutagenic activities of two sour cherry genotypes such as the SC genotype, a small-fruited wild sour cherry, and the LC genotype, a large-fruited cultivated sour cherry, were evaluated and compared. Moreover, total phenolic, total flavonoid, and total ascorbic acid contents and phenolic, sorbic, benzoic acids, and sugar contents of these sour cherries' genotypes consumed for different purposes by the local people were investigated and compared. The main reason for the preference of these samples is that sour cherry genotypes grown in Posof (Ardahan/Türkiye) have not been studied before.

### MATERIALS AND METHODS

### **Cherry Samples**

Two sour cherry genotypes such as the SC genotype which is a small-fruited wild sour cherry and the LC genotype which is a large-fruited cultivated sour cherry grown in Posof where there are suitable conditions for fruit-growing activities due to microclimatic properties of Ardahan in Eastern Anatolia region of Türkiye were used in this study. The SC genotype has fruit that is about two times smaller than the LC genotype. Fruit samples of two sour cherry were collected from genotypes Posof (Ardahan/Türkiye) in the harvest period of 2019 and brought to the laboratory under appropriate conditions and stored at +4 °C.

### **Extract Preparation**

The stems, leaves, and seeds were removed from fruit samples. Extraction was carried out as previously described (Erbil et al., 2020). The filter-sterilized aqueous extracts were stored at -20 °C and they were used in antibacterial and mutagenic/antimutagenic activity tests.

For the extract prepared to be used in total phenolic and flavonoid content and antioxidant activity tests, 2 g of fresh fruit sample was homogenized with 20 ml of methanol (80%), and the resulting mixture was kept in a shaker (ACMI 006) at +4 °C for 24 hours. Afterward, it was centrifuged for 10 min at 5000 rpm, and then the supernatant was collected. For the total ascorbic acid measurements, the supernatant obtained by using the same extraction method with oxalic acid (0.4%) was used instead of methanol.

### **Determination of Total Phenolic Content**

Using the Folin-Ciocalteu method (Spanos & Wrolstad, 1992), the total phenolic content was determined. The absorbance was measured at 750 nm in a UNICO S1205 Visible Spectrophotometer. Using the gallic acid standard, the total phenolic content was calculated (y=0.863x+0.134,  $R^2=0.998$ ). All tests were performed in triplicate.

### Determination of Total Flavonoid Content

The total flavonoid contents of sour cherry genotypes were determined according to the method developed by Quettier-Deleu et al. (2000) using a UNICO S1205 Visible Spectrophotometer at 415 nm. Using the routinely prepared calibration curve, the total amount of flavonoid substance was calculated  $(y=0.021x+0.040, R^2=0.999)$ . All tests were performed in triplicate.

### Determination of Total Ascorbic Acid Content

Total ascorbic acid contents of sour cherry genotypes were determined spectrophotometrically (UNICO S1205 Visible Spectrophotometer) (AOAC, 1990) and the absorbance values were measured at 520 nm. The total amount of ascorbic acid content was calculated using the calibration curve. All tests were performed in triplicate.

### **Determination of Antioxidant Capacity**

# DPPH (2,2-diphenyl-1-picrylhydrazyl) assay

DPPH free radical scavenging activity was performed according to methods suggested by Bakhshi and Arakawa (Bakhshi & Arakawa, 2006). The absorbance was read in a spectrophotometer (UNICO S1205 Visible Spectrophotometer) at 515 nm. Trolox was used as an antioxidant standard. The IC50 was calculated from a graph of inhibition against the different concentrations. All tests were performed in triplicate.

# ABTS [2,2'-azino-bis (3-ethylbenzothiazoline-6-6-sulfonic acid)] assay

ABTS free radical scavenging activity was determined according to the method suggested by Re et al. (1999). The absorbance was measured spectrophotometrically at 734 nm (UNICO S1205 Visible Spectrophotometer). Trolox was used as an antioxidant standard. The IC50 was calculated from a graph of inhibition against the different concentrations. All tests were performed in triplicate.

### FRAP (ferric ion reducing antioxidant power) assay

The FRAP method was performed according to Benzie & Strain (1996). The absorbance was measured at 593 nm. The standard curve was prepared using FeSO<sub>4</sub> solution (100-1000  $\mu$ l) (*y*=0.0011x 0.0029, R<sup>2</sup>:0.999). The results were calculated in terms of "µmol Fe (II) g<sup>-1</sup>". All tests were performed in triplicate.

# Phenolic Acid Analysis

Fruit samples were used fresh in the analysis and the

juice was squeezed. 5 ml of cherry juice samples were taken, and it was completed with methanol up to its volume in a 25 ml. The filtrate was filtered through a 0.45 µl syringe tip microfilter. Phenolic acid analyses were performed with SHIMADZU-LCMS/MS 8040 and ODS-4  $(3\mu mx2.1mmx50mm)$ Inertsil column. Methanol (B) and ammonium acetate (1mM)-acetic acid (0.02%) solution (A) were used as the mobile phase. The flow rate was 0.4 ml min<sup>-1</sup>, the column temperature was 40 °C, and the injection volume was 10 µl. Additionally, the nebulizing gas flow was 2.9 l min<sup>-1</sup>, the drying gas flow was 15 l min<sup>-1</sup>, the DL temperature was 400 °C, and the heat block temperature was 250 °C.

### Benzoic and Sorbic Acid Analyses

In the analysis, sour cherry samples were used fresh, and the juice was squeezed. 5 ml of cherry juice samples were taken. 17.5 ml methanol was added, and it was completed with ultrapure water up to its volume in a 50 ml. After mixing well, the solution was filtered through filter paper and then the filtrate was filtered through a 0.45  $\mu$ l syringe tip microfilter. Bezoic and sorbic acid analyses were made via Shimadzu/LC-20AD HPLC and DAD (Diode Array Detector) was used as the detector. Inertsil ODS-3 column (5 $\mu$ mx4.6mmx150mm) was used as the column. Acetonitrile/water (80/20) was used as the mobile phase. The injection volume was 20  $\mu$ l, the column temperature was 30 °C, the flow rate was 1 ml min<sup>-1</sup>, and the processing time was 10 minutes.

# Sugar Analysis

The sour cherry samples were used fresh in the analysis and the juice was squeezed. 3 ml of acetonitrile/water (80/20) solution was added to 1 ml of fresh cherry juice samples and the mixing solution was filtered through a 0.45  $\mu$ l syringe tip microfilter. Sugar analyses were made by Shimadzu/ LC-20AD HPLC. RID (Refractive Index Detector) and Inertsil NH2 column (5 $\mu$ mx4.6mmx250mm) were used. Acetonitrile/water (80/20) was used as the mobile phase. The flow rate was 1.3 ml min<sup>-1</sup>, the column temperature was 40 °C, the injection volume was 20  $\mu$ l, and the processing time was 25 min.

### **Determination of Antibacterial Activity**

Antibacterial activities of sour cherry extracts were studied by agar well diffusion, minimum inhibition concentration (MIC). bactericidal minimum maximum concentration (MBC), tolerable and concentration (MTC) methods. Pseudomonas aeruginosa ATCC 9027, Klebsiella pneumoniae ATCC 33495. Enterobacter aerogenes ATCC 13048. Escherichia coli ATCC 8739, Bacillus spizizenii ATCC 6633 (American Type Culture Collection, Manassas, VA) were used as test microorganisms.

Muller Hinton Agar (MHA) (Merck, Germany) was used as the medium for the agar well diffusion method (Hufford et al., 1975) and wells with a diameter of 10 mm were prepared. 150 µl of each sour cherry extract was added to the wells, and erythromycin was used as a positive control. Petri plates were incubated at 37 °C for 48 hours and inhibition zones were measured with the help of a digital caliper. All tests were performed in triplicate.

Minimum inhibition concentration (MIC) was determined by the broth microdilution method (Abbasoğlu et al., 1995; Duman et al., 2016). Muller Hinton Broth (MHB) (Merck, Germany) was used as the medium and sour cherry extracts were diluted (7.43-0.0290 mg ml<sup>-1</sup> for LC genotype, 8.2-0.0320 mg ml<sup>-1</sup> for SC genotype). 16-hour bacterial cultures were adjusted according to the 0.5 McFarland standard and all plates were incubated at 37 °C for 18 hours. After the incubation, 0.5% 2,3,5-triphenyl tetrazolium chloride (TTC) solution was added to each well and the well in which no colour change was observed was determined as MIC.

To determine the minimum bactericidal concentration (MBC), 10  $\mu$ l of samples were taken from each of the wells in which no bacterial growth was observed and inoculated on Muller Hinton Agar medium and incubated for 24-48 hours at 37 °C. The highest extract dilution providing 99% bacterial inhibition was determined as MBC (Erkmen, 2016).

The highest extract concentration, which does not affect bacterial growth, was determined as the maximum tolerable concentration (MTC) (Erkmen, 2016).

# Determination of Mutagenic and Antimutagenic Activities

The mutagenic activities of aqueous sour cherry extracts were performed according to the plaque incorporation method suggested by Maron and Ames (Maron & Ames, 1983) in the absence of an S9 mix. The pre-incubation test of the Salmonella/Microsome test was also applied to investigate the antimutagenic effects of extracts in the presence of S9 mix (Maron & Ames, 1983). To determine the mutagenic and antimutagenic activities, Salmonella typhimurium TA 98 and Salmonella typhimurium TA 100 strains were used as test microorganisms. While 4-Nitro-Ophenylenediamine (4-NPD; Product Number: 1088898-5G, Sigma Aldrich, St. Louis, MO, USA) was used as a positive control for TA 98 (10 µg/plate), sodium azide (SA; Cat. No. S 2002, Sigma Aldrich) was used as a positive control for TA 100 (100 µg plate<sup>-1</sup>). In addition, 2-aminofluorene (2-AF) (cat. no A-9031; Sigma) was used as a positive mutagen (20 µg/plate) in the presence of S9 mix in both TA 98 and TA 100 test strains. Plates were incubated at 37°C for 48-72 hours, and then his+ revertant bacterial colonies were counted on the plates. All tests were performed in triplicate.

# Statistical Analysis

The results of this study were analyzed by the SPSS statistical analysis package program (version 25) and results were presented as "mean  $\pm$  standard deviation". The statistical data were subjected to the analysis of the t-test. The differences at p<0.05 were considered statistically significant. According to the Shapiro-Wilk test, the data of the mutagenic and antimutagenic activity tests showed a normal distribution. Using the SPSS software, these data were analyzed by one-way ANOVA, followed by Dunnett's test, with  $p \le 0.05$ , being statistically significant.

# **RESULTS and DISCUSSION**

Sour cherry is rich in bioactive components, and its health-promoting effects are associated with these components (Yılmaz et al., 2019). The phenolic content of sour cherry is affected by factors such as temperature, light, and fruit maturity level (Ferretti et al., 2010).

The total phenolic, total flavonoid and total ascorbic acid contents were determined as  $176.9 \text{ mg } 100\text{g}^{-1}$ ,  $70.3 \text{ mg } 100\text{g}^{-1}$ , and  $342.7 \text{ mg } 100\text{g}^{-1}$ , respectively, and these values were statistically higher than the values of the LC genotype (p<0.05) in this study (Table 1). Piljac-Žegarac and Šamec (2011) established that while the total phenolic contents of sour cherry fruits were  $176.73-291.39 \text{ mg GAE } 100\text{g}^{-1} \text{ FW}$ , total flavonoid contents were found as  $97.45-130.13 \text{ mg CE } 100\text{g}^{-1} \text{ FW}$ . In another study, total phenolic content was reported as 235.81 and  $340.80 \text{ mg GAE } 100\text{g}^{-1} \text{ FW}$  in two sour cherry cultivars (Kazazic et al., 2022).

The phenolic compounds in herbal products have antioxidant activity as well as beneficial effects on health because thanks to the scavenging feature of free radicals, they can prevent the damage caused by reactive oxygen. In addition to their antioxidant properties, these compounds also affect many sensory properties of foods, especially colour, astringency, and flavour (Garcia-Parrilla et al., 2016). Polyphenols have potential antioxidant properties since they have a phenolic ring that can stabilize and replace the unpaired electrons in the aromatic ring (Qiu et al., 2010). Antioxidant activity may vary according to the number and binding sites of hydroxyl (-OH) groups attached to the structure (Heim et al., 2002).

Different plants may have different levels of antioxidant activity (Can Agca et al., 2023; Şeker and Karaçelik, 2023). In this study, in parallel with the phenolic contents, ABTS ( $IC_{50}$ ) and FRAP values were found to be statistically higher in the SC genotype (30.22 µg/ml and 234.8 µmolFeII g<sup>-1</sup>, respectively) than the LC genotype (20.77 µg/ml and 211.4 µmolFeII g<sup>-1</sup>,

respectively). However, there was no statistically significant difference between samples in DPPH radical scavenging activity results (p<0.05) (Table 1). It was also reported in previous studies that the sour

cherry had an antioxidant effect (Wang et al., 1999; Traustadottir et al., 2009; Damar and Ekşi, 2012; Bonerz et al., 2007; Blando et al., 2004).

Table 1. Bioactive components and antioxidant activities of sour cherry genotypes *Çizelge 1. Vişne genotiplerinin biyoaktif bileşenleri ve antioksidan aktivitesi* 

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	Total Phenolic	Total Flavonoid	Total Ascorbic	ABTS	DPPH	FRAP
	Content	Content	Acid Content	$(IC_{50})$	$(IC_{50})$	(µmolFeII g <sup>-1</sup> )
	(mg 100g <sup>-1</sup> )	(mg 100g <sup>-1</sup> )	$(mg \ 100g^{-1})$	μg/ml	μg/ml	
LC genotype	$143.7 \pm 6.13^{b}$	$65.1 \pm 2.9^{b}$	$298.2 \pm 12.8^{b}$	$20.77 \pm 1.6^{b}$	$23.74 \pm 1.3^{a}$	$211.4 \pm 12.3^{b}$
SC genotype	$176.9 \pm 13.5^{a}$	$70.3 \pm 0.99^{a}$	$342.7 \pm 18.5^{a}$	$30.22 \pm 0.9^{a}$	$25.36 \pm 1.5^{a}$	$234.8 \pm 13.9^{a}$
$D^{*}CC + 1 + 1$	(1) 1 $(1)$	1 1	1 1.00	1.	1	(

Different letters (a-b) shown in the same column show statistical differences according to the test (p<0.05)

Herbal phenolics have an antimicrobial effect on microorganisms, and this effect is significant in eliminating food-borne pathogens (Kołodziejczyk et al., 2013; Demirdöven et al., 2015). The results of the antibacterial activity of the LC genotype are presented in Table 2. As a result of the analyses, *Bacillus spizizenii* ATCC 6633 was the most sensitive bacterium among the test bacteria and the highest antibacterial effect was developed against this microorganism. It is observed that the antibacterial effects against other test bacteria were weaker.

Table 2. Antibacterial effects of the LC genotype *Cizelge 2. LC genotipinin antibakteriyel etkisi* 

	Inhibition Zone (mm)	MIC (mg ml <sup>-</sup> 1)	MBC (mg ml <sup>-</sup> 1)	MTC (mg ml <sup>-1</sup> )
Bacillus spizizenii ATCC 6633	$34.0067 \pm 0.23$	0.2321	0.2321	0.1160
<i>Escherichia coli</i> ATCC 8739	-	3.715	7.43	1.8575
Klebsiella pneumoniae ATCC 33495	$13.23 \pm 0.4464$	3.715	7.43	1.85.75
Enterobacter aerogenes ATCC 13048	-	3.715	7.43	1.8575
Pseudomonas aeruginosa ATCC 902'	7 $17.0667 \pm 0.4860$	3.715	7.43	1.8575

The results of the antibacterial activity of the SC genotype are presented in Table 3. It was observed that *Bacillus spizizenii* ATCC 6633 was also the most sensitive bacterium among test bacteria similar to the LC genotype and the antibacterial effects against the other test bacteria were weaker. Homoki et al (2018) reported that sour cherry extract was effective on *Streptococcus mutans* and that chewing gum with sour cherry extract may be beneficial in preventing dental

caries. In another study, it was reported that the oil obtained from the cold-pressed sour cherry seeds was effective on *Staphylococcus aureus* (Basyigit et al., 2021). Additionally, it was reported that sour cherry had an antifungal effect against some food-borne mould species (Tomar et al., 2022). When the results obtained from this study and the results obtained from previous studies are evaluated together, it is concluded that sour cherry has an antimicrobial effect against different microorganisms at different rates.

Table 3. Antibacterial effects of the SC genotype *Cizelge 3. SC genotipinin antibakteriyel etkisi* 

	Inhibition Zone (mm)	MIC (mg ml <sup>-1</sup> )	MBC (mg ml <sup>-1</sup> )	MTC (mg ml <sup>-1</sup> )
Bacillus spizizenii ATCC 6633	$34.88 \pm 0.3364$	1.025	1.025	0.5125
Escherichia coli ATCC 8739	-	4.1	8.2	2.05
Klebsiella pneumoniae ATCC 33495	-	4.1	8.2	2.05
Enterobacter aerogenes ATCC 13048	-	8.2	8.2	4.1
Pseudomonas aeruginosa ATCC 902	$7  14.58 \pm 0.6753$	4.1	8.2	2.05

Interest in bioactive phytochemicals obtained from natural sources is increasing day by day (Serra et al., 2010). Polyphenols have protective effects against some diseases such as cancer, coronary, and cardiovascular diseases and reduce the tendency to these diseases (Cilek et al., 2012). The phenolic acids of LC and SC genotypes of sour cherry were evaluated, and results were presented in Table 4. According to the results, succinic acid was the main phenolic acid in both SC and LC genotypes (75.3 mg  $L^{-1}$  and 45.4 mg  $L^{-1}$ , respectively) (Figure 1), followed by rutin trihydrate (27.15 mg  $L^{-1}$ , 22.2 mg  $L^{-1}$ , respectively) (Figure 2). In a previous study, rutin was determined as 0.22 mg  $L^{-1}$  in sour cherry fruit (Tomar et al., 2022). In another study, it was found that the main phenolics in sour cherry were flavonols such as catechin, epicatechin, quercetin-3-glucoside, quercetin-3-rutinoside, camferol-3-rutinoside (Goncalves et al., 2004).

Table 4. Phenolic acids of the LC and SC genotypes	
Cizelge 4. LC ve SC genotiplerinin fenolik asit iceriği	

Çizelge 4. LC ve SC genotiplerinin fenolik asit içeriği				
Phenolic Acid	LC Genotype	SC Genotype		
Caffeic acid	$493.7~\mu{ m g~L^{-1}}$	$485~{ m \mu g}~{ m L}^{\cdot 1}$		
Resveratrol	-	-		
Rutin trihydrate	$22.2~{ m mg}~{ m L}^{\cdot 1}$	$27.15~{ m mg~L^{-1}}$		
Sinapic acid	-	-		
Quercetin	-	$674~\mu g~{ m L}^{\cdot 1}$		
Gallic acid	-	-		
Transferulic acid	$255.7~{ m \mu g}~{ m L}^{-1}$	$184.3~\mu{ m g}~{ m L}^{\cdot1}$		
p-coumaric acid	$132.3~\mu{ m g}~{ m L}^{-1}$	$705.6~\mu\mathrm{g~L^{\cdot 1}}$		
Vanillic acid	$803.3~\mu{ m g~L^{-1}}$	$473~\mu\mathrm{g~L^{\cdot 1}}$		
Naringin	-	-		
Succinic acid	$45.4~{ m mg}~{ m L}^{-1}$	$75.3~{ m mg~L^{-1}}$		

While the SC genotype had higher amounts of rutin trihydrate (27.15 mg L<sup>-1</sup>), p-coumaric acid (705.6 µg L<sup>-1</sup>), and succinic acid (75.3 mg L<sup>-1</sup>); the LC genotype contains more caffeic acid (493.7 µg L<sup>-1</sup>), transferulic acid (255.7 µg L<sup>-1</sup>), and vanillic acid (803.3 µg L<sup>-1</sup>). SC genotype included 674 µg L<sup>-1</sup> of quercetin, but the LC genotype did not. Similarly, quercetin was found to be 0.63 mg L<sup>-1</sup> in *Prunus cerasus* fruit (Tomar et al., 2022). Quercetin is an important bioflavonoid (Lakhanpal et al., 2007) and because of its pharmacological effects on mammalian cells and tissues, it is considered health-promoting and diseasepreventing (Block, 1992).

Resveratrol, sinapic acid, gallic acid, and naringin were not detected in both sour cherry genotypes, in this study. The phenolic and flavonoid contents of plants and fruits can be easily affected and changed by many factors such as climatic conditions, soil structure, geographical factors, etc. So, it is expected that the bioactive components and their amounts of fruit samples grown in different geographical conditions at different times may vary.

According to the result of sorbic and benzoic acid analyses, both genotypes had no sorbic and benzoic acids. Different amounts of glucose and fructose were determined, and it was observed that both glucose and fructose values of the LC genotype (73.672 g L<sup>-1</sup> and 40.942 g L<sup>-1</sup>, respectively) were higher than SC genotype (68.270 g L<sup>-1</sup> and 34.046 g L<sup>-1</sup>, respectively). However, it was determined that neither genotype had any sucrose (Table 5). It was reported that the fructose contents of sour cherry cultivars were between 16.1-18.3 mg 100g<sup>-1</sup> DW and glucose contents were between 46.1-56.4 mg 100g<sup>-1</sup> DW (Wojdylo et al., 2021). In another study, fructose and glucose contents were determined as 3.98 µg g<sup>-1</sup> and 6.03 µg g<sup>-1</sup> (respectively) in wild sour cherry (Karaat et al., 2019).

Due to the various biological activities of plants, the interest in herbal products has increased considerably in recent years. However, plant cells contain varying amounts of different phytochemicals, and their consumption above a certain dose can create toxic or mutagenic activity in humans (Wan-Ibrahim et al., 2010). The mutagenicity tests of the LC and SC genotypes were investigated on *Salmonella typhimurium* TA 98 and TA 100 strains (Table 6). Four different concentrations of both sour cherry extracts (10, 20, 40, 80  $\mu$ L plate<sup>-1</sup>) were determined via preliminary works and used in analyses. As a result of the analyses, no mutagenic effect was detected against TA 98 and TA 100 strains at any doses of both sour cherry genotypes.

Table 5. Sugar contents of LC and SC genotypes	
Çizelge 6. LC ve SC genotiplerinin şeker içerikler	ri

<b>,</b>	LC Genotype (g L <sup>-1</sup> )	SC Genotype (g L <sup>-1</sup> )
Glucose	73.672	68.270
Fructose	40.942	34.046
Sucrose	-	-

Antimutagenicity tests of LC and SC genotypes of sour cherry were investigated on Salmonella typhimurium TA 98 and TA 100 strains in the presence of the S9 mix (Table 7). According to the results of the pretesting, four different concentrations of both sour cherry extracts (10, 20, 40, 80 µL plate<sup>-1</sup>) were determined and used in analyses. As a result of the analyses, no antimutagenic effect was detected against TA 98 and TA 100 strains at any doses of both sour cherry genotypes. It was reported that sour cherry inhibited tumour development in mice and the proliferation of colon cancer cells in humans (Kang et al., 2003; Traustadottir et al., 2009). The antimutagen activity was found in previous studies for different fruit samples. The different doses of aqueous extracts of Kızıl, Gügüm, Banda, and Deveci pears (Erbil et al., 2018) and different doses of methanolic extracts of apple leaves exhibited antimutagenic activity (Erbil et al., 2020).

### CONCLUSIONS

Because of their health-promoting and diseasepreventing effects, plants, plant-derived materials, and fruits have been used by humans for different purposes for centuries. Especially fruits have an essential place in the human daily diet. Nowadays, many fruits are considered functional foods due to their bioactive compounds and biological activities.

One of these fruits is sour cherry, which has a wide range of uses. In this study, the bioactive components and various biological activities of two sour cherry genotypes grown in Posof/Ardahan (Türkiye), which have not been studied previously, were evaluated. Succinic acid and rutin trihydrate were the most dominant phenolic acids in these sour cherry genotypes. In addition, sour cherry extracts possessed antioxidant and antibacterial activities but did not show any mutagen and antimutagen effects. Based on these results, it may be said that sour cherry has health-supporting effects for humans and may be considered a functional food.





kromatogramı, c: SC genotipinin kromatogramı, d: LC genotipinin kromatogramı



Figure 2. Chromatograms of rutin trihydrate of the LC and SC genotypes, a: Calibration curve, b: Chromatogram of standard solution, c: Chromatogram of SC genotype, d: Chromatogram of LC genotype
 Şekil 2. LC ve SC genotiplerinin rutin trihidrat kromatogramları, a: Kalibrasyon eğrisi, b: Standart solüsyonun kromatogramı, c: SC genotipinin kromatogramı, d: LC genotipinin kromatogramı

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### Author Contribution

The authors declare that they have contributed equally to the article.

### **Conflict of Interest**

N. Erbil, Z.T. Murathan, and M. Arslan declare that they have no conflict of interest.

Table 6. The mutagenic effects of LC and SC genotypes (in the absence of S9 mix)
Cizelge 7. LC ve SC genotiplerinin mutajenik etkisi (S9 mix vokluğunda)

ur Cherry Genotypes		TA 98	TA 100
		Mean	. <b>± Sd*</b>
	Control	$19.00 \pm 2.65$	$116.0 \pm 12.5$
	Positive Control	$6329 \pm 444$	$3969 \pm 220$
	10**	$14.00 \pm 2.89$	$79.33 \pm 7.22$
LC Genotype	20	$12.00 \pm 0.577$	$113.33 \pm 8.69$
	40	$14.00\pm2.52$	$115.7\pm10.4$
	80	$20.67 \pm 3.33$	$117.00 \pm 5.29$
SC Genotype	Control	$19.00 \pm 2.65$	$116.0 \pm 12.5$
	Positive Control	$6329 \pm 444$	$3969 \pm 220$
	10**	$23.00 \pm 3.21$	$135.7 \pm 30.1$
	20	$18.00 \pm 3.21$	$138.3\pm28.7$
	40	$21.00 \pm 2.08$	$77.33 \pm 4.63$
	80	$17.33 \pm 4.06$	$60.00 \pm 8.50$

\*Sd: Standard deviation; \*\*: Concentration of sour cherry extracts ( $\mu$ L plaque<sup>-1</sup>); 4-NPD for TA 98 and SA for TA 100 were used as positive controls

Table 7. The antimutagenic effects of LC and SC genotypes (in the presence of S9 mix)
Çizelge 8. LC ve SC genotiplerinin antimutajenik etkisi (S9 mix varlığında)

Sour Cherry Genotypes	3	TA 98	TA 100
		$Mean \pm Sd^*$	
	Control	$57.0 \pm 16.2$	$119.7 \pm 22.6$
LC Genotype	Positive Control	$402.0 \pm 60.4$	$571 \pm 122$
	10**	$463.0 \pm 72.9$	$442.0 \pm 22.5$
	20	$347.0 \pm 62.5$	$612.0 \pm 65.0$
	40	$265.0 \pm 54.6$	$414.0 \pm 36.9$
	80	$303.0 \pm 59.6$	$410.0 \pm 42.6$
SC Genotype	Control	$57.0 \pm 16.2$	$119.7 \pm 22.6$
	Positive Control	$402.0 \pm 60.4$	$571 \pm 122$
	10**	$699.0 \pm 33.4$	$690.0 \pm 39.0$
	20	$276.0 \pm 21.7$	$392.0 \pm 11.1$
	40	$525.0 \pm 52.8$	$403.0\pm79.2$
	80	$240.0 \pm 21.0$	$373.0\pm58.2$

\*Sd: Standard deviation; \*\*: Concentration of sour cherry extracts ( $\mu$ L plaque<sup>-1</sup>); 2AF was used as positive controls for TA 98 and TA 100

### REFERENCES

- Abbasoğlu, U., Tosun, F., & Aydınoğlu, A. (1995).
  Antimicrobial Activity of Gonocytisus angulatus
  (L.) Spach. FABAD Journal of Pharmaceutical Sciences, 20, 125-127.
- Anonymous (2008). Girasun'dan çıktık yola. Dört Mevsim Meyve Suyu, 6, 8-9.
- Association of Official Agricultural Chemistry (AOAC) (1990). *Official Methods of Analysis*, 15th ed., Arlington VA, USA, pp 1058-1059.
- Bakhshi, D., & Arakawa, O. (2006). Effects of UV-b irradiation on phenolic compound accumulation and antioxidant activity in 'Jonathan' apple influenced by bagging, temperature and maturation. *Journal of Food, Agriculture & Environment, 4*(1), 75-79.
- Basyigit, B., Saglam, H., Hayoglu, I., & Karaaslan, M. (2021). Spectroscopic (LC-ESI-MS/MS, FT-IR, NMR) and functional characterization of fruid seed oils extracted with green technology: a comparative study with *Prunus cerasus* and *Punica granatum*

oils. Journal of Food Processing and Preservation, 45(5), e15451.

- Benzie, I.F.F., & Strain, J.J. (1996). The ferric reducing ability of plasma (FRAB) as a measure of "Antioxidant power": The FRAB assay. *Analytical Biochemistry*, 239, 70-76.
- Blando, F., Gerardi, C., & Nicoletti, I. (2004). Sour cherry (*Prunus cerasus* L) anthocyanins as ingredients for functional foods. *J Biomed Biotechnol, 5*, 253-258.
- Block, G. (1992). The data support the role of antioxidants in reducing cancer risk. *Nutrition Reviews*, 50(7), 207–213.
- Bonerz, D., Würth, K., Dietrich, H., & Will, F. (2007). Analytical characterization and the impact of ageing on anthocyanin composition and degradation in juices from five sour cherry cultivars. *European Food Research and Technology*, 224, 355–364.
- Can Agca, A., Yılmaz Sarılatın, S., & Sever Yılmaz, B. (2023). Türkiye'de Yetişen *Helianthemum*

oelandicum subsp. incanum (Willk.) G. Lopez Bitkisinin Serbest Radikal Süpürücü, Antiinflamatuvar ve Hipoglisemik Aktivitelerinin Değerlendirilmesi. Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi, 26(2), 227-233.

- Chandra, A., Nair, M.G., & Lezzoni, A. (1992).
  Evaluation and characterization of the anthocyanin pigments in tart cherries (*Prunus cerasus* L.). J Agrlc Food Chem, 40(6), 967-969.
- Cilek, B., Luca, A., Hasirci, V., Sahin, S., & Sumnu, G. (2012). Microencapsulation of phenolic compounds extracted from sour cherry pomace: Effect of formulation, ultrasonication time and core to coating ratio. *Eur Food Res Technol, 235*, 587-596.
- Connolly, D.A., McHugh, M.P., Padilla-Zakour, O.I., Carlson, L., & Sayers, S.P. (2006). Efficacy of a tart cherry juice blend in preventing the symptoms of muscle damage. *Br J Sports Med*, 40, 679-683.
- Damar, İ., & Ekşi, A. (2012). Antioxidant capacity and anthocyanin profile of sour cherry (*Prunus cerasus* L.) juice. *Food Chemistry*, 135, 2910-1914.
- Demirdöven, A., Karabıyıklı, Ş., Tokatlı, K., & Öncül, N. (2015). Inhibitory effects of red cabbage and sour cherry pomace anthocyanin extracts on food-borne pathogens and their antioxidant properties. *Food Sci Technol, 63*, 8-13.
- Duman, R., Uysal, N.E., & Aladag, M.O. (2016). Evaluation of antibacterial activities of *Taraxacum farinosum* Hausskn. Et Bornm and *Taraxacum mirabile* Wagenitz Extracts. *International Journal of Scientific and Technological Research*, 2(2), 46-54.
- Erbil, N., Murathan, Z.T., Arslan, M., Ilcim, A., & Sayin, B. (2018). Antimicrobial, antioxidant, and antimutagenic activities of five Turkish pear cultivars. *Erwerbs-Obstbau*, 60, 203-209.
- Erbil, N., Arslan, M., Murathan, Z.T., Ilcim, A., & Borekci, B.S. (2020). Some biological effects of the fruits and leaves of different apple cultivars, including red-fleshed apples, grown in a microclimatic region of Türkiye: Part I. Erwerbs-Obstbau, 62, 399-410.
- Erkmen, O. (2016). *Laboratory Techniques in Microbiology*. Nobel Academic Publishing Education Consultancy, Ankara.
- Ferretti, G., Bacchetti, T., Belleggia, A., & Neri, D. (2010). Cherry antioxidants: from farm to table. *Molecules*, 15, 6993-7005.
- Garcia-Parrilla, M.C., Torija, M.J., Mas, A., & Cerezo,
  A.B. (2016). Vinegar and Other Fermented Condiments. In: Frias, J., Martinez-Villaluenga, C.,
  & Peňas, E. (eds). Fermented Foods in Health and Disease Prevention. Academic Press, London, pp 577-591.
- Goncalves, B., Landbo, A.K., Knudsen, D., Silva, A.P., Moutinho-Pereira, J., Rosa, E., & Meyer, A.S. (2004). Effect of ripeness and postharvest storage on the phenolic profiles of cherries (*Prunus avium*)

L.). J Agric Food Chem, 52, 523-30.

- Heim, K.E., Tagliaferro, A.R., & Bobilya, D.J. (2002). Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *Journal of Nutritional Biochemistry*, 13, 572-584.
- Homoki, J., Gyemant, G., Balogh, P., Stundl, L., Biro-Molnar, P., Paholcsek, M., Varadi, J., Ferenc, F., Kelentey, B., Nemes, J., & Remenyik, J. (2018). Sour cherry extract inhibits human salivary alpha-amylase and the growth of *Streptococcus mutans* (a pilot clinical study). *Food & Function*, 9(7), 4008-4016.
- Hufford, C.D., Funderburk, M.J., Morgan, J.M., & Robertson, L.W. (1975). Two antimicrobial alkaloids from heartwood of *Lirodendron tulipifera* L. *Journal of Pharmaceutical Science*, 64, 789-792.
- Kang, S.Y., Seeram, N.P., Nair, M.G., & Bourquin, L.D. (2003). Tart cherry anthocyanins inhibit tumor development in Apc (Min) mice and reduce proliferation of human colon cancer cells. *Cancer Lett*, 194, 13-19.
- Karaat, F.E., Gündüz, K., Saraçoğlu, O., & Yıldırım, H. (2019). Pomological and phytochemical evaluation of different cherry species: Mahaleb (*Prunus mahaleb* L.), wild sweet cherry (*Prunus avium* L.) and wild sour cherry (*Prunus cerasus* L.), sweet and sour cherry cultivars. *Acta Sci. Pol. Hortorum Cultus, 18*(4), 181-191.
- Kazazic, M., Mehic, E., & Djapo-Lavic, M. (2022). Phenolic content and bioactivity of two sour cherry cultivars and their products. Bulletin of the Chemists and Technologists of Bosnia and Herzegovina, 58, 1-6.
- Kim, D.O., Heo, H.J., Kim, Y.J., Yang, H.S., & Lee, C.Y. (2005). Sweet and sour cherry phenolics and their protective effects on neuronal cells. J Agric Food Chem, 53, 9921-9927.
- Kołodziejczyk, K., Sójka, M., Abadias, M., Viñas, I., Guyot, S., & Baron, A. (2013). Polyphenol composition, antioxidant capacity, and antimicribial activity of the extracs obtained from industrial sour cherry pomace. *Ind Crops Prod*, *51*, 279-288.
- Kuehl, K.S., Perrier, E.T., Elliot, D.L., & Chesnutt, J.C. (2010). Efficacy of tart cherry juice in reducing muscle pain during running: a randomized controlled trial. J Int Soc Sports Nutr, 7, 17.
- Lakhanpal, P., & Kumar, D. (2007). Quercetin: A versatile flavonoid. International Journal of Medical Update, 2(2), 22-37.
- Maron, D.M., & Ames, B.N. (1983). Revised methods for the *Salmonella* mutagenicity test. *Mutation Res*, *113*, 173-215.
- Martin, R.K., Bopp, J., Burrell, L., & Hook, G. (2011). The effect of 100% tart cherry juice on serum uric acid levels, biomarkers of inflammation and cardiovascular disease risk factors. *The FASEB Journal, 25*, 339.2.

- Pigeon, W.R., Carr, M., Gorman, C., & Perlis, M.L. (2010). Effects of a tart cherry juice beverage on the sleep of older adults with insomnia: a pilot study. J Med Food, 13, 579-83.
- Piljac-Žegarac, J., & Šamec, D. (2011). Antioksidant stability of small fruits in postharvest storage at room and refrigerator temperatures. *Food Research International*, 44, 345-350.
- Qiu, J., Ren, C., Fan, J., & Li, Z. (2010). Antioxidant activities of aged oat vinegar *in vitro* and inmouse serum and liver. *J Sci Food Afric*, 90, 1951-1958.
- Quettier-Deleu, C., Gressier, B., Vasseur, J., Dine, T., Brunet, J., Luyck, M., Cazin, M., Cazin, J.C., Bailleul, F., & Trotin, F. (2000). Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. *Journal of Ethnopharmacology*, 72, 35-40.
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, 26(9/10), 1231-1237.
- Serra, A.T., Seabra, I.J., Braga, M.E., Bronze, M.R., de Sousa, H.C., & Duarte, C.M. (2010). Processing cherries (*Prunus avium*) using supercritical fluid technology. Part 1: Recovery of extract fractions rich in bioactive compounds. *J Supercrit Fluids*, 55, 184-191.
- Spanos, G.A., & Wrolstad, R.E. (1992). Phenolic of apple, pear and white grape juices and their changes with processing and storage. J Agric Food Chem, 40(9), 1478-1487.
- Şeker, M.E., & Karaçelik, A.A. (2023). Giresun'da Yetişen Fındık Ağacı (Corylus avellana L.) Yapraklarında Bazı Fenolik Bileşiklerin ve Antioksidan Aktivitelerinin Araştırılması. Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi, 26(2), 234-244.

- Tomar, O., Akarca, G., Gök, V., & İstek, Ö. (2022). Chemical composition and antifungal potential of apricot, sour cherry, and cherry tree bio-products (resins) against food-borne molds. *Food Bioscience*, 47, 101627.
- Traustadottir, T., Davies, S.S., Stock, A.A., Su, Y., Heward, C.B., Roberts, L.J., & Harman, S.M. (2009). Tart cherry juice decreases oxidative stress in healthy older men and women. *J Nutr, 139*, 1896-900.
- Wan-Ibrahim, W.I., Sidik, K., & Kuppusamy, U.R. (2010). A high antioxidant level in edible plants is associated with genotoxic properties. *Food Chemistry*, 122(4), 1139-1144.
- Wang, H., Nair, M.G., Strasburg, G.M., Booren, A.M., & Gray, J.I. (1999). Antioxidant polyphenols from tart cherries (Prunus cerasus). J Agric Food Chem, 47, 840-844.
- Wojdylo, A., Figiel, A., Lech, K., Nowicka, P., & Oszmiański, J. (2014). Effects of convective and vacuum-microwave drying on the bioactive compound, color, and antioxidant capacity of sour cherries. *Food Bioprocess Tech*, 7, 829-841.
- Wojdylo, A., Nowicka, P., Turkiewicz, I.P., & Tkacz, K. (2021). Profiling of polyphenols by LC-QTOF/ESI-MS, characteristics of nutritional compounds and in vitro effect on pancreatic lipase, α-glucosidase, α-amylase, cholinesterase and cyclooxygenase activities of sweet (*Prunus avium*) and sour (*P. cerasus*) cherries leaves and fruits. *Industrial Crops & Products, 17*, 114214.
- Yılmaz, F.M., Görgüç, A., Karaaslan, M., Vardin, H., Bilek, S.E., Uygun, Ö., & Bircan, C. (2019). Sour cherry by-products: compositions, functional properties and recovery potential – a review. *Critical Reviews in Food Science and Nutrition*, 59(22), 3549-3563.