The antimicrobial and antioxidant properties of garagurt: traditional Cornelian cherry (*Cornus mas*) marmalade

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Abstract

The traditional Cornelian cherry marmalade, named as 'Garagurt', is usually consumed for nutritional purposes and health benefits. The objective of this study was to determine the antimicrobial and antioxidant activities of Cornelian cherry marmalade. Antioxidant activities of the sample as determined by ABTS, cupric ion-reducing antioxidant capacity (CUPRAC) and 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assays were 8,428 ± 1,206 mg TE/100 g, 1,599 ± 41.4 mg TE/100 g and 773 ± 206 mg TE/100 g respectively. The antimicrobial activity of the sample was determined by the disc diffusion method in minimum inhibitory concentration (MIC) against *Listeria monocytogenes*, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli, Escherichia coli* O157:H7, *Salmonella typhimurium, Pseudomonas fluorescens* and *Yersinia enterocolitica.* The MIC value of garagurt (0.66 mg phenol compound/g) was ≥256 mg/mL for *L. monocytogenes, S. aureus, Y. enterocolitica, E. coli* and *P. fluorescens* when gentamicin (10 µg) was used as positive control. Total phenolic content (TPC), which provides antimicrobial and antioxidant activities, was determined as 195 ± 6.35 mg GAE/100 g in aqueous methanolic extract of garagurt. This product in different forms could be used for its antimicrobial effect to increase the shelf life of different foods.

Keywords: *Cornus mas*, marmalade, antioxidant activity, antimicrobial activity

1. Introduction

Diseases originating from oxidative damage, such as cancer, coronary insufficiency, arthritis and diabetes, neurodegenerative diseases, such as Alzheimer's and Parkinson's, and many other diseases as well as signs of aging could be prevented or delayed by consumption of natural nutrients with high antioxidant contents (Dinda *et al*., 2016; Hosseinpour-Jaghdani *et al*., 2017; Moldovan *et al*., 2017; Polat *et al*., 2013; Szumny *et al*., 2015; Zargari, 1996, 1997). In this sense, several wild fruits with high levels of phytochemicals are becoming more and more important. Many of them are consumed as fresh, but they are also processed and preserved by different methods for off-season consumption. Production of jams or marmalades is one of the traditional processing methods used in Anatolia. This method is based on concentrating the fruit pulp with or without addition of sugar. Several wild fruits can be used for marmalade production, including Cornelian cherry (Şengül *et al*., 2018). Cornelian cherry (*Cornus mas* L.) is a temperate climate fruit growing in Anatolia and is commonly found as shrubs in coastal areas. It grows in wide geographic areas such as the south and southwest Asia and the temperate regions of the Northern Hemisphere (Çelik, 2009; Moldovan *et al*., 2017; Şengül *et al*., 2018). It has a sour taste and intense flavour, red in colour and oval in shape similar to olive. Cornelian cherry is highly tolerant to different environmental conditions, pests and diseases. Growing successfully in natural conditions without the use of

pesticides makes this fruit suitable for organic production (Bijelić *et al.*, 2011; Drkenda *et al.*, 2014). The production of Cornelian cherry in Turkey in 2017 was 10,012 tons from 699,422 trees (Turkish Statistical Institute (TUIK), 2018). On the other hand, the number of Cornelian cherry trees is reducing because of the low commercial value of fruits. Tontul *et al.* (2018) recommended the processing of value-added products for the sustainability of this fruit.

The chemical composition of the fruit depends on the genotypes and its propagation in a geographical location (Dinda *et al.*, 2016). For instance, 13 cultivars of Cornelian cherry fruits from Artvin, Turkey, were reported to contain 13.7–18.6% soluble solids, 31–70 mg/100 g vitamin C, 0.75–2.18% total protein, 0.36–1.08% cellulose, 0.57–1.28% tannin and 0.51–1.13% total ash (Ercisli *et al.*, 2011); 24 cultivars of Cornelian cherry fruits from Samsun, Turkey, were indicated to have 16–88 mg/100 g, fresh weight (FW) of vitamin C; 112–292 mg/100 g, FW of anthocyanins and 7.7–16.4 g/100 g, FW of sugars (Tural and Koca, 2008); 16 cultivars of Cornelian cherry fruits from Western Black Sea and inner Anatolia, Turkey, were reported to contain 29–112 mg/100 g, FW vitamin C, 148–237 mg/100 g, FW anthocyanins and 2.8–7.0 g/100 g, FW reducing sugars (Yilmaz *et al.*, 2009); 5 cultivars of Cornelian cherry fruits from Czech Republic were analysed to have 19.9–43.3 mg/100 g, FW vitamin C and 6.1–25.3 mg/100 g, FW anthocyanins (Cetkovska *et al.*, 2014). On the other hand, the phenolic, anthocyanin and tannin contents of Cornelian cherry fruits were found to depend on the ripeness of fruits; and the bluish stage of fruits show the highest phenolic and anthocyanin contents (Gunduz *et al.*, 2013). The above-mentioned physicochemical data on Cornelian cherry fruits show their high contents of phytochemicals (Dinda *et al.*, 2016). Major minerals contained in the fruits are established as potassium (K) (14300.984 ppm), calcium (Ca) (1560.095 ppm), magnesium (Mg) (715.231 ppm), phosphorus (P) (605.558 ppm) and sulphur (S) (436.754 ppm) (Kalyoncu *et al.*, 2009).

Phytochemicals such as flavonoids, phenolics, carotenoids and vitamins are very important nutritional compounds for human health (Bernal *et al.*, 2011; Kubola *et al.*, 2011; Rangkadilok *et al.*, 2007). These compounds exhibiting antioxidant, anticarcinogen, antimutagen and antimicrobial activities are known for their positive effects on health and therefore used for phytotherapy (Coşkun, 2006; Vareed *et al.*, 2006). Phenolics (i.e. gallic acid, ellagic acid, catechins, *p*-coumaric acid, rutin, quinones, flavonoids, coumarins and tannins) are very important for antimicrobial activities as well as antioxidative effects (Rangkadilok *et al.*, 2007; Şengün and Yücel, 2015; Turaland Koca, 2008).

In addition to strong antioxidants such as phenolic acids (Ercisli *et al.*, 2011; Kucharska, 2012; Serteser *et al.*, 2009), flavonoids (David and Moldovan, 2015; Pawlowska *et al.*, 2010; Yılmaz *et al.*, 2009) and vitamin C, Cornelian cherry also contains organic acids (Kucharska *et al.*, 2011; Seeram *et al.*, 2002) terpenoids (Güneş *et al.*, 2016) and iridoids (Deng *et al.*, 2013). Total phenolic and flavonoid contents and potent antioxidant activity are affected by the genotype of the fruit, variety, location, agronomic factors, post-harvest and storage processes. Mature Cornelian cherry fruits are utilised as food preservatives and for the treatment of diarrhoea and gastrointestinal diseases in traditional Turkish medicine (Celik *et al.*, 2006). This wild fruit, which is protective against oxidative stress (Vardin *et al.*, 2018), has got anti-allergic, antimicrobial, anti-inflammatory, antidiabetic, anti-obesity, anti-atherosclerotic, anticancer, hypolipidemic, hepatoprotective, neuroprotective, cardioprotective, diuretic and antimalarial properties (Alavian *et al.*, 2014; Dinda *et al.*, 2016; Hassanpour *et al.*, 2011; Jayaprakasam *et al.*, 2005; Moldovan *et al.*, 2016a, 2016b; Popović *et al.*, 2012; Vareed *et al.*, 2006; Yilmaz *et al.*, 2009). For determining biological activity and justifying the traditional use of *Cornus* species, the phytochemical composition was evaluated as an important factor (Czerwin´ska and Melzig, 2018). Xi *et al.* (2011) found that the use of *Cornus mas* powder in cured meat production at 1%, 2% and 3% levels reduced the number of *Listeria monocytogenes* by 2–4 log cfu/g. They studied the effect of Cornelian cherry and its products on seven different food pathogens (vancomycin-resistant *E. faecium*, *E. coli*, *E. coli* O157:H7, *L. monocytogenes, P. aeruginosa*, *S. typhimurium* and *S. aureus*) and indicated that the antimicrobial effect of phenolic fractions was reduced by the technological processing of Cornelian cherry juice (Côté *et al.*, 2011; Wu *et al.*, 2008).

Cornelian cherry has sweet-sour and slightly astringent taste. Fruits are consumed as fresh or used to produce jam, marmalade, fruit leather, pekmez (a kind of molasses), compotes, ice-cream, yogurt, chutney and several types of beverages, or are dried. Anthocyanins of the fruits could be used as natural food colourants (Bijelić *et al.*, 2011; Kazimierski *et al.*, 2019; Mohebbi *et al.*, 2015; Moldovan and David, 2014; Ozgen, 2015; Topdaş *et al.*, 2017; Tontul *et al.*, 2018). In Turkey, sour Cornelian cherry concentrate is used for salad, in soup and as appetiser (Bozdogan, 2017; Çakmakçı and Tosun, 2010; Demir and Kalyoncu, 2003). Fresh fruits are ideal ingredients for cakes and desserts. Recently, attention has been drawn to the possibility of applying the pickling method for Cornelian cherry fruits to obtain what is known as Polish olives. Roasted Cornelian cherry seeds were also used for preparing coffee (Kazimierski *et al.*, 2019). [AQ4]

Fruit preserves can be considered as important sources of phenolics with antioxidant capacity (Rosa *et al.*, 2015). Jam and marmalade production is one of the preservation methods of these perishables. Garagurt is a traditional marmalade prepared from Cornelian cherry fruit in

Balıkesir, Turkey. It has been consumed by local people for centuries for therapeutic effects against psoriasis, eczema and allergy problems and insulin resistance as well as cardiovascular diseases. Garagurt has a very long shelf life without the use of preservatives. It can be stored at room temperature for long periods without microbial [AQ5] growth (i.e. yeast or mould).

Within this context, the objective of this study was to determine the phenolic composition and antimicrobial and antioxidant properties of traditional garagurt marmalade for the first time.

2. Materials and methods

Materials

Traditional Cornelian cherry marmalade, named as 'garagurt', was obtained from Dursunbey district of Balıkesir province, Turkey. The following chemicals were obtained from Sigma-Aldrich GmbH in Sternheim, Germany: 2,2´-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS); the stable free radical, DPPH; 3-(2-pyridyl)-5,6-bis (4-phenyl-sulphonic acid)-1,2,4-triazine (Ferrozine); linoleic acid; α-tocopherol; polyoxyethylene sorbitan monolaurate (Tween-20) and trichloroacetic acid (TCA). The standards, including gallic acid (≥99%), ellagic acid (95%), catechin (≥98%), *p*-coumaric acid (≥98.0%) and rutin (≥94%), were of High-Performance Liquid Chromatography (HPLC) grade obtained from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany).

Production of traditional Cornelian cherry marmalade (garagurt)

The production of garagurt is shown in Figure 1. Cornelian cherry fruits were collected from Dursunbey district of Balıkesir province, Turkey. Fruits were washed after removal of stems and leaves. Fruits were boiled in tap water for 2 h. The pulp was obtained after the separation of skin and core. It was concentrated by boiling for 6–8 h (no sugar was added). Then it was sun-dried, cooled and filled in glass jars.

Physicochemical analysis of garagurt

The specific gravity and total soluble solids (°Brix) in garagurt samples were measured using a digital refractometer (Mettler Toledo RE50, Switzerland). The pH was measured using a digital pH metre (Hanna, HI 2020—02, USD). The total acidity was determined by titrating with 0.1 N NaOH and expressed as citric acid equivalent. The total protein and ash contents were analysed according to the Association of Official Analytical Chemists (AOAC, 1990) procedures. Vitamin C content was determined by a UV–Vis Spectrophotometer (Thermo) according to the method [AQ6] of Uylaşer and Başoğlu (2016). The lightness (L), red/green coordinate (a) and yellow/blue coordinate (b) values were measured using a colorimeter (Spectrophotometer CM-5, [AQ7] Konica Minolta Sensing Europe B.V.).

Extraction of traditional Cornelian cherry marmalade (garagurt)

For the sample extraction to be further used for antioxidant assays, 10 mL of ethanol (60%) was added to 2 g of marmalade. The mixture was homogenised using a vortex. Then the homogenised mixture was kept in ultrasonic bath for 15 min, and centrifuged at 4,000 rpm (+4°C) for 10 min and the obtained supernatant was separated. Then 10 mL of ethanol (60% v/v) was added to the remaining pellet and re-extracted as described previously. Supernatants obtained from both extractions were pooled and completed to 25 mL with aqueous ethanol

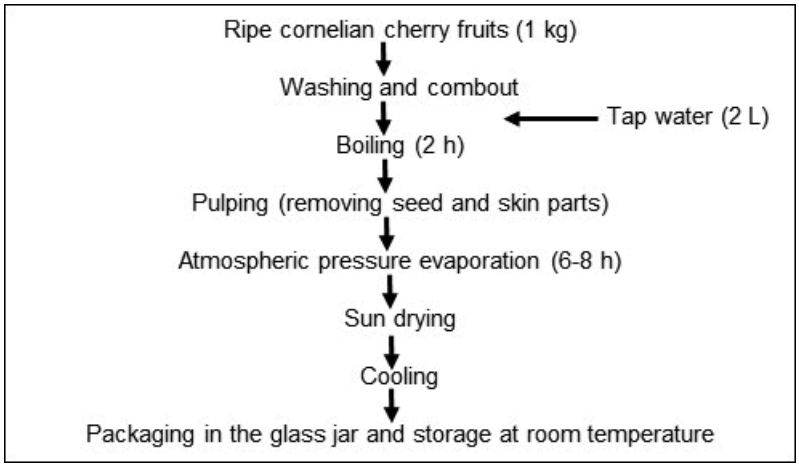

Figure 1. Production of traditional Cornelian cherry marmalade (garagurt).

[AQ8] (Kamiloglu *et al*., 2015b). Three independent extracts were prepared from the marmalade sample and measured in triplicate during analyses.

Determination of total phenolic content (TPC)

The TPC was determined by the Folin–Ciocalteu (FC) method (Spanos and Wrolstad, 1990) with some modifications. Briefly, 0.75 mL of FC reagent was mixed with 100-µL extract; after 5 min, 0.75-mL saturated Na_2CO_3 solution was added and the mixture was kept for 90 min in dark at room temperature. Gallic acid, 0.01–0.1 mg/mL, was used to prepare a standard curve and data were expressed in milligram gallic acid equivalents (GAE)/gram sample.

Determination of individual phenolics using HPLC

Briefly, extracts were filtered by 0.45-µm membrane filters and introduced into a Waters 2695 HPLC (Waters Co., Milford, MA, USA) connected to a photodiode array (PDA) detector (Waters 2996). A Supelcosil LC-18 25 cm × 4.60 mm, 5-µm column (Sigma-Aldrich) was used as the stationary phase. Two different mobile phases were used: mobile phase A, double distilled water with 0.1% (v/v) trifluoroacetic acid (TFA) and mobile phase B, acetonitrile with 0.1% (v/v) TFA. The linear gradient was used as follows: at 0 min, 95% solvent A and 5% solvent B; at 45 min, 65% solvent A and 35% solvent B; at 47 min, 25% solvent A and 75% solvent B and at 54 min turning into initial conditions. At a flow rate of 1 mL/min, 10 µL of sample was injected and spectral measurements were done at 280 nm, 312 nm and 360 nm (Kamiloglu *et al.*, 2015a).

Determination of total antioxidant activity/capacity

2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical scavenging assay

ABTS assay was employed as described by Miller and Rice-Evans (1997). Shortly, 100 µL of sample extract was mixed with 1 mL of $ABTS^+$ solution and after 1 min, the absorbance was determined at 734 nm against the extraction solution. The standard was plotted with different concentrations of standard Trolox solution (0.01–0.1 mg/mL) instead of the sample extract. The control sample was generated using an equivalent amount of extraction solvent instead of sample.

Cupric ion reducing antioxidant capacity (CUPRAC) assay

The CUPRAC method was used as described by Apak *et al*. (2004). The reaction mixture was prepared using 1-mL 0.01 mM $CuCl_2$, 1-mL 7.5 mM neocuproine, 1-mL 1 M NH_4Ac 1-mL water and 100-µL extract. After mixing, the tube was incubated in dark for 30 min and the final absorbance was monitored spectrophotometrically at 450 nm against a reagent blank. Different concentrations of Trolox (0.01–0.1 mg/mL) were used for the preparation of standard curve. The total antioxidant capacity of samples was expressed as milligram of Trolox equivalent (TE)/gram sample.

DPPH assay

Briefly, 100 µL of extract was added to 2 mL of 0.1 mM DPPH in methanol solution. The reaction mixture was vortexed and kept in dark at room temperature for 30 min before measuring its absorbance at 515 nm. The methanol solution was used as a blank (Kumaran and Karunakaran, 2006). A standard curve was plotted with Trolox (0.01–0.1 mg/mL) and total antioxidant capacity was expressed as milligram of TE/gram sample. [AQ9]

Determination of antimicrobial activity

Antimicrobial activity of garagurt was investigated by the Kirby–Bauer technique using the disc diffusion method on Mueller–Hilton agar (Oxoid CM337). The tested microorganisms (*Listeria monocytogenes* (ATCC 7644), *Bacillus cereus* (ATCC 11778), *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 25292), *Escherichia coli* O157:H7 (ATCC 43894), *Salmonella typhimurium* (ATCC 14028), *Pseudomonas fluorescens* (ATCC 13525) and *Yersinia enterocolitica* (ATCC 23715) were spread on media by adjusting density at 0.5 McFarland. Twenty marmalade solutions were impregnated on 6-mm diameter paper discs (Oxoid CT0998B), and discs were placed on the media. Gentamicin (10 µg, Oxoid CT0024B) was used as control. Antimicrobial activity was determined by measuring inhibition zones. Weak (≤12 mm), medium (12–16 mm) and strong (≥16 mm) was the scale used to evaluate the antimicrobial activity according to zone diameters (Bauer *et al*., 1966). All experiments were performed under aseptic conditions and as binary trials.

Determination of minimum inhibitory concentration (MIC)

The MIC was determined as described by the Institute of Clinical and Laboratory Standards (CLSI, 2012). Ten different concentrations (1–512 µg/mL) were tested by the MIC method; 100 µL of serial dilutions and 5 µL of bacterial solutions (108 CFU/mL) were added to the wells and incubated at 37 ± 2 °C for 24 h. One positive and one negative control well were used for each bacterium.

MICs were determined by observing the lowest concentrations that completely inhibited the growth of microorganisms in micro-dilution wells.

3. Results

Physicochemical composition

The physicochemical composition of garagurt, including its pH, total titratable acidity (citric acid equivalent), specific gravity, soluble dry matter, protein, ash, ascorbic acid and TPC as well as L, a and b values are shown in Table 1.

Antioxidant activity

The antioxidant activity of garagurt extract was analysed by employing three different methods. Antioxidant activities of the samples as determined by ABTS, CUPRAC and DPPH assays were 8,428 ± 1,206 mg TE/100 g, 1,599 ± 41.4 mg TE/100 g and 773 ± 206 mg TE/100 g respectively (Figure 2).

Table 1. Physicochemical properties of garagurt (Cornelian cherry marmalade).

pH	2.04
Total titratable acidity (g/100 mL)[1]	1.98
Specific gravity (g/cm^3, 20°C)	1.3052
Soluble dry matter (°Brix)	53.2
Protein (%N × 6.25)	1.78
Ash (%)	2.32
Ascorbic acid (mg/100 g)	48
Total phenolic content (mg GAE/100 g)	195 ± 6.35
L	9.35
a	7.12
b	7.22

[1]Citric acid equivalent.

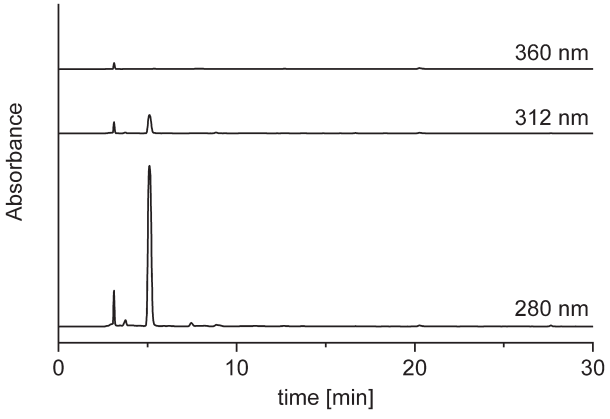

Figure 2. HPLC chromatograms of garagurt extract at 280 nm, 312 nm and 360 nm.

Phenolic profile of garagurt extract

The phenolic compounds in the samples were identified at the wavelengths of 280 nm, 312 nm and 360 nm by comparison with absorbance spectrum. Related chromatograms and the concentrations of identified phenolic compounds are presented in Figure 2 and Table 2 respectively.

Antimicrobial activity of extracts

The antimicrobial activity of garagurt, which had 0.66 mg/g total phenol compounds, was tested on 3 gram-positive and 5 gram-negative microorganisms, which are important food pathogens (Table 3). Gentamicin (10 µg) discs were used as positive controls. Although antimicrobial activity of the sample was weak against *E. coli*, *E. coli* O157:H7 and *S. typhimurium*, it had strong antimicrobial activities against *S. aureus, Y. enterocolitica, P. fluorescens, L. monocytogenes* and *B. cereus.*

Minimum Inhibitory Concentration of extract

The lowest concentration of garagurt, which has completely inhibited the bacterial growth, was determined as MIC value (Table 4). Although MIC values of garagurt

Table 2. Specific phenolic compounds identified and their quantities in garagurt extract.

Absorbance	Retention time (min)	Component	Concentration (mg/100 g)
280 nm	3.67–3.75	Gallic acid	33.4 ± 2.24
280 nm	20.2	Ellagic acid	16.9 ± 2.04
280 nm	27.58–27.64	Catechin derivative	12.6 ± 0.97
312 nm	16.664	*p*-Coumaric acid	1.40 ± 0.07
312 nm	18.339	*p*-Coumaric acid derivative	1.00 ± 0.02
360 nm	25.83	Rutin	0.70 ± 0.12

Table 3. Inhibition diameter zones (mm) on the tested bacteria of garagurt.

The tested bacteria	Zone of inhibition (mm)	
	512 mg/mL of garagurt	Gentamicin 10 µg
Listeria monocytogenes	18	18
Bacillus cereus	17	25
Staphylococcus aureus	25	25
Yersinia enterocolitica	22	25
Salmonella typhimurium	10	19
Escherichia coli	12	20
Escherichia coli O157:H7	12	13
Pseudomonas fluorescens	20	24

Table 4. Minimum inhibitory concentrations (MIC) of garagurt against the tested bacteria.

The tested bacteria	512	256	128	64	32	16	Gentamicin (80 mg/mL)
Listeria monocytogenes	-	+	+	+	+	+	0.003906
Bacillus cereus	+	+	+	+	+	+	0.001953
Staphylococcus aureus	-	+	+	+	+	+	0.000976
Yersinia enterocolitica	-	+	+	+	+	+	0.003906
Salmonella typhimurium	+	+	+	+	+	+	0.000976
Escherichia coli	-	+	+	+	+	+	0.003906
Escherichia coli O157:H7	+	+	+	+	+	+	0.003906
Pseudomonas fluorescens	-	+	+	+	+	+	0.003906

(0.66 mg phenol compound/g) were ≥256 mg/mL for *L. monocytogenes, S. aureus, Y. enterocolitica, E. coli* and *P. fluorescens*, reproduction was observed in all dilutions of *B. cereus, S. typhimurium* and *E. coli* O157:H7.

4. Discussion

Physicochemical composition

In a previous study, the pH and titratable acidity were reported as 2.09 and 2.1%, respectively, in Cornelian cherry concentrate (Bozdogan, 2017) and 2.68–2.80 and 1.39–2.00%, respectively, in its marmalade (Kökosmanlı and Keleş, 2000). The specific gravity and soluble dry matter content obtained in this study were higher than those for Cornelian cherry concentrate and marmalade reported in literature (Bozdoğan, 2017; Kökosmanlı and Keleş, 2000). Garagurt is a traditional product that was heat-treated for longer periods (10 h). Increase in soluble dry matter as a result of the concentration of the product also affects its density. Cornelian cherry fruits have been reported as a rich source of ascorbic acid (Tural and Koca, 2008) but decreases as a result of processing during marmalade or pulp production (Bozdoğan, 2017; Kökosmanlı and Keleş, 2000). The L, a, b values for garagurt were 9.35, 7.12, 7.22 respectively. In a previous study, similar values for L (9.22), a (7.14) and b (7.02) have been reported for the Cornelian cherry concentrate (Bozdoğan, 2017).

[AQ10]

Total Phenolic Content

Cosmulescu *et al.* (2019) reported TPC values between 163.69 and 359.28 mg GAE/100 g, FW for wild Cornelian cherry genotypes. In another study, TPC of polar fraction and methanolic extract for fresh Cornelian cherry was determined as 439.9 ± 34.6 mg GAE/100 g and 2,110 ± 84.0 mg GAE/100 g respectively (Karaaslan *et al.*, 2018). Similarly, Stankovic *et al.* (2014) reported the TPC values of different parts of Cornelian cherry (leaf, flower and fruits) ranging between 34,109 and 1,277 mg GAE/100 g. It has been suggested by previous studies that non-polar fractions of fresh Cornelian cherry extracts have higher TPC compared to polar fractions of their extracts (Caillet *et al.*, 2012). While Tural and Koca (2008) reported TPC of Cornelian cherry fruits changing from 2.81–5.79 mg GAE/g, Horasan Sağbasan (2015) determined this value as 1081.9 mg GAE/100 g. Capanoglu *et al.* (2011) also reported TPC of fruits as 4918.8 mg GAE/100 g dry weight (DW). The reason for differences in TPC in these studies could be related to the types of fruits used, the degree of maturity, different extraction methods and different solutions used for extraction. Lower TPC for garagurt in comparison to fresh Cornelian cherry could be related to the degradation of phenolic compounds due to heat treatment or exposure to sunlight during the sun-drying of marmalade. Likewise, Caillet *et al.* (2012) suggested that juice processing of cranberries could result in lower TPC of the final product.

[AQ11]

Şengül *et al.* (2018) determined TPC, phenolic composition and antioxidant activity of traditionally produced Cornelian cherry marmalade. Their TPC result (64.67 µg/GAE g) was lower than our result. Kamiloglu *et al.* (2015b) determined TPC, antioxidant activity and phenolic acids in black carrot jams and marmalades. Jam and marmalade processing significantly reduced TPC, antioxidant activity and phenolic acids as between 89.2% and 90.5%, 83.3% and 91.3%, and 49.5% and 96.7% respectively. However, processing of black carrot to jam and marmalade caused increase in the recovery of bioaccessible TPC, phenolic acids and antioxidant activity in the range of 7.2–12.6%, 1.4–8.1% and 4.7–31.5% respectively.

Rababah *et al.* (2011) evaluated the effect of jam processing on some fruits with respect to their TPC, antioxidant capacity and anthocyanin content during 5 months of storage at 25 °C. Jam processing reduced TPC, antioxidant capacity and anthocyanins of all fruits. Authors stated that despite decrement of TPC in jam processing, it could be considered as a suitable method to maintain the content of phenolics during storage. Howard *et al.* (2010) produced blueberry jams (sugar and sugar-free) and stored the samples for 6 months at 4 °C and 25 °C. Although jam processing caused losses of anthocyanins,

procyanidins, chlorogenic acid and oxygen radical absorbance capacity (ORAC) in both jam types, flavonols were retained well. Significant losses in anthocyanins and procyanidins occurred over 6-month storage. While chlorogenic acid content reduced during storage, flavonols and ORAC changed little. Jams stored at 4 °C retained higher amounts of anthocyanins, procyanidins and ORAC value than jams stored at 25 °C. Sugar-free jams retained higher amounts of anthocyanins than sugar-containing jams during late storage. They reported that blueberry jams should be cold-stored to better protect phenolic compounds and antioxidant activity. Even though the treatments used for jam and marmalade production caused reduction in TPC, flavonoids, ascorbic acid and carotenoids with respect to fresh fruits, it has been shown not to have a significant impact on the decline of antioxidant activity (Levaj *et al*., 2012; Rababah *et al*., 2011; Yildiz and Alpaslan, 2012).

Antioxidant activity

Şengül *et al.* (2018) determined antioxidant capacity (DPPH) of Cornelian cherry marmalade as 3.72%. Hassanpour *et al.* (2011) found the total antioxidant activity of fresh Cornelian cherry fruit as 38.98–82.37%. Many compounds that exhibit antioxidant effects are significantly degraded during food processing activities such as pasteurisation, sterilisation, dehydration, cooking and storage, and as a result, antioxidant activities are reduced. According to literature, antioxidant activities of foods decrease more during traditional production methods due to increase in applied temperature and processing time. Although the antioxidant activity of Cornelian cherry was not determined in the study conducted by Hassanpour *et al.* (2011), it was observed that the antioxidant capacity values reported for fresh Cornelian cherry fruits were higher than those determined in marmalade in the present study. It could be concluded that the lower antioxidant activity values in marmalades was due to the uncontrolled heat treatment conditions applied during marmalade production using a traditional method. Popović *et al.* (2012) reported considerable variation among *Cornus mas* genotypes in terms of antioxidant capacity. DPPH and ABTS values of acetone extracts of *Cornus mas* determined by Karaaslan *et al.* (2018) were 1,053 ± 38.1 mg TE/100 g, FW and 2,907 ± 152 mg TE/100 g, FW respectively. Cosmulescu *et al.* (2019) reported the antioxidant activities of six Cornelian cherry types. Their DPPH results varied between 1.24 and 2.71 mmol Trolox/100 g, FW. Horasan Sağbasan (2015) analysed the antioxidant activity of Cornelian cherry by DPPH, ABTS, CUPRAC and ferric-reducing antioxidant power (FRAP) assays, reported as 144.4, 124, 352.2 and 103.3 µmol Trolox/100 g respectively. Moldovan *et al.* (2017) reported ABTS radical scavenging activity of fresh *Cornus mas* samples as 2,420 mg TE/L and declared that heat treatment and high-temperature storage caused significant decrease in total antioxidant activity and total phenolic contents. Capanoglu *et al.* (2011) also studied the antioxidant activity of Cornelian cherries by ABTS, CUPRAC and FRAP assays and reported the values as 50.8, 76.3 and 22.3 g TE/100 g, DW respectively. On the other hand, West *et al.* (2012) investigated the antioxidant potential of Cornelian cherry juice and puree prepared from different varieties. According to their results, *Cornus mas* puree showed higher ORAC and reducing power compared to *Cornus officinalis* juice. Petridis *et al.* (2010) studied the antioxidant capacity of Cornelian cherry and other fruits grown in northern Greece. Their results showed that Cornelian cherry fruits had the highest FRAP value (80.15-µM ascorbic acid equivalent (AAE)/g, DW), followed by jujube (69.55 µM AAE/g, DW), cherries (32.60 µM AAE/g, DW), black grapes (31.40 µM AAE/g, DW) and blackberry (26.10 µM AAE/g, DW). The antioxidant power of Cornelian cherry fruits depends on the genotype, as well as the geographical region and climate of the area where it is cultivated, and its ripeness level. For instance, total antioxidant activity of 24 genotypes of Cornelian cherry fruits grown in Samsun, Turkey displayed a wide range changing from 16.21–94.43 µM AAE/g, FW as measured with FRAP assay (Tural and Koca, 2008).

Phenolic profile of garagurt extract

The most common phenolic compound in the samples was determined as gallic acid. Phenolic compounds in fresh Cornelian cherry fruit are considered good radical scavengers (Caillet *et al.*, 2012). It has been reported that strong antioxidant activities of fresh fruits are mostly caused by anthocyanins. However, no anthocyanin was detected in the garagurt sample, which could be due to the process effect.

Şengül *et al.* (2018) determined gallic acid, (+)catechin, (-)epicatechin, caffeic acid and ellagic acid in Cornelian cherry marmalade as 2.58, 1.22, 31.50, 8.91 and 2.40 mg/kg respectively. Rutin was not determined. Gallic acid and ellagic acid contents in our sample were found to be higher compared with these results. Cosmulescu *et al.* (2019) also examined the phenolic compounds of wild Cornelian cherry fruit genotypes. Among the individual phenolic compounds, gallic acid was determined in higher amount (14.49 mg/100 g), followed by coumaric acid (13.79 mg/100 g), ellagic acid (5.71 mg/100 g), salicylic acid (1.43 mg/100 g), ferulic acid (1.25 mg/100 g) and synaptic acid (0.19 mg/100 g). Milenkovic-Andjelkovic *et al.* (2015) indicated that ellagic acid was the predominant phenolic acid in Cornelian cherry fruit and leaf extracts, followed by chlorogenic and gallic acids.

Pyrkosz-Biardzka *et al.* (2014) indicated the presence of pelargonidin 3-*O*-galactoside, cyanidin 3-*O*-galactoside and delphinidin 3-*O*-galactoside, myricetin (26.54 mg/100 g) and rutin (3.07 mg/100 g) in Cornelian cherry fruit. Rudrapaul *et al.* (2015) reported the presence of a new β-hydroxychalcone (4-acetoxy-5,2′,4′,6′,β-pentahydroxy-3-methoxychalcone), a new flavanone (7,3′-dihydroxy-5,4′-dimethoxy flavanone) and seven known compounds: 2R, 3R-trans-aromadendrin, naringenin-7-*O*-methylether, myricetin, quercetin-3-*O*-rutinoside, ursolic acid, gallic acid and d-glucose in the methanolic extract of Cornelian cherry fruit. Wide variability was recorded for the same phenolic compound between different genotypes. The variation limits were quite high, indicating that the genotype and environmental factors are very critical for fruit composition. Moldovan *et al.* (2017) reported decreased antioxidant activities in fresh Cornelian cherry extracts stored at different temperatures and periods. These losses have been reported to be largely due to a decrease in the presence of anthocyanins (Pawlowska *et al*., 2010). Similarly, Kamiloglu *et al.* (2015b) reported that individual anthocyanins of black carrots were decreased up to 95% when they are processed into marmalade. Consequently, because the stability of anthocyanins depends on their types and source, Cornelian cherry anthocyanins could have been totally degraded during marmalade processing or their concentrations were lower than the limit of detection (LOD) of the used HPLC system. Factors such as Cornelian cherry genotype, ecological conditions of the region where the fruit is grown, cultivation technique, cultural measures, maturity level, transport, storage, differences in production methods of marmalades and differences in the methods used for extraction could affect the composition and antioxidant activity of Cornelian cherry marmalade.

Antimicrobial activity of extracts

There are some studies on the antimicrobial activity of Cornelian cherry fruit products obtained by different processing methods. Harich *et al*. (2017) reported that concentrated Cornelian cherry juice showed strong antimicrobial activity against *E. coli* O157:H7, *L. monocytogenes* and *S. typhimurium* using agar disc diffusion and MIC method. In another study, the same researchers reported that pressed Cornelian cherry fruit extracts have strong antimicrobial activity against *E. coli, E. coli* O157:H7*. S. typhimurium, S. aureus, Y. enterocolitica, P. aerugionosa, L. monocytogenes* and *B. cereus* according to MIC values; however, gram-positive microorganisms were more resistant against the Cornelian cherry puree extracts. The Cornelian cherry puree was used for the marination of pork meat, and the results indicated that reduction in the number of *E. coli, S. enteritidis, L. monocytogenes* and *S. aureus* was statistically significant (Gniewosz and Stobnicka, 2017). In our study, antimicrobial activity of garagurt was investigated by using the disc diffusion method as well as MIC dilution method. Although garagurt had weak antimicrobial activity against *S. typhimurium, E. coli* and *E. coli* O157:H7, strong antimicrobial activity was observed against *L. monocytogenes*, *S. aureus*, *Y. enterocolitica* and *P. fluorescens*. While *B. cereus* had a strong antimicrobial activity with 17-mm zone in the disc diffusion method, MIC dilution proliferation was detected at 512 mg/g dilution. This situation can be explained by the loss of disc properties or the pseudo-resistance of microorganism to antibacterial agent (Arıkan and Uysal, 2005).

The findings of our study were compared with other researchers (Gniewosz and Stobnicka, 2017; Harich *et al*., 2017), and even though the antimicrobial activity was found to be similar to other studies (strong against *L. monocytogenes, S. aureus* and *P. fluorescens*), the results indicating a weak antimicrobial activity against *S. typhimurium, E. coli* and *E. coli* O157:H7 were not comparable. This could be explained by variability in phenolic compounds due to different fruit processing steps. In addition, there were 40–80 times proportional differences in terms of active compounds between the antibiotic formulation which had the MIC values of the positive control gentamicin (10 μg) used in MIC dilution (0.001953–0.003906 mg/g) and the ratio of total phenolic compounds in the dilution of 256 mg/g (0.165 mg) and the marmalade. It is necessary to investigate the possibilities of incorporation of Cornelian cherry fruit/extract as an antimicrobial agent by increasing active ingredient content by using different food processing techniques.

5. Conclusion

The main purpose of garagurt production is to transform fresh Cornelian cherry fruits into a stable product with long shelf life. Traditional Cornelian cherry marmalade known as garagurt has been used for centuries for the treatment of many diseases. To the best of our knowledge, this is the first research investigating the antioxidant and antimicrobial effects of garagurt, and our aim was to increase the knowledge on this traditional product. According to the results, the antioxidant activity of garagurt was not provided by anthocyanins; they were lost during heat treatment; instead, gallic acid and ellagic acid were found to be the main antioxidant substances. Although Cornelian cherry marmalade had poor antimicrobial activity against *S. typhimurium*, *E. coli* and *E. coli* O157:H7, strong antimicrobial activity was observed against *L. monocytogenes*, *S. aureus*, *Y. enterocolitica* and P. *fluorescens*. This traditional product could be used as an ingredient with its natural antimicrobial effect in the

production of different foods and may have a positive effect to prolong shelf life. We conclude from the results that the consumption of garagurt might display a good antioxidant activity. However, further *in vivo* studies are recommended to confirm whether these *in vitro* activities could be confirmed *in vivo.* The future studies should also focus on identifying other specific phytochemicals in garagurt.

Conflict of interest

The authors have declared no conflict of interest in this article.

[AQ12] **Funding**

[AQ13] **Compliance with Ethical Standards**

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