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ORIGINAL PAPER

Effects of drying method on the phenolics content and antioxidant activities of cherry laurel (*Prunus laurocerasus* L.)

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Abstract
Cherry laurel fruit is a good source of phenolics together with considerable antioxidant activity. However, it is only available for a short period of time during the year. Drying could offer long-time preservation however it harms the bioactive compounds due to the high temperatures applied during the process. The aim of this study is to determine the most proper drying method for cherry laurel in terms of preserving its bioactive compounds. The phenolics contents, antioxidant and antibacterial activities, and color properties of cherry laurel dried via lyophilization, vacuum drying, and fan oven-drying were compared. Lyophilized cherry laurel had the highest phenolics content (315.51 Gallic acid equivalent mg/100 g dried cherry laurel) and color intensity (C*). The antioxidant activity was determined using both DPPH and ABTS assays. The highest antioxidant activity (6.16 mM TE/g in DPPH and 28.83 mM TE/g in ABTS) thus, the lowest half-maximal inhibitory concentration (IC_{50}) was detected for lyophilized samples in both DPPH and ABTS assays in methanolic (1.59 mg dried cherry laurel) and aqueous (0.30 mg dried cherry laurel) extracts, respectively. In general, vacuum- and fan oven-dried cherry laurel presented similar phenolics contents and antioxidant activities. None of the dried cherry laurel extracts showed antibacterial activity against the selected bacteria. Although lyophilization is a high-cost technology, it could be applied as an alternative method in the drying of cherry laurel since it provides high-quality products while maintaining its bioactivity.

Keywords Bioactive compounds · Cherry laurel · Color · Freeze-drying · IC_{50}

Introduction

Nowadays, there are numerous technologies currently in use for food preservation purposes from the primitive drying to the novel technologies such as irradiation, intelligent and modified atmosphere packaging. Aiming the long term uses and continuous availability, preservation of food products using different technologies has been a point of interest for both producers and consumers. While producers are in the pursuit of finding the most efficient and cheapest option for food preservation, consumers are chasing for the preserved foods presenting the most similar sensorial and nutritional qualities with the fresh ones. Fruits, being rich sources of bioactive compounds including phenolics, carotenoids, phytosterols, vitamins, antioxidants, and dietary fiber, are one of the most targeted foods for application of preservation methods [1, 2]. They have a quite short shelf life due to their high water content which makes them susceptible to microbial spoilage and mechanical damages [3]. Also, they have limited availability due to geographical and climatic factors. Hence, the preservation of fruits is important for both commercial and nutraceutical purposes.

Among all preservation methods, drying has a long history as a common practice for the preservation of fruits. However, with the awareness of consumers and the advancement of technology, drying technology is also developing and the final product quality is increasing. A variety of drying methods is currently available including solar-, oven-, vacuum-, microwave-, infrared-, spray-, fluid bed-, and freeze-drying (lyophilization). These methods may provide advantages over specific products and can be combined or modified depending on the characteristics of the target foods and desired end product qualities [3, 4].

The purpose of drying is to remove the water present in the product as much as possible in order to delay or completely prevent enzymatic and microbiological degradations. However, depending on the temperature and time applied in

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the drying process, decrease in various quality parameters, especially physical and sensory, may occur. Furthermore, the bioactive compounds such as anthocyanins and phenolics are affected negatively with increasing drying time. Zielinska and Michalska [5] reported a significant reduction in anthocyanin content in blueberries with drying, while low temperature-drying and prolonged exposure to oxygen resulted in great loss of polyphenols and other antioxidant compounds. On the other hand, hot air-drying is still one of the most common method used to dry fruits due to its low cost. Vacuum drying provides better quality food and less loss in bioactive compounds since it allows evaporation of water at lower temperatures compared to solar or convection oven drying [6]. The freeze-drying process follows the principle of sublimation after the water in the food has been converted into a solid form by freezing. The product is dried at temperatures below zero thus the loss of valuable components such as vitamins, phenolics, and other antioxidant substances is less compared to the other drying processes, and final products with physical and sensory properties close to fresh ones can be obtained [7]. In the study by Michalczyk et al. [8], they confirmed that freeze-drying resulted in higher polyphenolic and anthocyanin content together with better antioxidant properties compared to air-drying of selected berries.

Cherry laurel (*Prunus laurocerasus* L./*Laurocerasus officinalis* Roem), locally named *karayemis* or *taflan*, is a member of Rosaceae family with cherry-like fruits in bunches colored in pink to black depending on the ripening stage. Although the fruits from wild types have a bitter taste, the cultivated forms carry larger and sweet fruits that can be consumed not only fresh but also as a jam, marmalade, and molasses or in dried and pickled forms. It is mainly produced in Western Europe, Balkan countries, and some of the Mediterranean countries in the world while in Turkey it is mostly grown in north eastern coast [9–12]. Its leaves have been used to treat cough, hemorrhoid, eczema, asthma, and digestive system problems including ulcer since early times in Anatolia [13]. In addition to the leaves, the fruits of cherry laurel also became popular due to their health-promoting properties recently. They are rich in anthocyanins and phenolics [14]. Celik et al. [15] determined a good correlation of 0.945 between total phenolics content and antioxidant activity of cherry laurel. Cherry laurel is also a good source of procyanidins which are associated with reduced risk of cardiovascular diseases. Cherry laurel was found to contain 25 times more procyanidin than Cornelian cherry in a study by Capanoglu et al. [16].

Considering its mentioned health benefits and its potential commercial applications, preservation of cherry laurel via drying is important. Cherry laurel fruit is only available during summer for a short time (mostly in June and July). However, dried cherry laurel could be used all year long as a nutraceutical furthermore, it has a high potential as an intermediate product for functional food applications also as a natural food colorant and flavor-enhancer [17–19]. There are many studies available [14, 15, 20–24] regarding the antioxidant and phenolics contents. Also, there are studies aiming to determine drying kinetics of cherry laurel and effects of drying on characteristics of cherry laurel powder [25–28]. However, we haven't come across any studies on the effects of drying method on the phenolics content, antioxidant and antimicrobial activities of cherry laurel.

In this context, this study was designed to compare the color properties, phenolics contents, antioxidant and antimicrobial activities of cherry laurel dried by fan oven, vacuum oven, and lyophilization.

Materials and methods

Cherry laurel fruits

Cherry laurel (*Prunus laurocerasus*) fruits were collected from the orchards (Ordu, Turkey) in the summer of 2015. The fresh fruits were transported to the laboratory and seeds were removed manually, after washing. Then, the deseeded fruits were immediately stored at − 20 °C for further use.

Drying process

Frozen fruits were prepared with the similar method given by Ergüney et al. [26] and processed into pulp using a commercial blender (8011ES, Waring Commercial, Torrington, CT, USA). The pulp was dried with three different methods using (i) a fan oven (NST-120, Nükleon, Ankara, Turkey) (ii) a vacuum-oven (VO 200; Memmert, Schwabach, Germany), and a lyophilizer (FreeZone 2.5, Labconco, Kansas, MO, USA). The drying conditions were summarized in Table 1.

Dried samples were ground into powder using a coffee grinder (Sinbo SCM 2934, Istanbul, Turkey), then packed in glass jars to avoid any moisture absorption and stored at − 20 °C until analysis. Total dry matter was determined by calculation of weight loss after drying the samples in a convection oven (Ecocell EC 111; MMM Group, Planegg, Germany) at 105 °C until a steady weight was reached.

Table 1 Drying conditions for cherry laurel

Drying method	Temperature (°C)	Pressure (mbar)	Time (h)
Fan-oven (FO)	55	–	72
Vacuum-oven (VO)	55	100	72
Lyophilizer (LO)*	− 50	0.45–1	72

*Approximate values for the auto-mode

Following the same principle, dry samples were incinerated in a furnace (PLF 115 M; Protherm, Ankara, Turkey) at 550 °C until the black color disappears to determine the ash contents.

Preparation of extracts

Two types of cherry laurel extracts were prepared either with distilled water or acidified methanol (0.1% HCl, v/v). The cherry laurel powders (1 g) were extracted with 50 mL of earlier mentioned solutions using a homogenizer (T18; IKA-Werke GmbH & Co., Staufen, Germany) in an ice water bath at 9000 rpm for 15 min. Then, the extracts were centrifuged two times at 4000 rpm for 10 min to remove the pellets completely and obtain clear supernatants. They were used for further analyses of total phenolics contents, antioxidant and antimicrobial activities.

Total phenolics content and antioxidant activity/capacity

Total phenolics content was determined according to Folin-Ciocalteu assay as described by Demirkol and Tarakci [29]. The extracts (20 µL) were diluted (1:79) with the corresponding solvent, water or methanol, followed by the addition of Folin reagent (Merck, Darmstadt, Germany) and saturated Na_2CO_3 solution (20%). The final solution was mixed by inversion and kept in dark at room temperature for 2 h. The absorbance values at 760 nm were recorded using a spectrophotometer (UVmini-1240, Shimadzu, Japan). A standard curve was constructed using Gallic acid (Sigma-Aldrich Corp., St. Louis, MO) and the results are given as mg Gallic acid equivalents (GAE) per 100 grams of dried cherry laurel.

The antioxidant activity was determined based on the assays of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and 2,2-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) trolox equivalent antioxidant capacity. Varying concentrations of samples were prepared for both methanol (0.1% HCl) and water extracts. The absorbance readings were recorded at 515 nm and 734 nm wavelengths using the spectrophotometer for DPPH and ABTS assays, respectively. The standard curve was plotted using Trolox (Sigma-Aldrich Corp.) and the results were expressed as µg Trolox equivalent (TE) per milligram of dried cherry laurel.

Also, in order to calculate the amount of extract (mg) required for the inhibition of 50% of the DPPH (IC_{50}), a standard curve was plotted using the inhibition (%) values ($\text{Inhibition } (\%) = 100 \times [\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}]/\text{Abs}_{\text{control}}$) against different concentrations of dried cherry laurel.

Antibacterial activity

An agar-well diffusion assay was performed using *Escherichia coli* BL21, *Staphylococcus aureus* NCTC 8530, and *Listeria monocytogenes* ATCC 7644 as the model bacteria to determine antibacterial activity of dried cherry laurel extracts (in water and methanol with 0.1% HCl). All bacteria were cultivated in Tryptic Soy Broth (Merck, Darmstadt, Germany) at 37 °C and passaged at least two times prior to the assay. Fresh cultures (1%) were centrifuged and the cell pellets were re-suspended in sterile saline solution (0.85%, w/v). Their absorbances were adjusted to 0.09 ± 0.005 at 600 nm wavelength (corresponding to McFarland = 0.5) to standardize the inoculums. The cultures (1 mL) were pour-plated in 20 mL of Mueller–Hinton Agar (Merck, Darmstadt, Germany). Using a sterile cork borer, 4×6 mm-diameter holes were bored in a single 90 mm-diameter petri dish. Aliquots of 50 µL of the extracts transferred into the wells and plates were incubated at 37 °C for 24 h. The inhibition zones were measured using a digital caliper [30].

Color measurement

Colorimetric properties were revealed using a colorimeter (Minolta, Chromameter CR-400, Japan).The values were expressed according to the CIE Scale where L*, a*, and b* corresponds to brightness, redness, and yellowness, respectively. The chroma (C*) value was calculated using the Eq. 1 [28].

$$C* = \sqrt{a*^2 + b*^2} \quad (1)$$

Statistical analysis

All analyses were carried out at least in duplicate and the results are given as "mean ± standard deviation". Statistical analyses were performed using Minitab 17 (Minitab Inc., State College, PA, USA). One-way analysis of variance (ANOVA) through the general linear model was applied at $\alpha = 0.05$ significance level. Significant differences ($p < 0.05$) between the means were further analyzed using the Tukey Test.

Results and discussion

The dry matter and ash contents of cherry laurel powders are given in Fig. 1a and b. The vacuum-drying resulted in significantly lower total dry matter content (91.64%) than the fan oven (93.18%) and lyophilization (92.69%) suggesting a less effective drying ($p < 0.05$). Total ash content of LO cherry laurel was found to be significantly lower than that of the FO one ($p < 0.05$). Although some minerals could be degraded or evaporated at high temperatures the results indicate that

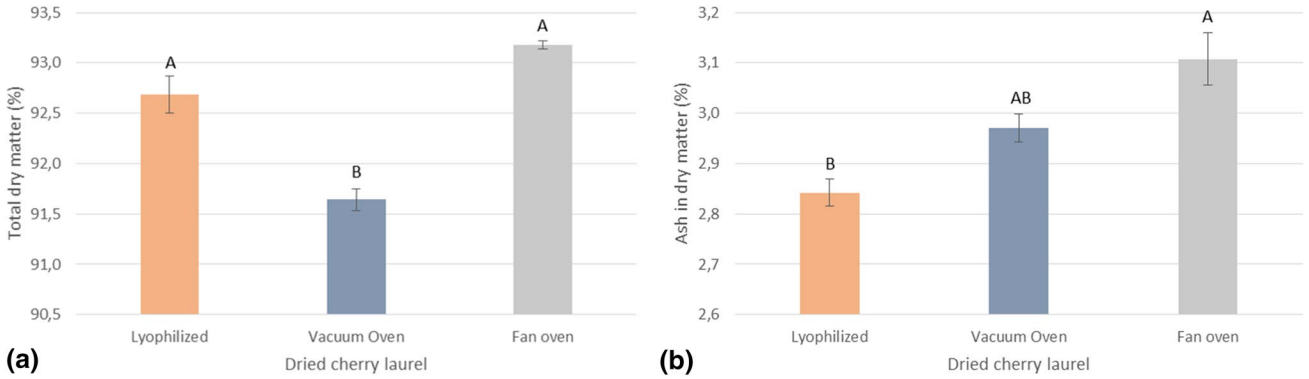


Fig. 1 Total dry matter (**a**) and ash (**b**) contents of cherry laurel dried by different methods. Different capital letters indicate significant differences between drying methods ($p < 0.05$)

high-temperature application affected ash concentration adversely. However, lower ash contents in lyophilized foods have also been encountered in different studies [31, 32].

Phenolics content

Phenolics contents were determined using water and methanolic extracts of the dried cherry laurel. Phenolics contents were calculated based on the standard curves plotted ($R^2 = 0.994$ and 0.991, respectively) and expressed as Gallic acid equivalent (GAE). Water extract of LO cherry laurel had significantly higher phenolics content (315.51 GAE mg/100 g dried cherry laurel) than the other two dried cherry laurels (Fig. 2a). Although there is no significant difference ($p > 0.05$), fan oven drying resulted in higher phenolics content (279.63 mg GAE/100 g dried cherry laurel) compared to vacuum drying (254.15 mg GAE/100 g dried cherry laurel). Similarly, methanolic extract of LO cherry laurel had the highest phenolics content (335.99 GAE mg/100 g dried cherry laurel). Except VO cherry laurel, methanolic extracts had slightly lower phenolics content than their water extracts (Fig. 2b). Celep et al. [33] investigated the phenolics content of three fruits, including cherry laurel. They determined 23.64 mg GAE/g of cherry laurel extract (80% methanol). In a study by Altuntas et al. [22], phenolics content of cherry laurel was found in the range of 1697–2210 µg GAE/g fresh cherry laurel at different stages of ripening. Beyhan et al. [24] tested different genotypes of cherry laurel in Sakarya (Turkey) and determined the phenolics contents as 1197–6801 mg GAE/100 g dried cherry laurel. However, they did not define which drying method was used. Also, Capanoglu et al. [16] tested phenolics content of sour cherry, laurel cherry, and cornelian cherry. Laurel cherry had the lowest phenolics content among all with 3129.2 mg GAE phenolics in 100 g freeze-dried sample.

Antioxidant activity/capacity

Antioxidant activities of water and methanolic extracts of dried cherry laurel were calculated using the standard curve ($R^2 = 0.994$) and expressed as Trolox equivalents (TE). Compared to the water extracts, methanolic extracts showed

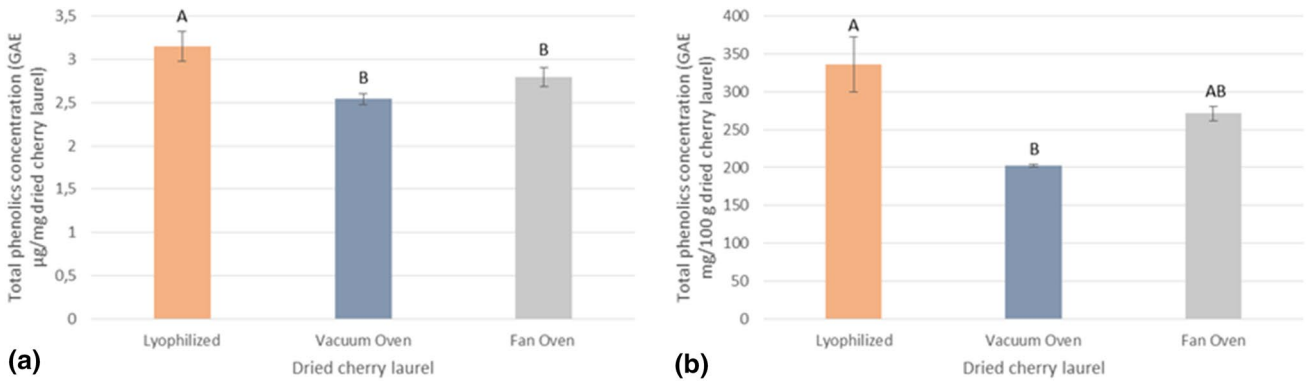


Fig. 2 Total phenolics contents of water (**a**) and methanolic extracts (**b**) of cherry laurel dried by different methods (GAE µg/mg dry cherry laurel). Different capital letters indicate significant differences between drying methods ($p < 0.05$)

higher antioxidant activities in the DPPH assay and vice versa for the ABTS assay. Methanolic extracts of LO cherry laurel had the highest antioxidant activity (2.99 mM TE/g dried cherry laurel) with significant differences ($p < 0.05$). VO (2.37) and FO (2.35) dried cherry laurel had similar antioxidant capacities and a similar scheme could also be observed for the water extracts (Fig. 3a). Although the trend of LO having the highest antioxidant activity is similar in ABTS assay, the antioxidant capacity values were considerably higher compared to the antioxidant activity values determined in the DPPH assay (Fig. 3b).

IC_{50} value is the amount of sample required for the inhibition of 50% of the radical compound (DPPH or ABTS). Therefore, there is an inverse relation meaning that a higher IC_{50} indicates a lower antioxidant activity. In the DPPH assay, aqueous extracts of dried cherry laurel presented higher IC_{50} values compared to methanolic extracts and vice versa in the ABTS assay (Fig. 4a, b). Although there are quite many studies on the antioxidant activity of cherry laurel, it is difficult to make a statement due to the different extraction methods and standards used as well as calculations applied. Celik et al. [15] determined the antioxidant activity of 11 fresh cherry laurel genotypes between 21.2 and 32.2 µmol TE/g. Confirming our findings, methanolic extracts having higher antioxidant activity, Sahan et al. [34] also found that methanolic extract of fresh cherry laurel had higher phenolics content (26.70 µmol TE/g fresh fruit) compared to the water extracts. Contrary to the results obtained in this study, the radical scavenging activity of freeze-dried *Vitis labrusca* L. was found lower compared to forced air oven-dried ones in a study by Demirkol and Tarakci [29]. Similar to the results obtained in this study, higher phenolics contents and antioxidant activities were determined in selected freeze-dried berry types compared to air-dried ones in a study by Michalczyk et al. [8].

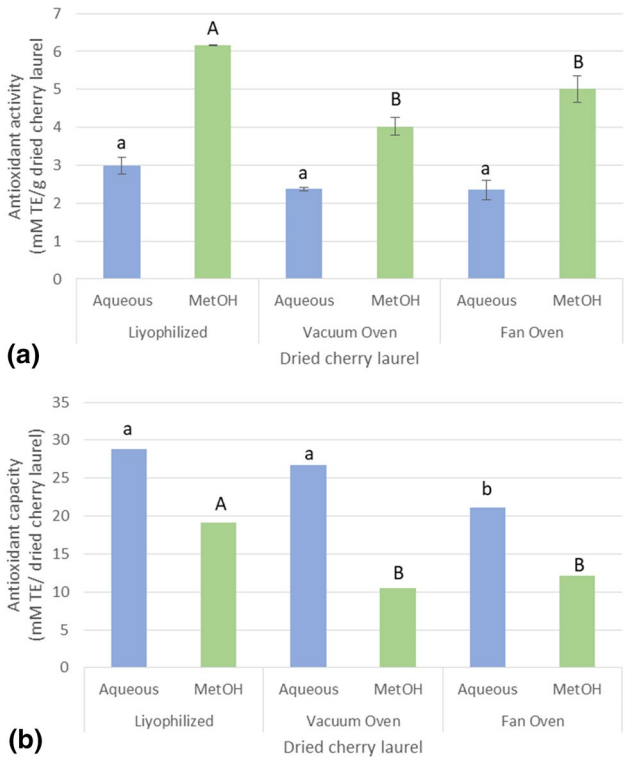


Fig. 3 Antioxidant activity/capacity (mM TE/g dried cherry laurel) of cherry laurel using (**a**) DPPH assay (**b**) ABTS assay. Different capital and small letters indicate significant differences between methanol and water extracts of cherry laurel dried by different methods, respectively ($p < 0.05$)

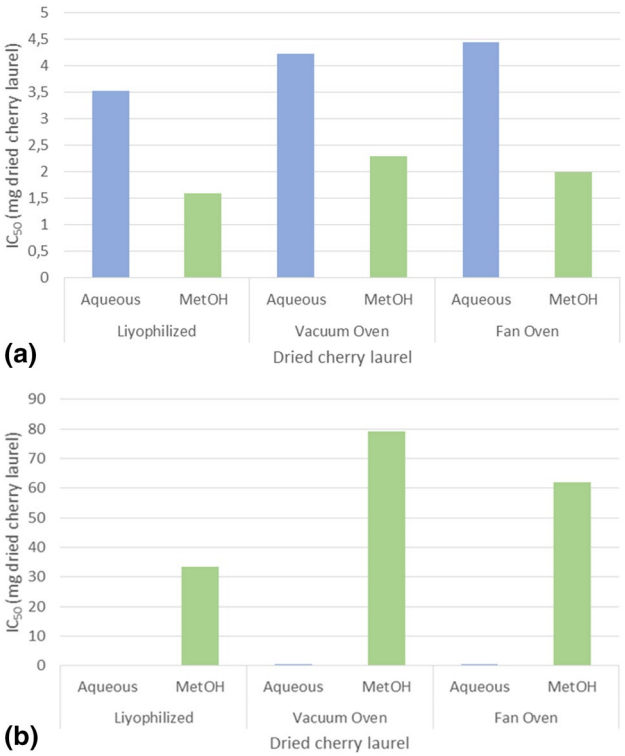


Fig. 4 IC_{50} value (mg dried cherry laurel) for dried cherry laurel using (**a**) DPPH (**b**) ABTS assays

Antibacterial activity

The leaf and seed extracts of cherry laurel were tested for presence of inhibitory activities in the earlier studies [35, 36]. Acetone and ethanolic extracts (among aqueous, methanolic, ethanolic, acetone, and chloroform extracts) of cherry laurel leaves were found most effective against the tested bread spoilage fungi in a study by Sahan [35]. Also, due to their amygdalin and prunasin, the cyanogenic glycosides, the seed extracts of cherry laurel were found to be inhibitory against a certain type of a mite, *Tetranychus urticae* [36]. In this study, the antibacterial activity of cherry laurel powders was tested using both

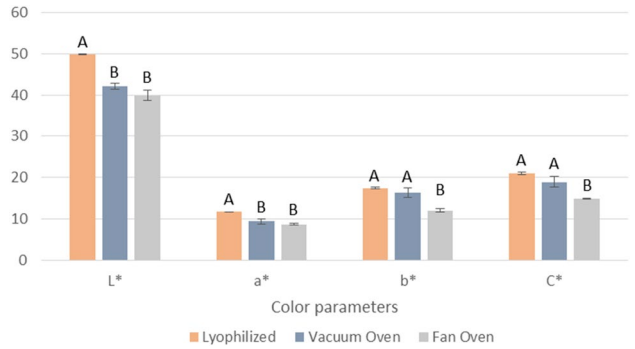


Fig. 5 Color parameters (L*, a*, b*, and C*) of cherry laurel dried by different methods. Different capital letters indicate significant differences between drying methods ($p < 0.05$). C*: Chroma

methanolic and aqueous extracts for each drying method. To the best of our knowledge, this is the first report on the antibacterial activity of dried cherry laurel extracts. However, none of the extracts exhibited any antibacterial activity against *E. coli* BL21, *S. aureus* NCTC 8530, and *L. monocytogenes* ATCC 7644. Antimicrobial activities are usually species or even strain specific. Furthermore, the extraction technique and solvent have a crucial role in the inhibitory effects of extracts prepared. Therefore, different types of extraction techniques and solutions, or more concentrated extracts could be tested against different types of microorganisms in future studies to make a more comprehensive interpretation.

Color

Color is an important parameter for the perception and preference of food products by the consumers. Although relatively low temperature (55 °C) was applied in vacuum-drying and fan oven drying, LO cherry laurel had significantly higher brightness (L*) and redness (a*) values ($p < 0.05$). The LO cherry laurel had the highest L* (49.84), a* (11.72), and b* (17.48) values while FO cherry laurel had the lowest (L*:39.88, a*:8.71, b*: 12.09). Similarly, Demirkol and Tarakci [29] found higher L* and a* but lower b* values for freeze-dried *Vitis labrusca* L. The chroma value (C*) represents the color intensity and calculated using the a* and b* values. Also, the highest C* value, the most intense color, was owned by the LO cherry laurel (Fig. 5). Compared to the current color values, Talih and Dirim [28] determined similar brightness, redness, and chroma values (L*:45.08, a*: 20.54, C*:20.78) for lyophilized cherry laurel but lower b* value (b*: 3.15).

Conclusion

The results revealed that lyophilized cherry laurel powder possessed the highest phenolics content and antioxidant activity/capacity with better color properties. Under the conditions applied in this study, the vacuum-drying and fan oven-drying resulted in similar phenolics contents and antioxidant activities. In compliance with the early studies, the antioxidant activity of methanolic extracts was significantly higher than those of the aqueous extracts. On the other hand, water extracts had higher antioxidant capacity than methanolic extracts. Unfortunately, no antibacterial activity was detected for any of the extracts against any bacteria tested.

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