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Research Paper / Araştırma Makalesi

# Effects of Freeze-Drying Process on Antioxidant and Some Physical Properties of Cherry Laurel and Kiwi Fruits

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### ABSTRACT

Freeze-drying is a trend method for the preservation of thermosensitive and nutritive food products. In this study, two different fruits, kiwi fruit with high ascorbic acid content and cherry laurel fruit with high phenolic content, were selected to study the freeze-drying effect on these compounds. Ascorbic acid content, total phenolic content and antioxidant capacity of kiwi and cherry laurel fruits were determined before and after freeze-drying process. Ascorbic acid content of kiwi and cherry laurel fruits were 205.14±21.33 and 3.00±1.02 mg/100 g dry matter, respectively. Total phenolic content of kiwi and cherry laurel fruits were 262.66±19.97 and 1056.78±90.73 mg GAE/100 g dry matter, respectively. Ascorbic acid contents did not change, while antioxidant capacities increased by freeze-drying process for both fruits. The total phenolic content of cherry laurel fruits increased significantly after freeze-drying in contrast to the total phenolic content of kiwi fruits. Color values changed with freeze-drying, the lightness and yellowness values increased significantly for both fruits (p<0.05). The rehydration ratios of freeze-dried kiwi and cherry laurel fruits were found similar at 25 and 40°C. The results of this study showed that freeze-drying method is highly recommended for the preservation of nutritive values of these fruits and off-season products.

Keywords: Ascorbic acid, Phenolic, Cherry laurel, Kiwi fruit, Freeze-drying

# Dondurarak Kurutma İşleminin Karayemiş ve Kivinin Fiziksel ve Antioksidan Özellikleri Üzerine Etkisi

### ÖΖ

Dondurarak kurutma besleyici ve sıcaklıktan kolay etkilenebilen gıda ürünlerinin korunmasında kullanılan yeni bir metottur. Bu çalışmada, dondurarak kurutmanın etkilerini gözlemlemek için yüksek askorbik asit (kivi) ve fenolik bileşen içeren (karayemiş) iki tip meyve seçilmiştir. Kivi ve karayemiş meyvelerinin dondurarak kurutulmasından önce ve sonra askorbik asit içeriği, toplam fenolik madde içeriği ve antioksidan kapasiteleri belirlenmiştir. Kivi ve karayemiş meyvesi için askorbik asit içerikleri sırasıyla 205.14±21.33 ve 3.00±1.02 mg/100 g kuru madde olarak bulunmuştur. Kivi ve karayemiş meyvesi için toplam fenolik madde içerikleri sırasıyla 262.66±19.97 ve 1056.78±90.73 mg GAE/100 g kuru madde olarak belirlenmiştir. Her iki meyve için de dondurarak kurutma işlemi ile birlikte antioksidan kapasiteleri artarken, askorbik asit içerikleri değişmemiştir. Toplam fenolik bileşen miktarında dondurarak kurutulmuş karayemiş meyvesinde önemli ölçüde artış olmuştur ancak kivi meyvesinde olmamıştır. Renk değerleri her iki meyve için dondurarak kurutma işlemi ile birlikte değişmiştir (p<0.05). Dondurarak kurutulmuş kivi ve karayemiş meyvelerinin 25 ve 40°C'deki rehidrasyon oranlarının benzer olduğu bulunmuştur. Rehidrasyon oranları, 25 ve 40°C'deki suyun içerisinde tutulma sürelerinin başlangıcında daha hızlı artış gözlenmiştir. Çalışmanın sonuçları dondurarak kurutma işleminin, besleyici kurutulmuş meyveler ve sezon dışı ürünler için uygun bir metot olduğunu göstermiştir.

Anahtar Kelimeler: Askorbik asit, Fenolik, Karayemiş, Kivi, Dondurarak kurutma

### INTRODUCTION

Oxygen is indispensable part of our lives and has toxic effect due to the formation of reactive oxygen species (ROS). Increase of ROS in cells leads to the generation of oxidative stress. Antioxidants act as defensive to damage induced with oxidative stress. Fruits have components, phenolic antioxidant especially compounds, carotenoids and ascorbic acid [1, 2]. Many studies show that intake of antioxidant-rich fruits is associated with reduce the risk of cardiovascular disorders. cancer, neurological diseases and atherosclerosis [3-5].

Phenolic compounds in fruits are diverse group of secondary metabolites. They donate an electron to free radical and prevent oxidative damage. Total phenolics are considered as the major contributor to the antioxidant activity of fruits, vegetables and herbs [6-9]. Besides, these compounds powerfully affect color of fruits [10]. Another electron donor is ascorbic acid known as vitamin C. Ascorbic acid which is a water soluble compound cannot be synthesis in human body; therefore, we must consume it from exogenous supplements [11].

Kiwifruit (*Actinidia deliciosa* Planch) is known as a good source of ascorbic acid. Because of the phytonutrient content of kiwifruit including carotenoids, lutein, phenolic, flavonoids and chlorophyll, it is also an excellent source of antioxidant [12-14].

Cherry laurel (*Laurocerasus officinalis* L.) also known as taflan or wild cherry is produced in the Black Sea Region in Turkey. It is a popular fruit for its characteristic taste and nutritional properties. In Turkey, it is consumed as fresh, dried or processed into jam, marmalade, canned, or pickled; also, it is known for its diuretic and antidiabetic properties and medical effects on stomach ulcers, digestive system problems, bronchitis, eczemas, and hemorrhoids [15, 16].

Since harvesting of fruits is not possible for every season, research studies are concentrated on different methods to make them accessible. Drying is the most common and traditional method. Due to the preservation of biological activity of thermosensitive components, freeze-drying is an important drying process [17]. Freeze-drying is a low temperature dehydration process and during this process water is removed by sublimation of ice from frozen food parts. Freeze-drying preserves the shape, taste, color, flavor, appearance, texture, dimensions and the nutritional compounds of the raw material. Also, it prevents the microbial spoilage, oxidation and extends the shelf life of foods. However, freeze-drying is a slow and consequently an expensive process. Therefore, the use of this process is restricted to high value products [17-19].

Freeze-drying process takes place in three stages: freezing, primary drying and secondary drying. In the first freezing step, the temperature of the food product is decreased below the water triple point. In the primary drying stage ice sublimation takes place with heating (below the triple point) under partial vacuum condition. With the increasing temperature, the bounded water is desorbed in the last step [17, 18].

In recent years, changes in consumer demand for high quality products have caused to use new technologies and produce more attractive, safe and nutritious products. The aim of this study was to preserve the nutritional components of kiwi fruit and cherry laurel fruits by freeze-drying. To reach this objective ascorbic acid content, total phenolic content and antioxidant activity of fresh and freeze-dried fruits were analyzed. Also, the color and rehydration capacity measurements of fruits were examined to understand the physical changes during the freeze-drying process.

### MATERIALS and METHODS

### Chemicals

In this study, chemicals with analytical purity were used. Vitamin C standard (ascorbic acid), oxalic acid, sodium carbonate, copper(II) chloride, ammonium acetate, ethanol, methanol, potassium dihydrogen phosphate and phosphoric acid were purchased from Merck (Darmstadt, Germany). Gallic acid standard was obtained from Acros Organic (Geel, Belgium). Trolox standard [(±)-6-hydroxy-2,5,7,8-tetramethylchroman-2carboxylic acid] was purchased from Aldrich (Steinheim, Germany). Folin-Ciocalteu reagent and DPPH (2, 2diphenyl-1-picryl-hydrazyl-hydrate) were obtained from (Buchs, Switzerland). Neocuproine was Fluka purchased from Carlo Erba Reagents (Milano, Italy).

### Freeze-drying Process

Fruits were purchased from major chain supermarkets in Istanbul. Fresh fruits were dried with a pilot scale freeze drier (VirTis Ultra 25 Super XL, New York, USA). Operation conditions of freeze-drying were selected as -20°C for freezing step, 10°C for drying step and 10 Pa for chamber pressure during the drying.

### Preparation of Aqueous Sample Extracts for Determination of Ascorbic Acid Content

Oxalic acid solution (0.5%) was used to prepare the extracts. Weighted samples were placed into a test tube and 10 mL of oxalic acid solution was added. Test tubes were sonicated with an ultrasonic bath (Elmasonic E 30 H, New Jersey, USA) for 15 minutes and centrifuged at 3200 rpm for 15 minutes. Then liquid phases were filtered through 0.45 µm filter.

### Preparation of Aqueous Sample Extracts for Determination of Total Phenolic Content and Antioxidant Activity Analyses

Extracts were prepared with 75% methanol solution. Weighted samples were transferred into test tubes and after mixing with methanol solution were sonicated with an ultrasonic bath. After centrifugation at 2500 rpm for 10 min, the upper supernatant was gathered in another tube. Remaining precipitate was mixed with 3 mL of methanol solution again and the extraction procedure was repeated until supernatant volume reached 10 mL. Supernatants were filtered through 0.45 µm filter.

# Determination of Ascorbic Acid by the HPLC Method

Ascorbic acid (AA) content was determined in triplicates by the Hitachi LaChrom Elite HPLC system (VWR-Merck, Vietnam) with a UV detector. HPLC column was a Phenomenex Luna 5u C18 column (250 x 4.6 mm ID) and separation was carried out isocratically at 25°C. Detection was performed at 254 nm. The mobile phase was 25 mM potassium dihydrogen phosphate (adjusted to pH 2.2 with phosphoric acid) with a flow rate of 1 mL/min. Sample injection volume was 10  $\mu$ L [20]. Quantification was done with respect to the standard curve of ascorbic acid.

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

The Folin-Ciocalteu colorimetric method defined previously by Wojdyło et al. [21] was carried out to measure the total phenolic content (TPC). Folin-Ciocalteu reagent (0.2 mL) and 2 mL of distilled water were added to the sample extract solution. After incubation at room temperature for 3 minutes, 1 mL of 20% sodium carbonate was added and spectrophotometric analysis was completed after 1 h of incubation in darkness. The blue color was determined in a spectrophotometer (Lambda 35, Perkin Elmer, Shelton, ABD) using a wave-length of 765 nm. Quantification was performed by the standard curve of gallic acid ( $R^2 = 0.998$ ). The mean results of triplicate analysis were expressed as gallic acid equivalents (GAE), milligrams per 100 g of dry matter (dm).

### DPPH radical scavenging activity (%) = [Abs<sub>(control)</sub> – Abs<sub>(sample)</sub>] / (Abs<sub>(control)</sub>) × 100 Eq.1

where  $Abs_{(control)}$  is absorbance of the blank (reacting mixture without the test sample) and,  $Abs_{(sample)}$  is absorbance of reacting mixture with the test sample. Triplicate analyses were performed and results were given as means ± standard deviation.

### **Color Measurements**

The color analysis of fruits was carried out by using a Konica Minolta CR-400 Chroma meter (Osaka, Japan) according to Hunter Lab system (L: lightness, a: redness, and b: yellowness). Measurements were evaluated at four points in center and lateral locations of samples and average results were given. The total color difference from the fresh fruits ( $\Delta E$ ) were calculated as defined in Eq. 2.

$$\Delta E = \sqrt{(L_o - L)^2 + (a_o - a)^2 + (b_o - b)^2}$$
 Eq. 2

where subscript "o" denotes to the color value of fresh samples, L; a and b indicate brightness, redness and yellowness of dried samples respectively. Fresh fruits

# Determination of Cupric Ion Reducing Antioxidant Capacity

Cupric ion reducing antioxidant capacity (CUPRAC) was determined using the normal sample measurement method described previously [22]. To a test tube 1 mL each of copper (II) chloride solution (10<sup>-2</sup> M, 0.4262 g of CuCl<sub>2</sub>·2H<sub>2</sub>O in 250 mL of distilled water), neocuproine solution (7.5 x 10<sup>-3</sup> M, 0.039 g of neocuproine in 25 mL of 96% ethanol) and ammonium acetate buffer solution (pH 7.0, 19.27 g of ammonium acetate in 250 mL of distilled water) were mixed. The sample or standard solution (x mL) and distilled water was also mixed ((1.1x) mL) with the reagent mixture until the final volume reached 4.1 mL. The absorbance was determined at 450 nm with a spectrophotometer after 1 h of incubation in darkness. The standard calibration curve of Trolox was used to quantify the antioxidant capacity of samples. The results were expressed as Trolox milligrams per 100 g of dry matter (dm). All determination values were given as means ± standard deviation of triplicate analysis.

# Determination of DPPH Radical-Scavenging Activity

The DPPH radical-scavenging activity was determined using the method described previously by Brand-Williams et al. [23] with a few modifications. DPPH (5  $\mu$ g) was dissolved in 70% methanol (250 mL). The radical stock solution was prepared fresh daily. The DPPH solution (3 mL) was added to 200  $\mu$ l of extracts. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min and the resulting color was measured spectrophotometrically at 517 nm against blanks. The DPPH radicalscavenging activity was subsequently calculated according to Eq. 1.

were used as the reference and a larger  $\Delta E$  means that greater color change from the reference material.

### **Rehydration Capacity**

Rehydration capacities of freeze-dried fruits were measured by immersing a pre-weighed sample into water at room temperature (25°C) and at 40°C. The weight of the rehydrated samples was measured after 1, 2, 3, 4, 5, 10, 20, 30, 40, 50, 70, 100 and 120 min immersion in water for experiments carried out at room temperature and after 1, 2, 3, 4, 5, 10, 20, 30, 40, 50, 70 min immersion in water for experiments carried out at 40°C. At time intervals, the samples were taken out and weighted after drying on paper towels to eliminate excess water. Triplicate analyses were done and the rehydration ratio was obtained from the ratio of the fruit weight after and before the rehydration procedure [24].

# Statistical Analysis

The measurements were conducted at least in triplicates and results were given as mean  $\pm$  standard deviations. Statistical analysis was performed with the SPSS software (version 15 for windows, SPSS, Inc., Chicago, IL, USA). The data were subjected to analysis of variance (ANOVA) and the significance level was set at p<0.05.

# **RESULTS and DISCUSSION**

# Ascorbic Acid Content

In this study, extracts of kiwi fruit and cherry laurel were analyzed by HPLC. Results from HPLC analysis are shown in Table 1. We observed that kiwi fruit had higher ascorbic acid content than cherry laurel. Kvesitadze et al. [25] reported that ascorbic acid content in different kiwi fruit cultivars was 414-434 mg/100 g of dry matter. There was no significant difference in contents of ascorbic acid of fresh and freeze-dried kiwi fruit (p<0.05). Ascorbic acid content of fresh cherry laurel in 100 g dry and wet base weight were 3.00±1.02 mg and 0.68±0.23 mg (recalculated), respectively. Celik et al. [15] reported that ascorbic acid contents ranged from 2.1 to 4.1 mg/100 g wet base weight for different cherry laurel genotypes. Similar results were obtained for freeze-dried samples of cherry laurel. Ascorbic acid contents of fresh and freeze-dried cherry laurel were not significantly different (p<0.05). Ascorbic acid is a very sensitive vitamin. Oxygen and high temperature can be cause degradation of ascorbic acid. Freeze-drying process is carried out under vacuum at a low temperature. For this reason, were not observed a statistically important decrease in ascorbic acid content after freeze-drying process. Asami et al. [26] and Gumusay et al. [27] reported that freeze-drying process preserved higher levels of ascorbic acid in comparison with air-drying process. Chang et al. [28] observed that amount of ascorbic acid in two tomato cultivars decreased with 8.2-10% compared to fresh ones.

# **Total Phenolic Content**

The impact of freeze-drying on total phenolic content was evaluated and compared to fresh samples. We found that cherry laurel had higher total phenolic content than kiwi fruit (Table 1). Total phenolic content of kiwi fruit and cherry laurel were determined as 262.66±19.97 and 1056.78±90.73 mg GAE/100 g dm. Gorinstein et al. [28] reported that total phenolic contents of kiwi extracts was 5.62-7.91 mg GAE/g dm. Celik et al. [15] reported that total phenolic contents for different cherry laurel aenotypes were found as 364-503 mg GAE/100 fresh weight. We found that impact of freeze-drying process on phenolic content of kiwi fruits was not significantly important (p<0.05). Gumusay et al. [27] observed similar results for freeze-drying of tomatoes. However total phenolic content in freeze-dried cherry laurel had higher than that in their fresh counterparts. Chan et al. [30] reported an increase in phenolic content of ginger leaves with freeze-drying process. The reason for an increase in total phenolic content of freeze-dried cherry laurel fruits is that freeze-drying process might be increased up by the extraction efficiency of phenolic compounds.

# **Cupric Ion Reducing Antioxidant Capacity**

The antioxidant capacity of fresh and dried fruit samples was determined by analysis of the CUPRAC assay. According to results given in Table 1, cherry laurel had the high cupric ion reducing power (1555.70±447.21 mg trolox/100 g dm), while kiwifruit had lower (326.59±13.61 mg trolox/100 g dm). Park et al. [31] found similar results for CUPRAC of kiwifruit. The CUPRAC values of freeze-dried kiwifruit samples increased from 326.59±13.61 to 370.20±60.31, but this change was not statistically significant (p≥0.05). However, CUPRAC values of freeze-dried cherry laurel increased sharply (p<0.05). Gümüşay et al. [27] indicated that increased extraction efficiency and decreased antioxidant contents loss during freeze-drying process caused the antioxidant activity increase.

# DPPH Radical Scavenging Activity

The DPPH radical scavenging activity assay was performed to evaluate the antioxidant potential of kiwi and cherry laurel fruits. The DPPH values of fresh and freeze-dried fruit samples were calculated for 10 mg and 0.1 mg of dry matter, respectively. Therefore, as can be seen from Table 1, freeze-dried kiwi and cherry laurel fruits had higher antioxidant capacities than fresh fruits. Das et al. [32] were reported that DPPH radicalscavenging activity of freeze-dried wheatgrass were higher more than fresh ones. Another study about drying methods of mushroom indicated that freeze-drying of mushroom showed higher DPPH values than fresh ones. Freeze-drying, which formed ice crystals and rupture cell structure, increases the extraction efficiency and increases the ability of cellular components transportation, and consequently extraction [33]. Similar tendency about antioxidant activity of freeze-dried samples were found in both methods, as a result, we can conclude that freeze-drying is an effective method to preserve antioxidant properties of food materials.

### Color Measurements

Color values (L, a and b) of fresh and freeze-dried kiwi and cherry laurel fruits are shown in Table 2. Color measurements of cherry laurel fruit were done for both inner and outer surface. Color data of outer surface of cherry laurel were not given, because there was no difference between fresh and freeze-dried samples ( $\Delta E$ ; 1.77±0.57). We found statistically differences between all color values of fresh and freeze-dried samples for two fruit (p<0.05). Lightness and yellowness were increased with freeze-drying process. Cui et al. [34] observed that freeze-dried apples had higher lightness and yellowness value than their fresh counterparts. Browning was enabled with oxygen and high temperature. Freeze-drying process was operated under vacuum at low temperature, so lightness was expectedly increased. The total color change values were calculated for understanding of changes in redness and yellowness. Freeze-dried kiwi fruit had higher ΔE value than freeze-dried cherry laurel.

		AA content	TPC	DPPH	CUPRAC values			
		(mg/100 g dm)	(mg GAE/100 g dm)	inhibition (%)*	(mg Trolox/100 g dm)			
Kiwifruit	Fresh	205.14±21.33	262.66±19.97	15.57±1.22	326.59±13.61			
KIWIITUIL	Dried	232.46±8.47	304.02±3.26	3.67±0.21	370.20±60.31			
Charry Joural	Fresh	3.00±1.02	1056.78±90.73	29.02±1.41	1555.70±447.21			
Cherry laurel	Dried	0.94±0.04	1552.74±44.02	35.48±0.87	3712.96±340.87			

Table 1. Ascorbic acid, total phenolic content and antioxidant properties of fresh and freeze-dried cherry laurel and kiwi fruit

\* DPPH measurements of fresh and freeze-dried fruit samples were calculated for 10 mg and 0.1 mg of dry matter, respectively.

Lable 2. Color properties of fresh and freeze-dried cherry laurel and kiwi fruit									
-			L	а	b	ΔE			
Kiwi fru	Kiwi fruit	Fresh	42.70±0.58	-6.18±0.04	11.54±0.07				
	NIWITUI	Dried	75.13±0.83	-9.23±0.19	21.86±0.40	34.17±0.12			
	Charmeloural	Fresh	25.72±1.18	2.60±0.18	7.05±0.01				

12.33±0.18

32.13±0.36

#### **Rehydration Capacity**

Rehydration capacity is an important quality property of dehydrated products. It shows the retain capability of the original form of the dried products and directly effects the consumer demands for dried foods [35]. The rehydration rate of freeze-dried kiwi and cherry laurel fruits at room temperature and 40°C were calculated and demonstrated in Figures 1 and 2. The rehydration ratio increased within the beginning of experiment and then slowed down. A similar tendency can be found in

Dried

Cherry laurel

the study of Demiray and Tulek [36]. As shown in the Figures 1 and 2, the freeze-dried kiwi fruit exhibited higher rehydration ratio and faster water absorption property than cherry laurel fruit for both temperatures because of the porous structure and wider contact area of kiwi fruit. The constant values of rehydration ratio of fruits were similar at room temperature and 40°C, but the increase of it was more rapid at the high temperature. Freeze-dried samples show high rehydration capacities because freeze-drying cause large voids and porous structure [37].

12.80±0.26

13.05±1.13

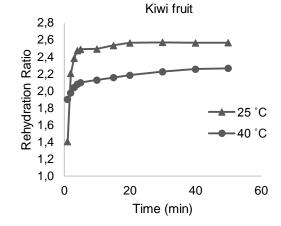


Figure 1. Rehydration rate of dried kiwi fruit at 25 and 40°C

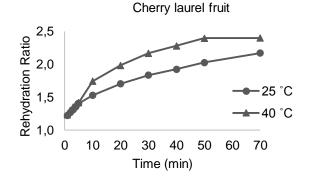


Figure 2. Rehydration rate of dried cherry laurel fruit at 25 and 40°C

### CONCLUSIONS

The results showed that freeze-drying method can be applied for high quality dried fruits and off-season products. Freeze-drying process reduces the postharvest losses and preserves the structure, color and flavor, so it can be satisfactorily applied to drying of cherry laurel, kiwi and other fruits. Besides, this process enhances the antioxidant properties of fruits, compared to fresh ones.

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