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

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

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 fruit 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 rehydration ratio in water at 40°C increased more quickly in the beginning of immersion than the ratio at 25°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

ÖZ

Dondurarak kurutma besleyici ve siçaklıklık kolay etkilenebilen gıda ürünlerinin korunmasına kullanılan yeni bir metottur. Bu çalışmada, dondurarak kurutmanın etkilerini gözlemlemek için yüksek ascorbik asit (kivi) ve fenolik bileşen içeren (karayemiş) iki tip meyyve seçilmiştir. Kivi ve karayemiş meyvelerinin dondurarak kurutulmasından önce ve sonra asorbik asit içeriği, toplam fenolik madde içeriği ve antioksidan kapasiteleri belirlenmiştir. Kivi ve karayemiş meyvesi için asorbik asit içeriği 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 içeriği sırasıyla 262.66±19.97 ve 1056.78±90.73 mg GAE/100 g kuru madde olarak bulunmuştur. Her iki meyye için de dondurarak kurutma işlemi ile birlikte antioksidan kapasiteleri artarken, asorbik asit içeriği değişmemiştir. Toplam fenolik bileşen miktarında dondurarak kurutulmuş karayemiş meyvesinde önemli ölçüde artış olmuştur ancak kivi meyvesinde olmamıştır. Renk değerleri her iki meyye için dondurarak kurutma işlemi ile birlikte değişmiştir, aydınlatıcı ve sarı renk değerleri önemli ölçüde artmıştır (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 süreslerinin başlangıcında daha hızlı artış göstermiştir. Çalışmanın sonuçları dondurarak kurutma işlemini, besleyici kurutulmuş meyveler ve sezon dışı ürünler için uygun bir metot olduğunu göstermiştir.

Anahtar Kelimeler: Ascorbik 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 antioxidant components, especially phenolic 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].

Kiwi fruit (Actinidia deliciosa Planch) is known as a good source of ascorbic acid. Because of the phytountrient content of kiwi fruit 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-2-carboxylic acid] was purchased from Aldrich (Steinheim, Germany). Folin-Ciocalteu reagent and DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) were obtained from Fluka (Buchs, Switzerland). Neocuproine was 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 centrifugated 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 µL [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, AB) 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 (%) = \[\frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100\]  
where $\text{Abs}_{\text{control}}$ is absorbance of the blank (reacting mixture without the test sample) and $\text{Abs}_{\text{sample}}$ 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 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 ± 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 genotypes 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 radical-scavenging 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 (ΔE; 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.
Table 1. Ascorbic acid, total phenolic content and antioxidant properties of fresh and freeze-dried cherry laurel and kiwi fruit

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

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

Table 2. Color properties of fresh and freeze-dried cherry laurel and kiwi fruit

<table>
<thead>
<tr>
<th></th>
<th>L</th>
<th>a</th>
<th>b</th>
<th>ΔE</th>
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<tbody>
<tr>
<td>Kiwifruit</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh</td>
<td>42.70±0.58</td>
<td>-6.18±0.04</td>
<td>11.54±0.07</td>
<td></td>
</tr>
<tr>
<td>Dried</td>
<td>75.13±0.83</td>
<td>-9.23±0.19</td>
<td>21.86±0.40</td>
<td>34.17±0.12</td>
</tr>
<tr>
<td>Cherry laurel</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh</td>
<td>25.72±1.18</td>
<td>2.60±0.18</td>
<td>7.05±0.01</td>
<td></td>
</tr>
<tr>
<td>Dried</td>
<td>32.13±0.36</td>
<td>12.33±0.18</td>
<td>12.80±0.26</td>
<td>13.05±1.13</td>
</tr>
</tbody>
</table>

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 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].

![Figure 1. Rehydration rate of dried kiwi fruit at 25 and 40°C](image1)

![Figure 2. Rehydration rate of dried cherry laurel fruit at 25 and 40°C](image2)
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.

REFERENCES


