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Araştırma Makalesi (Research Article)

Effect of Freezing and Drying Methods on Some Biochemical Properties of Prickly Fig (*Opuntia ficus-indica*) Fruit

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Abstract: In this study, the fruit of *Opuntia ficus-indica* was examined in the fresh, frozen, sun and in microwave dried samples. In fresh *Opuntia ficus-indica* samples, the total phenolic and flavonoid content was found to be 3.30 µg GAE/g DW and 1.46 µg QE/g DW, whereas in sun-dried samples found to be 2.60 µg GAE/g DW and 0.56 µg QE/g DW respectively. TEAC and IC₅₀ are indicators of antioxidant capacity, and in fresh fruits TEAC and IC₅₀ found to be 66.91 µmol Trolox/g DW and 45.95 mg ml⁻¹, while 39.01 µmol Trolox/g DW and 57.36 mg ml⁻¹ in sun-drying, respectively (p<0.05). While the amount of ghrelin, GSH, GSSG and MDA in fresh prickly fig were found to be 19.20; 372; 20.85; 3.00 µg/g DW, on the other hand in sun-dried samples were found to be 9.90; 210.00; 33.60; 4.78 µg/g DW, respectively. In addition, ghrelin and GSH in dried fruits decreased while GSSG and MDA increased in comparison to fresh sample (p<0.05). It can be concluded that the fruit of *Opuntia ficus-indica* is rich in ghrelin and GSH. The most suitable preservation techniques for *Opuntia ficus-indica* fruits is freezing to consume it in all season and microwave drying appears to be more advantageous than sun-dried in terms of time.

Dondurma ve Kurutma Yöntemlerinin Dikenli İncir (*Opuntia ficus-indica*) Meyvesinin Bazı Biyokimyasal Özellikleri Üzerine Etkisi

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Anahtar kelimeler

Antioksidan kapasite,
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Fenolik maddeler.

Öz: Bu çalışmada, dikenli incir (*Opuntia ficus-indica*) meyvesi taze, dondurulmuş, güneş ve mikrodalga ile kurutularak incelenmiştir. Taze *Opuntia ficus-indica* örneklerinde toplam fenolik ve flavonoid madde içeriği sırasıyla 3.30 µg GAE/g KM ve 1.46 µg QE/g KM bulunurken, güneşte kurutulmuş örneklerde ise 2.60 µg GAE/g KM ve 0.56 µg QE/g KM olarak bulunmuştur. Antioksidan kapasitenin göstergesi olan TEAC ve IC₅₀ değerleri taze meyvelerde sırasıyla 66.91 µmol Trolox/g KM ve 45.95 mg/mL iken, güneşte kurutulmuş örneklerde ise 39.01 µmol Trolox/g KM ve 57.36 mg ml⁻¹ olarak bulunmuştur (p<0.05). Taze dikenli incirdeki grelin, GSH, GSSG ve MDA miktarları sırasıyla 19.20; 372; 20.85; 3.00 µg/g KM iken, güneşte kurutulmuş örneklerde ise 9.90; 210.00; 33.60; 4.78 µg/g KM olarak bulunmuştur. Ayrıca, kurutulmuş meyvelerdeki grelin ve GSH miktarı azalırken, GSSG ve MDA miktarları artmıştır (p<0.05). Dikenli incir meyvesinin grelin ve GSH açısından zengin olduğu, bu meyvelerin tüm mevsimlerde faydalanılabilmesi için en uygun koruma metodu, dondurarak

saklamaktır. Mikrodalga ile kurutma, zaman açısından güneşte kurutma yöntemine göre daha avantajlı görünmektedir

1. Introduction

Opuntia ficus-indica is important in terms of nutrient values such as ascorbic acid, potassium, phosphorus, magnesium, sodium, calcium glutamine, proline, taurin, amino acids, phenolic substances, and antioxidants. It is also reported that it has a positive effect on health due to the richness of high antioxidant activity and phenolic substances (El-Mostafa et al., 2014). The antioxidants in *Opuntia ficus-indica* show anticancer, anti-inflammatory, hypoglycemic, hypolipidemic and hypocholesterolemic properties (Bensadón et al., 2010). *Opuntia ficus-indica*, fruits, and stems have been used in folk medicine for different medicinal purposes. *Opuntia ficus-indica* has been reported to be useful in the treatment of many chronic diseases (Osuna-Martínez et al., 2014). It has been proved that non-nutritive components such as active antioxidant and phytochemicals contained in the fruit of *Opuntia ficus-indica*, against oxidative stress have been found protective effects in rats (Livrea and Tesoriere, 2006).

Phenolic compounds found as secondary metabolites in plants consist of different compounds such as complex flavonoids, simple flavonoids, phenolic acids and anthocyanins (Babbar et al., 2014). Flavonoids constitute the largest group of phenolic compounds in the plant. They are among the substances responsible for the coloring of fruits and vegetables. Flavonoids are very important in the function of some enzymes. These substances were found to have anti-oxidative, anti-inflammatory, anti-cancer and anti-mutagenic properties (Panche et al., 2016).

Ghrelin is a peptide hormone that stimulate growth hormone release from the pituitary gland. It has affected on the brain to regulate the modulation of glucose metabolism. It has been proved that ghrelin, which is a hormone found in animals can also be found in various plants and fruits (Aydin et al., 2006).

The protective effects of *Opuntia ficus-indica* against oxidative damage can be explained by the presence of various antioxidants such as vitamins C, and E, carotenoids, reduced glutathione, flavonoids, and phenolic acids (Stintzing et al., 2001). Oxidized glutathione, is an indicator of oxidative stress, inhibits the protein synthesis in cells, while reduced glutathione has many physiological functions, such as reducing the toxicity of xenobiotics, transport of amino acids and act as co-enzymes in some enzymatic reactions. (Mendoza-Cózatl et al., 2005). Reactive carbon compounds are formed as a result of lipid peroxidation and malondialdehyde (MDA) is the indicator of this process (Gawel et al., 2004).

Opuntia ficus-indica is a seasonal fruit; to be able to consume it all year around different preservation methods applied (Fadda and Mulas, 2010). Preservation techniques have a profound effect on the nutritional value and medicinal benefits of the fruits. Although drying in the sun is widely used methods, they can also be dried in ovens, drying tunnels and under vacuum as well. Microwave drying is a relatively new technique to be investigated for many foodstuffs. The drying temperature, drying time, light intensity and humidity are important factors on the nutritional content of fruits (Maisnam et al., 2016).

In our previous work, water and oil-soluble vitamins with some elements in *Opuntia ficus-indica* fruits have been studied (Bakar et al. 2020). The purpose of this study to investigate the effect of processing methods such as freezing and drying on total phenolic, flavonoid content, antioxidant capacity, ghrelin, glutathione, malondialdehyde content of prickly pear fruit.

2. Materials and Methods

2.1. Materials

Ripe *Opuntia ficus-indica* (k17533923) fruits were harvested from Osmaniye province (GPS location: 37.272707, 36.122754), in August, 2018. Samples collected were identified by Faculty of Science, Department of Biology, at Firat University. Fruit samples were collected from different parts of the region for a good sampling. The prickles of the collected samples were cleared. Five samples of the cleaned fruit samples were randomly taken into each storage bag secured. The fresh fruit samples were analyzed as soon as the fruits were collected, while sun and microwave-dried samples with frozen

samples were analyzed after ten days of drying. Frozen samples were kept at -20 °C until assayed. Chemicals and equipment used by Bakar et al (2020), were also used in this study.

2.2. Microwave and sun drying

Drying processes were carried out according to the processes applied by Bakar et al. (2020)

2.3. Extraction of *Opuntia ficus-indica* fruit

The prickly pear fruits samples were homogenized in a blender and 15.0 gram of homogenized samples were Soxhlet extracted with 300 mL of methanol (1:20 g ml⁻¹) for four hours. The solvent was removed at 40 °C in a vacuum rotary evaporator. The weighed solid extract was dissolved in 50 mL of methanol. This solution was stored at 4 °C in the refrigerator until analyzed.

2.4. Determination of total phenolic content

The spectrophotometer was used to determine the total phenolic content according to the Folin-Ciocalteu method modified by Dewanto et al. (2002). 0.50 mL of distilled water, 0.250 mL of sample or Gallic acid and 0.125 mL of Folin-Ciocalteu reagent were mixed and shaken. After 6 minutes, 1.250 mL of 7 % sodium carbonate solution was added and the total volume completed to 3.00 mL with distilled water. After 90 minutes, the absorption was measured at 760 nm by a UV-Visible spectrophotometer. A working graph of Gallic acid solutions prepared at different concentrations was established. The total phenolic content of the samples was determined and the results were given as µg Gallic acid per g dry weight sample (µg GAE/g DW).

2.5. Determination of total flavonoid

The total flavonoid substance was determined by UV-visible spectrophotometer as described by Dewanto et al. (2002) 0.025 mL sample or quercetin, 1.250 mL distilled water and 0.075 mL 5% sodium nitrite solution, 0.150 mL 10% solution of aluminum chloride were mixed in a glass tube and allowed to stand for 5 minutes then 0.500 mL 1.0 M sodium hydroxide solution was added and total volume was completed to 2.500 mL with distilled water followed by measurement of absorbance at 510 nm. A working graph was formed with quercetin solutions prepared in different concentrations. The total phenolic content of the samples was determined using the working graph and the results were given as µg QE/g DW.

2.6. Total antioxidant capacity

Total antioxidant capacity was determined by two different methods DPPH and TEAC.

2.6.1. DPPH (2,2-diphenyl-1-picrylhydrazyl) method

The antioxidant capacity was measured according to the method based on the scavenging activities of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical as described by Nile et al. (2013). A solution of 25 µg ml⁻¹ DPPH in methyl alcohol was prepared, and the absorption of DPPH solution was measured at 510 nm. Then different amount of the sample extracts was added to DPPH solution and kept in dark for 30 minutes before measurement of absorbance at 510 nm. The percentage of DPPH scavenging effect was calculated using the following equation (1):

$$\text{DPPH Scavenging effects (\%)} \text{ or } \text{Percent Inhibition} = \frac{(A_0 - A_1)}{A_0} \cdot 100 \quad (1)$$

A₀ is the absorbance of the sample free DPPH solution, and A₁ is the absorbance of the sample containing DPPH solution after 30 minutes. The results of DPPH were given as IC₅₀ (µg ml⁻¹). The IC₅₀

values indicates the concentration of the antioxidant substance which inhibits 50% of the DPPH radical in the medium. Low IC₅₀ values indicate high antioxidant activity.

2.6.2. TEAC (Trolox Equivalent Antioxidant Capacity)

The ABTS free radical-scavenging activity was determined according to the method described by Re et al., (1999). The stock solutions including 7.0 mM ABTS solution and 2.4 mM potassium persulfate allowed to stand in the dark at room temperature for 12–16 h. The ABTS^{•+} solution was diluted with phosphate buffer (pH=7.4) to obtain an absorbance of 0.800 ± 0.010 at 734 nm. Then 20 µL of the sample or Trolox standard was added to 2.0 mL ABTS^{•+} solution and allowed to stand at room temperature for 15 minutes then absorption was measured at 734 nm. Previously prepared ABTS^{•+} solution used as the control group. All measurements were performed in four parallel for every sample. The percentage inhibition of ABTS^{•+} was calculated the formula given below (2):

$$\% \text{ Inhibition} = \frac{(A_{C(0)} - A_{A(t)})}{A_{C(0)}} \cdot 100 \quad (2)$$

where A_{C(0)} is the absorbance of the control at t=0 minute, and A_{A(t)} is the absorbance of the (Trolox + ABTS^{•+}) or (Sample + ABTS^{•+}) at t=15 minute. The free radical scavenging capacity of the sample was calculated as percent inhibition of ABTS^{•+}. The standard calibration curve was generated using Trolox concentration of 1.0-4.0 µg ml⁻¹. The antioxidant capacity of the sample was calculated as Trolox equivalent as µmol Trolox/g DW.

2.7. Determination of ghrelin, glutathione, and MDA

From each sample of *Opuntia ficus-indica*, 2.0 g of homogenized sample was weighed and 1.00 mL of 0.50 M perchloric acid was added to each homogenate to precipitate proteins then incubated for 10 minutes in the sonicator. Each sample was made up to 6.0 mL with distilled water and vortexed then centrifuged for 5 minutes at 6000 rpm. The supernatant was filtered off (Whatman No 1) and taken into 1.0 mL of HPLC vials to determine the ghrelin, glutathione (reduced and oxidized) and MDA levels. Ghrelin, glutathione, and MDA were determined according to the method of Ibrahim et al. (2017), by HPLC (Exsil column 100-5 ODS 25 cm, 4.6 mm ID, 5 µm), the mobile phase (pH=4.0) consisted of 50 mM NaClO₄ in 1% H₃PO₄ with a flow rate of 1.0 mL/minute. Ghrelin and glutathione were determined at 215 nm and MDA at 254 nm respectively.

2.8. Statistical Analysis

Statistical analysis was carried out on four different samples in parallel. A pc computer was used for variance analysis and LSD multiple comparison test was performed at p<0.05 level.

3. Results and Discussion

The total phenolic and flavonoids content with total antioxidant capacity, glutathione, ghrelin and stress biomarkers were investigated. The results are given in Figures 1-3. Phenolic compounds happened to be the most known secondary metabolites found in plants and their distribution is seen throughout the metabolic processes. These phenolic substances consist of different kinds of compounds that include: complex flavonoids, simple flavonoids, phenolic acids and anthocyanins (Babbar et al., 2014).

The total phenolic content in prickly pear samples found to be in between 2.60±0.15 to 3.30±0.25 µg GAE /g DW (Figure 1). According to the obtained results, the highest amount of phenolic substance was seen in fresh samples, then frozen and in microwave dried samples while the lowest amount was observed in the sun-dried sample. The results indicate that drying processes significantly decrease of phenolic content in the fruit samples (p<0.05).

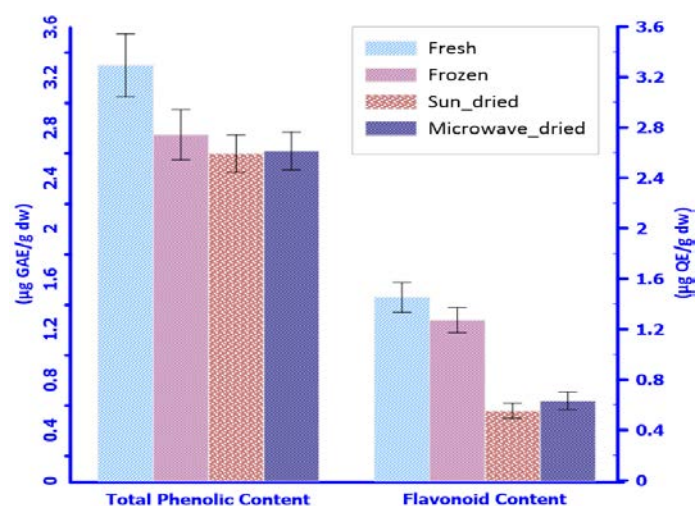


Figure 1. Total phenolic and flavonoid content in fresh, frozen, sun and microwave dried *Opuntia ficus-indica* fruits.

In the study conducted by Patil et al. (2019), the phenolic content of prickly pears was found as 42.454 mg GAE/100g in 50% methanol extract and 38.736 mg of GAE/100g in the water extract.

In the study conducted by Cha et al. (2013), the content of total phenolic and flavonoids in *Opuntia humifusa* reported to be 125.10 µg GAE/g and 48.90 µg CE g⁻¹, respectively. It has been reported that the total phenolic content in different seeds, fruits and vegetables varies between 169-1048 mg/100g DW (Velioğlu et al., 1998). There are different studies on the effect of drying on phenolic content in fruits. In some studies, they reported that drying process caused a decrease in the total phenolic content (Zanoelo et al., 2006; Meral, 2017) on the other hand a study conducted by Carranza-Concha et al., (2012) reported that the total phenolic content was increased as a result of drying. Because of the heat treatment, some phenolic compounds are converted and some are released to medium. Therefore, it can be said that the drying process on different products does not have the same effect on the total phenolic content (Miletic et al., 2013).

Fruits and vegetables can be consumed either fresh or processed. Flavonoids content in fruits and vegetables may be affected by different preservation methods including drying (Kamiloglu et al., 2015). In this study, the flavonoid content in fresh, frozen, sun-dried and microwave dried samples were found to be 1.46±0.12, 1.28±0.10, 0.56±0.06 and 0.64±0.07 µg QE/g DW respectively (Figure 1). The highest amount was observed in the fresh samples followed by frozen, microwave dried then sun dried samples.

In a study by Hahm et al. (2015), the total flavonoid in the fruit of *Opuntia ficus-indica* was reported to be 1.91±0.29 mg QE/g DM. It has been reported that flavonoid amounts have been decreased as a result of freezing and drying of fresh vegetables and fruits (Kamiloglu et al., 2015). When the fruits are subjected to various preservation processes the total flavonoid content might decrease as a result of some interactions in their structure. Some phenolic components and antioxidant substances are destroyed and reduced by heat treatment. Generally, it is expected that antioxidant activity and total phenolic content will decrease with heat treatment.

Two different methods, DPPH and TEAC, were used to determine the antioxidant capacity of the samples in this work. While calculating IC₅₀ values in the DPPH method, Trolox equivalent was calculated in TEAC method. The IC₅₀ value refers to the concentration of the antioxidant substance which inhibits 50% of the DPPH radical in the medium. The antioxidant capacity is inversely proportional to IC₅₀ value. The IC₅₀ recorded from our work in fresh, frozen, sun and microwave dried *Opuntia ficus-indica* fruit sample were 45.95±2.24, 50.24±2.84, 57.36±2.11 and 52.12±3.10 µg ml⁻¹ respectively (Figure 2). In the study conducted by Cha et al. (2013), they found the radical scavenging activity (DPPH) of methanol extract of *Opuntia humifusa* as 72.5%. Surinrut et al. (2005), found that IC₅₀ values of mangosteen, orange, pomelo, grape and papaya fruits ranged from 11.18 to 32.80 mg ml⁻¹. In another study, Reynertson et al. (2005), reported that IC₅₀ values between 15.9-247 mg ml⁻¹ in

seven different fruits of the Myrtle family. TEAC method is widely used in determining the antioxidant capacity of food samples due to easy of application in both aqueous and lipid phases.

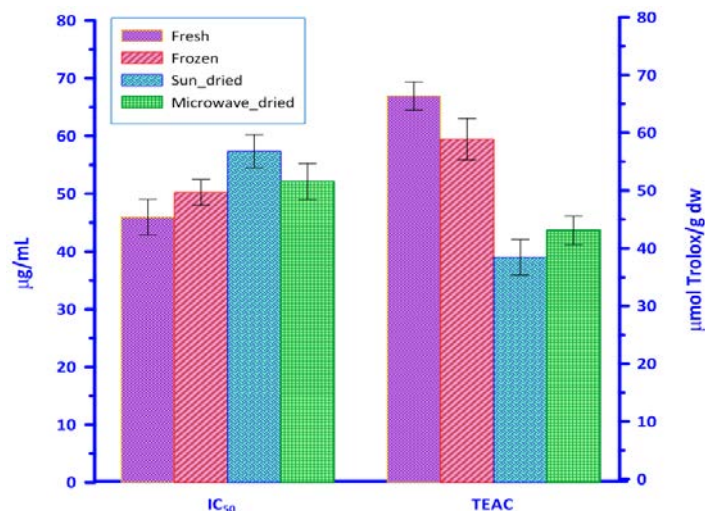


Figure 2. Total antioxidant capacity (TEAC and IC₅₀ value) in fresh, frozen, sun and microwave dried *Opuntia ficus-indica* fruits

In this study, free radical scavenging activity (TEAC) of the fresh, frozen, sun and microwave-dried *Opuntia ficus-indica* fruit samples were found to be 66.91±3.58, 59.47±3.10, 39.01±2.45 and 43.67±2.46 µmol Trolox/g DW, respectively (Figure 2). Su et al. (2007) found that antioxidant activity level of 80% methanol extracts of rosehip fruit was 190 ± 4.81 µmol TEAC g⁻¹. The trolox equivalent antioxidant capacities of fruit samples were found to be lower than that of rosehip fruit. As seen in Figure 2, the antioxidant capacity decreased as a result of drying processes. Experimental findings indicate that the changes in IC₅₀ and TEAC values as a result of drying are statistically significant (p < 0.05). The experimental result showed that the heat treatment decreases the total antioxidant capacity due to the decrease in the total phenolic, flavonoids and antioxidant vitamins in the fruits (Miletic et al., 2013).

Ghrelin, is also called growth hormone, present in the peptide structure can be affected by applied heat treatments. This study showed that the ghrelin content in fresh, frozen, sun and microwave dried fruit samples found to be 19.20±1.40, 18.80±1.31, 9.90±0.74, and 10.10±0.79 µg/g DW, respectively (Figure 3). The higher amount of ghrelin was observed in the fresh samples followed by frozen and microwave then sun-dried samples (p < 0.05). It was reported by İbrahim et al., (2017), that the amount of ghrelin in *Crataegus laevigata* fruits in different regions was found between 18.96±6.73 and 79.96±12.14 µg g⁻¹.

Glutathione, which is necessary for the immune system of the cells, has a peptide structure is the most important intracellular antioxidant molecule, also involve in the transport of amino acids in metabolism and reduction of sulfhydryl groups in proteins (Mendoza-Cózatl et al., 2005). In this study, the amount of GSH in fresh, frozen, sun and microwave dried samples of *Opuntia ficus-indica* fruit was found to be 372±31.90, 344±28.80, 210.00 ±15.00 and 235.00±17.00 µg g⁻¹, respectively. The amounts of GSSG in the same samples were 20.85±1.24, 21.70±1.85, 33.60±1.80 and 31.20±1.64 µg g⁻¹, respectively. While the amount of GSH decreased as a result of drying processes, the amount of GSSG increased (p < 0.05) (Figure 3). Tesoriere et al. (2005) reported that the amounts of GSH in three different cultures of prickly pears were found to be in between 3.40 to 8.10 mg/100 g.

Both glutathione and ghrelin are known as peptides. Preservation methods applied to foods can significantly affect the biological activity of peptides. Ultrasound, heat, and irradiation processing might affect protein structure and functions. In addition, these processes may cause Millard reactions in food (Davis et al., 2001). As a result of the factors mentioned above, might leads to changes in the amount of peptides.

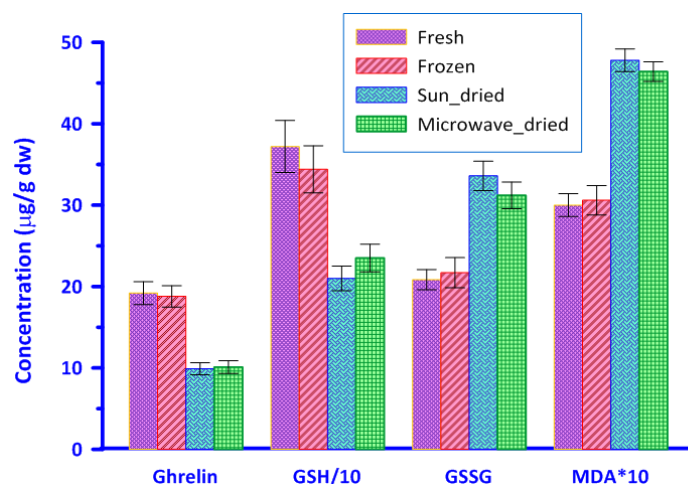


Figure 3. Ghrelin, GSH, GSSG and MDA content in fresh, frozen, sun and microwave dried *Opuntia ficus-indica* fruits (GSH values divided by 10, MDA values multiplied by 10).

Free radicals cause lipid peroxidation by affecting the unsaturated fatty acids in the membranes and MDA forms as a result of lipid peroxidation (Gaweł et al., 2004). In this study, the MDA content in fresh, frozen, sun and microwave dried *Opuntia ficus-indica* samples was found to be 3.00 ± 0.14 , 3.06 ± 0.18 , 4.78 ± 0.14 , and $4.64 \pm 0.12 \mu\text{g g}^{-1}$, respectively. The amount of MDA was found to be the highest in the sun-dried samples, while it was the lowest in fresh samples ($p < 0.05$) (Figure 3). The amount of MDA in ripe ber (*Ziziphus mauritiana Lam*) fruit was found to be 4.498 nmol/g (Kumar et al., 2011). It has been reported that the amount of MDA is increased in microwave and infrared dried apricot samples (Karatas and Kamisli, 2007).

The GSH/GSSG ratio and MDA are used as stress biomarkers. The stress is inversely proportional to the ratio of GSH/GSSG and is proportional to the amount of MDA. GSH/GSSG ratios of fresh, frozen, sun and microwave-dried fruits were calculated as 17.84, 15.85, 6.25 and 7.53 respectively. The increase in the amount of MDA in fruits and the decrease in the GSH/GSSG ratio indicate that oxidative stress occurs by drying processes. It can be said from these results that, preservation methods applied cause stress on the fruits.

Conclusion

The total phenolic, flavonoids and antioxidant capacity in fresh and frozen *Opuntia ficus-indica* fruit were higher than the amounts in the sun and microwave dried fruits. In addition, the amount of ghrelin and reduced glutathione in dried fruits decreased while the amount of oxidized glutathione and malondialdehyde increased. These results suggest that the best preservation methods for *Opuntia ficus-indica* fruit are freezing. In addition, microwave drying seems more advantageous than drying under the sun in terms of drying time.

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