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Effect of Frozen Storage and Modified Atmosphere Packaging Followed by Cold Storage on Some Quality Characteristics of Semi-Dried Persimmons

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ABSTRACT

Dried persimmon is a relatively new product for Turkish consumers. Thus, the quality characteristics and storage conditions of this product have not yet well-defined. In this study, the effect of frozen storage and modified atmosphere packaging (100% N₂ and 30% CO₂+70% N₂) followed by cold storage on microbiological, physicochemical and sensory quality of semi-dried persimmons was investigated. Both modified atmosphere packaging and frozen storage were effective in suppressing the growth of total aerobic mesophilic bacteria, yeast and mold. Increase in L color values, an indicator of sugar migration, was observed in air and modified atmosphere packaged samples. Increases in a and b color values were recorded during frozen storage and were not affected by any of the treatments. Sensory analysis results revealed that color, taste and general acceptability scores of modified atmosphere packaged and freeze-stored samples were significantly higher than those of air packaged ones. Therefore, both modified atmosphere packaging and frozen storage can be recommended to maintain the quality characteristics of semi-dried persimmons.

Keywords: Modified atmosphere packaging, Frozen storage, Semi-dried fruits, Shelf life

Yarı-Kurutulmuş Trabzon Hurmalarının Bazı Kalite Özellikleri Üzerine Donmuş Depolamanın ve Modifiye Atmosferde Paketleme Sonrası Soğuk Depolamanın Etkisi

ÖΖ

Kurutulmuş Trabzon hurması, Türk tüketiciler için nispeten yeni bir üründür. Bu nedenle, söz konusu ürünün kalite karakteristikleri ve depolama koşulları henüz net bir şekilde tanımlanmamıştır. Bu çalışmada, donmuş muhafazanın ve modifiye atmosferde (%100 N₂ and %30 CO₂+%70 N₂) paketleme sonrası uygulanan soğukta depolamanın yarı-kurutulmuş Trabzon hurmalarının mikrobiyolojik, fizikokimyasal ve duyusal özelliklerine etkisi incelenmiştir. Hem modifiye atmosferde paketleme hem de donmuş muhafaza toplam aerobik mezofilik bakteri, maya ve küf gelişimini baskılamada etkili bulunmuştur. Normal ve modifiye atmosferde paketlenen örneklerde, şeker göçünün bir göstergesi olarak L renk değerlerinde artış gözlenmiştir. Donmuş muhafaza edilen örneklerde, gerçekleşen esmerleşme reaksiyonları nedeniyle a ve b renk değerlerinde artışlar meydana gelmiştir. Örneklerin nem içerikleri ve sertlik değerleri depolama boyunca istatistiksel açıdan anlamlı düzeyde değişmemiş ve bu değerler gerçekleştirilen uygulamalardan etkilenmemiştir. Duyusal analiz sonuçları göstermiştir ki, modifiye atmosferde paketlenen ve donmuş muhafaza edilen örneklerin hava atmosferinde paketlenen örneklerin renk, tat ve genel kabul edilebilirlik skorları hava atmosferinde paketlenen örneklerinkinden yüksektir. Bu nedenle, gerek modifiye atmosferde paketleme gerekse donmuş muhafaza teknikleri yarı-kurutulmuş Trabzon hurmalarında kalitenin korunması için önerilebilir.

Keywords: Modifiye atmosferde paketleme, Donmuş muhafaza, Yarı-kuru meyve, Raf ömrü

INTRODUCTION

Persimmon (*Diospyros kaki* L.), belonging to the *Ebenaceae* family, was originated from Far East Asia. This plant is distributed from tropical to temperate climates in a wide range of areas around the world [1]. There are more than 400 species of the plant, but *Diospyros kaki* L., *D. lotus* L., *D. oleifera* and *D. virginiana* L. are the most commercially important ones. The most common cultivars grown worldwide are

astringent 'Hachia' and non-astringent 'Fuyu' [2]. China is the biggest producer of persimmon in the world. According to the FAO statistics, this country produced approximately 4.2 million tons of persimmons in 2017 [3]. Korea, Japan, Brazil and Azerbaijan are the other leading persimmon producer countries. Turkey also produces persimmons and the production amount has increased each year since the early 2000s [4]. This upward trend can be clearly seen in Figure 1.



Figure 1. Changes in annual persimmon production in Turkey over the years from 2004 to 2017 [4]

Persimmon is a climacteric fruit. The respiration of climacteric fruit increases at the onset of ripening and is related to the ethylene production of the fruit. Normally, persimmon produces a small amount of ethylene but the sensitivity of the fruit to ethylene is extremely high [5]. Storing the fruit at low temperatures (typically at 0°C) can be used to hold fruit for 1-3 months. However, the fruit is also very sensitive to chilling injury, a significant quality loss expressed as softening and gelling of flesh; flavor, odor, juice and sweetness losses; flesh darkening and mottling, and skin translucence which is generally observed in the fruit held at higher temperatures, e.g., 5°C [6]. Therefore, storing of persimmons as a fresh fruit can be challenging. For this reason, this fruit can be generally processed into products such as jam, marmalade, etc. or preserved by canning or drying techniques.

Regardless of which product is produced (jam, marmalade, fresh, canned or dried fruit), harvesting the fruit at the correct maturity is crucial in terms of product quality. Skin color, fruit flesh firmness, soluble solid and tannins contents are common parameters used for indicating harvest maturity. Kuzucu and Kaynas [7] indicated astringency caused by high tannin content and over-softening as the main problems in early- and lateharvested fruits, respectively. For persimmons for fresh consumption in the Çanakkale Region, the authors suggested the second half of November as the appropriate harvesting time. The best harvest time for persimmons to be dried should be just before this, in other words, the period in which the fruit color changes from green to yellow [8].

In Turkey, persimmons are dried conventionally in open air by some grower families with limited means, therefore the production amount are not very high. For dried persimmon production, harvested fruits are simply washed to remove dirt and dust, and peeled with a knife. The peeled fruit are tied consecutively with long strings, hung in a high position and then let dry as whole fruit in open air. The moisture levels of dried persimmons were reported as 35 and 19% by Tulek and Demiray [9] and Bolek and Obuz [10], respectively. Drying can be thought as a preservation method for persimmons and can also be employed as a way of producing "a new product" from this fruit. Actually, dried persimmons have been produced in the Far East countries for years; however production and consumption of this product has recently started in Turkey. Dried persimmon production has also appealed to many investigators and various research studies have been conducted by Turkish scientists in recent years [8-15]. Since it is a relatively new product and not known very well in the whole country, it has not been cited in Turkish legislations and thus any characteristics (even moisture content) of dried persimmons have not been regulated by legislation.

In domestic production, in order to prevent microbiological deterioration, persimmons are sometimes over-dried and consequently the moisture content of the final product is quite low (<25%). This low

moisture product is resistant to microbial spoilage but the sensory characteristics are usually undesirable. An alternative way to produce dried persimmon is drying the product to relatively high moisture content (\geq 30%). However, at this moisture level, the product is quite susceptible to microbial attack and frozen storage is inevitable. Obviously, it is not a very cost-effective preservation method and scientific community is in search of economical techniques that can maintain microbiological and physicochemical quality of semidried persimmons.

Storage conditions are critical issues in maintaining quality of the product after processing. Humidity is one of the most important parameters to be controlled since it is directly related with dehydration and/or rehydration of the product. Storage temperature is also of vital importance in maintaining quality of the product. Storage of dried fruits at refrigerated temperatures preserves sensorial qualities, thereby extending shelf life. Freezing dried fruits allows for extremely longer storage periods but generally thought as a costly process. Vacuum and modified atmosphere packaging techniques could work perfectly for maintaining the quality of dried fruits during storage.

The number of studies focused on the storage conditions of semi-dried fruits, especially of semi-dried persimmons, is very limited. The aim of this study was to investigate the effect of packaging in air and modified atmosphere (100% N₂ and 30% CO₂+70% N₂) and subsequent cold storage on microbiological, physicochemical and sensory quality of semi-dried persimmons and to compare the results with those of frozen storage.

MATERIALS AND METHODS

Materials

Plate count agar (PCA), dichloran rose bengal chloramphenicol (DRBC) and sodium chloride were purchased from Merck (Darmstadt, Germany). Microbiological media and the solutions used in the study were prepared in high purity water obtained from TKA Pacific UP/UPW water purification systems (Niederelbert, Germany).

The persimmons used in the study were harvested in late September 2017 from plants grown in an orchard located in Honaz Region in Denizli, Turkey. Fruits of which color was about to change from green to yellow were chosen for harvest. The fruits were washed under running tap water, peeled manually, tied with a string and hung on an overhead bar made of stainless steel. Then, the fruits were let dry in open air for 32 days until the moisture content fell down to 30±5%. Weather conditions during drying (September 30-October 31, 2017) were as follows: minimum temperature was 8°C, maximum temperature was 28°C and relative humidity ranged between 39.5-91.2% (Data obtained from Turkish State Meteorological Service).

Packaging and Storage

Semi-dried persimmon samples were packaged using a packaging machine (DZ-260, Seles, Wenzhou Xingye . Machinery Equipment Co. Ltd., Beijing, China). Laminated plastic pouches (polyethylene terephthalate coextruded ethyl vinyl alcohol, 25x25 cm, Sesa Ambalaj ve Plastic San. Tic. A.S., İzmir, Turkey) were used for air and modified atmosphere packaging of the samples. Based on technical data sheet provided by the supplier, O₂ transfer rate (23°C and 0% RH) and water transfer rate (38°C and 90% RH) of the pouches were less than 5 cm³/m² day and 5 g/m² day, respectively. The thickness of the bag was measured with a digital caliper (Mitutoyo Co., Kanagawa, Japan) and determined as 130±2 µm. Semi-dried persimmons (approximately 200 g) were placed in these plastic pouches. After evacuating the air inside the pouch to a partial pressure of 675.1 mm of Hg by vacuum and flushing the desired gas composition (100% N₂ or 30% CO₂+70% N₂) into the package, the pouches were heat-sealed. Then, these packages were placed in a fridge (Vestel GT 366, Manisa, Turkey) at + 4°C for the storage period.

The samples to be frozen were placed in freezer bags (low density polyethylene, 10 ± 2 µm thickness, 20x30 cm, Koroplast, İstanbul, Turkey). Then, the bags were heat-sealed using the same machine mentioned above and placed in a deep-freezer (Vestel FT 280, Manisa, Turkey) set at - 18°C. The CO₂ and O₂ concentrations in the packages were determined using a portable gas analyzer (Dansensor, Checkpoint, PBI, Ringsted, Denmark) at the beginning and end of storage.

Microbiological, Physicochemical and Sensory Analyses

For microbiological analyses, 10 g of semi-dried persimmon was put into a stomacher bag and 90 mL of sterile sodium chloride solution (0.85%) was added. The content of the bag was homogenized in a stomacher (Stomacher 400, Seward Medical, London, UK). Serial dilutions were prepared from this homogenizate. TAMB and yeast-mold counts were determined by spread-plating onto PCA and DRBC, respectively. Incubation periods were 24 h at 37°C for TAMB and 3 d at 25°C for yeast-mold.

A colorimeter (CSM 1, PCE Instruments, Southampton, UK) was used to measure L (lightness), a (redness) and b (yellowness) values of the samples in the Hunter color space. The light source was D65 and the measuring aperture was 4 mm. Measurements were taken from five different places on the surface of the fruits and averages of these measurements were reported. The firmness of the samples was measured using a fruit sclerometer (GY-3, Zhejiang Top Cloud-Agri Technology Co., Ltd., Zhejiang, China) with an 8-mm diameter penetrometer tip. The tip was inserted into 3 different places on the surface of the fruits and averages of these measurements were reported. The moisture contents of the samples were determined using a digital moisture analyzer (SMO 01, Scaltec, Göttingen, Germany). Five g of shredded fruit was put into the disk plate analyzer,

dried at 130°C to a constant weight and the moisture content was read out on the display. On each sampling day, three replicates were analyzed for each treatment.

Sensory analysis was conducted by 30 untrained panelists on each sampling day during storage. For each treatment, samples were portioned, coded with randomly chosen 3-digit numbers and served on plastic plates at room temperature. Frozen samples were served after thawing at room temperature within 1 h. Panelists evaluated color, odor, taste, chewiness and general acceptability of the samples on a scale ranging from very poor (1) to very good (5). Spring water was served for rinsing the mouth before testing each sample.

Statistical Analysis

An analysis of variance (ANOVA) was employed to determine significant differences among means using a statistical package program (MINITAB v. 13, MINITAB Inc., State College, PA, USA). Significant differences among means were identified by Duncan's Multiple Range Test ($p \le 0.05$) using MSTAT-C statistical software (MSTAT 1991, Michigan State University, MI, USA).

RESULTS AND DISCUSSION

Effect of Frozen Storage and Modified Atmosphere Packaging Followed by Cold Storage on Microbiological Quality of Semi-Dried Persimmons

The counts of TAMB in semi-dried persimmon samples packaged in air and modified atmosphere during storage for 4 months are shown in Figure 2. Initial TAMB counts of the samples were 1.96±0.24-2.16±0.45 cfu/g. The TAMB counts steadily increased throughout the storage in samples packaged in air. On each sampling day, TAMB counts of these samples were significantly higher than that on the previous sampling day (p<0.05). Significant increases were also observed in the TAMB counts of samples packaged in modified atmosphere, but these increases were much slighter when compared with the ones packaged in air. The TAMB counts of the frozen and modified atmosphere packaged samples were significantly lower than that of the samples packaged in air on each sampling day (p<0.05). There were not significant differences among the TAMB counts of the frozen and modified atmosphere packaged samples at the end of the second, third and fourth months of storage (p>0.05). Gatto et al. [16] investigated the effect of packaging and storage conditions on some quality parameters of semi-dry tomato. The authors indicated that the product stored in modified atmosphere

(30% CO₂+70% N₂) at + 4°C had best quality characteristics and good microbiological stability. On the contrary, Papoff et al. [17] reported that modified atmosphere packaging resulted in no additional improvement on the microbial quality of dried figs stored at + 20°C for 6 months. It can be due to O₂ (2.96%) remained inside the packages used in their study. In our study, the O₂ concentration did not exceed 0.1% in any of the package used for modified atmosphere packaging (data not shown). Certainly, performance of the packaging machine, permeability of the packaging material, O₂ demand of the microorganisms, storage conditions (temperature, etc.) and characteristics of the modified atmosphere packaging technique.

Similar to TAMB counts, yeast counts of the samples packaged in air significantly increased during storage (Figure 3). There was also a significant increase at the end of storage in yeast counts of the samples stored in 100% N₂ atmosphere (p<0.05). However, this increase was quite low when compared with that determined for the samples packaged in air. On the other hand, yeast counts of the samples packaged in 30% CO₂+70% N₂ did not increase significantly at the end of storage period. At the first month of storage, the yeast counts of these samples were even lower than the initial counts (p<0.05), however this effect disappeared over time. Antimicrobial effect of CO₂ was also reported in the past. Panagou et al. [18] reported that the death rate of yeasts in table olives was quite high in the presence of 40 and 100% CO₂ compared to aerobic conditions. El Halouat and Debevere [19] reported that the inhibitory effect of CO₂ against Zygosaccharomyces rouxii was pH-dependent. The strains of the yeast showed a high tolerance to CO_2 at high pH values (pH>4.0). The authors reported that even 80% CO2 did not inhibit the growth of the strains studied.

Mold counts in semi-dried persimmon samples packaged in air and modified atmosphere during storage for 4 months are shown in Figure 4. There were no significant differences in mold counts of the samples packaged in air for the first two months of storage. However, mold counts of these samples increased dramatically in the last two months of storage. Slight and insignificant increases were observed in mold counts of frozen and modified atmosphere packaged samples (p>0.05). On any sampling day, the differences among the mold counts of frozen and modified atmosphere packaged samples were not significant (p>0.05). These results are in agreement with the findings of Villalobos et al. [20] who reported the effectiveness of modified atmosphere packaging in controlling fungal growth.





Figure 2. Effect of modified atmosphere packaging and frozen storage on total aerobic mesophilic bacteria (TAMB) counts of semi-dried persimmons. Data represent mean values and standard errors from 3 replicates. Values with different small letters show significant differences among different treatments for the same sampling day (p<0.05). Values with different capital letters show significant differences among differences a



Figure 3. Effect of modified atmosphere packaging and frozen storage on yeast counts of semi-dried persimmons. Data represent mean values and standard errors from 3 replicates. Values with different small letters show significant differences among different treatments for the same sampling day (p<0.05). Values with different capital letters show significant differences among differences among different sampling days for the same treatment (p<0.05).



Figure 4. Effect of modified atmosphere packaging and frozen storage on mold counts of semi-dried persimmons. Data represent mean values and standard errors from 3 replicates. Values with different small letters show significant differences among different treatments for the same sampling day (p<0.05). Values with different capital letters show significant differences among different sampling days for the same treatment (p<0.05).

According to the results of our study, modified atmosphere packaging (especially the atmosphere containing CO₂) are quite effective in maintaining the microbiological quality of semi-dried persimmons. The counts of microorganisms in modified atmosphere packaged samples were lower than those of packaged in air in all experiments conducted in this study. Additionally; TAMB, yeast and mold counts of the samples packaged in 30% CO2+70% N2 were not significantly different than those of frozen samples. Therefore, it can be concluded that packaging in 30% CO₂+70% N₂ atmosphere followed by storage at 4°C were as effective as frozen storage in terms of microbiological control in semi-dried persimmons. It is probably a result of both limiting the survival of aerobic microorganisms due to reduced oxygen concentration in the package and inhibitory effect of CO₂ against microorganisms.

Effect of Frozen Storage and Modified Atmosphere Packaging Followed by Cold Storage on Physicochemical Properties of Semi-Dried Persimmons

The effects of modified atmosphere packaging and frozen storage on L color values of semi-dried persimmons are shown in Figure 5. The L values of all samples (except the frozen ones) increased during storage. The increase was more pronounced in the

samples packaged in air but observed as well as in the ones packaged in modified atmosphere. This can be explained by local accumulation of soluble solids. During drying and subsequent storage -if the humidity of the surrounding environment is relative low- moisture transfers from inner parts to the surface of the fruit. Soluble solids (mainly sugars) are also transported within the moisture as a solution through the fruit tissues. Free moisture evaporates from the fruit surface while soluble solids reside on the surface of the fruit, crystallize in time and form a white layer [21]. On the other hand, increases in L value were not observed for the samples stored at - 18°C. It shows that moisture transfer from inner parts of the fruit was restricted by immobilization of water molecules through phase transition. On the contrary, L values of these samples were significantly lower at the end of storage. Most probably, this can be a result of a browning reaction. It is known that enzymes in the frozen tissues continue to catalyze many biochemical reactions including browning reactions unless they are inactivated by a heat treatment such as blanching. Moreover, in frozen fruit, since a high amount of water is turned into ice the residual solution becomes more concentrated [22]. It means that the concentrations of the reactants of the reactions (e.g. non-enzymatic browning reactions) could increase and more severe reactions could take place in the frozen samples.



Figure 5. Effect of modified atmosphere packaging and frozen storage on the L color values of semi-dried persimmons. Data represent mean values and standard errors from 3 replicates. Values with different small letters show significant differences among different treatments for the same sampling day (p<0.05). Values with different capital letters show significant differences among different sampling days for the same treatment (p<0.05).

The effects of modified atmosphere packaging and frozen storage on the a and b color values of semi-dried persimmons are shown in Figure 6 and Figure 7, respectively. Both a and b values of the samples stored at - 18°C increased throughout the storage. It can be a result of browning reactions or pigment destruction process. According to Karaman et al. [13], during drying process and subsequent storage, the enzymes are released upon tissue injury of the plants and the reaction may proceed to form new compounds with different colors. Similar color changes were observed during storage of electron beam irradiation treated and non-treated sun-dried apricots at ambient temperature [23]. It is surprising that these color changes were not observed in any samples stored at + 4°C. Most probably, the same reactions occurred also in these samples (maybe more severely), however the white layer (formed due to local accumulation of soluble solids) covered the whole surface of the fruit masked these color changes.

The effects of modified atmosphere packaging and frozen storage on moisture contents of semi-dried persimmons can be seen in Figure 8. Moisture contents of the samples fluctuated within the range of 28.8-33.16% during the storage period. However, no significant differences were determined in the moisture contents of any samples at the beginning and at the end of storage. Moreover, there were no significant differences among the moisture contents of the samples treated with different techniques. In contrast to our results, Wani et al. [24] reported significant increases in dried apricots stored under ambient conditions. The authors attributed the change in moisture content to vapor pressure differential between apricots and the storage environment. Of course, water vapor permeability of the packaging material is a determining factor on this issue. For the samples stored at -18°C, possible explanation for maintaining moisture content could be the limiting the transportation of the water from the fruit to surrounding environment by changing its state from liquid to solid [25].



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Figure 6. Effect of modified atmosphere packaging and frozen storage on the a color values of semi-dried persimmons. Data represent mean values and standard errors from 3 replicates. Values with different small letters show significant differences among different treatments for the same sampling day (p<0.05). Values with different capital letters show significant differences among different sampling days for the same treatment (p<0.05).



Figure 7. Effect of modified atmosphere packaging and frozen storage on the b color values of semi-dried persimmons. Data represent mean values and standard errors from 3 replicates. Values with different small letters show significant difference among different treatments for the same sampling day (p<0.05). Values with different capital letters show significant difference among different sampling days for the same treatment (p<0.05).



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Figure 8. Effect of modified atmosphere packaging and frozen storage on moisture contents of semi-dried persimmons. Data represent mean values and standard errors from 3 replicates. Values with different small letters show significant differences among different treatments for the same sampling day (p<0.05). Values with different capital letters show significant differences among different sampling days for the same treatment (p<0.05).

Similar to moisture content, firmness values of the samples were not significantly different at the beginning and at the end of storage (Figure 9). Although slight differences were observed among the firmness values of the samples treated with different techniques, they were not significant for the last two months of storage (p>0.05). Increase in firmness could be a result of water loss from the samples. For instance, Sen et al. [26]

reported moisture loss and firmness increment in dried figs treated with phosphine and vacuum after a storage period of 2 months at ambient temperature. Since our results related with moisture content variation is parallel to firmness values, it could be concluded that there would be no water loss and firmness problem in semidried persimmons stored for four months preserved with the techniques used in the present study.



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Figure 9. Effect of modified atmosphere packaging and frozen storage on firmness of semi-dried persimmons. Data represent mean values and standard errors from 3 replicates. Values with different small letters show significant differences among different treatments for the same sampling day (p<0.05). Values with different capital letters show significant differences among different sampling days for the same treatment (p<0.05).

Effect of Frozen Storage and Modified Atmosphere Packaging Followed by Cold Storage on Sensory Properties of Semi-Dried Persimmons

Table 1 shows the average scores obtained for sensory characteristics of semi-dried persimmons packaged in air and modified atmosphere during storage for 4 months. Odor and chewiness characteristics of the samples did not change during storage, however significant decreases were observed in color, taste and general acceptability scores at the end of storage (p<0.05). Similarly, Elmaci et al. [27] observed significant decreases in the appearance, texture and flavor quality scores of unsulfured sun dried apricots during a storage period of 48 weeks. Meyvaci et al. [28] reported that the most significant changes were observed in skin color and flavor of dried figs during storage. The treatments tested in the present study (modified atmosphere packaging and frozen storage) did not significantly affect the odor and chewiness of the product however resulted in slight but significant increases in taste and general acceptability scores in the first month of storage. In general, color scores of modified atmosphere packaged and frozen samples were higher than those packaged in air and stored at + 4°C. Modified atmosphere packaging was also reported

to improve the sensory characteristics of other dried products like pistachios [29] and almonds [30].

CONCLUSIONS

In this study, we examined the effects of normal and modified atmosphere packaging and subsequent cold storage on microbiological, physicochemical and sensorv quality of semi-dried persimmons and compared the results with those of frozen storage. On microbiological aspect, modified atmosphere the packaging (especially packaging in 30% CO₂+70% N₂) can be an alternative preservation technique for semidried persimmons since low microbial loads were recorded at the end of storage period as if the product was frozen. According to sensory analysis; color, taste and general acceptability scores of modified atmosphere packaged and freeze-stored samples were similar and higher than those of packaged under normal atmosphere. On the other hand, parameters such as relative humidity and temperature during storage should be chosen carefully and kept stable. Otherwise, undesirable color changes caused by sugar migration, browning, pigment destruction, etc. could not be prevented.

Table 1. Results of the sensory analysis of semi-dried persimmons packaged in air and modified atmosphere during storage for 4 months*, **

Characteristic	Treatment	Initial	Month 1	Month 2	Month 3	Month 4
Color	Air, 4°C	3.8±0.9aA	3.3±1.1bAB	3.2±1.1bB	3.5±1.0aAB	3.0±0.9bB
	100% N ₂		3.9±0.8aA	3.9±0.9aA	3.5±1.0aA	3.7±0.9aA
	70% N ₂ +30% CO ₂		3.8±0.8abA	3.8±1.0aA	3.6±1.1aA	3.3±1.2abA
	Air, -18°C		3.8±1.0abA	3.7±1.2abA	3.6±1.2aAB	3.1±1.1bB
Odor	Air, 4°C	3.7±0.8aA	3.8±0.6aA	3.7±0.7aA	3.8±0.9aA	3.4±08aA
	100% N ₂		3.9±0.9aA	4.0±0.8aA	3.7±1.1aA	3.8±0.7aA
	70% N ₂ +30% CO ₂		3.9±0.7aA	3.8±0.8aA	3.7±0.8aA	3.6±0.7aA
	Air, -18°C		4.1±0.8aA	3.8±0.8aA	3.8±0.8aA	3.7±0.5aA
Taste	Air, 4°C	4.3±0.7aA	3.6±1.1bB	3.5±1.0aB	3.9±1.2aAB	3.8±0.7aB
	100% N ₂		3.9±0.9abABC	3.5±1.2aC	4.1±0.7aAB	3.8±0.8aBC
	70% N ₂ +30% CO ₂		4.2±0.6aA	3.7±0.9aBC	4.0±0.9aAB	3.5±0.9aC
	Air, -18°C		4.2±0.8aAB	3.8±1.2aB	3.9±1.1aAB	3.8±0.9aB
Chewiness	Air, 4°C	4.0±0.9aA	3.9±0.9aA	3.6±1.2aA	3.8±1.0aA	3.9±1.0aA
	100% N ₂		3.9±0.9aA	3.7±0.8aA	4.0±0.8aA	4.0±0.8aA
	70% N ₂ +30% CO ₂		4.0±0.7aA	3.9±1.0aA	3.8±1.1aA	3.8±0.9aA
	Air, -18°C		4.2±0.8aA	3.9±1.2aA	4.1±1.1aA	3.8±1.2aA
General acceptability	Air, 4°C	4.2±0.7aA	3.7±1.0bB	3.6±0.9aB	3.8±1.0aAB	3.6±0.9aB
	100% N ₂		3.9±0.8abAB	3.6±1.0aB	3.9±0.8aAB	3.9±0.7aAB
	70% N ₂ +30% CO ₂		4.2±0.6aA	3.8±0.9aAB	3.7±1.0aB	3.5±0.9aB
	Air, -18°C		4.2±0.9aA	3.7±1.0aB	4.0±1.1aAB	3.6±0.9aB

*: Values are the average of the evaluations of 30 panelists

**: For a specific characteristic, values with different small letters within a column and values with different capital letters within a row are significantly different (p<0.05)

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