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Research Paper / Araştırma Makalesi

Effect of Temperature and Packaging Method on Bioactive Compounds of Freeze-dried Red Beet Powder during Storage

Ozgur Aktok, Engin Demiray

Pamukkale University, Faculty of Engineering, Department of Food Engineering, Denizli, Turkey

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Corresponding author (Yazışmalardan Sorumlu Yazar): edemiray@pau.edu.tr (E. Demiray)
+90 258 296 3090 +90 258 296 3262

ABSTRACT

In this study, the effect of two different storage temperatures (4 and 25°C) and two different packaging techniques (normal atmosphere (NAP) and modified atmosphere (MAP)) on some quality properties of freeze-dried red beet powder during storage for 28 days. Color, total phenolic content, total antioxidant activity and betanin analyses were performed weekly during storage. The L*, a*, b*, chroma and hue angle values of all samples stored at low temperature and room temperature decreased during storage while their ΔE values increased. At the end of the storage, a loss between 1.20 and 2.30% occurred in the total phenolic contents of powder samples. The highest antioxidant activity value was determined in MAP samples stored at low temperature, and the lowest antioxidant activity value in NAP samples stored at room temperature. Losses in the betanin contents of NAP and MAP samples stored at room temperature were 12.02 and 10.14%, respectively. In samples stored at low temperature, their loss rates were 2.03 and 0.81%, respectively. In general, the storage condition in which the bioactive compounds of freeze-dried red beet powder suffered the least loss was at low temperature and in samples packaged with a MAP technique.

Keywords: Antioxidant activity, Betanin, Freeze-drying, Phenolics, Red beet

Depolanma Sırasında Sıcaklık ve Paketleme Yönteminin Dondurarak Kurutulmuş Kırmızı Pancar Tozunun Biyoaktif Bileşenleri Üzerine Etkisi

ÖZ

Bu çalışmada, iki farklı depolama sıcaklığının (4 ve 25°C) ve iki farklı paketleme tekniğinin (normal atmosfer (NAP) ve modifiye atmosfer (MAP)) dondurarak kurutulmuş kırmızı pancar tozunun 28 günlük depolanması sırasındaki bazı kalite özelliklerine etkisi incelenmiştir. depolama süresince haftalık olarak renk, toplam fenolik içerik, toplam antioksidan aktivite ve betanin analizleri yapılmıştır. Renk sonuçları incelendiğinde NAP ve MAP koşulları altında 4 ve 25°C'de depolanan tüm numunelerin L*, a*, b*, kroma ve hue açısı değerlerinin düştüğü ve ayrıca ΔE değerlerinin arttığı saptanmıştır. Depolama sonunda örneklerin toplam fenolik içeriğinde %1.20-2.30 oranında kayıp meydana gelmiştir. En yüksek antioksidan aktivite değerinin 4°C'de depolanan MAP numunelerinde, en düşük antioksidan aktivite değerinin ise 25°C'de depolanan NAP numunelerinde olduğu belirlenmiştir. 25°C'de depolanan NAP ve MAP numunelerinde sırasıyla %12.02 ve 10.14 betanin içeriği kaybı meydana gelmiştir. 4°C'de depolanan numunelerde bu kayıp oranları sırasıyla %2.03 ve 0.81 olarak hesaplanmıştır. Genel olarak biyoaktif bileşiklerin en az kayba uğradığı depolama koşulunun düşük sıcaklıkta ve MAP tekniği ile ambalajlanan örneklerde olduğu belirlenmiştir.

Anahtar Kelimeler: Antioksidan aktivite, Betanin, Dondurarak-kurutma, Fenolikler, Kırmızı pancar

INTRODUCTION

Red-colored vegetables are quite important in terms of nutrition with their essential vitamin and mineral contents. In addition, being a source of fiber has made it widely consumed in a variety of cultures and climates. Red-colored vegetables, containing high amounts of antioxidants, scavenge free radicals that are harmful to the body and have protective properties against cardiovascular diseases and cancer [1]. Red beet (*Beta vulgaris* L.) is a tuberous plant belonging to the *Chenopodiaceae* family which can grow in all climatic conditions. By means of the carotenoids, ascorbic acid, phenolic substances and betalains in its structure, the importance of red beet which is rich in minerals is increasing in human nutrition [2]. Red beet, which is consumed in various ways, is used in its fresh form to make salads, fermented carrot juice drinks and pickles, and as a natural colorant in baby foods, ready-made soup mixes and sauces in dried form. Red beet is grown in a wide variety of areas around the world extending to America, Europe and India [1, 2].

Betalains have strong antioxidant, antiviral, anticancer, antilipidemic and antibacterial activities having some positive health effects, and they are divided into main betacyanins (red-violet) and betaxanthins (yellow) according to their structures [2, 3]. The main betacyanins are specified as betanin in beet, and betaxanthins as vulgaxanthin I and II. The color of betanin is pH dependent; it is bright bluish red between 4-5, turning into a blue-violet color as the pH increases. When the pH value reaches levels above 7 (alkaline), betanin is decomposed by hydrolysis and a yellow-brown color is formed. Betanin, being an organic product, has a wide range of uses in the food industry; it is used as a colorant in meat, sausages, ice cream and powdered soft drinks and other confectionery [2, 3].

Being one of the traditional food preservation methods, drying has an important role in food processing. An extended shelf life is obtained for a standard product by the drying process, which basically aims to reduce the water content and water activity in the food. Undesirable enzymatic, chemical, biochemical, textural and sensory changes and microbial spoilage can be controlled by reducing the moisture content of the dried product by 1-5% during drying. It provides an economic advantage since the decrease in weight and volume after drying will reduce packaging, storage and transportation costs [4].

Freeze drying, also known as lyophilization, is a drying method widely used in biotechnology, chemistry, pharmacy and food industry. Freeze drying process is based on the principle of removing the free water from the frozen product by sublimation and the bound water by desorption under low pressure. In the freeze-drying process, during which the water is removed from the structure of the product with the help of vacuum in the solid phase, the texture and shape of the product is less damaged compared to other drying methods, and the valuable components losses such as minerals, vitamins and aroma in the structure of the product are minimized [5, 6].

In this study, it is aimed to obtain red beet powder by freeze-drying method, which could be a product that is easy to transport and store. This powder product can be used as a coloring and flavoring additive in food formulations. For this purpose, the physical and chemical properties of freeze-dried red beets and the obtained powder products were investigated. Besides, in the study, the effects of two different storage temperatures (4°C and 25°C) and two different packaging techniques (normal atmosphere (NAP) and modified atmosphere (MAP)) were also determined regarding the nutrient compounds of freeze-dried red beet during one-month storage.

MATERIALS and METHODS

Raw Material

Red beets used in the study were purchased from the local market in Pamukkale district of Denizli, Turkey. Those red beets were brought to the laboratory of the department in cold conditions and in a rapid manner. The samples, which were undergone the selection process, were kept in the refrigerator until freeze-drying. Before starting the freeze-drying process, the initial moisture content of the red beets used in the experiments was determined by keeping them in an oven at 100°C for 24 hours. The dry matter content was found to be 11.32% and the initial moisture content was calculated as 7.834 kg water/kg dry matter.

Chemicals

All chemicals were of analytical grade unless stated. Solvents used in antioxidant assays were of HPLC grade. Gallic acid and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Fluka (Switzerland) while sodium carbonate was from Riedel-de Haen (Germany). Folin-Ciocalteu reagent was purchased from Merck (Germany). Trolox® (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and betanin standards were obtained from Sigma (St. Louis, MO, USA).

Methods

Freeze-Drying, Packaging and Storage

At least 30 minutes before starting the drying experiments, refrigerated and selected red beets (500±10 g) were allowed to reach the ambient temperature. Then, after the red beets were washed and sorted, their stems were cut. Then the shells were peeled and sliced with a ring-shaped stainless steel knife. A portion of sliced samples was taken and their dry matter content was determined together with the initial amounts of bioactive compounds. The remaining samples were chopped for a minute with a Waring blender (Waring Products Inc., Torrington, CT, USA). Chopped samples were frozen in flasks at -80°C for 24 hours. At the end of this period, samples were placed in a laboratory scale freeze-drier (Labconco FreeZone 2.5 Plus, Kansas City, MO) and freeze-drying was performed. After 24 hours, powder product (about 100 g) was collected and packed in low-density polyethylene bags in NAP and MAP techniques.

Two experimental batches were prepared, namely, NAP: packed with air (20.4% O_2 + 0.83%CO_2 + 78.77%N_2); MAP: packed with 100%N_2. These samples were packed and sealed using a semi-automatic packaging machine (DZ-260Seles, Wenzhou Xingye Machinery Equipment Co. Ltd., Beijing, China). Packed samples were stored in a test cabinet (NUVE TK120, İstanbul, Turkey) for a month at different storage temperatures (4 and 25°C). The color values (L*, a*, b*, C, H° and ΔE), total phenolic content, total antioxidant activity and betanin contents of powder products were determined weekly during storage and the changes in these properties were monitored during storage.

Color Measurements

CIE (International Illumination System-Commission Internationale de l'Eclairage) L*, a* and b* color values of fresh and dried samples were determined from three different points with PCE-CSM1 (PCE Instruments, UK) colorimeter. From L*, a* and b* values measured, C (chroma-color saturation) calculated by the Equation 1, H° (hue angle-color intensity angle) by the Equation 2, and total color change (ΔE) by the Equation 3 [7].

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

$$H^{\circ} = tan^{-1}\left(\frac{b^*}{a^*}\right) \quad (2)$$

$$\Delta E = \sqrt{(L^*_0 - L^*)^2 + (a^*_0 - a^*)^2 + (b^*_0 - b^*)^2} \quad (3)$$

According to the CIE color coordinate system, L* value is an indicator of whiteness-blackness, ranges from 0 (black) to100 (white), a* value is an indicator of green-red and ranges from -60 (green) to +60 (red), and b* value is an indicator of blue-yellow ranges from -60 (blue) to +60 (yellow). H° value indicates the quality of the color and 0° or 360° represents red, 90° yellow, 180° green, 270° blue. The chroma value, on the other hand, expresses the vividness and saturation of the color, while the 0 value represents gray achromatic (colorless) colors, the more the value the higher the vividness of the color [7].

Extraction of Bioactive Compounds

For the analysis of total phenolic content and antioxidant activity in fresh and freeze-dried red beet samples, the extraction process was carried out by modifying the methods specified by Singleton et al. [8] and Thaipong et al. [9]. For the extraction process, firstly, the fresh sample was homogenized with a blender (Waring) and turned into pulp, and the dry samples were crushed into powder by grinding at 10,000 rpm for 20 seconds with a blade grinder (Isolab, Turkey). Fresh or dry samples (3 g) were weighed into Falcon tubes, distilled water (45 mL) was added as extraction solution, and they were extracted in an orbital shaker (SHO-1D, Daihan, Seoul, South Korea) at 200 rpm for 15 min. At the end of the period, the tubes were centrifuged at 9000 rpm for 60 min at 4°C and transferred to amber bottles with a Pasteur pipette. Extracts were stored at -18°C prior to analyses.

Determination of Phenolic Content

The phenolic content determination of fresh red beet puree and freeze-dried red beet powder was made according to the method suggested by Singleton et al. [8]. Extracts of prepared red beet puree and powders, Folin-Ciocaltaeu (Merck, Germany). reagent (10%, volume/volume (v/v), in water) and sodium carbonate solution (20%, weight/volume (w/v), in water) were mixed in a test tube and kept at room temperature for 2 hours, and the absorbance of the solutions were read at 760 nm in a spectrophotometer (EMC-11-UV Spectrophotometer, Duisburg, Germany). Gallic acid (3,4,5-trihydroxybenzoic acid) (Fluka, Switzerland) solution was prepared at different concentrations, and the steps performed in the samples were carried out in the same way and a calibration curve was created. By using the calibration curve prepared (y=0.0099x+0.0792 where x is concentration and y is absorbance; r^2=0.9999), the total phenolic content of red beet puree or powder was calculated as mg gallic acid equivalent (GAE)/100 g dry matter in red beet puree and powder.

Determination of Antioxidant Activity

DPPH stock solution was prepared in methanol with a final concentration of 24 mg/100mL. The working solution was prepared with methanol by diluting the stock solution so that the final absorbance to be 1.20±0.02. The calibration curve was obtained with Trolox®. Trolox® solution was prepared with a concentration of 12.5 mg/25mL and a final concentration of less than 50 µM in the spectrophotometer cuvette for the Trolox® calibration curve. In the experiments, 150 µL of sample or standard 2850 µL of DPPH working solution was mixed in test tubes and the reaction continued for 60 minutes in a dark environment. At the end of the period, the absorbance was read in a spectrophotometer (EMC-11-UV Spectrophotometer, Duisburg, Germany) at a wavelength of 515 nm. Samples that did not fall within the calibration curve range at the end of the reading were diluted until they fell in this range [9].

Determination of Betanin Contents

According to Slatnar et al. [10], the betanin content of samples was determined by HPLC (High performance liquid chromatography) device with some modifications. For this purpose, fresh or dried samples (3 g) were taken into plastic tubes, and the samples were homogenized for a minute with 45 mL of ultrapure water. Then tubes were centrifuged at 4°C in a centrifuge (Nüve, NF1200R, İstanbul, Turkey) for 60 min at 9000 rpm. After centrifugation, clear supernatant was taken into amber bottles with a Pasteur pipette. Supernatants collected in amber bottles were passed through a 0.45 µm pore diameter membrane filter (Cronus, SMI-Labhut Ltd, Gloucester, United Kingdom) before being injected into the HPLC device. Betanin was defined by comparing the arrival time of the peak in the chromatogram of the samples with the arrival time of the peak in the chromatogram of pure betanin standard. The betanin content of samples was calculated as mg/100 g dry matter according to the standard betanin curve prepared

in the concentration range of 1000-3000 mg/L and the equation defining this curve (y=220.95x-26473 where x is concentration in mg/L and y is absorbance; r^2=0.9970).

In analyses, a Shimadzu LC-20AD (Japan) model HPLC device and a PDA (photo-diode array) detector were used. In the separation of betanin, acetonitrile and formic acid/ultrapure water (1:99, v/v) were utilized as mobile phase. Separation was performed with a C-18 column (250 × 4.6 mm, ID, 5 mm) (Thermo Fisher Scientific, Waltham, MA, USA) at a wavelength of 320 nm at a flow rate of 0.25 mL/min. Injection volume was 20 µL, and the column temperature was 35°C. Elution was conducted by using a solvent gradient system containing solvent A (acetonitrile) and solvent B (formic acid and ultra-pure water solution 1:99, v/v). Gradient was as follows: 0–6 min: 97%-84% B, 6–10 min: 84%–50% B and 10–15 min: 50%–97% B.

Statistical Analysis

Storage studies of freeze-dried samples were carried out in 2 replications. Variance analysis was applied to determine the effects of storage conditions, storage time and their interactions on the color properties, total phenolic content, total antioxidant activity and betanin content of red beet powders, and significant differences were subjected to Duncan multiple comparison test. Statistical analyses were performed using the SAS statistical package program (Version 6.12, SAS Institute, Cary, NC, USA), and the results were given as mean ± standard deviation.

RESULTS and DISCUSSION

Effect of Storage Conditions on Color of Freeze-dried Red Beet Powder

Color is one of the most important parameters affecting consumer expectations in dried products. The color parameters (L*, a* and b*) of the samples are shown in Table 1. The L*, a* and b* color values of fresh red beet were 24.02±0.21, 22.13±0.42 and 5.07±0.33, respectively.

The L* value of freeze-dried red beet powder was 24.51±0.20 at the beginning of storage. L* values decreased for samples packed with both atmospheric air (NAP) and 100% N_2 gas (MAP) stored at 4 and 25°C. While the maximum decrease occurred in NAP samples stored at 25°C, the least decrease was determined in MAP samples stored at 4°C. In terms of L* value, it was shown that it could be appropriate to pack freeze-dried red beet powders with 100% N_2 gas and store them at low temperatures. The a* color value, which was 25.31±1.02 at the beginning of storage, decreased in all samples at the end of storage. But, it was determined that there was a statistically insignificant difference between the a* values on the 7th day of storage and the ones on the 28th day of storage, except for the MAP samples stored at 4°C ($p>0.05$). On the 21st and 28th days of storage, there was a statistically insignificant difference in all samples ($p>0.05$). When the b* values in Table 1 were examined, it was seen that there is no statistical difference between the 0th day and the 7th day of storage in all samples ($p>0.05$). But, from the 14th day, the b* values of all samples were statistically different from 8.38±0.71 determined at the beginning of storage ($p<0.05$).

Table 1. Changes in color parameters of freeze-dried red beet powder samples during storage

Color parameter	Storage Condition		Storage Period (day)				
	Temperature (°C)	Packaging	0	7	14	21	28
L*	4	NAP	24.51±0.20^{a}	22.60±0.39^{bA}	21.21±0.62^{cA}	21.97±0.78^{bcA}	20.81±0.43^{cdA}
		MAP	24.51±0.20^{a}	23.34±0.98^{abA}	22.88±1.43^{abA}	22.00±0.44^{bA}	21.80±0.57^{bcA}
	25	NAP	24.51±0.20^{a}	20.37±0.46^{bB}	20.45±0.54^{bB}	22.11±1.23^{cA}	19.65±0.55^{bdA}
		MAP	24.51±0.20^{a}	19.67±0.91^{bB}	25.66±0.57^{aC}	22.47±0.68^{cA}	20.31±0.45^{bA}
a*	4	NAP	25.31±1.02^{a}	23.62±0.67^{bA}	23.69±0.76^{bA}	23.11±1.15^{abA}	22.57±1.37^{bA}
		MAP	25.31±1.02^{a}	25.67±0.82^{aB}	23.19±1.22^{bA}	23.34±0.67^{bA}	23.72±0.66^{bB}
	25	NAP	25.31±1.02^{a}	23.56±1.04^{bA}	22.98±0.76^{bA}	24.54±1.15^{abB}	22.84±1.37^{bA}
		MAP	25.31±1.02^{a}	23.45±1.32^{bA}	21.80±0.81^{cB}	23.47±0.34^{bA}	23.87±0.80^{bB}
b*	4	NAP	8.38±0.71^{a}	7.56±1.51^{abA}	6.67±0.65^{bA}	6.48±0.11^{bA}	5.56±0.88^{bcA}
		MAP	8.38±0.71^{a}	7.32±1.77^{abA}	5.98±0.98^{bB}	6.16±1.61^{abA}	6.35±0.89^{bB}
	25	NAP	8.38±0.71^{a}	8.41±0.73^{aB}	6.67±0.65^{bA}	6.48±0.11^{bA}	5.56±0.88^{bcA}
		MAP	8.38±0.71^{a}	8.98±1.63^{aB}	6.70±0.82^{bA}	6.23±0.76^{bA}	6.39±0.78^{bB}
C	4	NAP	26.66±0.75^{a}	24.80±0.67^{bA}	24.49±0.53^{bA}	24.05±0.44^{bA}	23.38±0.58^{cA}
		MAP	26.66±0.75^{a}	26.69±0.78^{aB}	23.95±0.44^{bA}	24.14±0.68^{bA}	24.56±0.34^{bB}
	25	NAP	26.66±0.75^{a}	25.02±0.59^{bA}	23.93±0.29^{cA}	25.38±0.43^{bB}	23.51±0.49^{cA}
		MAP	26.66±0.75^{a}	25.11±0.50^{bA}	22.81±0.25^{cB}	24.28±0.76^{bA}	24.71±0.73^{bB}
H°	4	NAP	18.32±0.78^{a}	17.75±0.43^{aA}	14.71±0.57^{bA}	16.10±0.49^{cA}	15.10±0.65^{bA}
		MAP	18.32±0.78^{a}	15.92±0.52^{bB}	14.46±0.33^{cA}	14.78±0.39^{cB}	14.99±0.58^{cA}
	25	NAP	18.32±0.78^{a}	19.64±0.95^{aC}	16.19±0.62^{bB}	14.79±0.40^{cB}	13.68±0.37^{dB}
		MAP	18.32±0.78^{a}	20.95±0.64^{bC}	17.08±0.36^{cC}	14.87±0.44^{dB}	14.99±0.45^{dA}
ΔE	4	NAP	0	2.68±0.37^{aA}	4.26±0.45^{bA}	3.77±0.51^{bcA}	5.14±0.48^{bdA}
		MAP	0	1.62±0.27^{aB}	3.59±0.52^{bB}	3.89±0.32^{bA}	3.74±0.28^{bB}
	25	NAP	0	4.50±0.41^{aC}	4.98±0.76^{aC}	3.16±0.98^{bB}	6.14±0.44^{cC}
		MAP	0	5.22±0.45^{aD}	4.06±0.57^{bA}	3.49±0.88^{cB}	4.87±0.52^{bA}

$^{A-D}$ Means in the same row having a common letter are not significantly different ($p>0.05$). $^{a-d}$ Means in the same row having a common letter are not significantly different ($p>0.05$).

At the beginning of storage, the chroma value of samples was 26.66±0.75. At the end of storage, the chroma value of all samples was between 23 and 25. In general, the chroma value of all samples decreased. The greatest reduction was detected in NAP samples. There was no statistically significant difference in the chroma values of MAP samples calculated between the 21st and 28th days of storage ($p>0.05$). Considering the Hue angle values in Table 1, it was determined that this value, which was 18.32±0.78 at the beginning of storage, decreased in all samples at the end of storage. As in the chroma value, there was no statistically significant difference in Hue angle values between the 21st and 28th days of storage in the MAP samples ($p>0.05$). Considering the Hue angle values calculated for the NAP samples, it was seen that there was no statistical difference between the 0th day and the 7th day of storage. The ΔE value is an important parameter that indicates the total color change in freeze-dried and stored samples. At the end of storage, while the ΔE values of samples were calculated as 5.14±0.48 and 3.74±0.28, respectively, for NAP and MAP samples stored at 4°C, it was calculated as 6.14±0.44 and 4.87±0.52 for those samples stored at 25°C.

As can be seen, the ΔE value of samples stored at 4°C was lower than those stored at 25°C. This demonstrated that it would be appropriate to store red beet powder at temperatures lower than room temperature. In addition, the ΔE values of MAP samples were calculated lower than those of NAP samples at both storage temperatures. As a result, it is thought that the storage of red beet powder, packed with 100% N_2 gas and at 4°C, will be important in terms of product color quality.

These results were coincided with some references in the literature to dried some fruit and vegetables. The effects of different temperatures (4, 15, 25, and 35°C), package (vacuum and normal pressure) and light/dark conditions on the quality changes of dried apricots during storage for 6 months were investigated [11]. Color values (L*, a*, and b*) significantly ($p <0.05$) decreased with increase of storage temperature, especially at temperatures higher than 25°C [11]. Besides, the color deterioration accelerated by increased temperature during storage were also found in dried orange juice powder [12].

Effect of Storage Conditions on Total Phenolic Content of Freeze-dried Red Beet Powder

Table 2 shows changes in the total phenolic content of freeze-dried red beets during storage at different temperatures (4°C and 25°C) and in packages with different gas contents (NAP and MAP). The initial total phenolic content of freeze-dried red beets was 369.54±0.16 mg GAE/100 g dry matter. Hamid and Nour [13], in their study, dried red beet in the sun, an oven or a freeze-drier. They reported the total phenolic content of sun-dried samples as 34.74 mg GAE/g wet matter. The total phenolic content of oven-dried samples was 33.28 mg GAE/g wet matter while it was 30.19 mg GAE/g wet matter for freeze-dried samples.

At the end of 28 days of storage, a loss between 1.20 and 2.30% in the total phenolic content of freeze-dried red beet powder samples occurred. This decrease in samples stored at 4°C was less than the samples stored at 25°C. Namely, the decrease in the total phenolic content of samples increased with an increase in storage temperature. It was determined that the highest total phenolic content loss was in samples packaged with NAP and stored at 25°C. The least loss was determined in samples packaged at 4°C and 100% N_2 (MAP). After 28 days of storage, it was determined that the total phenolic content of NAP and MAP samples stored at 25°C were not statistically different from each other ($p>0.05$). At the end of the 28th day, the total phenolic content of NAP and MAP samples were statistically different from each other in those stored at 4°C ($p<0.05$).

Table 2. Changes in total phenolic content (mg GAE/100 g DM) during storage of freeze-dried red beets

Storage Period (day)	Storage Condition			
	4°C		25°C	
	NAP	MAP	NAP	MAP
0	369.54±0.16^{aA}	369.54±0.16^{aA}	369.54±0.16^{aA}	369.54±0.16^{aA}
7	366.67±1.82^{aA}	366.55±1.16^{aA}	367.50±2.22^{aA}	367.01±1.00^{aA}
14	361.44±2.17^{bA}	367.31±2.57^{aB}	366.86±1.91^{abB}	366.08±0.81^{bB}
21	362.57±2.06^{bA}	367.21±1.22^{aB}	365.47±2.54^{abB}	359.90±2.35^{cC}
28	361.45±3.01^{bA}	365.12±3.14^{aB}	361.03±2.36^{cA}	362.79±2.49^{cA}

$^{A-C}$ Means in the same row having a common letter are not significantly different ($p>0.05$). $^{a-c}$ Means in the same column having a common letter are not significantly different ($p>0.05$).

Effect of Storage Conditions on Total Antioxidant Activity of Freeze-dried Red Beet

Total antioxidant activity values of freeze-dried red beet samples were between 122.47±1.54 and 123.00±0.09 mmol TE/100 g dry matter after 28 days of storage (Table 3). The total antioxidant activity values of red beet powder samples packaged in two different atmospheres and stored at two different temperatures increased compared to the 0th day of storage. This may be due to the formation of secondary compounds with antioxidant activity formed during storage processes. Gokhale and Lele [14] stored red beet powder in polyamide bags at 27°C for 150 days. They stated that there was an increase of 9.37% in the antioxidant activity value at the end of storage.

Table 3. Changes in total antioxidant activity of freeze-dried red beet powder samples (mmol TE/100g DM) during storage

Storage Period (day)	Storage Condition			
	4°C		25°C	
	NAP	MAP	NAP	MAP
0	120.52±0.44^{aA}	120.52±0.44^{aA}	120.52±0.44^{aA}	120.52±0.44^{aA}
7	122.94±2.11^{bA}	121.92±0.67^{aA}	122.56±1.29^{bA}	121.55±0.81^{aA}
14	122.91±1.58^{bA}	123.24±1.11^{bA}	122.33±1.27^{bA}	123.04±1.10^{bA}
21	122.75±0.21^{bA}	123.88±0.67^{bB}	122.43±0.76^{bA}	123.11±0.49^{bB}
28	122.80±3.01^{bA}	123.00±0.09^{bA}	122.47±1.54^{bA}	122.92±0.18^{bA}

$^{A-B}$ Means in the same row having a common letter are not significantly different (p>0.05). $^{a-b}$ Means in the same column having a common letter are not significantly different (p>0.05).

According to the Table 3, the highest antioxidant activity value was determined at the end of 28-day storage for MAP samples stored at 4°C, and the lowest antioxidant activity value was for NAP samples stored at 25°C. It was determined that there was no statistically significant difference between the antioxidant activity values between the 14th and 28th days of storage in the samples stored at 4°C (p>0.05). The same is true for samples stored at 25°C.

Effect of Storage Conditions on Betanin Content of Freeze-dried Red Beet

The initial betanin content of freeze-dried red beet powder was 3582.67±0.21 mg /100 g dry matter. The betanin values of freeze-dried powder samples packaged in different forms during 28-day storage are given in Table 4. It was determined that decreases in their betanin contents were more for the samples stored at room temperature. In NAP and MAP samples stored at 25°C, losses in their betanin contents were 12.02 and 10.14%, respectively. In samples stored at 4°C, these loss rates were calculated as between 2.03 and 0.81%. By looking at the loss rates, it was seen that MAP samples have the least loss and the NAP samples the most. This showed that, in packaging of freeze-dried red beets it would be appropriate to prefer MAP conditions instead of normal atmosphere conditions. In addition, with the preference of low temperatures as storage temperature, the amount of loss in betanin content will be less. Betanin contents of NAP and MAP samples stored at 4°C after 28 days of storage were not statistically different from each other (p>0.05). This means that freeze-dried red beets should be packaged with modified atmosphere packaging technique and also stored at low temperatures in terms of low betanin loss.

Kaur et al. [15] stored red beet powder in HDPE bags at 4°C for 3 months, and betacyanin, betaxanthin and betalain analyses were performed during storage. They stated that there was a loss of 21.03, 17.95 and 21.13% in the betacyanin, betaxanthin and betalain contents of samples, respectively.

Table 4. Changes in betanin contents of freeze-dried red beet powder samples (mg/100g DM) during storage

Storage Period (day)	Storage Condition			
	4°C		25°C	
	NAP	MAP	NAP	MAP
0	3582.67±0.21^{aA}	3582.67±0.21^{aA}	3582.67±0.21^{aA}	3582.67±0.21^{aA}
7	3572.01±0.22^{aA}	3562.25±0.96^{aA}	3552.01±0.15^{bA}	3537.25±0.96^{aB}
14	3512.29±0.82^{cA}	3551.56±0.90^{aB}	3374.29±0.79^{cA}	3412.56±0.96^{bA}
21	3505.83±0.60^{aA}	3515.01±0.99^{aB}	3275.83±0.65^{dC}	3315.01±0.69^{cD}
28	3509.90±0.72^{aA}	3553.53±0.13^{aA}	3151.90±0.12^{eC}	3219.53±0.43^{dC}

$^{A-D}$ Means in the same row having a common letter are not significantly different (p>0.05). $^{a-d}$ Means in the same column having a common letter are not significantly different (p>0.05).

CONCLUSION

When the color values of samples packaged with the NAP and MAP technique, which were stored both at 4°C and at 25°C, were examined during storage, it was determined that there was a significant difference between color values of the 0th and 28th days. The highest total color change (ΔE) was calculated in samples packaged with NAP technique stored at 25°C, and the lowest in samples packaged with MAP technique stored at 4°C. At the end of 28 days of storage, a loss between 1.20 and 2.30% occurred in the total phenolic content of powder samples. The antioxidant activity values of freeze-dried red beet powder samples packaged in two different atmospheres and stored at two different temperatures increased compared to the 0th day of storage. In NAP and MAP samples stored at 25°C, losses in their betanin content were 12.02 and 10.14%, respectively. In samples stored at 4°C, these loss rates were 2.03 and 0.81%, respectively. Freeze-drying did not cause any significant reduction in color values and bioactive components of red beets. In general, it was determined that the storage condition in which the bioactive compounds suffered the least loss was low temperature and MAP technique.

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