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EFFECT OF FREEZING AND STORAGE ON ASCORBIC ACID AND B-CAROTENE CONCENTRATION OF FROZEN RED PEPPER (*CAPSICUM ANNUUM* L.)

*DONDURMA VE DONDURULMUŞ DEPOLAMANIN KIRMIZI BİBERİN (*CAPSICUM ANNUUM *L.) ASKORBİK ASİT VE B-KAROTEN KONSANTRASYONU ÜZERİNE ETKİSİ*

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ABSTRACT

In this study, ascorbic acid and β-carotene, changes of red peppers were measured during the period of freezing with different temperature applications and the follow-up storage period at -20ºC. For this purpose, samples were frozen at the environment temperatures of -30ºC, -35ºC and -40ºC by using 0.5 m/s of air velocity until the temperature of -20ºC was achieved in the sample core. During the freezing process, ascorbic acid and β-carotene levels were determined in defined temperature ranges in order to specify the changes in quality. Then, the frozen products stored for 3 months and at the end of each month, the amount of ascorbic acid and β-carotene concentrations were determined. According to the study results, the amount of ascorbic acid of fresh red pepper was 65.123 mg/100g of dry matter, while they were determined as 57.202, 61.305, and 63.949 mg/100g dry matter at the frozen temperature range of -30ºC, -35ºC and -40ºC in frozen red pepper samples, respectively. Thus, β-carotene amount was 31.092 mg/100g dry matter in fresh red pepper, and it decreased to the levels of 29.093, 29.952 and 30.233 mg/100g dry matter at the temperatures of -30ºC, -35ºC and -40ºC, respectively. Both ascorbic acid and β-carotene has been observed to be better preserved by lower ambient temperature of freezing in red pepper. In the storage period, the amount of ascorbic acid in red pepper was observed to decrease in time. However, β-carotene levels increased in red pepper samples depending on the time of frozen storage.

Keywords: Ascorbic Acid, Freezing, Frozen Storage, Red Pepper, β-Carotene.

ÖZET

Bu çalışmada, kırmızı biberlerin askorbik asit ve β-karoten değerlerinin farklı sıcaklık uygulamaları ile dondurulma ve -20ºC'de takip eden depolama süresi boyunca değişimi ölçülmüştür. Bu amaçla, numuneler -30ºC, -35ºC ve -40ºC ortam sıcaklıklarında 0,5 m/s hava hızı kullanılarak numune çekirdeğinde -20ºC sıcaklık elde edilene kadar dondurulmuştur. Dondurma işlemi sırasında, kalite değişimlerini belirlemek için askorbik asit ve β-karoten seviyeleri belirlenen sıcaklık aralıklarında belirlenmiştir. Daha sonra dondurulmuş ürünler 3 ay boyunca depolanmış ve her ayın sonunda askorbik asit miktarı ve β-karoten konsantrasyonları belirlenmiştir. Çalışma sonuçlarına göre, taze kırmızı biberin askorbik asit miktarı 65.123 mg/100g kuru madde iken, dondurulmuş kırmızı biber örneklerinde -30ºC, -35ºC ve -40ºC dondurulmuş sıcaklık aralığında sırasıyla 57.202, 61.305 ve 63.949 mg/100g kuru madde olarak belirlenmiştir. Böylece, taze kırmızı biberde 31.092 mg/100g kuru madde olan β-karoten miktarı -30ºC, -35ºC ve -40ºC sıcaklıklarda sırasıyla 29.093, 29.952 ve 30.233 mg/100g kuru madde seviyelerine düşmüştür. Kırmızı biberde hem askorbik asit hem de β-karotenin daha düşük dondurma sıcaklıklarında daha iyi korunduğu gözlenmiştir. Depolama sürecinde kırmızıbiberdeki askorbik asit miktarının zamanla azaldığı gözlenmiştir.

Anahtar Kelimeler: Askorbik Asit, Dondurma, Donmuş Depolama, Kırmızı Biber, β-Karoten.

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1. INTRODUCTION

Red pepper, *Capsicum annuum* L., is from the *Solanaceae* family, as a tomato and eggplant, and the Capsicum genus. Red pepper is one of the most extensively consumed and commercially important vegetables in the world. It is rich in ascorbic acid and carotenoid content and is a very valuable food for human nutrition. Although red pepper is consumed both fresh and dried, it is also involved in the food industry as canned food, tomato paste, pepper juice, pickles, frozen products, fried products, and sauces, and as an antibiotic raw material for pharmaceuticals, feedstuffs, and production of dyes. It can also be used in seasoning mix, soft drinks, and production of ice cream and chewing gum [1, 2].

In the world, the basic methods used for food preservation in today's technology include high and low-temperature applications, drying, various mechanical treatments, chemical addition, fermentation, and irradiation. These methods are effectively and widely used in several food industry sectors [3]. Freeze preservation, which is included in the low-temperature application, is one of the most common and effective preservation methods used in many stages of the food industry such as production, storage, marketing, and consumption. Freezing is an important food preservation method that prevents the physical, chemical, and microbiological deterioration of the food by turning the water in the food into ice crystals and thus, ensuring that the food remains durable and of high quality for a long time. This method is based on the principle of inhibiting the reproduction and activity of microorganisms in the food at low temperatures and decelerating biochemical and chemical reactions as much as possible [4-6].

In freeze preservation, fruits and vegetables can be preserved as close to their fresh and high-quality states. In other words, frozen fruits and vegetables are better either fresh or properly cold-stored. The objective of this study was to evaluate the effect of freezing temperatures (-30, -35 and -40ºC) and during frozen storage at -20ºC for four months on the chemical quality (ascorbic acid and β -carotene contents) of red pepper.

2. MATERIAL AND METHODS

2.1. Material

In the study, red peppers (*Capsicum annuum* L.) grown in Kale Region of the province of Denizli were used as material. Red pepper samples used in freezing experiments were purchased from the market in August, September, and October. During the laboratory study, the samples were stored in a polyethylene bag at 4ºC in the refrigerator.

2.2. Chemicals

β-Carotene and ascorbic acid standards used in HPLC (High-Performance Liquid Chromatography) analyses were purchased from Sigma (St. Louis, MO, USA). HPLC grade hexane, acetonitrile, methanol, ethanol and dichloromethane were purchased from Merck (Darmstadt, Germany). β-Carotene standard solution was prepared in methanol (Merck, Darmstadt, Germany). The ascorbic acid standard solution was prepared with ultrapure water.

2.3. Methods

2.3.1. Freezing Procedure

Red peppers to be used in the freezing process were purchased at 2 kg batches and brought to the department laboratory. Attention was paid that red peppers in each batch had the same maturity level and uniform size. A 2 kg batch was taken from the pepper samples for each temperature (-30ºC, -35ºC, and -40ºC) to be used in the freezing experiments. The red peppers were carefully washed. After washing, the red peppers were cleaned from their stems and seeds and vertically cut into two pieces. These pieces were sliced 30 mm (3 cm) wide. The sliced pepper samples were weighed at an average of 75 g in tared plastic containers, and they were closed with a plastic lid. The red pepper samples in plastic containers were prepared for quality analysis at 5 different core temperatures (0ºC, -5ºC, -10ºC, -15ºC, and -20ºC) during the freezing process and were aligned on trays. In order to monitor the core temperature with the data logger (MS6D, Comet system, Czech Republic), a temperature probe was placed into one of the plastic containers from the edge and placed in the center of the sample and the

container was closed with a lid. Another temperature probe was placed inside the freezer (Elcold DK-9500, Hobro, Denmark) to monitor the ambient temperature. The prepared trays were placed in the freezer and the freezing process was performed at an airflow of 0.5 m/s provided by the fan placed in the freezer for all temperatures (-30ºC, -35ºC, and -40ºC). The study was carried out in two parallels and two replicates.

2.3.2. Frozen Storage

The red pepper samples were prepared to be frozen at -30ºC, -35ºC, and -40ºC while the samples to be examined during storage were also taken into the freezer. The samples were monitored during the freezing stage. When the core temperatures reached -20ºC, the samples were transferred to the storage process and stored at -20ºC for 3 months. The samples were separated for analysis at the end of each month. After 3 months of storage, ascorbic acid and beta carotene concentrations were determined at the end of each month.

2.4. Analysis

2.4.1. Determination of Ascorbic Acid Changes

The extraction method suggested by Demiray et al. [7] was used after some modifications. For this purpose, fresh and frozen red pepper samples were first sliced into small pieces and then passed through blender in order to completely disintegrate the tissue. 1 g of the pulp obtained after blending was weighed into polypropylene centrifuge tubes. Then, 25 mL of water containing 1% metaphosphoric acid was taken and transferred into the centrifuge tubes. The pulp mixture was passed through the homogenizer for homogenization. Then, centrifugation was carried out at 9,000 × g at 5ºC for 15 minutes. At the end of this procedure, phase separation was achieved in the centrifuge tubes and the supernatants were collected in glass tubes with a Pasteur pipette. Supernatants in glass tubes were passed through a 0.45 µm membrane filter before injection into the HPLC device.

In the analysis, Shimadzu LC-20AD (Shimadzu Corporation, Kyoto, Japan) model HPLC device and the same model PDA (Photo-diode Array) detector were used. In the separation of ascorbic acid, ultrapure water whose pH was adjusted to 3 with H_3PO_4 solution was used as the mobile phase. Separation was performed with an ACE 5 C-18 column (250 × 4.6 mm, ID, 5 mm) (Advanced Chromatography Technologies, Aberdeen, Scotland) at a wavelength of 254 nm, isocratically at a flow rate of 0.5 mL/min. The injection volume used in the analysis was determined as 20 µL, and the column temperature was determined as 25°C.

2.4.2. Determination of β-Carotene Changes

Extraction process in samples for β-carotene analysis Demiray et al. [7] modified according to the method specified. For this purpose, seven grams of fresh, freezing, and frozen red pepper samples werecarefully weighed and mixed with 70 mL of ethanol-hexane solution (4:3, v/v) containing 1% BHT (w/v). The mixture was homogenized by a homogenizer (Miccra D-8, ART Prozess- & Labortechnik GmbH & Co. KG, Müllheim, Germany), and then transferred into a polypropylene centrifuge tube. As in the ascorbic acid analysis, the homogenized mixture was centrifuged at 9,000 × g for 15 minutes at 5ºC. At the end of this procedure, phase separation was achieved in the centrifuge tubes and the ethanol-hexane phase collected in the upper part was transferred into glass tubes using a Pasteur pipette. Supernatants collected in the glass tubes were passed through a 0.45 µm membrane filter before injection into the HPLC device (Shimadzu Corporation, Kyoto, Japan).

Chromatographic separations were carried out using a GL Sciences C18 (250×4.6 mm, ID 5 mm) column with isocratic elution. Mobile phase consists of 40% acetonitrile, 20% methanol, 20% ethanol and 20% dichloromethane. The column was equilibrated for 10 min prior to each analysis. Flow rate was 0.45 mL/min and injection volume was 20 µL.

2.4.3. Statistical Analysis

Statistical analyses of the data obtained as a result of the trials carried out in two parallels and two replications. Analysis of variance was applied to determine the effects of storage conditions, storage

time, and interactions between samples on ascorbic acid content and β-carotene content, and the significant differences were subjected to Duncan multiple comparison test ($p<0.05$) [8].

3. RESULTS AND DISCUSSION

3.1. Changes in the Amount of Ascorbic Acid and β-Carotene During Freezing of Red Peppers

During the freezing of red peppers at -30ºC, -35ºC, and -40ºC, samples were taken at certain temperatures and the amounts of ascorbic acid and β-carotene in the samples were determined. The values and the results of the analysis of variance are presented in Table 1.

The initial amount of ascorbic acid in red pepper was 65.12±4.82 mg/100g dry matter. In a study, it was reported that fresh red peppers contain 107.4±2.3 mg/100 g of dry matter ascorbic acid [9]. In another study in the literature, it was reported that fresh red pepper contains 41.7 mg/100 g of dry matter ascorbic acid As can be understood from the results, the ascorbic acid content of red peppers may vary depending on many factors such as the species, growth conditions, maturity level [10, 11].

When the ascorbic acid values of the red peppers frozen at different freezing temperatures until reaching the -20ºC core temperature were examined, it was determined that the ascorbic values of the red peppers frozen at -30 degrees were statistically different from the values at -35ºC and -40ºC during the freezing process. On the other hand, it was determined that ascorbic acid was preserved more as the freezing temperature decreased and that the lowest loss was at -40ºC among the freezing temperatures studied. This shows that the freezing process applied for the preservation of fruits and vegetables preserves the nutrient content with the minimum loss. In a similar study conducted by Leong and Oey [12], it was stated that there was a slight decrease in the amount of ascorbic acid in frozen red peppers; however, this decrease was not statistically significant.

As for ascorbic acid, the change in the amount of β-carotene at the determined temperature ranges during the freezing of red pepper at different temperatures was tested. In the analyses performed prior to the freezing process, the amount of β-carotene was 31.09±1.35 mg/100g of dry matter in fresh red pepper. As is known, the amount of carotenoid compounds in fruits and vegetables varies according to various factors such as variety, species, maturity, and growth conditions [13, 14]. It was seen that the amount of β-carotene contained in red peppers used in the study is consistent with the values given in the literature. In a study, it was reported that fresh red peppers contain 0.84±0.18 mg/g of dry matter β-carotene [12]. Topuz and Özdemir [15] stated in their study that the amount of β-carotene in fresh red pepper was 359.6±7.80 mg/kg dry matter.

Table 1. Average experimental data of ascorbic acid and β-carotene changes during the freezing process of red pepper samples at different temperatures*

Core Temperature (°C)	-30°C		-35°C		-40°C	
	Ascorbic Acid content (mg/100 g DM)	β-Carotene content (mg/100 g DM)	Ascorbic Acid content (mg/100 g DM)	β-Carotene content (mg/100 g DM)	Ascorbic Acid content (mg/100 g DM)	β-Carotene content (mg/100 g DM)
Fresh	65.12±4.82^{aA}	31.09±1.35^{aA}	65.12±4.82^{aA}	31.09±1.35^{aA}	65.12±4.82^{aA}	31.09±1.35^{aA}
0	61.61±0.59^{abA}	29.52±0.35^{aA}	65.07±3.77^{aB}	29.23±0.89^{aA}	65.03±2.24^{aB}	29.74±1.54^{aA}
-5	60.20±1.43^{abA}	28.99±0.74^{aA}	64.36±4.33^{aB}	29.25±0.60^{aA}	64.60±2.17^{aB}	30.51±1.02^{aA}
-10	58.38±5.38^{abA}	29.63±0.85^{aA}	63.66±1.31^{aB}	30.28±1.23^{aA}	64.47±2.54^{aB}	29.98±0.93^{aA}
-15	57.49±2.78^{bcA}	30.28±1.70^{aA}	61.75±2.13^{aB}	29.64±0.39^{aA}	64.57±6.89^{aC}	30.53±0.60^{aA}
-20	57.20±2.53^{bcA}	29.09±0.27^{aA}	61.31±6.12^{aB}	29.95±1.71^{aA}	63.95±0.92^{aC}	30.23±0.82^{aA}

*Values indicated with different letters in the same column are significantly different from each other ($p<0.05$).

Table 1 presents the experimental values regarding the amount of change in β-carotene during the processing of red pepper frozen at -30ºC, -35ºC, and -40ºC. It was determined that there was a slight decrease in the amount of β-carotene during the freezing of red pepper samples at different freezing temperatures and that this decrease was not statistically significant ($p>0.05$). In the freezing process carried out at -30ºC, the amount of β-carotene decreased from 31.09±1.35 mg/100 g of dry matter to

29.09±0.27 mg/100 g of dry matter. It was determined that the amount of β-carotene decreased from 31.09±1.35 mg/100 g of dry matter to 29.95±1.71 mg/100 g of dry matter in the freezing application performed at -35ºC and to 30.23±0.82 mg/100 g of dry matter at -40ºC. It was determined that the amount of β-carotene was higher in red pepper samples frozen at -40ºC than the samples frozen at -30ºC and -35ºC and that β-carotene was better preserved as the freezing temperature decreased. It was determined that β-carotene values of red peppers frozen at three different temperatures were not statistically significantly different during the freezing process. In a study, it was stated that the amount of β-carotene in fresh red peppers containing 0.84±0.18 mg/g dry matter β-carotene after freezing at -20ºC increased to 1.30±0.28 mg/g dry matter level, but this increase was not statistically significant [12]. In a study conducted on broccoli, one of the frozen vegetables other than red pepper, Alanis-Garza et al. [16] froze seven different commercial broccoli varieties and determined the β-carotene contents of fresh and frozen samples. As a result, it was reported that there was no statistically significant difference between the β-carotene contents of fresh and frozen samples ($p>0.05$).

3.2. Changes in the Amount of Ascorbic Acid and β-Carotene During Frozen Storage of Red Peppers

Red pepper samples were stored at -20°C for three months after being frozen at -30, -35, and -40°C until the core temperature reached -20°C. Ascorbic acid analyses were performed at the end of each month during storage and the amounts of ascorbic acid and β-carotene were determined. The obtained values are given in Table 2.

The amount of ascorbic acid in red pepper samples after freezing (0th month) was determined according to the freezing temperature (-30ºC, -35ºC, and -40ºC). The initial amount of ascorbic acid after freezing at -30ºC was 57.20±2.54 mg/100g of dry matter. During the storage of these samples at -20ºC, the amount of ascorbic acid decreased to 57.04±6.00 mg/100 g of dry matter at the end of the 1st month, to 52.67±2.73 mg/100 g of dry matter at the end of the 2nd month, and to 50.84±3.93 mg/100 g of dry matter at the end of the 3rd month. There was also a significant decrease in the amount of ascorbic acid in red pepper samples that were frozen at -35ºC and -40ºC and stored at -20ºC. As seen in Table 2, there was a statistically significant difference ($p<0.05$) between the amount of ascorbic acid in the fresh red pepper samples and the amount of ascorbic acid in the samples frozen at different temperatures and stored for 3 months. The amount of ascorbic acid in frozen red pepper samples changed to lower levels depending on the process during storage at -20ºC, however, as a result of the statistical analysis, it was determined that these changes were not at a significant level ($p>0.05$), beside a few exceptions. On the other hand, the amount of ascorbic acid in the samples frozen at -40°C and stored at -20°C was higher than in the samples frozen and stored at other temperatures.

Table 2. Average experimental data of ascorbic acid and β-carotene changes during frozen storage of red pepper samples at -20°C.*

Storage period (month)	-30°C		-35°C		-40°C	
	Ascorbic Acid content (mg/100 g DM)	β-Carotene content (mg/100 g DM)	Ascorbic Acid content (mg/100 g DM)	β-Carotene content (mg/100 g DM)	Ascorbic Acid content (mg/100 g DM)	β-Carotene content (mg/100 g DM)
Fresh	$65.12±4.82^{aA}$	$31.09±1.35^{a}$	$65.12±4.82^{aA}$	$31.09±1.35^{a}$	$65.12±4.82^{aA}$	$31.09±1.35^{a}$
0	$57.20±2.54^{abA}$	$29.09±0.27^{aA}$	$61.30±6.12^{abB}$	$29.95±1.71^{aA}$	$63.95±0.79^{abC}$	$30.23±0.82^{aA}$
1	$57.04±6.00^{abA}$	$29.99±0.47^{aA}$	$59.11±1.58^{abB}$	$29.22±0.56^{aA}$	$59.96±5.60^{abcB}$	$29.62±0.56^{aA}$
2	$52.67±2.73^{bcA}$	$29.24±0.49^{aA}$	$56.93±3.69^{abcB}$	$29.46±0.42^{aA}$	$57.65±1.55^{bcB}$	$29.33±0.45^{aA}$
3	$50.84±3.93^{bcA}$	$29.09±0.70^{aA}$	$49.65±1.85^{cA}$	$29.34±0.42^{aA}$	$55.34±4.90^{cB}$	$29.26±0.67^{aA}$

*Values indicated with different letters in the same column are significantly different from each other ($p<0.05$).

The findings obtained in the study are consistent with the literature data. Indeed, in the study of Oruna-Concha et al. [10], the frozen pepper samples were stored at -22ºC for 1, 2, 3, 5, 7, 9, and 12 months and it was stated that the amount of ascorbic acid in the samples decreased starting from the 1st month. Gębczyński and Lisiewska [17] stored frozen broccoli at -20 and -30°C for 12 months. Ascorbic acid

analyses of the samples were performed at the 4th, 8th, and 12th months of storage. As a result, it was determined that the amount of ascorbic acid in the samples decreased during storage at both storage temperatures.

Table 2 shows β-carotene contents of red pepper samples frozen at different temperatures during storage at -20°C for three months. The initial amount of β-carotene after freezing (0th month) at -40ºC was 30.23±0.82 mg/100 g of dry matter. During storage at -20ºC, the amount of β-carotene was 29.62±0.56 mg/100 g of dry matter at the end of the 1st month, 29.33±0.45 mg/100g of dry matter at the end of the 2nd month, and 29.26±0.67 mg/100g of dry matter at the end of the 3rd month. Considering the values, although the amount of β-carotene decreased during storage, this decrease was not statistically significant ($p>0.05$). A decrease in the amount of β-carotene was also observed in red pepper samples that were frozen at -30ºC and -35ºC and stored at -20ºC. However, this decrease was not statistically significant ($p>0.05$), as in the samples frozen at -40°C and stored. As a result, it was determined that there was no significant change in the amounts of β-carotene in red pepper samples frozen at different temperatures and stored at -20°C. Morais et al. [18] examined the effects of scalding and freezing storage (6 months) on the stability of β-carotene and capsanthin pigments in three different red pepper varieties and stated that the stability of pigments varies depending on scalding conditions, storage time and pepper cultures and that storage time has a higher effect on pigment stability than scalding conditions. In another study, Lisiewska and Kmiecik [19] stored sliced frozen tomatoes at -20 and -30°C for 12 months and reported that the amount of β-carotene in the samples decreased at the end of the storage.

4. CONCLUSION

The freezing process followed by a frozen storage is a good preservative process to maintain almost unchanged the ascorbic acid and β-carotene contents of red peppers. Frozen storage does not significantly affect the ascorbic acid and β-carotene contents of red peppers.

It can be seen in the literature that the effect of freezing on fruits and vegetables is still not clearly revealed and that there are different findings reported in the studies. However, it is important to examine the effect of the freezing process in more detail due to the preservation of nutritional properties of frozen and stored products, high levels of bioactive components, and the widespread consumption of these products by consumers.

According to the results of the study, it is seen that ascorbic acid is better preserved at -40ºC than the other two temperatures in the freezing of red peppers. When the results obtained in terms of β-carotene are examined, it is seen that there is no significant change in β-carotene value at all three freezing temperatures. As a result, it was determined that freezing at -40ºCwould be more appropriate when both components were taken into consideration. In the 3-month storage process, similar results were obtained.

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