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The effects of the novel home freezing system on microstructure, color, antioxidant activity, and microbiological properties of strawberries

Ahsen Rayman Ergün, Dr.*, Murat Yanat, PhD, Taner Baysal, Prof. Dr.

Ege University, Engineering Faculty, Food Engineering Department, İzmir, Turkey

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ABSTRACT

In these days people are more interested in frozen foods, especially home freezing fruits and vegetables. In this study, the effects of a new developed quick freezer system for home-type refrigerator on the freezing rates and some quality properties of strawberries were investigated. The freezing cabinet (at −30 °C with 1.2 ms^{-1} air) was designed and manufactured by Bosch und Siemens Hausgerate GmbH (Çerkezköy, Turkey) then the strawberrries were frozen in a novel quick freezer and compared with the samples frozen statically at classic home type refrigerator (at −18 °C without any air blown) via the freezing times at three different (bottom, middle and top) position. Microstructure, color, antioxidant content and microbiological quality during storage of 4 months at −25 °C compared. Strawberries reached -15 °C approximately 234 min shorter in the novel system. Freezing rates were determined as 0.32 cm h^{-1} for the static freezer and 1.51 cm h^{-1} for the quick freezer. Unlike the classic home-type refrigerator, quick freezing process took place in the novel home freezing system. SEM images showed that higher freezing rate in the novel system provides better protection in tissue structure and cell walls. The antioxidant activity of the strawberries frozen in the novel home freezing system was 8.96% higher after the freezing process. Brightness and redness of samples were protected better after quick freezing during storage. Nonetheless, no evident differences were observed in microbiological criteria.

Effets du nouveau systéme de congélation domestique sur la microstructure, la couleur, l'activité antioxydante et les propriétés microbiologiques des fraises

Mots-clés: Fraise; Congélation au domicile; Vitesse de congélation; Surgélation; Microscope électronique à balayage; Activité antioxydante

1. Introduction

Fruits have an important role in human nutrition due to their tastes and valuable nutrients. In former times, fruits were consumed in only their seasons, but now they can be eaten at all times of the year thanks to the developing international trade networks, greenhouse activities, and various preservation methods developed. Nowadays, consumers are more aware of the quality and the benefits of their foods than in the past, and prefer the foods that are most nutritious, healthy, closest to the fresh condition and almost no-treated (Danesi and Bordoni, 2008). For this reason, even though it is one of the ancient methods, freezing technology is still unrivaled and it has become more common in the last decade and for long-term storage, in order to use the products in the off-season (Poiana et al., 2010; Sorica et al., 2019). Deep freezing is one of the most widespread used preservation methods for foods. By this method the rate of most deteriorative reactions and microbial activities are significantly reduced (Zhan et al., 2019). Furthermore, the advantages including less nutrient loss and almost always available in the same standards have made frozen foods as an important part of daily life (Çurkan et al., 2012).

* Corresponding author.
E-mail addresses: ahsen.rayman.ergun@ege.edu.tr (A. Rayman Ergün), muratyanat@gmail.com (M. Yanat), taner.baysal@ege.edu.tr (T. Baysal).

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Nomenclature

a*	redness\Greenness value
AA	antioxidant Activity
b*	yellowness\Blueness value
CFU	colony Forming Unit
DPPH	1,1-diphenyl-2-picrylhydrazyl
L*	lightness\Darkness value
Mag	magnification
SEM	scanning Electron Microscope
ΔE	total color difference

Fresh vegetables are preferably stored in a refrigerator or freezer because they only remain fresh for a short time. (Çubukçu et al., 2019). Among the fruit species with the widest use for freezing can be remembered berries, strawberries, cherries, peaches and apricots (Sorica et al., 2019). Zhao et al. (2020) discussed the importance of freezing process that protects phenolics in berries. One of these fruit is strawberry which is rich in vitamin C and polyphenols, including anthocyanins, ellagic acid derivatives and flavonols (Kamiloğlu, 2019), and is used mostly in frozen state from in the industry. Poiana et al. (2010), emphasized the importance of freezing strawberries will increase flexibility for consumers via the availability. The effects of freezing process on the structure and quality of this fruit has been evaluated since old years due to its perishable structure (Sahari et al., 2004; Delgado and Rubiolo, 2005; Buggenhaut et al., 2006; Cheng et al., 2014; Kamiloğlu, 2019). Protecting the features of this fruit such as color, texture and aroma is the critical point in freezing because it has low solid content (approximately 10%) so susceptible to the damages. Buggenhout et al. (2006) studied the effect of infusion with pectinmethylesterase and calcium combined by different freezing conditions for strawberries to protect the stucture. Similarly, Suutarinen et al. (2000) used calcium chloride and sucrose against the tissue deteriotation of strawberry as a freezing pretreatment. Reno et al. (2011), described the importance of microscopy is being used to study the influences of processing conditions on the food structure who studied the microstructural changes of frozen strawberries. In addition it was mentioned that quick freezing provides small ice crytals by fast freezing rate and effective on the color, structure and nutritive value positively (Dima et al., 2012). The effect of the freezing process on the cell structure can differ due to the size of the ice crystals occur in the different freezing rates. Van der Sman (2020) explained the freezing operation and formation of ice crystals well and discussed how the freezing rate impacts the ice crystal size, and thus the food quality. If the freezing rate is sufficiently slow, the average temperature and solute/biopolymer concentration will follow closely the freezing line until the temperature of the cooling medium is reached. Large crystals resulting from slow freezing cause deformation, dislocation, and perforation of cell walls, and when defrosting these products, juice losses are high (Sorica et al., 2019). Therefore providing fast freezing is important for obtaining the frozen product in better quality so the researchers aimed to try the innovative ways for quick freezing. In the literature, novel freezing systems have been discussed that can improve the organoleptic quality or economics of production of frozen foods. Some of these novel freezing technologies are pointed to increase the rate of heat removal from the food while others change the physical/chemical structure of the product (James et al., 2015). Jo et al. (2014) stated the importance of novel quick freezing methods on the quality of the product and decided that in the case of vegetables and fruits, it is also possible to greatly reduce the overall freezing time. These methods include high-pressure freezing, dehydrofreezing, and applications of antifreeze protein and ice nucleation protein (James et al., 2015). Also electrostatic, ultrasound, microwave, and radiofrequency-assisted freezing methods have been proposed where new techniques need to improve (James et al., 2015).

The changes in the living conditions due to the disease Covid-19 in this year, people begin to make all of their needs and food at home. They wanted to store the food in refrigarator for a long time and the importance of freezing increased. Under the circumstances, home freezing gained popularity. Turkish people also find frozen products “time-saving” and “ease of preparation” (Bektaş et al., 2010). However, they do not prefer to purchase frozen foods; on the contrary, freeze at their homes. Frozen strawberries are typically produced commercially, although preparation of fresh strawberries for home-freezing is also common (Huang et al., 2019).

Freezing fresh strawberries at home is a simple process; strawberries should be washed, not soaked, allowed to dry, placed in freezer containers or bags, and then placed into a freezer (−20 °C) (Huang et al., 2019). Nevertheless, industrially frozen foods have better properties in many quality criteria after thawing compared to home freezing food, due to foods can be frozen more quickly at lower temperatures in the industrial freezers. At this point quick freezing system at home freezers has become a significant subject to be dealt with.

With all this in mind, the aim of the present study was to investigate the effect of the novel quick freezing cabinet which is installed inside the freezing unit of classic refrigerators (able to blow air at −30 °C with 1.2 ms^{-1} air velocity) on the freezing time and comparing the quality (microstructure, color, antioxidant activity, and microbiological properties) of strawberries after freezing and during the storage time for 4 months with classic home type freezer.

2. Material and methods

2.1. Strawberry

Strawberry samples were purchased from a local market in Bornova (Izmir, Turkey) and were stored in a case box at +4 °C with 90% relative humidity in the Fruit and Vegetable Processing Pilot Plant, Department of Food Engineering, before the freezing process. The samples with a minumum 3.0 cm, maximum 3.4 cm diameter (average diameter of 3.2±0.2 cm) with suitable ripeness degree and bright red color were used. Samples were washed with +5 °C mains water then dried with filter paper for removing the water from the surface prior to freezing.

2.2. Freezing and frozen storage

Slow freezing was achieved in home type freezer at −18 °C without blowing air to reach the center point of −15 °C. Modified freezer cabinet which was designed and produced by Bosch und Siemens Hausgerate GmbH (Çerkezköy, Turkey) was used for quick freezing. Schematic illustration of the freezing process was shown detailed in Fig. 1. This unit situated in the freezer section of home type refrigerator. It is capable of blowing air at a speed of 1.2 ms^{-1}, at −35 °C. This cabin had internal lengths of 18 × 14 × 24 cm. In all freezing processes, strawberries were frozen in a 10 × 5 × 22 cm sized, perforated stainless steel container. The cabin cooled and when the temperature was constant the samples were placed and temperature data was recorded by thermocouples. 400 g of fruit was frozen in each freezing process. During the all freezing operations, temperature changes occuring in the samples and the freezing ambient were collected by a computer and packaged software system (Dali08, Ordel, Turkey). Temperature measurements were monitored by T-type thermocouple

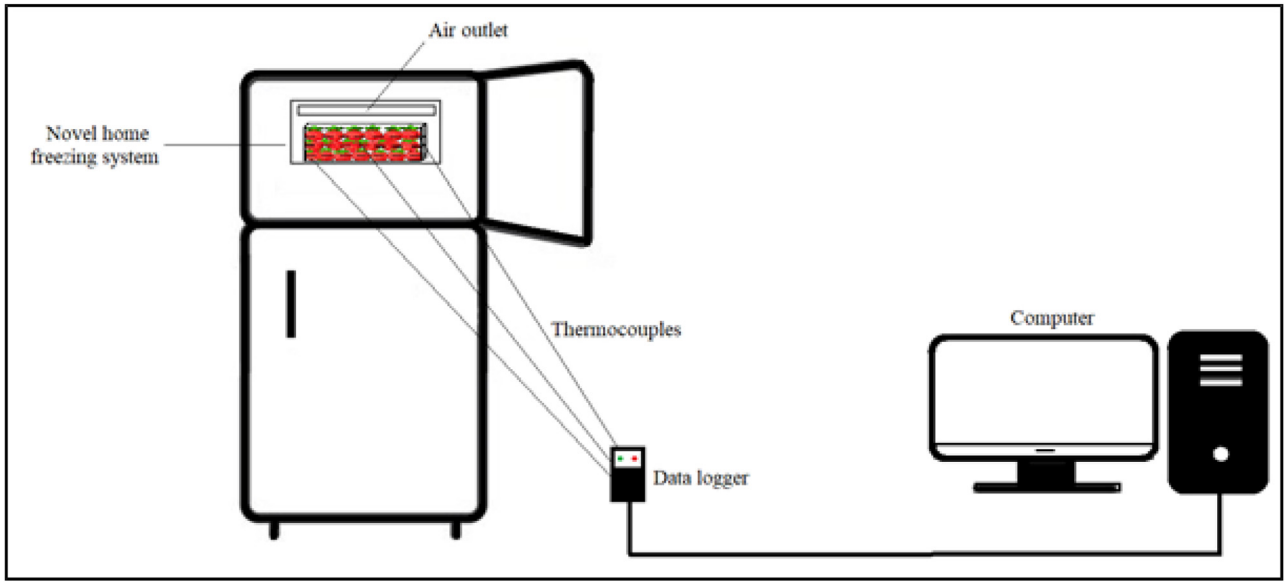


Fig. 1. Schematic illustration of the freezing process.

(OSTS, Ordel, Turkey) which were fixed to the top, bottom and center points of the individual samples (Fig. 1).

After freezing processes, the frozen strawberries packaged with low-density polyethylene (LDPE) bags (24 cm x 28 cm) were stored in a freezer (Uğur Deep Freezer, UCF 310 SSL, Turkey) at −25 °C for 4 month and analyzed at the end of 2nd and 4th month. The freezing process was performed in 2 replicates, and analyses were replicated at 3 times.

2.2.1. Freezing time and rate

The time required to decrease the temperature of the product from its initial value to a target value at its thermal center can be described as freezing time. The freezing rate was defined for the foods as the time to reach the temperature of −15 °C from 0 °C at the cold point. The freezing rate was calculated as the ratio of the distance of the center point in the frozen sample to the freezing time given in the Eq. (1) (Cemeroğlu, 2004). The distance was the radius matching the characteristic length. According to the calculations in Şahin and Şumnu (2006), the equivalent radius of strawberries was determined as 1.58 cm and freezing rates were calculated with the following Eq. (1). The measures were repeated minimum 3 times.

$$\text{Freezing rate} = \frac{\text{Distance (center to surface) cm}}{\text{Freezing time (0°C to } -15°\text{C) h}} \quad (1)$$

2.3. Scanning Electron Microscopy (SEM)

Frozen strawberries were freeze-dried by a freeze dryer (Armfield Refrigerant, R502, England) with a vacuum pump (Javal, 852, Brook Crompton Betts Ltd., Australia) during 24 h at −40 °C before taking images. The analysis was done by Scanning Electron Microscope (SEM) (SEM, Quanta 250 FEG-Carl Zeiss 300VP model) located in İzmir Katip Çelebi University, Central Research Laboratory to determine particle appearance of the cells according to Unakar et al. (1981). For this purpose, approximately 5 g of sample was adhered to one side of the double-sided tape and covered with gold, and after the plating process, the particle appearance (at 3 kV voltage) was determined by scanning electron microscope (60x-5000x).

2.4. Color determination

The color values (L *, a *, b *) of samples were measured with Hunter Lab Clor Flex CX1633 (Managment Company, USA) color measuring device. The total color difference (ΔE) was calculated from L *, a *, b * values using the following Eq. (2).

$$\Delta E = \sqrt{\left(L^* - L^*_{ref}\right)^2 + \left(a^* - a^*_{ref}\right)^2 + \left(b^* - b^*_{ref}\right)^2} \quad (2)$$

2.5. Antioxidant activity

The antioxidant activities were determined according to the method given by Brand-Williams et al. (1995). DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical (Merck, Germany) and a spectrophotometer (Varian Cary 50 Scan, Australia) were used. The standard curve was formed according to different concentrations of methanol in DPPH solution.

2.6. Microbiological analyses

Total mesophilic aerobic bacteria count, yeast and mold count analyses were done according to methods (990:12; 997:12) in Anonymous (2012a,b). In both analyses, 10 g of each thawed sample was taken into sterile bags and homogenized in a stomacher (BagMixer 400 CC, Interscience, France) by adding 90 ml of 0.1% peptone water. Suitable 3M Petrifilm medium plates (3M Cooperation, USA) were used for growth medium.

2.7. Statistical analysis

Duncan and T-tests on SPSS 18 (SPSS Inc., Chicago, III, USA) software program were used for evaluation of differences between treatments at levels of significance $p < 0.05$.

3. Results and discussion

3.1. Calculation of freezing rates

The freezing times of strawberries which located at different points of the stack are given in Table 1. On average, strawberries had reached from 0 °C to −15 °C in 63, and 297 min for quick-frozen and static-frozen samples, respectively. After the

Table 1
Freezing times of strawberries (min).

Freezing medium	Freezing time (0–(−15) °C)) (min)		
	Top	Middle	Bottom
QF-1	59	62	67
QF-2	55	60	70
QF-3	60	63	71
SF-1	289	301	299
SF-2	294	307	298
SF-3	291	299	295

QF: Quick freezer, SF: Static freezer.

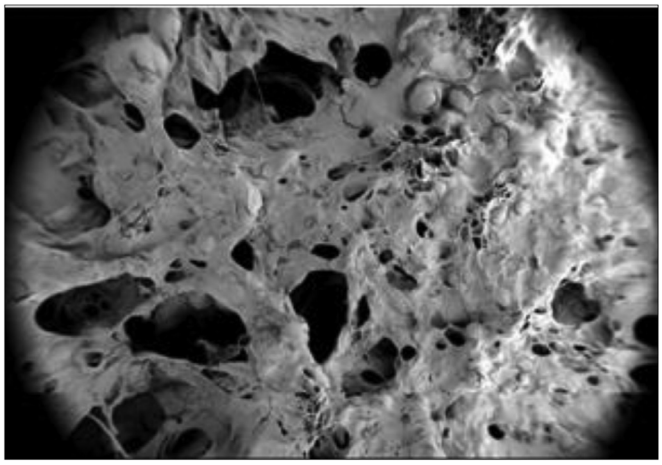


Fig. 2. Scanning electron microscope image of static-frozen strawberry after the freezing process (Mag=60X).

quick freezing process a significant reduction was determined in the freezing time. Because the position of air outlet in the novel freezer, strawberries located in the top of the freezing container reached to the temperature of −15 °C earlier than others. Freezing rate changed with the position. Freezing rates were determined as 0.32 cm h^{-1} for the static freezer and 1.51 cm h^{-1} for the quick freezer and they were considered as slow and quick freezing processes according to Sun (2011). Freezing proceeded slowly in static home type freezer but the novel system ensured fast freezing. In another study the freezing time for strawberry was found as 51.16 min at an air velocity of 1.71 ms^{-1} by Müftügil (1986). It was explained that the freezing process of strawberry has a rapid fall in temperature which takes place in the initial period. The temperature drop is much lower around the freezing point. This is due to the inhibition of temperature change because of the heat of phase conversion. The rate of temperature drop is again rapid in the final phase of freezing, as a consequence of further freezing and a simultaneous cooling of the samples (Müftügil 1986). Similar study showed that freezing applied at low temperatures increases the freezing rate and consequently the freezing time was shortened. It was mentioned that when higher freezing rates were applied, the rate of damage to the sample can also decrease (Ergün et al., 2020).

3.2. Scanning electron microscope (SEM) images

The cell walls and the cytoplasmic membrane generally seem as bright regions in the micrographs; ice and cell contents were in the darker areas (Bomben and King, 1982). Figs. 2 and 3 show SEM micrographs of strawberry tissue frozen in the static freezer at 0.32 cm h^{-1}. Collapsed and irregular shaped cells which point to tissue distortion were observed. These findings proved the extracellular freezing. Slow freezing rates generally create some alterations in the transport membranes that cause membrane deterioration which result in the loss of the ability to act as a diffusion barrier or semipermeable membrane. This process ends with the leaching of cellular substances from tissues and water transfer from intracellular to extracellular (Delgado, 1997). As a consequence of slow freezing, structural alterations were observed such as membrane deterioration, contraction of the cells and reduction in water retention capacity were higher in static-frozen strawberries. Also drip loss values could be explained with this structural property The data showed a significantly lower water loss for quickly frozen strawberries when compared to the slowly frozen strawberries during thawing ($p < 0.05$). Suutarinen et al. (2000) specified that the parenchymall cells were changed after freezing.

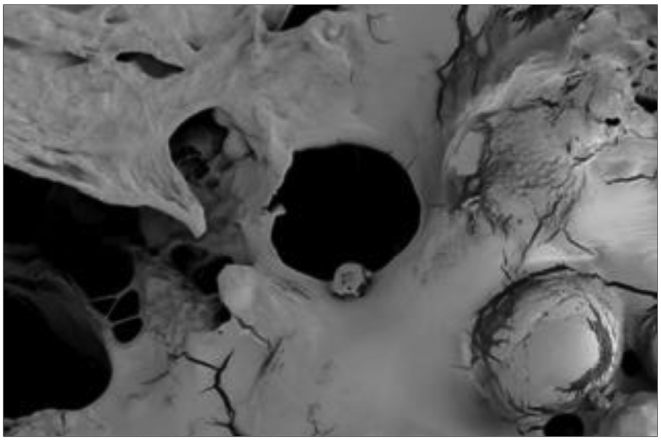


Fig. 3. Scanning electron microscope image of static-frozen strawberry after the freezing process (Mag=250X).

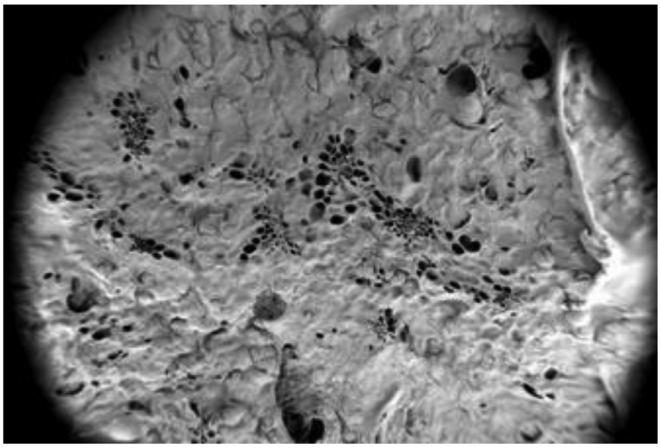


Fig. 4. Scanning electron microscope image of quick-frozen strawberry after the freezing process (Mag=60X).

On the contrary to static-frozen strawberries, SEM micrographs of quick-frozen (1.51 cm h^{-1}) samples which shown in Figs. 4 and 5 have much more uniform tissue and well-protected cell walls. The integrity of isodiametric cells and intact membranes were the results of the small-sized ice crystals and more numbers of nucleation zones occurred in quick freezing. Fig. 4 would indicate that freezing rate was rapid enough, the minimum effect of the crystal growth and ice nucleation were observed on cell walls and ice formation was mainly in intracellular (Allan-Wojtas et al., 1999). The loss of cellular fluid occurs during freezing, with the crystal growth, and the manifestation of these crystals occurs in the intercellular spaces (Reno et al., 2011).

Table 2
L∗ a∗ b∗ values of fresh and frozen samples.

	Fresh fruit	Quick freezer			Static freezer		
		After freezing	2.month	4.month	After freezing	2.month	4.month
L*	22.76±0.01	$28.84^{a,I}$±0.04	$27.16^{b,I}$±0.02	$23.23^{c,I}$±0.02	$27.79^{a,II}$±0.02	$26.88^{b,II}$±0.02	$22.14^{c,II}$±0.01
a*	28.60±0.01	$39.46^{a,I}$±0.01	$37.07^{b,I}$±0.10	$32.09^{c,I}$±0.01	$38.08^{a,II}$±0.03	$35.55^{b,II}$±0.01	$30.81^{c,II}$±0.01
b*	15.67±0.01	$24.88^{a,I}$±0.02	$19.87^{b,I}$±0.02	$18.48^{c,I}$±0.01	$23.50^{a,II}$±0.01	$18.90^{b,II}$±0.02	$17.03^{c,II}$±0.02

Significant differences ($p < 0.05$) were indicated with different letters (a, b) for samples frozen in the same equipment between months and different numbers (I, II) for samples frozen in the different equipment.

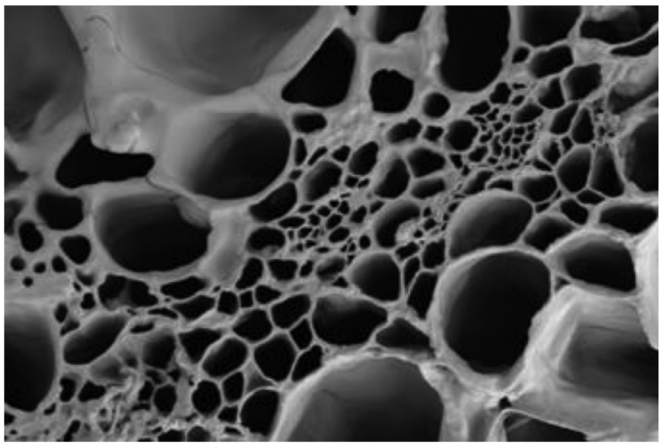


Fig. 5. Scanning electron microscope image of quick-frozen strawberry after the freezing process (Mag=1.00KX).

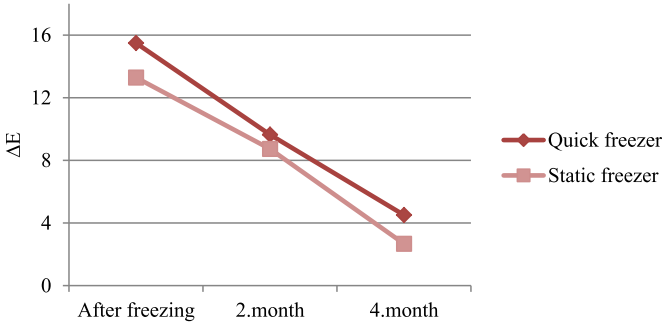


Fig. 6. Changes in the total color difference.

Delgado and Rubiolo (2005), reported that low ambient temperatures and high air velocities provide better protection of the tissue structure of strawberries. The amount and location of extracellular water and also properties of the ice crystals (size, shape, and number) are the main factors affecting the microstructure of frozen foods (Reid, 1990).

3.3. Color

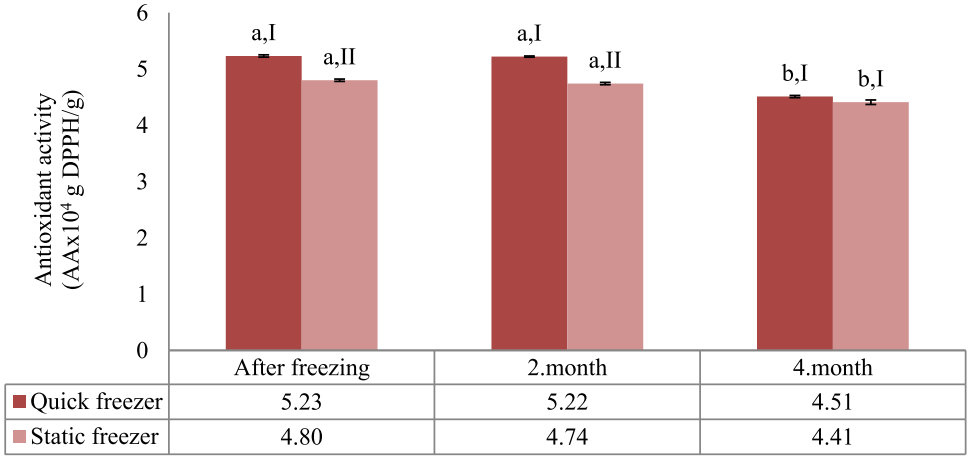
Color is one of the most important parameters for the acceptability and preferability of the product. It is also an important indicator of the quality and condition of the food. Chromatic changes are given in Table 2, also the changing trends of ΔE values are shown in Fig. 6. Generally, the pigments released after the thawing process from the damaged cells are the main reason for the change of color values in frozen fruits.

Compared to fresh fruit, evident rises of a* values were obtained in both samples according to anthocyanins diffusion from the center of the strawberry to surface layer ($p < 0.05$). Quick-frozen strawberries were more red and brighter, depending on higher L* and a* values ($p < 0.05$). Therefore, lower a* values were obtained in static-frozen sample regarding further tissue deterioration which caused more anthocyanin lost in thawing. In similar with the results of the present study, strawberries frozen in home type freezer had higher a* and b* values compared to fresh ones in the study carried by Bulut et al. (2018). Because of the recrystallization, and degradation of color pigments which occurred during the storage time, L*, a*, and b* values were getting close to color values of fresh fruit. For cherry tomatoes in the same cabinet the authors found after quick freezing, the brightness of samples to be higher than slow frozen samples. The b* value were lower in quick frozen products than that of slow frozen products (Ergün et al., 2020).

3.4. Antioxidant activity

Fruits and vegetables are rich in phenolic compounds such as tocopherols, ascorbic acid, carotenoids, flavonols and phenolic acids, which show antioxidant activity. These strawberries are rich in ellagitannins, which are large molecular weight polyphenols and phytochemicals protect plant cells against stress and disease (Can et al., 2005). The number of antioxidant substances can increase or decrease as a result of heat treatment and other technological processes applied to fruits and vegetables (Skrovankova et al., 2015).

Antioxidant activity values of strawberries are given in Fig. 7. A significant difference was found in the antioxidant activity of the samples after freezing processes ($p < 0.05$). It is thought that higher antioxidant activity value of quick-frozen strawberries owing to well-protected tissue and higher amount phenolic compounds. The phenolic substances, which are generally water-soluble compounds, move away from structure by drip loss. The previous research of the same authors confirmed this decrease by showing that the decrease in the phenolics were statistically significant at the end of 4th month and lower in quick freezing group than slow freezing samples. Some studies in the literature report the correlation between antioxidant activity and the number of phenolics in fruit (Kalt et al., 1999; Sun et al., 2002). Significant reductions occurred in the antioxidant activity of both samples after four-month storage ($p < 0.05$). 13.77% and 8.13% antioxidant activity losses were obtained during storage in quick-frozen and static-frozen strawberries, respectively. In adidition the decrease in antioxidants was because of anthocyanins and ascorbic acids. Ascorbic acid contents of these samples were given as 56.91, 53.73, 47.47 mg/100 g for slow frozen strawberry samples. 63.11, 63.77, 57.94 mg/100 g for quick frozen strawberry samples after freezing and during the storage, respectively in the previous study of this research (Yanat and Baysal 2018). Yanat and Baysal (2018) found that the content of monomeric anthocyanin in slowly frozen strawberry samples was lower than that of the quickly frozen sample group. They also stated that there was a significant reduction in the two sample groups in the total monomeric anthocyanin content after 4 months in storage. According to the study carried by Poiana et al. (2010), strawberris lost 22.90% of their antioxidant activity in the storage at −18 °C for 4 months.



Significant differences (p< 0.05) were indicated with different letters (a, b) for samples frozen in the same equipment and different numbers (I, II) for samples frozen in the different equipment.

Fig. 7. Antioxidant activity values of samples.

Table 3
Microbiological analyses results (log CFU/g).

	After freezing	2.month	4.month
Total mesophilic aerobic bacteria count (log CFU/g)			
Quick freezer	2.79±0.00 ^{a,I}	2.79±0.00 ^{a,I}	2.72±0.01 ^{b,I}
Static freezer	2.80±0.00 ^{a,I}	2.80±0.00 ^{a,I}	2.72±0.00 ^{a,I}
Mold and yeast count (log CFU/g)			
Quick freezer	3.61±0.00 ^{a,I}	3.61±0.00 ^{a,I}	3.60±0.00 ^{b,I}
Static freezer	3.66±0.00 ^{a,II}	3.63±0.00 ^{a,I}	3.61±0.00 ^{a,II}

Significant differences ($p < 0.05$) were indicated with different letters (a, b) for samples frozen in the same equipment and different numbers (I, II) for samples frozen in the different equipment.

3.5. Microbiological results

Due to its natural habitat of strawberry fruit grows in contact with various microorganisms in the soil. The number and composition of those microorganisms in the fruit can change with different processes and conditions (Archer, 2004).

Total mesophilic aerobic bacteria count, and yeast and mold count analyzes results are given in Table 3. The effect of freezing rate on total mesophilic aerobic bacteria count was not significant between the groups in this study ($p > 0.05$). However, a small lethal effect of 4 months storage at −25 °C was observed; even so, decreases in counts of both sample groups were not bigger than 1%. Some studies in the literature specify that only freezing process does not destroy the microorganisms, especially many bacteria have extreme resistant to the effect of freezing (Lund, 2000; Archer, 2004; Barbosa-Canovas et al., 2005).

The freezing process at different freezing rates created a slight difference in mold and yeast counts of samples ($p < 0.05$). After freezing, mold and yeast counts were obtained as 3.61 log CFU/g and 3.66 log CFU/g for quick-frozen and static-frozen strawberries, respectively. According to Beuchat (1984), a rapid decrease in temperature affect the cell integrity of molds and yeasts negatively and can reduce the number of colonies. Moreover, the effect of storage on the number of molds and yeasts were negligible ($p > 0.05$).

4. Conclusion

The results obtained in this study revealed that the novel home freezing system freezes the strawberry 234 min shorter than and at 1.51 cm h^{-1} freezing rate compared to the classic home type refrigerator. In addition novel system had positive effects on microstructure, antioxidant effect, and color of the samples. Lightness and redness was protected better during the storage in quick frozen samples. Quick-frozen strawberries had well-preserved tissue structure due to more numbers of nucleation zones occurred in quick freezing obtained from SEM analysis. Antioxidant activity was 10% higher in the 2nd month of storage in the saamples frozen at novel system. There was a small positive effect on yeast and mold count, no significant differences were found in both microbiological analyzes. It is considered that the novel home freezing system would be preferable by the consumers depending on its conclusive effects on frozen foods. The other fruits and vegetables could be studied in further researches.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijrefrig.2020.10.013.

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