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Original article

Determination of Antioxidant Activity and Total Anthocyanin Content of Frozen and Thawed Strawberries under Different Conditions[1]

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Abstract

Strawberries are among the summer fruits popularly consumed by consumers. High antioxidant activity, anticancer, anti-inflammatory effect and high bioactive substance content are also beneficial to human health. However, their shelf life is short due to their high water content and active metabolismThey can be kept frozen and processed in order to be consumable in all seasons. During freezing, when the water in the contents turns into ice crystals, the expansion occurs. For this reason, frozen and thawed fruit is generally softer than fresh fruit. These effects can vary with different types of freezing condition. In addition, freezing and thawing conditions can affect the stability of phenolic compounds, anthocyanins and antioxidant activity.

In this study, fresh strawberries were frozen at different temperatures; -18 °C, -86 °C, and individually quick frozen (IQF) as freezing methods. Frozen strawberries were thawed at 24 °C at room condition, +4 °C in the refrigerator and microwave oven with thawing mode. Total phenolic compounds, total flavonoid and total anthocyanins content, and total antioxidant activity were performed to examine the effect of freezing and thawing on biocompatibility. According to the results, the total phenolic compounds in the range of 0.77-2.76 mg gallic acid equivalent/g, flavonoid content 0.32-0.90 mg catechin equivalent/g, total anthocyanin content 0.02-0.16 mg/g and total antioxidant capacity 49.06 and 55.64% were found in strawberries. According to these results, it was determined that the loss of bioactive components was minimized by frozen with IQF and thawing in the microwave oven. In addition, the shortness of the thawing time in this process provides an extra advantage.

Keywords: DPPH, Freezing, Strawberry, Thawing.

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INTRODUCTION

Strawberry is rich in natural antioxidants as well as vitamins, mineral anthocyanins, flavonoids and phenolic acids. The red color of the fruit originates from pelargonidin 3-glucoside and cyanidin 3-glucoside. In recent years, efforts have been made to determine the positive effects of fruits with high anthocyanin content on health. Many studies have found that the total phenolic substance concentration is positively associated with the total antioxidant activity (Çam and Ersus, 2008). Phenolic compounds found in strawberry are ellagic acid, ellagic acid glucoside, quercetin 3-glucoside, quercetin 3-glucuronide and kaempferol 3-glucoside (De Ancos et al., 2000). Anthocyanins, which are natural plant pigments in the flavonoid group, are responsible for some nutraceutical benefits and attractive colors.

Strawberries are very attractive fruit for consumers, but their shelf life is short due to their active metabolism. They can be used mainly in processed form as food products or as a frozen product. (Janowicz et al., 2007). Freezing is one of the most common ways of preserving fruit over a long period of time. Frozen fruits are used as ingredients in many food formulations (Sablani, 2015). Freezing reduces the temperature of the food below the freezing point, causes minimal changes in sensory properties and nutritional values, preserved by a combination of reduced biochemical, enzymatic microbial activity and reduced water activity (Fellows, 2017).

During freezing, an expansion occurs with the formation of ice crystals which cause the cell wall to break down. For this reason, the structure of frozen fruits and vegetables is generally softer than that of the unfrozen product after they are thawed. Slow freezing methods cause significant softening due to extracellular ice formation (Bulut et al., 2018).

One of the important factors affecting frozen product quality is the freezing method. Freezing methods are classified into four main categories as freezing with cold air (freezing in stagnant air and freezing in air flow), plate freezing method, immersion freezing and freezing with cryogenic liquids. However, the only method that can be applied to freeze the food in domestic conditions is freezing with static cold air. Freezing with air current is the most used method in the industry and it is realized by application of air between -40 °C and -20 °C at 1-6 m/s. In freezing with static air, the air used is immobile and is applied in an isolated system at -15-30 °C (Biglia et al., 2016).

The thawing is one of the important points for frozen foods. In order to thaw fruits and vegetables, it is often thawed in microwave and refrigerator at home. Thawing in the microwave is a known method of reducing the thawing time to minutes as well as reducing microbial problems and chemical degradation (Oszmianski et al., 2009; Baysal et al., 2011). Thawing in the refrigerator is the process of placing the packaged frozen food in the refrigerator (4-6 ºC) and keeping the thawing process to completion. In this method, due to the long thawing time in the dissolution process, microorganisms may develop, drip loss, surface oxidation occur and color changes may occur (Bozkır et al., 2014).

Parameters such as temperature, duration are important in the thawing process. The chemical, physical and microbiological changes that occur during the thawing process cause damage to the product. The thawing rate should be slow enough to avoid liquid loss during passage of water to its original position. Rapid thawing at low temperatures to avoid appreciable heat rise and excessive dehydration in food; it is preferred in terms of preservation of food quality (Şengül, 2014).

Frozen foods are usually thawed in different forms, such as in the refrigerator, in the microwave, under warm water and in the room temperature. The most common and most preferred method is in the refrigerator. The refrigerator temperature must not exceed 5 °C during this thawing process. However, this method takes a long time to thaw (Atasever, 2000).

In this study, total phenolic, flavonoid and anthocyanin compound and total antioxidant activities of strawberry fruit which were frozen in three different methods: freezing in the freezer at -18 °C and -86 °C, and individual quick frozen at -30 °C; and three different thawing methods: in the refrigerator, in the microwave oven and at room temperature were determined. It is aimed to examine the variation of bioactive components in these different conditions.

Material and Methods

Material

Strawberry samples used as research material were obtained from a local grocery store in Manisa province. Samples were stored at 4 °C prior to analysis. The strawberries were washed and sized, before the freezing process.

Freezing process

To washed and sized strawberry samples (25 °C) were frozen in three different ways, in two different systems, in static air and air flow system. Freezing in the static air system 1.Freezing in the freezer at -18 °C (Bosch, Germany); 2. Freezing in the laboratory freezer at -86 °C (New Brunswick Scientific, USA); 3. In the air flow system; Individual Quick Frozen (IQF) at -30 °C. Strawberry samples were frozen in 50-g portions. In all experiments, the temperature of the ambient and the centre of the food was measured using a thermocouple. The temperature values were recorded and the freezing process was completed when the temperature of the centre of food reached -18 °C. Freezing times of frozen product in different systems were calculated. Strawberry samples reached a desired central temperature of -18 °C, for 25 minutes at IQF, 32 minutes at -86 °C and 90 minutes at -18 °C.

Thawing process

Frozen strawberry samples were thawed by three different methods. 1. In the refrigerator (Samsung, South Korea) for 4 hours at 4 °C; 2. In the microwave oven (Arzum, Turkey) thawing function for 3.5 minutes; 3. Thawing at room temperature for 2 hours.

Extraction Method

Total phenolic and total flavonoid content, total monomeric anthocyanin and antioxidant analysis were done with methanol extracts. Firstly, in order to carry out these analyses in the strawberry, phenolic substances were extracted. This method was made by modifying the method applied by Rodriguez et al., (2015) and Sağbasan, (2015).

Homogenized strawberry samples of 2 g were taken and mixed with 10 mL of methanol:water:acetic acid (73: 24.5: 2.5%; v:v:v) at 21 °C and were shaken in a 100% power ultrasonic bath for 15 min. Samples were transferred to a centrifuge tube and centrifuged at 4100 rpm for 15 min and the supernatant transferred to a 50 mL flask. The same operations were applied to the residue 2 more times. The supernatants were collected and completed with 50 mL of methanol:water:acetic acid (73 :24.5: 2.5%; v:v:v).

Total Phenolic Compounds Analysis

In the determination of total phenolic compounds, the phenolic materials were reacted with the Folin-Ciocalteu solution to form complexes and the resulting color was measured on a colorimetric basis (Çağındı, 2016; Rodriguez et al., 2015). For this purpose, 500 µL of extract mixed with a vortex for 30 seconds were taken. 250 µL of 2 N Folin-Ciocalteu reagent was added and mixed with vortex for 30 seconds. This mixture was then thoroughly mixed with the addition of 1.250 µL of 20% Na_2CO_3 solution. The resulting mixture was allowed to stand for 2 hours at room temperature and in the dark, then the resulting color absorbance was read at 760 nm on a Multiskan Go Microplate Spectrophotometer reader (Thermo Scientific, USA). The same method was applied to the gallic acid standards to generate a calibration graph (R^2= 0.998) and the results were given as gallic acid equivalent (GAE).

Total Flavonoid Content

Total flavonoid content was determined by a modification of the method by (Rodriguez et al., 2015). 500 µL of extracts were mixed with vortex for 30 seconds, and then 1.250 µL of distilled water and then 75 µL of 5% $NaNO_2$ were added to the test tube and waited for 6 minutes. In the end, 150 µL of 10% $AlCl_3$ was added, waited for 5 minutes and 500 µL of 1 M NaOH was added. The resulting mixture was read at 510 nm in a Multiskan Go Microplate Spectrophotometer reader (Thermo Scientific, USA) after the color mixture was allowed to stand at room temperature and in the dark for 30 minutes. The same method was applied to the prepared catechin standards and the calibration curve was drawn (R^2 = 0.998), and the results are given as the equivalent of mg catechin equivalent (CE).

Total monomeric anthocyanin content

The total monomeric anthocyanin content strawberry extracts samples were determined by the pH differential method (Cemeroğlu, 2013; AOAC, 2005).The extracts were mixed with buffer solutions with a pH value of 1.0 (0.025 M potassium chloride) and 4.5 (0.4 M sodium acetate), respectively. Take 1 mL of the extract transfer it to the test tube and, add 1 mL of pH 1.0 buffer solution. The other side, 1 mL of the same sample was added to the other tube and 1 mL of pH 4.5 buffer solution was added. The absorbance values of mixtures were determined at 528 and 700 nm in a Multiskan Go Microplate Spectrophotometer reader (Thermo Scientific, USA).

The absorbance value to be used in calculating the total amount of monomeric anthocyanins It was calculated according to equation 1 and equation 2.

$$\mathbf{A} = (\mathbf{A_{528}} - \mathbf{A_{700}})_{\mathbf{pH\ 1.0}} - (\mathbf{A_{528}} - \mathbf{A_{700}})_{\mathbf{pH\ 4.5}} \qquad (1)$$

A: The absorbance value used to calculate the total amount of monomeric anthocyanin

A528: absorbance value at 528 nm

A700: absorbance value at 700 nm

$$\mathbf{MA} = \frac{\mathbf{A \times MW \times DF \times 1000}}{\varepsilon \times \mathbf{L}} \qquad (2)$$

MA: Monomeric anthocyanins, mg/kg

MW: Molecular weight of cyanidin-3-glucoside (cyd-3-glu) anthocyanin (449.2 g / mol)

DF: Dilution factor

ε: molar absorption coefficient (26900)

L: Length of the light path of the spectrophotometer force (cm).

Determination of antioxidant activity by the DPPH method

Antioxidant activities of the strawberry samples were analysed by investigating their abilities in scavenging the DPPH% (Çağındı, 2009). 0.0154 g of DPPH was weighed and completed with 250 mL of methanol. 50 mL of this solution was taken and completed 100 mL with methanol. 200 µL of material extract was taken and added to 3.8 mL diluted DPPH solution. The solution was waited until stabilization and absorbance were read at 515 nm in a Multiskan Go Microplate Spectrophotometer reader (Thermo Scientific, USA). In addition, the absorbance values of the diluted DPPH solution were read at 515 nm and accepted as a control. The antioxidant activity was calculated according to the following formula.

$$Antioxidant\ activity(\%) = \left(1 - \frac{A_{Sample}}{A_{Control}}\right) * 100$$

A_{Sample}: Sample absorption at 515 nm

$A_{Control}$: Control absorbance at 515 nm

Statistical Analysis

All analytical determinations were performed in duplicate. Statistical analysis was performed using SPSS (version 22). Univariate analysis of freezing type and/or thawing type was done The differences of mean values among samples was determined using One-way analysis of variance (ANOVA) followed by Duncan.

Results and Discussion

The total phenolic and flavonoid content, total monomeric anthocyanin and total antioxidant activity of the strawberries subjected to different types of freezing and thawing are shown in Table 1. The statistical effect of freezing and thawing types is also shown in Table 2. All values are given on a fresh sample basis. The total phenolic content results of all the samples varied between 0.77 and 2.76 GAE/mL. The highest result was found in frozen and microwave-thawed samples while the lowest results were found in strawberries thawed at room temperature. When the effect of freezing and thawing types is examined, it is found that the thawing and interaction of thawing and freezing are significant ($p<0.05$), while the effect of freezing type on phenolic substance is not significant ($p>0.05$). Some values of treated strawberries were found to be high in the fresh sample as there was water loss during the thawing.

Holzwarth et al. (2012), examined changes in phenolic content by thawed frozen strawberry samples in IQF with different methods. Results of phenolic content values of the samples thawed at 37 °C for 2 hours was found to be the same as the control group. An increase of 20% was observed in the samples thawed at 37 °C for 24 hours. In samples thawed at 4 °C for 24 hours, a decrease of 13% was observed. The amount of phenolic substance in strawberry samples frozen at -20 °C was examined by household and liquid nitrogen application. The strawberry samples showed similarity to the obtained result, the amount of phenolic content is higher in strawberry samples where slow freezing (-20 °C) method.

Total flavonoid content ranged from 0.32 to 0.90 CE/g. The flavonoid values of all treated strawberry samples decreased compared to the control group. It has been reported that the process such as peeling, leaf separation applied to fruits and vegetables reduce the total flavonoid content (Çapanoğlu and Boyacıoğlu, 2009). The highest value for processed products was found to be frozen at -86 °C and thawed at the refrigerator. Strawberries that were thawed at room temperature in all types of freezing were found to be low. The effect of freezing type on flavonoid content was statistically insignificant, while thawing type and interactions were statistically significant ($p<0.05$).

The results of total monomeric anthocyanins ranged from 0.02 to 0.16 mg/g. The highest result was obtained frozen in IQF and thawed in the microwave. The effect of thawing type on the anthocyanin content was insignificant ($p > 0.05$), while the effect of freezing type and freezing-thawing interaction was significant ($p<0.05$).

In a study, strawberry samples were frozen in IQF and at -20 °C were thawed in the microwave at 4 °C, 20 °C and 37 °C to examine the amount of anthocyanin. Senga Sengana strawberries' amount of anthocyanin were reduced in microwave (10 min) by 12%, at 4 ° C (24h) by 21%, at 20 °C (8h) by 9% and 37 °C by 20%. (Holzwarth et al., 2012). Factors that cause the change in the amount of anthocyanin are temperature, light, oxygen, ascorbic acid, sugars, and enzymes (Asafi and Cemeroğlu, 2000).

The total antioxidant activity results ranged between 49.06 and 55.64% and the results were close to each other. The highest activity value is found frozen in IQF and thawed at the microwave. The only effect of freezing type on antioxidant activity was statistically significant ($p<0.05$). In a study, the freezing at -20 °C for 12 months and thawed at 7 °C produced raspberry fruit a decrease of the free radical scavenging activity in the four raspberry cultivars. Meanwhile, early raspberry cultivars suffered a minor decrease of radical-scavenging capacity with losses of 9 and 4%. The freezing process followed by a longterm frozen storage is a good preservative process to mantain almost unchanged the free radical scavenging capacity of raspberry fruits (de Ancos et al., 2000).

Table 1. The total phenolic and flavonoid content, total monomeric anthocyanin and total antioxidant activity of the strawberries subject to different types of freezing and thawing

Sample	Total phenolic compounds (mg GAE/mL)	Total flavonoid mg (CE/g)	Total anthocyanin (mg/g)	Totalantioxidant activity (%)
1QF-RD	$0.87^{d}\pm0.17$	$0.69^{ab}\pm0.14$	$0.13^{b}\pm0.01$	$54.96^{a}\pm1.13$
IQF-FD	$1.61^{bc}\pm1.61$	$0.45^{bc}\pm0.05$	$0.10^{bc}\pm0.00$	$52.94^{a}\pm2.33$
IQF-MW	$1.72^{b}\pm0.21$	$0.75^{a}\pm0.09$	$0.16^{a}\pm0.02$	$55.64^{a}\pm5.74$
DF-RD	$1.04^{d}\pm0.13$	$0.37^{c}\pm0.09$	$0.07^{d}\pm0.02$	$42.87^{b}\pm6.43$
DF-FD	$0.92^{d}\pm0.12$	$0.71^{a}\pm0.03$	$0.09^{cd}\pm0.01$	$49.24^{ab}\pm5.54$
DF-MW	$2.76^{a}\pm0.27$	$0.65^{ab}\pm0.09$	$0.09^{cd}\pm0.01$	$49.28^{ab}\pm4.42$
ULTF-RD	$1.15^{cd}\pm0.04$	$0.32^{c}\pm0.23$	$0.13^{b}\pm0.01$	$53.70^{a}\pm3.48$
ULTF-FD	$1.07^{d}\pm0.14$	$0.84^{a}\pm0.05$	$0.11^{bc}\pm0.00$	$55.35^{a}\pm1.80$
ULTF-MW	$1.91^{b}\pm0.46$	$0.37^{c}\pm0.05$	$0.10^{bc}\pm0.03$	$53.70^{a}\pm5.86$
Control	$0.77^{d}\pm0.22$	$0.90^{a}\pm0.01$	$0.02^{e}\pm0.00$	$49.06^{ab}\pm3.20$

IQF: Individual Quick Freezing

DF: Deep Freezing (-18)

ULTF: Ultra Low Temperature Freezer

RD: Room Temperature Thawing

FD: in Fridge Thawing

MW: in Microwave Thawing

Control: Raw material, fresh strawberry

Table 2. The statistical effect of freezing and thawing types on total phenolic and flavonoid content, total monomeric anthocyanin and total antioxidant activity

	Total phenolic compounds(mg GAE/g)	Total flavonoid mg (CE/g)	Total Antioxidant (%)	Total anthocyanin (mg/g)
Variance Source	Factor	Factor	Factor	Factor
Freezing Type (A)	1.12NS	1.44NS	5.57*	16.03*
Thawing type (B)	30.94*	4.42*	0.51NS	3.17NS
A X B	6.37*	7.19*	0.61NS	6.59*

NS statistically insignificant

*Statistically significant $p<0.05$

Conclusions

Fruits and vegetables are rapidly deteriorating due to the high water content. There are various food preservation methods applied to reduce the deterioration process. In the freezing process, which is among these methods, the water activity value is reduced to prevent the deterioration of fruits and vegetables. By freezing the temperature of the food during freezing, the free water in the product structure is converted from a liquid form to ice form and water activity is reduced. Thus, the biochemical, enzymatic and microbial reactions that occur in the structure of the product are reduced. Minimal changes in sensory qualities and nutritional values of the product occur. Another important factor for frozen foods is the thawing of frozen foods. In this study, total phenolic, total flavonoid, total anthocyanin content and total antioxidant activity results were examined to examine the effect of freezing and thawing on biocompatibility. When all values are examined, it is generally seen that the highest results are in IQF frozen and microwave-thawed products. Both of these methods have extra advantages in terms of time. When the effects of freezing or thawing on bioactive components are not statistically significant, methods can be selected, time and energy can be saved.

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