EFFECT OF DIFFERENT FREEZING RATES ON THE TEXTURE AND QUALITY PARAMETERS OF SELECTED FRUIT AND VEGETABLE

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EFFECT OF DIFFERENT FREEZING RATES ON THE TEXTURE AND QUALITY PARAMETERS OF SELECTED FRUIT AND VEGETABLES

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I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

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ABSTRACT

EFFECT OF DIFFERENT FREEZING RATES ON THE TEXTURE AND QUALITY PARAMETERS OF SELECTED FRUIT AND VEGETABLE

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The freezing rates of strawberry and green bean were determined at -23, -26 and -27°C in a home type freezer. For the estimation of freezing rate, the temperature-time data was obtained with a thermocouple embedded inside the sample. The freezing rates of strawberry were 0.75, 0.93 and 1.02 cm/h at -23, -26, and -27°C, respectively. For green bean, the freezing rates were 0.36, 0.50, 0.57 cm/h at -23, -26, -27°C, respectively. In three freezing rates studied, cellular integrity was examined with NMR relaxometry analysis. NMR relaxometry analysis showed that the freezing rates studied did not keep the cellular integrity in strawberries. However, cell integrity of green bean was kept better after freeze-thawing in comparison to strawberry.

Packaged strawberries and green beans were frozen stored at -27°C for 3 months to determine some quality parameters such as L-ascorbic acid, total phenolic content, antioxidant activity and color change. Total phenolic content and antioxidant activity change of selected samples did not decrease a value lower than 80% during the frozen storage at -27°C. L-ascorbic acid losses were approximately 42 and 27 % for 13 weeks of frozen storage of strawberry and green bean, respectively. The degradation rate of ascorbic acid was lower in green bean in comparison to strawberry. A significant change in color was not observed for the selected samples.

Home freezing should be done appropriately to preserve the nutritional content and texture of fruits and vegetables. The approximations and analyzes used in this study, can be a base to design and produce a convenient deep freezer systems.

Keywords: freezing rate, cellular integrity, phenolic compounds, antoxidant activity, ascorbic acid

ÖZ

FARKLI DONMA HIZLARININ SEÇİLMİŞ MEYVE VE SEBZENİN TEKSTÜR VE KALİTE PARAMETRELERİNE ETKİSİ

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Ev tipi derin dondurucuda, üç farklı dondurucu sıcaklığında (-23°C, -26°C ve -27°C) çilek ve taze fasülyelerin donma hızları hesaplandı. Donma hızlarının hesaplanması için örnek içine yerleştirilen termokupl ile sıcaklık-zaman verileri elde edildi. Çileklerin donma hızları -23, -26 ve -27°C 'de sırasıyla 0.75, 0.93 ve 1.02 cm/sa; taze fasülyelerin donma hızları ise -23, -26, -27°C 'de sırasıyla 0.36, 0.50, 0.57 cm/sa olarak hesaplandı. Çalışılan üç donma hızında, hücresel bütünlük NMR relaksometri analizi ile incelendi. NMR relaksometri analizi, çalışılan donma hızlarının çileğin hücresel bütünlüğünü korumadığını gösterdi. Bununla birlikte, dondurulup çözüldükten sonra taze fasülyenin hücre bütünlüğünün çileğe göre daha iyi korunduğu gözlemlendi.

Paketlenen meyve ve sebzeler, L-askorbik asit, toplam fenolik madde, antioksidan aktivitesi ve renk gibi bazı kalite parametrelerini belirlemek için. -27°C ’de 3 ay süre ile dondurularak muhafaza edildi. -27°C’de muhafaza süresince, seçilen örneklerin toplam fenolik madde miktarı ve antioksidan aktivitesi %80’den daha az bir değere düşmedi. 13 haftalık dondurarak depolama süresince, L-askorbik asit kaybı çilekte ve taze fasülyede sırasıyla %42 ve % 27 idi. Taze fasülyede askorbik asit degradasyon oranı çileğe gore daha azdı. Seçilen örneklerde, renkte önemli bir değişim gözlenmedi.

Evde dondurma işlemi, meyve ve sebzelerin besin içeriği ve tekstürünü korumak için uygun bir şekilde yapılmalıdır. Bu çalışmada kullanılan yaklaşımlar ve analizler, kullanışlı derin dondurucu sistemleri tasarlamak ve üretmek için temel olabilir.

Anahtar kelimeler: donma hızı, hücresel bütünlük, fenolik bileşikler, antioksidan aktivitesi, askorbik asit

To,

My beloved ones who bring out the best in me by being gentle, honest, judicious and acting as their own, and who encourage for the passion to overcome the challenges that make life meaningful and for achieving the goals that make the world go round in peace

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CHAPTER 1

INTRODUCTION

Major factors for consumer preference of fruits and vegetables are appearance, taste, culture, nutritive value and price. In our time, consumers have access to a diverse range of fruits and vegetables owing to international trade. Although fruits and vegetables are fresher and also cheaper in their season in comparison to imported food products, they are only available for a short time. Therefore, seasonal fruits and vegetables must be processed to prolong the shelf life (Leong and Oey, 2012). As a result of fast living conditions, the consumer awareness about frozen foods has increased due to ease of preparation. Hence, this has promoted the use of freezer at home. Freezing preservation of food at subfreezing temperatures usually between -18 and -35°C can extend the shelf life of fresh and perishable foods such as meats, fisheries, fruits and vegetables (Stoecker, 1998). Frozen fruits and vegetables are the most common frozen product type. Since, they rapidly decay due to their high water content (Barbosa-Cánovas et al., 2005). In Turkey, it is drawing attention that frozen food consumption becomes intense in big cities and western regions.

Freezing preservation of foods has been used for centuries for long-term storage (Kyureghian et al, 2010). Freezing process was established owing to progresses in technology. Ice-salt systems were developed to preserve fish and thereafter freezing was used as commercial preservation method in large scale operations. Major products frozen in storage chambers are fish, meat and butter (Barbosa-Cánovas et al, 2005).

As a beginning, small size fruits were frozen commercially in the east side of the U.S around 1905. Vegetables were frozen commercially later. In 1917, private firms started to conduct trials to achieve good quality frozen vegetable. Enzymatic reactions occurring in food required pretreatment of the vegetable. As a result, in 1929, blanching was implemented before freezing in order to prevent enzyme activity so that the enzymatic degradation and formation of off-flavors were prevented. Prevention of enzymatic degradation provided institutional appeal for frozen vegetables industry (Barbosa-Cánovas et al, 2005).

Clarence Birdseye, an American technologist revolutionized in 1928 by developing double-belt contact freezers so that the modern freezing industry started. The freezing concept was taken from Eskimos in the Arctic who used the method of rapid freezing for preservation of fish. The fish was fresh when thawed and eaten with the quick freezing process (Archer, 2004). According to this concept, a fast freezing is implemented so that small size ice crystals are formed which do not cause damage on food tissue and the texture of the food is protected. Further development was the blanching process in 1940 and so the frozen vegetable industry enlarged (Barbosa-Cánovas *et al.*, 2005). Freezing is advantageous when compared to other preservation methods from technological and economical point of view (Demiray and Tülek, 2010). The nutritional and sensory characteristics of frozen foods are kept better if freezing and storage conditions are appropriate. Among the long term preservation methos, frozen storage is considered to be preeminent when compared to canning and dehydration in terms of nutritional and sensorial characteristics (Fennema, 1977).

Figure 1.1 shows global fruit and vegetable industry products. Global fruit and vegetable industry includes canned, dried, frozen fruit and vegetables and juices, precut vegetables, ready-made salads.

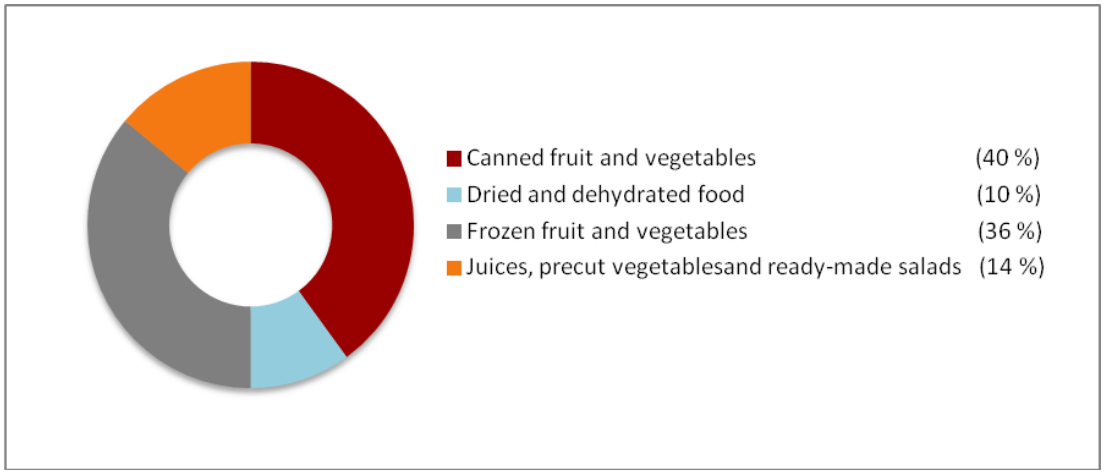

Figure 1.1 Products of Global Fruit and Vegetable Industry (IBIS World Industry Report, 2015).

Sales of frozen fruit and vegetables represent the second most important source of revenue. Approximately 36 % of revenue is provided from this segment. Frozen potatoes are the most popular product type followed by peas, carrots, beans and vegetable mixes. In the developed nations of the OECD (Organization for Economic Co-operation and Development), consumption of frozen fruit and vegetable has increased owing to rising consciousness for healthy food and also being time-poor. In the developing world, demand for home freezers also expanded due to economic growth and rising incomes (IBIS World Industry Report, 2015).

Home freezing preservation of fruits and vegetables has gained interest during the last decade (Poiana et al., 2010). According to Bektaş et al. (2010), study related to the purchasing behaviors of consumers and non-consumers of frozen food products in İzmir province, "time saving" and "ease of preparation" were among the highly effective reasons offered by the consumers in purchasing frozen food. It was found that frozen food consumers mostly preferred to purchase frozen pizza, millefeuille, potatoes, meat and meat products.

Since various vegetables and fruits are available in Turkey at any time of the year, the consumers did not prefer to purchase frozen vegetables and fruit. It was reported that study participants – who buy and do not buy frozen products - prefer to prepare vegetable group products by freezing them in their homes. Consumers with a higher personal income level had a higher demand for frozen products.

1.1 Freezing Methods

The freezing rate is the most important factor in freezing process to prevent damage on food tissue and drip loss in thawing. Freezing systems designed for food preservation may differ according to type of product, quality of frozen food, desire and economical reasons. The material of heat transfer medium specifies the freezing systems (Rahman, 1999):

- Freezing by contact with cooled solid or plate freezing: The foods (block or regular shaped) are placed between metal plates and then adjusted by pressure.
- Freezing by contact with cooled liquid or immersion freezing: The food product is submerged in this freezing media or solution (NaCl solutions, glycol, glycerol solutions and alcohol solutions) is sprayed as product conveyed through the freezer.

Because liquids are superior to air in terms of heat transfer characteristics, this system results with a quick freezing compared with air blast freezer and fluidized bed freezer. The main disadvantage of these freezers is the difficulty to find freezing media suitable for foods. In cryogenic freezers, product is subjected to an atmosphere below -60˚C. The refrigerants used in the food industry are liquid forms of CO_2 and N_2. The refrigerant can be sprayed and vaporized over the food. Also, product can be immersed in cryogenic liquid.

Because of the low freezing temperatures and high heat transfer rates, ultra-rapid freezing rate can be obtained. Because of the high cost of refrigerant of these freezers, this method is primarily intended for high value food products (Alexandre, Brandao and Silva, 2013).

- Batch and continuous freezing with a cooled gas in cabinet or air-blast freezing is used to freeze various irregular shaped foods even fruits and vegetables. Air-blast freezing provides quick freezing by circulating cold air over the food product at a velocity between 2.5 and 5 m/s and temperature between -30°C and -40°C (Hui, 2010; Alexandre, Brandao and Silva, 2013). Continuous air blast freezer has more advantages compared with batch air blast freezer due to being economic, easy to clean, reduced dehydration, space efficiency and superior quality. However, it is not flexible and economic as batch type (Fellows, 2000).

Fluidized-bed freezer is widely used to freeze small vegetables such as peas, diced carrots and potatoes, berry fruits, prawns, shrimp and diced meat (Chen and Rosenthal, 2009). Belt freezers are used for products such as fruit pulps, chicken, bagels, egg yolk, sauces and soups. Liquid nitrogen system is suitable for products such as fish fillets, seafood, fruits and berries (Alexandre, Brandao and Silva, 2013).

For a selected freezing system, there is more than one type of freezer. When choosing a freezer, a cost-benefit analysis must be carried out taking into account its functionality, feasibility and economic factors. Determination of the freezer system depends on the necessities to freeze a certain amount of product per hour (Barbosa-Cánovas *et al.*, 2005).

1.2 Freezing Mechanism

The freezing process comprises four main stages as shown in Figure 1.2. In the first stage, temperature of the product drops to freezing point by releasing sensible heat. Second stage is super-cooling stage where temperature falls below the freezing point, however, this stage is not always observed. Length of this stage is related to food variety and the freezing rate. Third stage is freezing stage where latent heat is removed and ice crystals are formed. At last stage, the food temperature is reduced to the storage temperature (Alexandre et al., 2013). The longest part of the freezing process is the third stage where water turns to ice crystals and it determines the freezing rate. In general, faster freezing methods result in small ice crystals and a better frozen food quality is obtained (Sun, 2011).

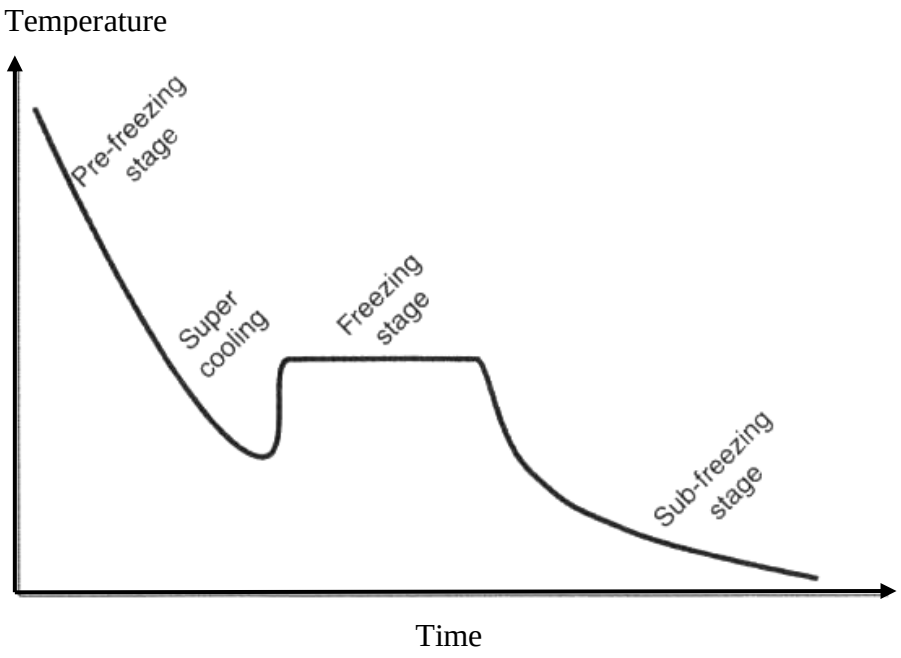

Figure 1.2 Typical cooling curve of freezing process (Alexandre et al, 2013).

During freezing of the food tissue, ice crystals are formed at extracellular and intracellular spaces. The location of ice crystal in food tissue is related to the freezing rate, nature of the cell, and the sample temperature. In general, crystallization is accepted to be initiated at extracellular fluid. Since, the cytoplasm is more concentrated and so the water in extracellular space freezes before cell contents. Crystallization continues particularly in extracellular spaces or it can proceed in intracellular spaces as well depending on freezing rate. Ice crystals are formed exclusively in extracellular areas in case of slow freezing (Sun, 2011).

Concentration of soluble solids rise and water activity is lowered in the unfrozen region as a result of ice formation in extracellular area. So the concentration of non-diffusible ions is higher in cell than the surrounding fluid resulting in higher amount of ionic particles inside the cell. Hence, freezing point is expected to be lower in internal region. In the case of high subfreezing temperatures, ice crystals do not occur in intracellular region giving rise to a supercooled fluid inside. In this case, water inside the cell may diffuse from the cells due to its higher water activity. Thus, the chemical potential in both fluids is equilibrated and result is the enlargement of ice crystals formed at extracellular spaces (Sun, 2011).

Slow freezing gives rise to large ice crystals particularly in exctracellular space and causes cell shrinkage. On the contrary, rapid freezing produces numerous small ice crystals that are uniformly distributed both in intracellular and extracellular spaces. Hereby, a frozen food product that looks alike unfrozen food is obtained (Sun, 2011).

1.3 Factors affecting Quality of Frozen Food

1.3.1 Freezing Rate

Continuous change in temperature occurs in freezing process. Therefore, it is uneasy to calculate freezing time when considering food systems. 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. Also, freezing time may be considered as the time elapsed between beginning of the freezing until whole product is frozen. In the literature there are different definitions of freezing rate.

Freezing rate (°C/h) can be described as the difference between initial and final value of product temperature divided by freezing time (Barbosa-Cánovas et al., 2005). A local freezing rate can be described for a particular location in the food due to the fact that temperature may differ at different points of the food. The local freezing rate can be described as the ratio of difference between initial and desired value of the product temperature to the time elapsed until the desired temperature at that location is reached (Persson and Lonhdal, 1993).

Foods can be considered as a solution including many dissolved compounds. The presence of free and bound water in food makes the freezing process complicated. Bound water cannot be frozen even at low freezing temperatures. The dissolved compounds in unfreezable water lower the freezing point of water below 0°C. The concentration of these compounds increase in the unfrozen water, hence variable freezing temperatures are observed in the freezing stage (Barbosa-Cánovas *et al*., 2005).

International Institute of Refrigeration (IIR) describes the freezing rate as the distance between the surface and thermal center divided by the time elapsed between the surface reaching at 0°C and the thermal center temperature of -15°C (cm/h). This wide range has been taken since at -15°C nearly all water is frozen out, even in fruits (Hawthorn and Rolfe, 1968).

Freezing rate is a generic term and is rather used to compare the freezing operation on a relative basis. Freezing rates are therefore, only average rates and they do not represent what happens in practice. Freezing rates differ in the range of 0.2 and 100 cm/h in commercial practice. Slow freezing ranges between 0.2–0.5 cm/h. Bulk freezing in cold chambers may be given as an example to slow freezing. Quick freezing ranges between 0.5–3 cm/h. Air blast and contact plate freezers operate in this range. Rapid freezing ranges between 5–10 cm/h. Freezing of small sized food products in fluidized beds is an example to rapid freezing. Ultra rapid freezing is in the range of 10-100 cm/h. This freezing rate is achieved by spraying or immersion in cryogenic fluids (Sun, 2011).

The factors affecting the freezing rate are the freezing system used, the initial value of the product temperature, packaging type, and product type. Frozen food quality is highly affected by freezing rate. Rapid freezing usually leads to higher quality food (De Ancos et al., 2006).

Cell wall integrity depends on the size, form and location of the ice crystal and it is determined by heat transfer rate i.e. freezing rate. Slow freezing rate lead to large ice crystals in the outer of the cells and intracellular water migrates out due to osmotic pressure which is known to cause mechanical damage to cell wall during thawing and finally drip loss. In addition, large sharp ice crystals formed as a result of slow freezing may injure delicate organelle and cell membrane which then causes release of enzymatic systems and their substrates. As a result, off-flavors, color and textural changes occur. Rapid freezing results in small size ice crystals, both inside and outside of the cell which protects the tissue structure from injuries (Hui et al. 2011).

Applying similar freezing rates to different foods even with similar dimensions lead to ice crystals with different sizes. The reason for that may be different water availability of foods. In addition, the same foods subjected to different pre-freezing treatments may have different size ice crystals (Alexandre et al., 2013).

Quick freezing is advantageous in fruit and vegetable products, however, temperature fluctuations during storage may cause recrystallization and quality loss in frozen product (Hui et al. 2011). Therefore, freezing process should be controlled thoroughly by paying attention to pre-freezing and post-freezing operations to obtain high-quality food products (George, 1993).

1.3.2 Storage Temperature

Quality of frozen food depends on storage temperature. The decrease in storage temperature leads to increase in frozen food quality (Sun, 2011). The upper limit of frozen storage temperature is widely accepted as -18 °C. The tissue of frozen food is not stable during storage and the quality decrease to a lower level depending on the type of product and the storage temperature (Sousa et al, 2005). Physical and chemical reactions occurring in food cause degradation of product quality.

Comprehensive information can be found in the literature about the effects of freezing and storage temperature on the nutrient content of fruits and vegetables. (Kyureghian et al, 2010). The reports about the studies on nutrient retention during frozen storage may be contradictory. The reason for that may be incomplete destruction of enzymes during pretreatment such as blanching or the variable degree of oxidation during packaging.

Also, raw material type and variety and freezing method may lead to variable results. Rapid freezing process itself does not affect nutrient content; however, frozen storage and thawing have small and variable effect on nutrient content (Mallett, 1993). Table 1 shows the practical storage life of several frozen fruits and vegetables at several storage temperatures.

Table 1.1 The practical storage life of several frozen fruits and vegetables at several storage temperatures (Singh and Heldman, 2014).

Product	**Storage time (months)**		
	-12°C	**-18°C**	**-24°C**
Raspberries/Strawberries (raw)	5	24	>24
Raspberries/Strawberries in sugar	3	24	>24
Peaches, Apricots, Cherries (raw)	4	18	>24
Peaches, Apricots, Cherries in sugar	3	18	>24
Asparagus (with green spears)	3	12	>24
Beans, green	4	15	>24
Broccoli	-	15	24
Brussels sprouts	6	15	>24
Carrots	10	18	>24
Peas, green	6	24	>24
Peppers, red and green	-	6	12
Potatoes, French fried	9	24	>24
Spinach (chopped)	4	18	>24
Onions	-	10	15

1.4 Effect of Freezing Rate and Frozen Storage on Quality Parameters of Frozen Fruits and Vegetables

Freezing and frozen storage have significant effect on food quality. During freezing and frozen storage, physical and chemical changes occur in food systems. The most common physical changes during freezing of food are modification in cell volume, dislocation of water, mechanical damage and freeze cracking. Physical changes during frozen storage are moisture migration, freezer burn, and ice recrystallization. Chemical changes are enzymatic reactions, protein denaturation, lipid oxidation and vitamin loss. The change in solute concentration and decompartmentation of cell contents during freezing may affect the rate of these reactions (Sun, 2011).

1.4.1 Total Phenolic Content and Antioxidant Activity

The main bioactive compounds in fruits are vitamins A and C, carotenoids, and phenolics. These compounds give the foods their antioxidant properties (Gardner et al., 2000). Having redox properties, phenolic compounds act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators (Rice-Evans et al., 1997). In previous studies, significant correlation was reported between total phenolics and antioxidant activity. Antioxidant activity may be affected by maturity at harvest, season of maturity, genetic differences, preharvest environmental conditions, postharvest storage conditions, and processing (Connor et al., 2002).

Connor et al. (2002) examined the changes in antioxidant activity for blueberry cultivars during cold storage temperature. The researchers found that the antioxidant activity did not significantly decrease from the harvest value during cold storage temperature and an increase in antioxidant activity and total phenolic content in berries during cold storage.

De Ancos et al. (2000) studied the effect of freezing and long-term frozen storage on total phenolic content. They found that freezing process slightly affected total phenolic content in raspberry fruit. However, a continuous decrease was observed in the total ellagic acid content which ranged between 14 and 21%. The losses were attributed to the enzyme polyphenol oxidase released from the cell wall of the fruit during storage.

Mullen et al. (2002) studied the effect of freezing and storage (-30°C) on the total flavonoids and antioxidant activity of raspberries. They reported that the total flavonol content and antioxidant capacity of raspberries were not significantly different in the fresh and frozen berries. Chaovanalikit and Wrolstad (2004) examined the total phenolics in cherries for 6 months of frozen storage. 25 % loss after 3 months and 50% loss at the end of frozen storage were observed at -23°C. However, the changes in phenolics were small at -70°C. According to the finding of Poiana et al. (2010), there was reduction in antioxidant capacity during the frozen storage (-18°C) of fruits. The reduction was small during 4 months of frozen storage. However, a significant reduction was observed in the following months. For 4 months storage, losses of antioxidant activity were approximately 15, 19 and 23 % in sour cherries, sweet cherries and strawberries, respectively. By the storage of 10 months, decreases of antioxidant activity up to 35, 38 and 42 % of initial value were observed for fresh fruit, sour cherries, sweet cherries and strawberries, respectively.

In the study of Hunter and Fletcher (2002), it has been demonstrated that frozen vegetables have similar antioxidant activity compared with the fresh vegetables. Furthermore, according to Murcia et al. (2009), antioxidant activity did not vary in frozen vegetables stored at -20˚C even 8 months. The analyses confirmed that frozen vegetables showed lower antioxidant activity and remain unchanged during long term frozen storage. Frozen storage of some vegetables such as broccoli and peas, caused some losses of phenolics.

In the review paper of Rickman et al. (2007), it is reported that frozen storage of fruits causes a small reduction in phenolic compounds. Increases in the phenolic content of some fruit varieties such as raspberries, were also reported with increase in total anthocyanins for early harvest cultivars. Freezing and long-term frozen storage of vegetables did not affect the radical scavenging activity i.e. antioxidant capacity, concluding that frozen storage of vegetables is also a favourable method to preserve phenolic compounds during long-term periods (Hui, 2011).

1.4.2 Ascorbic Acid

An extensive study has been performed on the nutrient composition of fruits and vegetables in freezing process, exclusively ascorbic acid (Jeremiah, 1996). Ascorbic acid is highly water soluble and heat-labile (Rickman, 2007). Considerable amounts of ascorbic acid may be lost through thermal processing, water leaching and oxidation. Hence, it is considered the most labile of vitamins and considered as an appropriate indicator for monitoring quality changes during food processing and storage (Serpen et al., 2006; Sinha, 2012).

Vitamin C is defined as the generic term for all compounds exhibiting the biological activity of ascorbic acid. The principal biologically active form of vitamin C is L-ascorbic acid (L-AA), but the first oxidation product of L-AA, i.e. dehydroascorbic acid (L-DHAA) also exhibits biological activity (Serpen and Gökmen, 2005). L-DHAA is then oxidized to 2,3-diketogluconic acid which does not have vitamin C activity (Jeremiah, 1996). There is reversible equilibrium between L-AA and L-DHAA, however L-DHAA is irreversibly oxidized to 2,3-diketogluconic acid under certain conditions such as oxidizing and reducing properties, heat, light etc. over time (Serpen et al., 2007).

The degradation of ascorbic acid is affected during frozen storage by several factors such as pretreatments, type of food and packaging, freezing process, time-temperature conditions, etc. (Skrede, 1996). Therefore, frozen storage period is limited by this quality index. Oxidation of ascorbic acid may be enzymic or nonenzymic. The enzyme mainly causing loss of Vitamin C is ascorbate oxidase. If this enzyme is not inactivated by pretreatments, it keeps its activity during the frozen storage of the product (Hui, 2011).

Enzymatic degradation of ascorbic acid can be prevented by eliminating ascorbic acid oxidase with heat treatment e.g. hot water blanching (Leong and Oey, 2012). Especially, water-soluble vitamins (e.g., vitamins C and B) are lost at sub-freezing temperatures. Other vitamins are lost as a result of drip loss. In addition, vitamin C losses are highly temperature dependent; a 10ºC increase in temperature causes a six fold to twentyfold increase in the rate of vitamin C degradation in vegetables and a thirty fold to seventy fold increase in fruits (Fellows, 2000).

Storage temperature is more effective than freezing method on the retention of ascorbic acid. It is stated by several studies that stability of ascorbic acid is higher in some fruits suc as berries, citrus, tomato, etc. when the frozen storage temperature decreases (Skrede, 1996; Lisiewska and Kmiecik, 2000). A study by Sahari et al. (2004) about the effect of low temperature and freezing methods on the ascorbic acid content of frozen strawberry showed that the loss of vitamin content was considerable at -12°C. However, vitamin content was higher at -18°C and -24°C which was reported to be accepted by consumers. After 90 days of storage, the decrease in ascorbic acid content was 64.5%, 10.7% and 8.9% at -12, -18 and -24 ˚C, respectively. Moreover, the major loss of ascorbic acid occurred during the first 15 days of storage at -12 ˚C (31.4%). Sahari et al. (2004) reported that ascorbic acid level decreases with time and with loss of 33-55% at the end of one year frozen storage.

Another study by Fraczac and Zalewska-Korona (1990) revealed that freezing method significantly affected the ascorbic acid content of strawberry and rasperry fruits. The greatest losses were found when the tunnel freezing (-30°C) was used. Highest vitamin C retention was obtained when strawberries were frozen in sugars. Vitamin C retention was highest for raspberries when they were frozen in liquid nitrogen (-180°C). Koyuncu and Dilmaçünal (2010) investigated vitamin C change in two strawberry cultivars (Dorit and Selva) during cold storage (0°C, 90-95% relative humidity for 10 days). The authors found that loss of vitamin C depends also on cultivar type. A slight increase was found in vitamin C content. The reason was explained as continuous ripening process of fruits.

Bahçeci et al. (2005) showed that the ascorbic acid is very sensitive to break down during frozen storage of green beans. Also, retention of this quality parameter is guaranteed by increasing blanching time and temperature. In the review paper of Rickman et al. (2007), refer to the study of Favell (1998), in general, losses of ascorbic acid as a result of thorough freezing process may differ within the range from 10 to 80%, with an average rate of 50%. Moreover, ascorbic acid also continues to degrade during prolonged storage of frozen products. After 1 year storage of fruits and vegetables at -18˚C to -20˚C, losses were within the range of 20 and 50%. Also, Gonçalves et al. (2011) found that frozen storage of broccoli for nearly 4 months reduced the amount of vitamin C significantly. In addition, main loss of vitamin C was observed at first 2 months of frozen storage. According to Lisiewska and Kmiecik (2000), long term frozen storage caused gradual pronounced losses in vitamin C content of tomato cubes. They fell to 71% and 45% of initial value after 12 months of storage at -20˚C and -30˚C. Similar losses were reported when tomato cubes were frozen for 9 months.

1.4.3 Color Change

Color is the first attribute that is perceived by the consumer and it determines the product acceptability. That is why it is accepted as the most important quality characteristic especially in fruits (Hui, 2006). Color changes in fruits and vegetables stem from chemical, biochemical, and physicochemical reactions. The major reactions that lead to color changes are degradation of chloroplasts and chromoplasts, alteration in natural pigments (chlorophylls, carotenoids, and anthocyanins) and progress of enzymatic browning. Chloroplast and chromoplast have fragile membrane which can be broken as a result of freezing process due to mechanical damage. Hence, chlorophylls and carotenoids are released and thereby making easier the formation of oxidation and enzymatic reactions (Sinha, 2012).

In green vegetables, lutein and β-carotene are the predominant carotenoids. Chlorophylls *a* and *b are* predominant chlorophylls. Oruña et al. (1997) studied the effects of freezing on the pigment content in green beans. They found that the pigment contents, the chlorophyll *a* and *b*, β-carotene and lutein contents in green beans decreased markedly during the first 2 months and then stabilized. Blanching before freezing pronouncedly minimized the decrease in lutein and β-carotene contents in green beans. Freezing in bags sealed under vacuum considerably decreases the β-carotene content in the blanched green beans. Chlorophyll degradation can occur as a result of loss of Mg and enzyme action. Hence, chlorophylls convert to pheophytins, and the enzyme chlorophyllase causes transformation of chlorophyll into pheoforbide resulting in brownish color in the plant product (Sinha, 2012). For instance, freezing and frozen storage of kiwi-fruit slices at -20°C for ca. 10 months reduced the chlorophyll concentration to an average value of 50% (Cano et al., 1993).

Chlorophyll is easily destroyed by acid, temperature, light, oxygen, and enzymes. Hence, temperature-time in blanching, freezing process and acidity should be controlled carefully for the preservation of chlorophylls (Sinha, 2012). Color changes during frozen storage were reported about some high-carotenoid foods including tomato products, and fruit slices of pineapple, kiwi, and papaya. Diminishing of the redness, increasing of the yellow character and a lighter color were the characteristic color changes in carotenoid foods during frozen storage (Lee and Coates, 2002). Surface color of food product can be affected by freezing rate. It is reported by Zaritzky et al. (1982) that faded colors are observed due to small ice crystals which lead to scattering of incident light.

Ferreira et al. (2006) analysed the color parameters of frozen and cooked beans. Effect of freezing rate on pigment content was also examined. They reported that freezing of beans before cooking considerably increased the lightness compared to unfrozen beans; however, freezing rate did not have significant effect. Color saturation was not different in frozen and cooked samples. Maximum saturation value was observed in beans frozen at -35 °C. Cooked beans presented higher coloration when compared to frozen-cooked beans. This result proved that lighter colors are obtained in cooked samples which are frozen prior to cooking. In the case of faster freezing rates, thawed and cooked samples had lighter color; however beans cooked directly after freezing presented darker color. The lowest greenness was detected in beans frozen at -24 °C. Since, slow freezing brought about severe damage on the cell which caused degradation of chloroplast. As a result, pigments may move to the inner parenchyma which causes reduction in chlorophyll concentration in the outer epidermis, hypodermis and outer parenchyma.

A recent study by Poiana et al (2010) showed that the color is highly unstable during long-term frozen storage. Conversion of monomeric form of anthocyanins to polymeric form reduced the color intensity. The decrease in color intensity of strawberries was 25% of initial value after 10 months of frozen storage.

The polymeric color increased from an approximate value of 11% to 27%. As for cherries, the color stability was superior during long term storage at -18°C when compared to strawberries. The authors concluded that the storage life of frozen fruits in a storage temperature of -18°C is ideal to keep down the decrease in color intensity and amount of bioactive compounds. In addition, Gonçalves et. al. (2011) found that color values decreased significantly with the increase of time and temperature of storage which reflects a change in broccoli green color.

1.4.4 Textural Properties

Cell wall and middle lamella components (pectins, hemicelluloses, and celluloses) of the plant tissue may undergo chemical or biochemical alterations which affect the texture of frozen fruits. The size and status of ice crystals may decompose cell wall promoting physical and chemical changes, and bring about mechanical injury in the cell wall. Water, constituting 90 % of total weight in most fruits and vegetables, gives support and texture to the plant tissue with dissolved solutes inside rigid plant cell walls.

During freezing process, an expansion occurs with the formation of ice crystals causing cell wall rupture. Consequently, the texture of frozen food is usually softer after thawing when compared to unfrozen product. This textural difference is perceivable particularly in products which are consumed raw, as in the case of fruits. Therefore, partial thawing of fruit is recommended so that texture loss is less noticeable. However, textural changes are not perceivable for the vegetables which are cooked before eating, since cell walls are also softened due to cooking (Schafer and Munson, 1990).

Plant cells have a membrane encircling them and also extensive membrane systems that divide the cell into many compartments. The plasma is surrounded by plasma membrane. This membrane is the interface between the cell and the extracellular surroundings. A cell wall encloses the plant cell. Plant cells may contain the plastids: chloroplasts, leucoplasts, amyloplasts, or chromoplasts. Vacuole corresponds to the largest volume of the cell. Turgor pressure is provided by vacuole.

It helps to maintain the high osmotic pressure of the cell and the content of different compounds in the cell namely inorganic ions, organic acids, sugars, amino acids, lipids, oligosaccharides, tannins, anthocyanins, flavonoids, and more. The tonoplast is a special type of membrane that encloses the vacuole. The cell wall of plants composes of many stacked cellulose microfibrils imbedded in a polysaccharide matrix. The cell wall is able to store water so that the cell volume may increase (hydration and absorption). Pectins, hemicelluloses, cellulose and lignin are the polysaccharides included in the matrix. Pectins are principally polygalacturonic acids and prevalent in the layer between cells. The deesterification process of pectin is associated with the softness of food tissue during ripening and processing (Hui et al, 2006).

Reduction of firmness in fruits during freezing and frozen storage was reported to be related to decrease of the pectin fraction (Lisiewska and Kmiecik, 2000). Firmness and textural quality was affected by destruction of cell wall pectins during processing of frozen carrots. While rapid freezing rates retain better texture and high degree of cellular integrity, slow freezing rates produced structural damage and considerable softening. Slow freezing of cellular tissues may bring about extracellular ice crystals. As a result, concentration of solutes increases causing cell dehydration and death through osmotic plasmolysis and membrane damage (Sinha, 2012).

Extracellular ice does not reenter the cells upon thawing and may result in drip loss and texture softening (Cheftel et al., 2000). Consumers desire food that has a firm, crispy and succulent texture. To keep the textural quality of the fresh foods, an extensive study was carried out on modifying processing techniques. Freezing rate is an important factor affecting textural quality of frozen products. The effect of freezing rate on some textural properties of food has been widely studied by various authors. Rapid freezing rates had beneficial effects on texture of frozen food. Since, ice crystals become larger at slower freezing rates, causing severe cell damage (Skrede, 1996).

According to the study of Brown (1967), very rapid freezing of green bean by immersion in liquid nitrogen had positive effect on texture even after cooking. Decreasing the freezing rate during freezing did not damage the portion of the bean already frozen. Textural differences were distinguishable in beans frozen at various rates. Marti and Aguilera (1991) evaluated three freezing methods (static freezing at -23±2°C, plate freezing at -50°C, immersion in liquid nitrogen) for blueberries and wild blackberries. Relative freezing rates between the three methods were 1:5:15 for static, plate and liquid nitrogen, respectively. They reported that the two fastest freezing methods produced the best texture. On the other hand, slow freezing resulted in severe damage to cell structure.

Strawberries frozen in liquid nitrogen resulted in better texture and lower drip loss than berries frozen by air blast freezing. High freezing rate had a positive effect during 6-12 months of storage at temperatures between -20 and -30°C. However, freezing rates higher than 1.5 cm/h didn't improve quality retention significantly (Jeremiah, 1996). Chourot et al. (2001) investigated the behaviour of green beans during immersion freezing (in brine at -17.5 °C) and conventional air-blast freezing (-30°C, 3m/s). Immersion freezing provided higher freezing rate (four times faster than air-blast freezing) and texture of green beans frozen by this method was preferred by sensory panelists.

Another study by Roy et al. (2001) indicated that the high temperature short time blanching followed by rapid freezing may be suggested as the optimum thermal processing conditions for a superior textural quality in frozen carrots. In a more recent study by Ferreira et al. (2006), the favourable effect of rapid freezing was proved; the merest damage was observed in green beans which were frozen at the highest freezing rate. The favourable effect of faster freezing was also observable in cooked green beans. The highest mechanical resistance of tissues of green beans was detected in samples frozen at the fastest rate (5.4 °C/min) and air-thawed at the slowest rate (0.35 °C/min).

The damage caused by freezing decreased with the increase in freezing rate. Also, in the study, microphotographs were taken to examine tissue structure. Gelatinized starch granules formed as a result of cooking were visible in microphotographs of beans. Tissue of the bean is known to be softened by freezing and cooking processes. Freezing gives rise to cell wall distortion. Cooking primarily brings about dissolution of intercellular pectin and considerable cell disconnection. Cooked control exhibited some cell distortion presumably due to cell wall dilatation.

Both textural and structural results imply that structural damage correlates inversely with the freezing rate. Since, the size and status of the ice crystal are determined by freezing rate. The number of nuclei determines the size of ice crystal. Faster freezing rates result in large number of nuclei. The mass of ice is shared between numerous small ice crystals. In the case of slow freezing rates, number of nuclei is small which increase the size of ice crsytal. The increase in freezing rate promotes nucleation and numerous small size crystals are formed (Sun, 2011). Findings of Ferreira et al. (2006) proved the positive effect of rapid freezing (5.4 °C/min) which improves texture of the product and negative effect of slow freezing (1.5 °C/min) which increases mechanical damage. In slow freezing rate, water molecules migrated from their location to the crystallization sites. As a result, internal tension increased leading to cell disconnection, cell wall rupture and decrease in turgidity.

Storage temperature is also an important factor that may affect textural quality of frozen food. The positive effect of rapid freezing may be lost if the conditions during frozen storage are insufficient. Temperature fluctuations below freezing point during frozen storage may cause dehydration of a product. Water evaporates from the fruit with the rise in temperature. If temperature drops again, the water vapor condenses again on the surface of packaging material since it cools more quickly than the fruit. Thawing during frozen storage should be prevented otherwise; freezing will be slow under these conditions, resulting in large ice crsytals and tissue damage (Jeremiah, 1996).

1.5 Objective of the study

In recent years, a diverse range of studies have been reported to expedite the freezing process and to form small and evenly distributed ice crystals throughout a frozen food product with the effects of freezing and frozen storage on quality characteristics of foods. Besides, the focus of interest related to home-frozen fruits and vegetables is increasing. The aim of this study is to determine the freezing rates and to analysis some quality parameters of green bean and strawberry in a home type freezer. Structural, nutritional and sensorial changes of these fruit and vegetable during freezing process will be analyzed. These analyses are color, cell integrity, ascorbic acid, total phenolic content and antioxidant activity. At the end of this study, freezing rate measurements and the estimations to show the effect of frozen storage conditions can be used for the development of new home-freezers.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

Strawberries (Festival variety) and green beans (Sırık variety) were purchased from a local market in Ankara (Turkey). Strawberries were sorted to eliminate fruits with defects including overripe or too small fruit. The stems of the berries were picked. LDPE bags (24 cm x 28 cm) were used for packaging of approximately 500 g of strawberries. Green beans were cut (2.5 cm in length) and blanched at 80–85°C for 2 min. After cooling by flowing tap water and removing excess water by using paper towel, LDPE bags (20 cm x 22 cm) were used for packaging (250 g sample /bag). Packaged samples were frozen in home type freezer (Arçelik 2572 D) and stored at -27°C for 3 months. Four replicates were used per treatment. Samples were left to thaw at room temperature (natural thawing) for about 1 h for quality control experiments. Samples were analyzed at 1 week intervals during frozen storage.

2.2 Measurement of the Sample Temperature

In this study, the sample time-temperature measurements were recorded by using 34972 A LXI Data Acquisition / Data Logger Switch Unit device (Agilent Technologies, Inc. Malaysia) with 30 gage (corresponding to 0.025 cm in diameter) T type thermocouples included 60 2-wire channels.

For the installation of the system, the support was taken from the Department of Mechanical Engineering, METU. Thermocouples were inserted into the center of food samples. Freezer temperature was adjusted to -23, -26 and - 27°C. Cooling curves were obtained by using this system for the estimation of freezing rates.

Freezing rate is calculated as (i) the distance between the surface to the center divided by the time elapsed between the surface reaching at 0°C and the center temperature of -15°C (cm/h) and also (ii) the difference between initial and final center temperature of product divided by freezing time (°C/h).

2.3. Determination of Ascorbic Acid Content

L-Ascorbic acid was measured by HPLC according to a slightly modified method described by Baardseth e*t al*. 3 g of blended sample was weighed in a screw-capped tube and 25 mL of 4.5 % metaphosphoric acid (MERCK 100546) is added for extraction. The samples were homogenized with IKA T-18 Ultra-Turrax Homogenizer at 14000/min for 1 minute. The mixtures are shaken for 1 hour at 4 °C and centrifuged at 15000 rpm for 10 minutes at 4°C. The supernatant was filtered through a Whatman no. 1 filter. Samples were kept on ice during preparation.

Analysis was performed using a Thermo Scientific Finnigan Surveyor HPLC eqipped with a Autosampler Plus, LC Pump Plus and UV-VIS Plus Detector (San Diego, CA). Reverse-phase seperation was attained using a VARIAN Chromsper5 C18 HPLC column (150 mm×4.6 mm I.D., 5 µm; MerckKGaA, Darmstadt, Germany). The mobile phase was mixture of buffer and methanol (MERCK 106007) with a ratio of 96:4. Buffer was aqueous solution of hexane sulfonic acid sodium salt (Merck 118305), potassium dihydrogen phosphate (MERCK 104877) and triethylamine (MERCK 808352) and brought to pH 3.0 with ortophosphoric acid (MERCK 100573).

The mobile phase flow rate was 1 mL min^{-1} and the injection volume was 25 µL. Column temperature was 35 ^{o}C and the samples were kept at 4^{o}C in the autosampler tray. Samples were filtered through Minisart NY 0,45 µm filters (Sartorius AG, Gottingen, Germany) prior to injection on the column. Samples were run in duplicate or triplicate. Peaks were detected at 254 with a retention time of 1.8 min. Quantification was done by using L-ascorbic acid (SUPELCO 47863) as the standard. Standards with 5 different concentrations were prepared by diluting the stock solution (1 mg/mL) with mobile phase.

A standard curve was prepared by basing on the peak areas obtained for different concentrations of the standard solution (Figure 2.1). The results were expressed as mg / 100 g fruit.

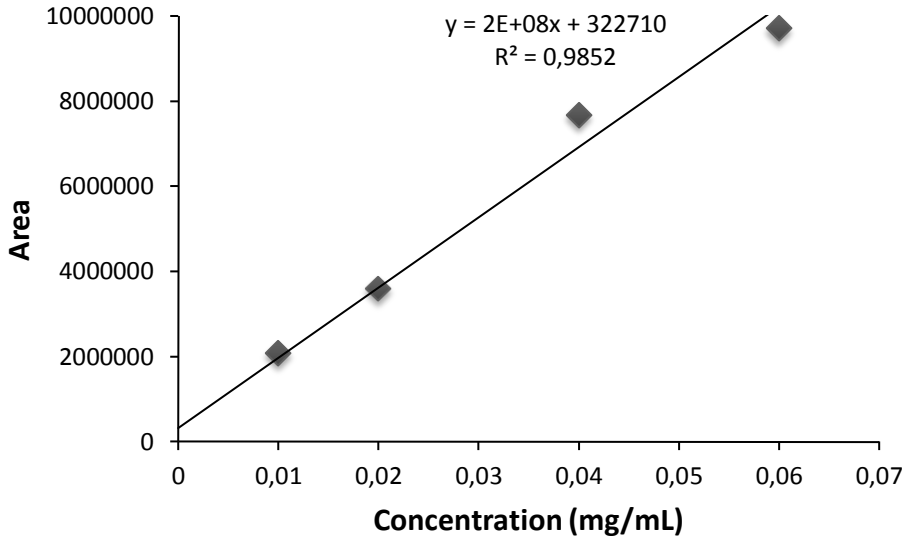

Figure 2.1 Standard curve of L-Ascorbic acid for determination of Vitamin C.

2.4 Determination of Total Phenolic Content and Antioxidant Activity

Sample Extraction: The fruit and vegetable samples were blended by using home type blender (ARZUM AR 186 CHOPPER, Turkey). 2 g of blended sample was weighed in a screw-capped tube. Extraction was done with 20 mL of (50:50 v/v) ethanol:water mixture (MERCK 100983) for total phenolic content analysis and (70:30 v/v) methanol:water mixture (MERCK, 106009) for antioxidant activity analysis. Then, samples were homogenized for 1 min using homogenizer (IKA T-18 Ultra-Turrax Homogenizer) at 14000/min for 1 minute and allowed to stand for 45 min at 4°C for complete solvent extraction. Following the extraction, samples were centrifuged at 15000 rpm and 4°C (Sigma 2-16 PK, Germany) for 10 min to remove the solid fraction. Filtering cloth was used to separate aqueous part from solid part. The filtered extract was used for the analysis.

Determination of total phenolic content: Total phenolic contents of the sample extracts were determined by Folin-Ciocalteu method (Singleton and Rossi, 1965) with further slight modifications. Sample extracts (0.5 mL) were placed in a test tube. 2.5 mL 0.2 N Folin-Ciocalteu's reagent (MERCK 109001) was added to the solution and allowed to react for 5 min. The reaction was neutralized with 2 mL 7 % of sodium carbonate (MERCK 106392). Blank sample contained 0.5 ml 50 % ethanol (MERCK 100983), 2.5 ml 0.2 N Folin-Ciocalteu's reagent (MERCK 109001) and 2.0 ml of 7.5 % of sodium carbonate (MERCK 106392). Samples were left to stand in dark place for 1 hour at ambient temperature (25°C). Then, absorbance at 760 nm was measured by using UV/VIS spectrophotometer (SHIMADZU UV–1700 PharmaSpec, Kyoto Japan). The samples were prepared in triplicate for each analysis and average value of absorbance was calculated. The same procedure was repeated for the standard solution of gallic acid (SIGMA 398225) and the calibration line was constructed using a six point standard curve (0–100 mg/L) shown in Figure 2.2.

Based on the measured absorbance, the concentration of phenolics was read (mg/L) from the calibration line. The content of phenolics in extracts was expressed in terms of gallic acid equivalent (mg of GAE/100 g of fresh weight).

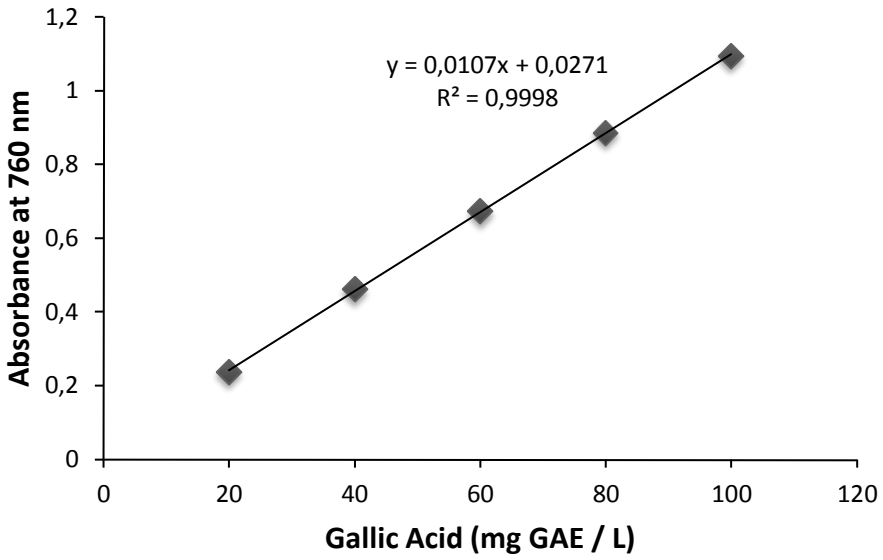

Figure 2.2 Calibration curve prepared by gallic acid in ethanol:water mixture (50:50 v/v) for determination of total phenolic contents.

Total antioxidant activity with DPPH˙ radical scavenging method: Total antioxidant activity was evaluated by using 2,2-diphenyl-1-picrylhydrazyl (DPPH•) (SIGMA, Germany) radical (Brand-Williams et al. 1995). DPPH is a free radical having free electron delocalization. It has a deep violet color in solution due to this delocalization which can be measured with UV-spectrophotometer in range of 515-520 nm wavelengths (Chen *et al*., 2013; Molyneux, 2004; Villano *et al*., 2007).

Antioxidants supply hydrogen to free radicals and scavenge radicals (Wang et al. 1999). Substances which have antioxidant characteristics alter the color of DPPH˙ solution into a pale yellow color. The basis of the method depends on the measurement of this decolorization.

DPPH solution (0.025g DPPH• /L methanol) is prepared with methanol. 0.1 mL of the sample extract and 2 ml of the DPPH solution are mixed. After shaking, the reaction mixture was kept in dark place for 1 hour at ambient temperature. The measurements of the absorbance values were performed on the spectrophotometer (SHIMADZU UV–1700 PharmaSpec, Kyoto Japan) at a wavelength of 517 nm. Methanol was used as blank. 2 mL of DPPH˙ radical solution (25 mg DPPH˙/ L MetOH) and 0.1 mL of methanol were mixed, and absorption at 517 nm was measured to find the amount of DPPH· at the beginning of reaction. Calibration curve was generated by preparing different concentrations of DPPH in methanol (Fig. 2.3). The DPPH• concentration in the reaction medium was calculated from the calibration curve, determined by linear regression.

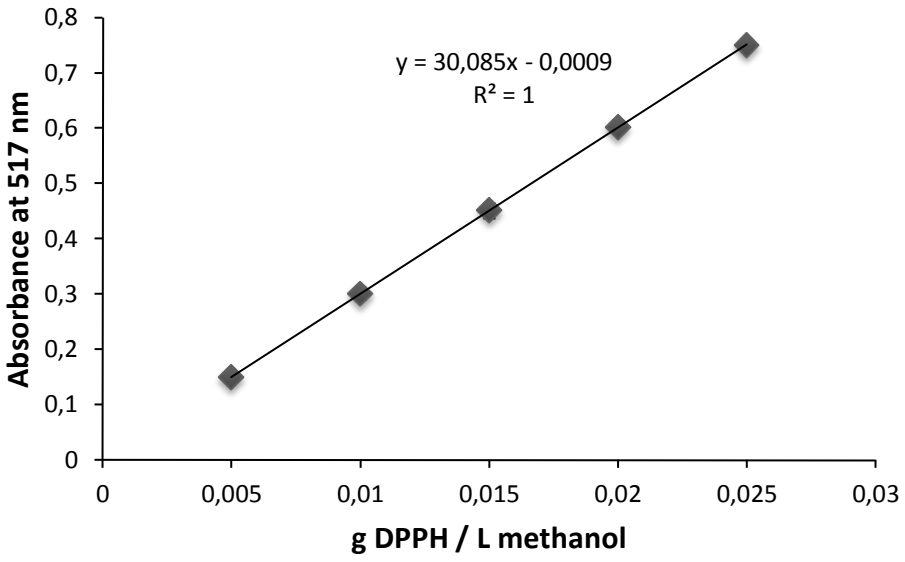

Figure 2.3 Calibration curve prepared by DPPH˙ radical in methanol for determination of antioxidant activity.

Results were examined by calculating the difference between the concentration of DPPH at the beginning and at the end of the reaction. Antioxidant amount was calculated by using following formula:

$$A = (DPPH^{\cdot}{}_{t=0\,min}) - (DPPH^{\cdot}{}_{t=60\,min})$$

Where A is the amount of antioxidant, $DPPH^{\cdot}{}_{t=0min}$ (C_1) is the amount of DPPH• at the beginning of the reaction and $DPPH^{\cdot}{}_{t=60min}$ (C_2) is the $DPPH^{\cdot}$ amount 60 min after the reaction starts. To find the antioxidant activity (AA) as mg DPPH/g fresh weight, the following formula could be used.

$$AA\ (mg\ DPPH/g\ fruit) = \left(\frac{C1-C2}{Wsample}\right) x\ V\ x\ d$$

Where d is the dilution rate, V is the volume of extract in mL, W_{sample} is the amount of sample in g.

To calculate remaining antioxidant percent (%), antioxidant activity was graded as % of the initial value of antioxidant activity where noted as 100 %, using the following formula:

$$A_R\ \% = \frac{A_{t=anytime}}{A_{t=0}} \times 100$$

Where A_R % is the remaining antioxidant percent, $A_{t=anytime}$ is the amount of antioxidant activity during the storage period and $A_{t=0}$ is the amount of antioxidant activity at the beginning of storage.

2.5 Color Measurement

Color measurements were performed on the surface of the food samples according to the CIE L* a* b* color notation system by using color measuring device DATACOLOR 110 spectrophotometer (Lawrenceville, NJ, USA) and its software. L* indicates the luminescence (lightness), a* means the color axis from green to red, and b* means the color axis from blue to yellow.

The following formula was used for the calculation of total color change (Billmeyer and Saltzman, 1981) and barium chloride ($BaCl_2$) was selected as reference (L_0*, a_0*, b_0*).

$$\Delta E = [(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2]^{1/2}$$

2.6 NMR Relaxometry Measurements

NMR relaxometry is a technique based on measuring the times T1 and T2. T1 and T2 times are the time constants that characterize decrease (T2) and increase (T1) of the signal at different planes resulting from the short-term implementation of the RF signal necessary for NMR. While T1 time is characterized by a signal increasing exponentially, T2 time is obtained from a signal curve decreasing exponentially.

NMR relaxation spectra, i.e. the output of NMR relaxometry experiments, is obtained by applying the inverse Laplace method to these signal curves. NMR relaxation spectra provides information about proton pools of the samples. With this technique, information about integrity of the cellular structure can be obtained.

For measurement of transverse proton relaxation times T2, strawberries and green beans were tested before freezing and during freezing (pre-freezing stage, freezing stage, sub-freezing stage, post-freezing stage) in all freezing rates. Approximately 1 cm from the center of the samples was cut to fit into the NMR tube. T2 experiments were performed in a 0.367 T (15.635 MHz) system (NMR Mobile Systems Inc., Russia) with a 16 mm r.f. coil. The transverse relaxation times (T2) were measured using a Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence with an echo time (TE) of 2.0 ms, 2500 echoes, and 8 scans. Relaxation spectra was obtained by applying Non-Negative Least Square (NNLS) to the T2 decay curves. For the purpose of 1D-NNLS analysis, PROSPA software (Magritek Inc., Wellington, New Zealand) was employed. Recycle delay was adjusted to 3 s for the analysis.

2.7 Statistical Analysis

The results were submitted to one-way Analysis of Variance (ANOVA). The statistical significance of results were determined by using the package program MINITAB (Version 16.1.1, Minitab Inc., Coventry, United Kingdom). Significant difference between means were tested using Tukey's test with a probability level fixed at $p<0.05$. Differences at $p<0.05$ were considered to be significant.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Determination of Freezing Rate

Cooling curves were obtained during freezing of unpackaged and packaged strawberries and green beans at possible freezer temperature sets of -23±1.1, -26±0.5 and -27°C±0.3. These curves were shown in Appendix B (Figures B.1-12). Formation of ice crystals occurs in the freezing stage and the frozen food quality is related to the freezing rate in this stage. In general, high freezing rate leads to the formation of small ice crystals and therefore a better frozen food quality is obtained.

Generally, freezing rate is defined as the ratio of the distance from center point to surface to the time that the centre temperature of the frozen product drops to -15°C from 0°C. In the literature, the freezing rate unit is generally given as cm/h. Characteristic parameters and freezing rates of non-packaged strawberries and green beans were presented in Tables 3.1-4 at different freezer temperatures. The distance between center and surface was defined as equivalent radius for strawberry and as characteristic length for green bean.

Strawberries have irregular shape, however the shape of the strawberry can be determined by examining its sphericity. Sphericity is described as the volume of solid divided by the volume of a sphere that has a diameter equal to the major diameter of the object thus, the solid sample is inclosed by the sphere. Sphericity equals to 1 for spherical objects (Mohsenin, 1980).

Volume of strawberries was measured by immersing the fruits in graduated cylinder. The dimensions (length and diameter of two ends) of the strawberries were meausered by using caliper gage. Considering the measured dimensions as 2*a*, 2*b*, and 2*c*, respectively, volume of the strawberry was determined from the following equation (Sahin and Sumnu, 2006):

$$Ve = \frac{4}{3}\pi abc.$$

Where *Ve* is the volume of a triaxial ellipsoid. Strawberries were considered as spherical since, the volume of the strawberry was nearly equal to the volume of the triaxial ellipsoid which has diameters equivalent to those of the sample.

$$\text{Sphericity} = \frac{\sqrt[3]{abc}}{a}$$

Considering the strawberry as spherical, sphericity equals to 1 and 2a is the equivalent diameter which is defined as the diameter of a sphere having the same volume as the strawberry. The half of the equivalent diameter was defined as equivalent radius (a) which was considered as the distance between surface and center of the strawberry.

For the green bean, characteristic length was taken as half of the thickness of the vegetable. Since, heat conduction is significant through the thickness of the green bean because of the large surface area and short conduction path.

Freezing time of strawberries were approximately 2.00, 1.68 and 1.60 hours at -23°C, -26°C and -27°C, respectively. For green beans, the freezing times were approximately 0.83, 0.65 and 0.63 h at -23°C, -26°C and -27°C, respectively. The freezing rates of strawberry were 0.74, 0.93 and 1.02 cm/h at -23, -26, and -27°C, respectively. For green bean, the freezing rates were 0.36, 0.51, 0.57 cm/h at -23, -26, -27°C, respectively.

Freezing rates corresponding to three freezer temperatures (-23, -26 and -27°C) were presented in Fig. 3.1. Freezing rates of the packaged samples were lower in comparison to non-packaged samples. Therefore, rate of heat transfer through package should also be considered in packaging frozen fruit and vegetables. Since, freezing time is related to surface heat transfer coefficient of the product (IIR, 1986). Although decreasing the freezer temperature provided higher freezing rates in the range of freezer temperatures tested, the calculated values of freezing rate shows slow freezing. Examining the freezing curves, it could be seen that the freezing stage is longest at -23°C. The freezing stage time is close to each other at -26 and -27°C (Figures B.1-12).

Moreover, degree of supercooling is differing as can be seen in cooling curves (Figs. B.1-12). Supercooling is defined as cooling a temperature below the freezing point of the sample without formation of ice. Once the crystal embryos exceed the critical radius for nucleation, the system nucleates at a point lower than freezing point. The temperature then increases to its freezing point. Freezing point depends particularly on the soluble solids content of the food (Arthey and Ashurst, 1996). Therefore, degree of supercooling may differ depending on the existence of many solid particles. It will be small if there are many solid particles in food (Haiying et al., 2006).

In some cooling curves, it is clearly realised that there are more than one freezing point in the freezing stage of food. This is due to existence of dissolved compounds. When the freezing starts at a point, the concentration of the remaining solution increases in unfrozen water. Then, this new solution has a new freezing point due to its increased concentration, and so the new freezing point is lower than the first freezing point. In this way, continuous ice crystallization occurs during freezing, and a new solution with higher concentration will occur. When the concentration of the solution rises to the saturation point at that temperature, it supercools for the last time and rises to its constant freezing point.

This point is the saturation point of the solution and the solution does not concentrate any more. Since the concentration of the solution remains constant, freezing continues at a constant freezing point until the end of freezing stage.

In this study, -27°C which referred to highest freezing rate between the freezer temperatures tested, was chosen in order to control quality parameters on food sample. Since, low storage temperature generally leads to higher quality frozen food in terms of nutritional characteristics.

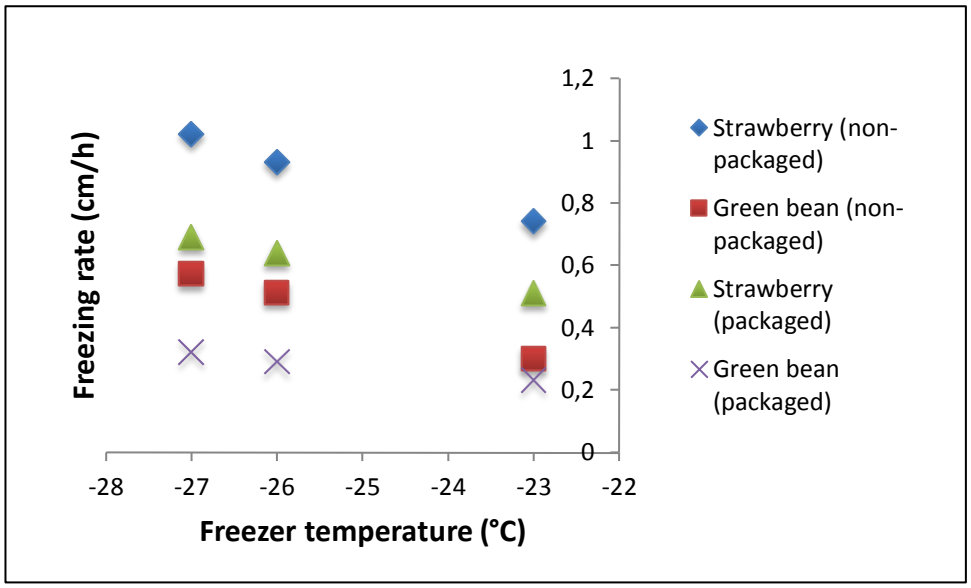

Figure 3.1 Freezing rates (cm/h) of strawberries and green beans versus freezer temperature (°C).

Table 3.1 Characteristic parameters and freezing rates of non-packaged strawberries at different freezer temperatures.

Freezer Temperature (°C)	Food sample	Initial Sample Temperature (°C)	$t_{0°C}$ and $t_{-15°C}$ (min)	Freezing time (h)	Equivalent radius (cm)	Freezing rate, R (°C/h)	Freezing rate, R (cm/h)
-23°C	Strawberry 1	16.62	18.50 and 132.83	1.90	1.37	7.89	0.73
	Strawberry 2	13.55	21.00 and 148.66	2.13	1.44	7.07	0.69
	Strawberry 3	13.68	19.16 and 133.00	1.89	1.49	7.94	0.79
	Strawberry 4	13.00	18.00 and 145.16	2.12	1.56	7.08	0.74
						R_{ave}=7.50	R_{ave}=0.74
-26°C	Strawberry 1	18.88	17.16 and 120.33	1.72	1.44	8.72	0.85
	Strawberry 2	16.75	15.66 and 117.00	1.69	1.58	9.50	0.95
	Strawberry 3	19.54	17.50 and 118.50	1.68	1.60	9.38	0.96
	Strawberry 4	17.01	15.16 and 115.66	1.68	1.58	9.50	0.95
						R_{ave}=9.28	R_{ave}=0.93
-27°C	Strawberry 1	15.42	14.83 and 109.50	1.57	1.58	9.56	1.02
	Strawberry 2	15.76	15.33 and 116.66	1.68	1.65	8.93	1.00
	Strawberry 3	15.84	15.33 and 117.00	1.69	1.67	8.87	1.00
	Strawberry 4	15.90	13.33 and 103.00	1.49	1.57	10.07	1.06
						R_{ave}=9.36	R_{ave}=1.02

Table 3.2 Characteristic parameters and freezing rates of non-packaged green beans at different freezer temperatures.

Freezer Temperature (°C)	Food sample	Initial Sample Temperature (°C)	$t_{0°C}$ and $t_{-15°C}$ (min)	Freezing time (h)	Characteristic length (cm)	Freezing rate, R (°C/h)	Freezing rate R, (cm/h)
-23°C	Green bean 1	16.9	7.66 and 59.33	0.86	0.3	17.44	0.35
	Green bean 2	17.09	7.50 and 60.00	0.87	0.3	17.25	0.34
	Green bean 3	21.22	6.83 and 52.50	0.76	0.3	19.74	0.39
	Green bean 4	16.83	7.16 and 57.66	0.84	0.3	17.86	0.36
						R_{ave}=18.07	R_{ave}= 0.36
-26°C	Green bean 1	9.36	3.50 and 43.00	0.65	0.31	23.08	0.48
	Green bean 2	7.25	2.83 and 42.33	0.66	0.27	22.73	0.42
	Green bean 3	7.27	2.83 and 43.50	0.67	0.35	22.39	0.52
	Green bean 4	10.04	3.66 and 42.00	0.64	0.39	23.44	0.61
						R_{ave}= 22.91	R_{ave}= 0.51
-27°C	Green bean 1	16.85	5.33 and 46.83	0.69	0.41	21.74	0.59
	Green bean 2	18.52	4.66 and 35.50	0.51	0.30	29.42	0.59
	Green bean 3	19.4	6.16 and 46.33	0.67	0.36	22.39	0.55
	Green bean 4	21.17	7.00 and 46.66	0.66	0.36	22.73	0.54
						R_{ave}= 24.07	R_{ave}= 0.57

Table 3.3 Characteristic parameters and freezing rates of packaged strawberries at different freezer temperature.

Freezer Temperature (°C)	Food sample	Initial Sample Temperature (°C)	$t_{0°C}$ and $t_{-15°C}$ (min)	Freezing time (h)	Equivalent radius (cm)	Freezing rate, R (°C/h)	Freezing rate, R (cm/h)
-23°C	Strawberry 1 Strawberry 2 Strawberry 3	18.49 18.77 18.72	33.66 and 262.00 27.33 and 211.66 24.83 and186.66	3.80 3.08 2.70	1.58 1.60 1.58	3.95 4.87 5.56 R_{ave}= 4.79	0.41 0.52 0.59 R_{ave}= 0.51
-26°C	Strawberry 1 Strawberry 2 Strawberry 3 Strawberry 4	18.90 15.75 16.78 19.86	22.00 and 154.66 15.83 and 139.66 23.66 and 169.00 29.00 and 173.00	2.21 2.06 2.42 2.40	1.47 1.46 1.42 1.46	6.79 7.28 6.20 6.25 R_{ave}= 6.63	0.66 0.71 0.59 0.61 R_{ave}= 0.64
-27°C	Strawberry 1 Strawberry 2 Strawberry 3	16.51 17.05 15.64	19.66 and 157.83 18.16 and 147.33 18.00 and 149.66	2.30 2.15 2.19	1.55 1.45 1.57	6.52 6.98 6.85 R_{ave}=6.79	0.67 0.67 0.72 R_{ave}= 0.69

Table 3.4 Characteristic parameters and freezing rates of packaged green beans at different freezer temperatures.

Freezer Temperature (°C)	Food sample	Initial Sample Temperature (°C)	$t_{0°C}$ and $t_{-15°C}$ (min)	Freezing time (h)	Characteristic length (cm)	Freezing rate, R (°C/h)	Freezing rate, R (cm/h)
-23°C	Green bean 1	15.85	11.00-144.50	2.22	0.37	6.76	0.17
	Green bean 2	17.28	16.66-146.66	2.16	0.36	6.95	0.17
	Green bean 3	14.14	7.50-84.00	1.27	0.27	11.82	0.22
	Green bean 4	15.36	7.66-69.66	1.03	0.42	14.57	0.35
							R_{ave}= 0.23
-26°C	Green bean 1	16.05	8.66-74.33	1.09	0.28	13.76	0.26
	Green bean 2	15.57	9.33-82.33	1.21	0.37	12.40	0.31
	Green bean 3	13.86	6.83-65.50	0.97	0.39	15.46	0.31
							R_{ave}= 0.29
-27°C	Green bean 1	14.42	5.83-60.00	0.90	0.42	16.67	0.47
	Green bean 2	14.51	10.00-90.83	1.34	0.31	11.20	0.24
	Green bean 3	14.37	8.00-78.33	1.17	0.30	12.82	0.26
							R_{ave}= 0.32

3.2. Change of Quality Parameters During Frozen Storage

3.2.1 Ascorbic acid

Ascorbic acid is one of the most heat sensitive components in fruits and vegetables and widely used as a nutritional quality indicator of fruit and vegetable processing. Ascorbic acid is readily oxidized by ascorbic acid oxidase or under strong oxidation conditions. The reversible equilibrium occurs between ascorbic acid and dehydroascorbic acid in which dehydroascorbic acid irreversibly oxidizes to diketogluconic acid. Strawberries have a relatively high content of vitamin C, which is around 65-84 mg/ 100 g, according to Turkish Food Composition Database (http://www.turkomp.gov.tr/). This range for green beans is 12- 18 mg/100g. The natural variation in vitamin content in fruits and vegetables is very large in the literature. Vitamin levels depend on the plant cultivar, growing conditions, maturity of the edible portion, post-harvest handling and storage conditions.

The experimental data and L-ascorbic acid retention for strawberry and green bean were presented in Table 3.5 and Figures 3.2 and 3.3. The ascorbic acid contents of the fresh and blanched green beans were 23 and 16 mg/l00 g, respectively. Blanching of green bean reduced the ascorbic acid content of about 30 %. The losses during hot water blanching may be assumed to be due to leaching and thermal degradation of ascorbic acid to L-dehydro-ascorbic acid and further oxidation which was also stated in a study by Tosun and Yücecan (2008). Blanching significantly protects ascorbic acid in vegetables during frozen storage. The blanching conditions that eliminate lipoxygenase or peroxidase can be used for the protection of ascorbic acid. However, blanching conditions are very important. Steam blanching provide better protection of ascorbic acid.

L-ascorbic acid content of the fresh strawberries was 38 mg/100g. The ascorbic acid level was higher in both frozen green bean and strawberry at the end of 1st week. The reason for the higher L-ascorbic acid level in frozen sample was attributed to the easier extraction of L-ascorbic acid after freeze-thawing. Since, ice crystals formed as a result of freezing disrupted the cell wall thereby improving the extraction of L-ascorbic acid. At the end of the frozen storage, the ascorbic acid content of both strawberry and green bean maintained a level above that of fresh samples. The same pattern was observed in the study of Favell (1998) who found that level of ascorbic acid in quick-frozen product was equal to or much better than fresh product. The results of that study also showed that ascorbic acid content of frozen beans at the end of 12 months was higher than initial content of whole green bean. Another study by Baardseth et al. (2010) found a higher level of ascorbic acid in blanched/frozen green bean than raw green bean. However, total ascorbic acid level was fully retained during blanching and freezing. The reason for lower content in raw sample was attributed to oxidation of the ascorbic acid prior to analysis causing higher level of L-dehydro-ascorbic acid in raw sample.

Table 3.5 L-Ascorbic acid content of strawberries and green beans during frozen storage.

Weeks	L-Ascorbic acid (mg/100 g sample)	
	Strawberry	Green bean
Fresh	38.78±6.47e	23.57±1.00e
Blanched	N.A	16.18±0.75f
1	82.49±3.35a	36.63±0.64a
3	77.90±3.56a	34.48±0.70ab
5	71.64±0.93ab	32.97±1.04ab
7	61.61±0.61bc	32.49±2.14b
9	55.41±0.75cd	31.39±1.51bc
11	51.22±3.57cd	28.90±0.38cd
13	47.85±0.16de	26.59±0.14de

All data were presented as mean value of replications ± standard deviation.

N.A: Not applicable

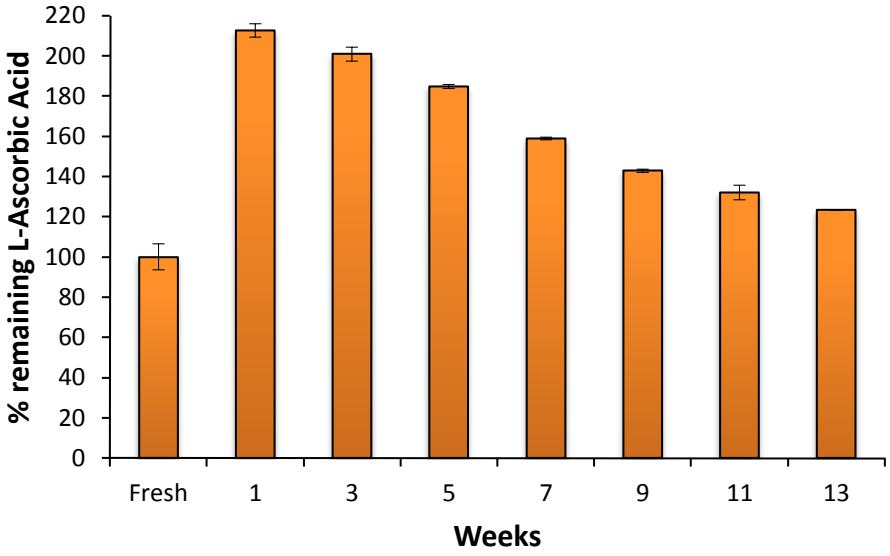

Figure 3.2 L-ascorbic acid content loss of strawberries through frozen storage. The error bars denote the standard deviation.

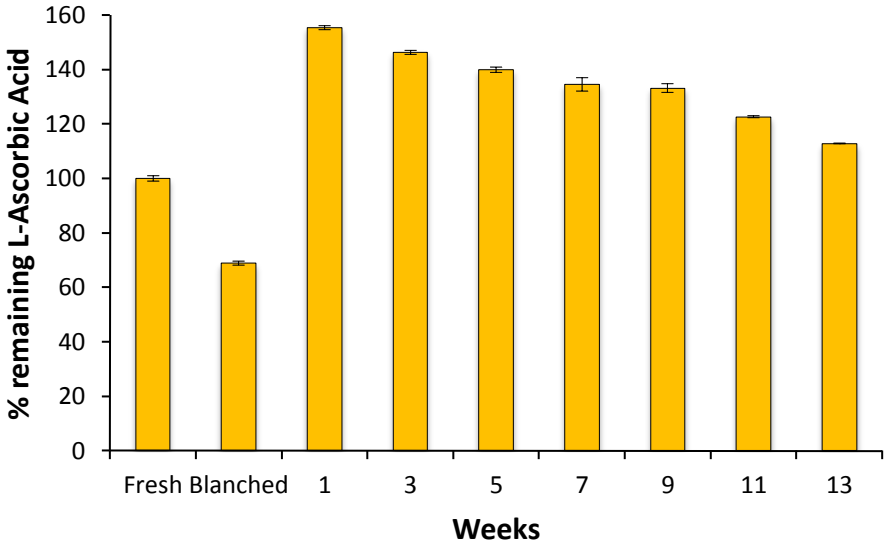

Figure 3.3 L-ascorbic acid content loss of green beans through frozen storage. The error bars denote the standard deviation.

In the study of Sahari et al. (2004), the effect of frozen storage temperatures on the vitamin C content of strawberries was examined. After 3 months of storage, loss of vitamin C was reported to be 64.5%, 10.7%, and 8.9% at -12, -18, and -24°C, respectively. The initial data was taken at 1st day after freezing. In the study of Martins and Silva (2003), significant loss of ascorbic acid was obtained while a negligible loss of L-dehydro-ascorbic acid (8%) was detected during 250 day storage of green beans at -7°C, -15°C and -30°C. The first measurement in their study was made immediately after freezing.

The average retention of ascorbic acid is expressed relatively to an initial, average value of 1st week of the frozen storage. Comparing the relative retentions of ascorbic acid, the deterioration rate of ascorbic acid during food processing follows first order kinetics. Ascorbic acid losses were approximately 42 and 27 % for 13 weeks frozen storage of strawberry and green bean, respectively. Ascorbic acid in green beans showed high stability ($k=0.0243$ week^{-1}, D-value= 95 week, $R^2 = 0.97$) compared to that of strawberry (k=0.0487 week-1, D-value= 47 week, $R^2 = 0.98$). The first week, the amount of ascorbic acid in strawberry and green bean was approximately two fold of that of the fresh samples. Optimum pH range for degradation of ascorbic acid is 3 to 4, and degradation rate is highly influenced by oxygen (Martins and Silva, 2003). Hence, lower pH degree of strawberry may be a reason of higher loss of ascorbic acid content in strawberry.

According to the study of Serpen et al. (2007) related to degradation kinetics of vitamin C in peas during frozen storage, the degradation of ascorbic acid is related to the balance of oxidation and reduction capacities. As a consequence of this study, it was reported that blanching has a significant effect on regeneration of dehydroascorbic acid to ascorbic acid and the oxidation of dehydroascorbic acid to diketogluconic acid. However, it does not have a leading effect on oxidation of ascorbic acid to dehydroascorbic acid.

Also, blanching significantly minimised the oxidation rate of dehydroascorbic acid into diketogluconic acid. Hence, retention of total vitamin C in peas increased during frozen storage. This increase was supposed to be due to elimination of oxidative enzymes and diminishing of atmospheric oxygen. As a result of this study, it is recommended that vitamin C content (L-ascorbic acid + L-dehydro-ascorbic acid) should be measured instead of L-ascorbic acid content.

3.2.2 Total Phenolic Content and Antioxidant Activity

Total phenolic content and antioxidant activity of strawberries and green beans were given in Table 3.6 and Figs. 3.4, 3.5, 3.6 and 3.7 during storage up to 3.5 months. Strawberry total phenolic content ranged between 168 mg GAE/ 100 g and 244 mg GAE/ 100 g in different studies (Poiana et al., 2010; Marinova et al., 2005). This value for green bean and yellow bean ranged between 35.5 -55.7 mg GAE/ 100 g (Marinova et al., 2005). Important phenolic compounds in strawberries are ellagic acid, ellagic acid glucoside, quercetin 3-glucoside, quercetin 3-gluronide and kaempferol 3-glucoside. Green beans contain high levels of flavonoids: quercetin, kaemferol, catechins, epicatechins, and procyanidins. The total phenolic content of fresh strawberry obtained in our study, was close to value of previous studies. However, fresh green bean variety (Sırık) showed relatively higher phenolic content than other studies. The variety, climatic conditions and harvest time are important parameters for total phenolic content.

Total phenolic content and antioxidant activity change of selected samples did not decrease a value lower than 80% during the frozen storage at -27°C. This decrease in phenolic content was thought to be due to polyphenol oxidase released from the plant cell wall during frozen storage which was also stated in a previous study by De Ancos et al. (2000).

Also increase in total phenolic content and antioxidant activity content is a well known concept as observed in this study during frozen storage of the fruits. This may be attributed to the cellular disruption caused by thawing of the fruit before the analysis which improved extraction of these compounds (De Ancos et al., 2000). Also, formation of the phenolics may occur after harvest through metabolism of phenolics which may lead to increase in phenolics during storage (Piljac- Zˇegarac and Sˇamec, 2011). Degradation of polyphenols is another reason of total phenolic content increase.

According to the study of Poiana et al (2010), the storage at -18°C up to 4 months did not have a significant effect on bioactive compounds. After a frozen storage time of 4 months, the decrease in antioxidant capacity was approximately 23% for strawberries. Phenolic content in frozen berries was reduced to 28-47% of its initial value after 10 moths of frozen storage. It is a long storage time since deep freeze storage time of fruits is generally maximum 6 months. Chaovanalikit and Wrolstad (2004) determined the total phenolics of the cherries during 6 months of frozen storage at -23^{o}C. As a result, 25% and 50% loss were reported after 3 and 6 months, respectively.

Berries are known to have high levels of phenols. In this study, strawberries showed higher total phenolic content and antioxidant activity in comparison to green beans. Since both the total phenolic content and antioxidant activity was approximately three times greater in strawberries when compared to green beans, this result may indicate that total phenolic content and antioxidant activity are in correlation with each other. However, individual fruit and vegetable sample did not show trend for total phenolic content similar to those for antioxidant activity. Since, different sample extracts and methods were used for antioxidant analysis. Nevertheless, the percentage of least and highest amount of total phenolic content and antioxidant activity were close to each other leading to the statement that no significant change was observed in total phenolics and antioxidant activity.

Our results are in accord with the studies of Gonzales et al. (2003) who studied on raspberry and blackberry varieties. They found that total phenolic and antiradical efficiency of blackberries was stable during 3 months of frozen storage at -24°C and declined thereafter and there was nearly 20% loss of phenols at the end of 12 months. However antiradical efficiency was stable during 12 months of frozen storage. The results of authors also showed that total phenolics and antiradical efficiency of raspberry varieties showed no significant change during 12 months of frozen storage.

Table 3.6 Total phenolic content (TPC) and antioxidant activities (AA) of strawberry and green bean during frozen storage at -27°C.

Weeks	Strawberry		Green bean	
	TPC (mg GAE/ 100 g fruit)	AA×10^4 (g DPPH˙/ g fruit)	TPC (mg GAE/ 100 g veg)	AA×10^4 (g DPPH˙/ g veg)
Fresh	210.14±12.40abc	4.77±0.09ab	69.16±12.76a	1.63±0.01a
1	213.15±6.53abc	4.30±0.25b	67.95±1.54a	1.85±0.20a
2	187.92±10.81bc	5.36±0.07a	63.31±1.52a	1.83±0.15a
3	214.07±7.10abc	4.38±0.19b	74.45±4.19a	1.89±0.04a
4	183.25±8.26c	5.20±0.25a	63.15±3.54a	1.49±0.22a
6	227.05±5.84a	4.27±0.19b	63.98±1.78a	1.50±0.23a
8	208.10±12.10abc	5.34±0.26a	71.67±5.01a	1.59±0.10a
10	208.57±17.44abc	4.87±0.30ab	76.70±6.39a	1.87±0.20a
12	216.78±13.51ab	4.35±0.29b	70.49±12.24a	1.77±0.23a
14	226.09±6.59a	5.31±0.26a	72.38±1.47a	1.82±0.10a

All data were presented as mean value of 3 replications ± standard deviation.

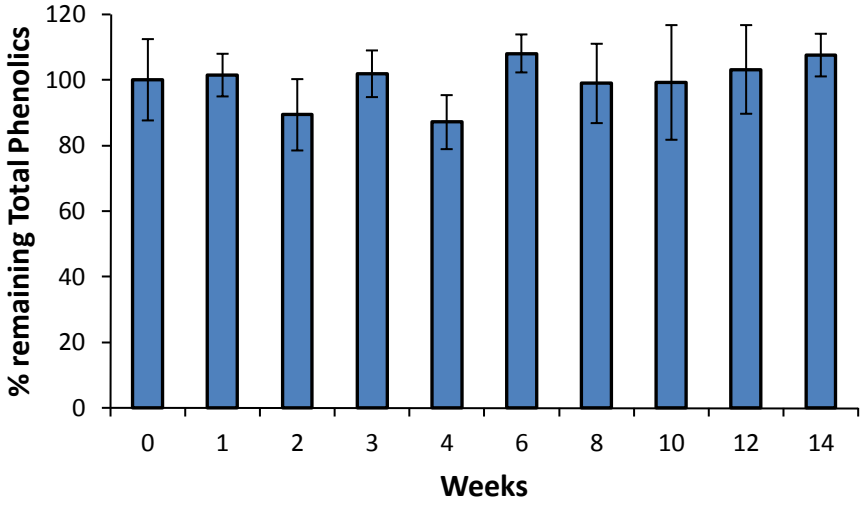

Figure 3.4 Total phenolic content retention (%) of strawberries through frozen storage. The error bars denote the standard deviation.

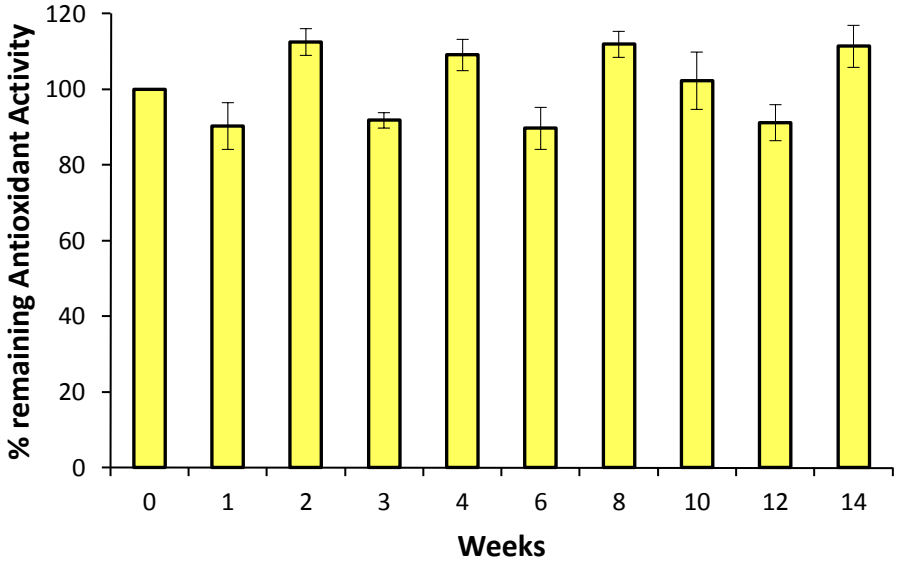

Figure 3.5 Antioxidant activity retention of strawberries through frozen storage. The error bars denote the standard deviation.

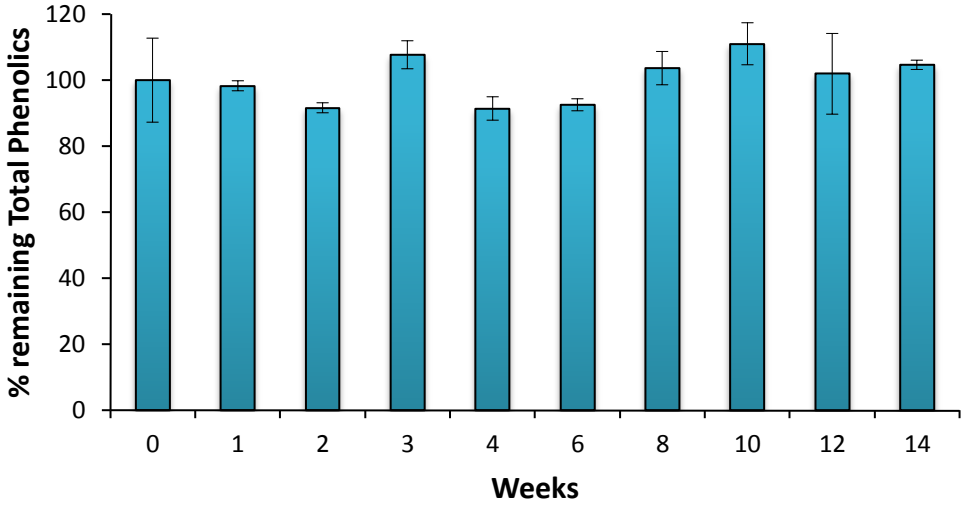

Figure 3.6 Total phenolic content retention of green beans through frozen storage. The error bars denote the standard deviation.

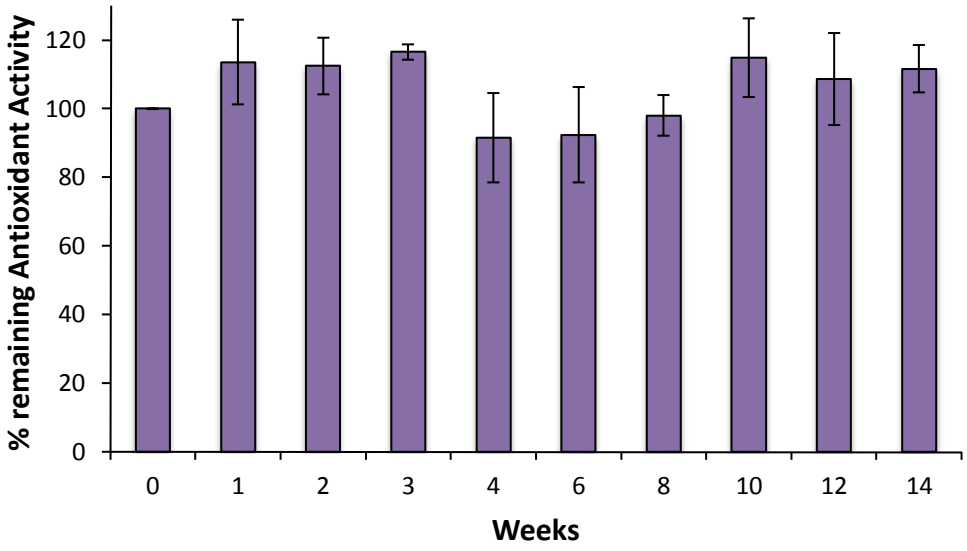

Figure 3.7 Antioxidant activity retention of green beans through frozen storage. The error bars denote the standard deviation.

3.2.3 Effects on Color

Color exhibits a paramount role in evaluation of food quality due to its effect on consumers' choice. Color was measured when the samples were partially thawed (approximately 15-20 min) to prevent drip loss and clear appearance. Chromatic changes of strawberry and green bean during frozen storage were shown in Tables 3.7 and 3.8. Total color changes (ΔE), which represent the magnitude of the color difference between fresh and stored samples, are shown in Figure 3.8. The color change of fruits is generally because of the destruction of anthocyanins causing rise in polymeric color. Since, monomeric anthocyanins convert into polymeric form.

Frozen storage did not lead to significant changes. The total color of strawberry showed a minor change at first week of frozen storage when compared to fresh sample, and then it maintained a stable value. Among color parameters, decrease in lightness (L*) and an increase in a* (greenness/redness) value were characteristic color changes which led to a higher value (ΔE) in frozen strawberry and green bean compared to fresh sample. For the green bean sample, the blached green bean was taken as control. In the study of Holzwarth et al. (2012), conventionally frozen (-20°C) and thawed strawberries exhibited higher a* and b* values and lower L* values compared to cryogenically (liquid nitrogen) frozen berries. Higher a* values were attributed to pigment diffusion from the center of the fruit to the outmost cell layers because of disrupted cell walls. Compared to conventionally frozen fruits, cryogenically frozen strawberries exhibited markedly higher L* values, which might be a result of small ice crystal formation on the fruit surface, as previously reported (Zaritzky et al, 1982).

Frozen vegetables are subjected to color modifications which take place during blanching and/or during frozen storage. In this study, total color change in green beans did not significantly change during frozen storage. The chlorophylls are responsible for the green color of many vegetables.

Conversion of chlorophylls to pheophytins and pyropheophytins cause color change. According to the study of Bahçeci et al. (2005), blanching at 70 °C for 2 min resulted in a decrease, while blanching at 90 °C for 3 min an increase in the half-life of chlorophyll during frozen storage. Another type of color deterioration is the removal of the phytol chain and formation of chlorophyllide from chlorophyll. According to the study of Martin and Silva (2002), chlorophyll and color loss at high storage temperatures are mainly attributed to pheophytisation. At lower storage temperatures, color is stabilized probably by the formation of metal–chlorophyll compounds and chlorophyll content does not give a reliable prediction of color retention.

Table 3.7 L* a* b* and total color change (ΔE) values of strawberries through frozen storage.

Weeks	L*	a*	b*	ΔE
Fresh (control)	41.11±2.49	21.04±1.29	8.55±1.44	64.39±0.50b
1	32.26±0.72	23.01±3.09	10.79±1.52	72.52±1.80a
2	36.68±0.66	22.12±1.00	8.73±0.78	67.32±0.45ab
3	34.47±4.66	22.57±3.78	9.76±4.89	73.05±1.98a
4	35.58±3.24	22.34±3.85	9.25±5.01	73.42±0.49a
6	31.23±1.43	24.29±1.45	9.70±0.05	73.54±1.82a
8	33.80±6.07	24.51±0.77	10.56±6.26	73.26±3.96a
10	29.67±1.73	27.24±4.50	9.85±0.52	73.31±2.88a
12	28.45±1.15	22.03±3.15	8.50±3.93	75.57±0.81a

All data were presented as mean value of 2 replications ± standard deviation.

Table 3.8 L* a* b* and total color change (ΔE) values of green beans through frozen storage.

Weeks	L*	a*	b*	ΔE
Blanched (control)	54.63±1.77	-14.47±0.99	30.92±2.16	56.81±1.96b
1	47.14±3.19	-15.37±1.76	25.22±5.84	60.75±0.09ab
2	47.99±2.01	-13.04±3.55	19.98±5.49	57.34±4.54b
3	39.92±1.65	-15.14±1.30	23.43±4.52	66.12±0.17a
4	54.52±1.00	-17.22±0.55	33.88±2.16	59.63±0.52ab
6	48.07±1.00	-15.43±0.16	26.30±1.00	60.25±0.35ab
8	52.00±1.15	-16.21±2.78	31.69±3.05	62.59±0.83ab
10	45.19±1.12	-16.46±2.02	26.76±2.15	62.03±0.04ab
12	46.36±1.00	-16.40±1.00	26.56±2.52	63.43±0.60ab

All data were presented as mean value of 2 replications ± standard deviation.

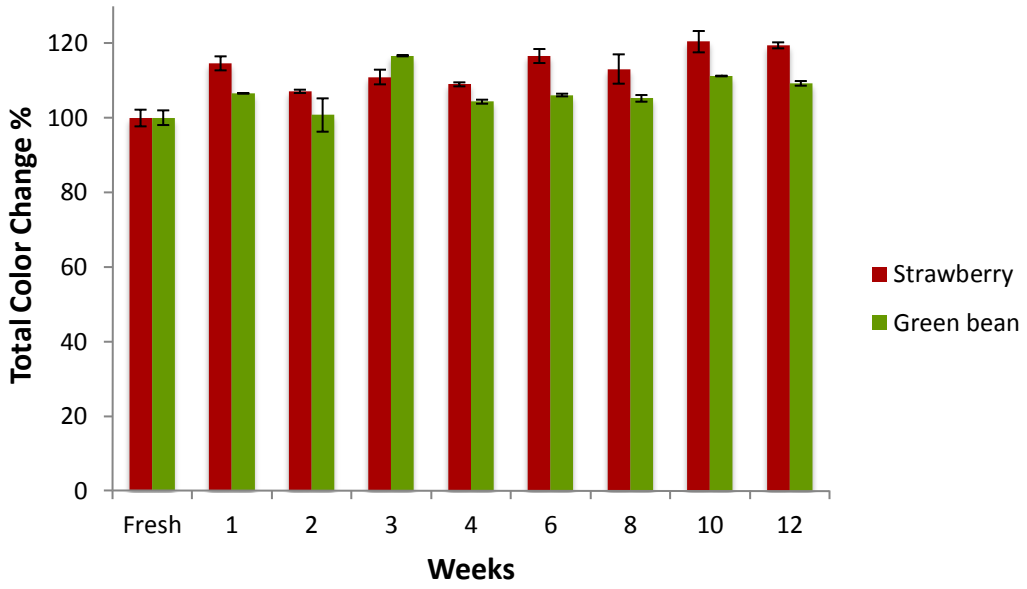

Figure 3.8 Total color change of strawberries and green beans through frozen storage. The error bars denote the standard deviation.

3.2.4 Effects on Cell Integrity

The basis of NMR relaxometry technique is nuclear magnetism and this magnetism stems from the spins of nucleons. For the analysis, NMR tube with the food sample inside is located into a large static magnetic field to acquire signal. In addition to this static magnetic field, a RF (radio frequency) pulse is applied and so a temporary disturbance on the sample is obtained. Thus, the relaxation of excited signal is observed to obtain diverse information about the food sample. We used NMR relaxometry based on T2 relaxation time (also known as spin–spin relaxation time). T2 is obtained by an exponentially decreasing signal curve. T2 time is related to energy transfer between surrounding spins, and is thought to be shorter for closer proximity between molecules. Therefore, T2 is shortest in solids. Since, molecules are packed closely leading to a higher energy transfer efficiency between spins. T2 relaxometry measurements along with relaxation spectra give information about physical properties of water such as quantity of water and interactive relation of water with the neighbouring compounds such as proteins or carbohydrates (Hashemi et al., 2010; Kırtıl et al., 2014; Bernstein et al., 2004; Zhang and McCharty, 2012).

T2 relaxometry was utilised in previous studies to find out the effect of freezing, drying, ripening and high pressure on the physiological change at sub-cellular level in apple, avocado, tomato, strawberry, banana, and onion (Ersus et al., 2010; Hills and Remigereau, 1997; Marigheto et al., 2005, 2009; Raffo et al., 2005). The change in spin–spin (T2) relaxation time value may be explained by the loss of moisture (Zhang and McCarthy, 2012). In addition to water content, T2 relaxation times are usually associated with physical properties of water, and interaction of water with macromolecules (Van As, 1992). Any change in the state and mobility of water (such as freezing) is expected to cause a change in T2 relaxation time of sample.

Water compartments in a plant cell have different relaxation times. Freezing and thawing may affect water compartmentation. Because, the water compartments do not freeze and thaw at the same temperature (Hills and Remigereau, 1997). Different proton transverse relaxation times may characterise the distribution of water in subcellular organelles (Belton and Ratcliffe, 1985).

T2 (spin–spin relaxation time) measurements were performed on strawberry and green bean at freezing stages (pre-freezing, freezing, and sub-freezing stage) for three freezing rates obtained at -23˚C, -26˚C and -27˚C. Also, T2 relaxation times of fresh and frozen-thawed samples were measured. Inversion of T2 value data resulted in relaxation spectrum. Comparison of T2 times and relative areas (RA) of fresh and frozen-thawed samples led to estimation of the level of cellular disruption in food tissue. Average T2 relaxation times at different freezing stages of strawberries and green beans for 3 freezing rates were shown in tables (Tables 3.9 and 3.10).

Table 3.9 Average T2 relaxation times at freezing stages of strawberries for 3 freezing rates.

T_f	-23°C		-26°C		-27°C	
	T(°C)	T2 (ms)	T(°C)	T2 (ms)	T(°C)	T2 (ms)
Fresh (Control)	22	581.78	22	553.91	22	553.29
Pre-freezing	2.9	466.33	3	509.16	5	535.86
Freezing	-1.5	228.05	-1.5	323.46	-1.6	476.88
Subfreezing	-12.5	123.31	-14.6	135.01	-15	117.77
Frozen	-23.2	85.22	-26	92.8	-27	75.63
Thawed	18	214.85	18	204.04	18	220.78

T_f : Freezer temperature corresponding to three freezing rates (-23°C, -26°C, -27°C)
T(°C): Sample temperature

Table 3.10 Average T2 relaxation times at different freezing stages of green beans for 3 freezing rates.

T_f	-23°C		-26°C		-27°C	
	T(°C)	T2 (ms)	T(°C)	T2 (ms)	T(°C)	T2 (ms)
Fresh (Control)	22	332.12	22	351.82	22	367.7
Pre-freezing	2.5	345.96	1.8	364.74	3.5	391.17
Freezing	-3.8	206.48	-3.8	291.33	-3.8	320.12
Subfreezing	-12	64.29	-14	67.3	-13	99.9
Frozen	-23.2	62.33	-26	61.37	-27	85.63
Thawed	20	284.91	20	206.60	20	204.29

T_f : Freezer temperature corresponding to three freezing rates (-23°C, -26°C, -27°C)
T(°C): Sample temperature

T2 relaxation times decreased significantly during freezing of strawberry at all freezing rates. In contrast to strawberry, T2 relaxation of green bean first increased in pre-freezing stage but then continued to decrease till subfreezing stage. According to these results, the freezing process with average T2 values was observed. During freezing process, temperature is decreasing and water is turning into ice crystals thereby affecting T2 value. Thus, it is clear that the decrease in water content along with decrease in temperature due to freezing resulted in lower relaxation degrees.

Multi-exponential inversion using the Non-Negative Least Squares (NNLS) method of the T2 data gives 1D NMR T2 relaxation spectrum. A spectrum of fresh green bean sample is shown in Fig. 3.8 as a representative. Relaxation spectrum differs in food systems. This divergence remarks the changes associated with proton. For example, new proton pools may be revealed due to proton exchange among the subcellular regions of the food, change in quantitiy of water and physiological events taking place in food. These proton pools are associated with water compartments inside viable cells which were displayed by a total of 3 peaks in relaxation spectra. These peaks refer to distinct water compartments (Belton and Capozzi, 2011).

In Tables 3.11 and 3.12, the spin–spin relaxation times (T2) and the percent relative areas (RA) that equal to each peak in the relaxation spectrum (Fig. 3.8) were presented. Magnitude of the signal intensity deriving from the proton pools is calculated which yields the relative area. Relative area implies the contribution of that cellular component to the whole signal (Oztop et al., 2010; Kırtıl et al., 2014).

Table 3.11 Average T_2 relaxation times and percent relative areas (RA) of green bean samples after freezing at -23°C, -26°C and -27°C.

T_f		Fresh-cut		Frozen-thawed (20°C)	
		T2 (ms)	RA (%)	T2 (ms)	RA (%)
	Peak 1	6.1	3.3		
-23°C	Peak 2	96	12.81	72	17.27
	Peak 3	360	77.2	310	77.05
	Peak 1	5.7	1.2		
-26°C	Peak 2	50	15.63	38	15.78
	Peak 3	380	76.06	210	75.87
-27°C	Peak 1	7.1	2.42		
	Peak 2	62	13.65	43	13.89
	Peak 3	380	77.34	210	78.47

T_f: Freezer temperature corresponding to three freezing rates (-23°C, -26°C, -27°C).

Table 3.12 Average T_2 relaxation times and percent relative areas (RA) of strawberry samples after freezing at -23°C, -26°C and -27°C.

		Fresh-cut		Frozen-thawed (18°C)	
T_f		T2(ms)	RA (%)	T2(ms)	RA (%)
	Peak 1	4.6	0.05		
-23°C	Peak 2	96	6.6	13	1.23
	Peak 3	590	90.08	210	92.93
	Peak 1	4.9	0.08		
-26°C	Peak 2	40	5.11	11	1.33
	Peak 3	550	87.83	200	90.44
-27°C	Peak 1	5.7	0.37		
	Peak 2	62	5.49	7.6	3.2
	Peak 3	550	89.18	210	90.2

T_f: Freezer temperature corresponding to three freezing rates (-23°C, -26°C, -27°C)

According to Fig. 3.9, Tables 3.11 and 3.12, peak 2 which has a small RA and short T2 was assigned to the protons that connect with the hard constituents of the cell wall. The water may be present in little pores of cell wall. The surrounding matrix and water-holding sites clutch this water leading to close proximity between water and neighbouring molecules and restricted water mobility. Due to these reasons, proton exchange increases between the molecules leading to decrease in T2 value (Black & Pritchard, 2002; Kırtıl et al., 2014). The 3rd peak with longer T2 times and high %RAs is most likely coming from water molecules that have a higher mobility than the ones entrapped inside the cell wall. Most of the water in plant cells is stored inside the vacuoles and cytoplasm. Thus, this compartment is assigned to free water of the cells either inside the vacuoles or the cytoplasm. Since T2 values of the first peak in fresh bean and strawberries are too short, this peak cannot be associated with a water compartment.

This value is close to the T2 relaxation times of solids (Hashemi et al., 2010). Therefore, the first peak may be assigned to protons related to starch or protein molecules.

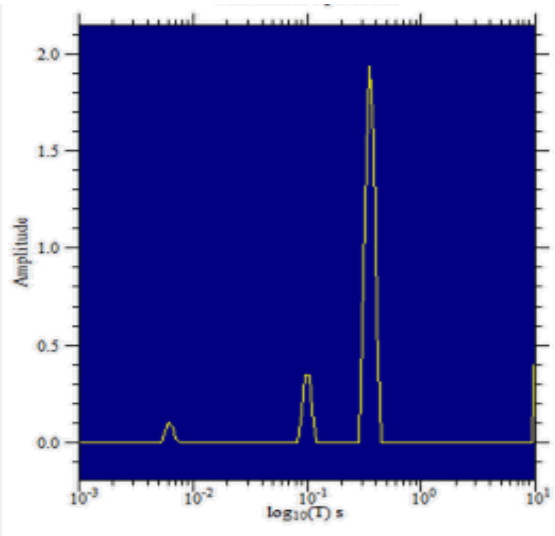

Figure 3.9 A representative 1D NMR spectrum of a fresh green bean sample.

In all freezing rates, there were 3 peaks in the relaxation spectrum before freeze-thawing which then decreased to 2 after thawing (Tables 3.11 and 3.12). The decrease in the number of peaks may point out the dissipation of the compartmentalisation of food cells (Kırtıl et al., 2014).

In frozen-thawed green beans, Peak 1 disappeared and T2 value of peak 2 slightly decreased for all freezing rates. Relative area of peak 2 (cell wall) slightly increased in slowest freezing rate (-23°C). The increase in RA may indicate the growth in size for that cellular compartment (Kırtıl et al., 2014). The increase in RA of peak 2 in slowest freezing rate may indicate a disruption in cell structure.

Besides, water soluble solids of peak 1 may penetrate into cell wall leading to increase in RA and being dissolved in cell wall component, this could reduce the T2 value of peak 2. This also explains the disappearance of peak 1, after the freezing stage. For other freezing rates, RA of cell wall peak of frozen-thawed vegetable was consistent with fresh sample. Hence, the cell wall appears to be preserved although there are some minor changes in T2 value and RA. T2 value of peak 3 decreased for all treatments; however RAs were not different from fresh sample. Still, the similarity of NMR relaxation spectrum of frozen-thawed vegetable with the fresh may suggest the little effect of higher freezing rates (-26°C and -27°C) on the cellular structure of green beans.

As for strawberries, there were significant decreases in T2 values and RAs of cell wall peak in all freezing rates. The decrease in both T2 value and RA is an indication of loss of water which may be due to drip loss after thawing. Also, the reduction in RA clearly indicated the disruption in cell wall component in all treatments. The fact that whether the cell organelles maintain a fresh texture after thawing depends on the formation and distribution of ice crystals in cellular tissue during freezing stage (Hills and Remigereau, 1997). Large ice crystals formed as a result of slow freezing may have caused the damage in cell wall as mentioned before. However, though damaged fastest freezing rate (-27°C) displayed the highest RA value (peak 2) meaning a lower disruption in cell wall compared to other freezing rates (-23°C and -26°C). There were slight increases in RAs of peak 3 which may indicate the increase in cytoplasm water content coming from disrupted cell wall component and penetrating into cytoplasm. Also, the significant decrease in T2 value of peak 3 without significant change in RA may indicate that water moves out from that cellular compartment (cytoplasm or vacuole), but still is present in extracellular space, since a signal (RA) is still being received from the compartments.

Cell turgor pressure is associated with the quantitiy of water in vacuole and textural characteristics of the food suffer a change due to loss of turgor pressure (Waldron et al., 1997). However, cell wall component is the most important parameter in assesing the cell integrity. As a result of slow freezing, the degree of structural changes such as membrane disruption, shrinkage of the cell and loss of water holding capacity were higher in strawberries if compared with that of green beans.

NMR relaxometry provides the non-destructive analysis of the freezing-thawing attitude of food cell components (Hills and Remigereau, 1997). The degree of cellular damage may be understood with interpretation of the relaxation spectrum. Therefore, NMR analysis could be used for further studies to see the effect of higher freezing rates on cell integrity.

CHAPTER 4

CONCLUSION AND RECOMMENDATIONS

Consumption of frozen fruits and vegetables has been increasing due to ease of preparation, time saving and little change in nutrient content when the freezing rate and storage temperature is well-maintained. Some of consumers prefer to prepare frozen fruits and vegetables at home rather than purchasing from market. Therefore, home freezing should be done appropriately to preserve the nutritional content and texture of fruits and vegetables. Long term frozen storage is not recommended for food products in frost-free home freezers. Since, these freezeres undergo warming cycles to defrost coils thereby warming up food products stored inside freezer. Beside this one, home freezers provide slow freezing rates as determined also in this study. Freezing rates of strawberries and green beans were estimated by setting the temperature of the home freezer to -23, -26 and -27 °C in a home-type freezer. Between the freezer temperatures tested, -27°C provided the highest freezing rate in the selected conditions; therefore this temperature was selected for freezing and frozen storage of the samples.

In this study, we also aimed to show the effect of selected freezing and frozen storage condition on some quality parameters of selected fruit and vegetable that the selected quality parameters can be further used to design and development of home freezers. During a storage period of 90 days at -27°C, a slight change was obtained in the total phenolic content, antioxidant activity and color with respect to the results obtained in the 1st week of frozen storage.

In all freezing rates studied, degradation in cell integrity was observed especially in the fruit sample due to large ice crystal formation as shown by NMR relaxometry analysis of selected fruit and vegetable. Higher freezing rates and frozen storage of food samples at a temperature below -18°C could be an efficient for freezing preservation of fruits and vegetables due to small ice crystals to protect textural quality attributes. To achieve a better textural quality, thawing rates should also be analyzed.

As a recommendation, to comprehend the effect of freezing rate on fruit and vegetables, a variety of foods can be examined with an industrially designed home freezer providing higher freezing rates, along with other food product types such as meats.

The approximations and analyzes used in this study, can be a base to design and produce a convenient deep freezer systems with two compartment in a home type freezer, in which a high freezing rate to obtain a large number of small size ice crystal is possible for producing frozen fruits and vegetables for home usage, by examining the effect of freezing rate and frozen storage temperatures on the selected representative samples. The freezer with two compartments may be produced: in one compartment the quick freezing will be performed and in the second compartment the storage of the frozen samples will be held. The textural, nutritional and sensory changes in the samples frozen by cold air stream at different freezing rates (0,2-5 cm /h) and stored at different temperatures (-18, -24 and -30°C) can be analyzed.

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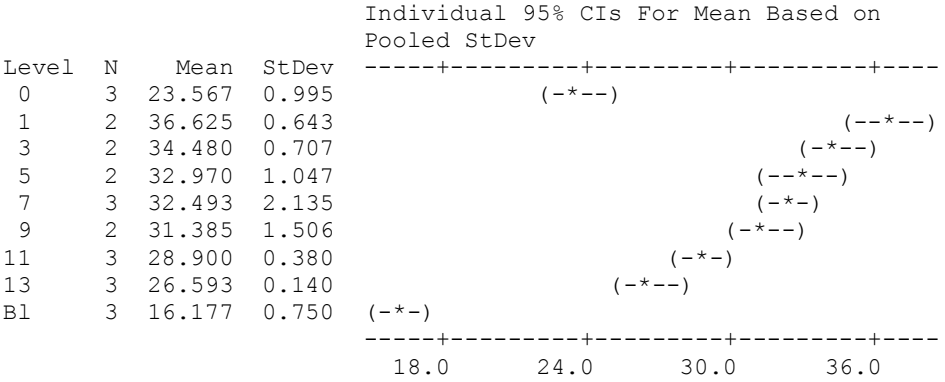
APPENDIX A

STATISTICAL ANALYSES

Table A.1 One way ANOVA and Tukey's Comparison Test for L-ascorbic acid content of green bean samples through frozen storage time of 13 weeks.

One-way ANOVA: L-ascorbic acid versus Time

```
Source  DF      SS      MS      F      P
Time     8  847.91  105.99  88.19  0.000
Error   14   16.83    1.20
Total   22  864.74

S = 1.096   R-Sq = 98.05%   R-Sq(adj) = 96.94%
```




```
Pooled StDev = 1.096

Grouping Information Using Tukey Method

Time  N    Mean  Grouping
 1    2  36.625  A
 3    2  34.480  A B
 5    2  32.970  A B
 7    3  32.493    B
 9    2  31.385    B C
11    3  28.900      C D
13    3  26.593        D E
```

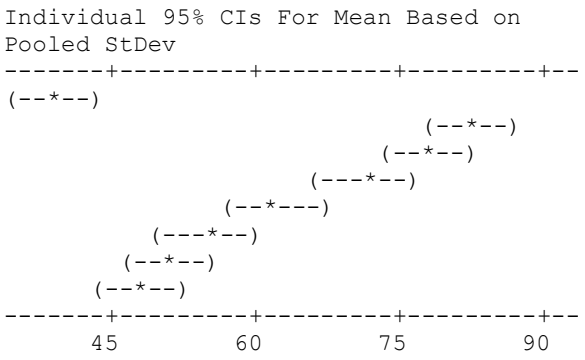
Table A.1 (Continued)

```
 0    3  23.567          E
Bl    3  16.177            F

Means that do not share a letter are significantly different.
```

Table A.2 One way ANOVA and Tukey's Comparison Test for L-ascorbic acid content of strawberry samples through frozen storage time of 13 weeks.

One-way ANOVA: L-ascorbic acid versus Time

```
Source  DF      SS     MS      F      P
Time     7  4816.0  688.0  56.34  0.000
Error   13   158.7   12.2
Total   20  4974.7

S = 3.494   R-Sq = 96.81%   R-Sq(adj) = 95.09%
```

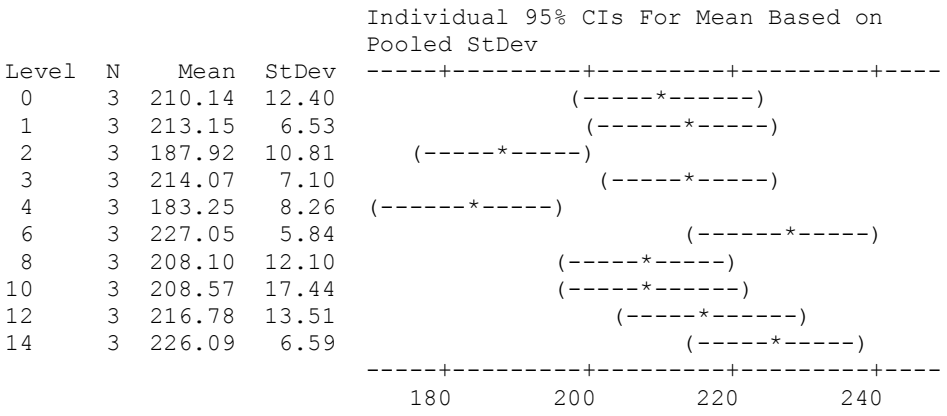



```
Pooled StDev = 3.494

Grouping Information Using Tukey Method

Time  N    Mean  Grouping
 1    3  82.490  A
 3    3  77.900  A
 5    2  71.645  A B
 7    2  61.615    B C
 9    2  55.410      C D
11    3  51.220      C D
13    3  47.847        D E
 0    3  38.777          E

Means that do not share a letter are significantly different.
```

Table A.3 One way ANOVA and Tukey's Comparison Test for Total Phenolic content of strawberry samples through frozen storage time of 14 weeks.

One-way ANOVA: Total Phenolic Content versus Time

```
Source  DF    SS   MS     F      P
Time     9  5484  609  5.34  0.001
Error   20  2284  114
Total   29  7769

S = 10.69   R-Sq = 70.59%   R-Sq(adj) = 57.36%
```




```
Pooled StDev = 10.69


Grouping Information Using Tukey Method

Time  N    Mean  Grouping
 6    3  227.05  A
14    3  226.09  A
12    3  216.78  A B
 3    3  214.07  A B C
 1    3  213.15  A B C
 0    3  210.14  A B C
10    3  208.57  A B C
 8    3  208.10  A B C
 2    3  187.92    B C
 4    3  183.25      C

Means that do not share a letter are significantly different.
```

Table A.4 One way ANOVA and Tukey's Comparison Test for Total Phenolic content of green bean samples through frozen storage time of 14 weeks.

One-way ANOVA: Total Phenolic Content versus Time

```
Source       DF      SS    MS     F      P
Time          9   576.0  64.0  1.56  0.194
Error        20   818.7  40.9
Total        29  1394.7

S = 6.398   R-Sq = 41.30%   R-Sq(adj) = 14.88%


                          Individual 95% CIs For Mean Based on Pooled StDev
Level  N    Mean   StDev     +---------+---------+---------+---------
 0     3  69.159  12.762           (--------*---------)
 1     3  67.951   1.539         (---------*---------)
 2     3  63.310   1.520     (--------*---------)
 3     3  74.451   4.192                 (---------*---------)
 4     3  63.983   1.775     (---------*---------)
 6     3  63.983   1.775     (---------*---------)
 8     3  71.671   5.012               (---------*--------)
10     3  76.704   6.387                     (---------*---------)
12     3  70.486  12.241              (---------*---------)
14     3  72.378   1.470                 (--------*---------)
                          +---------+---------+---------+---------
                         56.0      64.0      72.0      80.0

Pooled StDev = 6.398


Grouping Information Using Tukey Method


Time    N    Mean  Grouping
10      3  76.704  A
 3      3  74.451  A
14      3  72.378  A
 8      3  71.671  A
12      3  70.486  A
 0      3  69.159  A
 1      3  67.951  A
 6      3  63.983  A
 4      3  63.983  A
 2      3  63.310  A
```

Table A.5 One way ANOVA and Tukey's Comparison Test for Antioxidant Activity of strawberry samples through frozen storage time of 14 weeks.

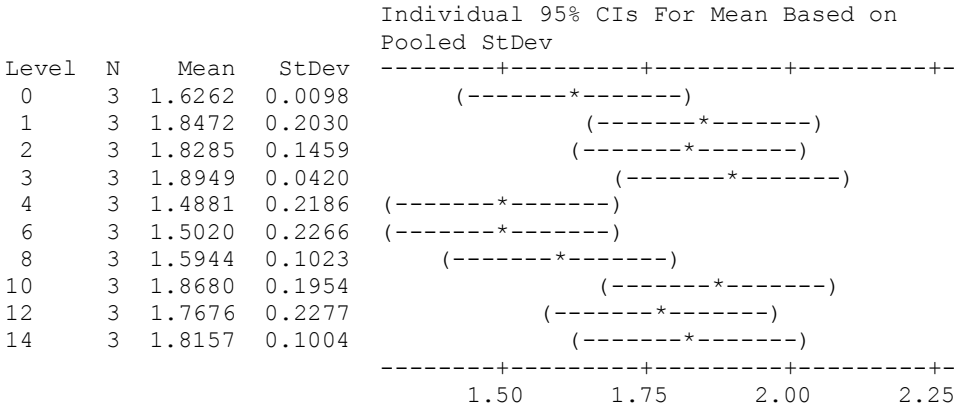
One-way ANOVA: Antioxidant Activity versus Time

```
Source  DF      SS      MS      F      P
Time     9  5.8332  0.6481  12.85  0.000
Error   20  1.0087  0.0504
Total   29  6.8419

S = 0.2246   R-Sq = 85.26%   R-Sq(adj) = 78.62%


                          Individual 95% CIs For Mean Based on Pooled StDev
Level  N    Mean   StDev    +---------+---------+---------+---------
 0     3  4.7674  0.0885              (----*-----)
 1     3  4.2995  0.2423     (----*----)
 2     3  5.3615  0.0682                          (----*-----)
 3     3  4.3779  0.1841      (-----*----)
 4     3  5.2003  0.2495                       (----*----)
 6     3  4.2714  0.1827    (----*-----)
 8     3  5.3395  0.2555                         (-----*----)
10     3  4.8724  0.2984                 (----*-----)
12     3  4.3474  0.2851      (----*----)
14     3  5.3106  0.2607                         (----*-----)
                          +---------+---------+---------+---------
                         4.00      4.50      5.00      5.50

Pooled StDev = 0.2246


Grouping Information Using Tukey Method

Time  N    Mean  Grouping
 2    3  5.3615  A
 8    3  5.3395  A
14    3  5.3106  A
 4    3  5.2003  A
10    3  4.8724  A B
 0    3  4.7674  A B
 3    3  4.3779    B
12    3  4.3474    B
 1    3  4.2995    B
 6    3  4.2714    B

Means that do not share a letter are significantly different.
```

Table A.6 One way ANOVA and Tukey's Comparison Test for Antioxidant Activity of green bean samples through frozen storage time of 14 weeks.

One-way ANOVA: Antioxidant Activity versus Time

```
Source  DF      SS      MS     F      P
Time     9  0.6528  0.0725  2.65  0.034
Error   20  0.5482  0.0274
Total   29  1.2010

S = 0.1656   R-Sq = 54.36%   R-Sq(adj) = 33.81%
```



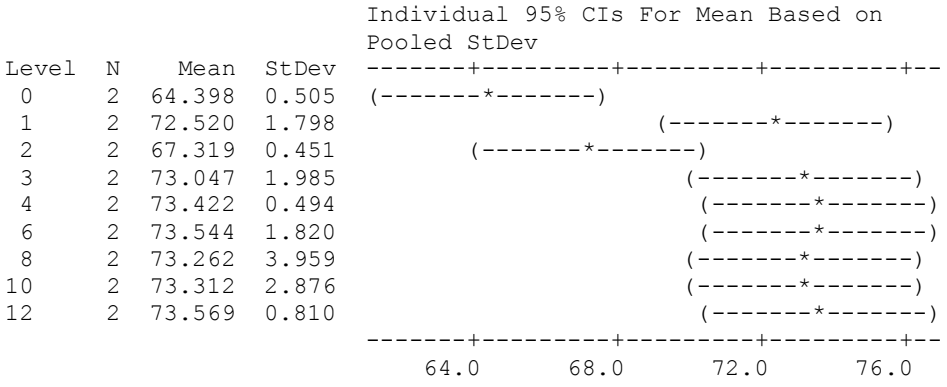

```
Pooled StDev = 0.1656
```

```
Grouping Information Using Tukey Method

Time  N    Mean  Grouping
 3    3  1.8949  A
10    3  1.8680  A
 1    3  1.8472  A
 2    3  1.8285  A
14    3  1.8157  A
12    3  1.7676  A
 0    3  1.6262  A
 8    3  1.5944  A
 6    3  1.5020  A
 4    3  1.4881  A
```

Table A.7 One way ANOVA and Tukey's Comparison Test for Total Color Change of strawberry samples through frozen storage time of 12 weeks.

One-way ANOVA: Total Color Change versus Time

```
Source  DF      SS     MS     F      P
Time     8  179.60  22.45  5.65  0.009
Error    9   35.78   3.98
Total   17  215.39

S = 1.994   R-Sq = 83.39%   R-Sq(adj) = 68.62%
```




```
Pooled StDev = 1.994
```

```
Grouping Information Using Tukey Method

Time  N    Mean  Grouping
12    2  73.569  A
 6    2  73.544  A
 4    2  73.422  A
10    2  73.312  A
 8    2  73.262  A
 3    2  73.047  A
 1    2  72.520  A
 2    2  67.319  A B
 0    2  64.398    B

Means that do not share a letter are significantly different.
```

Table A.8 One way ANOVA and Tukey's Comparison Test for Total Color Change of green bean samples through frozen storage time of 12 weeks.

One-way ANOVA: Total Color Change versus Time

```
Source  DF      SS     MS     F      P
Time     8  142.93  17.87  5.46  0.010
Error    9   29.43   3.27
Total   17  172.36

S = 1.808   R-Sq = 82.93%   R-Sq(adj) = 67.75%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev  ------+---------+---------+---------+---
 0     2  56.540  2.701  (------*-------)
 1     2  60.748  0.089             (------*------)
 2     2  57.337  4.544    (------*-------)
 3     2  66.120  0.170                          (------*-------)
 4     2  59.634  0.517           (------*------)
 6     2  60.250  0.353            (-------*------)
 8     2  62.588  0.831                  (------*-------)
10     2  62.031  0.044                 (------*------)
12     2  63.425  0.601                    (-------*------)
                          ------+---------+---------+---------+---
                             56.0      60.0      64.0      68.0

Pooled StDev = 1.808


Grouping Information Using Tukey Method

Time  N    Mean  Grouping
 3    2  66.120  A
12    2  63.425  A B
 8    2  62.588  A B
10    2  62.031  A B
 1    2  60.748  A B
 6    2  60.250  A B
 4    2  59.634  A B
 2    2  57.337    B
 0    2  56.540    B

Means that do not share a letter are significantly different.
```

APPENDIX B

COOLING CURVES

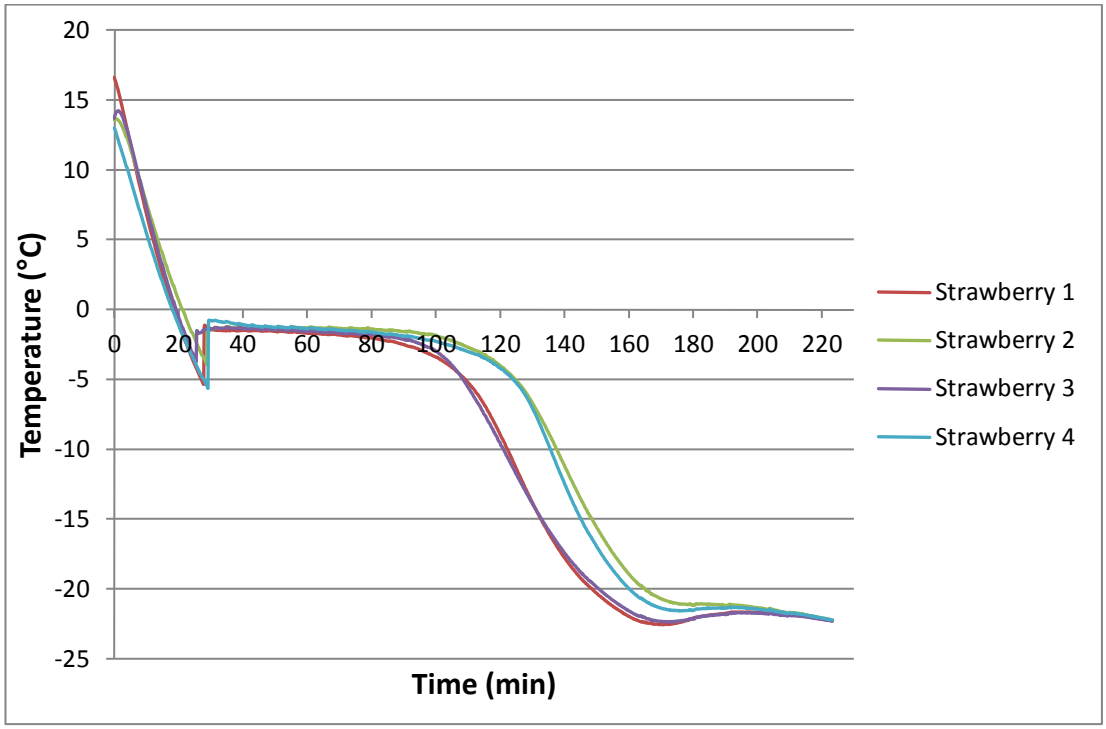


Figure B.1 Cooling curve of non- packaged strawberries frozen at -23°C.

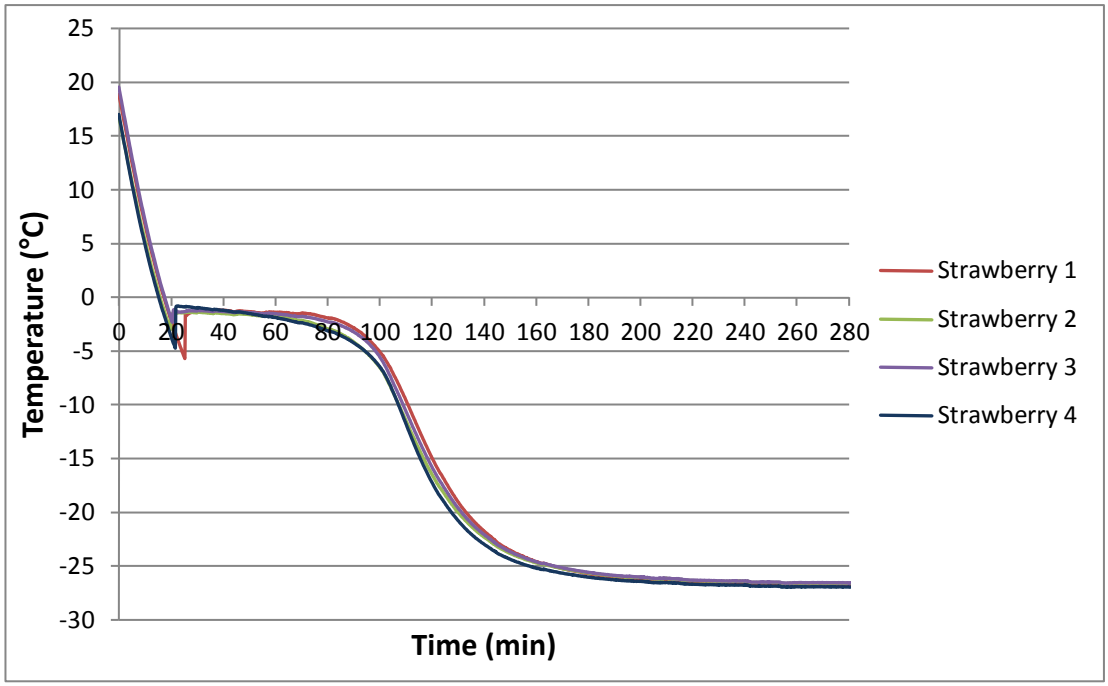


Figure B.2 Cooling curve of non-packaged strawberries frozen at -26°C.

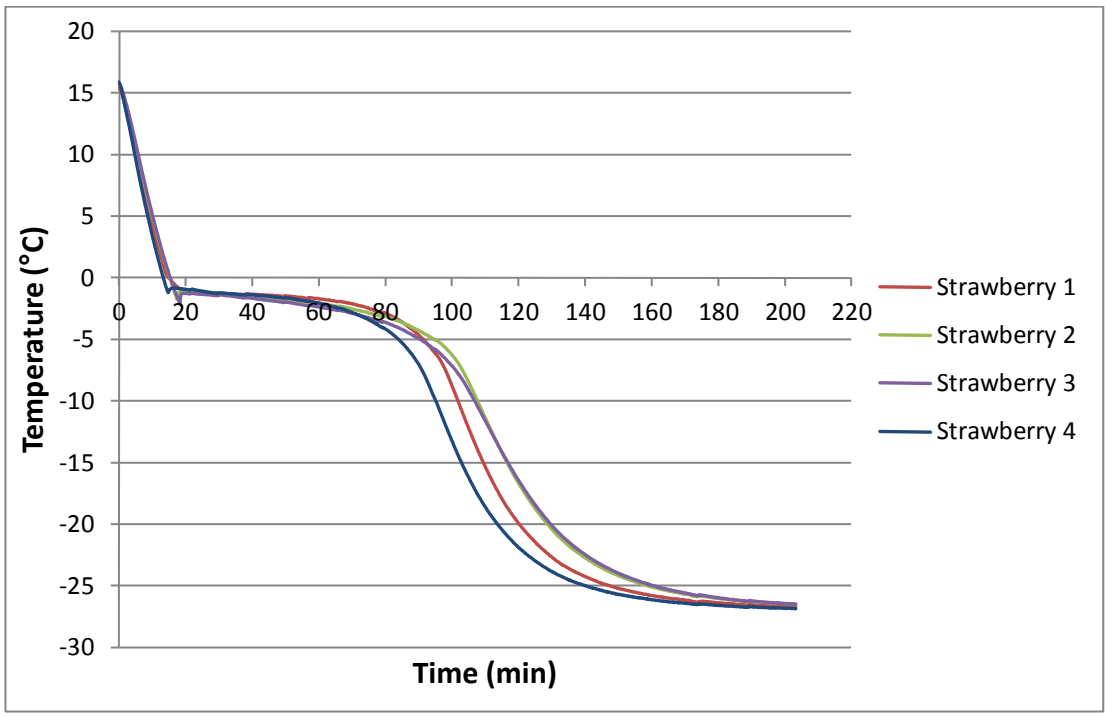


Figure B.3 Cooling curve of non-packaged strawberries frozen at -27°C.

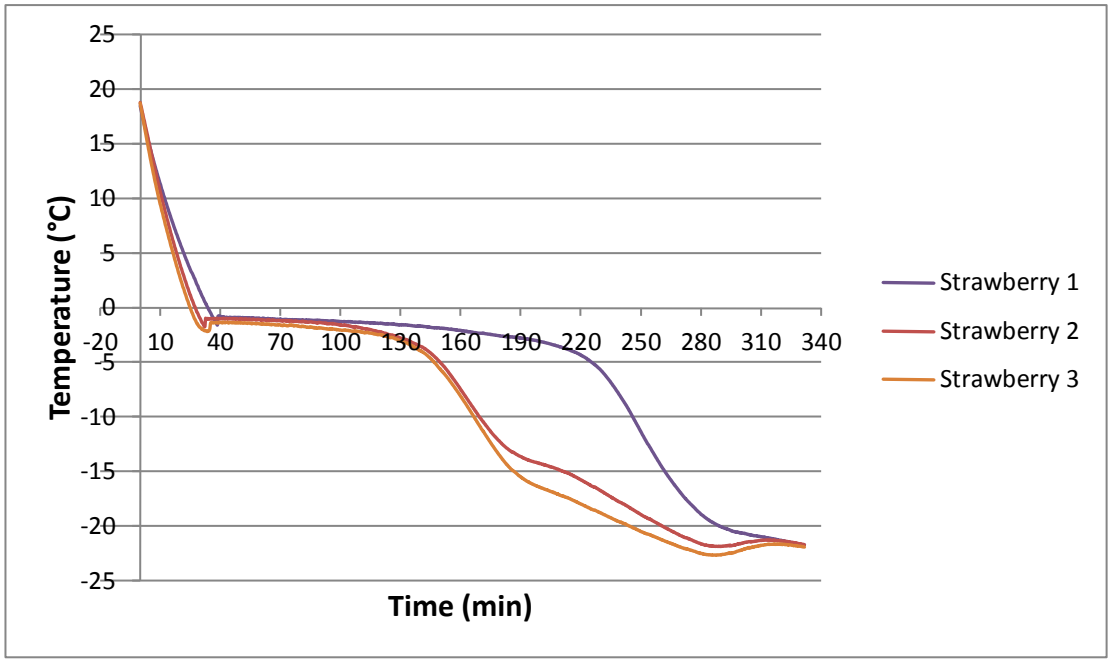


Figure B.4 Cooling curve of packaged strawberries frozen at -23°C.

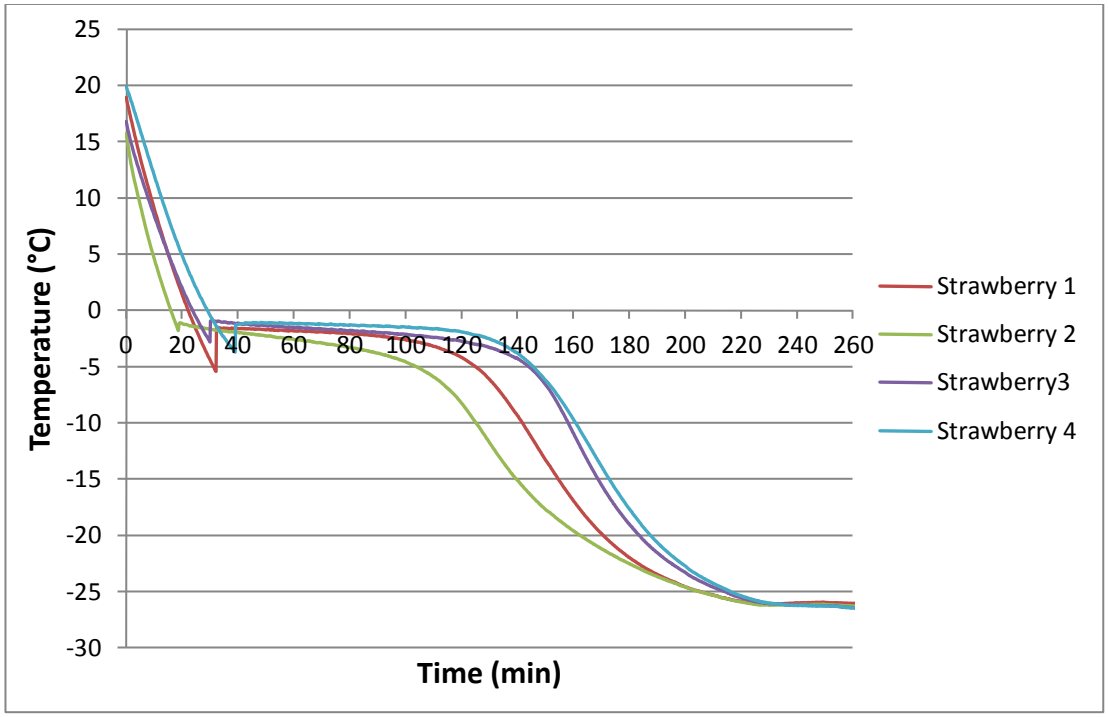


Figure B.5 Cooling curve of packaged strawberries frozen at -26°C.

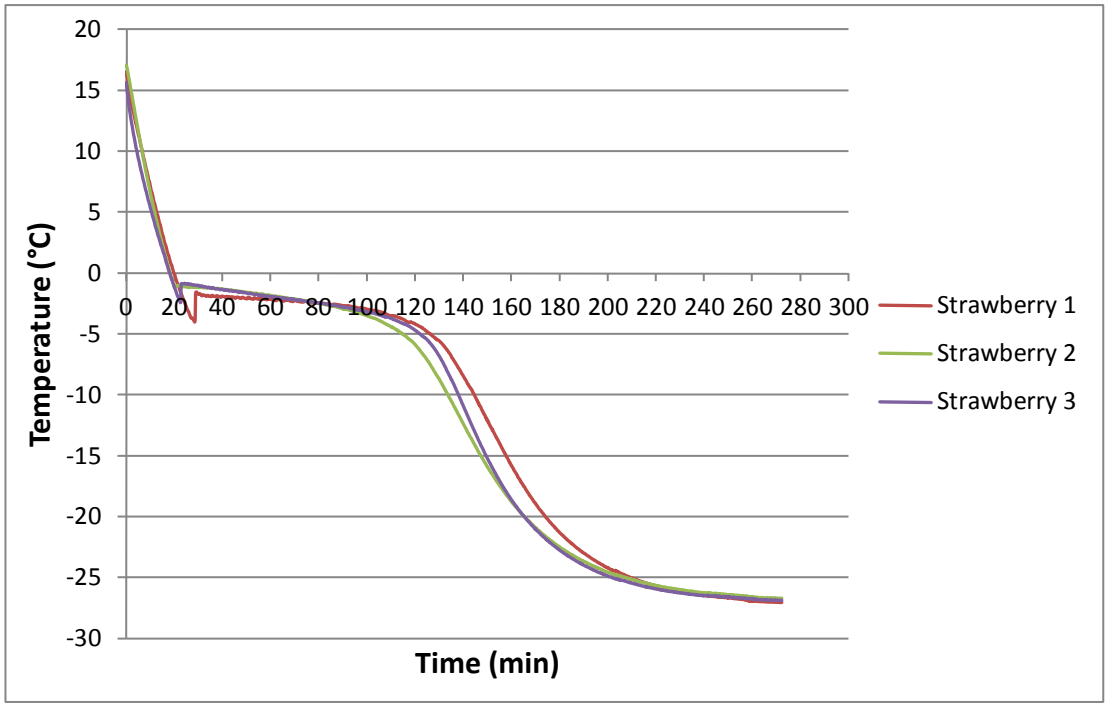


Figure B.6 Cooling curve of packaged strawberries frozen at -27°C.

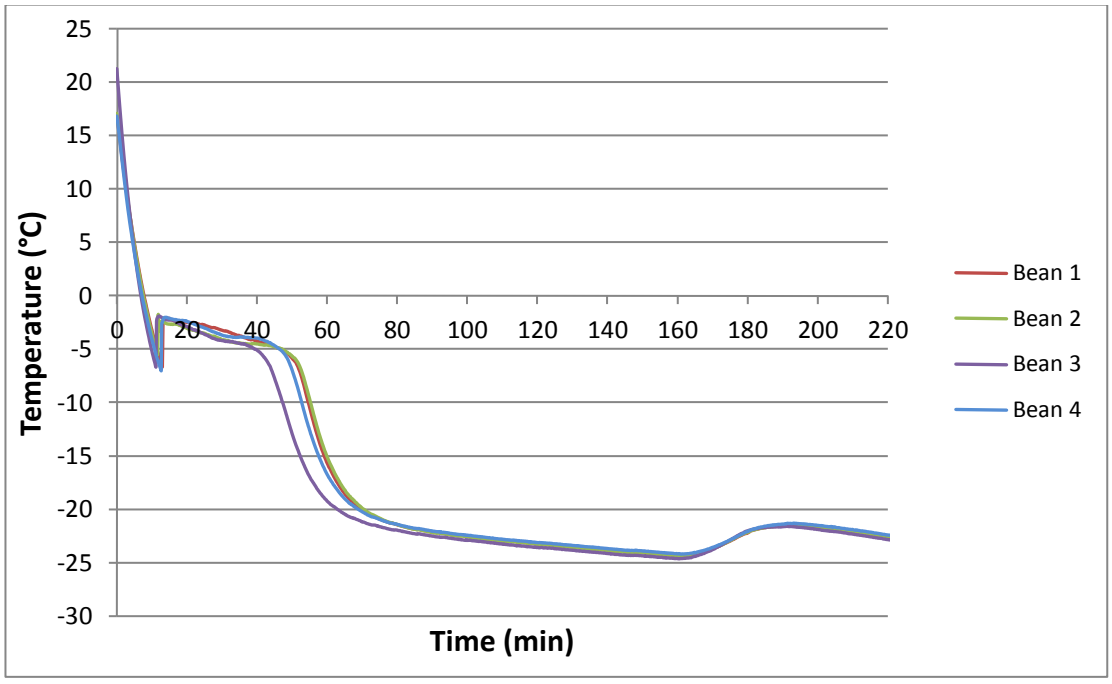


Figure B.7 Cooling curve of non-packaged green beans frozen at -23°C.

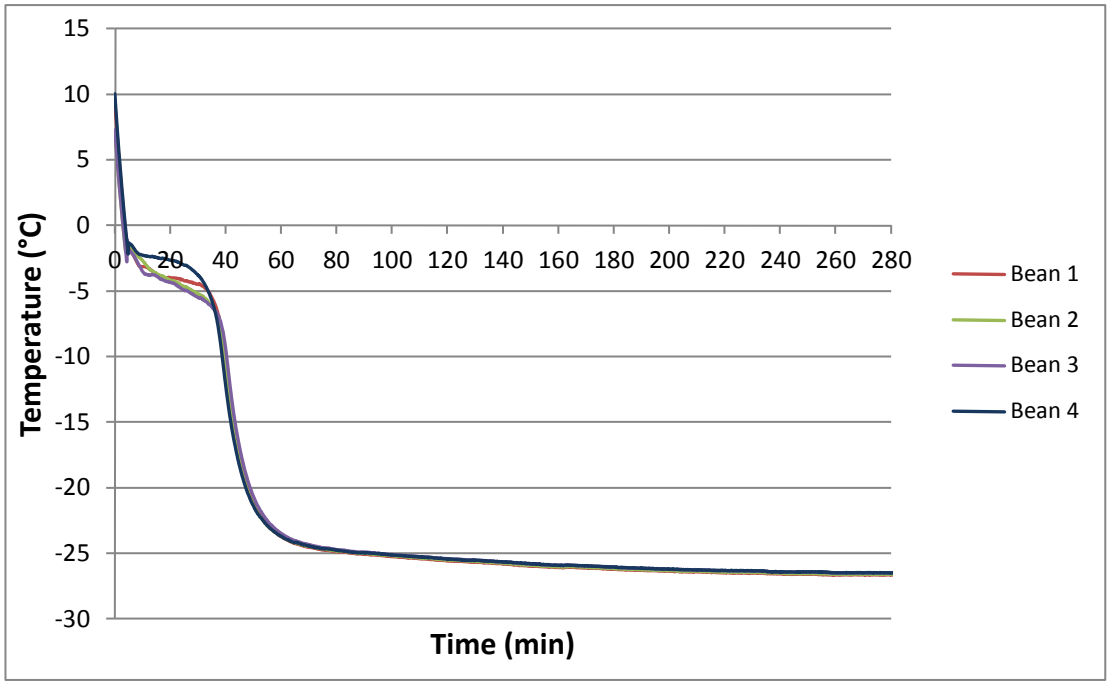


Figure B.8 Cooling curve of non-packaged green beans frozen at -26°C.

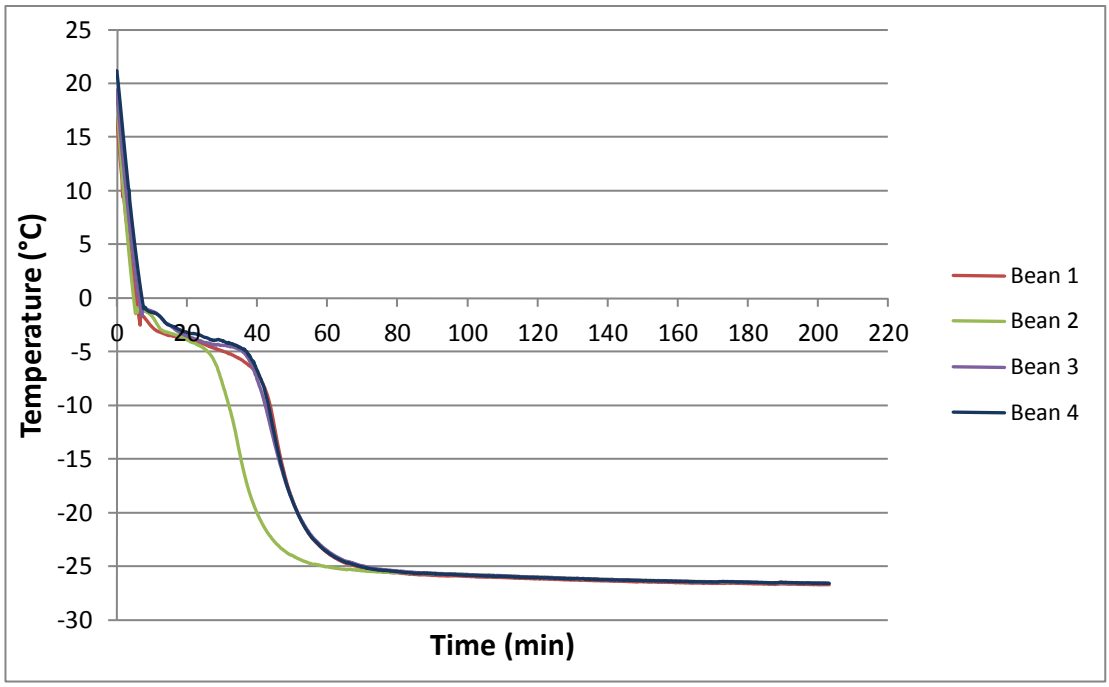


Figure B.9 Cooling curve of non-packaged green beans frozen at -27°C.

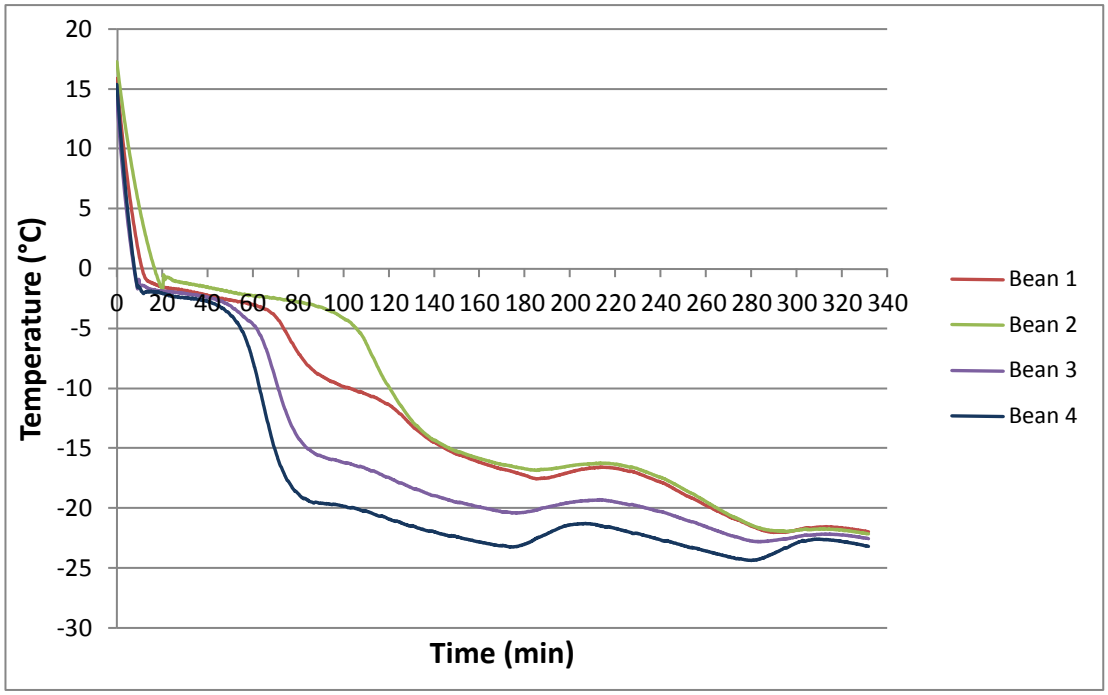


Figure B.10 Cooling curve of packaged green beans frozen at -23°C.

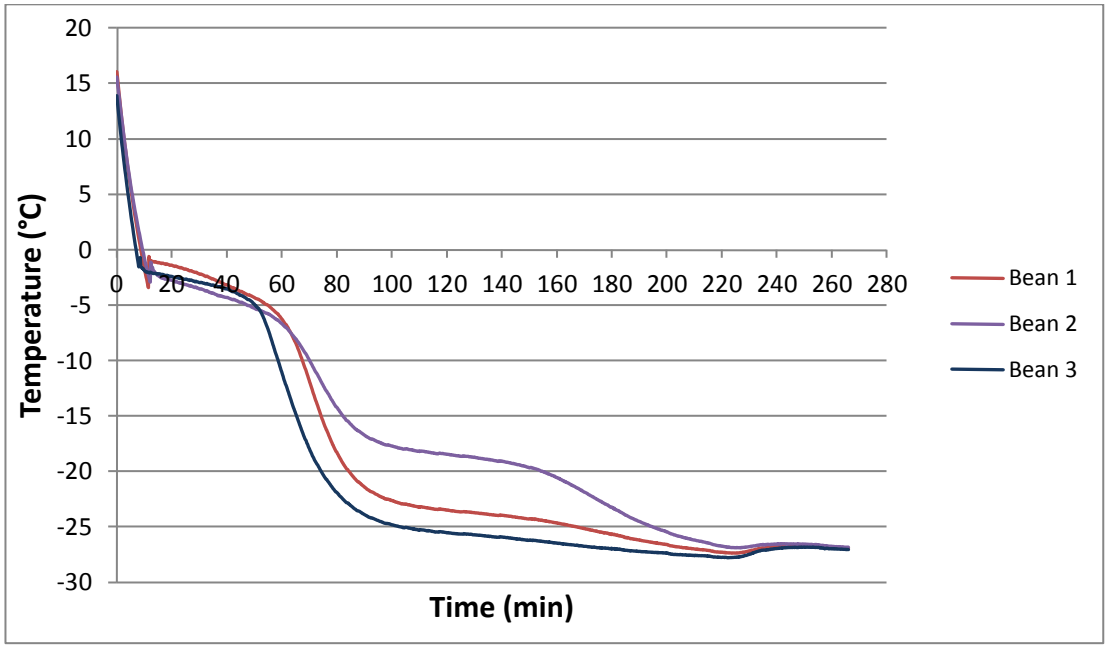


Figure B.11 Cooling curve of packaged green beans frozen at -26°C.

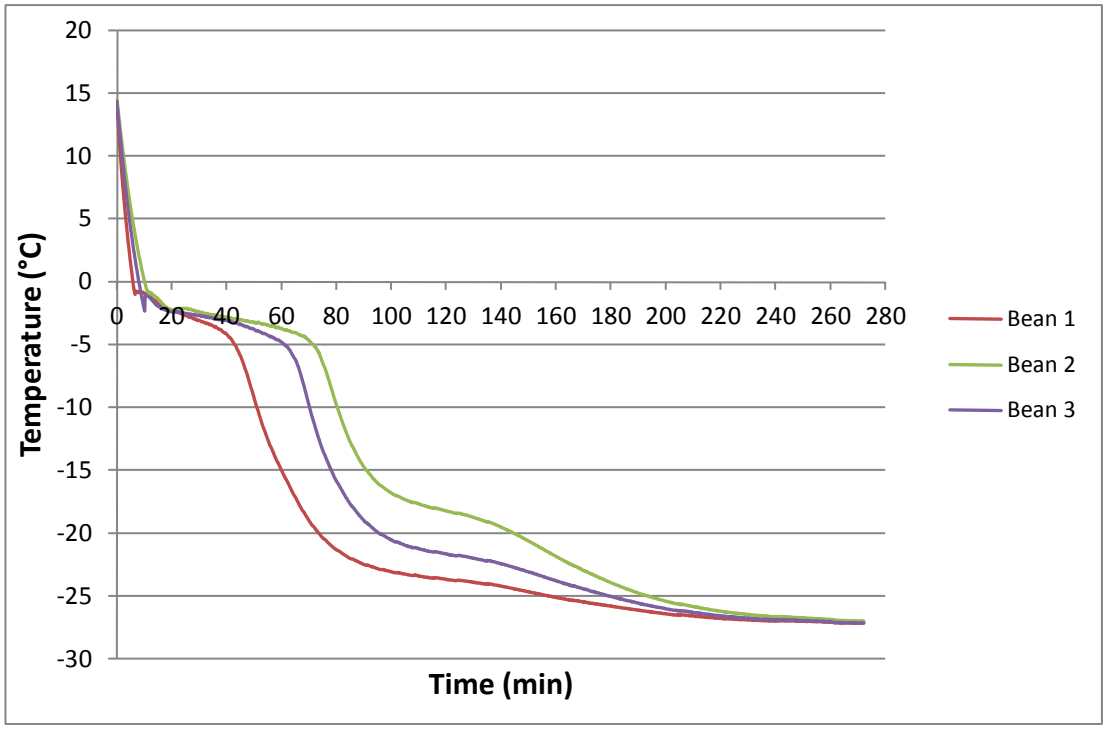


Figure B.12 Cooling curve of packaged green beans frozen at -27°C.