Client Technical Report

Title of Project:

Antioxidants in Fresh and Frozen Fruit and Vegetables: Impact Study of Varying Storage Conditions.

Client:
British Frozen Food Federation; Pelican PR.

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SUMMARY

Antioxidants and other key nutritional components of food are known to be affected by factors such as handling and storage conditions. The purpose of this study was to evaluate the impact of storage conditions on a range of fresh and frozen fruit and vegetables. Fresh produce were stored in a general household refrigerator at 4^{o}C for up to 3 days and a comparison made with equivalent frozen produce stored in a domestic freezer at -20^{o}C.

Six fresh and frozen produce were sourced from local supermarkets in the Chester area which included:

- Blueberries (*Vaccinium angustifolium*)
- Raspberries (*Rubus idaeus)*
- Peas (*Pisum sativum)*
- Green Beans (*Phaseolus vulgaris)*
- Cauliflower (*Brassica oleracea)*
- Baby Sweetcorn (*Zea mays var. saccharata)*

The fresh and frozen produce were analysed using published methods for:

- Vitamin C
- Total polyphenols
- Total anthocyanins:
- Carotenoids: β-carotene and lutein

From the initial data obtained, the following general conclusions can be made:

- The concentrations measured in frozen produce generally resembled those observed in the corresponding fresh produce prior to refrigerated storage.

- Analyte concentrations in fresh produce frequently exhibited a decrease during refrigerated storage, to levels below those observed in the corresponding frozen produce. The effects were most noticeable in the soft fruits.

- Concentrations were frequently lowest after 3 days of refrigerated storage..

Whilst general trends could be derived from the analysis undertaken, further investigation is considered desirable. In particular, the use of fruit and vegetables where their provenance is fully understood is considered highly desirable.

1. INTRODUCTION

1.1 Antioxidants in fruits and vegetables

Fruit and vegetables are typically over 90% water and once they are harvested, begin to undergo higher rates of respiration, resulting in moisture loss, quality deterioration and microbial spoilage which can further accelerate the spoilage process. Many fresh fruits and vegetables have a shelf life of only days before they become unsafe or undesirable for consumption. Washing, peeling and blanching steps prior to processing are responsible for some of the loss of water soluble nutrients. However, the thermal processing often associated with pre-freezing blanching treatments can be especially detrimental to heat-sensitive nutrients such as ascorbic acid (Vitamin C).

The primary purpose of blanching prior to freezing is to inactivate naturally occurring enzymes that may be still active in the frozen product. Blanching is an important preservation step in the freeze process of many vegetables. Soft fruits, on the other hand are usually not blanched prior to freezing owing to their delicate nature and inherent acidity (Rickman *et al* 2012).

Retention of nutrients is highly dependent on the cultivar, production location, maturity stage, season and processing conditions. By the time a consumer consumes fresh purchased produce, the frozen equivalent may be nutritionally similar to fresh owing to oxidative degradation of the nutrients during handling and storage of the fresh produce (Gebczynski & Kmiecik, 2005). Nutritional qualities may also vary according to season and growing location, so individual results may not be representative of yearly averages or regional availability.

The retention of vitamin C is often used as an estimate for the overall nutrient retention of food products. Vitamin C is by far the least stable nutrient during processing; it is highly sensitive to oxidation and leaching into water-soluble media during processing and storage. Retention of vitamin C can vary tremendously in all products depending on cultivar and processing conditions. In general, losses due to the freezing process can range from 10 – 80%, with averages around 50%. (Howard *et al* 1999).

Freshly picked vegetables consistently contain the greatest amount of vitamin C in all vegetables studies. However, vitamin C begins to degrade immediately after harvest. Green peas for example, have been shown to lose up to 51% WW of vitamin C during the first 24 – 48 hours after harvesting, (Philips *et al* 2010). Furthermore, vitamin C degrades steadily during prolonged storage, although refrigeration can slow the degradation rate. Fresh storage at ambient temperature can also result in greater loss of vitamin C, for example, fresh peas stored for one week can lose 50% of vitamin C. The loss of vitamin from prolonged frozen conditions remains problematic, and losses after 1 year from fruits and vegetables stored at -18^{o} to -20^{o}C can average 20-50%. (Tarrago-Trani *et al* 2012).

The phenolic compounds and flavonoids are ubiquitous in the plant kingdom. Phenolic compounds are among the most efficient antioxidant molecules and to date more than 6,400 structures for these compounds have been established. Phenolic compounds show positive correlations between a diet rich in phenolic compounds and a reduced risk of chronic diseases such as, cancer and cardiovascular disease (Castaneda-Ovando *et al* 2009). In general phenolic compounds are thus considered beneficial compounds as their antioxidant capacity is high. Research suggests that phenolic compounds are responsible for stalling or stopping the ‘initial trigger’ of chronic disease by serving as a sacrificial antioxidant to damaging free radicals in the body. Overall, there are hundreds of different phenolic compounds, and consequentially the reported results will be composite total phenolic values.

The phenolic composition of fruit and vegetables is also dependent on commodity, cultivar, maturity stage and post-harvest storage handling and storage conditions. Since phenolic compounds are antioxidants, they are subject to oxidation during storage and processing. The blanching processes prior to freezing in commercial operations can inactivate enzymes that cause oxidation of phenolics. However, chemical degradation can still occur during storage, depending on available oxygen and exposure to light (Turkmen 2005).

Phenolic compounds are water-soluble, rendering them susceptible to leaching. Furthermore, phenolic compounds and other phytochemicals are found in significant amounts in the peels of fruits and vegetables, so some content can be lost during the peeling step of processing.

In general, freezing causes minimal destruction to phenolic compounds in fruits, with retention levels dependent on cultivar. As an example, raspberries have been shown to lose up to 12% of phenolics in an early harvest cultivar, but a 12% gain of phenolics in a late harvest cultivar (Gonzalez *et al* 2003). Late harvest raspberries have also been shown to contain higher levels of antioxidants in particular, total anthocyanins after freezing. Anthocyanins pigments are important contributors to the colour and aoppeaerance of foodstuffs, in addition to their health benefits.

β-carotene as an antioxidant is present in high quantities in fruit and vegetables. Previous studies of β-carotene show no definite trend of nutrient retention within fruit and vegetables during prolonged storage. Loss of β-carotene during processing may be due to leaching of nutrients rather than by chemical degradation, although β-carotene is highly sensitive to light degradation. It has been shown that vegetables taken directly from field to the processing plant for steam blanching and fast freezing can retain over 70% of their total phenolic and β-carotene content during long-term storage (Howard 1999).

Levels of total phenolics in produce stored in refrigerated conditions have been shown to increase during the first 3 days of storage suggesting that secondary metabolic activity in the stored fruit and vegetables may be responsible for the increases observed. This has been attributed to probable increased activity of enzymes involved in phenolic synthesis. Changes in total phenolics are also dependent on cultivar and post-harvest conditions. Since retention of phenolic compounds appears to be erratic during frozen storage, further research is clearly recommended (Silva 2007).

1.2 Aims and objectives

The aims of this study were to:

- Compare the concentrations of selected antioxidants in a range of fresh and frozen fruits and vegetables

- Examine changes in the concentrations of these selected antioxidants present in the fresh fruits and vegetables during their subsequent storage.

The objectives of the study were to

- Evaluate the potential benefits of the use of frozen fruits and vegetables in comparison to the fresh equivalents.

- Determine the impact on selected antioxidants during refrigerated storage by domestic consumers of fresh produce

2. ANALYTICAL PROCEDURES

2.1 Chemicals

All chemicals were obtained from Sigma Chemical Ltd (Poole, Dorset).

2.2 Sampling and storage

Fresh and frozen produce were sourced from local supermarkets in the Chester area. These included:

- Blueberries (*Vaccinium angustifolium*)
- Raspberries (*Rubus idaeus)*
- Peas (*Pisum sativum)*
- Green Beans (*Phaseolus vulgaris)*
- Cauliflower (*Brassica oleracea)*
- Baby Sweetcorn (*Zea mays var. saccharata)*

After purchase, the fruits and vegetables were taken to the laboratory for storage and either refrigerated in the dark at 4°C, or in held in a freezer at -20^{o}C. The appliances used for storage were typical of domestic environments. Fresh produce was processed on the same day as it was acquired. Refrigerated fresh produce was stored for 3 days prior to subsequent processing. Analysis of the fruit and vegetables samples was undertaken in triplicate through standard methods as described below.

2.3 Vitamin C

High performance liquid chromatography (HPLC) was used to determine total vitamin C concentrations (in the form of ascorbic acid) as described by Fontannaz *et al.* (2006). In this method, food samples were initially homogenised with an extraction buffer which included a reducing agent (tris [2-carboxyethyl] phosphine hydrochloride) to convert dehydroascorbic acid (the oxidised form of ascorbic acid) to ascorbic acid.

Sample extracts were subsequently examined through use of an HPLC mobile phase that contained an ion pairing reagent (decylamine). Separation was performed using a reversed phase C_{18} column (Waters Spherisorb; 5µm; 150 x 4.6 mm) and a mobile phase flow rate of 1.0 ml/min. Detection of the eluted components was performed at λ265 nm.

Quantification of the vitamin C in the sample extracts was performed by means of an external calibration curve in the concentration range 1- 500 µg/ml. The calibration curve was linear (r^2 = 0.9977) with an approximate limit of detection of 0.1 µg/ml. Concentrations of Vitamin C in the fresh and frozen produce was determined as mg/kg fresh weight.

2.4 Total polyphenols

The concentration of total phenolics was determined through use of the Folin-Ciocalteau colorimetric method (Singleton & Rossi, 1965). Calculations were based on a calibration curve obtained with gallic acid. Concentrations of total polyphenols (as gallic acid equivalents) in the fresh and frozen produce were determined as mg/kg fresh weight.

2.5 Total anthocyanins

Samples of fruit and vegetables were processed before measurement by spectrophotometric analysis, as per AOAC International Official Method 2005.02. as described by Lee *et al.* (2005).

In this method, total monomeric anthocyanin was determined through use of a pH differential method, where absorbance at λ520 nm is determined at both pH 1.0 and pH 4.5. The difference in absorbance is proportional to total anthocyanin concentration. In this method, the total anthocyanin concentration of the samples (as the cyanidin-3-glucoside equivalents) were determined as mg/kg fresh weight.

2.6 Carotenoids: β-carotene and lutein

HPLC was used to determine β-carotene and lutein concentrations as described by Olives Barba *et al*. (2006). In this method, carotenoids were extracted from the fruit and vegetable samples using a hexane/methanol/acetone mixed solvent (50:25:25 v/v/v). The hexane layer containing the carotenoids was removed, dried with a stream of dry nitrogen and re-suspended in tetrahydrofuran/acetonitrile/methanol (15:30:55 v/v/v).

Sample extracts were subsequently examined through use of an HPLC mobile phase consisting of methanol/acetonitrile (90:10 v/v) and 9µM triethylamine. Separation was performed using a reversed phase C_{18} column (Waters Spherisorb; 5µm; 150 x 4.6 mm) and a mobile phase flow rate of 0.9 ml/min. Detection of the eluted components was performed at λ475 nm.

Quantification of the β-carotene and lutein in the sample extracts was performed by means of external calibration curves in the concentration range 1- 100 µg/ml. The calibration curves for β-carotene and lutein were linear (r^2 = 0.9977 and r^2 = 0.9944, respectively) with approximate detection limits of 1 µg/ml for β-carotene and lutein. Concentrations of β-carotene and lutein in the fresh and frozen produce were determined as mg/kg fresh weight.

3. RESULTS AND DISCUSSION

3.1 Vitamin C

3.1.1 Blueberry

The vitamin C concentrations measured in frozen and fresh blueberries are shown in Figure 3.1. The concentrations measured in fresh blueberries were not significantly different from those that had been stored frozen. A significant decrease in vitamin C concentrations was found after 3 days of storage.

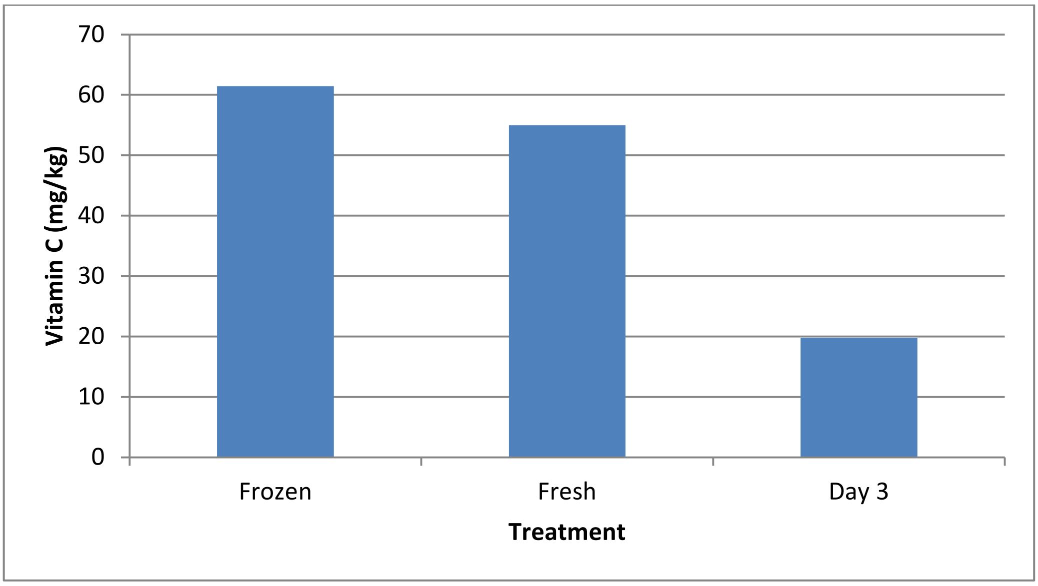

Figure 3.1 Vitamin C concentrations in frozen and fresh blueberry.

3.1.2 Raspberry

The vitamin C concentrations measured in frozen and fresh raspberries are shown in Figure 3.2. The concentrations measured in fresh raspberries were higher than those that had been stored frozen. Vitamin C concentrations subsequently decreased during refrigerated storage, with concentrations after 3 days comparable to those in the frozen produce.

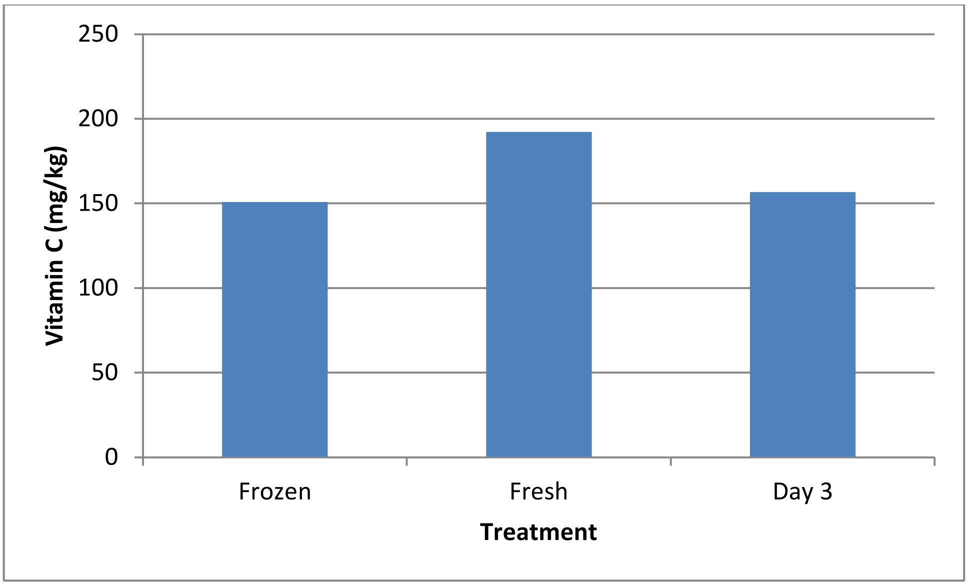

Figure 3.2 Vitamin C concentrations in frozen and fresh raspberry.

3.1.3 Pea

Vitamin C concentrations in fresh peas were higher than those recorded in the frozen produce (Figure 3.3). The concentrations subsequently decreased during refrigerated storage, with the lowest values recorded after 3 days.

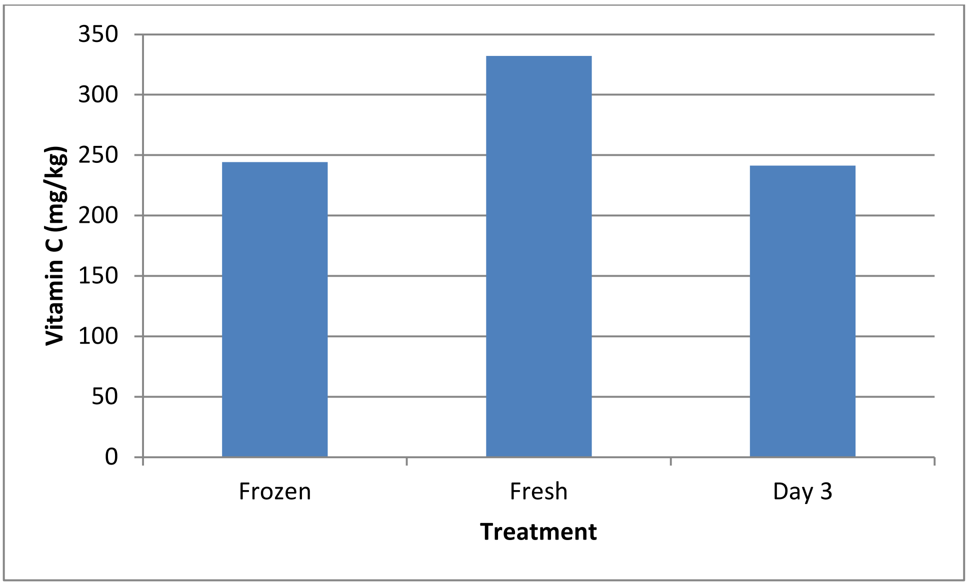

Figure 3.3 Vitamin C concentrations in frozen and fresh pea.

3.1.4 Green Bean

Vitamin C concentrations in fresh green beans were lower than those recorded for the frozen produce (Figure 3.4). A significant concentration decrease was observed in fresh produce during refrigerated storage. Final concentrations after 3 days were lower than those observed in the frozen produce.

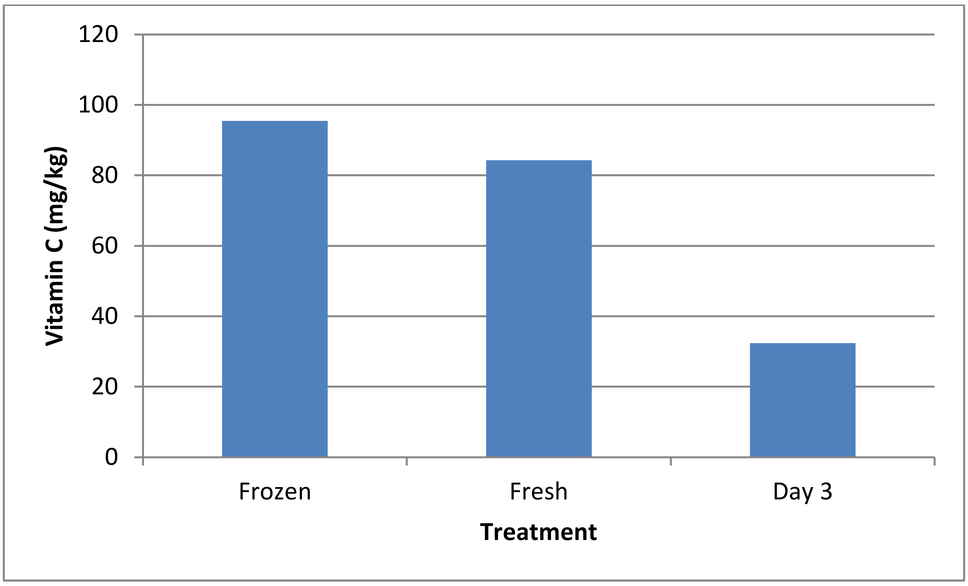

Figure 3.4 Vitamin C concentrations in frozen and fresh green bean.

3.1.5 Cauliflower

Vitamin C concentrations obtained for fresh cauliflower were significantly higher than those measured in the frozen samples (Figure 3.5). Concentrations decreased after 3 days of refrigerated storage.

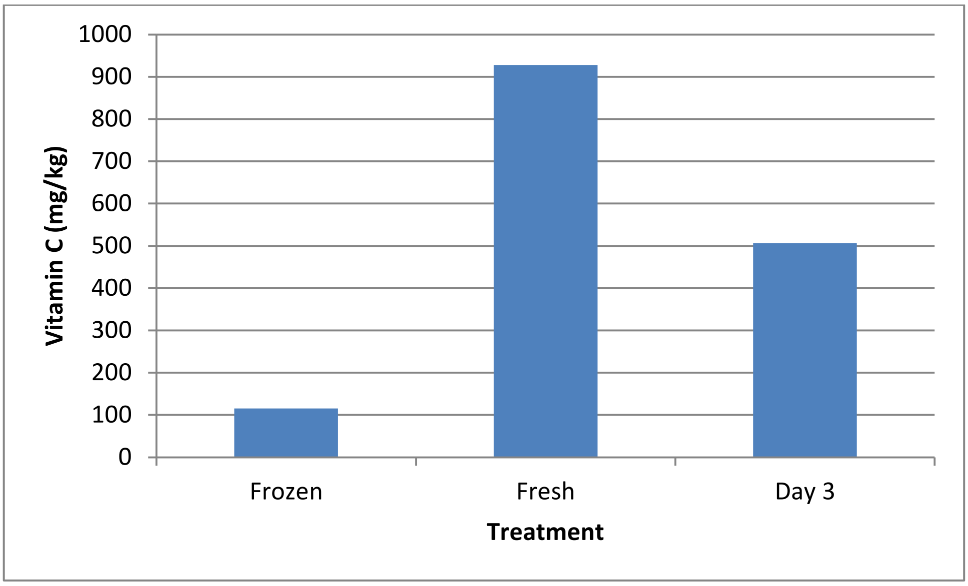

Figure 3.5 Vitamin C concentrations in frozen and fresh cauliflower.

3.1.6 Sweetcorn

Concentrations of vitamin C in fresh sweetcorn were lower than in the frozen produce. During refrigerated storage, a progressive decrease in concentrations occurred with the lowest concentrations recorded after 3 days (Figure 3.6).

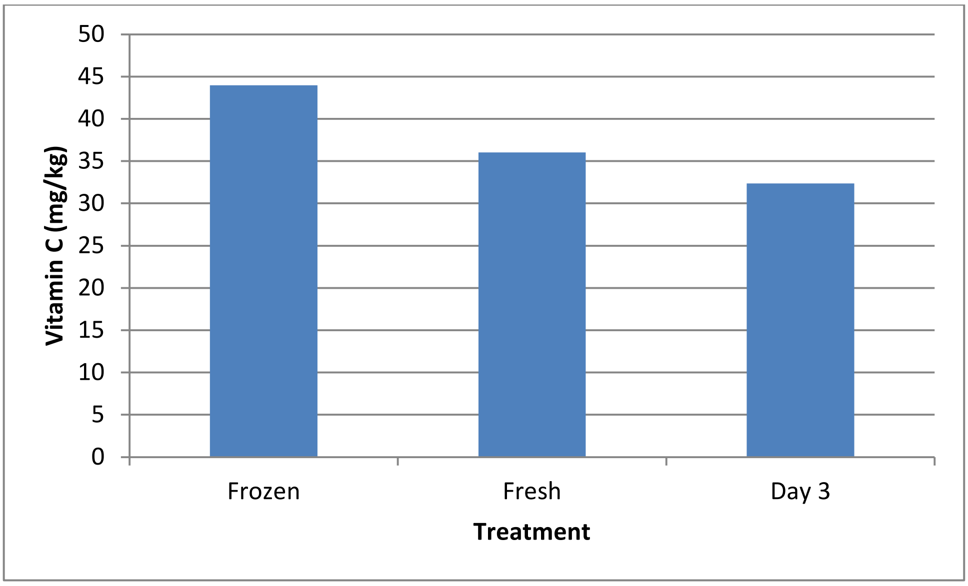

Figure 3.6 Vitamin C concentrations in frozen and fresh sweetcorn.

3.1.7 Discussion

With the exception of cauliflower, vitamin C concentrations in the fresh and frozen produce were generally comparable. In the case of blueberries and green beans vitamin C was slightly lower in fresh. Although the provenance of the frozen cauliflower was not known, it is possible that the significantly lower concentrations might have been as a result pre-treatment such as blanching.

Decreased vitamin C concentrations were frequently observed as a result of refrigerated storage of the fresh produce, with lowest concentrations observed after 3 days. In the case of blueberry, pea and cauliflower, lowest concentrations of vitamin C were observed after 3 days.

3.2 Total polyphenols

Total polyphenols were measured with reference to external gallic acid standards. Consequently, the total polyphenol concentrations present within the fruits and vegetables are expressed as gallic acid equivalents.

3.2.1 Blueberry

Total polyphenol concentrations recorded for fresh blueberry were significantly lower than in the frozen samples (Fig. 3.7). During refrigerated storage of the fresh produce, a significant increase in concentration was observed after 3 days.

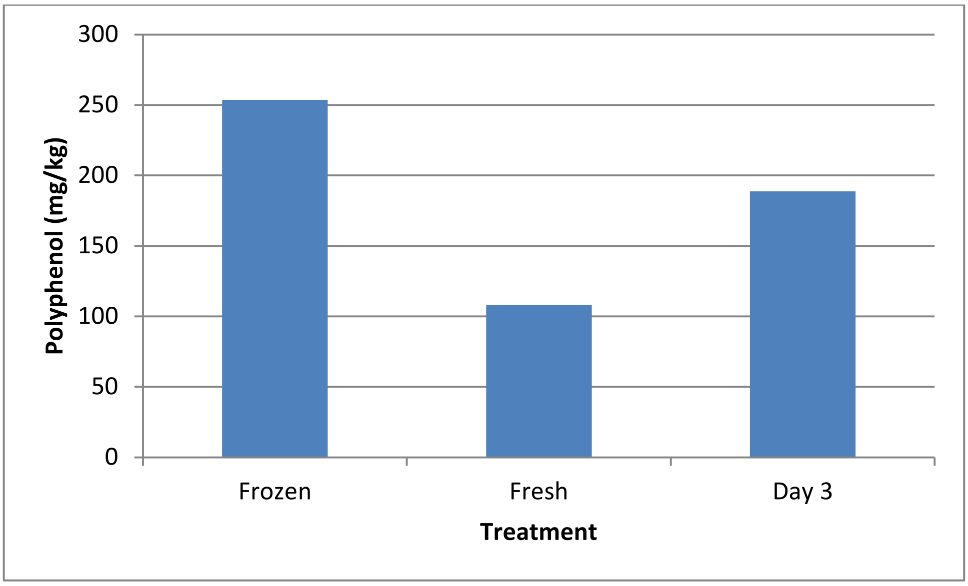

Figure 3.7 Total polyphenol concentrations in frozen and fresh blueberry.

3.2.2 Raspberry

Fresh raspberry exhibited total polyphenol concentrations lower than the frozen samples. Similar to blackberry, concentrations showed an initial increase after 3 days of refrigerated storage. Concentrations at the end of the storage period were higher than those in the fresh produce (Fig 3.8, below).

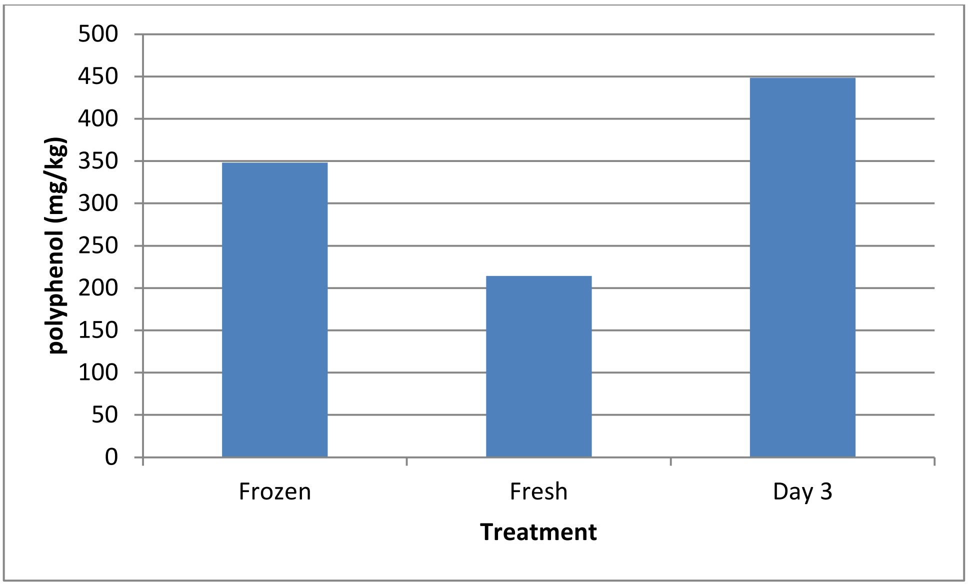

Figure 3.8 Total polyphenol concentrations in frozen and fresh raspberry.

3.2.3 Pea

Total polyphenol concentrations in fresh peas were greater than in the frozen samples (Fig. 3.9). During subsequent refrigerated storage, concentrations decreased after 3 days storage.

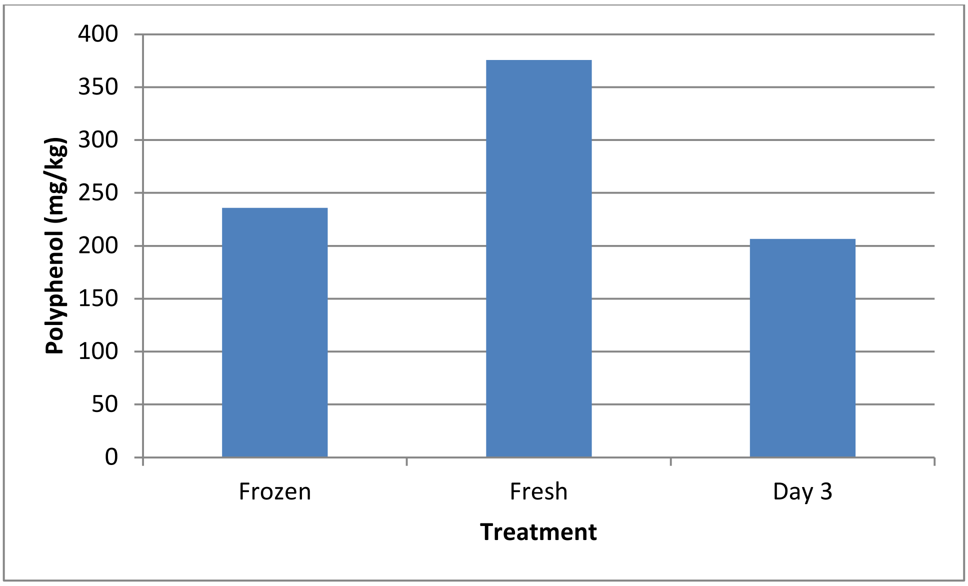

Figure 3.9 Total polyphenol concentrations in frozen and fresh pea.

3.2.4 Green Bean

Total polyphenol concentrations recorded in frozen and fresh green beans were not significantly different. During subsequent refrigerated storage there was a decrease in concentrations with the lowest concentrations measured after 3 days of refrigerated storage (Fig 3.10).

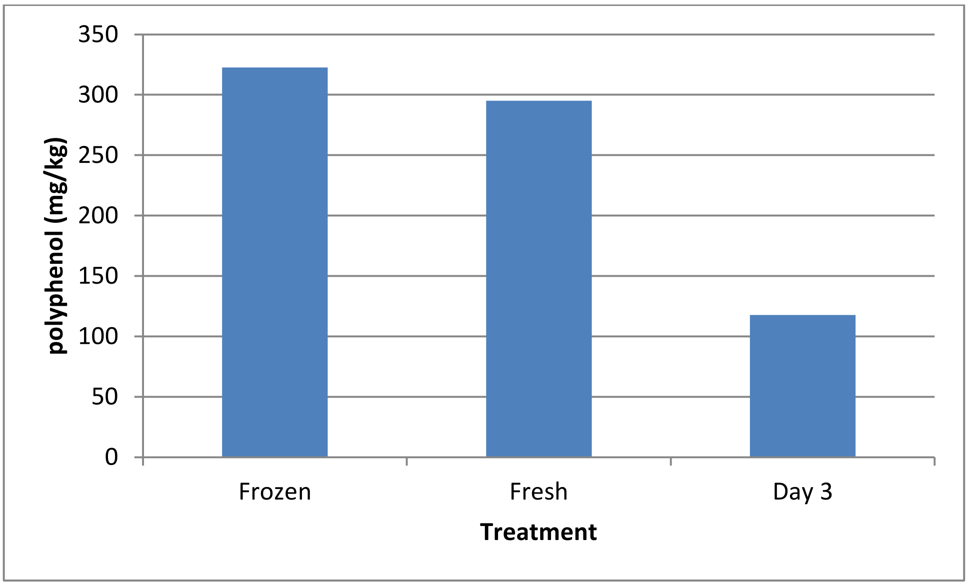

Figure 3.10 Total polyphenol concentrations in frozen and fresh green bean.

3.2.5 Cauliflower

Total polyphenol concentrations measured in fresh cauliflower were significantly lower than those in the frozen produce (Fig. 3.11, below). In contrast to the soft fruits, peas and green beans, no significant changes in total polyphenol concentrations were observed during subsequent refrigerated storage.

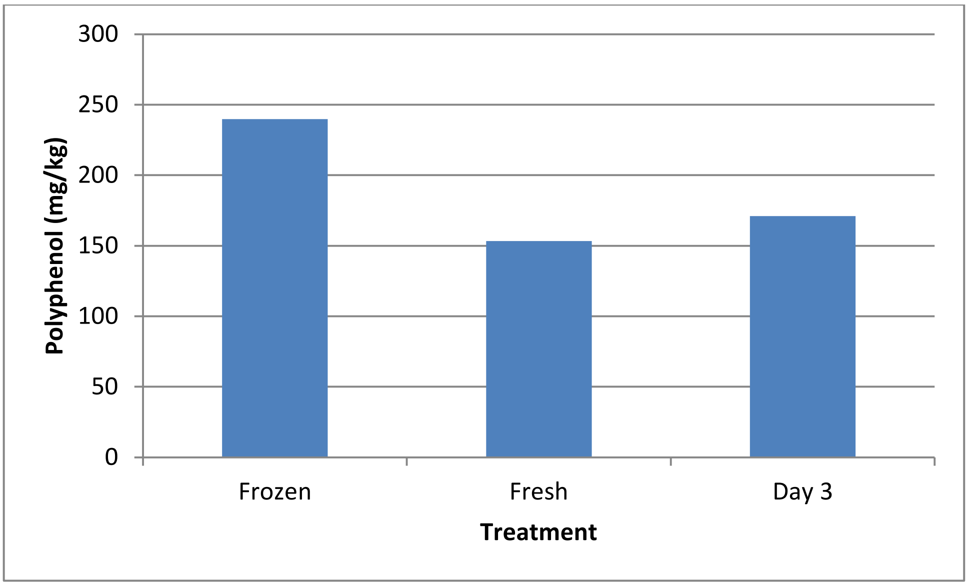

Figure 3.11 Total polyphenol concentrations in frozen and fresh cauliflower.

3.2.6 Sweetcorn

Total polyphenol concentrations in fresh sweetcorn were significantly greater than those recorded in the frozen sweetcorn samples. During subsequent refrigerated storage there was no significant change in the concentrations (Fig 3.12).

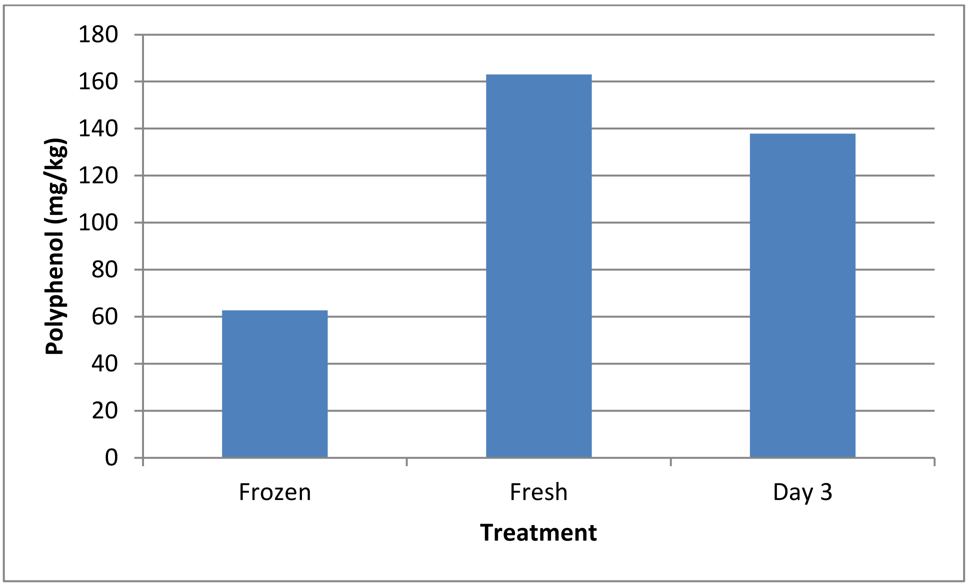

Figure 3.12 Total polyphenol concentrations in frozen and fresh sweetcorn.

3.2.7 Discussion

Total polyphenol concentrations varied between fresh and frozen samples of the various fruits and vegetables examined, as well as during refrigerated storage for up to 3 days. Examination of the berry fruits suggested that total polyphenol concentrations were lower in the fresh produce and after an initial decrease, the concentrations increased to levels similar to, or greater than those observed initially. It is considered that this represents changes due to ripening which in turn, results in an increase in the extractability of the polyphenols. Similar trends were not observed in the vegetables during refrigerated storage. For these materials, total polyphenol concentrations either decreased or remained stable during refrigerated storage.

3.3 Total anthocyanins

The total anthocyanin concentrations measured for each fruit or vegetable are based on cyaniding -3-glucoside equivalents.

3.3.1 Blueberry

The total anthocyanin concentrations in frozen and fresh blueberries were not significantly different (Fig. 3.13). During refrigerated storage, the concentrations dropped significantly after 3 days.

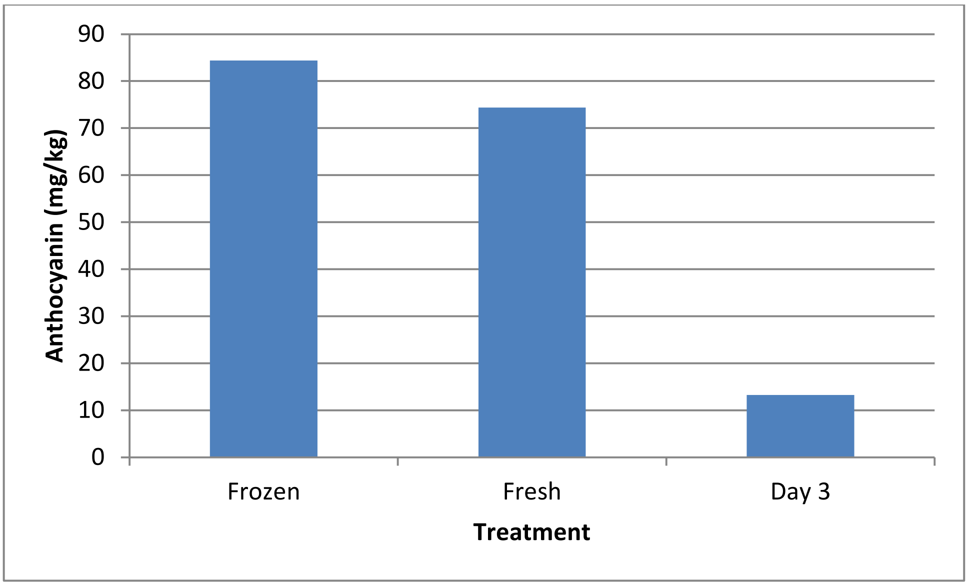

Figure 3.13 Total anthocyanin concentrations in frozen and fresh blueberry.

3.3.2 Raspberry

Total anthocyanin concentrations in fresh and frozen raspberries were also not significantly different (Fig. 3.14). Similar to that observed for blueberries, the concentrations dropped significantly after 3 days of refrigerated storage.

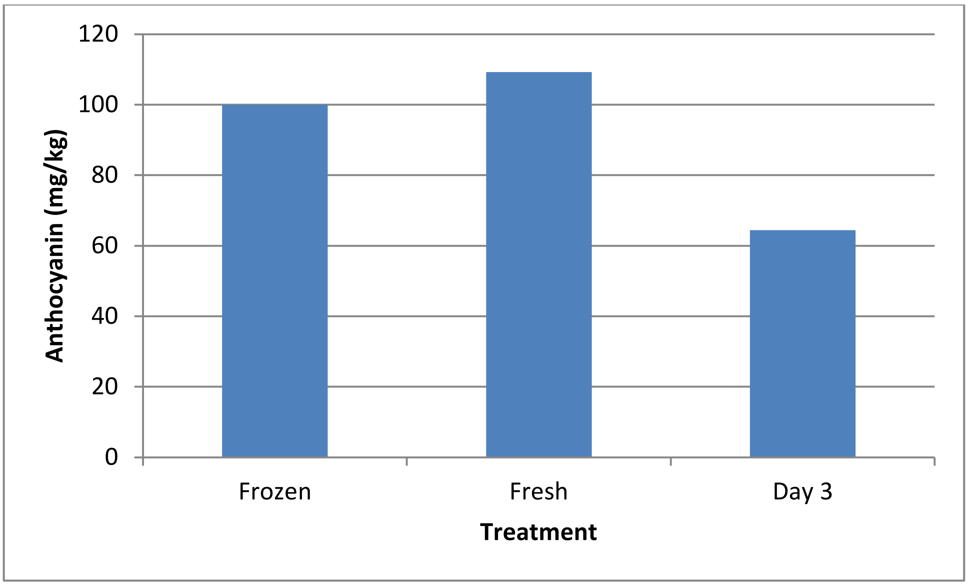

Figure 3.14 Total anthocyanin concentrations in frozen and fresh raspberry.

3.3.3 Pea

Fresh and frozen peas exhibited similar total anthocyanin concentrations (Fig. 3.15). During refrigerated storage, the total anthocyanin concentrations showed a significant decline, with the lowest concentrations recorded after 3 days refrigerated storage.

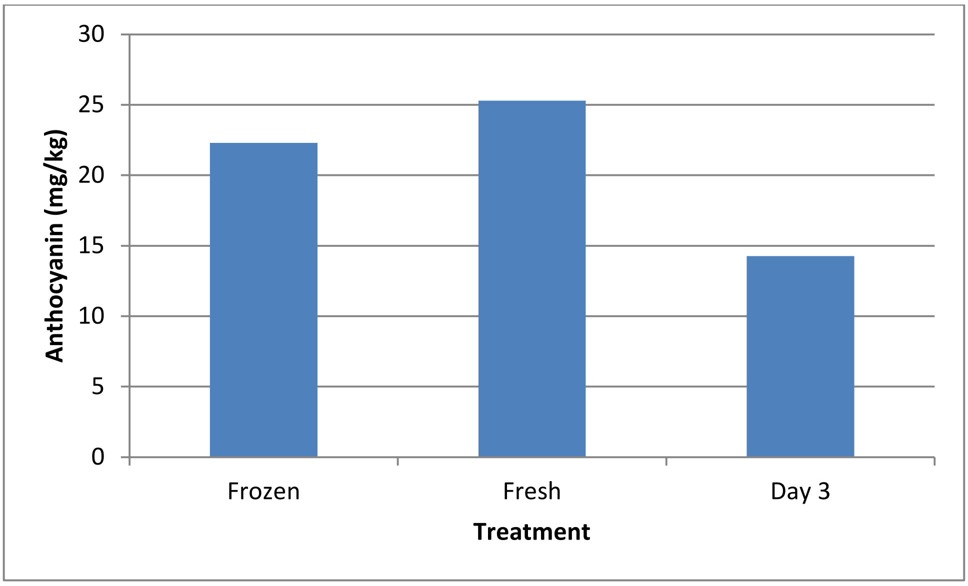

Figure 3.15 Total anthocyanin concentrations in frozen and fresh pea.

3.3.4 Green Bean

Total anthocyanin concentrations in the fresh green beans were higher than those of the frozen produce (Fig. 3.16). During refrigerated storage, the total anthocyanin concentrations decreased to levels below the detection limits of the analytical procedure.

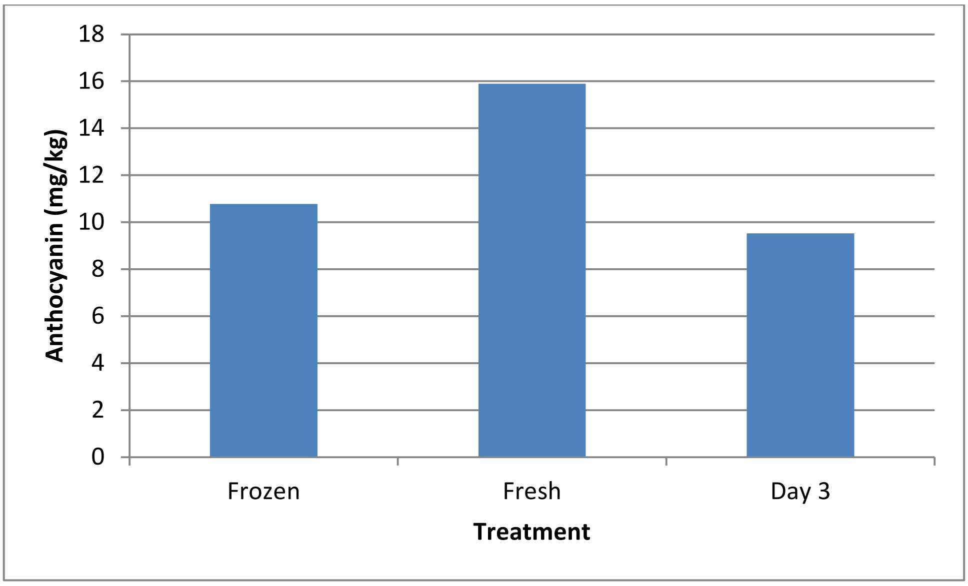

Figure 3.16 Total anthocyanin concentrations in frozen and fresh green bean.

3.3.5 Cauliflower

In contrast to the green beans, total anthocyanin concentrations in frozen cauliflower were significantly higher than fresh cauliflower (Fig. 3.17). During refrigerated storage of the fresh cauliflower, the total anthocyanin concentrations decreased significantly and were below detection limits after 3 days.

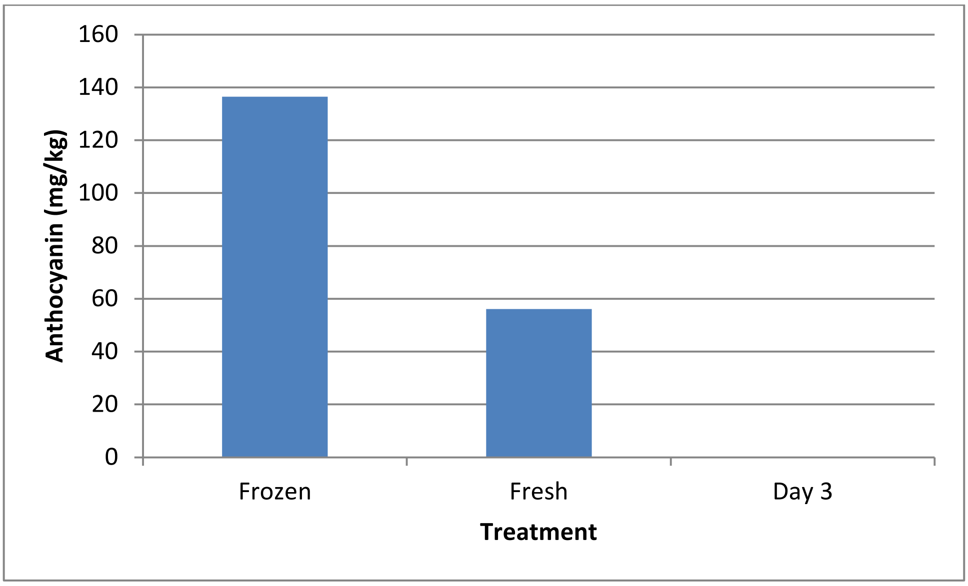

Figure 3.17 Total anthocyanin concentrations in frozen and fresh cauliflower.

3.3.6 Sweetcorn

Total anthocyanin concentrations in fresh sweetcorn were greater than those recorded for the frozen sweetcorn. During subsequent refrigerated storage the concentrations in the fresh produce showed an initial increase then a subsequent decrease. The overall change in total anthocyanin concentration was not significant.

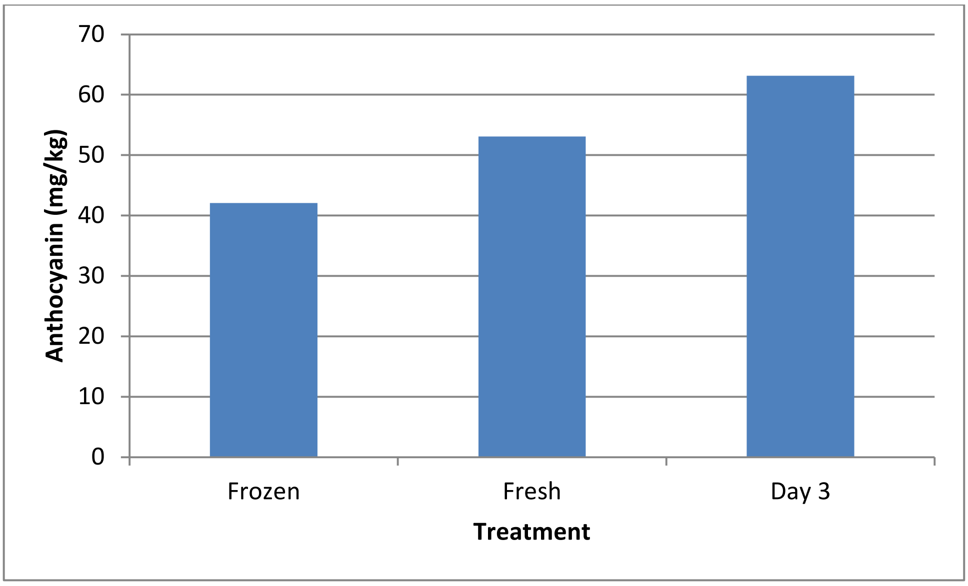

Figure 3.18 Total anthocyanin concentrations in frozen and fresh sweetcorn.

3.3.7 Discussion

Total anthocyanin concentrations recorded in the fruit and vegetable samples showed much variability, however, it was possible to identify some general similarities between the soft fruits and the vegetables samples. Total anthocyanin concentrations appeared to decrease during refrigerated storage of soft fruits. The total anthocyanin concentrations in the vegetables showed either no change or a decrease during storage. As in the case of total polyphenols, it is thought that any increase in the level of anthocyanin concentrations may be due to softening during the ripening process. No consistent differences between fresh and frozen produce could be identified.

3.4 Carotenoids: β-carotene

3.4.1 Blueberry

The concentrations of β-carotene measured in blueberry were low and were not significantly different between the frozen and fresh produce. During subsequent refrigerated storage there was a decrease in the β-carotene concentrations and after 3 days these were the same as those recorded in the frozen produce (Fig. 3.19).

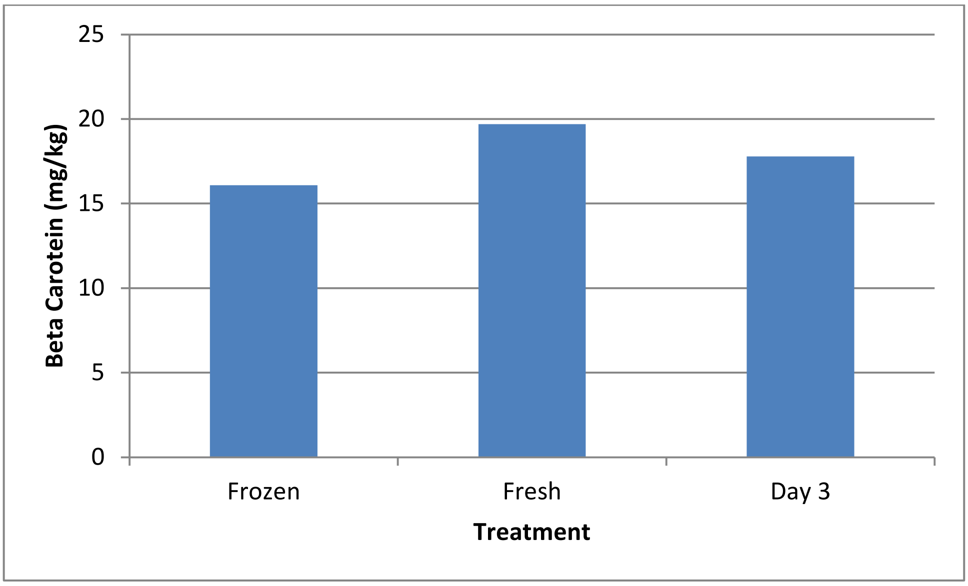

Figure 3.19 β-carotene concentrations in frozen and fresh blueberry.

3.4.2 Raspberry

β-carotene concentrations measured in the fresh and frozen samples were lower than those recorded for blueberry. Although the concentrations measured in fresh blueberry appeared to be lower than the frozen samples and to subsequently increase during refrigerated storage, the differences were not significant (Fig. 3.20, below).

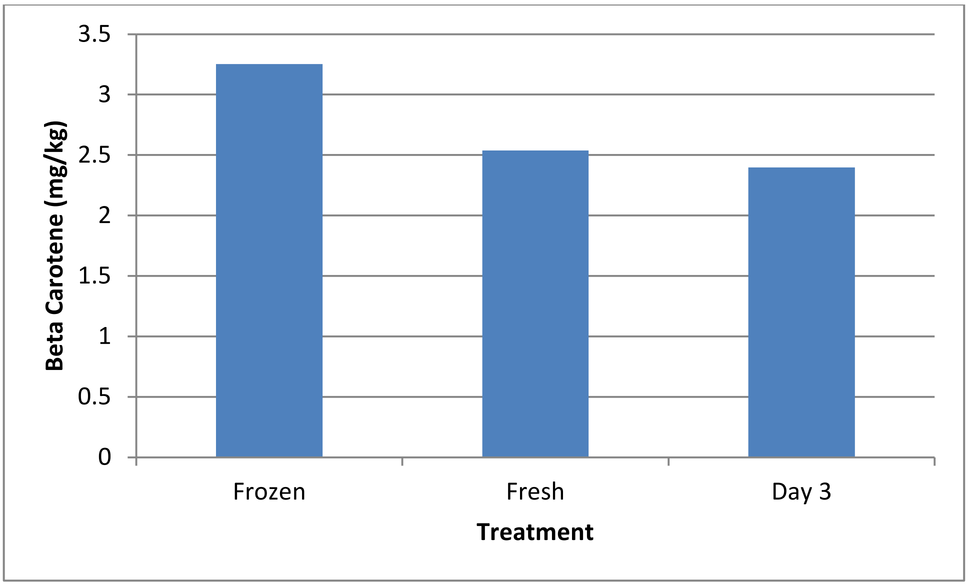

Figure 3.20 β-carotene concentrations in frozen and fresh raspberry.

3.4.3 Pea

The concentrations of β-carotene measured in the fresh peas were significantly higher than those recorded for the frozen samples. During the subsequent refrigerated storage there was a marginal increase in β-carotene concentrations (Figure 3.21).

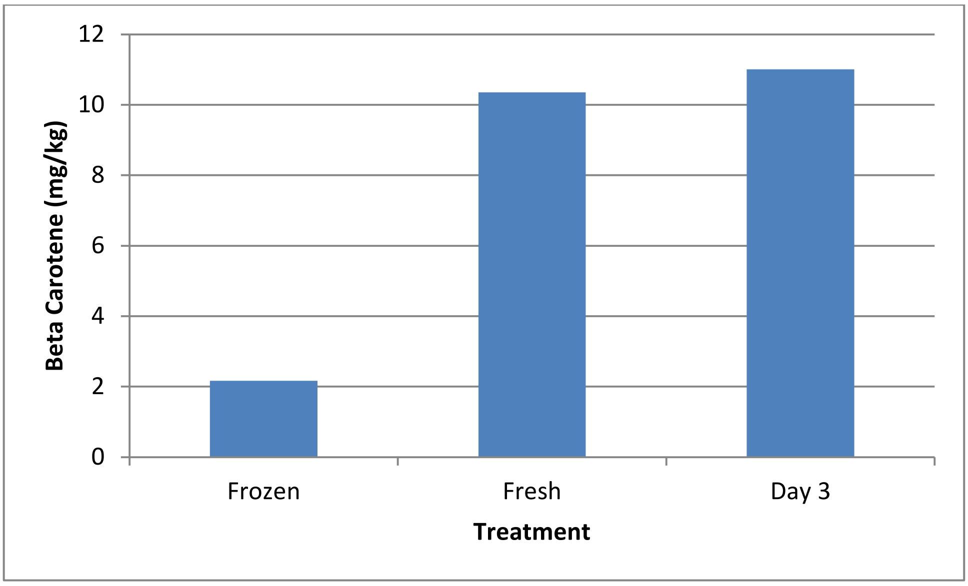

Figure 3.21 β-carotene concentrations in frozen and fresh pea.

3.4.4 Green Bean

β-carotene concentrations in the fresh and frozen samples analysed were below the detection limits of the method.

3.4.5 Cauliflower

Similar to green beans, the β-carotene concentrations in the fresh and frozen samples analysed were below the detection limits of the method.

3.4.6 Sweetcorn

The analytical method used was also unable to quantify β-carotene concentrations in the fresh and frozen sweetcorn samples.

3.4.7 Discussion

β-carotene concentrations in the fresh and frozen fruits and vegetables analysed were either very low or below the detection limits of the method. Where β-carotene concentration data were obtained, these indicated that the fresh and frozen berry fruits were similar and that there were only marginal changes during refrigerated storage for up to 3 days.

In general, the concentrations recorded using the HPLC method were low. It is considered that further optimisation of the method is necessary, combined with analysis of larger numbers of replicates.

3.5 Carotenoids: Lutein

3.5.1 Blueberry

Similar to β-carotene, lutein concentrations were also low in blueberry. Significantly higher concentrations were observed in the fresh blueberry compared to the frozen samples (Fig 3.22). During refrigerated storage there was a decrease in the lutein concentrations.

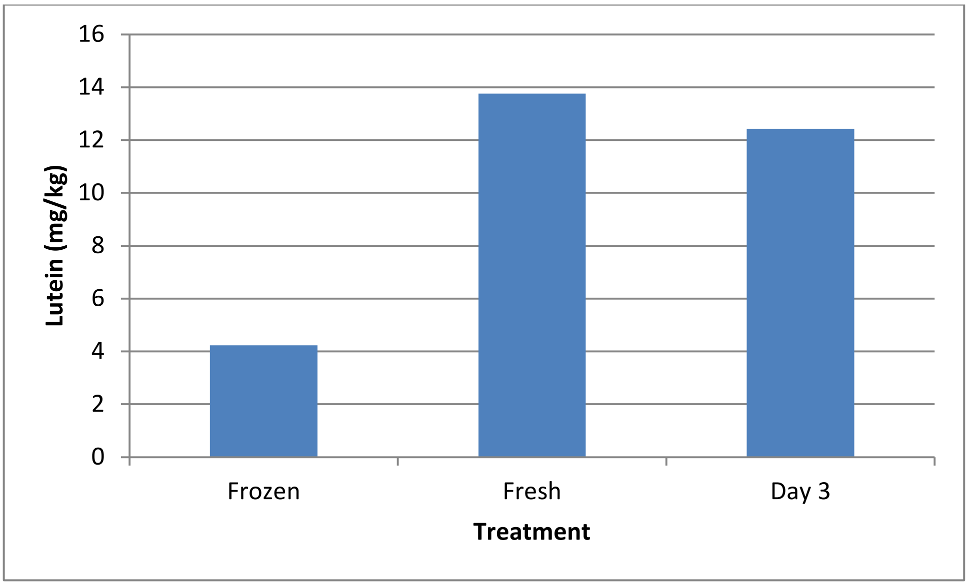

Figure 3.22 Lutein concentrations in frozen and fresh blueberry.

3.5.2 Raspberry

Lutein concentrations in raspberry were also uniformly low (Fig 3.23, below). Concentrations in the fresh and frozen samples were not significantly different. During refrigerated storage there was an apparent increase in concentrations after 3 days but this was also not significant.

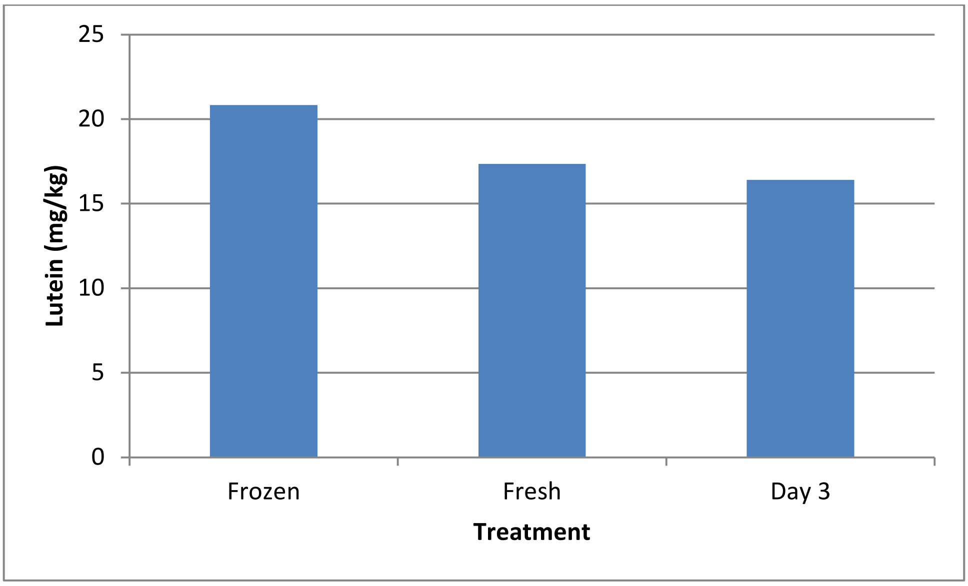

Figure 3.23 Lutein concentrations in frozen and fresh raspberry.

3.5.3 Pea

Lutein concentrations in fresh and frozen pea were also low and not significantly different (Fig. 3.24). During refrigerated storage there was also no significant change in the measured concentrations.

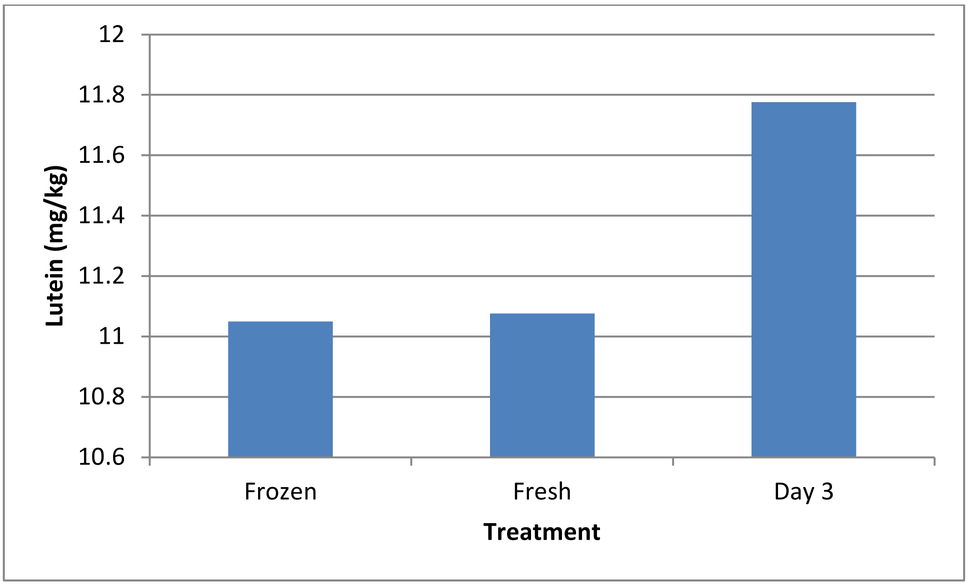

Figure 3.24 Lutein concentrations in frozen and fresh pea.

3.5.4 Green Bean

Lutein concentrations measured in the green bean samples were uniformly low. No significant difference was found between fresh or frozen samples and there were no observable effects due to storage for up to 3 days.

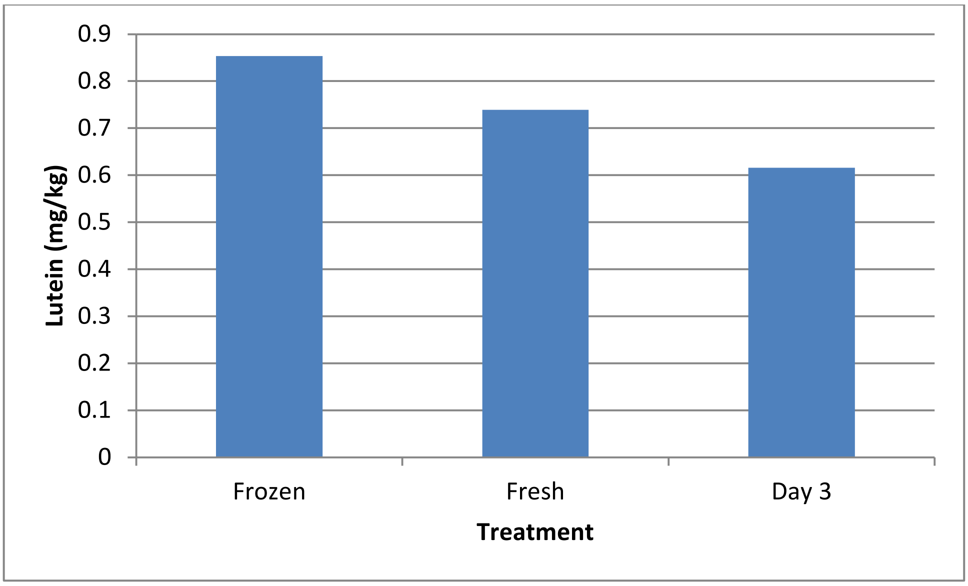

Figure 3.25 Lutein concentrations in frozen and fresh green bean.

3.5.5 Cauliflower

Lutein concentrations in the fresh and frozen cauliflower samples analysed were below the detection limits of the method.

3.5.6 Sweetcorn

Similar to cauliflower, Lutein concentrations in the fresh and frozen sweetcorn samples analysed were below the detection limits of the method.

3.5.7 Discussion

Lutein concentrations in the fresh and frozen fruits and vegetables analysed were either very low or below the detection limits of the method. Where lutein concentration data were obtained, these indicated that with the exception of blueberry, the fresh and frozen fruits and vegetables were similar and that there were no changes during refrigerated storage for up to 3 days. In contrast, the data obtained for blueberry indicated that concentrations were higher in the fresh samples and subsequently decreased during refrigerated storage, such that the concentrations resembled those found in the frozen produce.

In general, the concentrations recorded using the HPLC method were low. It is considered that further optimisation of the method is necessary, combined with analysis of larger numbers of replicates.

4. GENERAL DISCUSSION & CONCLUSIONS

Changes in vitamin C content and phenolic compounds during processing and storage appear to be variable and may depend highly on commodity, cultivar and post-harvest conditions. Future studies may clarify some of the reported discrepancies. Thermal treatments such as blanching performed pre-freezing may contribute to the loss of total phenolics; however, this may also contribute to increased extractability of phenolic compounds. Furthermore, since phenolic compounds are both water-soluble and sensitive to oxidation through exposure to light, degradation of total phenolics is possible during fresh and frozen storage conditions. However, since, the reported data in this study show variable results, future evaluation of vitamins and total phenolics may be germane.

Losses of nutrients during fresh storage may be more substantial than consumers realise. Dependent on commodity, freezing may actually preserve nutrient value. Frozen produce would appear to lose fewer nutrients initially because of the short heating time in the blanching process, but they may lose more nutrient value during long-term storage due to oxidation.

Overall, variations in nutrient content of starting material will influence final vitamin and anthocyanin content as concentrations can vary depending on vegetable type, maturity at harvest, genetic variation in the cultivars, pre-harvest conditions, post-harvest conditions, handling and storage and processing and preparation.

Some of the nutrient losses reported in this study may not be statistically significant in terms of human nutrition, as the apparent increases in nutrient value may be due to enzymatic activity (for example the action of pectin methyl esterases during ripening) increasing as a factor of time, therefore making the determination of these nutrients highly detectable instrumentally. However, the anomalies may be attributed to sampling variations, which are difficult to control for in non-uniform fruit and vegetables of unknown provenance.

Anthocyanins and vitamin C are all highly unstable and are very susceptible to degradation. Their stability can be affected by several factors such as pH, storage temperature, chemical structure, concentration, light, oxygen exposure, solvent exposure, the presence of enzymes, other flavonoids, proteins and metallic ions. The results in this study indicate that the observed levels of Vitamin C are generally higher in frozen produce than in fresh fruit and vegetables that have been stored for 3 days.

Anthocyanins may be released from the cellular matrix by freezing, although this may appear as an increase in values due to efficient extraction, it is possible that analysis of these released anthocyanins may render the nutrient more detectable instrumentally although there is no indication that these increased levels will be available biologically.

Since nutrients such as vitamin C and phytochemical content are highly dependent on commodity, cultivar and growing practices, more studies following the same food throughout the consumer chain would be beneficial. Analysis of fresh and frozen fruits and vegetables available in retail markets would also be more appropriate for understanding the nutritional content of fruits and vegetables available to the consumer.

Not knowing the true provenance of the fruit and vegetables, i.e., cultivar, country of origin, growing conditions and harvesting conditions could account for the wide range of variability within the results presented. Whilst, the fresh produce provenance is somewhat easy to determine due to labelling of produce, the same cannot be said for the frozen produce. From the perspective of the general consumer, there is very little information available on packaging of frozen produce that states how long the product has been frozen and in storage. These issues may very well determine the overall nutritional availability of vitamins and antioxidants within the frozen products. Furthermore, within this study, relatively small sample sizes were analysed. In future studies a larger sample of all fruit and vegetables of known provenance would be investigated.

From the initial data obtained during this study, the following general conclusions can be made:

- The concentrations measured in frozen produce generally resembled those observed in the corresponding fresh produce prior to refrigerated storage.

- Analyte concentrations in fresh produce frequently exhibited a decrease during refrigerated storage, to levels below those observed in the corresponding frozen produce. The effects were most noticeable in the soft fruits.

- Concentrations were frequently lowest after 3 days of refrigerated storage. was Any increase was considered to reflect the effects of ripening, in which the action of enzymes such as the pectin methyl esterases enhanced the softening of the tissue matrices and the subsequent extractability of the analytes.

Whilst general trends could be derived from the analysis undertaken, further investigation is considered desirable with larger sample sizes. Because of potential changes in the extractability of the target analytes leading to misleading assessments of antioxidant content, it is considered that an assessment of the bioaccessible antioxidants within the fresh and frozen fruit and vegetables would also be of interest. This would require processing of the fruit and vegetables using an in vitro digestion system to mimic human digestive processes. Current research conducted at the University of Chester developed this strategy to more fully evaluate the effects of novel food processing technologies on food constituents and nutrients.

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Leatherhead
Food Research

Company Name: British Frozen Food Federation

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Project Title: Nutritional content of fresh vs frozen foods

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Contents

Background

Leatherhead Food Research (Leatherhead) was approached by British Frozen Foods Federation to carry out analyses to determine the nutritional value (specifically antioxidant-type compounds) in fresh vs frozen fruits and vegetables.

The proposed approach was to focus on a range of analytes in a limited number of samples. The vegetables and fruit selected were those which could be obtained in a supermarket in both fresh and frozen forms on the same day.

Leatherhead suggested that composite samples should be prepared from vegetables obtained from different supermarkets. In this way some account might be taken of factors such as variety, growing conditions and country of origin, which may all affect the composition of natural samples such as these.

Objective

The key objectives of this project are as follows:

Determine a number of nutritional parameters in fresh vs frozen fruits and vegetables.

Approach

Materials

Samples
Samples of broccoli, Brussels sprouts, spinach, carrots and blueberries were purchased fresh and frozen from four local supermarkets.

Storage
Frozen samples were stored frozen between purchase and analysis.
Fresh samples were stored in a refrigerator and sampled 3 days after purchase.

Sample preparation
Fresh samples were prepared as they might be in a domestic kitchen.

Composite samples were prepared as follows: 50g portions from different supermarkets were combined and chopped/blended in a homogeniser.

Samples were transferred to screw-top containers and either analysed on the day of preparation or stored frozen until analysis.

Methods

Vitamin C:
Samples were extracted into metaphosphoric acid in the presence of dithiothreitol as reducing agent. Extracts were analysed by HPLC with UV detection, and quantified against external standards.

Total polyphenols:
Total polyphenols were estimated using the Folin-Ciocalteu method, quantified against gallic acid. Results are given in gallic acid equivalents (GAE).

Total anthocyanins:
Total anthocyanins were estimated spectrophotometrically and calculated as cyanidin-3-glucoside according to AOAC official method 2005.02 (pH differential method).

Lutein and β-carotene:
Samples were extracted into a mixture of ethanol:hexane in the presence of BHT as antioxidant. After concentration of the hexane extract the samples were analysed by HPLC with visible detection and quantified against external standards.

Moisture determination:
Moisture content of the samples was determined by loss on drying at 101 ºC using the sand in dish method.

Results

Raw data is given in Appendix 1. Table 1 shows a summary of the average values expressed on dry matter. Results have been expressed on dry matter to take into account any differences in moisture content between the samples. In particular it was noted that frozen broccoli and spinach had higher water content than the fresh samples.

		Total polyphenols mg/kg gallic acid equivalents, expressed on dry matter	Total anthocyanins as cyanidin-3-glucoside equivalents mg/kg dry matter	Vitamin C mg/100g dry matter	Lutein mg/kg dry matter	β-carotene mg/kg dry matter
Broccoli	Frozen	4810	N/A	640	52.7	48.03
	Fresh, 3 days refrigerated storage	6290	N/A	600	37.8	10.3
Carrots	Frozen	2275	N/A	25	22.9	815
	Fresh, 3 days refrigerated storage	2015	N/A	21	6	425
Brussels Sprouts	Frozen	N/A	N/A	625	17.2	16.4
	Fresh, 3 days refrigerated storage	N/A	N/A	450	10.8	6.85
Spinach	Frozen	7165	N/A	81	625	285
	Fresh, 3 days refrigerated storage	10755	N/A	240	515	270
Blueberries	Frozen	12035	7925	N/A	N/A	N/A
	Fresh, 3 days refrigerated storage	13515	9995	N/A	N/A	N/A

Table 1: Average results of analysis of broccoli, carrots, Brussels sprouts, spinach and blueberries for selected parameters. Values expressed on dry matter.
N/A = not analysed

Total polyphenols
Figure 1 shows the total polyphenols in broccoli, carrots, spinach and blueberries. The duplicate analyses are shown as separate bars in order to show the differences between replicate analysis of the same sample. On average the frozen carrots appear to have a very slightly higher level of polyphenols than the fresh carrots, although more samples would need to be analysed to determine if there was a statistically significant difference. The conclusion from the data obtained in this study is that fresh and frozen carrots have similar levels of total polyphenols. For the broccoli and spinach samples the trend was for the frozen samples to be lower in total polyphenols than the fresh samples. For the blueberry samples, again the spread of data suggests that more analysis would be necessary to draw a definite conclusion, but the trend appears to be for the frozen sample to be lower in polyphenols than the fresh samples.

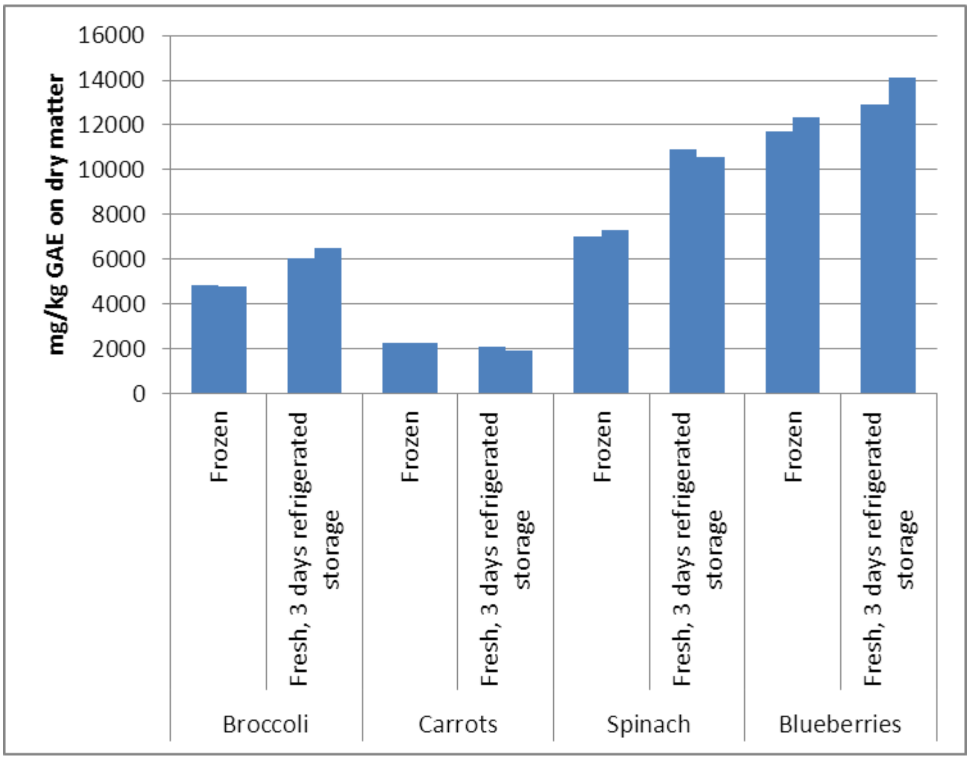

Fig 1. Total polyphenols in fresh and frozen vegetables and fruit, expressed on dry matter as gallic acid equivalents. Individual results of duplicate analyses shown to demonstrate variability of data.

Vitamin C
Figure 2 shows the measured vitamin C levels in samples of broccoli, carrots, Brussels sprouts and spinach. Taking into account analytical variability, the broccoli and carrots showed similar levels of vitamin C in the fresh and frozen samples. The frozen Brussels sprouts, however, do show considerably greater levels of vitamin C than the fresh samples. In contrast, the frozen spinach showed considerably less vitamin C than the fresh samples.

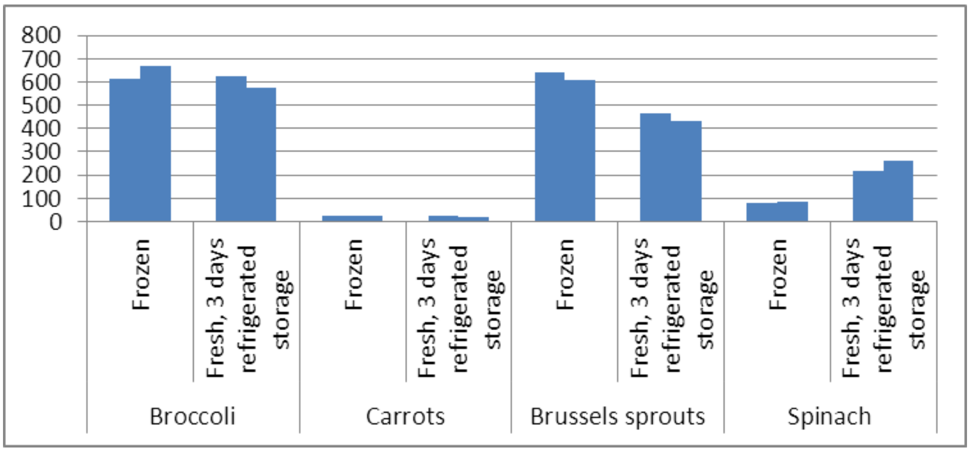

Fig 2. Vitamin C in fresh and frozen vegetables and fruit, expressed on dry matter. Individual results of duplicate analyses shown to demonstrate variability of data.

Lutein

Fig. 3 shows lutein in broccoli, carrots and Brussels sprouts, and Fig. 4 shows lutein in spinach (shown on a different scale). Triplicate analyses were carried out on the frozen and three day refrigerated samples, enabling the standard deviation to be calculated and shown as an error bar. It can be seen for all of the vegetables analysed that the trend was for the lutein in the frozen samples to be higher than in the fresh samples. The greatest difference was seen in the carrots where the levels were considerably higher in the frozen sample.

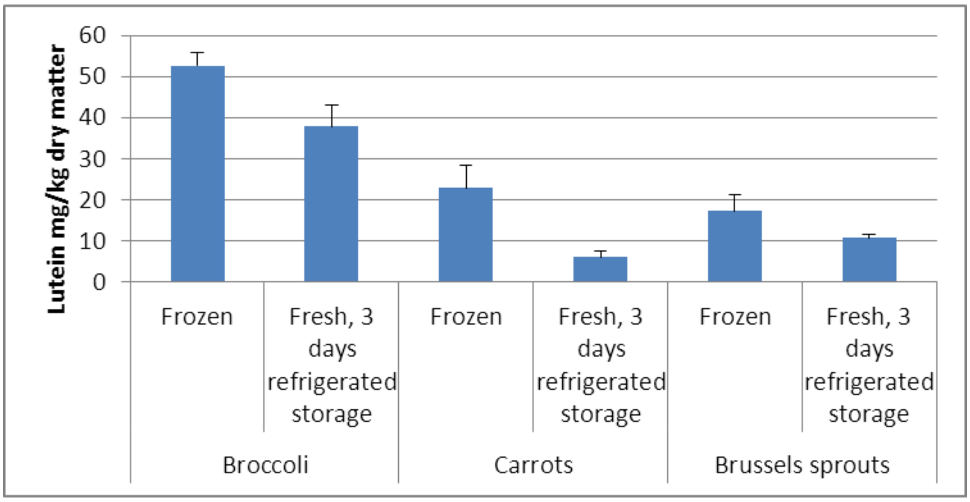

Fig 3. Lutein in fresh and frozen broccoli, carrots and Brussels sprouts, expressed on dry matter. Average of three replicates shown for frozen samples and fresh samples stored for three days. Error bars show one standard deviation.

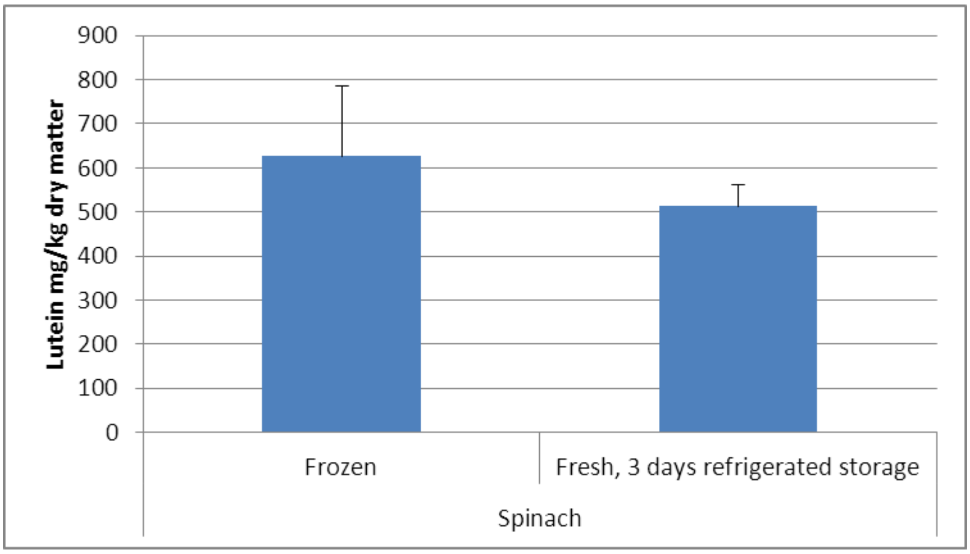

Fig 4. Lutein in fresh and frozen spinach, expressed on dry matter. Average of three replicates shown for frozen samples and fresh samples stored for three days. Error bars show one standard deviation.

β-carotene

Figures 5 and 6 show β-carotene results. In order to reduce analytical variability all samples were extracted and run as one batch, therefore not all samples were analysed in duplicate. Figures show results from individual duplicates, where obtained, and show good agreement. It can be seen that for carrots, broccoli and Brussels sprouts the frozen samples were considerably higher in β-carotene than the fresh samples. Frozen spinach had similar levels of β-carotene to the fresh samples.

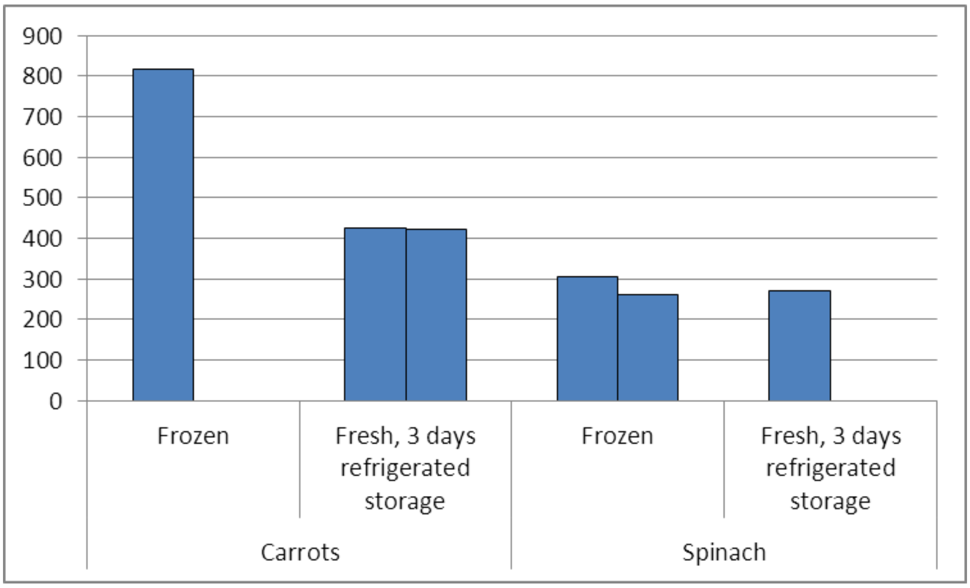

Fig. 5 β-carotene in carrots and spinach. Individual duplicate results shown, where obtained, to show variability between duplicate analyses

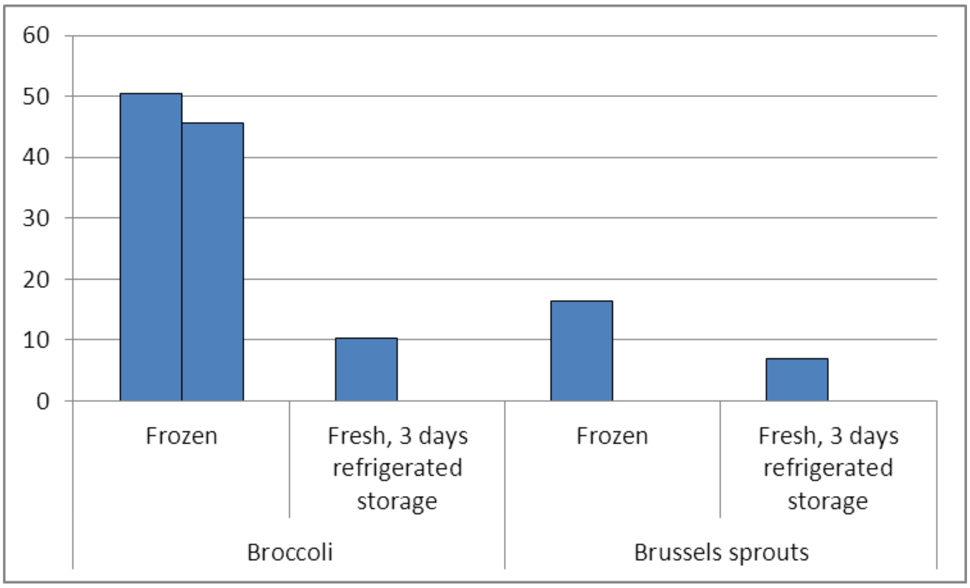

Fig. 6 β-carotene in broccoli and Brussels sprouts. Individual duplicate results shown, where obtained, to show variability between duplicate analyses

Anthocyanins
Anthocyanin analysis was carried out on extracts of frozen and fresh blueberries, and the results are shown in Fig. 7. The agreement between replicates was very close, and it can be seen from the graph that the frozen blueberry sample has lower total anthocyanins than the fresh samples.

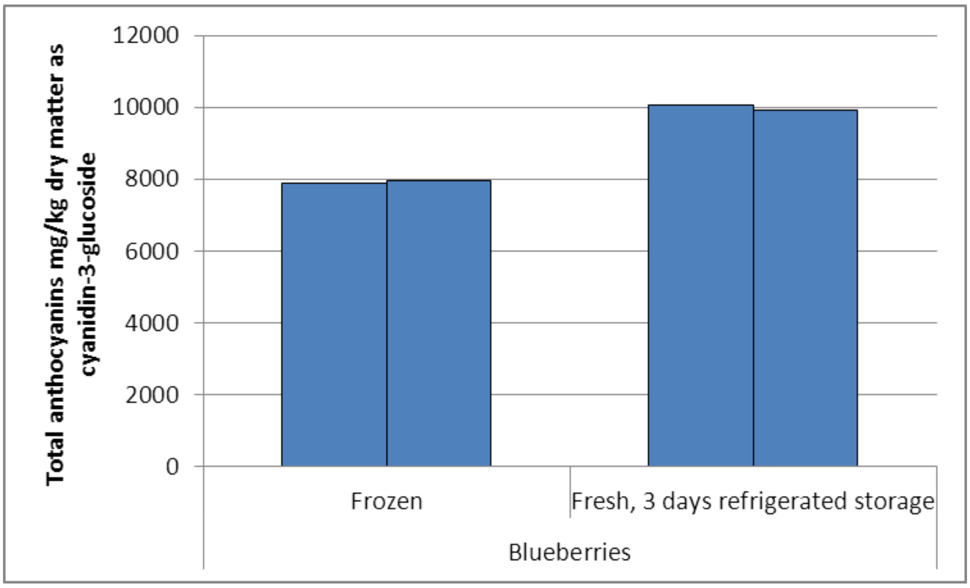

Fig. 7. Anthocyanins in fresh and frozen blueberries, individual results of duplicate analyses shown.

Discussion and Conclusions

The following conclusions have been drawn based on the data obtained:

- The fresh and frozen carrot samples had similar levels of polyphenols
- The trend for the frozen broccoli, blueberry and spinach samples was that they were lower in polyphenols than the fresh samples
- The frozen Brussels sprouts samples were higher in vitamin C than the fresh samples
- The frozen broccoli and carrots had similar levels of vitamin C to their fresh equivalents
- The frozen spinach had less vitamin C than the fresh samples
- Lutein tended to be higher in frozen samples than fresh (broccoli, carrots, Brussels sprouts, spinach), with the greatest difference seen in the carrots
- β-carotene was higher in frozen carrots, broccoli and Brussels sprouts than fresh samples
- Frozen spinach had similar levels of β-carotene to the fresh samples
- Total anthocyanin content of frozen blueberries was lower than the fresh samples

As discussed above, further work would be required to discover the reasons behind these differences, but factors such as nutrient levels in the crops before processing, and the effect of any processing steps themselves (blanching, for example) would need to be taken into account.

Collection of the samples for this study was carried out in one day from four supermarkets local to the laboratory at which the analysis took place. The study therefore provides a snapshot of a small number of the samples available at that time. The study was not designed to survey all available products, and did not cover all factors which might affect the nutrient content of the fruit and vegetables selected. Additionally the study was not set up to look at the effects of freezing on fruits and vegetables; the frozen vegetables in this study may well have been a different variety from the fresh products available, and may have been grown in different countries or at different times of the year. The results of this study are simply a measure of the nutrients available in those samples purchased commercially at that time.

Appendix 1

		Sample No.	Moisture g/100g	Dry Matter g/100g	Dry Matter g/100g ave	Total Anthocyanins mg/kg	Total Anthocyanins mg/kg dry matter	Ave Total Anthocyanins mg/kg dry matter	Total polyphenols mg/kg GAE	Total polyphenols mg/kg GAE on dry matter	Ave. Total polyphenols mg/kg GAE on dry matter	Vitamin C mg/100g fresh weight	Vitamin C mg/kg dry matter	Ave. Vitamin C mg/kg dry matter	lutein mg/kg fresh weight	lutein mg/kg dry matter	ave. lutein mg/kg dry matter	b-carotene mg/kg fresh weight	b-carotene mg/kg dry matter	ave. b-carotene mg/kg dry matter
Broccoli	Frozen	1301464	93.1	6.9	6.85				332	4848	4810	41.9	611	639	3.69	53.9	52.7	3.45	50.4	48.0
			93.2	6.8					327	4771		45.7	667		3.37	49.2		3.13	45.7	
															3.76	54.9				
	Fresh, 3 days refrigerated storage	1301465	88.4	11.6	11.65				707	6064	6289	72.6	623	598	3.87	33.2	37.8	1.2	10.3	10.3
			88.3	11.7					759	6513		66.8	574		4.29	36.8				
															5.06	43.4				
Carrots	Frozen	1301467	91.7	8.3	8.35				191	2290	2273	2.0	24	25	1.55	18.6	22.9	68.2	816.8	816.8
			91.6	8.4					188	2256		2.2	26		2.43	29.1				
															1.76	21.1				
	Fresh, 3 days refrigerated storage	1301468	90.7	9.3	9.3				193	2077	2013	2.3	25	21	0.47	5.1	6.0	39.7	426.9	424.2
			90.7	9.3					181	1950		1.6	17		0.5	5.4		39.2	421.5	
															0.71	7.6				
Brussels Sprouts	Frozen	1301470	86.7	13.3	13.35							85.3	639	623	1.93	14.5	17.2	2.19	16.4	16.4
			86.6	13.4								81.1	607		2.03	15.2				
															2.93	21.9				
	Fresh, 3 days refrigerated storage	1301471	87.4	12.6	12.55							58.6	467	450	1.23	9.8	10.8	0.86	6.9	6.9
			87.5	12.5								54.4	433		1.37	10.9				
															1.46	11.6				
Spinach	Frozen	1301473	92	8	7.95				560	7045	7163	6.1	77	81	63.5	798.7	626.0	24.4	306.9	284.3
			92.1	7.9					579	7281		6.7	84		47	591.2		20.8	261.6	
															38.8	488.1				
	Fresh, 3 days refrigerated storage	1301474	89.9	10.1	10.05				1099	10934	10754	21.7	216	238	47.6	473.6	512.8	27.1	269.7	269.7
			90	10					1063	10575		26.1	260		57.2	569.2				
															49.8	495.5				
Blueberries	Frozen	1301476	84.4	15.6	15.6	1229	7878	7923	1828	11718	12034									
			84.4	15.6		1243	7968		1927	12351										
	Fresh, 3 days refrigerated storage	1301477	85	15	14.95	1506	10074	9997	1930	12912	13514									
			85.1	14.9		1483	9920		2110	14116										