Public Health Nutrition MSc

Nutritional comparison of cooked fresh and frozen vegetables

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Abstract

Dietary antioxidants (AO) are believed to contribute to the overall health benefits seen from fruit and vegetables. Despite increased public awareness of the health benefits of fruit and vegetables through campaigns such as 5 A DAY, consumption remains low. Freezing is usually regarded as destructive to AO and ascorbic acid (AA) and this has fostered a belief that fresh vegetables are nutritionally superior to frozen. In this study, AO and AA activity in commercially bought fresh and frozen vegetables were investigated and compared after a typical home cooking practice (boiling). Five different vegetables were examined: carrots, broccoli, green beans, peas and spinach. Each vegetable was bought four times from a selection of local supermarkets and green grocers in the Wirral, United Kingdom to account for variation. The oxygen radical absorbance capacity (ORAC) assay and 2, 6-dichlorophenolindophenol (DCPIP) assay were utilised to measure total antioxidant capacity (TAC) and AA content respectively. The results showed both fresh and frozen vegetables to contain AO and AA after cooking. Cooked fresh spinach and peas contained significantly ($p<.05$) higher levels of total AO than cooked frozen spinach and peas. However the remaining fresh and frozen vegetables (broccoli, carrots and green beans) did not appear to differ in AO content after cooking. Furthermore there was no difference between AA content in fresh and frozen cooked vegetables. The current study provides evidence against the misconception that fresh is always nutritionally superior to frozen at the point of consumption. Frozen vegetable promotion may be the way forward to increase fruit and vegetable consumption as they are generally nutritionally comparable to fresh but, cheaper, result in less waste, are more convenient and, if packaged correctly, taste the same as fresh. Further work, on a larger scale, is needed, to measure AO and AA content of fresh and frozen vegetables bought and cooked by the consumer.

Declaration of original work

'I hereby declare that work contained herewith is original and entirely my own work (unless indicated otherwise). It has not been previously submitted in support of a Degree, qualification or other course'.

Signed:

Anna Simpson

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2.0 Tables/Figures

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3.0 Notations

µL = microlitre

g = gram

M = molar

mg = *milligrams*

mL = millilitre

µM = micrometre

nm = nanometre

mM = millimolar

± = standard deviation

Kg = kilogram

£ = great British pound

% = percentage

4.0 Acronyms

CVD = cardiovascular disease

FR = free radicals

OS = oxidative stress

AO= antioxidants

TAC = total antioxidant capacity

AA = ascorbic acid

TP = total phenolics

ORAC = oxygen radical absorbance capacity

DCPIP = 2, 6-dichlorophenolindophenol

DIP = 2, 6-dichloroindophenol

DHAA = dehydroascorbic acid

PBS = phosphate buffered saline

Trolox = 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2 carboxylic acid

AAPH = 2, 2'-azobis(2-amidinopropane) dihydrochloride

HCL = hydrochloric acid

CV = coefficient of variation

SD = standard deviation

HPLC = high performance liquid chromatography

5.0 Introduction

5.1 General Introduction

Today there is vast interest by health organisations, the public and consumers in improving health and wellbeing through diet. The Eatwell plate is a key public health message that illustrates the type and proportions of foods we should be eating as part of a well balanced, healthy diet: it recommends a persons diet should consist of 33% fruit and vegetables (Department of Health, 2012). Fruit and vegetables have been shown to have a protective effect against some cancers (Wargovich, 2000; Dragsted, Strube & Larsen, 1993). Data provides strong support for fruit and vegetables and their protective role against cancer of the pancreas, bladder and breast (American Institute of Cancer Research, 1997). Additionally a prospective cohort study in Boston that followed 84, 251 women aged 34-59 years for 14 years and 42,148 men aged 40-75 years for 8 years investigated the effect of fruit and vegetable intake on risk for coronary heart disease (CHD) (Joshipura et al., 2001). The outcome measure was non fatal myocardial infarction or fatal CHD and outcomes showed those who ate most fruit and vegetables had a relative risk (RR) for CHD of 0.8 compared to those who ate the least fruit and vegetables. Each 1 portion increase per day of fruit and vegetables was linked with a 4% lower risk of CHD (Joshipura et al., 2001). Furthermore 690 participants aged 25-64 years were involved in a 6 month randomised control study of brief negotiation method to encourage consumption of 5 portions of fruit and vegetables a day (John, Ziebland, Yudkin, Roe & Neil, 2002). The findings revealed those who increased fruit and vegetable intake by 1.4 portions per day had a reduction in systolic ($p<0.0001$) and diastolic blood pressure ($p = 0.02$) (John et al., 2002). Findings by Appel et al., (1997) also showed a diet rich in fruit and vegetables decreased systolic ($p<0.001$) and

diastolic blood pressure (p = 0.07). This is relevant as cardiovascular disease (CVD) is the most common cause of death in the UK, responsible for 34% of deaths and cancer is the second most common cause, accounting for 27% of deaths (World Health Organisation, 2011). Additionally, insufficient consumption of fruit and vegetables is now described as one of the ten global disease burden risk factors (World Health Organisation, 2009). The World Health Organisation (WHO) (2002) has estimated as many as 2.7 million lives worldwide could be saved every year if people consumed a sufficient amount of fruit and vegetables. Unfortunately the Department for Environment, Food and Rural Affairs (DEFRA) (2012) has found a downward trend in the purchase of fruit and vegetables in the United Kingdom since 2006. The known importance of fruit and vegetables led to the United Kingdom's 2003 5 A DAY campaign to promote the consumption of a variety of at least 5 portions of fruit and vegetables within a day (Department of Health, 2010). Despite increased public awareness of the health benefits of fruit and vegetables, consumption remains low; it was reported in 2010 that only 25% men, 27% women and 20% children (5-15 years) were consuming 5 portions a day (DEFRA, 2012).

5.2 Fruit and Vegetables, Antioxidants and Health

The way in which fruit and vegetables improve health is not yet fully understood. Fruit and vegetables contain a huge variety of vitamins and minerals as well as bioactive constituents such as phytochemicals and phytoestrogens. Many of the above also act as AO. Dietary AO are believed to contribute to the overall health benefits seen from fruit and vegetables consumption due to their ability to alleviate the consequences of oxidative stress

(OS) in development of disease and the ageing process (Joseph, Denisova, Bielinski, Fisher & Shukitt-Hale, 2000; Meydani, 2001; Ames, Shigenaga & Hagen, 1993).

OS results from an imbalance between the production of reactive oxygen species (free radicals) and AO defences. Free radicals (FR) are unstable highly reactive and energized molecules having unpaired electrons; they react quickly with other compounds in an attempt to capture an electron and thus become stable. When a molecule has been attacked and lost an electron it becomes a free radical itself, resulting in a chain reaction. FR thus adversely alter proteins, lipids and DNA (Young & Woodside, 2001). Some phytochemicals act as AO and they neutralize FR by donating one of their electrons (hydrogen), ending the chain reaction. They do not become FR as they are stable in either form.

Oxidative damage to biological molecules is associated with the cause and function of health conditions such as CVD (Steinberg, 1997), neurodegenerative disease (Joseph et al., 2000), some cancers (Ames et al., 1993), diabetes (Laaksonen & Sen, 2000) and others. The build up of oxidative lesions in biological molecules is thought to cause the degenerative processes seen in ageing (Meydani, 2001). There is an increasing body of evidence to support that fruit and vegetables boost endogenous AO within the body and consequently alleviate oxidative damage and its adverse effects of on health (Hertog et al., 1995; Joseph et al., 2000; Aviram et al., 2004).

There are many thoroughly investigated components within fruit and vegetables that perform as AO, such as fibre, polyphenols, flavonoids, isoflavones, vitamin A, B, C, E, calcium, catechins, uric acid and thiocyanates (Kararkaya & Kavas, 1999). These may work alone or together to impose their protective function against FR. Phytochemical AO are

preferentially oxidised by free radicals and therefore protect the biological molecules and systems from oxidative damage. Kalt (2005) reported that the most ample AO in fruit and vegetables are vitamin C, carotenoids and phenolics and they work effectively due to their structure which is electron rich in the form of oxidized double bonds and hydroxyl groups. Therefore the influence of different factors on total AO within vegetables will focus mainly on vitamin C, carotenoids and phenolics as they will contribute hugely to TAC.

5.2.1 Vitamin C

A well-known AO is vitamin C which is otherwise known as AA. It is a water-soluble AO known to be important to health (Benzie & Szeto, 1999; Davey et al., 2000). Human cells cannot perform the last step of vitamin C biosynthesis, hence, in humans AA has to be supplemented through food and/or as artificial supplements. Fruit and vegetables contain vitamin C however the amount varies depending on type (Favell, 1998) (see Table 5.1). The UK has a reference nutrient intake for vitamin C of between 25-30mg/d for children and between 35-40mg/d for men and women depending on age (Committee on Medical Aspects of Food Policy, 1991). Plasma vitamin C concentrations of less than 11umol/L (0.2 mg/100ml) indicates biochemical depletion (Sauberlich, 1975). Scurvy, which is linked to poor vitamin C status, has been a major health problem throughout human history (Carpenter, 1986). In the early stages of scurvy symptoms can include fatigue, weight loss, irritability, generalized myalgias, and weakness (Wang and Still, 2007). Later symptoms include the classic findings of petechiae, purpura, bleeding gums, hemarthrosis, corkscrew hairs, and poor wound healing (Hodges, Hood, Canham, Sauberlich & Baker, 1971). This is due to the role vitamin C plays in the formation of mature collagen, and deficiency impairs the synthesis of connective tissue causing delayed wound healing and formation of fragile

granulation tissue (Peterkofsky, 1991). If untreated, scurvy is fatal. The most commonly believed health benefit of AA is its role in prevention/relief of the common cold (Pauling, 1970). However, vitamin C potentially has a much more important role to play in health promotion. A study which investigated the relation between a diet high in vitamin C and coronary mortality studied a cohort of 5,133 Finnish men and women aged 30-69 years who were all free from heart disease at the start. They found dietary intake of vitamin C to reduce the risk of CHD mortality in women (Knekt et al., 1994). Ample AA may also have some sort of role in reducing the risk of oesophageal, pancreatic and lung cancer (Wargovich, 2000). Finally Gale, Marytn, Winter and Cooper (1995) researched whether vitamin C status was related to death from stroke in those aged 65+ years. The 20 year follow up study in Britain found those in the highest third of the distribution of vitamin C ingestion had a RR of stroke mortality of 0.5 compared to those in the lowest third (Gale et al., 1995).

Table 5.1: U.S Department of Agriculture (USDA) nutrient data for vitamin C (mg) content in select raw fruit and vegetables (g)

Description of vegetable	Weight (g)	Vitamin C content (mg)
Apple, raw with skin	138	6.3
Broccoli	31	27.7
Carrot, raw with skin	72	4.2
Cauliflower	100	48.2
Spinach	30	8.4
Kiwi fruits, green, raw	76	70.5
Orange	131	69.7
Tomatoes, red, ripe, raw, yearly average	123	16.9
Celery	40	1.2
Raspberries	123	32.2

(USDA Nutrient Database, 2013)

5.3 Factors affecting AO in Vegetables

Vegetables are generally low in fat, energy and protein but high in carbohydrates and fibre and they contain a large variety of micronutrients. These attributes are readily associated by the consumer with fresh vegetables but there has been a popular misconception that frozen and canned vegetables are lacking in micronutrients. However Hunter and Fletcher (2002) found frozen vegetables to be comparable to their fresh equivalents in AO content but to contain substantially higher amounts than levels found in canned vegetables. Increasingly frozen food is being shown to not be nutritionally inferior to

fresh (Favell, 1998). Both fresh and frozen vegetables are susceptible to the same effect of nutrient loss due to factors including storage, processing and cooking.

Vegetables are grown in different parts of the world, at a variety of temperatures and at different times of year. In addition the major constituent of vegetables is water and, following harvest, they undergo increased rates of respiration causing moisture loss, quality deterioration and potential microbial spoilage (Rickman, Barrett & Bruhn, 2007). As soon as fruit and vegetables have been harvested they are no longer attached to their source of nutrients (plant or tree) and practically begin using themselves as a source of energy (Rickman et al., 2007). Therefore the shelf life of many vegetables is days. Consequently storage and processing was developed and has been used for many years to transform perishable vegetables into safe, appealing and stable products.

5.3.1 Storage

Whether raw or processed, vegetables are handled and stored by both the producer and consumer which may alter their nutritional profile. Before storage even begins operations such as cutting and slicing may occur and this could provoke fast enzymatic reductions of some naturally occurring AO due to cellular disturbance (Davey et al., 2000). The storage method of refrigeration slows down respiration which in turn increases shelf life of vegetables, however AO are still affected.

Ascorbic Acid

Compared with other AO AA is more vulnerable to experience losses during handling and storage after harvesting due to being a less stable vitamin (Kalt, 2005). Freshly picked vegetables contain the highest amount of AA (Favell, 1998) however AA degrades straight

after harvesting, for example, AA in raw green peas reduced by around half wet weight after just 24-48 hours (Fellers and Stepat, 1935). Additionally, a study discovered broccoli florets that contained high AA after harvest suffered an AA loss of 52% after 3 weeks storage (Howard, Wong, Perry & Klein, 1999). Refrigeration can slow its degradation rate (Rickman et al., 2007). Due to AA degradation, which happens between harvest and consumption, there are suggestions that processing such as freezing, canning and jarring may have a preserving effect (Howard et al., 2007; Hunter & Fletcher, 2002; Fellers & Stepat, 1935). In support of this an investigation found AA within peas and spinach stored at 4^{o}C was lower than the amount found in their frozen equivalents after 10 days. In the same study fresh spinach stored at ambient temperatures lost its entire AA content by day 4 and after 21 days in chilled samples (Hunter & Fletcher, 2002). In frozen vegetables the blanching process caused a loss of 20% and 50% of AA in spinach and peas respectively but AA levels then remained constant during storage of 21 days (Hunter and Fletcher, 2002). Puupponen-Pimiä et al., (2003) findings supported that blanching causes the major losses of AA but that during storage AA suffers minimal loss - they found through a 12 month storage period, losses were 10% or less in 20 different vegetables.

Phenolics

In terms of phenolic compounds in fresh vegetables studies have shown similar results to AA with large losses of phenolic compounds in broccoli after 10 days storage (Vallejo, Tomas-Barberan and Garcia-Viguera, 2003).Data on phenolic compounds within vegetables after frozen storage is lacking. However, a study was conducted in Finland by Puupponen-Pimiä et al., (2003) that investigated the impact of freezing and long term freezer storage on various compounds found in 20 frequently consumed vegetables. They

found some small losses of total phenolics (TP) on a dry weight basis in broccoli, carrots, cauliflower, peas and potatoes during a year of frozen storage. Overall losses in TP content following blanching and long term storage was ranged from 20-30% (Puupponen-Pimiä et al., 2003).

Carotenoids

There are few studies that describe spoilage of carotenoids during fresh storage. Salunkhe, Bolin and Reddy (1991) consider carotenoid losses during storage of fresh products to be low and that in some products post-harvest carotene production occurs. Howard et al., (1999) discovered a 10% β-carotene increase in carrots in 2 harvest years after storage in a refrigerator for 14 days. Howard et al., (1999) acknowledged that although it is conceivable that some interconversion of carotenoids occurred as the plant cells diminished whilst being stored, the seeming increase in b-carotene levels may have been due to variation in the chromatographic system due to the standard being unstable. Simonetti, Porrini and Testolin (1991) found substantial losses of β-carotene in peas after 3 weeks of storage. Similar to the findings of fresh vegetables an increase in β-carotene has been observed in stored frozen vegetables too (Elkins, 1979; Guerra-Vargas, Jaramillo-Flores, Dorantes-Alvarez & Hernandez-Sanchez, 2001) as have decreases (Bunea et al., 2008). Additionally modified atmosphere packaging appears to be effective in helping to retain vitamin C, phenolics and carotenoids in fruit and vegetables (Howard et al., 1999; van der Sluis, Dekker, de Jager & Jongen, 2001; Barth & Zhuang, 1996). Howard et al., (1999) discovered modified atmospheric packaging to slightly increase vitamin C between 3-7 days and fall back down to pre-storage levels after 7 days. Modified atmospheric packaging also

prohibited any loss of carotene in broccoli florets when stored between 6 ^{o}C and 5 ^{o}C whereas those unwrapped lost 50% of carotene content under the same storage conditions (Barth and Zhuang, 1996). Finally flavonoid phenolics in apples were retained when stored at 1.5 ^{o}C or 4 ^{o}C with or without controlled atmosphere however storage life was nearly doubled when stored in a controlled atmosphere.

5.3.2 Processing

Processing was developed and is now used to maintain foods fresh features whilst retaining vitamins and AO over a substantial period of time (Prochaska, Nguyen, Donat & Piekutowski, 2000). The preservation method of freezing is viewed as superior to techniques such as canning as freezing allows produce to retain more nutrients (Hunter & Fletcher, 2002). The freezing process includes pre freezing treatments, freezing, frozen storage and thawing. Freezing helps to preserve food by slowing enzymatic reactions, ageing and microbial growth (Bahceci, Serpen, Gokmen & Acar, 2005). However nutrient loss still occurs during the freezing process and, according to Fennema (1982), this is due to physical separation (peeling and trimming), leaching (during blanching), thermal (during blanching) and chemical degradation (during storage). Puupponen-Pimiä et al., (2003) consider the blanching step to be the cause of nutrient loss. Some research supports the idea that blanching can impact on AO content within vegetables (Hunter & Fletcher, 2002; Puupponen-Pimiä et al., 2003). Blanching occurs prior to freezing to inactivate enzymes that damage colour, flavour and nutritive value during the frozen storage (Fennema, 1982). However blanching can cause a loss of such features. Generally the blanching process involves subjecting vegetables to steam or hot water for 1-10 minutes at 75^{o}C-95^{o}C: time

and temperature is dependent on type of vegetables (Cao, 1996). After blanching, the product should be cooled quickly to reduce the breaking down of heat sensitive nutrients (Barbosa-Cánovas, Altunakar & Mejía-Lorío, 2005).

AA and Phenolics

Nutrient loss due to freezing is particularly true in the case of vitamin C and phenolic AO (Kalt, 2005). AA is heat labile and water soluble and although blanching is required to inactivate enzymes, it is harmful to the vegetables, causing vitamin C losses by thermal degradation and leaching (Negi & Roy, 2000). Like vitamin C phenolic AO are water soluble and can be lost from vegetable tissue due to leaching by processing in water (Kalt, 2005). In terms of AA, Favell (1998) described small losses of AA in frozen carrots yet significant losses in broccoli (20%) and peas (30%). The freezing process as a whole appears to account for an average of 50% AA loss (Fennema, 1982). Vitamin C is highly water-soluble and sensitive to heat and these factors are likely to be associated to seen losses (Rickman et al., 2007). Interestingly Bunea at al., (2008) found TP in spinach to decrease during processing whilst individual phenolic compounds acted differently. Three of the main phenolic acids, namely *ortho*-coumaric acid, ferulic acid and *para*-coumaric acid, all significantly ($p<.05$) increased during the blanching process. This indicates stability of some phenolic compounds. Conversely blanching and freezing has been shown to result in losses ranging from 20-30% of TP in peas, cauliflower and potatoes (Puupponen-Pimiä et al., 2003). Yet the same study discovered no change in TP in carrots and a 26% increase in cabbage.

Carotenoids

Carotenoids seem to be less adversely altered by processing compared with phenolics and AA. In the case of carotenoids processing can cause separation of AO from the plant matrix, an increase in carotenoid AO and enhanced digestive absorption (Shi & le Maguer, 2000). Talcott, Howard and Brenes (2000) noted that processing improved the bioavailability of β-carotene within carrots which caused an increase in overall AO. This study found that processed carrots had higher AO levels than their fresh equivalent. In the same study that examined vitamin C content in carrots, spinach, brassicas and potatoes there was little reduction in α-carotene and β-carotene following blanching (Puupponen-Pimiä et al., 2003).

5.3.3 Cooking

In Western countries vegetables are usually consumed as semi cooked or boiled (Sultana, Anwar & Iqbal, 2008). Cooking alters the chemical composition which potentially changes bioavailability, nutrient content and health promoting compounds namely AO (Pellegrini et al., 2010). There has been extensive research on the effect of cooking on fresh vegetables (Turkmen, Poyrazoglu, Sari & Sedat Veliioglu, 2006; Miglio, Chiavaro, Visconti, Fogliano & Pellegrini, 2008; Sultana et al., 2008). However, there is little research available on cooked frozen vegetables, despite the fact that they are usually eaten this way.

A study investigating TAC in vegetables found that frozen cooked green vegetables usually contained higher TAC than their fresh equivalents whereas red/yellow frozen vegetables suffered higher losses of AO activity than fresh due to cooking (Danesi & Bordoni, 2008). Recently Pellegrini et al., (2010) evaluated the consequences of different

cooking techniques (boiling, microwaving and basket and oven steaming) on phytochemical concentration and AO capacity of fresh and frozen brassica vegetables. Overall findings showed fresh brassicas retained phytochemicals and TAC better than frozen during cooking. The above study found both fresh and frozen brassica vegetables lost AA in large amounts during all cooking methods (Pellegrini et al., 2010). Another investigation found that boiling, regardless of whether the product was fresh, frozen or tinned, reduced plant tissue AA in spinach and green beans but not lutein (carotenoid antioxidant) concentrations (Delchier, Reich & Renard, 2012). A more recent study (Mazzeo et al., 2011) investigated the impact of cooking (boiling and steaming) on three frozen vegetables phytochemical content (carotenoids, chlorophylls, polyphenols and AA) and TAC. Findings revealed steaming increased some phenolic compounds within spinach, carrots and cauliflower, reduced the loss of overall carotenoids and increased β-carotene in spinach and TAC was maintained or increased in both steamed carrots and spinach. With regard to boiling, cooking time began when the water started to boil, spinach was cooked for 10 minutes, carrots for 12 minutes and cauliflower for 9 minutes. Following boiling all vegetables were drained and cooled rapidly on ice. Findings revealed that boiling caused a reduction in TAC and phytochemical content for all of the frozen vegetables assayed (Mazzeo et al., 2011). However, another study found that cooked vegetables, whether canned, fresh or frozen didn't always contain less AA than the equivalent uncooked vegetables (Howard et al., 1999).

Findings have shown that fresh is generally better than frozen for optimal vitamin C content providing fresh is only stored for a minimal period in refrigerated temperatures. Blanching and freezing appears to be less damaging to AA yet prolonged storage and cooking can result in significant losses (Howard et al., 1999). Changes in TP during storage,

processing and cooking appear to be diverse and tends to be dependent on the product. Extractability of phenolics appears to increase due to thermal treatments (cooking and blanching) and due to their properties (water soluble and sensitive to oxidation) phenolic compounds losses are likely during fresh and frozen storage. Finally, compared to the water soluble AO carotenoids appear to be more stable to storage, processing and cooking. The increases seen in carotene may have resulted from moisture changes during processing or due to carotene separating from the cell matrix during thermal treatments (Rickman et al., 2007). The major AO within both fresh and frozen vegetables, which contribute to total AO capacity, are effected in a variety of ways during storage, processing and cooking.

5.4 Direct studies comparing TAC and AA within fresh and frozen vegetables.

The amount of research that directly compares fresh and frozen vegetables is limited and most of the data is not recent. The findings from the studies that have been conducted are inconsistent. A Spanish study that used 25 vegetables (artichoke, asparagus, beetroot, broad bean, broccoli, Brussels sprout, carrot, cauliflower, celery, chicory, cucumber, eggplant, endive, garlic, green bean, leek, lettuce, maize, onion, pea, pepper, radish, spinach, Swiss chard and zucchini) to investigate AO activity in vegetables that had been processed or refrigerator stored proposed that consumption of raw vegetables contributed a higher AO intake to the diet than consumption of the same amount in frozen form (Murcia, Jiménez & Martínez-Tomé, 2009). This suggestion was supported by a survey of commercially available spinach, peas, carrots and green beans which discovered higher AO content in fresh rather than frozen (Hunter & Fletcher, 2002). However, after prolonged storage, nutritional losses occur in fresh vegetables even when stored in refrigeration. For

example, Favell (1998) discovered vitamin C degradation in some fresh vegetables stored in a refrigerator for several days; the losses were greater than that of vegetables stored in freezers. This is particularly relevant as fresh vegetables usually spend 3-7 days in storage before consumption (Bushway, Helper, King, Perkins & Krishnan, 1989). Additionally, Howard et al., (1999) found that, although the initial blanching of broccoli and green beans resulted in AA loss, retention remained steady after freezing. Furthermore Makhlouf et al., (1995) found frozen vegetables such as green beans, sweetcorn and peas contain similar levels of vitamin C as fresh vegetables. Finally, an investigation found frozen green vegetables often contain higher AO activity than their fresh equivalents after cooking but red/yellow frozen vegetables suffer greater AO losses than fresh after cooking (Danesi & Bordoni, 2008).

5.5 Present Study

Previous studies of vegetable AO levels have been mainly concerned with unprocessed and uncooked samples (Cao, Sofic & Prior, 1996). As vegetables are usually consumed after storage, processing and cooking it is more relevant to the consumer to discover whether fresh or frozen vegetables contain more AO at the point of consumption. The studies mentioned above indicate the varying effects these factors have on different AO and consequently total AO content within vegetables. Additionally, despite increased public awareness of the health benefits of fruit and vegetables, consumption remains low with only 25% men, 27% women and 20% children eating 5 portions of fruit and vegetables a day (DEFRA, 2012). Therefore it is essential to investigate whether fresh or frozen vegetables contain more AO at the point of ingestion so that, even if consumption remains low, public

health workers can advise about the vegetables that offer maximum AO and consequently maximum health benefits. Additionally, if fresh and frozen vegetables contain comparable amounts then recommending frozen may increase consumption due to the lower costs (see Table 8.1 and Table 8.2), convenience and reduced waste (WRAP, 2008). The findings from the few studies that have directly compared TAC and vitamin C content within fresh and frozen vegetables have been varied. Therefore this study will investigate TAC and AA levels of processed (frozen) and non-processed vegetables (fresh) after cooking. The results will reflect the AO content that the consumer actually ingests. The vegetables that will be assayed are 5 vegetables widely available in both fresh and frozen form to the European consumer; peas, green beans, broccoli, carrots and spinach (Favell, 1998). Each vegetable will be bought in fresh and frozen form from four different shops to account for possible variation in AO and AA content within the same type of vegetable (Kurilich & Juvik, 1999), differing storage times and conditions and varying freezing techniques (steam or boiling blanching).

As mentioned previously vegetables contain a variety of AO and it is unfeasible to measure them individually. Therefore, it is more realistic to use techniques that quantify total AO activity of food. This allows a direct comparison to be made between different types of vegetables, both fresh and processed. The present study will measure total AO content of a variety of cooked vegetables in fresh and frozen form. The assay to measure TAC will be ORAC which was developed by Cao and co-workers (1993, 1995).

AA is an AO micronutrient that most consumers associate with vegetables (and fruit) and plays an important role in health (Benzie & Szeto, 1999; Davey et al., 2000). The content of AA varies between different vegetables, for example, brassicas contain high levels and root

vegetables contain low levels (Favell, 1998). Vitamin C content even differs between the same types of vegetable, for example, a survey of 50 broccoli varieties showed AA to content to range between 56 and 120mg/100g fresh weight (Kurilich & Juvik, 1999). Consequently vitamin C is not a gauge of quality as such, but since it is a vitamin sensitive to chemical and enzymic oxidation and is extremely water soluble, it is an ideal marker to monitor difference between cooked unprocessed (fresh) and processed (frozen) vegetables (Favell, 1998). In this study the DCPIP titration assay will be conducted to measure oxidised vitamin C.

5.6 Aims

The aim of this study was to compare total AO and AA within a variety of fresh and frozen cooked vegetables.

5.7 Objectives

a) to investigate and compare the AO properties of fresh and frozen cooked vegetables.
b) to investigate and compare the AA content of fresh and frozen cooked vegetables.

5.8 Hypothesis

5.8.1 Null Hypothesis (H_0)

There will be no significant difference in the TAC of fresh and frozen cooked vegetables.

5.8.2 Alternative (research) hypothesis (H_1)

There will be a significant difference in the TAC of fresh and frozen cooked vegetables.

5.8.3 Null hypothesis (H_0)

There will be no significant difference in AA content of fresh and frozen cooked vegetables.

5.8.4 Alternative hypothesis (H_1)

There will be a significant difference in AA content of fresh and frozen cooked vegetables.

6.0 Methods

6.1 Design

The research project was a laboratory investigation. The study followed an independent measures design. Measurements were taken in duplicate for AO assays and in triplicate for AA assays. Means and standard errors were calculated.

6.2 Materials and Procedures

6.2.1 Plant Materials

The samples to be assayed in this project were vegetables reported as being widely available in fresh and frozen form to European consumers. These vegetables were peas, green beans, broccoli, carrots and spinach (Favell, 1998). Fresh was defined as unprocessed vegetables that were available at the time of purchase. Frozen was defined as conventionally quick frozen vegetables that were available at the time of purchase. Products bought were whole. This is because operations such as cutting and slicing can cause fast enzymatic reduction of some naturally occurring AO as a result of cellular disturbance (Davey et al., 2000). Surveys have found the content of certain AO within vegetables to have substantial variation (Kurilich & Juvik, 1999). Consequently, to account for the variation of the products, each individual vegetable (in both fresh and frozen form) was bought four times from four different shops in the Wirral, United Kingdom. The products were bought the night before testing, fresh vegetables were stored in a fridge at 5^{o}c and frozen vegetables were stored in a freezer at -18^{o}c. All vegetable products were own brand, but not value, apart from frozen broccoli bought in Sainsburys which was Birdseye due to lack of stock. Table 6.1 and Table 6.2 show the type of product, the outlets they were bought from and when possible the origin of the produce.

Table 6.1: Description of fresh vegetable samples

Vegetable	Outlet	Where
Broccoli	Tesco	UK
	Morissons	Spain
	Sainsburys	Spain
	Fine Fruits of Pensby	UK
Spinach	Tesco	Spain
	Morissons	Spain
	Sainsburys	Spain
	Fine Fruits of Pensby	Spain
Carrots	Tesco	UK
	Morissons	UK
	Sainsburys	UK
	Fine Fruits of Pensby	UK
Green beans	Tesco	Morocco
	Morissons	Kenya
	Sainsburys	Kenya
	Fine Fruits of Pensby	Kenya
Peas	Tesco	Guatemala
	Morissons	Kenya
	Sainsburys	Guatemala
	Fine Fruits of Pensby	Kenya

Table 6.2: Description of Frozen Vegetable Samples

Broccoli	Tesco	EU
	Morissons	Unknown
	Sainsburys	Unknown
	Asda	Spain
Spinach	Tesco	EU
	Morissons	Unknown
	Sainsburys	Spain
	Asda	Belgium
Carrots	Tesco	Unknown
	Morissons	Unknown
	Sainsburys	Belgium
	Asda	Unknown
Green beans	Tesco	UK
	Morissons	Unknown
	Sainsburys	UK
	Asda	Unknown
Peas	Tesco	UK
	Morissons	Unknown
	Sainsburys	UK
	Asda	UK

6.2.2 Procedures

Two assays were utilised in this study: the ORAC assay and DCPIP titration method.

Two different extraction methods were required for AA and AO.

Antioxidant Extraction

Fresh samples were weighed to precisely 2.0g and then cleaned and washed with water. This was done to reflect what the consumer would do before cooking the vegetables. Additionally the skin from the carrots was peeled off. The skin had also been removed in the carrots frozen form. Frozen samples were thawed and weighed to 2.0g. The vegetables were cooked individually in the laboratory. 200mL of boiled water was poured into a glass measuring beaker and then placed onto a pre heated hot plate. Once water in the beaker began to bubble a sample was placed into the beaker. If the sample was fresh it was cooked until tender as instructed by Jiménez-Monreal, García-Diz, Martínez-Tomé, Mariscal and Murcia (2009). If the sample was frozen the manufacturer's instructions were followed. The cooking methods were a reflection of how fresh/frozen vegetables would be cooked by the consumer. The antioxidant extraction method was adapted from Pellegrini et al., (2007). Once samples were cooked any excess water was drained off and they were homogenized using a pestle and mortar. The homogenized samples were then put into 15mL centrifuge tubes along with 8mL of distilled water and extracted under agitation for 15 minutes at room temperature. The samples were then centrifuged at 1000g for 10 minutes. The supernatants were then collected and stored in a 30mL plastic container

Equipment:

- Kettle
- Hotplate (Stuart CB500 1-9)
- Glass measuring beaker
- Precision balance (Denver S-203 capacity 200g, readability 0.001g)
- Pestle and mortar
- 15mL centrifuge tube
- Orbital incubator (Stuart SI50)
- Centrifuge (ALC PK 120)

Materials:

- Samples
- Distilled water

The ORAC assay

The ORAC assay is the most popular method to assess overall AO capacity in fruit and vegetables and was produced and developed by Cao and co-workers (1993, 1995). The ORAC assay used in this investigation was based on the protocol described by Zulueta, Esteve and Frigola (2009). This assay works by monitoring the decay of fluorescence of the molecule fluorescein caused by free radicals generated by the compound AAPH (2, 2'-azobis(2-amidinopropane) dihydrochloride). The decay in fluorescence can be slowed down by any AO present in a sample. The assay is calibrated using TROLOX (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2 carboxylic acid) which is a vitamin E analogue. The ORAC assay measures all traditional AO and combines both inhibition time and inhibition percentage of

the free radical or oxidant action by an antioxidant into a single value using an area under the curve method for quantitation of the data.

Equipment:

- 96-Well Plate (sterile, non-binding and flat bottom)
- Whirlimixer (Fisons)
- Platform shaker (Heidolph instruments, Titramax 100)
- Incubator (Binder)
- Plate reader

Materials:

The following chemicals were provided by Chester University lab stock:

- Fluorescein
- TROLOX
- PBS (Phosphate buffered saline)
- AAPH

Coefficient of Variation:

Before assaying the samples the coefficient of variation (CV) was calculated. This was to show whether the ORAC assay methodology provided consistent results. To do this the ORAC assay was performed using 42 samples of 20µM TROLOX (water-soluble analogue of *vitamin E*). The mean (SD±) of the 42 TROLOX samples was calculated.

The CV as a percentage was calculated by applying the formula below in a Microsoft Excel spread sheet (Microsoft, Washington, USA):

$$CV = \frac{\text{standard deviation}}{\text{mean}} \times 100$$

Procedure:

On each plate a control for no fluorescein was conducted to check for fluorescence in the samples and a no sample control was conducted to check the fluorescein fluorescence was stable. Finally a no AAPH control was conducted on one product to ensure the samples would not affect the fluorescence.

This assay is based in the protocol described by Zulueta et al., (2009).
1. The chemicals and samples required for the ORAC assay were prepared (see Appendix A).

2. A plate template was used to label where samples, PBS controls and TROLOX would be in the plate.

3. 50µL of working fluorescein was added into the wells for samples and TROLOX standards.

4. 50µL of PBS was put into wells to act as blanks.

5. 50µL of the different dilutions of sample (1/10, 1/100, 1/1000 and 1/10,000) were put into wells in duplicate.

6. 50µL of standards (20µM TROLOX) and controls (PBS in both fluorescein and PBS-blank wells) were put into wells.

7. The plate was shaken using a lab platform shaker and then placed in a 37^{o}c incubator for 15 minutes.

8. The plate reader was pre heated to 37^{o}c.

9. 25µL of AAPH solution was added into all wells quickly before the plate was put into the plate reader.

10. The plate was left in the plate reader for an hour. The plate reader measured fluorescence at 535nm (emission) using 485nm (excitation) every 5 minutes until samples had reached 5% of original fluorescence.

11. When analysing the output it was important that the kinetic curves chosen for each sample showed an endpoint (see Appendix B). Additionally the blank (PBS) value should be smaller than TROLOX. Once kinetic curves were chosen for each sample the mean of the duplicates needed to be calculated as did the mean of PBS and TROLOX.

The ORAC values, which are expressed as µM trolox equivalents (µM TE) were calculated by applying the formula below in a Microsoft Excel spread sheet (Microsoft, Washington, USA):

$$\mathrm{ORAC}(\mu\mathrm{M\ TE}) = \frac{\mathrm{C_{Trolox}} * \left(\mathrm{AUC_{Sample}} - \mathrm{AUC_{Blank}}\right) * \mathrm{K}}{\left(\mathrm{AUC_{Sample}} - \mathrm{AUC_{Blank}}\right)}$$

C_{Trolox} is the concentration (µM) of trolox (20µM)

k is the sample dilution factor (1/10, 1/100, 1/1000, 1/10,000)

AUC is the area underneath the fluorescence decay curve of the sample, blank and trolox

AA extraction method

Fresh samples were weighed to precisely 5.0g and then washed with water; this was done to reflect what the consumer would do before cooking the vegetables. Additionally the skin from the carrots was peeled off. The skin had also been removed in the carrots frozen form. Frozen samples were thawed and weighed to 5.0g. The vegetables were cooked individually. 200mL of boiled water was poured into a glass measuring beaker and then placed onto a pre heated hot plate. Once water in the beaker began to bubble a sample was placed into the beaker. If the sample was fresh it was cooked until tender as instructed by Jiménez-Monreal et al., (2009). If the sample was frozen the manufacturer's instructions were followed. The cooking methods were a reflection of how fresh/frozen vegetables would be cooked by the consumer. The AA extraction method was adapted from Rizzolo, Brambilla, Valsecchi and Eccher-Zerbini (2002). After cooking, any excess water was drained off and the samples were homogenized with 15mL of distilled water using a pestle and mortar. The samples were then put into a 20mL centrifuge tube and centrifuged for 20 minutes at 2000g and the supernatants were collected.

Equipment:

- Kettle
- Hotplate (Stuart CB500 1-9)
- Glass measuring beaker
- Precision balance (Denver S-203 capacity 200g, readability 0.001g)
- Pestle and mortar
- 15mL centrifuge tube
- Pipette (1-5mL, 200-1000µl)

- Pipette tips
- Orbital incubator (Stuart SI50)
- Centrifuge (ALC PK 120)
- 30mL plastic container

Materials:

- Samples
- Distilled water

The DCPIP Titration Assay

AA is active as the L ismor. Its stability is lost when it is oxidized to dehydroascorbic acid (DHAA). DHAA readily hydrolyses to diketogulonic acid. Therefore the assay of AA is based on its reducing properties. AA will be titrated with 2, 6-dichloroindophenol (DIP). When titrating an acid solution of AA against blue DIP, the blue reagent will turn colourless when it is reduced by the AA. When all AA has been oxidised, excess DIP will turn the solution pink, thereby showing the endpoint. The DCPIP titration assay measures oxidised AA.

Materials:

- 0.25M Oxalic acid
- 1M HCL (hydrochloric acid)
- DIP dye (250mg NaDIP and 210mg $NaHCO_3$ per litre)
- Distilled water

Coefficient of Variation:

Before assaying the samples the CV was calculated. This was to show whether the DCPIP titration assay methodology provided consistent results. To do this the DCPIP titration assay was performed using 12 samples of 0.5mL of AA. The mean (SD±) of the 12 AA samples was calculated.

The CV as a percentage was calculated by applying the formula below in a Microsoft Excel spread sheet (Microsoft, Washington, USA):

$$CV = \frac{\text{standard deviation}}{\text{mean}} \times 100$$

Standard Calibration curve:

A standard solution of AA (0.5mg/cm3) was prepared. Different measures of AA (0.25, 0.5, 0.75, 1.0mg) were tested in triplicate using the DCPIP titration assay to measure volume of DIP dye (cm3) required to oxidise AA (pink end point) (see Table 6.3). Using the results a calibration curve was produced (see Appendix C). The formula obtained from the calibration curve was used to estimate AA content of the samples.

Table 6.3: Concentrations of different solutions used for the AA triplicate standard curve and mean DIP dye required to oxidise AA

	A	B	C	D
0.25M Oxalic acid cm3	9	9	9	9
1M HCL cm3	1	1	1	1
Ascorbic acid 0.5mg/cm3	0.25	0.5	0.75	1.0
DIP dye cm3	2.8, 2.8, 2.8	5.6, 5.7, 5.6	8,7.8, 8	10.6, 10.6, 10.6
Mean DIP dye cm3	2.8	5.6	7.9	10.6

Procedure:

1. 9mL oxalic acid (0.25M) and 1mL (1M) HCL were pipetted into 3 conical flasks (A, B, C).

2. 0.5mL of sample was added into each of the 3 conical flasks.

3. A vertical burette was filled with blue DIP dye and the first burette reading was taken from the bottom of the meniscus. The funnel was removed from the top of the burette.

4. In turn each flask was titrated with DIP dye fairly rapidly whilst shaking continuously.

5. The end point was when a pale pink persisted for 5 seconds.

6. The second burette reading was recorded and the volume of dye used for titration was noted.

7. The mean amount of DIP dye used was calculated from the three recordings.

8. The formula generated from a calibration curve was used to work out the amount of oxidised vitamin C within the 0.5mL of sample.

AA per 100g

AA per 100g of sample was calculated as it is more meaningful to the consumer and an easier value to compare with other study findings. 0.5mL of the sample was measured in the assay and the extraction used 5g of sample plus 15mL of distilled water. Therefore to work out vitamin C in a 100g the following steps were conducted;

0.5 x 30 = vitamin C in 5g of sample

vitamin C in 5g x 20 = vitamin C in 100g of sample

6.3 Data management and data analysis

Experiments were performed in duplicate for the ORAC assay and in triplicate for the DCPIP titration assay. The means (±SD) of the four samples of each cooked vegetable in fresh and frozen form were calculated.

The CV of the ORAC assay was 4.3% and was 1.8% for the DCPIP titration assay. Thus showing methodology to be consistent.

Individual statistical analysis was performed on the mean TAC/AA content in cooked fresh/frozen vegetables. If Shapiro Wilks was not significant ($p>.05$) then the sample was normally distributed so an Independent Samples T test was performed. When conducted the Independent Samples T test if the Levene test was not significant ($p>.05$) then equal variance was not assumed and if the test was significant ($p<.05$) then equal variance was assumed .If Shapiro Wilks was significant ($p<.05$) then the data was not normally distributed and a non-parametric Mann Whitney 'U' Test was performed. Statistical analyses was undertaken using SPSS version 16 and the level of significance was set to $p<0.05$.

7.0 Results

7.1 TAC of samples

In order to investigate and compare the antioxidant properties of fresh and frozen cooked vegetables an ORAC assay was conducted.

Table 7.1: Mean *(±SD) TAC* $(\mu M\ TE)$ of cooked fresh broccoli and green beans

Type of vegetables	$\mathbf{TAC\ (\mu M\ TE)}$
Broccoli	850 ± 578
Green beans	930 ± 675

Table 7.2: Mean (±SD) TAC $(\mu M\ TE)$ of cooked frozen broccoli and green beans

Type of vegetables	$\mathbf{TAC\ (\mu M\ TE)}$
Broccoli	211 ± 90
Green beans	204 ± 70

A T test for independent samples was conducted on broccoli and green bean samples. Cooked fresh broccoli (Mean (±SD) = 850 ± 578) did not appear to differ in total antioxidant levels from cooked frozen broccoli (Mean (±SD) = 211 ± 90), p = .113. Levene's test indicated unequal variances (F = 61.51, p<.001), so degrees of freedom were adjusted from 6 to 3. Cooked fresh green beans (Mean (±SD) = 930 ± 675) did not appear to differ in total antioxidant levels from cooked frozen green beans (Mean (±SD) = 204 ± 70), p = .120. Levene's test indicated unequal variances (F = 155, p = <.001), so degrees of freedom were adjusted from 6 to 3.

Table 2.3: Mean *(±SD) TAC* $(\mu M\ TE)$ of cooked fresh spinach, peas and carrots

Type of vegetables	$\mathbf{TAC(\mu M\ TE)}$
Spinach	2531 ± 833
Peas	1398 ± 408
Carrots	743 ± 955

Table7.4: Mean (±SD) TAC $(\mu M\ TE)$ of cooked frozen spinach, peas and carrots

Type of vegetables	$\mathbf{TAC(\mu M\ TE)}$
Spinach	418 ± 259
Peas	249 ± 89
Carrots	211 ± 90

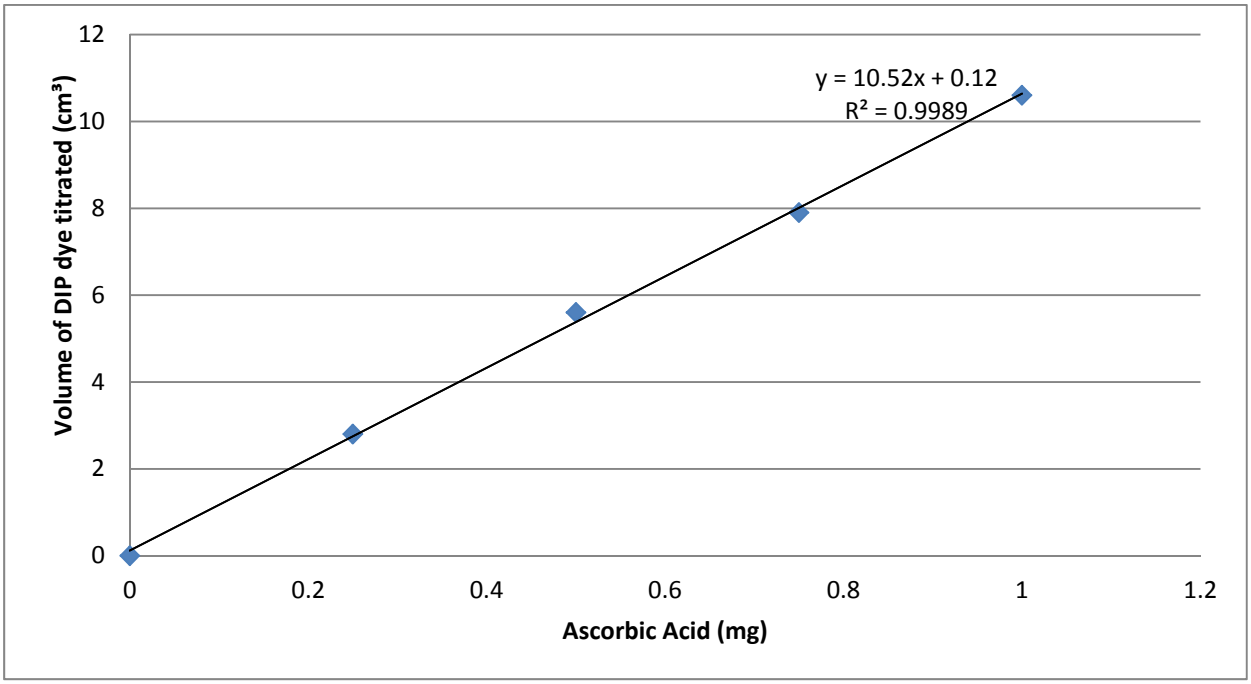
A Mann Whitney 'U' Test was performed on spinach, peas and carrots. Cooked fresh spinach (Mean (±SD) = 2531 ± 833) contains significantly higher levels of total antioxidant than cooked frozen spinach (Mean (±SD) = 418 ± 259), p = .029. Cooked fresh peas (Mean (±SD) = 1398 ± 408) contain significantly higher levels of total antioxidant than cooked frozen peas (Mean (±SD) = 249 ± 89), p = .029. Cooked fresh carrots (Mean (±SD) = 743 ± 955) did not appear to differ in total antioxidant levels from cooked frozen carrots (Mean (±SD) = 211 ± 90), p =.200.

7.2 AA content of samples

7.2.1 Ascorbic acid calibration curve

A calibration curve (see Figure 7.1) was produced so that the concentration of AA within the vegetables could be determined when assaying.

Figure 7.1: **Ascorbic acid standard curve**



7.2.2 AA content

In order to investigate and compare the AA content of fresh and frozen cooked vegetables the DCPIP titration assay was conducted.

Table 7.5: Mean *(±SD)* AA content (mg) of cooked fresh and frozen carrots

Type of vegetables	**AA content(mg)**
Fresh carrots	0.27 ± 0.047
Frozen carrots	0.26 ± 0.050

A T-Test for independent samples was conducted on carrots. Cooked fresh carrots (Mean (±SD) = 0.27 ± 0.047) did not appear to differ in total AA content from cooked frozen carrots (Mean (±SD) = 0.26 ± 0.050), p = .816.

Table 7.6: Mean *(±SD)* AA content (mg) of cooked fresh broccoli, green beans, spinach and peas

Type of vegetables	$\mathbf{AA\ content(mg)}$
Broccoli	0.61 ± 0.017
Green beans	0.27 ± 0.027
Spinach	0.35 ± 0.043
Peas	0.30 ± 0.047

Table 7.7: Mean (±SD) AA content (mg) of cooked frozen broccoli, green beans, spinach and peas

Type of vegetables	$\mathbf{AA\ content(mg)}$
Broccoli	0.53 ± 0.096
Green beans	0.27 ± 0.038
Spinach	0.27 ± 0.067
Peas	0.31 ± 0.057

A Mann Whitney 'U' Test was performed on broccoli, green beans, spinach and peas. Cooked fresh broccoli (Mean (±SD) = 0.61 ± 0.017) did not appear to differ in total AA content from cooked frozen broccoli (Mean (±SD) = 0.53 ± 0.096), *p* = .286. Cooked fresh green beans (Mean (±SD) = 0.27 ± 0.027) did not appear to differ in total AA content from cooked frozen green beans (Mean (±SD) = 0.27 ± 0.038), p = 1.00. Cooked fresh spinach

(Mean (±SD) = 0.35 ± 0.043) did not appear to differ in total AA content from cooked frozen spinach (Mean (±SD) = 0.27 ± 0.067), *p* = .114.Cooked fresh peas (Mean (±SD) = 0.30 ± 0.047) did not appear to differ in total AA content from cooked frozen peas (Mean (±SD) = 0.31 ± 0.057) p = .866.

7.2.3 AA per 100g

The mean AA content within each vegetables sample was converted into amount per 100g as this is better understood by the consumer and easier for comparisons. When compared the current studies findings with the findings of the Food Standard Agency (FSA) (2002) the amount of AA per 100g appears similar (see Table 6.8 and Table 6.9).

Table 7.8: AA content per 100g of assayed cooked fresh vegetables compared with the FSA (2002) values for AA per 100g in cooked fresh vegetables

Type of vegetable	Ascorbic acid per 100g (mg)	FSA Reference
Broccoli	28	44
Green beans	8	-
Spinach	13	8
Peas	10	12
Carrots	8	2

Table 7.9: AA content per 100g of assayed cooked frozen vegetables compared with the FSA (2002) values for AA per 100g in cooked frozen vegetables

Type of vegetable	Ascorbic acid per 100g (mg)	FSA Reference
Broccoli	23	-
Green beans	8	7
Spinach	8	6
Peas	11	16
Carrots	8	-

8.0 Discussion

When comparing the nutritional profile of fresh and frozen cooked vegetables it is necessary to define the terms used. In this study fresh vegetables referred to vegetables available for purchase by the consumer from the supermarket and green grocers rather than vegetables straight from harvest. The term frozen is used to describe vegetables that have been harvested, prepared, blanched and quick frozen commercially followed by standard frozen storage (-18oc) in supermarkets and which are available to the consumer. The term cooked differed between fresh and frozen in the present study. Cooking methods used aimed to reflect the cooking styles of the consumer. Therefore fresh vegetables which generally lack cooking instructions were cooked (boiled) until tender following the protocol of Jiménez-Monreal et al., (2009). Frozen vegetables were cooked (boiled) following the manufacturer's instructions on the packaging of each product. Thus cooked fresh and frozen vegetables assayed reflected both vegetables that would be available to purchase by the consumer and cooking methods that would be adopted.

This study aimed to compare the nutritional composition of a variety of fresh and frozen cooked vegetables by assaying for TAC and AA content. The five vegetables assayed were green beans, spinach, broccoli, carrots and peas. The results obtained from the ORAC assay illustrated that fresh cooked spinach and peas contained a higher TAC (see Table 7.3) than frozen cooked spinach and peas (see Table 7.4). This meant the alternative hypothesis for TAC could be accepted. However, there was no difference in TAC between cooked fresh broccoli, green beans and carrots (see Table 7.1 and Table 7.3) and their cooked frozen counterparts (see Table 7.2 and Table 7.4). Thus providing support for the TAC null hypothesis. Therefore the present study has shown cooked frozen vegetables can offer the same nutritional benefits as cooked fresh vegetables, but this is dependent on the vegetable

considered. There was no difference in the AA content of all cooked fresh vegetables (see Table 7.5 and Table 7.6) and their cooked frozen counterparts (see Table 7.5 and Table 7.7). Therefore the AA null hypothesis could be accepted. Hence it appears that cooked frozen and fresh vegetables provide comparable AA levels to the consumer at the point of consumption.

Previous research on nutritional comparisons of cooked fresh and frozen vegetables is limited. Therefore the present study provides much needed extra information on the nutritional quality of food bought and cooked as per the consumer. The few findings regarding nutritional value within fresh and frozen vegetables have been inconsistent. Overall this study supports the idea that frozen vegetables at the point of consumption are just as nutritious as fresh.

8.1 Ascorbic acid

Similarly to the present study Favell (1998) investigated AA content within fresh and frozen peas, green beans, spinach, broccoli and carrots. However, despite acknowledging that the quality of the final cooked vegetable is most important, Favell (1998) did not assay vegetables after cooking. Favell (1998) design aimed to cover differing modes of storage during transportation, retail and at home prior to use of the vegetables. Samples were stored in ambient (20 ^{o}C), chilled (4 ^{o}C), chilled/ambient and frozen (-18 ^{o}C) temperatures. Prior to cooking fresh peas stored at ambient, chilled and ambient chilled contained more AA than frozen at 2 days storage. The blanching process reportedly reduced AA by approximately 30% (Favell, 1998). However by day 7 AA in peas stored in ambient and ambient/chilled conditions had fallen below that contained in frozen peas, but at day 14 fresh peas in chilled storage contained equal amounts of AA to frozen (Favell, 1998). So, the

nutritional status of frozen peas was similar to that of typical market purchased vegetables. Within the present study the storage time and conditions of the fresh peas may have impacted on the final AA content within the samples but this was not controlled in order to reflect the vegetables that the consumer buys and cooks. After the initial losses from blanching (30%) AA within frozen peas appears to stay relatively constant during storage (only 10% over 12 months) (Favell, 1998). Few studies have directly compared the impact of cooking on fresh and frozen peas but an Australian study conducted in 1984 reported that fresh cooked peas contain slightly higher AA than cooked frozen peas (Willis, Evans, Lim, Scriven & Greenfield., 1984). The difference in AA between cooked fresh and frozen peas was 0.03g per kilogram of AA (Willis et al., 1984). The differences between AA content reported by Willis et al., (1984) was minimal and may differ to the findings from the present study due to variation in AA content within the market purchased vegetables (Kurilich & Juvik, 1999) due to factors such as differing storage times and conditions and varying freezing techniques (steam or boiling blanching). From previous findings the results of the present study, that fresh peas contain similar amounts of AA to frozen peas, is unsurprising due to the clear effects time and temperature of storage may have had on AA content before purchase. Furthermore Makhlouf et al., (1995) found similar results to the present study; they found boiled frozen peas to contain similar levels of AA to boiled fresh peas.

After blanching/freezing process broccoli still contained 80% of its AA (Favell, 1998). However, AA content within broccoli stored in chilled conditions remained higher than its frozen counterpart past day 14, in fact even after 21 days 80% AA remained. Yet storage in ambient conditions caused AA levels to fall below that seen in frozen broccoli samples, even

after day 1 of storage. After the initial rapid loss of AA broccoli stored in ambient conditions had a steady loss with 44% of AA remaining after day 7. Favell (1998) suggests that the slow rate of AA loss in broccoli is due to protection that storage as a whole floret provides. AA content within fresh broccoli appears stable as long as it's stored in chilled temperatures and in whole florets. The present study found no difference in AA content between cooked fresh and frozen broccoli. There is a lack of previous studies investigating the impact of boiling. Howard et al., (1999) looked at AA content in fresh and frozen broccoli after microwave cooking in water. Conversely they found the mean AA difference between cooked frozen broccoli and cooked fresh broccoli to be 36.3 mg/100g. Additionally, they observed only small changes between the uncooked and the cooked broccoli in fresh and frozen samples. Howard et al., (1999) study offers a much needed direct comparison of the AA content post cooking in fresh and frozen. However, this study assayed vegetables grown specifically to be tested rather than vegetables available to the consumer for purchase. Additionally fresh broccoli was stored as whole florets, which Favell (1998) stated offers protection from AA losses, whereas frozen broccoli was cut into small florets prior to freezing. Operations such as cutting and slicing can provoke fast enzymatic reductions of some naturally occurring AO due to cellular disturbance (Davey et al., 2000). In the present study only broccoli stored as a whole was assayed. Therefore the differences between the two studies findings may have been due to methodological differences.

Spinach has a high surface to volume ratio compared with the other vegetables and is therefore vulnerable to blanching which can reportedly cause 20–70% AA loss (Favell, 1998). Despite the negative impact of blanching, fresh spinach stored in chilled/ambient and ambient conditions contained less AA after 2 days than frozen spinach, however, chilled

spinach contained equal amounts (Favell, 1998). Favell (1998) found AA content to be so unstable within fresh spinach that the samples stored in ambient temperatures only had 10% AA left after 3 days and the chilled samples only had 20% remaining after 7 days, with no AA before day 14 (Favell, 1998). Furthermore, an investigation found that boiling, regardless of whether the product was fresh, frozen or tinned, reduced plant tissue AA in spinach (Delchier et al., 2012). Recent research on the impact of cooking on AA in spinach appears limited but Klein, Kuo and Boyd (1981) assayed fresh spinach before and after boiling and established AA to decrease significantly after cooking with retention of AA only being 51%. There appears to be little literature on the impact of cooking on AA in frozen spinach but the present study offers new findings that indicate frozen and fresh spinach after cooking contain similar amounts of AA.

An investigation studying AA content in a variety of fresh and frozen vegetables before cooking found the blanching/freezing process caused no loss in AA content in carrots, in fact a slight increase in AA occurred (Favell, 1998). Consequently from day 1 carrots stored in chilled and ambient temperatures contained less AA than frozen (Favell, 1998). Conversely Puupponen-Pimiä et al., (2003) found AA content in carrots to decrease by around 30% during blanching but with no significant further losses during storage. Again little literature has been conducted on the effect of boiling on AA within frozen carrots although Howard et al., (1999) investigated the AA content of fresh refrigerated, frozen and canned carrots uncooked and microwaved cooked in 1994 and 1995 and compared the results. They found that when AA was averaged over total storage time for cooked and uncooked samples, fresh refrigerated samples contained comparable levels of AA to microwave cooked (carrots cooked in water) fresh refrigerated carrots ($p>.05$) (Howard et

al., 1999). Additionally frozen carrots AA levels were also unaffected by microwave cooking (Howard et al., 1999). This study therefor suggests that the AA in fresh and frozen carrots is unaffected by cooking. Consequently findings from Favell (1998) indicate that it is the period and conditions of fresh carrots storage that will influence whether fresh or frozen carrots contain higher AA after cooking as although AA losses may occur during blanching (Puupponen-Pimiä et al., 2003) levels remain fairly constant during frozen storage and cooking does not appear to effect AA content in either form of carrot. Previous studies lend some support to the idea that AA levels in fresh and frozen boiled carrots may be similar as was found in the present study but again more research is required.

In Favell (1998) investigation, AA within green beans stored in chilled and ambient temperatures fell below that of frozen green beans after 1 day. Favell (1998) found the freezing process/blanching had little impact on AA whereas Howard et al., (1999) conversely found AA to be less stable after blanching of green beans, consequently reporting losses of about 50%. A 1984 Australian study found cooked market-purchased green beans contained greater AA than cooked frozen (Willis et al., 1984). Whereas Makhlouf et al., (1995) found boiled frozen green beans to contain similar levels of AA to fresh boiled green beans. Those findings are in agreement with the findings of the present study.

8.2 TAC

Within the present study fresh peas contained higher TAC than frozen peas after boiling. This differs to a study conducted in Italy in 2008 that investigated the changes in AO activity in popular fresh and frozen vegetables using 3 cooking methods (steaming, boiling and microwave cooking). They established that frozen cooked peas contained higher TAC

than their fresh counterparts (Danesi & Bordoni, 2008). Other studies have looked at the impact of storage and cooking on different AO within peas that contribute to TAC. Weits, van der Meer, Lassche, Meyer, Steinbuch and Gersons (1970) found small but significant losses of total carotene during frozen storage of peas and Simonetti et al., (1991) found a 46% wet weight decrease of β-carotene within fresh peas after 3 weeks storage. Nevertheless, post cooking, fresh and frozen peas appeared to contain similar amounts ofβ-carotene (Willis et al., 1984). Furthermore Puupponen-Pimiä et al., (2003) studied the impact of blanching and freezing on phenolic compounds in a selection of vegetables, including peas, and found average losses of 20-30% dry weight of TP in most vegetables. With regards to phenolics Turkmen, Sari and Sedat Velioglu (2005) found boiling, steaming and microwaving to cause some losses of TPs within fresh peas, green beans and broccoli. AA also contributes to TAC and after 14 days storage in chilled conditions (4oC) AA content within fresh peas was equal to frozen (Favell, 1998). Additionally Willis et al., (1994) found fresh cooked peas to contained only 0.03g of AA per kilogram extra than cooked frozen peas. The previous studies offer some support for the present study finding of no difference in TAC between cooked fresh and frozen peas. Finally, the difference between the present study and that of Danesi and Bordoni (2008) may be due to alternative cooking methods. Within the present study the fresh vegetables were cooked until tender (Jiménez-Monreal et al., 2009) and the frozen for as long as instructed on packaging in order to reflect cooking methods of the consumer, whereas Danesi and Bordoni (2008) cooked both fresh and frozen vegetables for 20 minutes in salty water. Furthermore Danesi and Bordoni (2008) used home frozen peas rather than commercially frozen thus missing out the blanching step, which Puupponen-Pimiä et al., (2003) considers to be the cause of nutrient loss.

A Spanish study that tested 25 vegetables, one being broccoli, investigated the AO activity in vegetables that had been processed or refrigerator stored. They found after 7 days refrigerated (4 ^{o}C) storage broccoli had suffered AO losses of 41.3% with regards to the first day of analysis (Murcia et al., 2009). Refrigerated broccoli after 7 days had suffered higher AO losses than frozen broccoli stored for 8 months at -20 ^{o}C. However after 24 hours refrigeration stored fresh broccoli showed highest AO activity. Therefore indicating that time broccoli is stored for can impact negatively on AO content. The findings from Murcia et al., 2009 study are for broccoli before cooking and, interestingly, Gawlik-Dziki (2008) found boiling to reduce the AO caffeic acid and kaempferol in both fresh and frozen broccoli samples but found boiling to significantly decrease phenolics in fresh samples but increase polyphenols by 38% in frozen broccoli. Additionally, both fresh and frozen broccoli boiled for 5 minutes experienced losses in AA, with findings revealing AA retention to generally be higher for fresh than frozen (Hudson, Dalal & Lahance, 1985). The present study found after cooking there was no significant difference between fresh and frozen broccoli AO content. Despite a lack of previous studies that directly compare AO within fresh and frozen cooked broccoli the findings above suggest that fresh broccoli contains the highest TAC in the first 24 hours of refrigerated storage post harvest but by 7 days large AO losses occur and that depending on whether the broccoli is at its freshest the impact of cooking may lead fresh and frozen cooked broccoli samples to contain similar levels of AO.

Spinach appears to be very sensitive to the blanching/freezing process. Puupponen-Pimiä et al., (2003) found AA to decrease by 30% during blanching and by a further 30% during frozen storage. Additionally Bunea et al., (2008) discovered TP in spinach decreased during the freezing process although the same study that examined AA content in frozen

spinach found little reduction in α-carotene and β-carotene following blanching (Puupponen-Pimiä et al., 2003). With regards to boiling, a study conducted in Turkey found total AO activity in spinach to increase significantly ($p<0.05$) during 5 minutes boiling compared to total AO in fresh uncooked spinach (Turkmen et al., 2005), however, Mazzeo et al., (2011) measured total AO in frozen spinach before and after boiling using two different assays (FRAP and TEAC) and found significant ($p<0.05$) losses after cooking. Previous research offers some support for the findings in the present study, that fresh spinach contains higher AO than frozen after cooking due to the losses seen in frozen spinach from blanching and cooking.

Studies on TAC within fresh and frozen carrots has varied. A study that investigated the impact of blanching and freezing on phenolic compounds in carrots discovered no change in content after freezing (Puupponen-Pimiä et al., 2003). With regards to carotenoids carrots have shown 10% increases inβ-carotene after 14 days refrigeration (Howard et al., 1999) whilst frozen carrots in storage have also experienced totalβ-carotene gains (Guerra- Vargas et al., 2001). In support of Guerra- Vargas et al., (2001) a further study also found that freezing appears to increase the bioavailability of β-carotene and consequently increases overall total AO within carrots (Talcott et al., 2000). These findings could be due to the isomerization that occurs or to the greater extractability of carotenoids after heat treatment (Rickman et al., 2007). Again few studies have investigated TAC in carrots after cooking, especially TAC within frozen carrots. Yet Mazzeo et al., (2000) who investigated the impact of cooking on frozen carrots viewed losses in the AO polyphenols and carotenoids but, despite these losses, TAC suffered minimal loss after boiling. Finally, a study found no impact of boiling on TAC in fresh carrots (Mazzeo et al., 2000). The present

study found there to be no significant difference between fresh and frozen cooked carrots TAC. The above studies offer some support to the findings in the present study due to the counteracting impact of freezing that increase AO that make up TAC in carrots, the loss of AO in frozen carrots due to boiling and the increases or steadiness of TAC after cooking procedures.

According Danesi and Bordoni (2008) AO activity was found to be significantly ($p<.001$) higher in boiled frozen green beans than their boiled fresh counterparts (Danesi & Bordoni, 2008). The findings from the present study differed to Danesi and Bordoni (2008) as no difference between cooked fresh and frozen green beans TAC was discovered. However, Danesi and Bordoni (2008) study findings are surprising as Howard et al., (1990) found the green beans to be sensitive to the blanching process, consequently causing the AO AA to reduce by as much as 50% in their study. As mentioned earlier Danesi and Bordoni (2008) assayed home frozen vegetables rather than commercially quick frozen and therefore the blanching step was missed out. In terms of fresh green beans, storage appears to negatively affect AO levels (Favell, 1998). Interestingly another study (Makhlouf et al., (1995) found that despite potential losses from blanching at the point of consumption, boiled frozen green beans contained similar levels of AA to fresh green beans. Additionally, Delchier et al., (2012) found that, regardless of whether green beans were fresh or frozen boiling did not reduce lutein concentration (carotenoid AO). Finally, Turkmen et al., (2005) studied the effect of boiling, steaming and microwaving on fresh produce and green beans experienced increases in TP during all procedures. Further research is needed on the impact of cooking on frozen products and their TP content. The research available again suggests that storage time of green beans is the factor that dictates AO content at the point of consumption as after the blanching process green beans TAC remains fairly steady (Murcia

et al., 2009) and, from research available, cooking appears to have similar effects on AO in fresh and frozen products.

8.3 Limitations of the present study

The present study offers much needed information on the nutritional quality of fresh and frozen vegetables bought and cooked by the consumer. However, increasing the scale of the investigation may increase data reliability. Additionally, testing products over the course of a year would have accounted for the potential effect that season may have on agricultural practice and storage (Danesi & Bordoni, 2008) which may have impacted on AA and AO in fresh and frozen vegetables.

This study only investigated the effects of one common cooking method (boiling) on AO and AA properties. Other methods were not used due to time constraints. Evidence from previous studies indicates that steaming of vegetables retains and increases AO properties (Gawlik-Dziki, 2008; Roy, Juneja, Isobe & Tsushida, 2009). Therefore the effects of steaming and other popular consumer cooking methods need to be investigated further to establish whether frozen and fresh vegetables are still nutritionally comparable at the point of consumption when different cooking methods are employed. Furthermore, future research on AO and AA content within a variety of fresh and frozen boiled vegetables is important as the nutritional content of vegetables at the point of consumption is the most relevant to the consumer in terms of health.

The sensitive ORAC assay of Cao et al., (1995) is seen as the 'gold' standard of AO testing and is used to report AO content of consumer products. However, the DCPIP assay used to measure AA has some limitations but, due to the assay being simple, quick and the reagents being inexpensive and available (equipment was available within University lab),

this method was used. Subjectivity is associated with the DCPIP assay as the experimenter must judge when the solution turns a persistent pink, as this indicates the endpoint which means the AA has been oxidised by the DIP dye. If the present study was repeated it would be worth using a reference colour chart to ensure each assay ends when the same colour pink persists, thus making results more accurate. A further limitation of the DCPIP assay is that vegetables contain compounds other than AA that will reduce the dye (Watada, 1982). The problems mentioned above do not occur when AA content is determined by the high performance liquid chromatography (HPLC) method (Watada, 1982), therefore this may be a suitable alternative assay to measure AA.

8.4 Implications for practice

Although the NHS promotes the consumption of fresh, frozen, tinned and dried fruit and vegetables within their 5 A DAY campaign (DOH, 2010) there is still a popular misconception that frozen vegetables are lacking micronutrients due to processing. AA has been used in studies to indicate the impact of processing and storage, due to its susceptibility to chemical and enzyme oxidation and water solubility (Clegg, 1974; Morrison, 1974). The outcome of the present study rejects the above misconception after discovering that both fresh and frozen cooked vegetables (spinach, peas, carrots, green beans and peas) contained similar levels of AA. Furthermore the present investigation illustrated that TAC within fresh cooked green beans, broccoli and carrots were similar to their cooked frozen counterparts.

8.4.1 Waste

Research by WRAP (2008) revealed 6.7 million tonnes of food per year is discarded by UK shoppers and 40% (by weight) is made up of fruit and vegetables that are still edible. Approximately 730,000 tonnes of edible vegetables are thrown away every year (DEFRA, 2010). Fruit and vegetable waste is worth almost £3 billion and 90% of this waste is fresh (1.4 million tonnes) that has not been used before the 'best before' and 'display until' date (WRAP, 2008). Most frozen vegetables will maintain a high quality for 12 to 18 months at -18 ^{o}C storage (Martinez-Romero, Castillo & Valero, 2004) and thus the waste is less than that of fresh. With regards to frozen vegetables, consumers can use the amount they need and the remains of the packet can then easily be stored until next use. Being able to store frozen leftover vegetables could also save money through lack of waste.

8.4.2 Cost

As previously mentioned since 2006 there has been a downward trend in the purchase of fruit and vegetables in the UK and only 25% mean, 27% women and 20% children (5-15 years) are consuming 5 portions a day (DEFRA, 2012). This is unsurprising as food prices have risen by 12% over the last 5 years and fruit and vegetable costs have risen by around 25% since 2007 (DEFRA, 2012). With falling incomes (after housing costs) and increased food prices, food affordability has reduced by 20% for lowest income households (DEFRA, 2012). Purchases of 5 A DAY across all households in 2011 was an average of 4.0 portions per person per day, the same amount as 2001-2002, however, the lowest income households reportedly buy the least fruit and vegetables with the average person only consuming 2.9 portions of their 5 A DAY in 2011 - this was 14% lower than 2007 (DEFRA,

2012). This is unsurprising as the main factor which influences consumer food choice is cost (DEFRA, 2012).

The present study found that most of the frozen cooked vegetables were comparable to cooked fresh in terms of AA and AO after cooking. AA is such an important micronutrient AO that the UK has a RNI (Committee on Medical Aspects of Food Policy, 1991) which can be met through food and or as artificial supplements. It plays a role in preventing scurvy (Carpenter, 1986), reducing risk of death from CHD in females (Knekt et al., 1994; Manson et al., 1992), reducing the risk of oesophageal, pancreatic and lung cancer (Wargovich, 2000) and decreasing the risk of stroke compared with those with the lowest AA intake (Gale et al., 1995). Additionally, dietary AO are believed to contribute to the overall health benefits associated with fruit and vegetables due their ability to alleviate the consequences of OS in the development of disease and the ageing process (Joseph et al., 2000; Meydani, 2001; Ames et al., 1993). As cost is a major factor when purchasing food it is necessary to promote fruit and vegetables, that as well as being nutritious, cost less. Therefore a price comparison (£/Kg) between the fresh and frozen products assayed within the present study was conducted using three local supermarkets in the Wirral, United Kingdom (see Table 8.1). The products used for costing were the supermarkets own middle range products.

Table 8.1: Cost comparison (£/Kg) of fresh and frozen vegetables

	ASDA		SAINSBURYS		TESCO	
	FRESH	FROZEN	FRESH	FROZEN	FRESH	FROZEN
GREEN BEANS	£4.88/Kg	£1.19/Kg	£5.71/Kg	£1.40/Kg	£5.89/Kg	£1.20/Kg
BROCCOLI	£2.00/Kg	£1.15/Kg	£2.50/Kg	£1.20/Kg	£2.50/Kg	£1.15/Kg
CARROTS	£0.90/Kg	£1.00/Kg	£0.90/Kg	£1.00/Kg	£1.00/Kg	£1.00/Kg
PEAS	£6.67/Kg	£1.25/Kg	£9.38/Kg	£1.24/Kg	£8.34/Kg	£1.25/Kg
SPINICH	£6.25/Kg	£1.39/Kg	£7.50/Kg	£1.39/Kg	£7.30/Kg	£0.91/Kg

Table 8.2: Percentage (%) of difference in price between individual fresh and frozen vegetables in each supermarket, the average percentage difference in cost between all fresh and frozen vegetables within each supermarket and the percentage difference in cost between fresh and frozen vegetables across all supermarkets

	ASDA			SAINSBURYS			TESCO		
	FRESH	**FROZEN**	**% DIFF**	**FRESH**	**FROZEN**	**% DIFF**	**FRESH**	**FROZEN**	**% DIFF**
GREAN BEANS	£4.88	£1.19	311%	£5.71	£1.40	308%	£5.89	£1.20	391%
BROCCOLI	£2.00	£1.15	74%	£2.50	£1.20	108%	£2.50	£1.15	117%
CARROTS	£0.90	£1.00	-10%	£0.90	£1.00	-10%	£1.00	£1.00	0%
PEAS	£6.67	£1.25	434%	£9.38	£1.24	656%	£8.34	£1.25	567%
SPINACH	£6.25	£1.39	350%	£7.50	£1.39	440%	£7.30	£0.91	702%
		Average	231%		Average	300%		Average	356%
								Average % difference across all supermarkets	296%

The price comparison revealed that on average frozen vegetables were lower in price per Kg than fresh vegetables across all supermarkets investigated (see Table 8.2). Additionally frozen green beans, broccoli, peas and spinach were all cheaper per Kg than their fresh counterparts in each individual supermarket (see Table 8.2). Interestingly carrots were cheaper in price per Kg in fresh form than frozen form in all supermarkets considered (see Table 8.2).

8.4.3 Taste

Little research on direct taste comparisons between fresh and frozen vegetables has been completed. However, with regards to the taste of fresh and frozen foods a project was carried out by the Manchester Food Research Centre (2010) on behalf of British Frozen Food Federation (BFFF). The families within the study commented within food diaries and during focus groups and revealed a mixed response to the question of taste. On average, families preferred the taste of freshly cooked meals however, there were some comments referring to frozen as 'as good as fresh'. Flavour and aroma in frozen vegetables should be preserved if the product is wrapped in adequate material that prevents air passing into the vegetables or by removing as much air as possible from freezer bag/container prior to freezing, as oxygen in the air can cause rancid oxidative flavours in frozen products (Martinez-Romero et al., 2004). A good quality freezer package should be used to maintain the quality of frozen fruits and vegetables. Such packaging could be heavyweight aluminium foil, plastic coated freezer paper, and other plastic films which are effective at excluding oxygen (Food and Agriculture Organization of the United Nations, 2005). All products bought in frozen form from the supermarkets in the present study were packaged in plastic sealed bags.

8.4.5 Texture

One of the most important quality factors of frozen vegetables from the point of view of the consumer is texture (Martinez-Romero et al., 2004). Blanching is a thermal process used to inactivate enzymes that are the cause of off flavours and odours and attain maintenance of texture and nutritional quality and destruction of microorganisms (Bahceci et al., 2005). However, blanching is a thermal process and this can cause changes to texture. Processing operations can produce loss of turgor, weakening of cell wall and some degree of cell separation (Martinez-Romero et al., 2004). The effects of freezing on structure and integrity are especially noticeable in leafy vegetables due to high moisture and thin cell walls (Fuchigami, Yakumoto & Miyazaki, 1995).

8.4.6 Convenience

Finally in terms of convenience frozen vegetables are favourable to fresh. Frozen vegetables have been prepared before blanching and packaging. Generally the carrots have been peeled, broccoli trimmed, peas shelled and spinach washed. This is unlike fresh which require more preparation. Frozen vegetables only require cooking and additionally, they require less cooking time since most of the vegetables have been blanched before freezing. Consequently the time taken to prepare, cook and serve frozen vegetables is less than that of fresh vegetables.

To conclude, frozen vegetables are generally nutritionally comparable to fresh, but they are cheaper, result in less waste, are more convenient and should, if packaged correctly, taste the same as fresh. If the consumer can overcome potential differences in texture compared with fresh vegetables then frozen products are as desirable, if not more desirable

than fresh. As only 25% men, 27% women and 20% children (5-15 years) were consuming 5 portions of fruit and vegetables a day in 2010 (DEFRA, 2012) and as the lowest income households are buying the least fruit and vegetables (DEFRA, 2012) it would be beneficial for public health messages to promote frozen vegetables as generally comparable to fresh in nutritional benefits but at a lower price. This may then help to increase fruit and vegetable consumption by the whole population but particularly by those on the lowest incomes.

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10.0 Appendices

10.1 Appendix A

Preparation of chemicals for ORAC assay:

Preparation:

Fluorescein: - 44mg in 100mL of d.H20 (store at 4oc)
- 1/10 dilution of Fluorescein stock was prepared (1mL + 9mL of d.H20) and stored in the dark at 4oc

TROLOX: - 1mM solution in PBS
-Stored at -4oc

Preparation on the day:

AAPH: -
3mL PBS was added to 0.36g of AAPH to make enough solution for 25µL in each plate well.

Fluorescein: -
A 1/100 dilution of the 1/10 pre made stock was prepared by adding 50µL fluorescein into 5mL PBS. This made enough solution for 50µL into all the wells bar two rows.

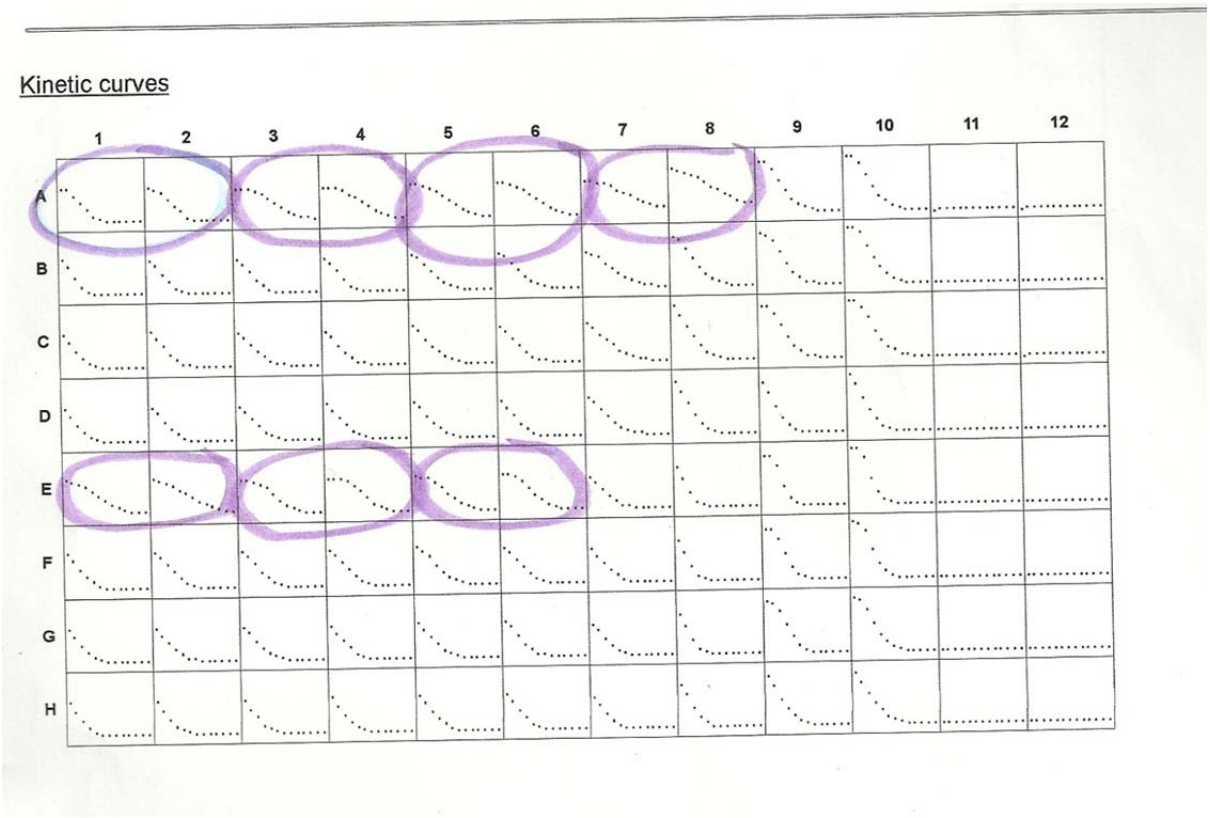
TROLOX: -
A 20µM solution of TROLOX was prepared by making a 1/50 dilution of stored stock. Enough was made to use as a standard in 12 wells (50µL per well) by adding 30µL of stored stock with 1470µL PBS which was then mixed and 750µL of the solution was removed.

SAMPLES: -
1/10, 1/100, 1/1000 and 1/10,000 dilutions of sample were made by adding 450ul PBS into four 1.5mL eppendorfs then adding 50µL into the first eppendorf labelled 1/10, shaking and then transferring 50µL from the first eppendorf into the second eppendorf to make 1/100 dilution. 50µL was transferred across the eppendorfs until a dilution of 1/10,000 is made.

10.2 Appendix B

The circled kinetic curves in the figure below are curves that show an endpoint.

Kinetic curve end points



10.3 Appendix C

Concentrations of different solutions used for AA triplicate standard curve

	A	B	C	D
0.25M Oxalic acid cm3	9	9	9	9
1M HCL cm3	1	1	1	1
Ascorbic acid 0.5mg/cm3	0.25	0.5	0.75	1.0
DIP dye cm3	2.8, 2.8, 2.8	5.6, 5.7, 5.6	8,7.8, 8	10.6, 10.6, 10.6
Mean DIP dye cm3	2.8	5.6	7.9	10.6

AA standard curve