Factors affecting the composition and quality of broccoli juice

A thesis presented in partial fulfilment of the requirements for the degree of

Master of Technology

in

Food Technology

at Massey University, Palmerston North, New Zealand.

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2009

Abstract

A shelf life trial using a fully balanced factorial experimental design was used to analyse the effects of acidity and light on broccoli juice made on a semi commercial scale over an eight week period in simulated retail refrigerated storage conditions. The research focused on making broccoli juice on a pilot scale, and what happens to the colour, composition and flavour during storage.

A pilot scale production of pasteurised broccoli juice was conducted and the juice satisfied microbiological safety limits for the eight week shelf life trial in retail storage conditions. The stability of the green colour of fresh broccoli through processing and storage was assessed. Neutral broccoli juice remained green for four weeks before the colour became more yellow. The acidified juice became yellow on acidification and did not change significantly during storage.

Dietary fibre and pectin levels did not change during storage. Chlorophyll and carotenoids levels decreased during storage and were directly influencing the colour changes in the juices. Ascorbic acid levels decreased significantly during processing resulting in low ascorbic acid levels (12 - 15 mg /100ml of juice) at the start of the shelf life trial and dropped further to 2-6 mg /100ml of juice after eight weeks. Acidification and storage in the dark had a protective effect on the degradation of ascorbic acid with only a 58% reduction in ascorbic acid levels compared to an 84% reduction in neutral light stored broccoli juice.

The effect of processing and storage on the flavour of the beverage was assessed using a trained sensory panel providing descriptive analysis. The sensory profiles for neutral and acidified juices were extremely different with the unbalanced acidity suppressing the perception of the basic tastes, sweet, salty and bitter. The neutral juice sensory profile only changed slightly in aroma attributes during storage for seven weeks. The astringent aftertaste of the acidified juice increased while the broccoli smell decreased during storage.

The results from this research indicate that the production of a broccoli juice with a yellow green colour and some retained nutritional components is achievable with a refrigerated (4 °C) shelf life of 30 days in light excluding glass packaging. The neutral juice is recommended as it was greener and had a broccoli flavour.

Acknowledgements

I would like to thank my supervisors, Drs Marie Wong, Erin O'Donohgue and Lee Huffman, for their advice, patience, guidance and support throughout this project.

Gratitude goes to Plant and Food Research (formerly Crop and Food Research). Their financial support and investment in my research made this possible.

To my colleagues Carl Massarotto, Shiji Nair, Virginia Corrigan, Allan Hardacre, Suzanne Clark, Rebecca Zou and Annie Teoh for their tolerance, helpful advice and support.

I would like to extend my appreciation to:

Erin O'Donohgue and Sheryl Somerfield for their help and guidance with the analytical testing and HPLC analysis and scientific rigor.

Garry Radford and Warrick Johnson at Institute of Food Nutrition and Human Health (IFNHH) Palmerston North for their support and help taming the UHT equipment, Malcolm Reeves from EIT for the use of the water press and Kapiti Green Ltd for the generous supply of broccoli.

Duncan Hedderly and Andrew McLaughlan for their help and guidance with the statistical analysis.

Virginian Corrigan and John Grigor for their advice on the sensory training, assessment and analysis and to the sensory panellists for their dedicated efforts.

Ann-Marie Jackson, Laboratory services manager, School of Engineering and Advanced Technology, Massey University, and Weiping Liu, Laboratory Technician, IFNHH, Massey University for the microbial analysis.

Lastly but my biggest thanks go to my family for their patience and tolerance, support and sacrifice, especially Stuart and little Jessica whose life to date has revolved around broccoli juice.

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CHAPTER 1

Introduction

Broccoli is a popular vegetable and an excellent source of antioxidants, vitamin C, fibre and folate. It also contains good levels of calcium, iron, potassium and vitamins A & E (Gourley 2003; Lister & Bradstock 2003; Zhang & Hamauzu 2004). Broccoli contains phytonutrients such as chlorophyll and carotenoids for which evidence of perceived and proven health benefits is accumulating (Kassie et al. 1996; Gourley 2003; Lister & Bradstock 2003; Lamikanra et al. 2005; Weatherspoon et al. 2005; Jahangir et al. 2009). However, the majority of these health benefits can only be attained by consuming fresh or minimally processed broccoli due to the deleterious effects of processing on these nutritional components (Gourley 2003; Weatherspoon et al. 2005; Sun et al. 2007; Tosun & Sevinc 2008; Jahangir et al. 2009). Today consumers are increasingly seeking variety and convenience (Moskowitz et al. 2006). Optimizing the use of broccoli in different product formats, such as juices, provides consumers with more options as well as potentially increasing the crop's economic value for growers by utilizing parts of the crop that would otherwise be discarded.

The decision to investigate the quality parameters of a beverage from broccoli was made due to the nutritional and functional benefits of broccoli and the potential to utilise a waste stream. Generally only the broccoli head with a small portion of stalk is harvested for consumer consumption. The majority of the edible stalk and out of size specification broccoli is not harvested and ploughed back into the field. Since 2004 total vegetable juice exports in New Zealand have increased 47% from $5.4 million to $10.2 million dollars in 2008 (Kerr et al. 2004; Aitken & Hewett 2008). In 2008 there were 18000 tonnes of broccoli harvested and consumed on the domestic market (Aitken & Hewett 2008). To make the nutritional and functional benefits of broccoli more accessible to a wider population it is worth exploring a commercially feasible method(s) to minimise the flavour and odour components in a broccoli beverage which are undesirable to the consumer.

Fruit and vegetable juices are produced using combinations of physical destruction and/or enzyme-assisted reactions of the fruit or vegetable to expel the juice, in some cases leaving large amounts of insoluble waste pomace. Depending on the processing method the juice can

take a variety of forms e.g. clear clarified juices, cloud juices containing cellular material in suspension, pulpy juices and nectars made by pulping whole fruits or vegetables. Different juicing methods may contribute to or reduce flavour and odour development.

The aim of this project was to investigate the properties of broccoli juice and identify factors that influence a stable colour and nutritional components so that a commercially viable process to produce a broccoli beverage can be recommended.

The following objectives will be investigated to produce a stable green juice from broccoli using physical extraction techniques:

- The stability of the green colour of fresh broccoli through processing and storage will be assessed.
- The identification of key nutrient components in broccoli juice and monitor their changes during processing and storage with a view to maintaining the nutritional benefits of broccoli.
- The effect of processing and storage on the flavour of the beverage will be determined. This will play an important part in the consumer appeal and potential commercial success of the beverage as broccoli has a distinct flavour and associated odour.

CHAPTER 2

Literature review

2.1 Broccoli

2.1.1 Broccoli

Broccoli, *Brassica oleracea* var*. italica*, belongs to the family Brassicaceae or Cruciferae, genus and subgroup Oleracea (Dixon 2007; Higdon et al. 2007; Perera et al. 2007; Podsedek 2007; Jahangir et al. 2009). 'Brassica' (Latin for cabbage) (Higdon et al. 2007) is only one of 350 genera in the Brassicaceae family (Fahey et al. 2001; Jahangir et al. 2009). Broccoli exists in many forms some with single compact large heads, some with sprouting or relatively small flower heads, and in a variety of colours form dark purple, green or yellow/green. The flowers have four equal sized petals in the shape of a cross hence the name 'crucifer' or cross bearer. (Dixon 2007)

In New Zealand all of the 18,000 tonnes of broccoli produced annually is consumed on the domestic market (NZFVI 2009). The broccoli head is harvested shortly before the flowers open. The actual duration (approximately 10-12 weeks from planting) of the crop depends on the weather conditions, air temperature and cultivar planted (Rosa et al. 2001; Dixon 2007). Different broccoli cultivars are planted throughout the year to ensure an annual supply to meet consumer demand. In New Zealand the most popular broccoli varieties for the autumn, winter and spring season are 'Marathon' and 'Legacy' with 'Arcadia' and 'Liberty' grown in the summer and autumn season (McKee 2009).

2.1.2 Nutritional components in broccoli

Fruits and vegetables, when viewed within the context of the total food supply contribute a significant amount of the micro nutrients compared to the macro nutrients; 90% of the dietary vitamin C, more than 50% of the vitamin A and more than 35% of the vitamin B6. The importance of fruit and vegetables in human nutrition is clearly evident (Weatherspoon et al. 2005). Numerous epidemiological studies indicate that *Brassica* vegetables in general, and broccoli in particular, may decrease the risk of cardiovascular diseases and protect humans against some cancers (Fahey et al. 2001; Jeffery et al. 2003; Lister & Bradstock 2003; Higdon et al. 2007; Podsedek 2007; Vig et al. 2009). They contain a number of nutrients and phytochemicals with cancer and chemo protective properties. The effects of combinations of chemo protective phytochemicals present in the Brassica vegetables or their overall action mechanisms in providing protective effects are still far from being understood (Fahey et al. 2001; Moreno et al. 2006; Higdon et al. 2007).

Broccoli is an excellent source of dietary essential minerals (Ca, Mg, Na, K, Fe, Zn), folate and fibre. They are also rich sources of dietary antioxidants (i.e. vitamins C and K1, flavonoids) and phytochemicals , carotenoids, chlorophyll and glucosinolates (and glucosinolate-breakdown products) (Baik et al. 2003; Jeffery et al. 2003; Lister & Bradstock 2003; Siomos et al. 2004; Moreno et al. 2006; Galgano et al. 2007; Jagdish et al. 2007; Perera et al. 2007; Podsedek 2007; Jahangir et al. 2009).

The composition of raw broccoli is presented in Table 2.1. It shows variation in source of broccoli depending on country (USA, Australia or New Zealand). The composition changes with processing and consumer preparation method (USDA 2009). Fructose and glucose were found to be the major soluble sugars in broccoli. Sucrose was only present at 20% of the glucose level. An investigation of 11 broccoli cultivars for differences between growing season (summer/winter or spring/summer) and between cultivars on the synthesis of glucose, fructose and sucrose has shown that glucose levels are lower in summer/winter broccoli. Rosa et al (2001) suggest this is linked to the production of glucosinolates (secondary plant metabolites), which are found to be higher in the summer/winter crop compared to the spring/summer crop. Overall higher sugar contents were found in summer/winter crops (Rosa et al. 2001; Siomos et al. 2004).

Table 2.1 Composition of raw Broccoli (*Brassica oleracea* var*. italica*) (Athar et al. 2006; FSANZ 2009; USDA 2009) Carbohydrate, (a: by difference, b: available carbohydrates).

Broccoli, raw (*Brassica oleracea* var. *italica*)		**USA**	**Australia**	**New Zealand**
Proximates	Units	Value / 100g	Value / 100g	Value / 100g
Energy	kJ	141	124	134
Water	g	89.3	89.4	88.0
Protein	g	2.8	4.4	4.4
Total lipid (fat)	g	0.4	0.3	0.6
Ash	g	0.9	0.9	-
Carbohydrate, (a, b)	g	6.6a	0.4b	2.3b
Fibre, total dietary	g	2.6	3.6	3.8
Starch	g	0.0	0.0	0.0
Sugars, total	g	1.7	0.4	2.3
Minerals				
Calcium, Ca	mg	47	33	42
Iron, Fe	mg	0.7	0.9	1.2
Magnesium, Mg	mg	21	22	-
Phosphorus, P	mg	66	81	-
Potassium, K	mg	316	345	487
Sodium, Na	mg	33	22	5
Zinc, Zn	mg	0.4	0.6	0.7
Copper, Cu	mg	0.05	0.05	-
Manganese, Mn	mg	0.21	0.22	-
Selenium, Se	µg	2.5	0	0.3
Vitamins				
Vitamin C, total ascorbic acid	mg	89	99	57
Thiamin	mg	0.07	0.08	0.09
Riboflavin	mg	0.12	0.21	0.35
Niacin	mg	0.64	0.50	1.30
Pantothenic acid	mg	0.57	0.47	-
Vitamin B-6	mg	0.18	0.09	0.21
Folate, total	µg	63	49	75

2.1.3 Composition variance within the broccoli plant

The edible broccoli head is made up of the stalk and florets (which are bunches of tiny flowers) (Figure 2.1). The bottom section of the stalk in Figure 2.1 is usually not consumed as it is too fibrous. The composition of each section varies because of the different roles they play in the development of the plant (Siomos et al. 2004). Florets are made of rapidly developing tissue of immature flowers, where as the stems are made of more mature tissue with an extensive pith area. Siomos et al (2004) found the floral portion of the broccoli head has higher levels of soluble solids, dry matter, total soluble phenols, more water soluble carbohydrates and organic acids compared to the stem portion, which has higher levels of nitrates and reducing sugars (Siomos et al. 2004).

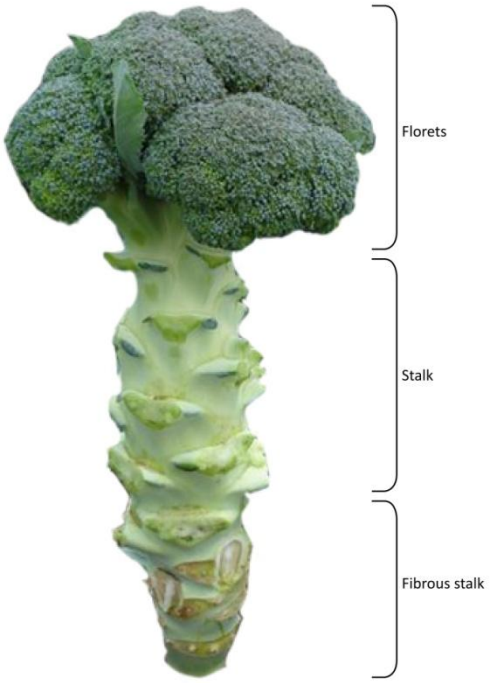


Figure 2.1 A broccoli plant with the outer leaves removed before harvesting.

Jagdish et al (2007) analysed six different broccoli cultivars for major antioxidant phytochemicals and reported variability in the vitamin C content (25 to 82mg/100g) and the beta-carotene content (48 to 113mg/100g). Correspondingly Podsedek (2007) found variances over 4-fold in vitamin C levels in different broccoli cultivars. These results were comparable to other studies, which all indicate variation in vitamin C and other polyphenols due to variation

in cultivars, environmental conditions, post harvest handling, processing conditions and methods of extraction (Moreno et al. 2006; Jagdish et al. 2007; Podsedek 2007).

2.1.4 Phytochemicals

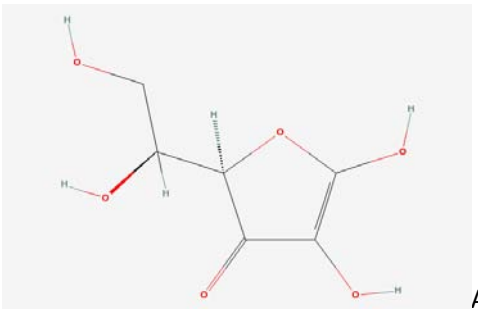
The phenolic compounds are a large group of the secondary metabolites widespread in the plant kingdom. They are categorised into classes depending on their structure and subcategorised within each class according to the number and position of hydroxyl groups and the presence of other components. The most widespread and diverse group of polyphenols are flavonoids which are built on C6-C3-C6 flavone skeleton (Podsedek 2007). Flavonoid compounds in broccoli florets include two major compounds (quercetin 3-*O*-sophoroside and kaempferol 3-*O*-sophoroside) and three minor components (isoquercetin, kaempferol 3-*O*-glucoside and a kaempferol diglucoside) (Podsedek 2007; Sun et al. 2007).

Antioxidants can scavenge free radicals and protect the human body from oxidative stress and prevent cardiovascular diseases, cancers, diabetes, lowering cholesterol and reducing inflammations (Gourley 2003; Lister & Bradstock 2003; Russell 2004; Sun et al. 2007; Martinez-Sanchez et al. 2008; Xu 2008). Antioxidants inhibit the generation of free radicals, which initiate lipid oxidation, or reduce the number of free radicals in a system and delay lipid oxidation (Xu 2008).

2.1.5 Vitamin C

Vitamin C or ascorbic acid is vital for good health and the functioning of many biological processes in humans. Fruit and vegetables are the most common sources of vitamin C (Russell 2004; Galgano et al. 2007; Jagdish et al. 2007; Podsedek 2007). The biological function of L-ascorbic acid is an enzyme cofactor, a radical scavenger, and as a donor/acceptor in electron transport at the plasma membrane and as such functions as an antioxidant (Russell 2004; Podsedek 2007). Vitamin C exists naturally as two biologically active compounds: L-ascorbic

acid (AA) and its oxidised form dehydro-L-ascorbic acid (DHAA) (Figure 2.2). Total vitamin C is the sum of the AA and the DHAA content in foods (Zapata & Dufour 1992; Vallejo et al. 2003; Russell 2004; Gebczynski & Kmiecik 2007).


AA

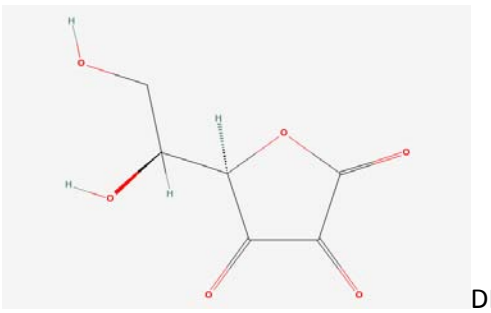

DHAA

Figure 2.2 L-ascorbic acid (AA) and its oxidised form dehydro-L-ascorbic acid (DHAA)(Anon 2009d).

AA and DHAA are readily oxidised in solution, especially when exposed to elevated temperatures, some divalent cations (e.g. copper and iron), dissolved oxygen, alkaline pH, light, or degradative (oxidizing) enzymes. The oxidation of AA to DHAA is reversible, the oxidation of DHAA to biologically inactive products is not (Russell 2004). Vitamin C is the most labile of all vitamins and is often used as an indicator of overall vitamin stability in foods (Zapata & Dufour 1992; Russell 2004; Podsedek 2007; Tosun & Sevinc 2008; Coultate 2009).

2.1.6 Glucosinolates

Glucosinolates all share a similar basic structure consisting of a β-d-thioglucose group, a sulphonated oxime group and a side chain derived from methionine, phenylalanine, tryptophane or branched-chain amino acids (Moreno et al. 2006). Glucosinolates are stable water-soluble precursors of isothiocyanates (Figure 2.3) (Fahey et al. 2001; Vig et al. 2009). The sulphate group of a glucosinolate molecule is strongly acidic and plants accumulate glucosinolate by sequestering them as potassium salts in plant vacuoles (Moreno et al. 2006).

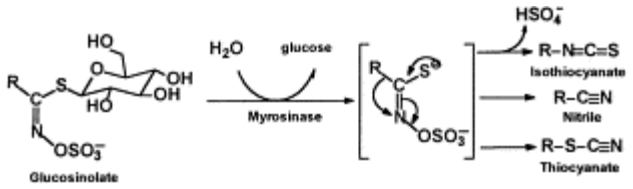


Figure 2.3 Glucosinolate breakdown process (Fahey et al. 2001).

Glucosinolates are numerous and the most extensively investigated ones are found in *Brassica* vegetables (Fahey et al. 2001). More than 120 glucosinolates have been characterised (Moreno et al. 2006). One third of all glucosinolates contain a sulphur atom in various states of oxidation (Fahey et al. 2001). *Brassica* vegetables each exhibit a characteristic profile of glucosinolates, differing substantially, even though they are all members of *Brassica oleracea* (Fahey et al. 2001). An examination of 50 broccoli varieties by Jeffery et al (2003) showed an approximately 20 fold variation in glucosinolates (Jeffery et al. 2003). Glucosinolates vary in their distribution throughout a plant and are present in seeds, roots, leaves and stems. The age of the plant also provides variation with higher levels of glucosinolates being found in the young sprouts rather than the mature plants (Fahey et al. 2001; Jeffery et al. 2003).

Glucosinolates in their natural state are inactive or some can have a detrimental effect on our health, but after hydrolysis by myrosinase, active compounds such as isothiocyanates or indoles can be formed (Figure 2.3), which have a positive effect on our health such as cancer protection (Brandi et al. 2005; Moreno et al. 2006; van Eylen et al. 2007; Hounsome et al. 2008; Vig et al. 2009). The potential protective role of *Brassica* vegetables and active compounds present in these vegetables such as isothiocyanates and indole-3-carbinol has been extensively studied in *in vitro* and *in vivo* carcinogenesis models. The metabolic breakdown products of the glucosinolates enhance the metabolising enzymes that protect DNA from damage. Results have consistently shown that the chemo-protective agents influence carcinogenesis during initiation and promotion phases of cancer development (Kassie et al. 1996; Vallejo et al. 2003; Brandi et al. 2005; Moreno et al. 2006; Higdon et al. 2007). The most characterised glucosinolate compounds in broccoli are sulforaphane and indole-3-carbinol, but many other isothiocyanates that are present in lower quantities may also contribute to the anti-carcinogenic properties (Kassie et al. 1996; Jeffery et al. 2003; Brandi et al. 2005; Moreno et al. 2006).

Glucosinolates are not bioactive in the animal that consumes them until they have been enzymatically hydrolysed by the endogenous myrosinase enzyme (thioglucoside glucohydrolase). As shown in Figure 2.3 glucose is released leaving an unstable thiono compound that can be rearranged to form the active isothiocyanate or less active nitriles or thiocyanates (Fahey et al. 2001; Baik et al. 2003; Jeffery et al. 2003; Galgano et al. 2007; van Eylen et al. 2007; Vig et al. 2009). The kinetics of the myrosinase reaction differs widely from species to species and even within the same plant (Fahey et al. 2001) The composition of the breakdown products depends on pH, metal ions (Fe^{2+} ions) and other protein elements such as the presence of epithiospecifier protein (ESP) (Baik et al. 2003; Bones & Rossiter 2006; van Eylen et al. 2007). Myrosinase is released by disruption of the plant cell. This can occur during cultivation, harvesting, chopping or crushing during processing, freeze-thawing or mastication (Fahey et al. 2001; Jeffery et al. 2003; Moreno et al. 2006; Galgano et al. 2007). The function of glucosinolates in plants is still unclear, but their potent odour and taste suggest a role in herbivore and microbial defence (Moreno et al. 2006).

2.1.7 Dietary Fibre

Dietary fibre is essentially the structural material of plant cells that is resistant to the digestive enzymes in the human stomach or intestines (Nelson 2001; Weatherspoon et al. 2005; Gray 2006; Rickman et al. 2007). The definition of dietary fibre has been debated in recent years. Previously it used to be simply intrinsic plant cell wall polysaccharides (Gray 2006), however it has since been expanded to include the structural polysaccharides of the cell wall such as cellulose, hemicelluloses, lignin, gums, pectins and mucins with different chemical physiochemical and physiological properties (Weatherspoon et al. 2005; Gray 2006; Hounsome et al. 2008).

Soluble fibre includes noncellulosic polysaccharides, pectins, gums and mucilages. Soluble fibre increases the viscosity in foods due to their capacity to absorb water and swell, causing stomach distension leading to an increased feeling of satiety (Nelson 2001; Weatherspoon et al. 2005). Soluble fibres are soluble in aqueous solutions of enzymes that are typical of the human digestive system (Nelson 2001). Insoluble dietary fibre provides a carbohydrate

substrate, which stimulates bacteria growth in the large bowel. It also increases the faecal bulk and reduces transit time, both of which are important in cancer risk reduction (Weatherspoon et al. 2005; Hounsome et al. 2008). Total dietary fibre refers to the total amount of both soluble and insoluble dietary fibre in a food (Nelson 2001).

2.1.8 Pectin

Pectin is a component of dietary fibre and can be soluble or insoluble. Pectin is a high molecular weight carbohydrate polymer which is an essential structural component in fruit and vegetables where in combination with hemi-cellulose binds single cells to form the cell walls and intracellular tissue and represents 15-20% of the dietary fibre in vegetables (Nelson 2001; Gray 2006). The term pectin covers a number of polymers that vary according to molecular weight, chemical configuration, neutral sugar content, and plant type (Flutto 2003).

Pectin exhibits a range of textural and rheological functional properties in plant based foods but these depend on both enzymatic and non-enzymatic degradation during maturation and processing (Fraeye et al. 2007). Knowledge of the pectin interactions can be used to tailor juice cloud stability and juice extraction yields (Ashurst 2004; Sila et al. 2009).

Pectins are polysaccharides composed of galacturonic acid chains interspersed with rhamose units and branched chains of pentose and hexose units (Figure 2.4). The chain is completely or partially esterified with methanol (Nelson 2001; Flutto 2003; Ashurst 2004; Gray 2006). The number of components and their position on the pectin backbone influence the functional properties of the pectin.

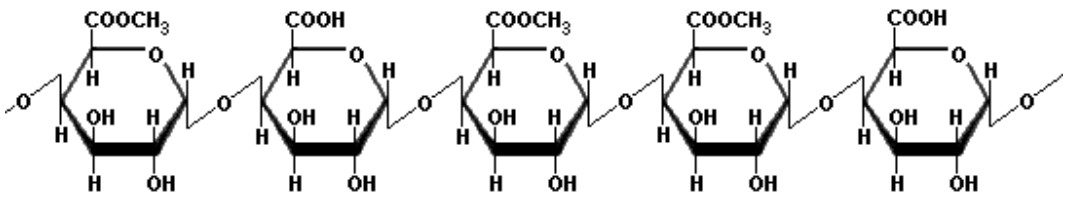


Figure 2.4 Structure of pectin (Anon 2009e).

The relationship between the pectin and environment (pH, dissolved solids, specific metal ions, ionic strength, and temperature) strongly influence the functionality due to molecular interactions. Any process capable of modifying molecular parameters can lead to significant functional changes in pectin (Sila et al. 2009).

Homogalacturonan is a main structural component of pectin made up of α(1,4)-linked galacturonic acid residues, part of which are methyl esterified (Fraeye et al. 2007). Changes in galacturonic acid levels provide evidence for the inter-conversion of pectin fractions (Sila et al. 2009).

When pectin is heated at neutral or weakly acidic pH, non-enzymatic degradation of pectin occurs in the homogalacturonan region via the β-elimination reaction which involves base catalysed splitting of the pectin chains and occurs in parallel with de-esterification (Sila et al. 2009). The difference in the thermal sensitivity of pectin fractions depends on the degree o f methoxylation (DM), with the water soluble pectin fractions being highly methoxylated (Sila et al. 2009).High DM pectin is more susceptible to the β-elimination reaction than low DM pectin (Fraeye et al. 2007; Van Buggenhout et al. 2009). The rate of β-elimination increases with increasing pH and DM (Fraeye et al. 2007; Van Buggenhout et al. 2009).

Pectin is very stable around its pKa value (acid disassociation constant)of pH3.5 (Sila et al. 2009). Plant based foods with pH >4.5 are processed above 80°C so are very susceptible to the β-elimination reaction which can occur as low as pH 3.8. Between pH 4 and 6 acid hydrolysis of pectin is negligible but β-elimination rates are significant and in this pH range some chemical demethoxylation of pectin can occur, which further reduces the extent of depolymerisation (Fraeye et al. 2007; Sila et al. 2009; Van Buggenhout et al. 2009).

Isolated pectin is soluble in hot water and has known gel forming properties (Gray 2006) and is widely used as a food ingredient. Pectin is water soluble with pH, temperature and ionic strength of the solution determining the rate of dissolution. The calcium content of water reduces the pectin solubility. Heat stability improves when water activity of the system is lowered with sugar.

2.2 Juice production

2.2.1 Processing techniques for juice production

Fruit and vegetable juices are produced using combinations of physical destruction and enzyme assisted reactions of the fruit or vegetable to expel the juice, in some cases leaving large amounts of insoluble waste. Commercial fruit or vegetable juices are primarily defined by the post-extraction processing. The juice can take a variety of forms such as clear clarified juices, light cloud or heavy cloud juices containing cellular material in suspension, pulpy juices and nectars made by pulping whole fruits or vegetables. Different methods of juicing may affect flavour and odour development.

Single strength juices are unmodified after extraction. They have a typically short shelf life and may have only minimal further processing. Typically single strength juices are made by juice bars and cafes in single serve volumes for immediate consumption. The commercially available single strength juices need preservation for distribution and storage but typically have a shelf life of days or weeks, rather than months (Varnam & Sutherland 1994). Hyun-Pa et al (2007) suggest that fresh vegetable juices should generally be consumed within 24 hours due to their potential for microbial growth.

Nelson and Tressler (1980) grouped vegetable juice into six classes as follows:

1) Juices prepared from acidic products (tomato and rhubarb) can be processed at relatively low temperature.
2) Vegetable or blends that are acidified with highly acid products (citrus, pineapple, tomato and rhubarb)
3) Vegetable juices acidified with organic acids to allow processing at relatively low temperatures
4) The excess juice obtained from fermented vegetables (e.g. sauerkraut juice)
5) Juices freshly extracted from non-acid vegetables immediately before consumption and are not heat treated or acidified.
6) Vegetable juices or blends when not acidified must be processed at relatively high temperatures to kill spores of spore-forming microorganisms.

2.2.2 Juice processing

Vegetable juice processing is similar to fruit juice processing and involves a common basic process that varies according to the type and structure of vegetable being juiced. The five elements associated with juice production are harvesting, transport and storage, pre-treatments, juicing and post treatments.

2.2.2.1 Harvest, transport and storage

The vegetable is harvested in a manner to minimise physical damage. Likewise with storage and transport, produce should be handled with care and stored at temperatures appropriate for the produce.

2.2.2.2 Pre treatments

The vegetables can be prepared for juice extraction using several different processes. Pre treatments are designed to ensure the quality of the raw ingredients and reduce surface microbial contamination and particle size reduction to optimise juice extraction.

Once received at the factory the produce is graded and washed and to ensure the produce is sound, free from gross damage and contamination and remove foreign objects (Varnam & Sutherland 1994). Washing can be in water flumes or spray washing depending on the product. The water can be hot or cold and /or contain a sanitiser such as chlorine or pass through a UV light tunnel.

Temperature pre treatments

Produce with short growing seasons is often stored frozen and defrosted when required. This can improve the juice yield as freezing damages the plant cells resulting in juice being released easier.

Blanching

Blanching is used as a pre-treatment to inactivate enzymes that will adversely affect the desired juice outcome. Peroxidase enzymes are the most stable and widespread plant enzymes. Their rate of destruction is used to indicate the effectiveness of blanching treatments in conjunction with the loss of the colour and textural changes of fruit and vegetables (Polata et al. 2009). The primary function of peroxidases in plants is the reduction of hydrogen peroxide at the expense of oxidation of phenolic compounds. Peroxidase enzymes are vegetable specific and thermally resistant. They are responsible for the mechanical properties of cell walls during extension, cell adhesion and disease resistance (Polata et al. 2009). Polata et al (2009) found the deactivation of peroxidases for broccoli followed a first order kinetics between 58°C and 74°C range studied using conventional heating. Murcia et al (2000) determined that the optimal blanching conditions for broccoli florets and stems that minimised organoleptic and nutritional changes as well as achieving acceptable reduction in peroxidase activity was 94°C for 60 sec, amino acid content was maintained by blanching up to 90 sec (Murcia et al. 2001). Jones et al (2006) showed blanching broccoli at 93°C for 2 min resulted in a 47-65% reduction in sulforaphane in the broccoli tissue but suggest that it is possible to produce juices from brassica vegetables that contain significant levels of isothiocyanates providing the juice is made within 24 hours of harvest and refrigerated (Jones et al. 2006). Sato et al (2003) used blanching (80 - 95°C for 5 – 20 min) prior to chopping, juicing and an anion exchange treatment with organic acids to remove the ‘discomfort odour’ in brassicaceous vegetables. This process prevents the formation of the allyl isothiocyanates by inactivating the myrosinase enzymes.

Enzyme pre treatments

In general clarified juices require enzyme treatment at some stage in the juicing process (Ashurst 2004). In immature fruit the pectin is mainly insoluble, but as the fruit ripens there is a gradual breakdown of some of the pectic substances in the skin and flesh cell walls, resulting in formation of polysaccharide component materials and this varying composition affects the efficiency of separation and yield of the juice (Ashurst 2004). Enzymes are usually dosed into pre-warmed mash, mixed well and left to stand for a recommended time and constant (optimum) temperature. Enzyme pre-treatment with pectolytic enzymes involves dosing pulp

and holding for 24 hrs at 5°C or 1 hr at 15-30°C before pressing (Varnam & Sutherland 1994). *Amylase* enzymes are used in fruit and vegetables with high starch content, to break down any residual starch that can gelatinise during juice production. Residual starch can result in precipitation and haze effects in the final product (Ashurst 2004). *Cellulase* enzymes are used for the total liquefaction of plant tissues to increase yield by reducing press energy (Ashurst 2004).

Milling

Some vegetable require physical size reduction prior to juice extraction. Hammer mills are widely used as they give high juice yield. Fixed knife mills are also used. They are a circular gravity fed chamber containing a three armed rotor that spins at high speed forcing the produce against fixed shredding knives. The pulp falls into a hopper and is pumped to the press through screens where stalks and other debris are removed (Varnam & Sutherland 1994).

2.2.2.3 Juicing

Juice is extracted from the produce using several different methods according to the starting raw material and the type of juice required. Juice is either pressed from the raw material or spun (centrifuged) or a combination of both. The efficiency of these methods is improved by the use of other pre-treatment processes to reduce the particle size and/or cause damage to the plant cell. Different techniques, including thermal, electrothermal, pulsed electric fields (PEF), chemical and enzymatic pre-treatment are suggested to enhance pressure assisted juice extraction (Praporscic et al. 2007).

In fruit and vegetable tissues cells are surrounded with elastic membranes and rigid walls, which limit efficiency of the pressing extraction (Praporscic et al. 2007). Juice can be extracted by pressing using either batch or continuous methods depending on the scale of juice to be extracted (Varnam & Sutherland 1994).

The rack and frame press is one of the oldest methods and often used by small processors. Pulp of soft produce is placed into a sterile heavy nylon or cotton bag in a frame on a wood or metal rack. Multiple bags are stacked up, the frame removed and pressure is applied to the

stack with a hydraulic ram to express the juice. Although very labour intensive this process produces high quality juice (Varnam & Sutherland 1994). For larger volumes automated hydraulic presses are used but require press aids to provide firmness to the mash and to form channels for the juice to exit. Press aids reduce cloudiness and require long fibres for efficient action such as ground wood pulp or sterilised rice hulls (Varnam & Sutherland 1994).

A screw press is a heavy graduated pitch screw fitting closely within a cylindrical screen. In a two stage process the easily removed juice is drained off then the pulp passes through the screw where it is compressed. The action of the screw is aided by the interaction with compressor bars incorporated into the press. Screw presses require press aids and efficiently produce cloudy juice (Varnam & Sutherland 1994). Belt presses are used effectively with firm fruit. Pulp blended with press aid is pressed between two mesh belts which pass through a series of rollers and the expelled juice is collected in a channel below (Varnam & Sutherland 1994).

Direct pressure or pressing has been the traditional juice processing method however the use of centrifugal separation of juice is becoming more common. The decanter centrifuge can be used in conjunction with a pressing system as a preliminary step to increase efficiency. Two units used in conjunction provide complete separation with the first used as a coarse primary stage and the second as a final clarification stage. The decanter is a horizontal scroll centrifuge with a cylindrical-conical solid-wall bowl for the continuous separation of solids out of suspensions (Ashurst 2004).

Compared to pressing, centrifugation produces single strength cloudy juices with a high percentage (60%) of small particles (1µM or smaller) in suspension compared to only 20% in pressed juice (Ashurst 2004). A major factor in the production of “naturally cloudy” juices is the rate of processing and to ensure stability the juicing stage should be followed immediately by pasteurisation in order to deactivate the enzymes naturally present in the fruit (Ashurst 2004). Decanters are frequently used in conjunction with disk-stack- type centrifuges in the pre-preparation of clear juices and juice concentrates, where the initial decanter treatment results in a partially clarified juice with a low level of suspended solids. This is followed by a clarification stage using a disk centrifuge whereby the solids are thrown outwards from the through-flow juice stream into a solids holding space and automatically discharged from there, as and when an optimum level of solids is reached (Ashurst 2004).

2.2.2.4 Post treatments

Once juice is extracted from the vegetable what happens to it is determined by or defined by its end use and shelf life. Juice can undergo any or a combination of the processes including clarification, heat treatments, non thermal processing, mixing and homogenisation, concentration and packaging.

Clarification is normally a combination of enzyme treatment, fining, centrifugation and filtration. Treatment with pectolytic enzymes is useful but not essential (Varnam & Sutherland 1994). Enzyme treatment in a holding tank for 8 hrs at 15-20°C or 1 hr at 45°C is recommended for apples but will vary according to produce and enzyme but temperatures between 20 and 40°C should be avoided to minimise yeast growth (Varnam & Sutherland 1994).

Centrifugation is used as a preliminary treatment to remove high or low density material. It is also applied after fining with bentonite and gelatine. Bentonite forms flocs with proteins and gelatine creates an insoluble floc with the phenolics and proteins. The insoluble flocs are removed by decanting, centrifuging or filtration. Water soluble chitosan is an alternative to gelatine (Varnam & Sutherland 1994).

Heat Treatments

Liquid products are relatively easy to pasteurise. The flow properties permit fast heat transfer by turbulent mixing using convection and conduction. The three types of process are, batch, continuous or in-container (Wilbey 2003a). Small scale juice processors cold fill juice into bottles and heat in a hot water bath. This method is inefficient and has adverse effects on taste and colour. Plate or shell and tube heat exchangers followed by hot fill into bottles and aseptic sealing is the most common method used (Varnam & Sutherland 1994). The temperature time conditions are process and product specific.

Pasteurisation of citrus juice at 90°C for 10 s or 85° for 4 min denatures pectinase preventing cloud breakdown in fresh juice (Wilbey 2003a). Pasteurisation of apple juice at 89°C for 90 s destroys potential spoilage organisms and denatures polyphenols oxidase, the enzyme that causes browning (Wilbey 2003a).Tomato juice is processed at 115°C for 15 s (Wilbey 2003a, 2003b).

Thermal treatment of carrots prior to juice extraction has been found to be an important step in producing cloud stable juices. Acidification is also deemed to be essential when using blanched carrots. Clarification can only be prevented by acidifying the mash before juice extraction. Acidification after juice extraction will result in poor cloud stability (Reiter et al. 2003).

How a juice is packaged impacts on its shelf life, whether it is hot or cold filled, aseptically or not, with or without gas flushing, depending on the type of gas used and the packaging material. Protection from the environment, reduced exposure to light and oxygen all can maintain the quality of a juice.

2.2.2.5 New and emerging technologies

Today the food industry is driven by the consumer need for high quality, minimally processed, additive free, shelf stable, and convenient and safe food products (van Loey et al. 1998). To meet these needs existing thermal preservation processes are being improved (e.g. better process control) or adjusted (e.g. aseptic processing, Ohmic and microwave heating), or new physical food preservation methods are introduced such as pulsed electric fields (PEF) and high hydrostatic pressure processing (HPP) (van Loey et al. 1998).

Pulsed Electric Fields (PEF)

The Pulsed Electric Fields process exposes flowing liquid products to high electrical field pulses for very short time periods causing the cell membranes to develop temporary or permanent pores either by enlargement of existing pores or the formation of new ones. The pores increase the cell permeability, allowing loss of cell contents or intrusion of surrounding media, either of which result in cell death in microorganisms and when applied to fruit or vegetable cell walls, juice extraction from the cells is improved (Clark 2006; Walkling-Ribeiro et al. 2009).

The use of pulsed electric fields treatment in conjunction with juice extraction by traditional pressing has a number of benefits. Traditional pressing produces turbid juices requiring further clarification whereas including PEF treatment eliminates the introduction of enzymes and can be done at moderate temperatures without undesirable changes in pigments, vitamins and

flavouring agents (Praporscic et al. 2007). The PEF treatment gives also a possibility of microbiological and enzymatic inactivation of the released juice. Relevant investigations of the quantitative and qualitative characteristics and variation of expressed juice after PEF treatment is still lacking. The study by Praporscic et al (2007) was aimed at investigating of the effects of PEF treatment on kinetics of colour evolution and the dry matter content in juices expressed from cellular tissues from apples and carrots (Praporscic et al. 2007).

Gamma irradiation

Gamma irradiation is a non-thermal pasteurisation technology designed to reduce the deleterious effects that heat treatments have on flavour, colour and nutrient value of vegetable juices (Hyun-Pa et al. 2007). The nutritional and sensory evaluation carried out by Hyun-Pa et al (2007) indicated that irradiation at 3-5 kGy improved the microbial shelf stability of fresh carrot and kale juices, and prolonged their shelf life up to three days compared to the one day shelf life of the non-irradiated control.

High Pressure Processing

High pressure processing (HPP) uses the medium in which the products are held, in most cases water, to evenly and instantaneously transmit the pressure throughout the product irrespective of the size and shape of the product thus avoiding the long times to heat the core and uneven heating of particulate products commonly observed with thermal treatments (Huppertz 2007). HPP is a similar process to a batch retort except using high pressure (100-1000MPa, which equals 1000-10000 times atmospheric pressure), not high temperature (Huppertz 2007). Liquid products need to be pre-packaged in flexible packaging with a headspace less than 10% of the total pack volume (Huppertz 2007).

HPP is attractive as it inactivates food deteriorative enzymes and microorganisms but smaller molecules such as fats, salts, and many flavour and nutrient compounds, remain largely unaffected (van Loey et al. 1998; Huppertz 2007). There are considerable differences in the pressure resistance between the microorganism inactivation rates as well as the product matrix in which the microorganisms are pressure treated (Huppertz 2007). Generally yeasts

and moulds are pressure sensitive but vegetative bacterial cells require higher inactivation pressures and elevated temperatures. Bacterial spores have extreme pressure stability and require a combination of pressure with other, ideally synergistic preservation technology (such as elevated temperature or enzymes) for inactivation (van Loey et al. 1998).

Approximately 20% of the current industrial scale HPP units worldwide are used for juice and beverage products and another 20% are used for fruit and vegetable products. One of the successful fruit and vegetable applications is shelf stable guacamole. The high pressure is used to inactivate the polyphenoloxidases which cause enzymic browning in avocados. HPP is also used for orange juice, retaining the vitamins and flavour while inactivating the spoilage microorganisms and enzymes (pectin methylesterase) (Huppertz 2007).

2.2.2.6 Broccoli juice processing

HPP and Broccoli juice

Examples of HPP juices and beverages in the market are found at www.nchyperbaric.com . The most recent is a broccoli and apple juice from the Czech Republic in 2004 (Anon 2006). Juice (Houska et al. 2006) containing 20% broccoli was packed in PET bottles with indirect HPP used for pasteurisation achieving a 21 day shelf life retaining the sensory and anti-cancer properties of a fresh juice (Anon 2006; Huppertz 2007). The high relative cost of the HPP compared to standard pasteurisation technologies confines the technology to use for the preservation of nutritional substances such as sulforaphane in broccoli juice. Houska et al (2006) compared high pressure pasteurisation of broccoli juice for 10 min at 500 MPa with heat pasteurisation at 80 °C for 20 min and found that the acidification of broccoli juice with citrus juice from pH 6.5 to pH 4.2 for microbial safety also helped to increase the sulforaphane content. Freezing the broccoli before juicing was also found to significantly reduce the sulforaphane content in the juice as freezing was thought to disturb the transformation reaction of glucosinolates into the isothiocyanates or caused the breakdown or inactivation of the enzyme myrosinase necessary for the creation of sulforaphane (Houska et al. 2006). Broccoli juices with pulp were found to have higher concentrations of sulforaphane than the filtered juice with the effect of acidification on sulforaphane content only evident in juices with pulp (Houska et al. 2006).

Glucosinolates in broccoli juice

Cutting fresh plant tissues creates optimal conditions for hydrolysis of glucosinolates by myrosinase. In the case of pulping of plant tissues, the complete breakdown of glucosinolates by autolysis occurs (Jeffery et al. 2003; Moreno et al. 2006; Higdon et al. 2007). Cooking reduces glucosinolate levels by as much as 30-60% depending on the cooking method (e.g. conventional, microwave, high pressure), cooking intensity (e.g. temperature, time), and on the type of compound. Also thermal degradation and washing leads to large losses of intact glucosinolates (Bones & Rossiter 2006; Moreno et al. 2006; Higdon et al. 2007). Van Eylen et al (2007) studied the stability of myrosinase in broccoli juice during combined temperature and pressure treatments. It was found that the glucosinolate breakdown products, sulforaphane and phenylethyl isothiocyanates were temperature labile but pressure stable. A mild pressure treatment to stabilise myrosinase was found to be advantageous (van Eylen et al. 2007).

Effect of processing on broccoli juice composition and quality

Processing and preparation methods such as blanching, canning, sterilization and freezing, as well as domestic cooking and their effect on the nutritional properties of broccoli have been widely studied. Comparative vitamin C retention between broccoli florets and stem was assessed by Galgano et al (2007) after blanching, freezing and cooking. Like Jagdish et al (2007) and Podsedek (2007) they found cooking procedure had a direct influence on the retention of vitamin C. In particular the amount of cooking water influenced the level of water soluble components such as vitamin C present with boiling resulting in the greatest losses of vitamin C. The combination of pressure and microwave cooking did not affect the vitamin levels as much as steaming. Freezing was found to preserve vitamin C (Galgano et al. 2007). The level of vitamin C in broccoli decreases with mechanical breakdown of tissue (chopping) and heating and on a lesser extent during frozen storage (Podsedek 2007; Tosun & Sevinc 2008). The stability of vitamin C in fruit juices is enhanced by the presence of citrate and flavonoid compounds, but eliminating oxygen is very important. Both citrates and flavonoids complex with metal cations and hence reduce reactions with vitamin C (Coultate 2009).

2.3 Juice safety

Microbial growth in a food product can be prevented or minimised using a variety of different factors relating to the product and the environment it is processed and stored in. Many of these factors work in synergy such as pH and temperature. A pH reduction or increase (from the microorganism's optimum) can allow the product to be heat treated at lower temperatures while still resulting in the same level of microbial destruction.

2.3.1 Fruit and Vegetables

Raw fruit and vegetables are sources of microorganisms mainly derived from soil, water and air including saprophytes such as coryneforms, lactic acid bacteria, spore-formers, coliforms, micrococci and pseudomonas. Bacteria are the predominant micro organism with significant numbers of yeasts and moulds and a small number of fungi (Bari et al. 2005). The bacteria include many potential food borne pathogens such as: *Listeria monocytogenes, Salmonella* spp*, Staphlococcus botulinum, Shigella* spp*, Bacillius cereus* and *Staphlococcus aureus* (Bari et al. 2005). The enteric pathogens (*Escherichia coli* 0157:H7, *Listeria monocytogenes, Salmonella* spp*)* are of most concern as they have a low infectious dose or have the potential for growth in food prior to consumption even when stored in refrigerated conditions (Ukuku et al. 2005).

The microbial contamination of raw vegetables usually occurs on exposed surfaces while the internal tissues remain essentially free of microorganisms (Bari et al. 2005). The post harvest growth of fungi in subsurface tissue can alter the pH of plant tissues allowing pathogenic bacteria to grow where it usually would not in healthy vegetables or fruit (Bari et al. 2005). The presence of microorganisms in a vegetable product reflects the effectiveness of anti-microbial treatments at any step from planting, processing through to consumption (Jay 1996; Ukuku et al. 2005). Ukuku et al (2005) recommend up to 200-300ppm chlorine as a wash water sanitiser, depending on whether the vegetable is whole or cut.

2.3.2 Microbial control factors

Microbial growth can be influenced by factors that are characteristic to the product (intrinsic) and / or from factors in the surrounding environment (extrinsic). These factors are detailed in Table 2.2. Extrinsic factors affect both the product and the microorganisms (Jay 1996; Bari et al. 2005).

Table 2.2 Intrinsic and Extrinsic microbial growth factors (Jay 1996; Bari et al. 2005).

Intrinsic factors	Extrinsic factors
• Moisture content and water activity (a_w)	• Temperature of storage
• pH and acidity	• Relative humidity of the environment
• Nutrients (water, energy source, nitrogen, vitamins and minerals)	• Presence and concentration of gases: carbon dioxide (CO_2), ozone (O_3) and oxygen (O_2) are toxic to certain microorganisms
• Biological structure: structure of the microorganism and physical barriers of the plant e.g. skin	• Modified atmosphere packaging (MAP): the use of CO_2, Nitrogen (N_2) and ethanol to change the environment in a package
• Oxidation-Reduction (Redox) potential; a measure of the ease by which a substance gains or losses electrons	• The presence and activities of other microorganisms
• Naturally-occurring antimicrobials	
• Competitive microflora	

The ability of microorganisms, including pathogens, to grow depends greatly on the combinations of intrinsic and extrinsic factors that are naturally present or imposed at any stage of the life of a plant product from growing, harvesting, production, processing, distribution and preparation at the site of consumption (Jay 1996; Bari et al. 2005). Hurdle technology uses combinations of multiple intrinsic and extrinsic factors as “barriers” against the growth of microorganisms in a product (Jay 1996). Many of the factors have synergistic interactions. For example the pH of the product significantly affects the lethality of heat treatment. As the pH is reduced or increased from the pH required for optimum growth of microorganisms (generally pH 7), then less heat is required to inactivate the microorganisms (Jay 1996; Bari et al. 2005).

2.3.2.1 Heat treatments for microorganisms

The use of high temperatures (all temperatures above ambient) to preserve food is based on the destructive effect it has on microorganisms. Food products can be either sterilised or pasteurised. Sterilisation is the destruction of all viable organisms in a food product. Pasteurisation by heat implies the destruction of all disease-producing organisms (in milk) or the destruction of spoilage organisms in other food products.

Pasteurisation by heat results in the destruction of spoilage organisms in food products (Jay 1996). The pH of milk (pH 6.7) is similar to the pH of neutral broccoli juice. The most common milk pasteurisation method is the high temperature short time (HTST) process, 72°C for 15 sec. The alternative temperature-time combinations for milk pasteurisation could include 89°C / 1.0 sec, 90°C / 0.5 sec, 94°C, 0.1 sec or 100°C / 0.01 sec (Jay 1996). Heat treatments to eliminate yeasts and lactobacilli are more severe than for elimination of vegetative pathogens. In citrus juices heat treatment at 70°C for 60 sec or 85°C for 30 sec is used to eliminate yeasts (Wilbey 2003a). Fruit juices generally have a pH<4.5, so growth of pathogenic bacteria will not be supported, but some survive for some time. Yeasts and lactobacilli may grow and cause juice spoilage. Moulds may grow on the surface (Wilbey 2003a).

2.3.2.2 Effect of freezing on microorganisms

For multiple reasons, freezing cannot be solely relied on as a method to destroy food borne microorganisms for food preservation. These include the type, state and strain of microorganism, the type of freezing used, the nature and composition of the food, the length of time of freezer storage, and the freezing temperature (Jay 1996). More microorganisms are destroyed at -4°C than at -15°C with temperatures below -24°C having no additional deteriorative effect (Jay 1996). Furthermore Jay (1996) suggests that it is the time temperature pattern characteristic of thawing that is potentially more detrimental than freezing. During thawing, the temperature rises rapidly to near the melting point and remains there throughout the long thawing period. This provides considerable opportunity for chemical reactions, re-crystallisation, and even microbial growth if thawing is extremely slow (Jay 1996).

2.4 Juice visual quality

2.4.1 Colour measurement of beverages

Three components are required to see colour: light source, object and observer (Leggett 2008; Schmehling 2008). The CIE (Commission Internationale de l'Eclairage) scale offers a well defined system for the colour measurement of opaque and transparent materials. It quantifies colour as a person perceives it. Human perception requires a source of white light, an object that modifies the light and a person who perceives colour from the object stimuli (Leggett 2008; Schmehling 2008). CIE colour values are calculated using a mathematical model based on a white light source, object and human observer that represent all colours in terms of L* lightness, a* redness-greenness and b* blueness-yellowness, Chroma colour intensity and hue angle colour are also measured (Leggett 2008). The CIE L*a*b* and CIE L*C*h systems are designed to quantify the colour of an object as it is perceived by a person in a specific light environment (Leggett 2008). The changes in the values over time are calculated as differences from the time zero results. They are presented as ΔL*, Δa*, Δb* and ΔC* and Δh values. The total colour difference (ΔE*) is also calculated from ΔL*, Δa*and Δb* (Shellhammer & Bamforth 2008).

2.4.1.1 Light source

Colour perception can change dramatically based on the light source. The way each light source renders colour is based on the energy present in the light source at each wavelength (Schmehling 2008). The illuminant is represented by a standardized set of numbers called the spectral power distribution (SPD) (Leggett 2008; Schmehling 2008). The SPD for daylight with a colour temperature of 6500 Kelvin (D65), indicates more evenly balanced amounts of spectral energy (red, orange, yellow, green, blue, indigo and violet – commonly referred to as ROYGBIV) and is often the primary light source under which products are evaluated (Schmehling 2008). Cool white fluorescent has a SPD that spikes in the blue and green regions of the spectrum. This excess of blue and green energy tends to dull the appearance of red and is typically used as a secondary or tertiary light source to simulate colour rendering in a retail environment (Schmehling 2008).

2.4.1.2 Observer

A person is defined as a standard set of numbers called the CIE standard observer (Leggett 2008). Observers can have perceptual differences or physiological conditions that can affect an observer's accuracy or discrimination of colour (Schmehling 2008).

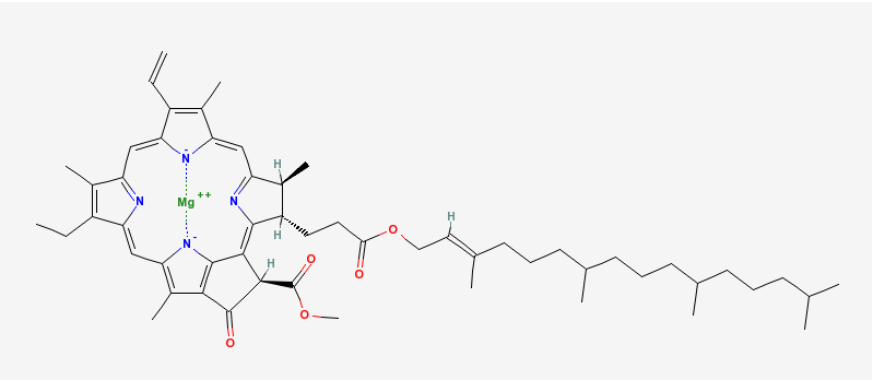
2.4.1.3 Object

Food products range from transparent to opaque. Products within this range are classed as translucent or light trapping. In all cases the product measurement will be quantified as a reflectance or transmission spectrum (Leggett 2008). The use of physical samples and instrumental measurements such as a spectrophotometer are often used separately or in conjunction with each other (Schmehling 2008). The cell path length is selected based on the chroma of the sample. The more chromatic the sample is, the shorter the path length of the cell (Leggett 2008). The scattering of light due to non-soluble particles in the liquid sample is a separate optical phenomenon from the absorbance of the colorant that causes the perception of colour, yet can affect the appearance of the food product. The hemispherical collector of a sphere instrument collects both regular and slightly scattered signal, negating the effects of any minor scattering inherent even in clear samples (Leggett 2008).

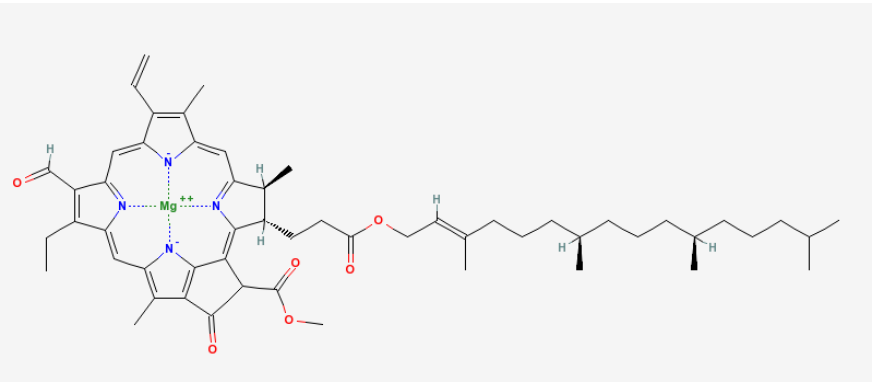
2.4.2 Chlorophylls

Chlorophylls are the major green pigments associated with photosynthesis in green plants, algae and photosynthetic bacteria and also contribute to the green colour of vegetables such as broccoli (Fennema 1996; Weemaes et al. 1999b; Lamikanra et al. 2005; Coultate 2009). In vegetables chlorophylls *a* and *b* (Figure 2.5) are found in the chloroplasts. The chlorophyll molecules interact with each other, as well as the proteins and lipids (Fennema 1996; Lamikanra et al. 2005). Chlorophylls are derived from the basic structure of porphin, which is a fully unsaturated macro cyclic structure containing four pyrrole rings linked by single bridging carbons. A porphyrin is a substituted porphin. Chlorophyll is considered a porphyrin with a

centrally located Mg^{2+} ion. The chlorophyll molecule is hydrophobic (Fennema 1996; Lamikanra et al. 2005; Coultate 2009).



(a)



(b)

Figure 2.5 Structure of (a) chlorophyll *a*, (b) chlorophyll *b* (Anon 2009d).

The isomer chlorophyll *a* contains a methyl group in position 3. The chlorophyll *b* isomer has an aldehyde group in position 3. Isomers of chlorophyll *a* and *b* are formed by the inversion of the carbomethoxy group at C-10 in products that are heat treated (Fennema 1996; Lamikanra et al. 2005).

Chlorophyll *a* and *b* are typically present in a ratio of 3:1 with chlorophyll *a* degrading faster than chlorophyll *b* (Fennema 1996; Weemaes et al. 1999b; Lamikanra et al. 2005; Turkmen et al. 2006; Coultate 2009). The initial degradation products are pheophytin and pheophorbide.

Heat treatment, storage or freezing produces the most common chlorophyll degradation resulting in the production of the olive-brown pheophytins. Chlorophyll in heated vegetable tissue is stable at pH 9.0, but unstable at acidic pH 3.0 (Fennema 1996). The olive brown pheophytin is formed by magnesium ions being displaced by two hydrogen ions. This reaction

is irreversible in aqueous solutions. Pheophytins can be further changed to pyropheophytin by replacing the C-10 carbomethoxy group with a hydrogen atom (Fennema 1996; Weemaes et al. 1999b; Lamikanra et al. 2005; Turkmen et al. 2006).

The effect of temperature on chlorophyll and colour in broccoli juice was investigated by Weemaes et al (1999b). Chlorophyll *b* is more heat stable than chlorophyll a (Fennema 1996; Weemaes et al. 1999b; Turkmen et al. 2006). Colour measurements (L, *a** and *b*)* of the heated broccoli juice were taken using a spectrophotometer. Weemaes et al (1999b) concluded that the loss of greenness after heating was due to the degradation of both chlorophyll *a* and *b* to pheophytin and other degradation products shown by a large decrease in the –a* colour value. The lightness also greatly decreased and the b* value had a minor decrease with heat (Weemaes et al. 1999b).

In its natural form chlorophyll is protected from photo degradation by its surrounding carotenoids and lipids but once the protective cells are ruptured chlorophyll is irreversibly bleached by light and oxygen exposure (Fennema 1996). The visible spectra of chlorophyll *a* and *b* and their derivatives are characterised by sharp light absorption bands between 600 and 700 nm (red regions) and between 400 and 500 nm (blue regions) (Fennema 1996).

2.4.3 Carotenoids

Carotenoids also contribute to the colour of vegetables, are present in the chloroplasts of green leafy vegetables, but are often masked by the more dominant chlorophyll pigments. The largest concentrations of carotenoids are found in the photosynthesis tissues with large amounts of chlorophyll pigments (Fennema 1996; Lister & Bradstock 2003; Coultate 2009; Farnham & Kopsell 2009).

Carotenoids (carotenes and xanthophylls) range in colour from red to yellow. They can be measured in the visible light spectrum between 430 to 480 nm (Fennema 1996; Podsedek 2007). Carotenoids are antioxidants due to their structure of conjugated double bonds which are radical scavengers and quenchers of singlet oxygen. Several carotenoids are precursors to vitamin A. Lutein and β-carotene are the dominant carotenoids in green brassica vegetables (Figure 2.6), with broccoli having total carotenoid levels of 1.6mg/100g (Podsedek 2007).

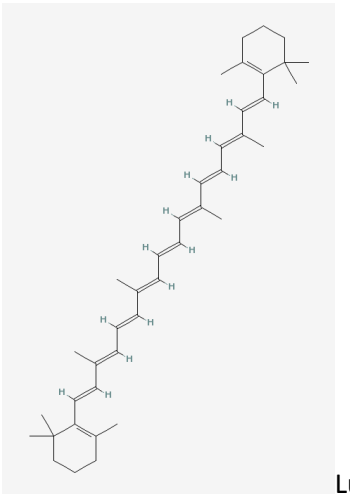
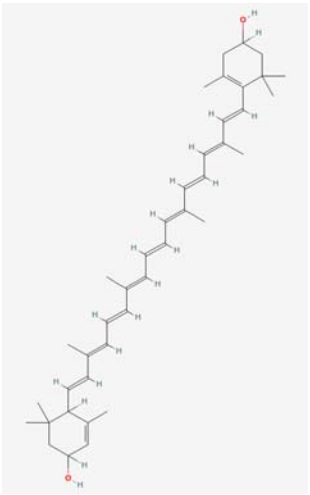
β-Carotene  Lutein 

Figure 2.6 Structure of carotenoids β-Carotene and lutein (Anon 2009d).

Carotenoids are soluble in lipids and organic solvents (Lister & Bradstock 2003; Podsedek 2007). Isomers of carotenoids are created by heat, light or acid. Carotenoids are relatively stable during post harvest storage in cool dark places, and freezing (Lister & Bradstock 2003). Carotenoids become more bio available after cooking (Fennema 1996; Lister & Bradstock 2003). Cooking broccoli significantly increases the lutein and beta-carotene content. Steaming is preferable to boiling as this minimizes leaching of water soluble antioxidants in to the cooking water (Lister & Bradstock 2003; Coultate 2009). Heating carotenoids in the absence of air isomerises some of the *trans* double bonds into *cis* double bonds, sometimes called neocarotenes. The colour intensity decreases with increasing *cis* double bonds (Podsedek 2007; Coultate 2009).

2.5 Juice flavour perception

The flavour of a product is an important quality attribute and linked to whether it is accepted or rejected by a consumer (Cadwallader 2005). Flavour is the integrated perception of aroma (odour) and taste, and to a lesser extent pain or nerve response (e.g. heat of capsaicin), texture and mouth feel and overall appearance (Cadwallader 2005).

Fruit and vegetable flavours are composed of a wide range of chemical compounds from non-volatile taste-active (including both inorganic and organic compounds) to volatile aroma-active organic molecules. Most often it is the aroma components that are the predominant contributors to the distinct flavour of fruit or vegetables (Cadwallader 2005).

Some non-volatile compounds such as sugars and organic acids impart sweet and sour tastes respectively. The percentage of soluble solids (°Brix) and °Brix to acid ratio are often used as indices of ripeness and flavour quality (Cadwallader 2005).

The sensory profile of raw and cooked broccoli has been extensively studied. Fresh cut grass (Kim et al. 1999; Jacobsson et al. 2004) and cooked cabbage (Jacobsson et al. 2004) were identified as aromas associated with broccoli. Sweetness and bitterness (Kim et al. 1999; Baik et al. 2003; Schonhof et al. 2004), grassy/fresh flavour of grass, sourness and summer (Jacobsson et al. 2004; Schonhof et al. 2004) were the most common attributes used for broccoli flavour. Pungent, spicy, kohlrabi, cauliflower, leek, spicy, broccoli like, green like fresh pea pod were also used (Schonhof et al. 2004) as was vegetable juice flavour (Kim et al. 1999) and raw potato earthy and off flavour (Baik et al. 2003). Cooked broccoli flavour was identified as methanethiol (Baik et al. 2003; Cadwallader 2005) and the cooked cabbage flavour as dimethylsulfide (Baik et al. 2003; Jacobsson et al. 2004; Schonhof et al. 2004; Cadwallader 2005). The astringent aftertaste (Kim et al. 1999) and burn sensation similar to horseradish (Baik et al. 2003) were identified as mouthfeel attributes associated with broccoli.

Glucosinolate content has been linked to influencing the broccoli flavour and consumer acceptance (Baik et al. 2003; Sato et al. 2003; Moreno et al. 2006; Higdon et al. 2007; van Eylen et al. 2007). The glucosinolates are sulphur-containing compounds that are responsible for the pungent and ‘discomfort ‘ aromas and spicy, bitter taste of raw broccoli (Baik et al. 2003; Sato et al. 2003; Cadwallader 2005; Higdon et al. 2007). The intensity of the bitterness and pungency identified in raw broccoli reduced once the enzyme myrosinase is inactivated

(Baik et al. 2003; Sato et al. 2003; Cadwallader 2005). Research by Baik et al (2003) concluded that Broccoli does contain sulphur containing amino acids identified in previous studies that contribute to heat induced flavours, but there was little evidence that glucosinolates are directly responsible for the distinctive flavour of cooked broccoli. However as mentioned previously Sato et al (2003) have patented a method to prevent the discomfort aroma from occurring in processed brassicaceous vegetable juices using blanching prior to chopping and anion exchange treatments with organic acids.

2.6 Summary

Broccoli is a significant source of essential vitamins, minerals and dietary components in the diet with processing impacting and in most cases reducing their levels and availability. The processing conditions are essential to achieving acceptable levels of nutrients in a processed juice.

Chlorophyll degradation in broccoli juice is well defined on lab scale and heat treatments (Weemaes et al. 1999b; Moreno et al. 2006; Galgano et al. 2007). The impact of high pressure processing on an acidified beverage containing broccoli juice has been investigated in large volumes (van Loey et al. 1998; Weemaes et al. 1999a; van Eylen et al. 2007).

Methods for the reduction of the broccoli odour using blanching prior to cutting and anion exchange treatments using organic acids has been patented (Sato et al. 2003). Sensory profiling of raw and cooked broccoli and broccoli juice has been extensively investigated (Kim et al. 1999; Jacobsson et al. 2004; Cadwallader 2005).

The current research that follows will contribute to an overview of factors that impact on the processing of broccoli juice for sale in a retail environment, not for immediate consumption. Broccoli juice will be made in a large semi commercial volume using basic juice semi industrial processing techniques and heat treatments followed by a shelf life study simulating retail refrigerated storage conditions comparing the effect of acidification and light on broccoli juice. Both biochemical and sensory analyses will be investigated.

CHAPTER 3

Materials and Methods

3.1 Juice Production

All juice was produced in the Institute of Food, Nutrition and Human Health (IFNHH) pilot plant, Massey University, Palmerston North. The details of the equipment used in the production processes are found in Table 3.1.

Table 3.1 Resources used for juice production.

Resource	**Model and Manufacturer**	**City, Country**
Wash water sanitiser	Tsunami 100, Ecolab	Minnesota, USA
Mincer	Meat Master Junior ML16, Berry's Ltd	Tauranga, New Zealand
Water press	Misurina 20 Litre Idropresse, Enotecnica Pillan	Vicenza, Italy
Pilot plant pasteuriser	Alpha Laval	Lund, Sweden
Laminar flow workstation	AC1100, Air Care Technologies Ltd	Cambridge, New Zealand
Clear glass bottles	300 ml condiment bottle with gold twist cap, supplied by Arthur Holmes Limited	Wellington, New Zealand
Retail display refrigerator	SCC-300RAX Refrigerated Showcase, Sanden Intercool	Singburi, Thailand
Fluorescent tube used in the retail fridge	TLD 30W / 865 cool daylight, Phillips Tube	Thailand
Fluorescent tubes used in the storage cold room	36 W / 865 daylight, Spectra Lux Plus, Radium 58 W / 865 cool daylight (5600 Kelvin), Spectra Lux Plus, Radium	Netherlands
Light meter	LI-250 with a LI-210SA photometric sensor, LI-COR	Nebraska, USA

3.1.1 Methods for Preliminary Experiments

Two preliminary experiments were under taken in order to determine the optimal juice acidification and pasteurisation parameters.

3.1.1.1 Acidification

Two different acids at five concentrations were used to acidify raw broccoli juice. The treatments were compared for their effectiveness in reducing pH and minimizing the colour change in the raw broccoli juice. Ascorbic acid was chosen as it is used commercially as an antioxidant and/or vitamin additive. Citric acid is a commonly used commercial acidity regulator.

The juices for preliminary acidification experiment were made according to the following process:

- Broccoli (*Brassica oleracea* var*. italica* cultivar *Liberty*) from Kapiti Green Limited (Levin, New Zealand) was washed, separated into 5 cm pieces of floret or stalk, blanched separately in water for 1 min at 100 ± 2°C and chilled in ice water. Separate blanching ensured consistent heating due to similar particle sizes and shapes. Drained florets and stalks were vacuum packed separately and frozen for preliminary experiments.
- Frozen bags of broccoli florets and stalks were defrosted and separately pulped using a mincer with a fine pore (2 mm) screen, then pressed with a water press. The floret juice (6.8 L) and stalk juice (4.5 L) were filtered through a 150 µm mesh and combined.
- The juice was acidified according to the experimental plan (Table 3.2) using variable amounts of either ascorbic acid or citric acid (BDH Laboratory Supplies, Poole, England) as a percentage of final weight. Acids were dissolved in water for effective dispersion. The juice was stored refrigerated at 4 ± 2°C

Three replicates of the 12 treatments (36 samples in total) were measured daily for three consecutive days and the results averaged for pH (Section 3.3.2.2), soluble solids (Section 3.3.2.4), titratable acidity (Section 3.3.2.3), and colour (Section 3.3.2.6).

Table 3.2 Experimental plan for acidification per 100 g of juice of raw broccoli juice.

Treatment	Acid type	Acid (g)	Water (g)	Broccoli juice (g)
1	Ascorbic	0	10	90
2	Ascorbic	1	9	90
3	Ascorbic	2.5	7.5	90
4	Ascorbic	5	5	90
5	Ascorbic	7.5	2.5	90
6	Ascorbic	10	0	90
7	Citric	0	10	90
8	Citric	1	9	90
9	Citric	2.5	7.5	90
10	Citric	5	5	90
11	Citric	7.5	2.5	90
12	Citric	10	0	90

3.1.1.2 Pasteurisation

The optimal conditions for pasteurisation were investigated using three different temperature and time combinations. These were evaluated using neutral and acidified broccoli juice to determine the optimal heat treatment according to the impact on colour, chemical properties, microbial safety and flavour.

The juice required for the pasteurisation experiment was made as follows:

- Whole broccoli (50 kg) (local retail vegetable supplier) was washed, chopped into 5 cm pieces then blanched as florets and stalks in water for 1 min at 100°C and chilled in ice water.
- Blanched broccoli was pulped using a mincer with a fine pore (2 mm) screen and the juice extracted with a water press. The raw broccoli juice (20 L) was filtered through a 150 µm mesh. Half of the juice was acidified to pH 4.0 with citric acid.

- The pH of the remaining "neutral" juice was pH 6.5 ± 0.3. Each juice was divided into duplicates and these were each pasteurised for 15 sec at three different temperatures (72 ± 1°C, 85 ± 1°C and 95 ± 1°C) according to the experimental plan in Table 3.3. The pasteurised juice was poured into sterile plastic 120 ml containers in a laminar flow unit.

Each treatment was analysed for pH (Section 3.3.2.2), soluble solids (Section 3.3.2.4), titratable acidity (Section 3.3.2.3), colour (Section 3.3.2.6), microbiological analysis (Section 3.3.1) and flavour. The flavour was assessed by four Plant and Food staff in an informal tasting.

Table 3.3 Experimental plan for heat treatment optimisation of neutral and acidified broccoli juice pasteurised for 15 sec.

Treatment	**Duplicate**	**Acidity**	**Temp °C**
1	-	Raw neutral	-
2	-	Raw acid	-
3	A	Neutral	72
4	B	Neutral	72
5	C	Acid	72
6	D	Acid	72
7	A	Neutral	85
8	B	Neutral	85
9	C	Acid	85
10	D	Acid	85
11	A	Neutral	95
12	B	Neutral	95
13	C	Acid	95
14	D	Acid	95

3.1.2 Final shelf life trial

3.1.2.1 Juice ingredients

Broccoli (*Brassica oleracea* var. *italica* cultivar *Ruben*) was used for the shelf life trial juice and the juice for sensory training. The 600 kg required for both the production runs was supplied by Kapiti Green Limited (Levin, New Zealand) from the same planting, but harvesting one week apart. The broccoli was harvested and chilled to 2 ± 1°C, transported overnight, stored covered at 2 ± 1°C (Jones et al. 2006) and processed within 72 h. Broccoli for pasteurisation experiments was sourced from local retail vegetable suppliers.

Citric acid for lowering the pH of the broccoli juice (pH 6.5 to pH 4.0) was prepared as a 10 % w/w solution using anhydrous citric acid (BDH Laboratory Supplies, Poole, England) and tap water.

3.1.2.2 Process flow diagram

The production process used for the shelf life trial and sensory training broccoli juice is shown in Figure 3.1.

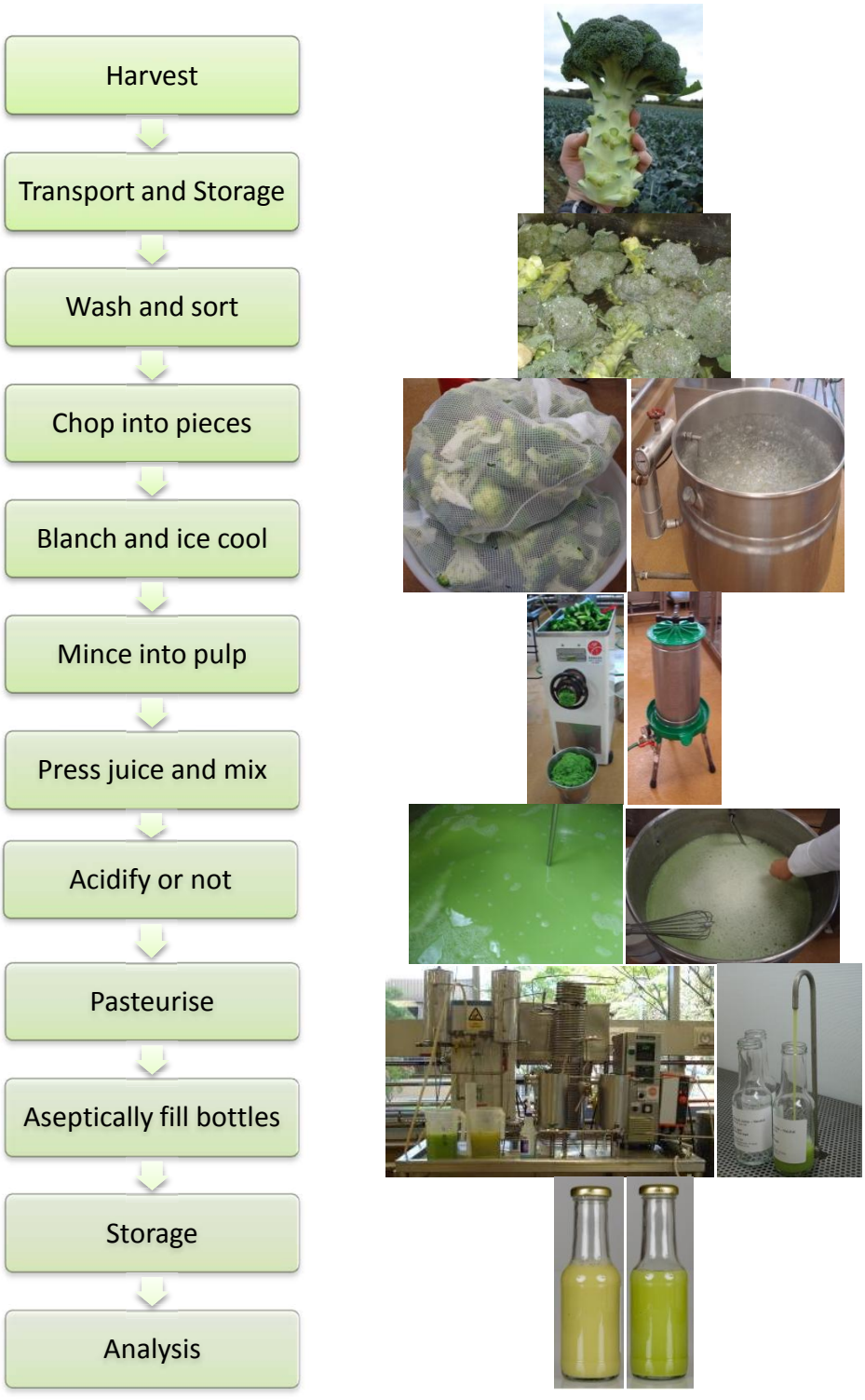


Figure 3.1 Process flow diagram and photos for the production of broccoli juice.

3.1.2.3 Shelf life trial juice

Fresh broccoli (400 kg) was juiced using the process described in Figure 3.1 and the equipment detailed in Table 3.1 to produce broccoli juice (161 L) for the shelf life study.

Broccoli (400 kg) was washed in water with 200 ppm peroxyacetic acid sanitiser (Table 3.1) to reduce the microbial load. Broccoli florets and stalks were chopped into 5 cm pieces, blanched in water for 1 min at 100 ± 2°C in a jacketed pan to reduce peroxidase activity (Murcia et al. 2000), chilled in ice water, drained and stored in closed buckets in a 4 ± 1°C chiller until required.

Blanched broccoli pieces were mashed using a mincer with a fine pore size (2 mm) screen and the juice extracted with a water press. The water press was lined with organza cloth to prevent fines from being pressed out. The raw juice was collected in buckets and the pressed pomace was weighed and discarded. The raw broccoli juice (20 L) was filtered through a 150 µm mesh and all extracts combined to ensure homogeneity. Half of the juice was acidified to pH 4.0 with a 10 % citric acid solution and the remaining juice was unmodified neutral broccoli juice (pH 6.5). The acidification of the broccoli juice increased the soluble solids of the raw broccoli juice from 5.1 ±0.0 °Brix to 5.3 ±0.1 °Brix in the raw acidified juice due to the soluble solids added as citric acid.

The neutral broccoli juice was pasteurised at 95°C for 15 sec and the acidified broccoli juice was pasteurised at 85°C for 15 sec as determined by the preliminary pasteurisation experiment. All the broccoli juice was pasteurised using the Massey University pilot plant pasteuriser, cooled to 4 ± 1°C and 250 ml of juice was aseptically dispensed into each glass bottle in the laminar flow workstation.

Each bottle was assigned to a neutral or acid juice then to a light or dark treatment and a storage time according to the experimental design and sampling requirements detailed in Section 3.2. Each bottle was labelled with its designation and filled with juice in a randomised order.

3.2 Shelf life trial conditions and experimental design

3.2.1 Experimental design

A full balanced factorial experimental design (Figure 3.2) was used to analyse the effects of acidity and light on broccoli juice over an eight week period in simulated retail refrigerated storage conditions.

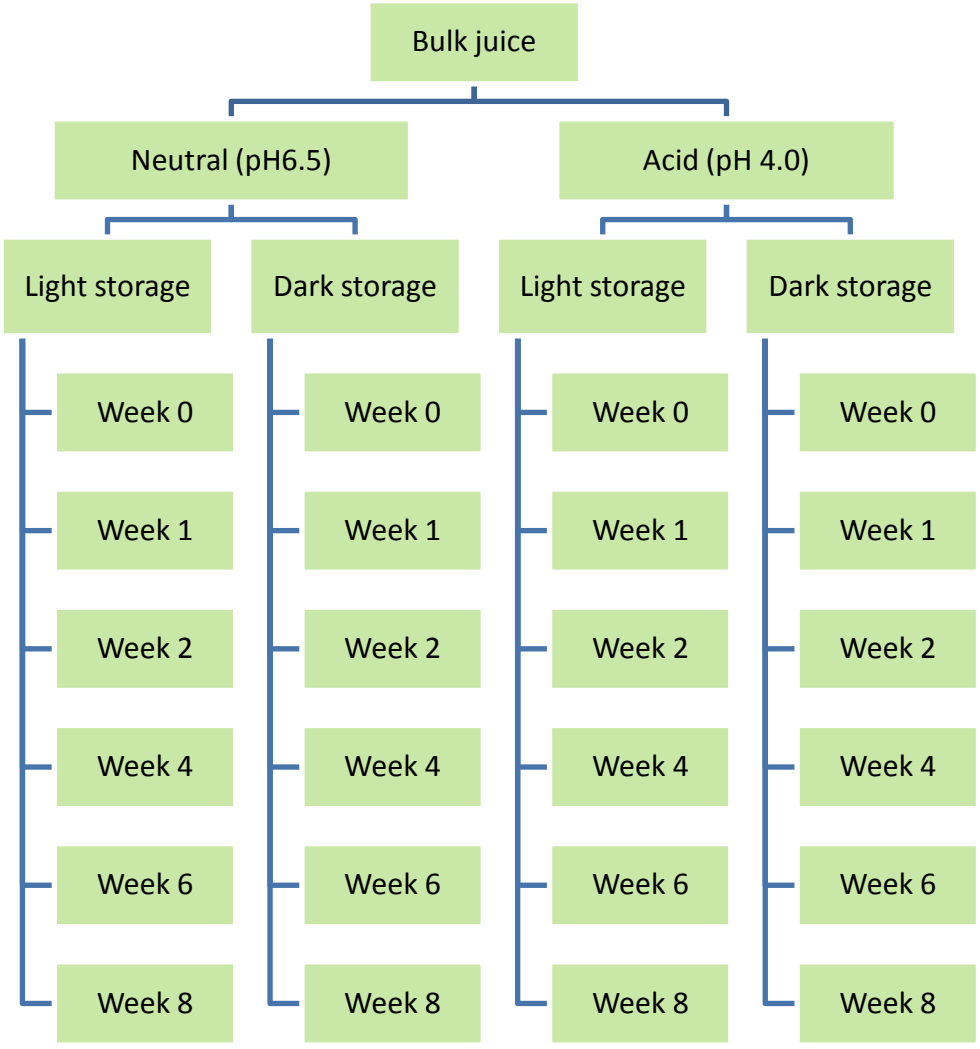


Figure 3.2 Shelf life trial full balanced factorial experimental design for refrigerated (4±1°C) storage time in retail lighting conditions. After set time periods juice was frozen at -20±2°C until the experiment was concluded and analyses commenced.

3.2.2 Storage conditions

Broccoli juice for light storage was stored in clear glass bottles with metal twist top caps (Table 3.3). The same clear glass bottles with metal twist top caps were used for dark storage treatments but they were completely covered in aluminium foil to exclude light.

For the shelf life trial a 4 ± 1°C cool room (10.5 m^3) was modified with additional lighting to simulate the storage conditions bottled juice would be exposed to in a retail display fridge (Table 3.1). All the bottles of juice were stored in open cardboard trays the same distance from the light source (435 Lux). The lights in the cool room were constantly on as is the case in the retail environment.

After each time period in Figure 3.2 bottles were removed from refrigerated storage for sub-sampling and/or immediate analysis and then frozen at -20 ± 2°C in closed cardboard boxes to exclude light until required for further analysis. The sub-sampled juice from the dark treatments was continuously protected from light in amber coloured tubes or with aluminium foil.

3.2.3 Storage time

Biochemical samples were assessed at 0, 1, 2, 4, 6, 8 weeks of refrigerated storage. Samples for sensory and microbiological testing were assessed at 0, 1, 2, 3, 5, 7 weeks of refrigerated storage due to time limitations on obtaining microbiological test results prior to sensory assessments to assure product safety.

3.2.4 Juice sample randomisation

The shelf life trial consisted of 24 treatments (Figure 3.2). A total of 492 bottles of juice were required for the microbiological, biochemical and sensory analyses. The allocation of bottles according to analyses (Table 3.4) was as follows:

- Microbiological analysis required one bottle per treatment per sampling time plus an additional two bottles for Week 0.
- Six bottles per treatment for biochemical analysis were sampled as sets of two bottles at the beginning, middle and end of the production run.
- Sensory analysis required four bottles per treatment per sampling time with two replicates (4 x 2).
- The sensory reference standards were Week 0 samples. Four bottles were required for each of the four assessment panels (4 x 4) with two replicates (x 2).

Table 3.4 Description of juice required for analysis for each type.

Analysis	**Bottles / treatment/sampling**	**Bottles / trial**
Microbiological	1	24 (+ 2 extra week 0)
Biochemical	6	144
Sensory assessment	8	192
Sensory references	-	64
Spare sensory samples	-	64
Total number of bottles required		492

All the bottles required for the neutral juice and acidified juices were labelled in the order in which they were to be filled. The randomised filling order is detailed in Appendix 1.

3.2.5 Juice sampling for analysis (microbiological, sensory and biochemical)

At each time period in Figure 3.2 bottles were removed from the refrigerated trial and treated according to their pre-labelled designation.

- Microbiological samples were tested at weeks 0, 1, 2, 3, 5 and 7 to allow time for the receipt of test results prior to sensory analysis. The microbiological analysis bottles were not sub-sampled to reduce contamination of samples, were maintained at 4 ± 1°C and sent for immediate analysis.
- Bottles labelled for sensory assessment were frozen at -20 ± 2°C in closed cardboard boxes for assessment at the end of the trial. All sensory assessment was done at the same time at the end of the storage trial.
- Each bottle for biochemical analysis was mixed and divided into five 45 ml sub-samples. Four of the sub-samples were frozen immediately for future analyses.
- The remaining biochemical sub-sample was analysed immediately at room temperature for soluble solids (Section 3.3.2.4) and colour analysis (Section 3.3.2.6). The sub-sample was then frozen and subsequently used for pH (Section 3.3.2.2) and titratable acidity (Section 3.3.2.3) measurements.
- Chlorophyll (Section 3.3.2.7), pectin, (Section 3.3.2.8) vitamin C (Section3.3.2.10) and dry matter (Section 3.3.2.5) were all measured from the same frozen sub sample. The resulting dry matter was used for determination of total dietary fibre (Section 3.3.2.9).

Three bottles were tested for biochemical analyses from each treatment (1 bottle each from the beginning, middle and end of the process run). The results for each treatment are the average of the three bottles. Depending on the analytical method replication of the test method was also conducted.

3.3 Juice analysis

3.3.1 Microbiological quality

Broccoli juice bottles labelled as microbiological samples were tested at the School of Engineering and Advanced Technology microbiology laboratory, Massey University, Palmerston North. The test methods used were followed from the 141.393 Food Microbiology & Safety Lab Instruction Manual (Flint & Bajaj 2008). The tests used were:

- Total aerobic plate count (cfu/ml)
- Yeasts and moulds (cfu/ml)
- The most probable number (MPN) method for the estimation of total and faecal coliforms and *Escherichia coli (E.coli)*

NB: The samples from the preliminary pasteurisation experiment were only tested for total aerobic plate count and yeast and moulds.

The Microbiological Reference Criteria for Food, (Section 5.8 Foods – cooked, ready to eat (or with subsequent minimal heating <70°C), part a), where all components are cooked in manufacturing process) and Section 5.21 (Packaged waters (including mineral waters and those bottles from natural underground sources)) were referred to for maximum limits of the chosen microbial tests. No limits could be found for yeasts and moulds as they are spoilage organisms and do not pose a food safety concern. (New Zealand Food Safety Authority 1995)

Recommended limits (New Zealand Food Safety Authority 1995).

- Aerobic plate count at 35°C 10^5 cfu/g
- Coliform 10^2 cfu/100ml
- *Escherichia coli* 0 cfu detected

3.3.2 Biochemical assays

3.3.2.1 Experimental Materials

The equipment used in the biochemical analyses is detailed in Table 3.5. All the chemicals, reagents and enzymes used in the biochemical analyses are detailed in Table 3.6.

Table 3.5 Equipment used in biochemical assays.

Instrument	**Model**	**Manufacturer, city, country**
Auto titrator	DL21 Titrator	Mettler Toledo, Melbourne, Australia
pH meter with refillable combination electrode	CG837 and N1042A	Schott Instruments, Mainz, Germany
Handheld refractometer	Pocket Pal-1	Atago, Tokyo, Japan
Freeze drier	Freezone 18	Labconco, Kansas City, USA
Handheld spectrophotometer with liquid cell holder attachment	CM-2600d and 7600-0000-1720	Konica Minolta Sensing Inc, Tokyo, Japan
Bench top centrifuge (fixed rotor)	Model 5417R	Eppendorf, Hamburg, Germany
Centrifuge (swing out rotor)	Model 4K15C	Sigma Laboratory Centrifuges, Steinheim, Germany
Plate reader	Spectra Max Plus384	Molecular Devices Pty Ltd, California, USA
96-well flat-bottom micro plates	Nunc®	Thermo Fisher Scientific, Roskilde, Denmark

Table 3.6 Enzymes, chemicals and reagents used in biochemical assays.

Enzymes	**Details**	**Supplier**
α-Amylase	Heat stable; 3,000 Ceralpha Units/ml	Total Dietary Fibre Kit, K-TDFR 01/05, Megazyme International Ireland Limited, Wicklow Ireland
Protease solution	350 Tyrosine Units/ml	
Amyloglucosidase solution	200 ρ–NP β-maltoside Units/ml	

Chemical	**Grade**	**Brand or Supplier**
Ethanol (ethyl alcohol)	99%	Anchor Grade, Polychem Marketing Ltd, NZ
L-Ascorbic acid	AnalaR	BDH Laboratory Supplies, Poole England
Citric acid	AnalaR	
Formic acid	AnalaR	
Potassium hydrogen phthalate	AnalaR	
Acetone	GR	Merck, Damstadt, Germany
Di-sodium tetraborate decahydrate	GR	
Methanol (methyl alcohol)	GR	
Hydrochloric acid	GR	
Sodium hydroxide	GR	
Sulphuric acid	GR	
Acetonitrile	LiChrosolv®	
Potassium acetate	Extra pure	
Galacturonic acid	Biochemika	Fluka, Sigma-Aldrich, Steinheim, Germany
MES (monohydrate (2-morpholinoethanesulfonic acid monohydrate))	Biochemika	
3-phenylphenol	Reagent	Sigma-Aldrich, Steinheim, Germany
TRIS - Trizma® Base (Tris[hydroxymethyl]aminomethane)	Reagent	
DL-Dithiothreitol (DTT)	Reagent	

Reagents	**Chemical**
MES/TRIS buffer 0.05 M, (x10 stock solution)for dietary fibre assay	53.312 g of (MES) and 39.4 g (TRIS) made up to 500 ml with water, with the pH adjusted to 8.2 at 24°C with 6 N NaOH.
Sulphuric acid/borax reagent for soluble pectin assay	4.767 g Di-sodium tetraborate dissolved in exactly 1 L concentrated sulphuric acid.
Phenylphenol reagent for soluble pectin assay	50 mg phenylphenol, 0.5 g NaOH, dissolved in 100 ml water

3.3.2.2 pH

Juice pH was determined using a pH meter with temperature adjustment and a refillable combination electrode. Samples were brought to room temperature (25 ± 2 °C) and stirred during pH measurement. For each juice treatment the final pH values were the average of the three separate juice replicates each measured twice and the temperature recorded.

3.3.2.3 Total titratable acidity

Titratable acidity of the broccoli juice samples was determined using the method described by Friedrich (2001), with modifications. Broccoli juice (10 ml) was diluted with distilled water (20 ml) and titrated against 0.02 M NaOH to an end point of pH 8.2. Each solution of NaOH was standardised against 0.02 M potassium hydrogen phthalate. The titratable acidity was calculated as g citric acid per 1 L of juice and is the average of the three juice replicates, titrated twice, for each juice treatment.

NB: The titratable acidity for the preliminary experiments (Section 4.1 and 4.2) were determined using 1 ml broccoli juice diluted in 50 ml water titrated against 0.1 M NaOH to an end point of pH 8.2 using an auto titrator.

3.3.2.4 Total soluble solids

The total soluble solids of the broccoli juice samples were measured using a handheld refractometer with automatic temperature adjustment. The total soluble solids were expressed as °Brix $_{(sucrose)}$, and results are the average of the three juice replicates for each juice treatment combination.

3.3.2.5 Dry matter

Aliquots (30 ml) of broccoli juice from each treatment were weighed into pre-weighed 50 ml tubes. The samples were frozen then freeze-dried until a constant weight was achieved. The freeze-dried juice samples were not exposed to high temperatures therefore enabling the dry matter to be used for subsequent dietary fibre analysis.

3.3.2.6 Juice colour

The colour of the juice treatments at each time period was measured using a hand held spectrophotometer with a 1 cm path length liquid cell holder attachment. The illuminant was D65 and calibrations were made against a white tile reference. Juice samples were mixed on a vortex mixer and dispensed into the cells. For each juice treatment the final colour readings were the average of the three juice replicates each measured three times, using auto averaging.

The colour readings were reported as lightness (L*), redness-greenness (a*), blueness-yellowness (b*), chroma (C*) colour intensity and hue angle (h) values using the CIE system(Culver & Wrolstad 2008). The changes in the colour over time were calculated as differences from the time 0 results and are presented as ΔL*, Δa*, Δb*, ΔC* and Δh values. The total colour difference (ΔE*) was also calculated as described by Shellhammer and Bamforth (2008).

$$\Delta E = \sqrt{[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]}$$

Where:
$\Delta L^* = L^*_T - L^*_{T0}$
$\Delta a^* = a^*_T - a^*_{T0}$
$\Delta b^* = b^*_T - b^*_{T0}$
$\Delta C^* = C^*_T - C^*_{T0}$
$\Delta h = h_T - h_{T0}$
T = Storage time (weeks) in refrigerated storage
T0 = Week 0, no refrigerated storage

3.3.2.7 Chlorophyll and carotenoid content

The chlorophyll and carotenoid content in the broccoli juice samples was determined according to the method described by Lichtenthaler (1987) with some modifications to suit analysis of juice and reduced volumes to suit analysis using a microplate reader.

Juice samples (1 ml) were centrifuged at 12 000 rpm (15 300 x *g*) for 10 min at 4°C. The colourless supernatant was discarded and the green pellet mixed with methanol (1 ml). Samples were incubated in darkness for 48 h followed by centrifugation at 12000 rpm (15300 x *g*) for 10 min at 4°C. The methanolic supernatant (200 µL) was dispensed into a 96-well

microplate and absorbance was read in a plate reader at wavelengths of 665 nm, 652 nm and 470 nm. Chlorophyll and carotenoid concentrations were calculated using the following formulas with methanol as the solvent (Lichtenthaler 1987).

Chlorophyll *a* (mg/ml) = $16.72 \times A_{665.2} - 9.16 \times A_{652.4}$

Chlorophyll *b* (mg/ml) = $34.09\ A_{652.4} + 15.28 \times A_{665.2}$

Chlorophyll *a* + *b* (mg/ml) = $1.44 \times A_{665.2} + 24.93 \times A_{652.4}$

Carotenoids (mg/ml) = $(1000 \times A_{470}$ -1.63 × Chl *a* -104.96 ×Chl *b*) ÷ 221

3.3.2.8 Soluble pectin

Soluble pectin content of the broccoli juices was analysed using the uronic acid method of Blumenkrantz and Asboe-Hansen (1973) but with reduced volumes to suit analysis using a micro plate reader.

Broccoli juice samples were centrifuged at 12 000 rpm (15 300 x *g*), for 5 min at 4°C and 40 µl of supernatant dispensed into a 96-well flat-bottomed micro plate followed by 200 µl of a 0.0125 M solution of di-sodium tetraborate dissolved in concentrated sulphuric acid (Table 3.6). The covered plate was incubated in 95°C oven for 1 h then pre-read in a plate reader at 520 nm. The plate was chilled on ice for 20 min and then phenylphenol reagent (10 µl) was added, the plate covered with sticky sealable plate covers and mixed vigorously. After exactly 5 min the absorbance was read again at 520 nm.

The quantity of uronic acid was calculated using a standard curve of galacturonic acid solution (0-200 µg/ml). To calculate the results as pectin, the final amount of uronic acid was multiplied by (176.1/194.1) to account for the water lost when galacturonic acids are linked in pectin chains (Blumenkrantz & Asboe-Hansen 1973). Results are expressed on a mg/100ml basis.

3.3.2.9 Total dietary fibre

The dietary fibre content of broccoli juice was analysed using the Megazyme Total Dietary Fibre Kit (Table 3.6), following the kit instructions but with reduced volumes.

Samples of freeze dried broccoli juice (0.5 g) were weighed into 50 ml plastic centrifuge tubes and completely dispersed in 10 ml of 0.05 M MES/TRIS buffer solution (pH 8.2). The samples were incubated with heat-stable α-amylase solution (12.5 µl) in a 98°C water bath for 35 min. Samples were cooled to 60°C, protease solution (25 µl) added and incubated in a 60°C water bath for 30 min. The sample pH was lowered to pH 4.1-4.8 at 60°C with 0.561 N HCL (1.25 ml for acid juice samples and 1.65 ml for neutral juice samples). Amyloglucosidase solution (50 µl) was added and the samples incubated again in a 60°C water bath for 30 min. After incubation, dietary fibre was precipitated in four volumes of 99% ethanol for 1 h at room temperature then centrifuged at 5000 rpm (5311 x *g*) for 10 min. The pellets were washed twice with 78% ethanol (5 ml), twice with 99% ethanol (5 ml) and finally once with acetone (5 ml), with centrifugation (5000 rpm, 5311 x *g*) for 5 min and draining between each washing. Washed residues were dried overnight at 65°C then at 95°C for 2 days until a constant weight was achieved. The total dietary fibre was calculated according to the following calculation:

$$\text{Total dietary fibre (\%)} = \frac{\text{residue weight} - \text{blank weight}}{\text{sample weight}} \times 100$$

3.3.2.10 Ascorbic acid analysis

Three replicates from each of the 24 juice treatments were thawed and diluted 1 in 5 with 100 mM potassium acetate, pH 3.0 (buffered with formic acid). Samples were centrifuged at 12000 rpm (15 300 x *g*), for 5 min at 4°C. Diluted samples were analysed as is, or with addition of DL-Dithiothreitol (DTT) (60 mg/ml, 20 µl added to 200 µl diluted juice). This enabled both available and total ascorbic acid to be determined.

High performance liquid chromatography (HPLC) analysis was performed with assistance. A Dionex Ultimate 3000 solvent delivery system and a Dionex 3000 PDA detector were used in conjunction with an Econosil C18 (10 µm, 250 mm x 4.6 mm) column (Alltech) fitted with a guard column. Column temperature was maintained at 25°C. The mobile phase consisted of 100 mM potassium acetate buffer, pH 5.0 (adjusted with formic acid), acetonitrile and water. Solvent flow was maintained at 1.5 ml/min, with running proportions adjusted according to a solvent delivery programme. Chromeleon software was used to identify and quantify ascorbic acid. Peak identification was based on the retention time of standards (0–100 µg/ml ascorbic

acid ± DTT as per the samples) monitored at 254 nm. Chromeleon software was used for peak integration and quantification.

3.3.3 Sensory testing

3.3.3.1 General (recruiting, panel and test method choice)

A trained sensory panel used a generic descriptive analysis method (Lawless & Heymann 1998) to provide an in-depth descriptive profile of neutral and acidified broccoli juice. The 12 panellists were recruited from Plant and Food's sensory panel staff list. Panellists were trained in 12 sessions of 1.5 h duration, held over a 6 week period. Training was carried out for neutral and acidified juices at the same time, using the same descriptors, rating scale and score sheet. Neutral and acidified broccoli juices were assessed as individual juices by the panel and each juice type had their own reference sample profile.

3.3.3.2 Panel training procedure

Broccoli (200 kg) was juiced using the process described in Figure 3.1 and the equipment detailed in Table 3.3 to produce broccoli juice (108 L) for sensory panel training. The acidified broccoli juice was pasteurised at 72°C for 15 sec and the neutral broccoli juice at 85°C for 15 sec. The bottled juice was frozen at -20°C until required.

All the training sessions were held around a large table with individual samples presented to each panellist. All tasting and rating was done in silence with the combined results being discussed and agreed upon. The training programme consisted of:

- Introduction and familiarisation with the aroma, taste, flavour, mouthfeel and after taste of both neutral and acidified broccoli juices.
- The development using panel consensus, of identifiable attributes with descriptors and references for the aroma, taste, flavour, mouthfeel and after taste that are applicable to both the neutral and acidified broccoli juices.

- The development of a rating scale and score sheet for use in the assessment of both the neutral and acidified broccoli juices.
- Checking the panel results for reproducibility and consistency.

Panellists were familiarised with neutral and acidified broccoli juice using the training samples described above. These were defrosted and either served on the same day as fresh samples or stored for varying time periods at 4 ± 1°C and presented as aged samples. Juice samples for training were served chilled (7 ± 2°C). Initially panellists tasted the juices and recorded their own descriptions of them. Individual responses were combined and a list of attributes was developed by consensus that described both neutral and acidified broccoli juice.

The 24 attributes finally agreed upon were:

- **Aroma** (6 attributes): broccoli smell, snow pea sweet smell, peppery / radish aroma, chemical aroma, metallic aroma, other aroma.
- **Flavour and taste** (9 attributes): overall flavour intensity, fresh broccoli flavour, peppery / radish flavour, sweet taste, sour taste, salty taste, bitter taste, chemical flavour, other flavour.
- **Mouthfeel** (3 attributes): tingly, astringent, other mouthfeel.
- **Aftertaste** (6 attributes): peppery / radish, sweet aftertaste, sour aftertaste, bitter aftertaste, astringent, other aftertaste.

The attribute “Other” was used for aroma, flavour, mouthfeel and after taste for recording a component that can be identified but is not in the general attribute list. When the “other” attribute was rated the panellist also described what they could detect.

Once the attributes were identified the panellists developed the terminology for describing all the attributes relating to aroma, flavour and taste, mouth feel and after taste to suit both the different profiles of the neutral and acidic broccoli juice. Table 3.7 details the attribute descriptors for broccoli juice and the specific attribute reference agreed upon by the panel. The reference samples were tasted by the panellists.

Table 3.7 Attributes, descriptions and references for neutral and acidified broccoli juice.

Aroma	**Description**	**Reference**
Broccoli smell	Raw broccoli	Raw broccoli
Snow pea sweet	Smells like young green snow pea pods	Snow peas
Peppery - radish	Smells like cut red radish	Grated red radish
Chemical	Artificial, solvent or paint smell. Smells foreign to food.	
Metallic	Smells like the inside of a washed Dole pineapple can	A empty Dole pineapple can filled with water and held overnight
Other		
Taste	**Description**	**Reference**
Overall flavour intensity	Overall intensity or strength of flavour of the drink	
Fresh broccoli flavour	Taste associated with raw broccoli and raw green stalky vegetables (cabbage, peas, beans, cauliflower)	Raw broccoli
Peppery – radish flavour	Tastes peppery like cut red radish	Grated red radish
Sweet taste	Taste associated with sugar (sucrose)	Sucrose solution (2%)
Sour taste	Taste associated with lemon juice	Lemon juice
Salty taste	Tastes like table salt (Sodium Chloride)	Salt solutions
Bitter taste	Taste associated with soda water	Schweppes soda water
Chemical flavour	Taste associated with artificial, solvent or paint or tastes foreign to food.	
Other		
Mouth feel	**Description**	**Reference**
Tingly	The effect of fizzy drink or sherbet sweets on the first half of your tongue	Schweppes soda water and fizzy sweets
Astringent	Mouth drying and or puckering	Tonic water
Other		
Aftertaste	**Description**	**Reference**
Peppery/ radish aftertaste	Tastes peppery like cut red radish	Grated red radish
Sweet aftertaste	Taste associated with sugar (sucrose)	Sucrose solution (2%)
Sour aftertaste	Taste associated with lemon juice	Lemon juice
Bitter aftertaste	Taste associated with soda water	Schweppes soda water
Astringent aftertaste	Mouth drying and or puckering	Schweppes tonic water
Other		

The intensity scale in Figure 3.3 was developed based on an unlabelled 15 point box scale. The numbered scale combines anchor descriptors at intervals along the scale in increasing intensity from none (1) to extreme (15). The scale (Figure 3.3) is included at the top of the score sheet used for assessment as shown in Appendix 2. The same scale was used for assessment of both neutral and acid broccoli juices.

Scale	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Descriptors	None	Threshold / barely there	Slight / just there		Low			Medium / moderate		Moderately strong		Strong		Very strong	Extreme

Figure 3.3 Intensity scale for rating neutral and acidified broccoli juice.

The order of appearance of the 24 attributes on the score sheet was determined by panel consensus and relates to order of detection in the broccoli juices. The score sheets developed through the sensory training were used for the final sample assessments (Appendix 2).

Once the attributes, score card and scale were developed the descriptive profile of the neutral and acidified broccoli juice reference juices were determined. Samples of neutral and acidified broccoli juice were modified with different levels of reference material (Table 3.7 and Appendix 3) according to the attribute being investigated. The modified samples were rated by the panel using the scale and the intensity of each attribute was determined. As a result, a descriptive profile was determined by consensus for both the neutral and acidified broccoli juices. The appropriate reference profile sheet was then supplied for both the neutral and acid sensory panel assessments (Appendix 4).

The panel was checked for reproducibility and consistency by assessing duplicate samples or either reference or modified juices and the results compared using means, standard errors and standard deviations.

3.3.3.3 Procedure for broccoli juice assessment by trained sensory panel

Juice assessments were held in the assessment booths at Plant and Food Research, Palmerston North, New Zealand. Assessment sessions for the neutral juice samples were held over 4 days in one week and the acid juice samples were assessed over 4 days the following week. Each day there were two assessment sittings held at 12.30 pm and 1.30 pm with six panellists in each sitting. Panellists evaluated six samples during each assessment session.

Sample Preparation

Frozen juice bottles were defrosted in cold water for two hours on the morning of the assessments. For each assessment session two 250 ml bottles were needed per treatment. The thawed juices were mixed in a 1 L plastic jug then stored in and served directly from the refrigerator at 7°C ±1°C. Serving the sample directly from the fridge represents the beverage drinking temperature if purchased from a retail display unit. Reference juice samples were served at the start of the session and kept in a cup of ice for the duration of the session so panellists could recalibrate themselves against the reference sample (Lawless & Heymann 1998).

Assessment

Each panellist received 30 ml juice samples served in 60 ml clear plastic sample cups (Galantai Plastics Group Limited, Auckland, New Zealand) coded with a three digit random number. The juice samples were presented one at a time following a complete block Williams Latin squared design balanced and randomised for carry-over effects to avoid artefacts due to presentation order and sensory adaptation due to continuous exposure (Lawless & Heymann 1998).

Juice samples were served on a white serving tray with bottled spring water and Carr's water crackers as palate cleansers between samples. Tooth picks were provided for stirring samples. Each panellist was provided with an instruction sheet, scoring sheets, the attribute definitions sheet and the broccoli juice reference sheet. These are all found in Appendices 2 and 3. There was a separate reference sheet each for neutral and for acidified broccoli juices (Appendix 4).

Assessments were performed in individual booths with controlled lighting and temperature. Red lighting (Osram 40 W) was used to reduce the colour differences between samples. Assessments were done in silence in a closed booth area to reduce external noise. Panellists were asked to refrain from wearing perfume and to not eat, drink coffee or smoke for one hour prior to assessments.

3.3.4 Statistical analysis

Microsoft Excel (2007) was used to calculate means, standard errors, and standard deviations and graphically present the results. Statistical analysis was done using GenStat for Windows 12th Edition (Payne et al. 2009).

Three-way factorial analysis of variance (ANOVA) was conducted on all data from biochemical analyses and where necessary the data were log-transformed to equalize variances. A significant statistical difference between the means was taken as $p<0.05$. Each treatment was assessed in triplicate (n=3).

All sensory assessment results were subjected to analysis of variance (ANOVA) with acidity, light, storage time and panellist as factors, main effects and linear and non linear trends were reported. A significant statistical difference between the means was taken as $p<0.05$.Each treatment was assessed in duplicate (n=2). All the sensory training results were interpreted using Panel check (2009, Nofima Mat, Osloveien, Norway).

CHAPTER 4

Preliminary experiments

Two preliminary experiments were undertaken to determine the most effective juice acidification method and then the best pasteurisation parameters for neutral and acidified broccoli juices.

4.1 Acidification

Citric and ascorbic acids were investigated as the acidifying agents for broccoli juice. Ascorbic acid was chosen because it is used commercially as an antioxidant and/or vitamin additive. Due to it being very labile, excess amounts are often added to compensate for losses during processing. Citric acid is a commonly used commercial acidity regulator. As detailed in Section 3.1.1 and Table 3.2, citric and ascorbic acid (dissolved in water) at varying concentrations were used to acidify raw broccoli juice. The treatments were compared for their effectiveness in reducing pH while minimizing the colour change in the raw (unpasteurised) broccoli juice.

The results presented in Table 4.1 show that the soluble solids of the juice increased with higher concentration acid solutions. This may be due to the contribution of citric and ascorbic acid to the soluble solids, and the dilution of the juices prepared at lower acid concentrations. Juices containing ascorbic acid had higher levels of soluble solids than juices containing citric acid. Less citric acid was required to achieve the desired pH 4.0 from the starting pH of broccoli juice (pH 6.5) and the titratable acidity was significantly greater than the juices containing ascorbic acid. Citric acid reduces the pH more effectively than ascorbic acid at the same concentrations due to the greater number of hydrogen ions (H^+) available.

Table 4.1 The pH, titratable acidity and °Brix of raw broccoli juice acidified with increasing amounts (% w/w) of ascorbic acid or citric acid. Data are means ±SE (n=9).

Juice pH	% w/w	pH	Titratable acidity (g citric /100ml)	°Brix
Ascorbic	0	6.6 ±0.05	0.11 ±0.001	4.4 ±0.09
Ascorbic	1	6.2 ±0.06	0.14 ±0.000	4.6 ±0.12
Ascorbic	2.5	5.3 ±0.03	0.19 ±0.006	5.0 ±0.24
Ascorbic	5	4.8 ±0.00	0.28 ±0.004	4.9 ±0.03
Ascorbic	7.5	4.4 ±0.08	0.37 ±0.003	5.2 ±0.02
Ascorbic	10	4.4 ±0.02	0.48 ±0.014	5.5 ±0.01
Citric	0	6.6 ±0.06	0.13 ±0.018	4.5 ±0.04
Citric	1	5.4 ±0.01	0.20 ±0.008	4.7 ±0.07
Citric	2.5	4.7 ±0.00	0.33 ±0.006	4.7 ±0.17
Citric	5	4.3 ±0.04	0.55 ±0.004	4.7 ±0.04
Citric	7.5	4.0 ±0.04	0.78 ±0.013	5.0 ±0.02
Citric	10	3.8 ±0.11	1.03 ±0.018	5.2 ±0.05

With the exception of the controls and the 1 % ascorbic acid juice, all the acidified juices turned the olive green colour characteristic of pheophytin conversion in the presence of acid (Coultate 2009). The colour measurements detailed in Table 4.2 show that at the 10 % concentration both juices had reached similar colour endpoints with the10% citric having a slightly darker lightness values. The juices became darker as the lightness values increased, the colour more saturated with decreasing chroma values and the decreasing hue angle saw the vibrant green become more yellow.

Table 4.2 Colour measurements of lightness, chroma and hue angle for raw broccoli juice acidified with increasing concentrations (% w/w) of ascorbic acid or citric acid. Data are means ±SE (n=9).

Juice pH	**%w/w**	**Lightness**	**Chroma**	**Hue angle**
Ascorbic	0	33.0 ±0.36	7.0 ±0.58	112 ±2.2
Ascorbic	1	33.6 ±0.10	7.5 ±0.30	111 ±1.7
Ascorbic	2.5	34.1 ±0.05	7.1 ±0.30	107 ±1.7
Ascorbic	5	34.9 ±0.00	6.7 ±0.43	108 ±0.1
Ascorbic	7.5	35.3 ±0.05	6.4 ±0.43	109 ±0.3
Ascorbic	10	35.4 ±0.04	6.4 ±0.47	109 ±0.5
Citric	0	33.1 ±0.37	7.2 ±0.57	113 ±2.1
Citric	1	33.6 ±0.40	6.6 ±0.70	105 ±3.4
Citric	2.5	34.9 ±0.24	6.3 ±0.74	107 ±1.1
Citric	5	35.8 ±0.06	6.2 ±0.47	111 ±0.8
Citric	7.5	36.0 ±0.04	6.2 ±0.39	110 ±0.6
Citric	10	35.8 ±0.11	6.4 ±0.34	109 ±0.4

From these results citric acid at a 10 % w/w concentration was chosen as the acidity regulator for broccoli juice in further experimental work. At this concentration the target pH of 4.0 was reached most effectively, while the change in the juice colour although not ideal was similar for both citric acid and ascorbic acid.

4.2 Pasteurisation

The most common milk pasteurisation method, the high temperature short time (HTST) process, 72°C for 15 sec was used as the starting point to determine the pasteurisation parameters for broccoli juice. The optimal pasteurisation conditions for neutral and acidified broccoli juice were investigated using three different temperatures as described in Section 3.1.1.2 and Table 3.3. The impact on microbiological safety, chemical properties, colour and flavour was evaluated.

Broccoli juice samples from each treatment were tested for the total aerobic plate count (TPC) and yeasts and moulds (Y&M) and the results are presented in Table 4.3. Raw unpasteurised neutral and acidified broccoli juice samples were also tested to provide information on the starting microbial contamination level. All three pasteurisation treatments (72 ± 1°C, 85 ± 1°C and 95 ± 1°C) for a duration of 15 sec produced very low microbial levels for both the neutral and acidified broccoli juices. The recommended limit for aerobic plate counts according the Microbiological Reference Criteria for Food (New Zealand Food Safety Authority 1995) is 100000 cfu/ml of sample at 35°C (Section 3.3.1). All the results in Table 4.3 are well within this limit, even the raw unpasteurised samples.

Pasteurisation at over 70°C for 15 sec should inactivate vegetative spoilage organisms such as yeasts, mould, *Lactobacillus fermentum* in a high acid juice. Fruit juices have a natural pH below 4.5 so are not high risk, but low acid juices such as pear juice, banana puree, tomato juice and unacidified broccoli juice (ph 6.5) would be a higher risk. More severe conditions such as 87°C for 15 s would be needed for inactivation of spoilage organisms (Wilbey 2003a, 2003b).

Table 4.3 Microbiological test results for neutral and acidified broccoli juice pasteurised for 15 sec at specified temperatures.

Treatment	Duplicate	Juice pH	Temperature (°C)	TPC (cfu/ml)	Y&M (cfu/ml)
1	-	Neutral	Raw	4000	3000
3	A	Neutral	72	<10	<10
4	B	Neutral	72	<10	<10
7	A	Neutral	85	<10	<10
8	B	Neutral	85	<10	10
11	A	Neutral	95	<10	<10
12	B	Neutral	95	<10	<10
2	-	Acid	Raw	4000	1200
5	C	Acid	72	<10	<10
6	D	Acid	72	10	<10
9	C	Acid	85	10	<10
10	D	Acid	85	<10	10
13	C	Acid	95	10	<10
14	D	Acid	95	<10	<10

Data are means ±SE (n=3). TPC = Total aerobic plate count assay, Y&M = yeast and moulds assay, cfu/ml = colony forming units per ml of sample.

The time taken for a 10 fold reduction in survivors at a given temperature is known as the decimal reduction time (or D) value (expressed in min or sec). The D values decrease with increasing temperatures. The rate of change in temperature required to give a 10 fold change in D value is known as the 'z' value (Wilbey 2003a). The lethal rate (L) is the time at the reference temperature required to give the same sterilising effect as one minute at the treatment temperature and is dimensionless (McCarthy 2000). The lethality (F) is the equivalent time of the process at the reference temperature. An ideal thermal process is where the temperature of the food is raised uniformly and instantaneously to a temperature lethal to the target microorganism at time zero (McCarthy 2000). A square process was assumed for the current processing conditions in Table 4.4. The pH of the product significantly affects the lethality of heat treatment. Less heat is needed to inactivate micro organisms as the pH is reduced or increased from their optimum pH of growth which is generally pH 7.0 (Jay

1996; Bari et al. 2005). The aim of thermal processing is to prevent microbial and enzymatic activities in the final product. Food with a pH greater than pH 4.5 is considered to be a low acid food and microorganisms such as *Clostridium botulinum* can grow under these conditions. This organism requires heating to 121.11°C for the required length of time for destruction. If the pH of the product is lower than pH 4.5 then milder heat treatments (100°C or less) are effective (Floros 1993). Table 4.4 details the lethality calculations for the destruction of *C.botulinum* using the temperature conditions of this trial and indicate that the lethality increases with increasing temperature but does not achieve the lethality required for inactivation of *C.botulinum* of F = approx 3 min. The calculated lethality values are quite low in respect to product sterilization however the processing conditions in Table 4.4 will all achieve a minimum effective pasteurisation modelled on the pasteurisation standard for milk (pH 6.5) at 72°C for 15 sec. Processing the neutral juice at higher temperatures (85 and 95 °C for 15 sec) will exceed minimum pasteurisation requirements as will the pasteurisation of the acidified juice at 72, 85 or 95 °C for 15 sec.

Table 4.4 Microorganism lethality calculations.

T (°C)	t (sec)	RT (°C)	z (°C)	L	F (sec)
72	15	121.11	10	0.00001	0.0002
85	15	121.11	10	0.00024	0.0037
95	15	121.11	10	0.00245	0.0367

All data refer to *C.botulinum.* T = product temperature, t =pasteurisation time (sec), RT = reference temperature, z= slope of thermal death time curve of microorganism. $L = 10^{(T-RT)/z}$. L is the lethal rate for a reference temperature of 121.11°C and a z value of 10°C. F = Lethality = $L \times t = t \times 10^{(T-RT)/z}$ which is the equivalent time of the process at the reference temperature (McCarthy 2000).

The results in Table 4.5 show that the titratable acidity of the neutral and acidified juices did not vary significantly according to the increasing heat treatments (p=0.361) although as expected there was a significant difference in titratable acidity between the neutral and acidified juices (p<0.001). The increasing treatment temperatures did not result in increased soluble solids in either the neutral and acidified juices (p=0.690). However increasing temperature did have a significant effect on increasing total chlorophyll levels for both neutral and acidified juices (p=0.003).

Table 4.5 Titratable acidity (g/100ml citric), soluble solids (°Brix) and total chlorophyll (mg/ml) content in neutral and acidified broccoli juice pasteurised for 15 sec at three different temperatures. Data are means ± SE (n=3).

Juice pH	Temperature (°C)	Titratable acidity	°Brix	Total chlorophyll
Neutral	Raw	0.08 ±0.001	4.8 ±0.00	6.2 ±0.25
Neutral	72 °C	0.05 ±0.011	3.7 ±0.80	5.3 ±0.23
Neutral	85 °C	0.05 ±0.001	4.0 ±0.07	5.9 ±0.08
Neutral	95 °C	0.05 ±0.000	4.1 ±0.20	6.1 ±0.11
Acid	Raw	0.35 ±0.000	4.7 ±0.00	5.4 ±0.01
Acid	72 °C	0.26 ±0.011	3.8 ±0.00	4.7 ±0.07
Acid	85 °C	0.25 ±0.017	3.8 ±0.17	4.5 ±0.03
Acid	95 °C	0.22 ±0.018	4.0 ±0.12	5.4 ±0.21

Table 4.6 Colour measurements, lightness, chroma and hue angle for neutral and acidified broccoli juice pasteurised for 15 sec at different temperatures. Data are means ±SE (n=3).

Juice pH	Temperature (°C)	Lightness	Chroma	Hue angle
Neutral	Raw	36.2 ±0.00	11.6 ±0.01	117.3 ±0.02
Neutral	72	35.6 ±0.13	10.8 ±0.18	117.4 ±0.05
Neutral	85	35.6 ±0.01	10.7 ±0.02	117.6 ±0.04
Neutral	95	35.6 ±0.11	11.0 ±0.14	117.5 ±0.02
Acid	Raw	38.6 ±0.00	9.9 ±0.01	105.1 ±0.01
Acid	72	39.4 ±0.00	10.0 ±0.02	105.2 ±0.03
Acid	85	39.4 ±0.02	10.0 ±0.01	104.8 ±0.03
Acid	95	39.3 ±0.08	10.3 ±0.00	104.8 ±0.03

The changes in the colour profile of the broccoli juice with differing heat treatments are shown in Table 4.6. Acidified juices were significantly lighter than the neutral juices (p<0.001). There was a significant linear increase in the lightness of both the neutral and acidified juices with increased heat treatment (p=0.003). The hue angle increased with higher temperatures, indicating the juices became slightly greener than the raw broccoli juice. The acidified juice hue differed (h= 105.1) compared to the hue of the neutral juice (h= 117.3) due the effect of acidification.

In addition to beneficial health properties, chlorophyll, in high concentrations is a visually appealing green colour (Litchentaler 1987). At neutral or alkaline pH, heat (e.g. blanching or pasteurisation) removes the phytol side chains from chlorophyll resulting in a conversion to chlorophyllides *a* and *b*, which are a brighter green colour than the chlorophyll original pigments (Daood 2003; Coultate 2009) which explains the increase in green colour after a mild heat treatment.

The flavour of the juices pasteurised at different temperatures was informally assessed by four Plant and Food staff who could not distinguish a change in flavour with the different pasteurisation temperatures. In comparison to the raw juices however, there was a difference in flavour in the neutral juices with pasteurisation resulting in more cooked notes and an increased pepper heat sensation particularly for the neutral juices.

After evaluation of the results the most suitable pasteurisation conditions for both neutral and acidified broccoli juice were judged to be 85°C for 15 sec as these parameters provide adequate heat treatment to maintain food safety whilst still maintaining a juice colour and flavour closely resembling raw broccoli juice. However a higher temperature (95°C for 15 sec) was chosen for the treatment of neutral broccoli juice to provide a greater safety margin during a shelf life trial and the associated sensory analysis. The acidified broccoli juice was not treated at the higher temperature (95°C for 15 sec) as the acidification to pH 4.0 was thought to provide an additional safety margin.

CHAPTER 5

Shelf Life Trial Results and Discussion

5.1 Introduction

A comprehensive investigation of the shelf life of broccoli juice stored in conditions approximating that of the retail environment for beverages was conducted over an eight week period. As detailed in Section 3.2, two broccoli juices (neutral, pH 6.5 and acidic pH 4.0) were stored refrigerated (4 ± 1°C) in light or dark conditions for different time periods (0, 1, 2, 4, 6 and 8 weeks). The broccoli juice treatments were analysed for microbiological safety, physical appearance, chemical and biochemical changes and differences in the sensory profile. Details of all methods used are provided in Section 3.3.

The results of the broccoli juice shelf life quality investigation are divided into the following sections:

- Processing factors: yields and constraints (yield, dry matter) (Section 5.2).
- Standard juice properties: chemical and microbiological characteristics of the broccoli juice (pH, titratable acidity, soluble solids, microbiology) (Section 5.3).
- Composition: the effect of storage conditions and time on the content of selected components related to juice nutritional quality (chlorophyll, carotenoids, vitamin C, dietary fibre, pectin) (Section 5.4).
- Colour: observations of the change in colour over time (lightness, chroma, hue and total colour difference ΔE) (Section 5.5).
- Sensory Evaluation: an evaluation of the changes in flavour profile over time of neutral and acidified broccoli juice using descriptive analysis (Section 5.6).

5.2 Processing factors

5.2.1 Processing parameters

Broccoli was sourced from the same farm was used in two juice production runs to produce the 267 L of juice required for training the sensory panel and the shelf life trial. Table 5.1 shows the yield calculation (0.43 L/kg wet weight of broccoli) for the shelf life trial juice. The same procedure was used in both production runs, with a similar juice yield (0.46 L/kg wet weight of broccoli) achieved the previous week in the smaller 108 L run for the sensory training juice. The 7% difference in yield between the sensory and shelf life trial production runs is due to pressing technique on the water press.

Table 5.1 Broccoli juice yield.

	Shelf life trial
Raw broccoli (kg)	375
Waste pomace (kg)	214
Juice (L)	161
Yield (L/kg wet weight of broccoli)	0.43

Table 5.2 Composition of neutral and acidic broccoli juice for the shelf life trial. % = content w/w, neutral juice (pH 6.5), acid juice (pH 4.0).

	Neutral	**Acid**
Ingredients	**%**	**%**
Juice	100	94.6
Water	0	4.9
Citric acid	0	0.5

Once the juice was pressed, it was combined and divided into two portions. One half of the juice was acidified as detailed in Table 5.2, and then both juice types were pasteurised and bottled according to the experimental plan (Section 3.2) in either clear or aluminium foil covered bottles. A solution of citric acid was added to acidify one batch of juice, rather than addition of solid citric acid crystals was preferred to ensure full solubilisation. This diluted the juice to 95% of its original concentration from 5.2 ±0.10 °Brix for neutral juice to 4.9 ±0.04 °Brix in the acidified juice.

5.2.2 Dry matter

The dry matter of the juice treatments was measured as an indicator of how well mixed the juice was at filling and how evenly distributed the composition was across the production process and the shelf trial treatments. All the neutral juice treatment samples were randomly filled from the same juice supply and likewise with the acidified juice samples.

Table 5.3 Dry matter (g/100ml) of broccoli juice treatments during storage time at 4°C. Data are presented as means ± SE (n=6).

Storage Week	Acid Light	Acid Dark	Neutral Light	Neutral Dark
0	5.3 ±0.02	5.3 ±0.02	5.0 ±0.01	5.0 ±0.03
1	5.3 ±0.01	5.4 ±0.01	5.0 ±0.03	5.2 ±0.12
2	5.3 ±0.08	5.3 ±0.03	5.0 ±0.01	5.0 ±0.02
4	5.3 ±0.03	5.0 ±0.28	5.0 ±0.01	5.0 ±0.04
6	5.3 ±0.06	5.4 ±0.00	5.0 ±0.01	5.0 ±0.01
8	5.2 ±0.13	5.1 ±0.19	5.1 ±0.06	5.0 ±0.00

The mean dry matter for the neutral broccoli juice was 5.1 ± 0.03 g/100ml with the dry matter for the acidified broccoli juice 5.3 ± 0.07 g/100ml being significantly different (p<0.001). There was no significant difference in dry matter between the light and dark treatments for both the neutral and acidified broccoli juice (p=0.753). The time in refrigerated storage did not significantly alter the dry matter in either the neutral or acidified broccoli juice treatments (p=0.194).

The process control and randomization of the samples produced relatively homogeneous dry matter for all juices in the shelf life trial with the only factor influencing this being the acidification with a 10 % citric acid solution (Table 5.2). This diluted the juice with water but the citric acid contributed to increased dry matter.

5.3 Standard juice properties

5.3.1 Microbiological testing

Microbiological testing was carried out primarily to ensure the broccoli juice from the shelf life trial was safe to be used for sensory evaluation. In addition the microbiological results provide information on the effectiveness of the pasteurisation and bottling process used.

The results presented in Table 5.4 show the levels of microbiological contamination in the neutral and acidified broccoli juice prior to pasteurisation. Three samples each of neutral and acidified juices were tested for microbial contamination prior to pasteurisation (Table 5.4). Freshly prepared (un-stored) juice as well as juices stored for 8 weeks either at 4 ± 1°C or -20 ± 2°C were tested to provide information on the maximum contamination likely under the conditions for the storage and sampling trial.

Table 5.4 Microbiological test results for raw (un-pasteurised) neutral and acidified broccoli juice.

Juice pH	Juice description	TPC (cfu/ml)	Y&M (cfu/ml)	Coliform (MPN/ml)	*E. coli*
Neutral	Raw	42000	20000	1100	Positive
Neutral	Raw frozen for 8 weeks	$>1x10^8$	$>1x10^8$	45	0
Neutral	Raw refrigerated for 8 weeks	$>1x10^8$	$>1x10^8$	78	0
Acid	Raw	280	140	0	0
Acid	Raw frozen for 8 weeks	10	100	0	0
Acid	Raw refrigerated for 8 weeks	900	1400 (fungi)	43	0

Juice description: Raw unpasteurised juice, a raw juice stored frozen (-20 ± 2°C) for 8 weeks and a raw juice refrigerated for 8 weeks (4 ± 1°C). TPC = Total aerobic plate count. Y&M = yeast and mould count. cfu/ml = colony forming units per ml of sample. Coliform = coliform count. MPN /ml = most probable number per ml of sample. ND = No bacteria were detected. Positive = bacteria were detected. *E.coli* = *E.coli* pathogen test, results reported as presence (positive) or absence (ND or 0). Fungi = fungi was also detected.

The acidification of raw juice results in a large reduction in microbiological population compared to the neutral juice. Freezing did not halt microbial growth, but resulted in higher counts in the neutral total plate count and yeast and mould tests. The long-term refrigerated storage of unpasteurised neutral or acidified broccoli juice provided good conditions for bacterial growth and therefore produced unacceptable juices. Coliforms were present in all neutral samples tested and the week eight acidified sample. The pathogen *E. coli* was detected in the fresh unpasteurised neutral juice.

The detection of fungi in the week 8 raw broccoli juice sample (Table 5.4) could be the result of cross contamination from the storage environment. The refrigerated cool room where the bottles were stored was not sterilized prior to use, and this in combination with high air flows may have provided conditions conducive to cross-contamination from previous contents.

According to the experimental plan (Section 3.2) broccoli juice bottles labelled for microbiological testing were removed from the shelf life trial storage, retained at 4 ± 1°C and sent for analysis according to the methods in Section 3.3. The shelf life trial results are detailed in Table 5.5. The results for all the juices (Table 5.5) were within the acceptable limits for human consumption as outlined in Section 3.3.1 and were therefore safe to use for sensory analysis.

Table 5.5 Microbiological test results for neutral and acidified broccoli juice sampled according to the shelf life trial experimental design in Section 3.2.

Juice pH	Treatment	Age (Weeks)	TPC (cfu/ml)	Y&M (cfu/ml)	Coliform (MPN/ml)	*E.coli*
Neutral	Wk0 Light	0	<10	<10	0	0
Neutral	Wk1 Light	1	10	13	0	0
Neutral	Wk2 Light	2	<10	<10	0	0
Neutral	Wk4 Light	3	50	90	0	0
Neutral	Wk6 Light	5	10	10	0	0
Neutral	Wk8 Light	7	30	<10	0.4	0
Neutral	Wk0 Dark	0	10	<10	0	0
Neutral	Wk1 Dark	1	16000	2000	0	0
Neutral	Wk2 Dark	2	<10	<10	0	0
Neutral	Wk4 Dark	3	10	<10	0	0
Neutral	Wk6 Dark	5	<10	10	0	0
Neutral	Wk8 Dark	7	10	<10	0.4	0
Acid	Wk0 Light	0	20	<10	0	0
Acid	Wk1 Light	1	10	10	0	0
Acid	Wk2 Light	2	<10	<10	0	0
Acid	Wk4 Light	3	10	<10	0	0
Acid	Wk6 Light	5	40	20	0	0
Acid	Wk8 Light	7	20	<10	0.4	0
Acid	Wk0 Dark	0	20	<10	0	0
Acid	Wk1 Dark	1	60	20	0	0
Acid	Wk2 Dark	2	10	10	0	0
Acid	Wk4 Dark	3	10	<10	0	0
Acid	Wk6 Dark	5	10	<10	0	0
Acid	Wk8 Dark	7	10	10	0	0

Key to Table 5.5: Juice pH = neutral (pH 6.5) or acidified (pH 4.0). Treatment = broccoli juice sampled according to the shelf life trial experimental plan in Section 3.2. Age = age of the juice (weeks) when it was tested. Test descriptions are the same as for Table 5.4.

Microbiological counts for all organisms were very low regardless of the pH of the broccoli juice. The light condition during storage did not influence the microbial population. After 8 weeks of storage, three out of four of the week 8 juice samples had coliform counts of 0.4 MPN/ml which, while still acceptable, does provide evidence that the conditions for microbial growth changed in both the neutral and acidified broccoli juices. Alternatively the juices could have been contaminated, if coliforms were present in the juice it is unlikely that it would take 8 weeks for detection. No *E. coli* were detected in any of the shelf life trial samples tested.

The juices stored for 0 or 1 weeks had higher than expected microbial counts. The testing for these samples was affected by the unavailability of the University testing laboratory facility. The juices were frozen for a period of 5 days until the lab reopened. Test results on raw juices shown in Table 5.4 indicated that freezing may not completely halt microbial growth. The most likely explanation is the failure of the testing laboratory's refrigeration unit during juice defrosting meant that the samples were at 11°C for 72 hours. These circumstances may have lead to the unusually high (but still acceptable) counts for these juices.

The pasteurisation conditions for neutral juice (95 ±1°C for 15 sec) and acidified broccoli juice (85 ±1°C for 15 sec) were effective at reducing the microbiological levels to well within acceptable limits which were maintained for the eight week shelf life trial.

5.3.2 Juice pH

The pH of the broccoli juice is an indicator of process quality, with variations within juice type indicating possible microbiological activity. The pH of the juice was measured at room temperature according to Section 3.3.2 and the results for the 24 broccoli juice shelf life trial treatments are presented in Table 5.6.

Table 5.6 pH values of broccoli juice treatments following storage at 4°C for the times indicated. Data are presented as means ± SE (n=3).

Storage Week	Acid Light	Acid Dark	Neutral Light	Neutral Dark
0	3.9 ±0.02	3.8 ±0.01	6.2 ±0.01	6.2 ±0.01
1	3.9 ±0.01	3.9 ±0.01	6.2 ±0.01	6.2 ±0.01
2	3.8 ±0.02	3.8 ±0.02	6.1 ±0.01	6.2 ±0.04
4	3.8 ±0.03	3.8 ±0.00	6.1 ±0.00	6.1 ±0.01
6	3.8 ±0.01	3.8 ±0.01	6.1 ±0.00	6.1 ±0.01
8	3.8 ±0.01	3.8 ±0.01	6.0 ±0.01	6.0 ±0.03

There was a significant difference (p<0.001) between the mean pH of the neutral treatments (pH 6.1±0.01) and the mean pH of the acidified treatments (pH 3.8±0.01). This indicates that acidification of juice prior to storage was maintained throughout the experiment. There was no significant effect of light on the pH of stored neutral or acidified broccoli juices (p=1.000). There was, however, a statistically significant effect of storage on juice pH (p<0.001). Results in Table 5.6 show that neutral juice pH decreases from week 0 to week 8, although this decrease was small. Microbial counts (Table 5.5) were also slightly higher in the neutral juice stored for 8 weeks, and this may be a potential source of the subtle change in pH.

5.3.3 Titratable acidity

The acid taste of a food is dependent on the amount of acid (titratable acidity), the pH and the type of acid present (Friedrich 2001). The pH of a juice is an indication of the concentration of free H_3O^+ dissociated from the acids in the juice whereas the titratable acidity is the total acid content of a juice, determined by titration of all the acid in the juice with a standard base, usually NaOH (Friedrich 2001). A number of organic acids are present in a plant-based juice, but to allow comparison with other juices the titratable acidity is expressed in terms of a standard acid, in this case citric acid.

The titratable acidity results of the shelf life treatments are found in Table 5.7. The titratable acidity of the acidified juice (5.8 ± 0.04 g/L) was significantly greater (p<0.001) than the neutral juice (0.8 ± 0.01 g/L) due to acidification during juice preparation. The titratable acidity of the broccoli juice treatments stored in the light was greater (p=0.031) than the titratable acidity of the treatments stored in the dark.

Table 5.7 Titratable acidity (g citric acid /L) of broccoli juice following storage at 4°C for the times indicated. Data are presented as means ± SE (n=3).

Storage Week	Acid Light	Acid Dark	Neutral Light	Neutral Dark
0	5.9 ±0.04	5.7 ±0.05	0.8 ±0.00	0.8 ±0.01
1	5.8 ±0.05	5.8 ±0.05	0.8 ±0.01	0.8 ±0.00
2	5.9 ±0.06	5.7 ±0.06	0.8 ±0.01	0.8 ±0.01
4	5.8 ±0.00	5.8 ±0.04	0.8 ±0.01	0.8 ±0.00
6	5.8 ±0.04	5.8 ±0.05	0.8 ±0.01	0.8 ±0.00
8	5.8 ±0.04	5.9 ±0.04	0.9 ±0.00	0.8 ±0.01

The average titratable acidity of both the neutral and acidified juices fluctuated during refrigerated storage (p<0.001). The variability during storage shown in Table 5.7 was not significant in a processing environment as the standard errors are similar to the error of the pH meter (±0.02 units) and the error associated with titration protocols (±0.1 ml 0.02 M NaOH).

5.3.4 Soluble solids

A refractometer calibrated with a sugar concentration (°Brix) scale was used to measure the soluble solids of the broccoli juice (Section 3.3.3). The refractive index is the ratio of the speed of light in a vacuum to its speed in a substance and is used as a measure of concentration of solutes in solution (Peris-Tortajada 2004). The °Brix is numerically equivalent percentage (w/w) of sucrose and only accurate for pure sucrose solutions, but as it is determined by refractive index, °Brix is used as an indicator of total soluble solids in the juice of fruits and vegetables (Varnam & Sutherland 1994).

Table 5.8 Soluble solids (°Brix) of broccoli juice treatments following storage at 4°C for the times indicated. Data are presented as means ± SE (n=3).

Storage Week	Acid Light	Acid Dark	Neutral Light	Neutral Dark
0	5.0 ±0.06	5.0 ±0.13	5.8 ±0.13	5.2 ±0.14
1	4.9 ±0.00	4.9 ±0.03	5.1 ±0.12	4.9 ±0.09
2	4.9 ±0.03	4.9 ±0.03	4.8 ±0.17	4.7 ±0.03
4	4.8 ±0.03	5.0 ±0.01	5.4 ±0.03	5.6 ±0.06
6	4.9 ±0.03	4.9 ±0.03	5.2 ±0.10	5.4 ±0.12
8	4.9 ±0.03	4.9 ±0.03	5.1 ±0.09	4.9 ±0.10

The mean soluble solids of the neutral broccoli juice (5.2 ±0.10 °Brix) was significantly greater (p<0.001) than the mean soluble solids for acidified juice (4.9 ±0.04 °Brix). This difference was a likely consequence of the acidification process which resulted in slight dilution of the juice (Table 5.2). Storage in light or dark conditions did not significantly change the soluble solids content (p=0.107). Storage in the light or dark did not affect the soluble solids for the neutral or acidified juice. The results in Table 5.8 show that there is a variation in soluble solids during storage. This was statistically significant (p<0.001).

5.4 Composition

5.4.1 Total dietary fibre

Dietary fibre is the structural material of plant cells that is resistant to the digestive enzymes in the human digestive system (Nelson 2001; Weatherspoon et al. 2005; Gray 2006). There are beneficial links between dietary fibre, increases in satiety and faecal bulking which are linked to the promotion of the growth of beneficial gut micro flora reducing the risk of some cancers (Nelson 2001; Weatherspoon et al. 2005). Total dietary fibre refers to the total amount of both soluble and insoluble dietary fibre in a food (Nelson 2001).

The total dietary fibre of broccoli juice was measured in triplicate (Section 3.3.2.9) for each of the 24 treatments in the shelf life trial (Table 3.2) with the results presented in Figure 5.1.

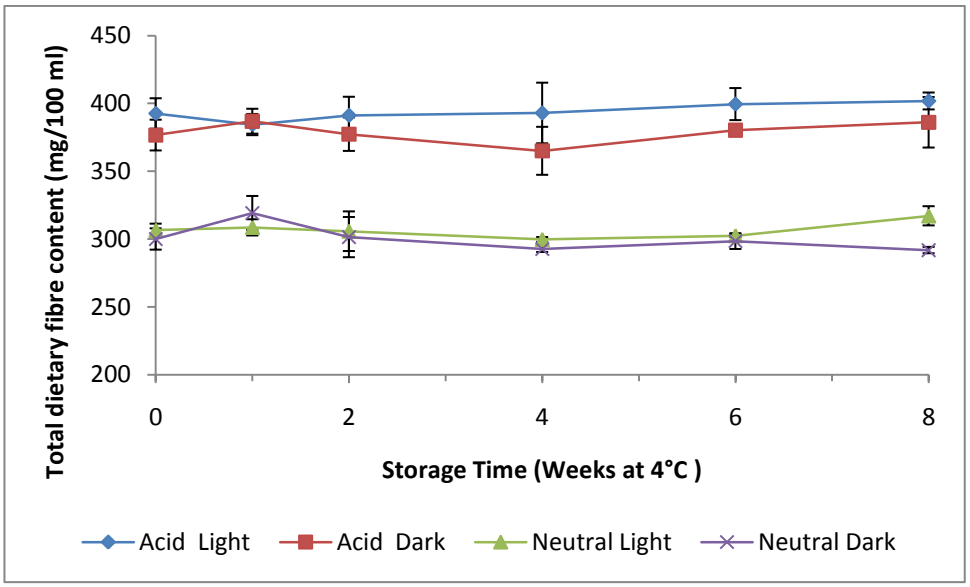


Figure 5.1: Total dietary fibre content (mg/100ml) for broccoli juice treatments over storage time at 4°C. Data are presented as means ± SE (n=3).

There was a significant increase (p<0.001) in dietary fibre content from the neutral juice (304 ± 6.7 mg/100 ml) to the acidified juice (386 ± 12.1 mg/100 ml). The only difference in the dietary fibre assay of the neutral and acidified juices was the neutral juice had more HCl added for pH adjustment (Section 3.3.2.9). Acid broccoli juice was diluted by 5% during acidification therefore it would be expected that acidified broccoli juice would have less dietary fibre than the neutral juice, Figure 5.1 shows the opposite to be true. These results follow the same overall trend shown in the dry matter content (Table 5. 3).

All the broccoli juice was passed though a 150 micron mesh screen prior to separation into acid and neutral samples, before pasteurisation. After the citric acid solution was added to the broccoli juice a precipitate formed and a colour change occurred. The acid samples were mixed and the precipitate was not visually evident in the juice after pasteurisation but the colour change remained. There was additional sediment observed in the acid samples compared to the neutral samples on storage but this was not quantified and there was no visual clearing in any of the juices. The unidentified precipitate could be an explanation for the increase in dietary fibre in the acid broccoli juice samples compared to the neutral broccoli juice samples.

There was a statistical difference (p=0.024) in dietary fibre between the broccoli juice (both neutral and acid) treatments stored in the light and treatments stored in the dark. Theoretically this is unexpected and could be due to the sensitivity of the statistical analysis or large standard errors in the analytical results.

Figure 5.1 shows the dietary fibre content in the broccoli juice did not change significantly during storage (p=0.659). It was not expected that the dietary fibre content would change greatly over time unless there was some deteriorative process occurring that would break down insoluble fibre. Insoluble fibre by definition does not break down in the human digestive system (Weatherspoon et al. 2005; Gray 2006) so it is unlikely that it would break down readily in a neutral (pH 6.5) or acidic (pH 4.0) broccoli juice.

5.4.2 Soluble pectin

Pectin is a structural plant cell wall polysaccharide (Fraeye et al. 2007; Sila et al. 2009) and is classed as a soluble dietary fibre representing 15-20% of the dietary fibre in vegetables (Nelson 2001; Gray 2006). The soluble pectin in broccoli juice was measured by uronic acid determination as described in Section 3.3.2.8 with results for all the broccoli juice treatments presented in Figure 5.2.

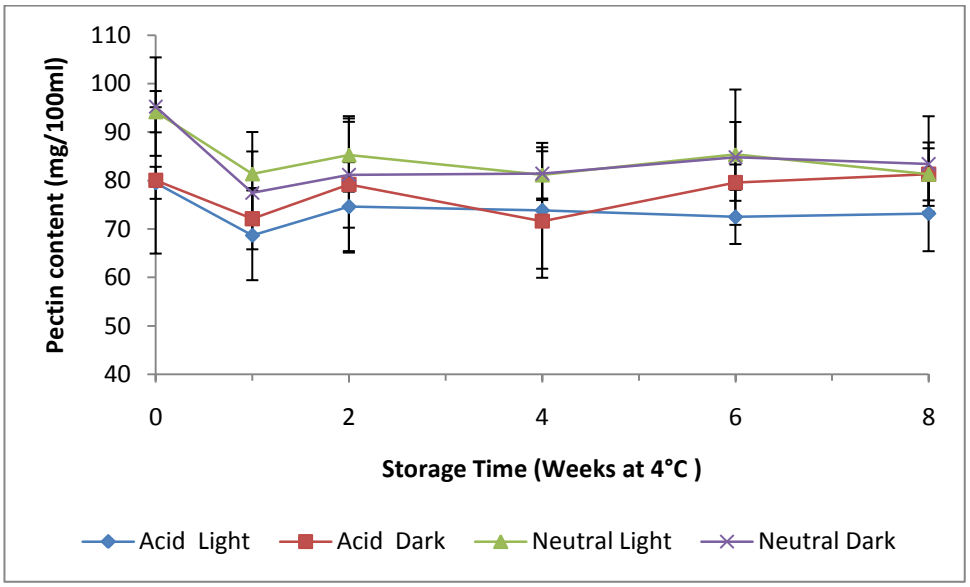


Figure 5.2 Pectin content (mg/ml) for broccoli juice treatments over storage time at 4°C. Data are presented as means ± SE (n=3).

The neutral broccoli juice had an average of 84 ± 8.1 mg/100ml of pectin which was significantly more than the 76 ± 8.6 mg/100ml in the acidified broccoli juice (p=0.020). The difference in the pectin content of broccoli juice stored in light or dark conditions was not significant (p=0.714). The length of time the broccoli juice was stored for did not alter the pectin content in the broccoli juice treatments significantly (p=0.512). The large standard errors (SE) across all the results do not alter the overall trends in treatments, but could be attributed to difficulty with analysing this component. The quantities of pectin present in the broccoli juice were at the lower end of the standard curve used for calculation.

5.4.3 Ascorbic acid

Vitamin C is vital for good health and functioning of many biological pathways in humans (Galgano et al. 2007; Jagdish et al. 2007; Podsedek 2007). Vitamin C exists naturally as two biologically active compounds: L-ascorbic acid (AA) and its oxidised form dehydro-L-ascorbic acid (DHAA). Total vitamin C is the sum of the L-ascorbic acid and the dehydro-L-ascorbic acid content in foods (Zapata & Dufour 1992; Vallejo et al. 2003; Gebczynski & Kmiecik 2007). L-ascorbic acid and dehydro-L-ascorbic acid are readily oxidised in solution, especially when exposed to elevated temperatures, dissolved oxygen, alkaline pH, light, or degradative (oxidizing) enzymes (Russell 2004). Vitamin C is the most labile of all vitamins and is used as an indicator of overall vitamin stability in foods (Zapata & Dufour 1992; Russell 2004; Podsedek 2007; Tosun & Sevinc 2008; Coultate 2009).

Samples were collected and stored according to protocols in Sections 3.2 and 3.3. All sub samples were protected from light during frozen storage. Total ascorbic acid or vitamin C content results are shown in Figure 5.3.

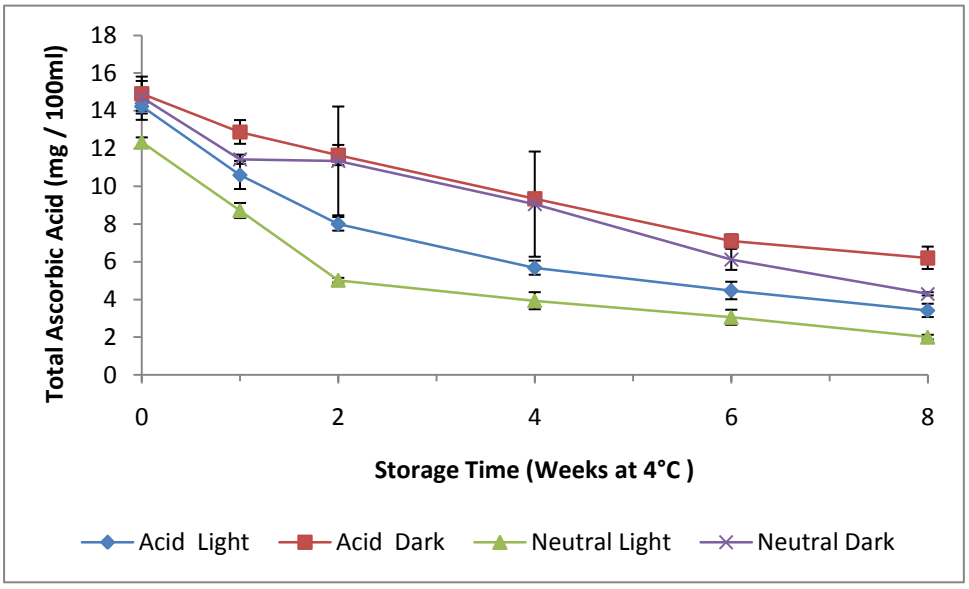


Figure 5.3 Ascorbic acid (mg/ml) for broccoli juice treatments over storage time at 4°C. Data are presented as means ±SE (n = 3).

The acidification of the broccoli juice appears to have had a protective effect on the ascorbic acid content (Figure 5.3). Acidified juice had significantly higher mean ascorbic acid (9.0 ± 0.51 mg/100ml) level than neutral juice (7.7 ± 0.76 mg/100ml) (p<0.001). The samples protected from light during storage had higher ascorbic acid content than the treatments with no protection. Light stored juice had significantly lower mean ascorbic acid than the dark stored juice (p<0.001). The acidified dark broccoli juice had greater ascorbic acid content than the neutral dark treatments and likewise with the acidified light treatments had greater ascorbic acid contents than the neutral light broccoli juice treatments. The mean ascorbic acid level in all broccoli juices decreased significantly with storage time (p<0.001). Figure 5.3 shows a downward trend in ascorbic acid content of all treatments over time.

There was a lot of variation in the data set and the standard errors for some data points were very high which repeat analysis did not change. The variation appears to be due the varying quantities of ascorbic acid in the samples.

Over eight weeks of storage the ascorbic acid levels in broccoli juice decreased from 14.9 ± 0.9 to 3.4 ± 0.4 mg/100ml for acidified juice and 14.7 ± 0.9 to 2.0 ± 0.1 mg/100ml for neutral juice. Compared to the ascorbic acid contents in raw broccoli, these values are relatively low but this was not unexpected as processing, exposure to light and storage time are known to decrease ascorbic acid. The reported ascorbic acid content of raw broccoli varies across variety 54.0 - 119.8 mg/100g fresh weight (Kurilich et al. 1999) and 25.5 - 82.3 ± 17.7 mg/100g fresh weight broccoli (Singh et al. 2007), and between floret 103 ± 3.5 mg/100g fresh weight and stem 124 ± 3.8 mg/100g fresh weight (Zhang & Hamauzu 2004). Raw and cooked ascorbic acid levels were compared by (Davey et al. 2000) with levels of 113 and 90 mg/100g fresh weight respectively.

Overall the acidified broccoli juice stored in the dark had the least reduction (58%) in ascorbic acid content over the eight weeks of storage and the highest residual ascorbic acid at week 8 (6.2 ± 0.6 mg/100ml juice). Light stored acid broccoli juice had a 76% reduction in ascorbic acid. The worst treatment conditions resulting in an 84% reduction in ascorbic acid from week 0 levels was for the neutral broccoli juice exposed to light for eight weeks. The ascorbic acid in dark stored neutral juice only reduced 71% over 8 weeks. From the results low pH and dark storage had a protective effect on ascorbic acid degradation.

5.4.4 Chlorophyll

Chlorophyll is a functional pigment of photosynthesis in green plants and is found with carotenoids in the chloroplast of plant cells. Chlorophyll *a* and *b* are found in a ratio of 3:1 in green plants (Coultate 2009; Farnham & Kopsell 2009). Light, heat, and acid are well known catalysts that produce large changes in the greenness of stored and processed foods as a result of chlorophyll instability, other enzymatic conversions also occur (Daood 2003; Turkmen et al. 2006; Coultate 2009).

The chlorophyll *a* and *b* and total chlorophyll were measured and calculated as described in Section 3.3.2.7. The results of the mean total chlorophyll content (mg/100ml) over eight weeks of storage are presented in Figure 5.4. Tables 5.9 and 5.10 show the mean chlorophyll content for neutral and acidified broccoli juice and the *p* values for the interactions of pH, light and storage time where a *p* value of <0.05 is a significant difference.

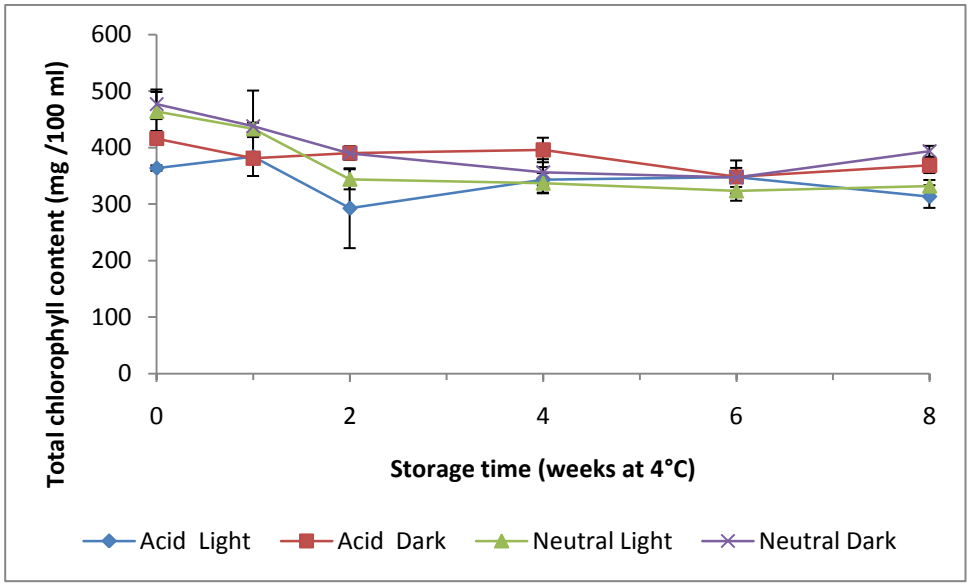


Figure 5.4 The change in mean total chlorophyll content (mg/100ml) of broccoli juice treatments over storage time at 4°C. Data are presented as means ±SE (n=3).

Table 5.9 (mg/100ml) total chlorophyll, chlorophyll *a* and chlorophyll *b* for broccoli juice treatments over storage time at 4°C. Data is means ± SE (n=36).

Juice pH	**Total chlorophyll**	**Chlorophyll *a***	**Chlorophyll *b***
Neutral	386±21.2	301±14.3	85±7.6
Acid	362±20.9	269±14.2	93±9.3

Table 5.10 ANOVA significant difference (*p* values) of total chlorophyll, chlorophyll *a* and chlorophyll *b* for all neutral or acidified broccoli juice treatments.

***p* values**	**Total chlorophyll**	**Chlorophyll *a***	**Chlorophyll *b***
pH (neutral vs. acid)	0.031	<0.001	0.056
Light (Light vs. dark)	0.002	<0.001	0.050
Storage time (week)	<0.001	<0.001	<0.001

The juice pH has a significant effect on the total chlorophyll level (Table 5.10) with the neutral broccoli juice treatments having more total chlorophyll (p=0.031) and a greater chlorophyll *a* (p<0.001) content than acidified broccoli juice which had greater levels of chlorophyll *b* (p=0.056).

The juice stored in the dark had significantly higher mean chlorophyll *a* (p=0.002), chlorophyll *b* (p<0.001) and total chlorophyll (p=0.050) than in juice exposed to light in clear bottles. The chlorophyll content of the broccoli juice significantly reduced according to the length of time the broccoli juice was stored (p<0.001) (Table 5.10). The same trend in chlorophyll content reduction over time for total chlorophyll shown in Figure 5.4 was apparent for chlorophyll *a* and *b* (data not shown). The mean chlorophyll content was significantly lower in weeks 2-8 than it was in weeks 0 and 1. There is an initial reduction in chlorophyll levels from weeks 0 to week 1 and 2. From week 2 to week 8 there is no significant difference in chlorophyll levels for chlorophyll *a* or *b* and hence total chlorophyll. This suggests the deterioration reactions for chlorophyll occurred in the first two weeks of storage, after which the levels remain relatively unchanged.

Figure 5.4 contains some large standard errors which may be due to the chlorophyll content being determined from samples that had been frozen. The chlorophyll to pheophytin conversion has been documented in frozen foods stored lower than -18°C. It is responsible for the colour change from a bright green to a dull olive green in frozen vegetables (Daood 2003). This could be responsible for the variation in the broccoli juice results at all time periods.

Turkmen et al (2006) measured chlorophyll *a* (4.39±0.27 mg/g dry matter) and chlorophyll *b* (2.55±0.13 mg/g dry matter) in fresh broccoli. In comparison chlorophyll levels in broccoli juice are very low. Neutral broccoli juice contained chlorophyll *a* (0.15±0.00 mg/g dry matter) and chlorophyll *b* (0.04±0.00 mg/g dry matter). Similarly acidified broccoli juice has chlorophyll *a* (0.14±0.00 mg/g dry matter) and chlorophyll *b* (0.05±0.00 mg/g dry matter).

5.4.5 Total carotenoids

Carotenoids are found universally in the chloroplasts of photosynthetic tissues but are often masked by the green chlorophylls (Lister & Bradstock 2003; Rodriguez-Amaya 2003; Schieber & Carle 2008; Coultate 2009; Farnham & Kopsell 2009). Chlorophyll is lost naturally from the plant leaves at the end of the plants active life in conjunction with the general breakdown of the chloroplast membranes, but carotenoids are more stable and are responsible for producing the residual yellow colour in autumn leaves and aging vegetables (Coultate 2009). The intensity and hue of a plant is influenced by which of the 600 carotenoids are present, their concentration and physical state (Rodriguez-Amaya 2003; Schieber & Carle 2008). There are two main groups of carotenoids: xanthophylls, which contain oxygen, and carotenes, which are hydrocarbons. Xanthophylls are the dominant pigment in yellow tissues where carotenes produce orange or red pigments (Coultate 2009).

The dominant carotenoids in green leafy plants are β-carotene and xanthophyll lutein (Lister & Bradstock 2003; Schieber & Carle 2008; Coultate 2009; Farnham & Kopsell 2009). In broccoli carotenoids were present even though the dominant juice pigment was green. Carotenoids are classed as antioxidants, are the most important source of provitamin A in the human diet with increasing evidence supporting their protective effect against chronic ailments such as cardiovascular disease and age-related macular degeneration (Daun 2005; Farnham & Kopsell 2009).

The total carotenoids were measured and calculated as described in Section 3.3.2.7 and the results shown as mg/100ml in Figure 5.5.

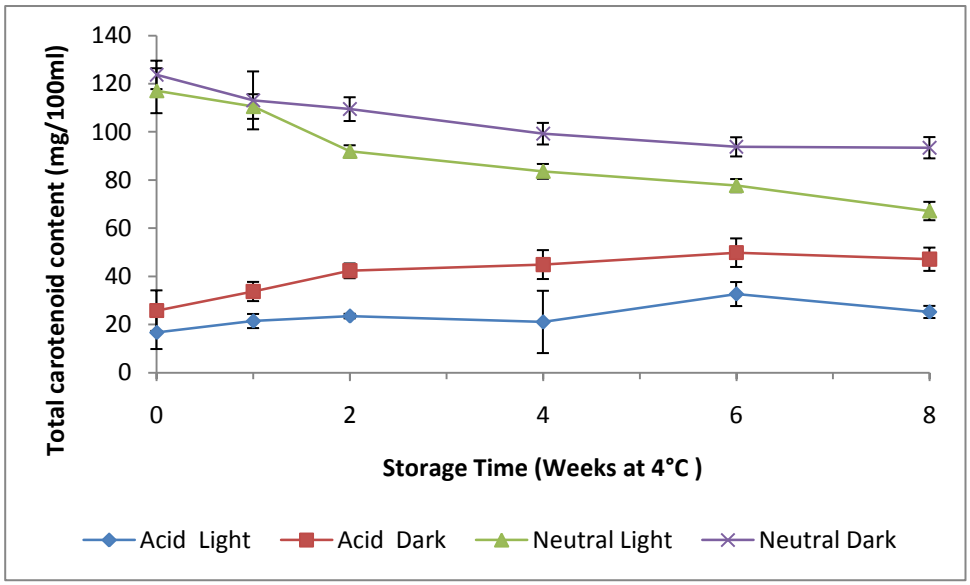


Figure 5.5 Total carotenoids (mg/100ml) for broccoli juice treatments over storage time at 4°C. Data are presented as means ±SE (n=3).

Figure 5.5 shows that there was a significantly greater mean carotenoid content in neutral broccoli juice (98±5.2 mg/100ml) than in the acidified broccoli juice (32±5.3 mg/100ml) (p<0.001). The carotenoid content of the broccoli juice treatments stored in the dark were significantly greater than the broccoli juice treatments stored exposed to light in clear bottles (p<0.001). Exposure to light therefore reduced the carotenoid content in broccoli juice. The time the broccoli juice spent in storage also reduced the carotenoid content. The acid treated juice generally had a significantly lower mean level of carotenoids than the neutral treatments.

Figure 5.5 shows the neutral juices all decreased in carotenoid content during storage and while the carotenoid content of the acidified treatments remained relatively similar if stored in the light but increased in the dark (p<0.001) due to the antioxidant activity of carotenoids (Daun 2005).

The average carotenoid content over all treatments in neutral and the acidified broccoli juice were relatively low. Zhang and Hamauzu (2004) measured total carotenoid levels in raw broccoli floret 3.75±0.25 mg/100g fresh weight and stem 0.010±0.03 mg/100g fresh weight.

5.5 Colour

The purpose of this investigation was to observe the changes in colour over time and compare the different broccoli juice treatments.

The lightness, chroma and hue angle colour measurements of the broccoli juice treatments were determined according to the methods in Section 3.3.2.6. Lightness (L*) measures the luminosity of a colour. This relates the colour to a number on the grey scale between black (0) and white (100). Chroma (C*) is the saturation of the colour starting from 0 and increasing in colour saturation. Values increase according to the purity of the hue. Hue angle (h) is the colour of a sample as defined by its location a 360° axis. The scales starts at 0° (or 360°) and denotes red, 90° is yellow, green at 180° and blue at 270°. The total colour difference value (ΔE*) is a single dimensionless value that takes into account differences between the L*, a* and b* of the sample and the standard. In this case the standard was the week 0 measurements.

Photographs of the 24 thawed juice treatments after shelf life storage and subsequent freezing are shown in Figure 5.6. Photos were all taken at the same time using the same light conditions as variations in the light conditions influence the colour of an object significantly (Leggett 2008). The photos were used as a point of reference when interpreting the instrumental results found in Table 5.11 with the trends presented in Figure 5.7 lightness, Figure 5.8 chroma, Figure 5.9 hue angle and Figure 5.10 total colour difference.

There was a visual difference between the neutral broccoli juice treatments (a Neutral Light and b Neutral Dark) and the acid broccoli juice treatments (c Acid Light and d Acid Dark) (Figure 5.6). The acidified broccoli juice treatments (L = 40.0 ± 0.02) were statistically significantly ($P<0.001$) lighter than the neutral broccoli juice treatments (L= 37 ± 0.02) however Table 5.11 and Figure 5.7 show only a small difference in initial values at week 0. The chroma of the neutral broccoli juice treatments (C = 9.0±0.02) was statistically significantly ($P<0.001$) more saturated than the acidified juice (C = 8.7±0.02) despite there being only small fluctuations in values overall in Figure 5.8 and Table 5.11. The hue angle was significantly ($P<0.001$) greater in the neutral broccoli juice (h^o = 112.23±0.04) making them more green than the acidified broccoli juice treatments (h^o = 110.23±0.04) (Table 5.11).

a

b

c

d

Figure 5.6 Photos of broccoli juice shelf life trial treatments.
The bottles are representative of sub-samples taken from all 24 treatments of the shelf life experimental design. All samples (A, acidified, N, neutral) were stored under the same light source at 4°C. All the dark samples (D) were covered in aluminium foil to exclude the light. The foil has been removed for the photos. All the Light samples (L) were stored as shown in clear glass bottles. The bottles were stored at 4°C for different time periods (0, 1, 2, 4, 6 or 8 weeks), then frozen until analysed. a) Neutral Light, b) Neutral Dark, c) Acid Light, d) Acid Dark.

Table 5.11 Colour values for neutral and acidified broccoli juice in light or dark storage over an eight week period.

Juice pH	Storage	Week	Lightness		Chroma		Hue angle		ΔE	
Neutral	Light	0	37	±0.1	9	±0.1	118	±0.1	0.0	±0.2
Neutral	Light	1	37	±0.0	9	±0.0	115	±0.1	0.4	±0.2
Neutral	Light	2	37	±0.0	9	±0.0	113	±0.1	0.9	±0.2
Neutral	Light	4	37	±0.0	8	±0.1	110	±0.1	1.6	±0.2
Neutral	Light	6	37	±0.0	8	±0.0	109	±0.1	1.8	±0.2
Neutral	Light	8	37	±0.0	8	±0.0	107	±0.1	2.1	±0.2
Neutral	Dark	0	37	±0.2	10	±0.1	118	±0.2	0.0	±0.4
Neutral	Dark	1	37	±0.0	9	±0.1	115	±0.1	1.2	±0.3
Neutral	Dark	2	37	±0.0	9	±0.0	113	±0.2	1.6	±0.3
Neutral	Dark	4	35	±0.2	9	±0.0	111	±0.2	1.8	±0.4
Neutral	Dark	6	37	±0.0	9	±0.0	109	±0.1	2.1	±0.3
Neutral	Dark	8	37	±0.1	9	±0.0	108	±0.1	2.2	±0.3
Acid	Light	0	40	±0.1	8	±0.2	112	±0.2	0.0	±0.6
Acid	Light	1	40	±0.0	9	±0.0	112	±0.0	0.5	±0.5
Acid	Light	2	40	±0.0	8	±0.1	111	±0.2	0.5	±0.5
Acid	Light	4	40	±0.0	8	±0.1	110	±0.3	0.4	±0.5
Acid	Light	6	40	±0.0	9	±0.1	108	±0.1	0.7	±0.5
Acid	Light	8	40	±0.0	9	±0.1	107	±0.1	0.9	±0.5
Acid	Dark	0	39	±0.2	9	±0.1	111	±0.1	0.0	±0.4
Acid	Dark	1	40	±0.0	9	±0.0	111	±0.1	1.0	±0.4
Acid	Dark	2	40	±0.1	9	±0.0	111	±0.1	1.0	±0.4
Acid	Dark	4	39	±0.1	9	±0.1	111	±0.3	0.6	±0.4
Acid	Dark	6	40	±0.0	9	±0.0	109	±0.1	0.8	±0.4
Acid	Dark	8	40	±0.0	9	±0.0	108	±0.1	0.9	±0.4

Figure 5.9 shows that the difference is greatest in the first three weeks of storage then both neutral and acidified juices follow a similar downward trend. The neutral broccoli juice treatments had the greatest change in total colour difference overall compared to the acidified broccoli juice treatments as shown in Figure 5.10.

Visually in Figure 5.6, the difference between light and dark treatments is less dramatic with the dark treatments being slightly more vibrant than the light treatments for both neutral and acid broccoli juices. Figure 5.10 shows that exposure to light did have an effect on the colour of the juices at both pH profiles with the neutral juices becoming significantly darker than the juices stored in the dark (p<0.001). The chroma significantly changed with the dark stored juices becoming more saturated than the juices exposed to light (p<0.001). The hue angle of the juice stored in the dark was significantly more green than the juice exposed to the light (p<0.001) although this is not really obvious in Figure 5.9 but a difference in hue values can be found in Table 5.11. Storage in light conditions produced slightly lower total colour difference than treatments stored in the dark. With the neutral dark having the greatest total colour difference.

The visual change in colour over time Figure 5.6 (from left to right) is most evident in the neutral broccoli juice treatments. The acid broccoli juice treatments appear to have little change over storage time. The lightness measurements shown in Figure 5.7, Table 5.11 and the photos in Figure 5.6 show that all the broccoli juice treatments got significantly darker over storage time (p<0.001). In Figure 5.7 the lightness results for both neutral and acid dark stored juices at week 4 of storage at 4°C show an uncharacteristic drop in value. This suggests a mixing error. The data points are treated as outliers and the Figure 5.7 assumes the trend is followed. Table 5.11 shows the chroma of the neutral and acidified juices became significantly less saturated with storage time (p<0.001). Both the neutral and acidified broccoli juice treatments become more yellow as the hue angle decreased significantly over storage time (p<0.001). Figure 5.10 shows all the broccoli juice treatments changed over storage time with the neutral treatments having the greatest overall change.

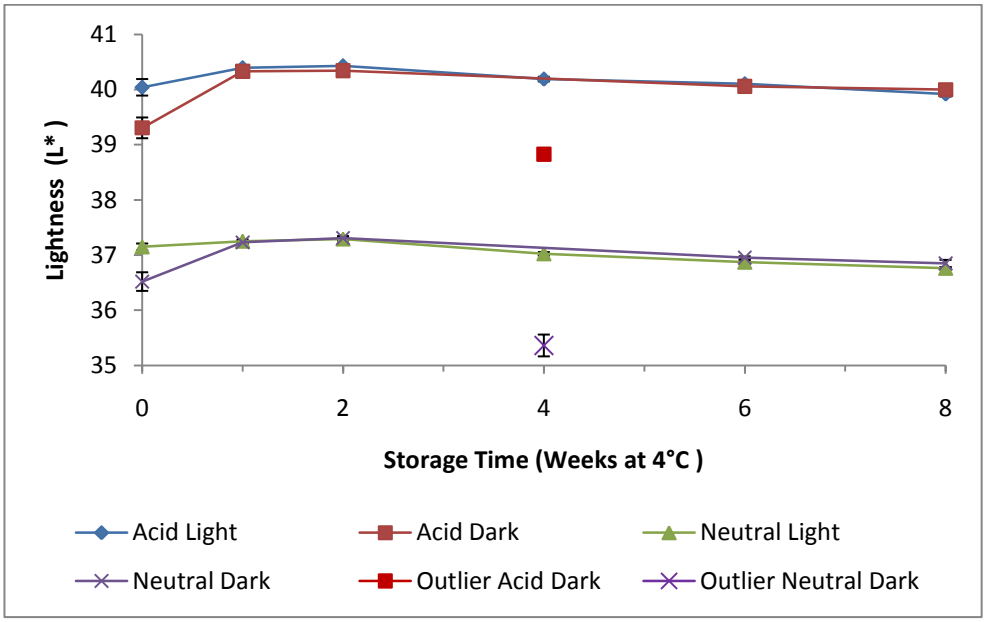


Figure 5.7 Lightness values for broccoli juice treatments over storage time at 4°C. Data are presented as means ± SE (n=6). Values relate to the difference in lightness, with 0 black and 100 = white.

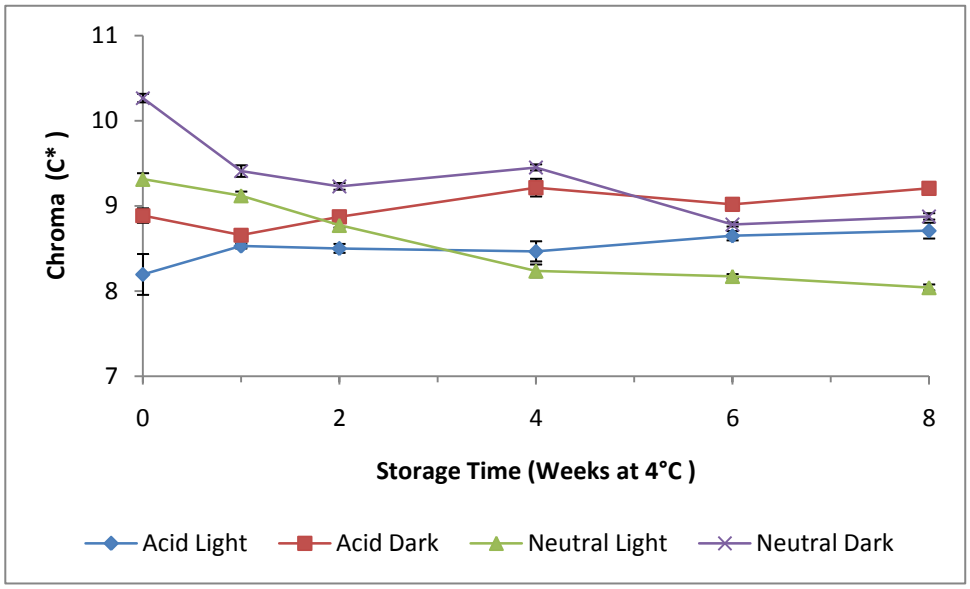


Figure 5.8 Chroma values for broccoli juice treatments over storage time at 4°C. Data are presented as means ± SE (n=6). Values increase in purity of hue from 0.

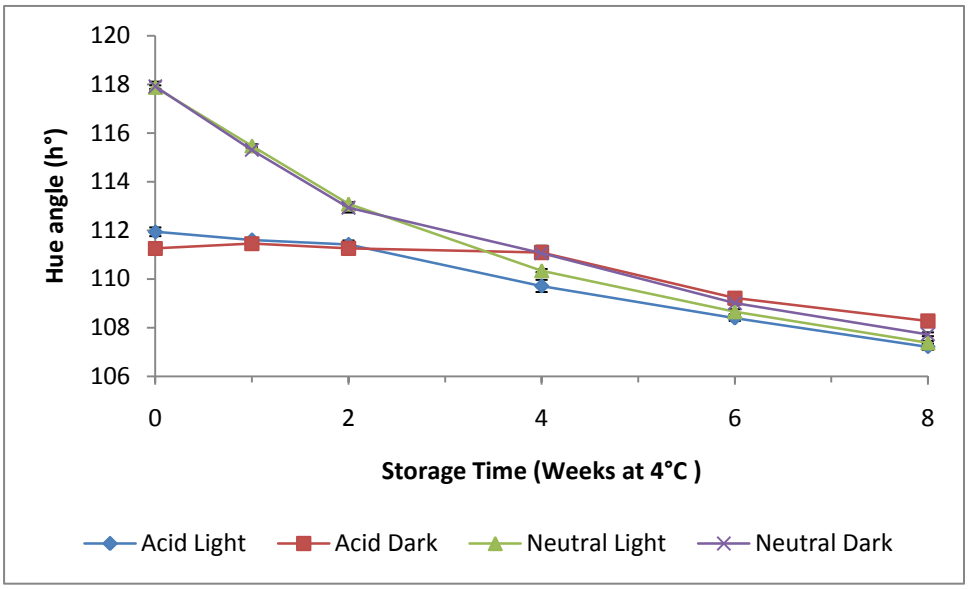


Figure 5.9 Hue angle values for broccoli juice treatments over storage time at 4°C. Data are presented as means ± SE (n=6). 60° = yellow, 120° = green.

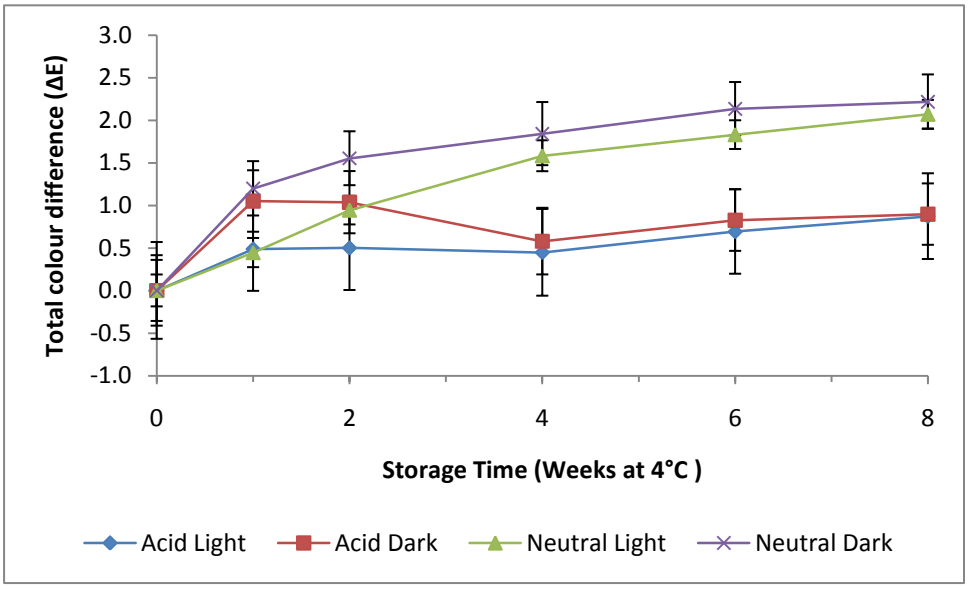


Figure 5.10 ΔE the total colour difference for broccoli juice treatments over storage time at 4°C. Data are presented as means ± SE (n=6).

5.6 Sensory Evaluation

Vegetable flavours are composed of a wide range of chemical compounds from non-volatile taste-active (including both inorganic and organic compounds) to volatile aroma-active organic molecules. The aroma components often determine the distinct flavour of the vegetable (Cadwallader 2005). Flavour is defined as the integrated perception of aroma (odour) and taste, and to a lesser extent pain or nerve response (e.g. heat of capsaicin), texture and mouth feel and overall appearance (Cadwallader 2005).

The objective of the sensory evaluation was to determine if the sensory profile of neutral and acidified broccoli juice changed over time during storage in either light or dark conditions.

The sensory attributes of neutral and acidified broccoli juice were evaluated by a trained sensory panel (n=12). Panel recruitment and training are described in Section 3.3.3. Broccoli juice used for the sensory assessment was sampled and stored as detailed in Section 3.2. An ANOVA was conducted on the assessment data with the panellist variability used as a blocking factor. The main effects of light and storage time were determined and included linear and non linear effects. Figure 5.11 presents the sensory profiles of the neutral and acidified reference juices used as reference samples during the training and assessment of the shelf life samples. The average results for the assessment data follow similar profiles as the reference juices (Figures 5.12 and 5.13).

A summary of attributes with significant results (p<0.05) from ANOVA for neutral broccoli juice treatments are displayed in Table 5.12 and Figure 5.12 with the acidified juice results found in Table 5.13a and Table 5.13b and Figure 5.13. The tables' detail the average attribute scores for each time period, the least significant differences of means at a 5% level and probability values. All the average attributes scores for each time period and the associated ANOVA results for the neutral juice are found in Appendix 5 and in Appendix 6 for the acidified juice.

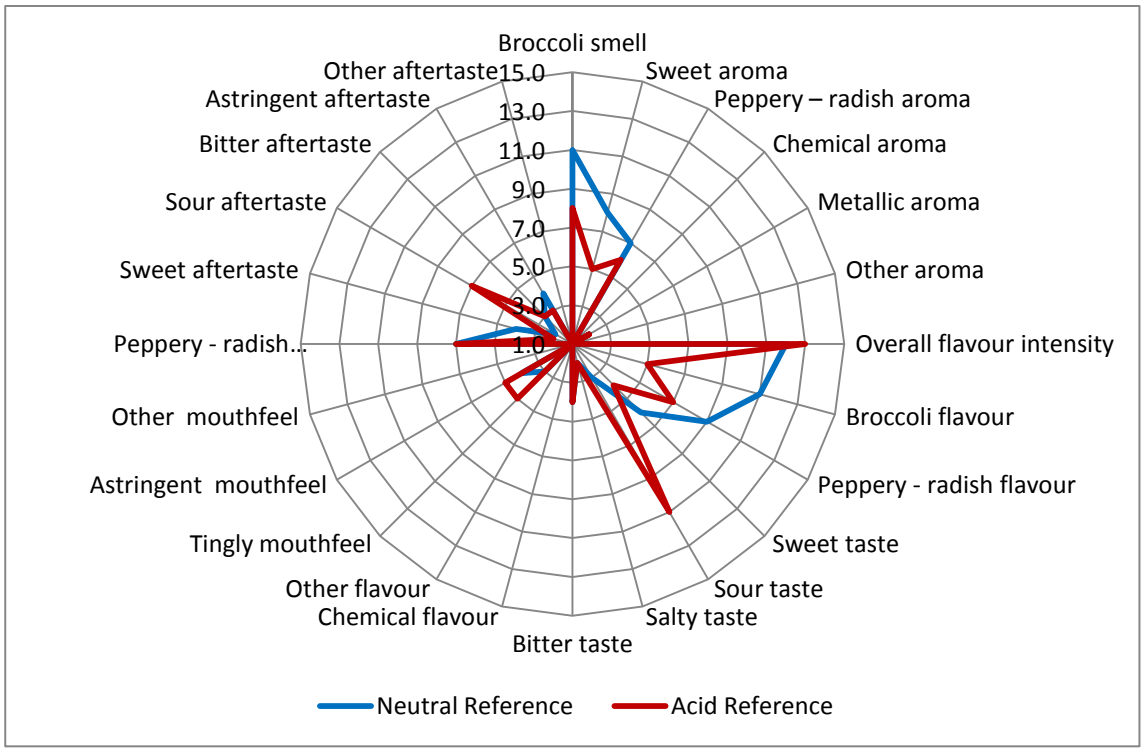


Figure 5.11 Spider plot of the sensory profiles of neutral and acidic reference broccoli juices.

The reference juice profiles were determined using consensus panel training as described in Section 3.3.3. The neutral broccoli juice had more intense broccoli, sweet and peppery radish aromas and flavours. The overall flavour intensity was slightly greater in the acidic broccoli juice. Sourness (taste and aftertaste) dominates the acidified broccoli juice sensory profile. There are also differences between the juices in astringent mouthfeel, sweet aftertaste and bitter aftertaste. Due to the tremendous differences between the profiles of the neutral and acidified broccoli juices it was decided to hold separate assessment sessions for neutral and acidified broccoli juices as shown in Figure 5.11. It should be noted that although neutral and acidified broccoli juices were not statistically compared directly with each other, they were scored using the same descriptors and rating scale.

5.6.1 Neutral broccoli juice

The spider plot of the neutral broccoli juice (Figure 5.12) shows the mean attribute scores for each of the six sampling periods. The light and dark samples have been combined due to their lack of variation (Table 5.12). The profiles for each week are similar indicating very little difference in the sensory profile of the neutral juice over the 8 weeks in refrigerated storage. Overall the aroma attributes; broccoli smell (10.6 to 11.1), sweet aroma (7.3 to 7.8) and peppery-radish aroma (7.0 to 7.3) became slightly more intense during the refrigerated storage of the neutral broccoli juice. The flavour, taste and after taste attributes showed little variation throughout the shelf life trial.

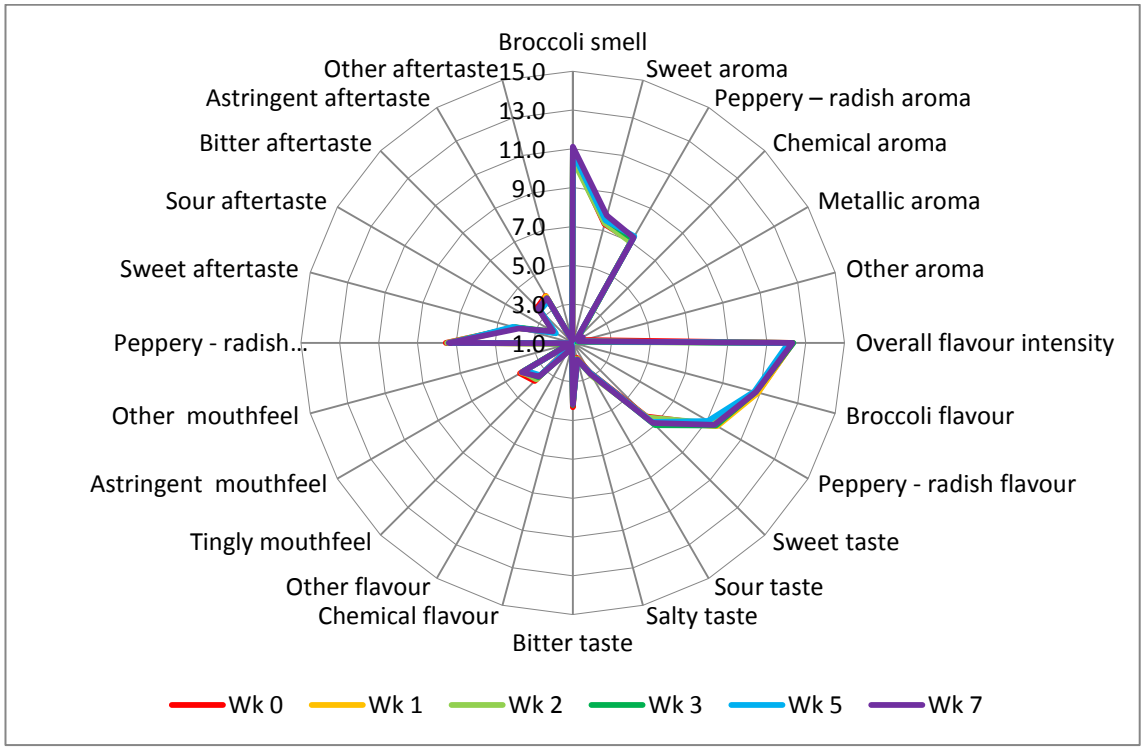


Figure 5.12 Spider plot of the means for neutral broccoli juice over 8 weeks storage time. Data is means of duplicate assessments and trained panellists (n=24).

The attributes for neutral broccoli juice with statistically significant results from the ANOVA are displayed in Table 5.12. Full details for all the 24 attributes can be found in Appendix 5.

The peppery-radish flavour was found to be stronger in juices stored in dark compared to the juice exposed to the light (p=0.049) as highlighted in Table 5.12. During storage the intensity of the metallic aroma (p=0.014), the broccoli smell and sweet aroma increased. Broccoli smell (p=0.013) (10.6 to 11.1) and sweet aroma (p=0.040) (7.3 to 7.8) increased in intensity following a linear trend over time whereas the metallic aroma increased following both a linear (p=0.046) and a nonlinear (p=0.031) variable trend as shown in Table 5.12 with scores increasing from 1.5 to 1.6 in light and 1.4 to 1.5 in dark but the scores fluctuated over time rather than increasing at a steady rate. The tingly mouthfeel decrease during storage time following a linear (p=0.043) pattern.

The assessment of chemical aroma resulted in a statistically significant interaction between light and dark storage and the time in storage (p=0.023). This is a non linear trend (p=0.011) with chemical aroma being stronger in light stored juices (1.5) but decreasing over storage time at a variable rate in both light (1.2) and dark juices (1.3).

Table 5.12 Summary of significant attributes from ANOVA for neutral broccoli juice treatments. Significance = $p<0.05$ (shown in **bold**).

Storage time (Neutral)	Broccoli smell	Sweet aroma	Chemical aroma	Metallic aroma	Peppery - radish flavour	Tingly mouthfeel
Light						
0	10.5	7.3	1.5	1.5	9.1	3.8
1	10.8	7.4	1.2	1.4	9.5	3.4
2	10.7	7.5	1.5	1.3	9.1	3.6
4	11.0	7.7	1.5	1.4	9.1	3.3
6	11.0	7.9	1.5	1.4	9.0	3.5
8	11.2	7.6	1.2	1.6	9.5	3.4
Dark						
0	10.7	7.4	1.3	1.4	9.8	3.8
1	10.6	7.5	1.6	1.4	9.7	3.7
2	10.2	7.2	1.3	1.4	9.5	3.7
4	10.8	7.5	1.4	1.5	9.9	3.7
6	10.6	7.2	1.2	1.3	9.0	3.3
8	11.0	8.0	1.3	1.5	9.4	3.5
Least significant differences of means (5% level)						
Light- dark	0.27	0.27	0.12	0.08	0.32	0.21
Store- time	0.47	0.47	0.21	0.15	0.55	0.36
Light- dark. store- time	0.67	0.67	0.30	0.21	0.78	0.51
F test probability values (significance $p<0.05$)						
Light- dark	0.141	0.381	0.328	0.722	**0.049**	0.220
Store- time	0.092	0.430	0.798	**0.014**	0.431	0.260
Linear	**0.013**	**0.040**	0.238	**0.046**	0.405	**0.043**
Nonlinear	0.493	0.963	0.917	**0.031**	0.382	0.658
Light- dark. store- time	0.799	0.283	**0.023**	0.654	0.574	0.633
Linear	0.476	0.818	0.797	0.502	0.206	0.563
Nonlinear	0.767	0.187	**0.011**	0.585	0.695	0.542

5.6.2 Acidified broccoli juice

The spider plot of the acidified broccoli juice (Figure 5.13) shows the mean attribute scores for each of the six sampling periods. The light and dark samples have been combined due to their lack of variation. The profiles for each week are similar indicating only a few little changes in the sensory profile of the neutral juice over the 8 weeks in refrigerated storage. Overall the aroma attributes; broccoli smell (8.0 to 6.9), sweet aroma (4.9 to 4.3) and peppery-radish aroma (6.3 to 5.9) reduced in intensity during the refrigerated storage of the neutral broccoli juice.

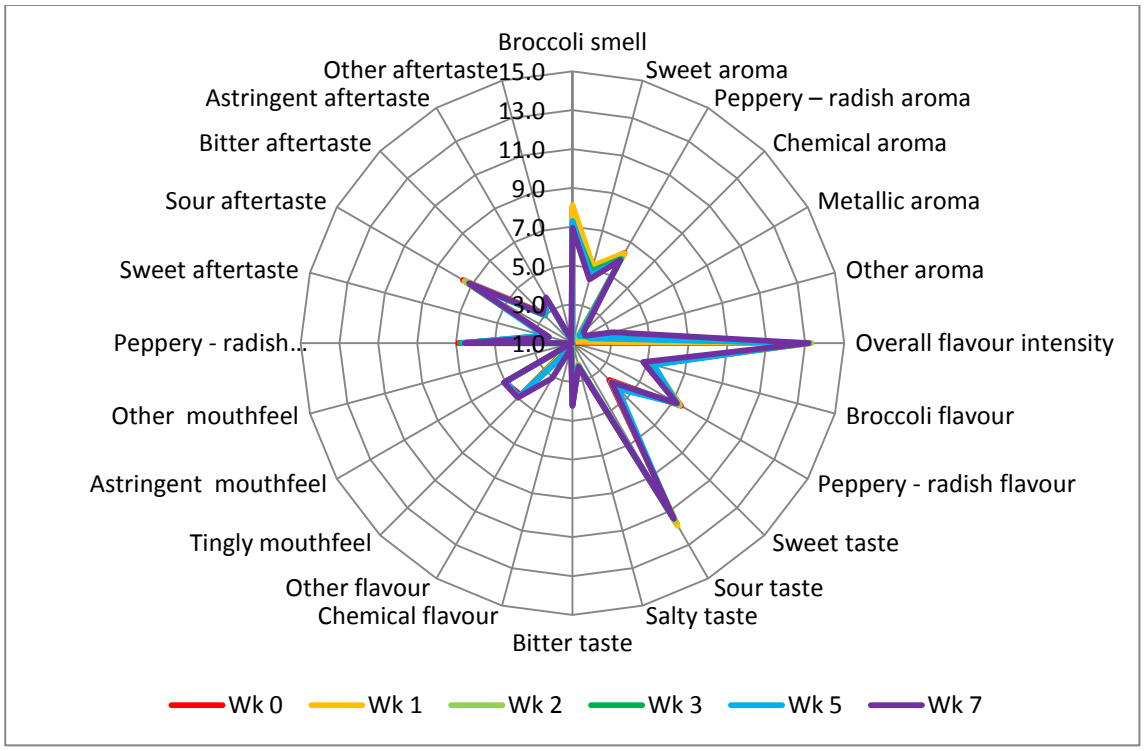


Figure 5.13 Spider plot of the means for acidified broccoli juice over 8 weeks storage time. Data is means of duplicate assessments and trained panellists (n=24)

Table 5.13a and Table 5.13b detail the attributes with significant results from the ANOVA for the acidified broccoli juice profile. Full details for all the attributes can be found in Appendix 6.

As with the neutral juice there are significant changes in the aroma attributes during the shelf life trial. During storage the intensity of broccoli smell ($p<0.001$) decreased from 7.8 to 6.8 in the light stored juice and from 8.1 to 7.0 in the dark stored juice. The sweet aroma was statistically more intense in the dark stored acidified juice compared with the juice stored in the light ($p=0.045$) but overall the sweet aroma decreased with storage ($p=0.003$) however this was only a decrease less than one point from 5.0 to 4.3 in the dark stored juice and even less from 4.7 to 4.3 in the light juice. The peppery radish aroma also decreased over time following a linear trend ($p=0.011$).

Both the chemical aroma ($p=0.003$) and chemical flavour ($p=0.022$) increased in intensity over time in storage with the dark stored juice having the stronger chemical aroma ($p=0.001$) (from 1.3 to 2.4) and chemical flavour ($p=0.031$) (from 1.1 to 1.5) compared to light storage over the whole eight week storage period.

The intensity of the broccoli flavour ($p=0.038$) and sweet taste ($p=0.026$) fluctuated over storage time with an overall decrease in strength. The sour taste decreased at a variable rate over storage time ($p=0.052$) and was stronger in the dark stored juice ($p=0.032$).

The bitter taste of the acidified juice was stronger in juice stored in the dark ($p=0.052$) compared to juice stored in the light. The tingly mouthfeel was stronger overall in the dark stored juices ($p=0.041$) compared to the light stored juice and the tingly mouthfeel varied in intensity over time ($p=0.026$). Sour after taste reduced during storage time ($p=0.013$) whereas the astringent aftertaste became more intense ($p=0.049$) during storage time with the light stored juices being stronger than the dark stored juices.

Table 5.13a Summary of significant attributes from ANOVA for acidified broccoli juice treatments. Significance= $p<0.05$ (shown in **bold**).

Storage time (Acid)	Broccoli smell	Sweet aroma	Peppery – radish aroma	Chemical aroma	Sour aftertaste	Astringent aftertaste
Light						
0	7.8	4.7	6.4	1.4	7.3	3.2
1	8.0	5.2	6.2	1.4	7.3	3.5
2	6.9	4.5	6.0	1.4	7.4	3.6
4	6.7	4.5	5.7	1.8	7.3	3.6
6	7.4	4.6	5.9	1.5	7.2	3.6
8	6.8	4.3	5.9	1.5	6.9	3.8
Dark						
0	8.1	5.0	6.3	1.3	7.8	3.8
1	8.2	4.9	6.3	1.4	7.6	3.5
2	7.5	5.1	6.1	1.4	7.4	3.7
4	7.4	5.1	6.2	1.4	7.0	3.6
6	7.1	4.5	5.8	1.7	7.1	3.5
8	7.0	4.3	5.9	2.4	7.5	3.7
Least significant differences of means (5% level)						
Light- dark	0.30	0.22	0.23	0.18	0.21	0.15
Store- time	0.53	0.38	0.40	0.31	0.36	0.26
Light- dark. store- time	0.74	0.53	0.57	0.44	0.50	0.36
F test probability values (significance $p<0.05$)						
Light- dark	0.080	**0.045**	0.407	0.592	0.074	0.309
Store- time	**<0.001**	**0.003**	0.114	**0.003**	0.074	0.443
Linear	**<0.001**	**<0.001**	**0.011**	**<0.001**	**0.013**	0.151
Nonlinear	**0.019**	0.715	0.648	0.730	0.414	0.606
Light- dark. store- time	0.504	0.096	0.555	**0.003**	0.080	0.116
Linear	0.364	0.559	0.863	**0.001**	0.999	**0.049**
Nonlinear	0.478	0.061	0.417	0.088	**0.044**	0.285

Table 5.13b Summary of significant attributes from ANOVA for acidified broccoli juice treatments. Significance= $p<0.05$ (shown in **bold**).

Storage time (Acid)	Broccoli flavour	Sweet taste	Sour taste	Bitter taste	Chemical flavour	Tingly mouthfeel
Light						
0	5.2	3.6	11.7	4.2	1.1	4.6
1	5.3	3.6	12.0	3.9	1.2	5.2
2	5.0	3.9	11.1	4.0	1.1	4.9
4	5.0	4.3	11.3	3.9	1.3	5.0
6	5.4	4.6	11.5	4.3	1.2	5.2
8	5.0	3.7	11.3	4.0	1.2	4.6
Dark						
0	5.1	3.7	11.8	4.3	1.1	5.0
1	5.2	4.0	11.6	4.4	1.2	4.8
2	5.2	3.7	12.2	4.0	1.1	5.2
4	5.5	3.9	11.3	4.1	1.2	4.9
6	5.2	4.1	11.2	4.2	1.4	4.7
8	4.5	3.9	11.6	4.5	1.5	5.4
Least significant differences of means (5% level)						
Light- dark	0.21	0.25	0.30	0.21	0.13	0.24
Store- time	0.37	0.44	0.52	0.36	0.22	0.42
Light- dark. store- time	0.52	0.62	0.73	0.52	0.32	0.59
F test probability values (significance $p<0.05$)						
Light- dark	0.846	0.649	0.490	**0.052**	0.206	0.568
Store- time	**0.038**	**0.026**	0.166	0.607	0.238	0.825
Linear	0.056	0.062	**0.052**	0.827	**0.022**	0.533
Nonlinear	0.082	0.051	0.393	0.471	0.824	0.777
Light- dark. store- time	0.102	0.338	0.060	0.459	0.310	**0.041**
Linear	0.256	0.491	1.000	0.977	**0.031**	0.473
Nonlinear	0.094	0.267	**0.032**	0.326	0.864	**0.026**

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CHAPTER 6

Discussion

A shelf life trial was designed as detailed in Section 3.2 to evaluate the effect of acidification and retail beverage storage conditions on broccoli juice made on a semi commercial scale. The key questions to be answered were: can broccoli juice be made on a pilot scale and then what happens to the colour, composition and flavour during storage.

6.1 Pilot scale production of broccoli juice

Broccoli juice was successfully produced using the production process detailed in Section 3.1.2. Two process runs produced the 267 L of juice required to train the sensory panel and supply the shelf life trial.

Due to the experimental nature of the broccoli juice and the volumes required a pilot scale process was developed using the resources available in the Massey University pilot plant. The basic processes of cleaning, size reduction, juice extraction, mixing and pasteurisation produced 0.43 L broccoli juice per kg of wet weight raw broccoli. The use of different equipment may increase the yield and improve the efficiency of the process.

To produce the 161 L of juice for the shelf life trial, with the resources available it took three days to process the required 375 kg of raw broccoli. There were several constraints in the process resulting in delays that may have introduced sources of potential contamination and reduced the quality in the final juice. Blanched broccoli, pulp and juice all had storage delays (at 4°C) until they were combined prior to pasteurisation and aseptic bottling.

The physical structure of broccoli required it to be hand cut into florets and stalks which took significant time. Broccoli florets and stalk pieces needed to be evenly sized to allow for even blanching and small enough to be fed into the mincer. Research by Galgano et al (2007) suggests that developing a method of evenly blanching whole broccoli heads would not be feasible. This is due to the compositional differences between the florets and the stalk and their degradation reactions to heat occurring at different rates. If hand cutting remains the

only option then increasing the size of downstream equipment could be beneficial or making the blanching and cooling step continuous.

The biggest time and process constraint was the capacity of the water press. The 20 kg water press was too small for the volume of pulp to be pressed. Only 5 kg of pulp could be pressed per load, with each press cycle taking five minutes; it took 10 hours to press all the pulp to achieve 161 L of juice. A larger 80 kg press that could extract the juice in one or two pressings is recommended.

The overall process design lends itself to a batch process due to the fact that part of the juice needed to be acidified. At some stage of the process there will be time delays, effectively managing these will minimize contamination and degradation potential. The processing conditions used had several limitations resulting in low juice yield and delays may have compromised the microbial and compositional quality of the broccoli juice. Nevertheless, the broccoli juice produced for the shelf life trial met microbiological safety requirements and was palatable.

Frozen storage at -20°C was used to retard any degradation processes that occurred during the storage at 4°C. Frozen juice from different time periods could also be assessed at the same time. Some degradation processes still occur at -20°C and for highly sensitive samples storage in a -80°C freezer is recommended. Due to the large number of juice samples and storage in glass bottles, -80°C frozen storage was not feasible and also not a realistic commercial practice.

6.2 Broccoli juice in a retail environment

The results in Figure 6.1 summarise what happened to the colour, composition and flavour of broccoli juice during simulated retail storage over the storage time of the four treatments in the shelf life trial.

	Neutral		Acid	
	Light	**Dark**	**Light**	**Dark**
				
	0NL	0ND	0AL	0AD
Microbiological	No Δ	No Δ	No Δ	No Δ
Juice pH	No Δ 6.1±0.01	No Δ 6.1±0.02	No Δ 3.8±0.02	No Δ 3.8±0.01
Dry matter (g/100ml)	No Δ 5.0±0.02	No Δ 5.1±0.04	No Δ 5.3±0.05	No Δ 5.2±0.09
Soluble solids (°Brix)	↔ 5.2±0.11	↔ 5.1±0.09	↔ 4.9±0.03	↔ 4.9±0.05
Titratable acidity (g/L citric)	No Δ 0.8±0.00	No Δ 0.8±0.01	No Δ 5.8±0.04	No Δ 5.8±0.05
Sugar acid ratio	6.5±0.22	6.4±0.22	0.8±0.00	0.9±0.01
Dietary fibre (mg/100ml)	No Δ 307±5.9	No Δ 301±7.6	No Δ 394±12.2	No Δ 379±12.1
Pectin (mg/100ml)	No Δ 85±6.4	No Δ 84±9.8	No Δ 74±8.2	No Δ 77±9.1
Ascorbic acid (mg/100ml)	↓84% 12-2	↓71% 15-4	↓76% 14-3	↓58% 15-6
Total chlorophyll (mg/100ml)	↘→ 464-332	↘→ 477-394	↘→ 364-313	↘→ 416-369
Total carotenoids (mg/100ml)	↓117-67	↓124-93	17↔25	26↔47
Colour (L= lightness, C= chroma, h = hue angle)	↓ L =37±0.0 C= 9±0.0 h=112±0.1	↓ L =37±0.1 C= 9±0.0 h=112±0.1	↘→ L =40±0.0 C= 9±0.1 h=110±0.1	↘→ L =40±0.1 C= 9±0.1 h=110±0.1
Sensory (Broccoli smell) (1-15 intensity scale)	10.9	10.7	7.6	7.3

Figure 6.1 Shelf life trial summary of trends

Key: Data are presented as means ± SE (n=24)

↓ Decreasing trend over time
↘→ Initial decrease then no change over time
No Δ No change over time
↔ Variable data over time but not significant and no increasing or decreasing trend

6.2.1 Effect of acidity on composition

The pH and titratable acidity of the neutral and acidified juices remained constant throughout the eight weeks of storage, the soluble solids fluctuated over time but there was no increasing or decreasing trend. The dry matter, dietary fibre and soluble pectin content did not change from their original levels during storage. The acidified juices had more dry matter and dietary fibre, but the neutral juice had greater levels of soluble pectin.

During the juice production two heat treatments were used, the broccoli was blanched prior to juicing and then the juice was pasteurised at either 95°C for neutral juice or 85°C for the acidified juice. Heat causes substantial changes in pectin solubility in water with water soluble pectin content increasing with increasing heat treatment temperature. The changes in pectin solubility as a results of thermal processing is linked to the β-elimination depolymerisation of pectin (Fraeye et al. 2007; Sila et al. 2009; Van Buggenhout et al. 2009). Increasing water soluble pectin at higher treatment temperatures is an explanation for the increased soluble pectin content in the neutral juice as it was pasteurised at a higher temperature than the acidified juice with lower soluble pectin levels.

All the juice was filtered through a 150µm mesh to minimise the fouling in the pasteuriser. The filtering removed the total solids and dietary fibre not already discarded as pomace which still included a mixture or whole and ruptured plant cells as the process only yielded 0.43 L juice per kg of wet weight raw broccoli. The resultant broccoli juice was a homogeneous cloudy juice rather than a juice with noticeable particulates like freshly squeezed orange juice. Cloud stability is a key visual characteristic and quality attribute of cloudy juices that influences consumer acceptance. The cloud particles influence the mouthfeel, flavour and colour. To be a stable suspension a juice cloud must have the appropriate specific gravity, particle size and charge. Cloud loss is associated with enzymatic precipitation of the cloud however prediction of the point at which the cationic precipitation occurs is difficult to determine (Sila et al. 2009).

The precipitation on acidification in the acid broccoli juices is a potential reason for the increase in total dietary fibre in the acid samples compared to the neutral samples, although the precipitate was unidentified. Although the neutral juices had greater levels of soluble pectin, when compared as a percentage of total dietary fibre, an average of 16% of the total dietary fibre in the acidified juices was soluble pectin and only 8% of the neutral juice total dietary fibre was soluble pectin.

6.2.2 Effect of light on composition

Glass bottles were chosen to reduce the oxygen transfer into the juice. In a retail environment juice is constantly exposed to light. The comparison of using clear bottles and covered or dark bottles in the same storage conditions is to determine impact of light on the shelf life of the broccoli juice, neutral or acidified.

The level of ascorbic acid at least halved over the eight weeks of storage. Both acidification and protection from light had a limiting effect on degradation of vitamin C with dark stored acidified juice only having a 58% decrease in ascorbic acid compared to neutral juice stored in the light with an 84% decrease in levels from week 0. The acidification of broccoli juice appeared to have a protective effect on the ascorbic acid content with the levels in the acidified juice being on average greater than the level of ascorbic acid in the neutral broccoli juice. The initial total ascorbic acid content in neutral juice was 14.7 ±0.9 mg/100ml and 14.9 ±0.9 mg/100ml in the acidified juice. After four weeks of refrigerated storage these levels dropped to 6.5 ±1.6 mg/100ml in the neutral juice and 7.5 ±0.2 mg/100ml in the acidified juice. The levels of total ascorbic acid or vitamin C in either the neutral or acidified broccoli juice were compared to the content in raw broccoli but processing, light, oxygen, alkaline pH and storage are all known to degrade ascorbic acid (Tosun & Sevinc 2008; Coultate 2009) Improvements in the processing efficiency would result in increased initial ascorbic acid levels would then need to be managed through light and oxygen protective packaging and chilled storage. In Australia and New Zealand the recommended daily intake (RDI) of vitamin C is 45 mg/day for both men and women (Anon 2005). Most fruit juices on the market have vitamin C added and the labels claim vitamin C levels of 40 mg/100ml (Anon 2009b, 2009c, 2009a). A 200ml glass of neutral or acidic broccoli juice with 6 mg/100ml of vitamin C would still provide 27% of an adults' daily requirement for vitamin C. Like other juices broccoli juice could have vitamin C added to increase the levels.

The exposure of the broccoli juice to light during storage reduced the carotenoid content with the carotenoid content in the dark stored juices being significantly greater than in the light stored juice ($p<0.001$). Multiple investigations by Schieber and Carle (2008) into the heating and illumination effects on carotenoid isomerisation and degradation suggest that data from model experiments are not applicable to complex food matrices and individual carotenes and pigments need measurement in their specific environments. Exposure to light predominantly

leads to the formation of 9-*cis*-β-carotene, where as heat treatments produce 13-*cis*-β-carotene, each of these isomers have different physical, chemical and biological properties (Schieber & Carle 2008).

6.2.3 Flavour discussion

The sensory profiles of the neutral and acidified juices were scored using the same descriptors and rating scale developed during the training sessions using both neutral and acidified reference juices. During the training period it was determined that as the neutral and acidified juices had such different sensory profiles that they could not be compared directly with each other in an assessment session without the acidified juice distorting the subtle changes in the neutral juice. Separate assessment sessions for neutral and acidified broccoli juice were held and as such the data from each was not statistically compared but as the same rating scale was used comparative observations have been made.

The greatest changes over time in both the sensory profiles were in the aroma attributes with the broccoli smell strongest in the neutral juices. There is a dramatic difference in the sensory profiles of neutral and acidified broccoli juice with the sourness of the acidified juice overpowering the perception of the other attributes such as sweetness. The ratio of sugar (°Brix) to acid (titratable acidity) influences the overall taste and sensory acceptance of a product and is often used as a measurement of the flavour quality and ripeness indicator in fruit, vegetables and their juices (Varnam & Sutherland 1994; Valentova & Panovska 2003; Cadwallader 2005).

The average sugar acid ratio of 6.5 in the neutral juice compared to 0.8 in the acidified juice is an effective representation scale of differences between the two juices. The smaller the ratio, the more unbalanced the juice becomes with the acid dominating the sensory impact. In comparison, acidified carrot juice (pH 4.4) has a sugar:acid ratio of 1.8 and non acidified (pH 5.6) carrot juice has a ratio of 5.5. Both juices were standardised to 8 °Brix and had titratable acidity in terms of citric acid of 4.40±0.07 g/L for acidified carrot juice and 1.48±0.02 g/L for non-acidified carrot juice (Reiter et al. 2003; Liang et al. 2006). The acidified carrot juice is

more balanced than the acidified broccoli juice as the higher soluble solids resulted in a sweeter perception (Schifferstein & Frijters 1990).

The acidified broccoli juice was acidified to less than pH 4.6 as a food safety measure. The sensory perception of the acidity of the acidified broccoli juice needs to be more balanced. The addition of salt, sugar and or different fruit juices is recommended to balance the flavour profile whilst still maintaining the desired pH <4.6. The addition of salt may actually increase the food safety potential of the juice by adding another microbiological hurdle into the system (Jay 1996).

Valentova and Panovska (2003) found sour and sweet tastes can inhibit the perception of each other and other results showed that the sourness of the acidified broccoli juice did appear to mask the perception of the sweetness (Schifferstein & Frijters 1990). The ratio of soluble solids to titratable acidity varied greatly between the neutral and acidic juice. This supports the sensory results which show the sourness of the acidic broccoli juice was very intense and distorted the sensory profile, suppressing the broccoli flavour, sweet flavour and peppery-radish flavour compared to the neutral juice profile. The detection of the bitter compounds increased in the acidified broccoli juice compared to the neutral broccoli juice. Bitter and acid compounds can influence the perception of each other depending on the concentration (Valentova & Panovska 2003).

A more desired sensory profile could be developed by acidification of the broccoli juice with other fruit juice rather than just acid may be beneficial in providing a more balanced flavour with the addition of the additional sugar and flavour compounds. Organic acids occur naturally in plant cells and are rarely found alone, and although all acids are sour they differ in the degree of sourness and in non-sour aroma and flavour characteristics according to their plant source (Hartwig & McDaniel 1995) such as citric acid from citrus fruit, malic acid from apples and tartaric from grapes. Hartwig and McDaniel (1995) found the flavour profiles of various food acids vary according to pH level from little difference between acid flavour profiles at pH 6.5 to large differences in the overall intensity and sourness of the different acids at pH 4.5. A dilution effect may also occur, reducing the sourness as more fruit juice would be required to achieve the same acidification compared to using a citric acid solution.

Carbon dioxide dissolves into food lowering the pH with the solubility increasing as the temperature decreases (Bari et al. 2005; Hewson et al. 2009). This may be a more subtle

method of acidification of the broccoli juice and it would be interesting to see if the degree of precipitation and colour change occurs compared to acidification with citric acid.

The final breakdown of β-carotene in leaves produces a residue β-ionone, a volatile compound which gives sun-dried and partially bleached hay its characteristic odour (Coultate 2009). Production of this compound in the beverages could be responsible the increase in the intensity score of "other aromas" towards the end of the storage time in both the light (1.0 – 3.7) and dark (1.1 – 2.6) stored acidified juice. The scores for "other" attributes were not statistically analysed as they were individual panellist comments.

6.2.4 Appearance and colour

The colour and appearance of a food influences the enjoyment and appetite prior and during its consumption (Hutchings 2003). Hutchings (2003) suggests our expectations of a food can be divided into five broad visual assessments. "Is it safe to consume?" "what is it?" (the foods identity including flavour and texture), "how useful is it for my needs?", "how pleasant will the drinking/eating experience be?" And finally "how satisfied will I be after drinking /eating it?"

Particular colours are associated with particular expectations, such as greenness and freshness in vegetables, and up to certain levels the association is stronger, such as increasing ripeness however beyond a certain colour the expectations becomes unacceptable as in yellow green vegetables being less fresh (Hutchings 2003).

The colour changes in the neutral juice showed a gradual change from green hue angles to a more yellow green as the juice aged, where as in the acidified juice this colour change occurred immediately upon acidification and was relatively consistent over time as shown by the total colour difference in Figure 5.10. The green colour of the neutral juice did evoke a perception of green vegetable and hence and association with broccoli and over time as the colour became less green the "freshness" perception could influence the neutral juice's shelf life. However the colour of the acidified juice did not look "fresh" and it was yellow green and its association with a green vegetable juice would be reduced.

A lower level of chlorophyll in the acidified broccoli juice (Table 5.10) compared to the neutral broccoli juice can be explained by the addition of acid to chlorophyll which replaces the Mg^{2+} ion with hydrogen ions resulting in pheophytins *a* and *b* producing the characteristic colour change from green to olive brown (Daood 2003; Coultate 2009). Research has been carried out with alkaline cooking water with positive results in retaining the chlorophyll green colour but at the expense of flavour changes, softened texture and reduction in vitamin C (Coultate 2009). Chlorophyll preservation in a green vegetable juice of 1:1:1 ratio of Chinese cabbage, romaine lettuce and spinach was achieved by blanching in alkali at 85°C for 2 min (Min et al. 2004) but this was not on a commercial scale.

Fennema (1996) describes the efforts to preserve the green colour of canned vegetable has been focused on retaining chlorophyllides, the green derivatives of chlorophylls or the formation of acid stable green coloured metallo complexes by displacing hydrogen ions on pheophytins and pyropheophytins with zinc or copper ions. Copper complexes of pheophytin and pheophorbide are available commercially. The Food and Agriculture Organization (FAO) of the United Nations has certified their use in foods at levels less than 200 ppm of free ionizable copper. They are not permitted for food use in the US (Fennema 1996; Lamikanra et al. 2005). Coultate (2009) suggests the use of chlorophyll as a food colour is difficult as it is insoluble in water and unstable unless it is in high fat foods, however a chlorophyll derivative, sodium copper chlorophyllin can be used. It is a sodium salt of chlorophyllin (chlorophyll with the phytol side chain has been removed) and has had the magnesium replaced by copper. The amount of copper it contains is too low to be toxic. Both chlorophyll (E140) and copper chlorophyllin (E141) are permitted food additives in Europe (Coultate 2009).

The colour measurements support the trends observed in the chlorophyll and carotenoid levels. The neutral juices had the greatest change in colour over storage and the acidified juices after an initial decrease in colour the levels remained constant for the remainder of the trial. The colour pigments chlorophyll and carotenoids had variable reactions to storage. The total chlorophyll content decreased rapidly in the first two weeks of storage and then levels remained stable for the duration of the shelf life trial. Neutral juices had the highest levels of chlorophyll with dark stored juice having the greatest level. The total carotenoids decreased over time in the neutral juice however in the acidified juice the carotenoid levels did not change from the initial week 0 level. Chlorophyll and carotenoids may have influenced colour

but it should be noted the results obtained are for total chlorophyll and total carotenoids, individual pigments may have differing trends.

Reports on carotenoid retention during processing and storage are conflicting, some claim no loss or increase in carotenoid content while other show considerable reductions. Rodriguez-Amaya (2003) suggests increases in carotenoid are likely to be artefacts of the analytical process due to loss of carotenoids in fresh samples, unaccounted loss of water, and leaching of soluble solids. Carotenoid losses during analysis to processing and storage effects must also be considered. Carotenoids are subject to isomerisation and oxidation during processing and storage (Rodriguez-Amaya 2003; Schieber & Carle 2008). Isomerisation to *cis* isomers is provoked by the release of constituent acids during slicing and pureeing of foods, heat treatment and exposure to light which all result in some loss of colour (Rodriguez-Amaya 2003; Schieber & Carle 2008). The time lag between peeling, cutting or pureeing and processing should be kept to a minimum so as not to allow enzymatic oxidation of carotenoids, which can cause more serious problems than thermal decomposition (Rodriguez-Amaya 2003). Processing under good manufacturing practices should have a small effect on carotenoids. Stability is good to excellent in frozen heat sterilised foods throughout the normal shelf life with oxygen content minimized by ascorbic acid scavenging properties (Rodriguez-Amaya 2003).

6.2.5 Neutral juice safety

The general microbiological reference criteria for *Listeria monocytogenes* as detailed by the New Zealand Food Safety Authority (1995) specify that foods which are generally eaten in the state that they are sold or given a mild (non-listeriocidal) heat treatment before consumption need be free from *Listeria monocytogenes*, i.e. to contain zero colony forming units per 25 g of product Although the neutral broccoli juice is manufactured in accordance with good manufacturing practices based on the principles of Hazard Analysis Critical Control Points (HACCP), its pH of 6.5, in addition to storage at refrigeration temperatures (4°C) provides conditions that could support the growth of *Listeria monocytogenes*. Acidified broccoli juice however has a pH of less than pH 4.6 and as such can be excluded from these criteria.

These reference criteria do not prevent the manufacture of a chilled neutral broccoli juice for retail sale, they highlight the need for additional microbiological testing of the process and the product to ensure a listeriocidal treatment is achieved. Based on the standard pasteurisation criteria for milk (pH 6.5) of 72°C for 15 sec, pasteurisation of neutral broccoli juice at 95°C for 15 sec would deliver a listeriocidal treatment (Mazzotta 2001b; Shearer et al. 2002), this is in addition to the effective initial blanching process of 100°C for 60 sec (Mazzotta 2001a), however this would need to be validated for the individual process equipment as estimating inactivation rates based on models is difficult (van Asselt & Zwietering 2006).

Many food products use a heating step to increase the shelf life and enhance the safety of the product by reducing its microbiological content. The temperature time combinations are determined based on challenge tests, legislation and experience or estimations based on the log reductions of bacteria using the *D/z* concept that assumes a log-linear inactivation over time (Mazzotta 2001b; Shearer et al. 2002; van Asselt & Zwietering 2006). The heat resistance of pathogens are influenced by strain variations, presence and concentration of salt or acid, growth stage of cells, experimental conditions (Mazzotta 2001b; van Asselt & Zwietering 2006).

6.2.6 Shelf life determination

To ascertain the shelf life of a product to meet consumer expectations, a multifaceted evaluation involving consistency of quality, food safety, retention of nutritional value and analysis of sensory attributes is required (Charalambous 1993; Man 2001). The definition of shelf life is the period of time under defined storage conditions, after manufacturing, for which a food product will remain safe and be fit for use. During this period of time the product should retain its desired sensory, chemical, physical, functional or microbiological characteristics, and comply with any label declaration of nutritional information when stored according to the recommended conditions (Man 2001).

Consumers are interested in thermally processed foods in which nutritive compounds are retained (Polata et al. 2009). Heat treatment of vegetable products should ensure their microbiological safety (to inactivate any soil borne pathogenic bacteria and spoilage

organisms), prevent browning and loss of colour, and should not affect the sensory and nutritional characteristics to obtain products similar to fresh vegetable juice (Hyun-Pa et al. 2007; Polata et al. 2009).

Chlorophylls are known to have anti-carcinogenic health effects and although the levels of chlorophyll in the broccoli juices did degrade during acidification, exposure to light and over time, Turkmen et al (2006) suggest that the nutritional properties of vegetables are maintained even if the chlorophyll is degraded to pheophytin *a* and pheophytin *b*, as these also have similar health effects.

On the whole the appearance and colour of the neutral broccoli juice remained appealing and visually green for four weeks (or 30 days) in refrigerated storage until it became a yellow green similar to the acidified broccoli juice. Both the neutral and acidified broccoli juices retained their initial homogeneous cloudiness for the entire eight week storage period.

Overall the sensory profile of the neutral broccoli juice smelled and tasted like slightly blanched broccoli and was pleasantly drinkable. The acidified broccoli juice, however in a blind tasting would not be distinguishable as specifically broccoli juice as it had a sour savoury vegetable juice flavour. The neutral juice could be consumed on its own with the acidified broccoli juice providing a savoury profile to a juice blend.

Both the neutral and acidified broccoli juices maintained microbiological safety limits for the eight week refrigerated storage period. Although a microbiological kinetic study was not done, based on this current experimental work (summarised in Table 6.1), the colour, sensory and compositional results indicate a shelf life of 30 days is achievable in refrigerated (4 °C) storage in glass packaging with shrink wrap labels for light protection. This is comparable to Arano® chilled fruit juice products including carrot and tomato juice which have a 40 day refrigerated shelf life and would exceed the Arano® premium 100% squeezed orange juice range which only has a shelf life of 14-18 days (Anon 2009a).

CHAPTER 7

Conclusions and recommendations

7.1 Conclusions

The results from this research indicate that the production of a broccoli juice with a stable colour and some retained nutritional components is achievable with a refrigerated (4°C) shelf life of 30 days in light protective packaging. The neutral juice is preferable as it is greener and pleasant distinguishable broccoli flavour. Although unrecognisable as broccoli juice the acidified juice could still provide benefit as a savoury vegetable flavour profile to a juice blend.

- Neutral broccoli juice required heat treatment at 95°C for 15 sec.
- Acidified broccoli juice required 5 g/L citric acid to reduce pH to 4.5 followed by heat treatment at 85°C for 15 sec.
- Neutral and acidified broccoli juice stored for eight weeks in retail storage conditions maintained microbiological safety levels below the minimum standard for ready to eat food products (New Zealand Food Safety Authority 1995)
- Neutral broccoli juice remained “green” for 4 weeks before the colour became more yellow. The acidified juice became yellow on acidification and did not change during storage.
- Dietary fibre and pectin levels did not change during storage.
- Chlorophyll and carotenoids levels decreased during acidification, light and storage and were directly influencing the colour changes in the juices.
- Ascorbic acid levels decreased significantly during processing. Acidification and storage in the dark had a protective effect on the degradation of ascorbic acid.
- The effect of processing and storage on the flavour of the beverage was assessed using a trained sensory panel providing descriptive analysis. The sensory profiles for neutral and acidified juices were very different with the unbalanced acidity masking the perception of sweetness, bitterness and the distinguishing broccoli aroma and flavour.

- The neutral juice sensory profile only changed slightly in aroma attributes during storage for seven weeks. The aroma and flavour profile of the acidified juice intensified over the storage period.

7.2 Recommendations

Process optimisation and development work is recommended to make it commercially viable. This research was carried out using traditional processing technologies. This can be used as the baseline to develop processing conditions that will optimise the nutritional components in a broccoli juice. Combinations of non-thermal processing such as high pressure processing and advances in packaging technologies would be starting points for further work.

Fortification with vitamin C will increase the levels of vitamin C deliverable to the consumer for a 4 weeks shelf life.

The acidification of broccoli juice with other organic acids or other fruit juices would enhance the flavour profile of the acidified broccoli juice by increasing the perception of sweetness and suppressing the current overwhelming sourness.

This research shows it is possible using basic processing and storage methods to produce a neutral and acidified broccoli juice whilst still retaining a usable level of Vitamin C, which is one of the most labile water soluble vitamins. Further research into the retention of other nutritional beneficial compounds such as glucosinolates and their breakdown products would be recommended.

Relatively low levels of dietary fibre were retained primarily as a result of the processing methods. Further research into thicker and more particulate broccoli juices may provide more beneficial levels of dietary fibre however levels of flavour compounds may also be increased.

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Appendix

Appendix 1: Bottle randomisation tables.

Appendix 2: Sensory panel assessment instructions and score sheet.

Appendix 3: Broccoli juices definitions for sensory assessments.

Appendix 4: Sensory profiles for neutral and acidified broccoli juice reference samples.

Appendix 5: Average sensory attribute scores and ANOVA results for neutral juice.

Appendix 6: Average sensory attribute scores and ANOVA results for acidified juice.

Appendix 1: Bottle randomisation tables.

No	Acid randomsiation				No	Neutral randomsiation		
1	Acid	Wk4 Light	Phys Chem1		1	Neutral	Wk2 Dark	Phys Chem1
2	Acid	Wk4 Light	Phys Chem1		2	Neutral	Wk2 Dark	Phys Chem1
3	Acid	Wk0 Light	Phys Chem1		3	Neutral	Wk4 Dark	Phys Chem1
4	Acid	Wk0 Light	Phys Chem1		4	Neutral	Wk4 Dark	Phys Chem1
5	Acid	Wk0 Dark	Phys Chem1		5	Neutral	Wk0 Light	Phys Chem1
6	Acid	Wk0 Dark	Phys Chem1		6	Neutral	Wk0 Light	Phys Chem1
7	Acid	Wk2 Dark	Phys Chem1		7	Neutral	Wk0 Dark	Phys Chem1
8	Acid	Wk2 Dark	Phys Chem1		8	Neutral	Wk0 Dark	Phys Chem1
9	Acid	Wk6 Light	Phys Chem1		9	Neutral	Wk8 Light	Phys Chem1
10	Acid	Wk6 Light	Phys Chem1		10	Neutral	Wk8 Light	Phys Chem1
11	Acid	Wk6 Dark	Phys Chem1		11	Neutral	Wk1 Dark	Phys Chem1
12	Acid	Wk6 Dark	Phys Chem1		12	Neutral	Wk1 Dark	Phys Chem1
13	Acid	Wk4 Dark	Phys Chem1		13	Neutral	Wk2 Light	Phys Chem1
14	Acid	Wk4 Dark	Phys Chem1		14	Neutral	Wk2 Light	Phys Chem1
15	Acid	Wk8 Dark	Phys Chem1		15	Neutral	Wk1 Light	Phys Chem1
16	Acid	Wk8 Dark	Phys Chem1		16	Neutral	Wk1 Light	Phys Chem1
17	Acid	Wk1 Dark	Phys Chem1		17	Neutral	Wk6 Light	Phys Chem1
18	Acid	Wk1 Dark	Phys Chem1		18	Neutral	Wk6 Light	Phys Chem1
19	Acid	Wk8 Light	Phys Chem1		19	Neutral	Wk6 Dark	Phys Chem1
20	Acid	Wk8 Light	Phys Chem1		20	Neutral	Wk6 Dark	Phys Chem1
21	Acid	Wk1 Light	Phys Chem1		21	Neutral	Wk4 Light	Phys Chem1
22	Acid	Wk1 Light	Phys Chem1		22	Neutral	Wk4 Light	Phys Chem1
23	Acid	Wk2 Light	Phys Chem1		23	Neutral	Wk8 Dark	Phys Chem1
24	Acid	Wk2 Light	Phys Chem1		24	Neutral	Wk8 Dark	Phys Chem1
25	Acid	Wk4 Dark	Sens wk4 fresh rep1		25	Neutral	Wk0 Dark	Sens wk4 froz rep1
26	Acid	Wk4 Dark	Sens wk4 fresh rep1		26	Neutral	Wk0 Dark	Sens wk4 froz rep1
27	Acid	Wk4 Dark	Sens wk4 fresh rep1		27	Neutral	Wk0 Dark	Sens wk4 froz rep1
28	Acid	Wk4 Dark	Sens wk4 fresh rep1		28	Neutral	Wk0 Dark	Sens wk4 froz rep1
29	Acid	Wk1 Dark	Sens final rep1		29	Neutral	Wk4 Light	Sens wk4 fresh rep1
30	Acid	Wk1 Dark	Sens final rep1		30	Neutral	Wk4 Light	Sens wk4 fresh rep1
31	Acid	Wk1 Dark	Sens final rep1		31	Neutral	Wk4 Light	Sens wk4 fresh rep1
32	Acid	Wk1 Dark	Sens final rep1		32	Neutral	Wk4 Light	Sens wk4 fresh rep1
33	Acid	Wk0 Light	Sens wk4 froz rep1		33	Neutral	Wk1 Light	Sens final rep1
34	Acid	Wk0 Light	Sens wk4 froz rep1		34	Neutral	Wk1 Light	Sens final rep1
35	Acid	Wk0 Light	Sens wk4 froz rep1		35	Neutral	Wk1 Light	Sens final rep1
36	Acid	Wk0 Light	Sens wk4 froz rep1		36	Neutral	Wk1 Light	Sens final rep1
37	Acid	Wk0 Dark	Sens wk4 froz rep1		37	Neutral	Wk8 Dark	Sens final rep1
38	Acid	Wk0 Dark	Sens wk4 froz rep1		38	Neutral	Wk8 Dark	Sens final rep1
39	Acid	Wk0 Dark	Sens wk4 froz rep1		39	Neutral	Wk8 Dark	Sens final rep1
40	Acid	Wk0 Dark	Sens wk4 froz rep1		40	Neutral	Wk8 Dark	Sens final rep1
41	Acid	Wk0 Light	Sens final rep1		41	Neutral	Wk1 Dark	Sens final rep1
42	Acid	Wk0 Light	Sens final rep1		42	Neutral	Wk1 Dark	Sens final rep1
43	Acid	Wk0 Light	Sens final rep1		43	Neutral	Wk1 Dark	Sens final rep1
44	Acid	Wk0 Light	Sens final rep1		44	Neutral	Wk1 Dark	Sens final rep1
45	Acid	Wk2 Dark	Sens final rep1		45	Neutral	Wk8 Light	Sens final rep1
46	Acid	Wk2 Dark	Sens final rep1		46	Neutral	Wk8 Light	Sens final rep1
47	Acid	Wk2 Dark	Sens final rep1		47	Neutral	Wk8 Light	Sens final rep1
48	Acid	Wk2 Dark	Sens final rep1		48	Neutral	Wk8 Light	Sens final rep1
49	Acid	Wk8 Dark	Sens final rep1		49	Neutral	Wk6 Light	Sens final rep1
50	Acid	Wk8 Dark	Sens final rep1		50	Neutral	Wk6 Light	Sens final rep1
51	Acid	Wk8 Dark	Sens final rep1		51	Neutral	Wk6 Light	Sens final rep1
52	Acid	Wk8 Dark	Sens final rep1		52	Neutral	Wk6 Light	Sens final rep1
53	Acid	Wk6 Dark	Sens final rep1		53	Neutral	Wk4 Light	Sens final rep1
54	Acid	Wk6 Dark	Sens final rep1		54	Neutral	Wk4 Light	Sens final rep1
55	Acid	Wk6 Dark	Sens final rep1		55	Neutral	Wk4 Light	Sens final rep1
56	Acid	Wk6 Dark	Sens final rep1		56	Neutral	Wk4 Light	Sens final rep1
57	Acid	Wk4 Dark	Sens final rep1		57	Neutral	Wk0 Light	Sens wk4 froz rep1
58	Acid	Wk4 Dark	Sens final rep1		58	Neutral	Wk0 Light	Sens wk4 froz rep1
59	Acid	Wk4 Dark	Sens final rep1		59	Neutral	Wk0 Light	Sens wk4 froz rep1
60	Acid	Wk4 Dark	Sens final rep1		60	Neutral	Wk0 Light	Sens wk4 froz rep1
61	Acid	Wk4 Light	Sens final rep1		61	Neutral	Wk2 Light	Sens final rep1
62	Acid	Wk4 Light	Sens final rep1		62	Neutral	Wk2 Light	Sens final rep1
63	Acid	Wk4 Light	Sens final rep1		63	Neutral	Wk2 Light	Sens final rep1
64	Acid	Wk4 Light	Sens final rep1		64	Neutral	Wk2 Light	Sens final rep1
65	Acid	Wk8 Light	Sens final rep1		65	Neutral	Wk0 Light	Sens final rep1

No	Acid randomsiation				No	Neutral randomsiation		
66	Acid	Wk8 Light	Sens final rep1		66	Neutral	Wk0 Light	Sens final rep1
67	Acid	Wk8 Light	Sens final rep1		67	Neutral	Wk0 Light	Sens final rep1
68	Acid	Wk8 Light	Sens final rep1		68	Neutral	Wk0 Light	Sens final rep1
69	Acid	Wk2 Light	Sens final rep1		69	Neutral	Wk6 Dark	Sens final rep1
70	Acid	Wk2 Light	Sens final rep1		70	Neutral	Wk6 Dark	Sens final rep1
71	Acid	Wk2 Light	Sens final rep1		71	Neutral	Wk6 Dark	Sens final rep1
72	Acid	Wk2 Light	Sens final rep1		72	Neutral	Wk6 Dark	Sens final rep1
73	Acid	Wk0 Dark	Sens final rep1		73	Neutral	Wk4 Dark	Sens final rep1
74	Acid	Wk0 Dark	Sens final rep1		74	Neutral	Wk4 Dark	Sens final rep1
75	Acid	Wk0 Dark	Sens final rep1		75	Neutral	Wk4 Dark	Sens final rep1
76	Acid	Wk0 Dark	Sens final rep1		76	Neutral	Wk4 Dark	Sens final rep1
77	Acid	Wk4 Light	Sens wk4 fresh rep1		77	Neutral	Wk2 Dark	Sens final rep1
78	Acid	Wk4 Light	Sens wk4 fresh rep1		78	Neutral	Wk2 Dark	Sens final rep1
79	Acid	Wk4 Light	Sens wk4 fresh rep1		79	Neutral	Wk2 Dark	Sens final rep1
80	Acid	Wk4 Light	Sens wk4 fresh rep1		80	Neutral	Wk2 Dark	Sens final rep1
81	Acid	Wk6 Light	Sens final rep1		81	Neutral	Wk4 Dark	Sens wk4 fresh rep1
82	Acid	Wk6 Light	Sens final rep1		82	Neutral	Wk4 Dark	Sens wk4 fresh rep1
83	Acid	Wk6 Light	Sens final rep1		83	Neutral	Wk4 Dark	Sens wk4 fresh rep1
84	Acid	Wk6 Light	Sens final rep1		84	Neutral	Wk4 Dark	Sens wk4 fresh rep1
85	Acid	Wk1 Light	Sens final rep1		85	Neutral	Wk0 Dark	Sens final rep1
86	Acid	Wk1 Light	Sens final rep1		86	Neutral	Wk0 Dark	Sens final rep1
87	Acid	Wk1 Light	Sens final rep1		87	Neutral	Wk0 Dark	Sens final rep1
88	Acid	Wk1 Light	Sens final rep1		88	Neutral	Wk0 Dark	Sens final rep1
89	Acid	Wk0 Light	Sens std freeze now		89	Neutral	Wk0 Light	Sens std freeze now
90	Acid	Wk0 Light	Sens std freeze now		90	Neutral	Wk0 Light	Sens std freeze now
91	Acid	Wk0 Light	Sens std freeze now		91	Neutral	Wk0 Light	Sens std freeze now
92	Acid	Wk0 Light	Sens std freeze now		92	Neutral	Wk0 Light	Sens std freeze now
93	Acid	Wk0 Light	Sens std freeze now		93	Neutral	Wk0 Light	Sens std freeze now
94	Acid	Wk0 Light	Sens std freeze now		94	Neutral	Wk0 Light	Sens std freeze now
95	Acid	Wk0 Light	Sens std freeze now		95	Neutral	Wk0 Light	Sens std freeze now
96	Acid	Wk0 Light	Sens std freeze now		96	Neutral	Wk0 Light	Sens std freeze now
97	Acid	Wk0 Light	Sens std freeze now		97	Neutral	Wk0 Light	Sens std freeze now
98	Acid	Wk0 Light	Sens std freeze now		98	Neutral	Wk0 Light	Sens std freeze now
99	Acid	Wk0 Light	Sens std freeze now		99	Neutral	Wk0 Light	Sens std freeze now
100	Acid	Wk0 Light	Sens std freeze now		100	Neutral	Wk0 Light	Sens std freeze now
101	Acid	Wk0 Light	Sens std freeze now		101	Neutral	Wk0 Light	Sens std freeze now
102	Acid	Wk0 Light	Sens std freeze now		102	Neutral	Wk0 Light	Sens std freeze now
103	Acid	Wk0 Light	Sens std freeze now		103	Neutral	Wk0 Light	Sens std freeze now
104	Acid	Wk0 Light	Sens std freeze now		104	Neutral	Wk0 Light	Sens std freeze now
105	Acid	Wk0 Light	Sens std freeze now		105	Neutral	Wk0 Light	Sens std freeze now
106	Acid	Wk0 Light	Sens std freeze now		106	Neutral	Wk0 Light	Sens std freeze now
107	Acid	Wk0 Light	Sens std freeze now		107	Neutral	Wk0 Light	Sens std freeze now
108	Acid	Wk0 Light	Sens std freeze now		108	Neutral	Wk0 Light	Sens std freeze now
109	Acid	Wk0 Light	Sens std freeze now		109	Neutral	Wk0 Light	Sens std freeze now
110	Acid	Wk0 Light	Sens std freeze now		110	Neutral	Wk0 Light	Sens std freeze now
111	Acid	Wk0 Light	Sens std freeze now		111	Neutral	Wk0 Light	Sens std freeze now
112	Acid	Wk0 Light	Sens std freeze now		112	Neutral	Wk0 Light	Sens std freeze now
113	Acid	Wk0 Light	Sens std freeze now		113	Neutral	Wk0 Light	Sens std freeze now
114	Acid	Wk0 Light	Sens std freeze now		114	Neutral	Wk0 Light	Sens std freeze now
115	Acid	Wk0 Light	Sens std freeze now		115	Neutral	Wk0 Light	Sens std freeze now
116	Acid	Wk0 Light	Sens std freeze now		116	Neutral	Wk0 Light	Sens std freeze now
117	Acid	Wk0 Light	Sens std freeze now		117	Neutral	Wk0 Light	Sens std freeze now
118	Acid	Wk0 Light	Sens std freeze now		118	Neutral	Wk0 Light	Sens std freeze now
119	Acid	Wk0 Light	Sens std freeze now		119	Neutral	Wk0 Light	Sens std freeze now
120	Acid	Wk0 Light	Sens std freeze now		120	Neutral	Wk0 Light	Sens std freeze now
121	Acid	Wk1 Dark	Phys Chem2		121	Neutral	Wk8 Dark	Phys Chem2
122	Acid	Wk1 Dark	Phys Chem2		122	Neutral	Wk8 Dark	Phys Chem2
123	Acid	Wk8 Light	Phys Chem2		123	Neutral	Wk6 Light	Phys Chem2
124	Acid	Wk8 Light	Phys Chem2		124	Neutral	Wk6 Light	Phys Chem2
125	Acid	Wk0 Light	Phys Chem2		125	Neutral	Wk6 Dark	Phys Chem2
126	Acid	Wk0 Light	Phys Chem2		126	Neutral	Wk6 Dark	Phys Chem2
127	Acid	Wk6 Dark	Phys Chem2		127	Neutral	Wk1 Light	Phys Chem2
128	Acid	Wk6 Dark	Phys Chem2		128	Neutral	Wk1 Light	Phys Chem2
129	Acid	Wk8 Dark	Phys Chem2		129	Neutral	Wk8 Light	Phys Chem2
130	Acid	Wk8 Dark	Phys Chem2		130	Neutral	Wk8 Light	Phys Chem2

No	Acid randomsiation				No	Neutral randomsiation		
131	Acid	Wk1 Light	Phys Chem2		131	Neutral	Wk1 Dark	Phys Chem2
132	Acid	Wk1 Light	Phys Chem2		132	Neutral	Wk1 Dark	Phys Chem2
133	Acid	Wk2 Light	Phys Chem2		133	Neutral	Wk0 Dark	Phys Chem2
134	Acid	Wk2 Light	Phys Chem2		134	Neutral	Wk0 Dark	Phys Chem2
135	Acid	Wk4 Dark	Phys Chem2		135	Neutral	Wk4 Dark	Phys Chem2
136	Acid	Wk4 Dark	Phys Chem2		136	Neutral	Wk4 Dark	Phys Chem2
137	Acid	Wk0 Dark	Phys Chem2		137	Neutral	Wk2 Light	Phys Chem2
138	Acid	Wk0 Dark	Phys Chem2		138	Neutral	Wk2 Light	Phys Chem2
139	Acid	Wk4 Light	Phys Chem2		139	Neutral	Wk0 Light	Phys Chem2
140	Acid	Wk4 Light	Phys Chem2		140	Neutral	Wk0 Light	Phys Chem2
141	Acid	Wk2 Dark	Phys Chem2		141	Neutral	Wk2 Dark	Phys Chem2
142	Acid	Wk2 Dark	Phys Chem2		142	Neutral	Wk2 Dark	Phys Chem2
143	Acid	Wk6 Light	Phys Chem2		143	Neutral	Wk4 Light	Phys Chem2
144	Acid	Wk6 Light	Phys Chem2		144	Neutral	Wk4 Light	Phys Chem2
145	Acid	Wk4 Dark	Micro		145	Neutral	Wk4 Dark	Micro
146	Acid	Wk1 Dark	Micro		146	Neutral	Wk0 Dark	Micro fresh test
147	Acid	Wk1 Light	Micro		147	Neutral	Wk1 Light	Micro
148	Acid	Wk6 Light	Micro		148	Neutral	Wk6 Light	Micro
149	Acid	Wk6 Dark	Micro		149	Neutral	Wk8 Dark	Micro
150	Acid	Wk8 Dark	Micro		150	Neutral	Wk6 Dark	Micro
151	Acid	Wk2 Dark	Micro		151	Neutral	Wk0 Dark	Micro
152	Acid	Wk0 Dark	Micro		152	Neutral	Wk1 Dark	Micro
153	Acid	Wk2 Light	Micro		153	Neutral	Wk0 Light	Micro fresh test
154	Acid	Wk0 Dark	Micro fresh test		154	Neutral	Wk2 Dark	Micro
155	Acid	Wk4 Light	Micro		155	Neutral	Wk8 Light	Micro
156	Acid	Wk0 Light	Micro		156	Neutral	Wk4 Light	Micro
157	Acid	Wk8 Light	Micro		157	Neutral	Wk0 Light	Micro
158	Acid	Wk0 Light	Micro fresh test		158	Neutral	Wk2 Light	Micro
159	Acid	Wk2 Dark	Sens final rep2		159	Neutral	Wk8 Dark	Sens final rep2
160	Acid	Wk2 Dark	Sens final rep2		160	Neutral	Wk8 Dark	Sens final rep2
161	Acid	Wk2 Dark	Sens final rep2		161	Neutral	Wk8 Dark	Sens final rep2
162	Acid	Wk2 Dark	Sens final rep2		162	Neutral	Wk8 Dark	Sens final rep2
163	Acid	Wk6 Light	Sens final rep2		163	Neutral	Wk4 Light	Sens wk4 fresh rep2
164	Acid	Wk6 Light	Sens final rep2		164	Neutral	Wk4 Light	Sens wk4 fresh rep2
165	Acid	Wk6 Light	Sens final rep2		165	Neutral	Wk4 Light	Sens wk4 fresh rep2
166	Acid	Wk6 Light	Sens final rep2		166	Neutral	Wk4 Light	Sens wk4 fresh rep2
167	Acid	Wk0 Dark	Sens wk4 froz rep2		167	Neutral	Wk4 Dark	Sens final rep2
168	Acid	Wk0 Dark	Sens wk4 froz rep2		168	Neutral	Wk4 Dark	Sens final rep2
169	Acid	Wk0 Dark	Sens wk4 froz rep2		169	Neutral	Wk4 Dark	Sens final rep2
170	Acid	Wk0 Dark	Sens wk4 froz rep2		170	Neutral	Wk4 Dark	Sens final rep2
171	Acid	Wk4 Dark	Sens wk4 fresh rep2		171	Neutral	Wk6 Dark	Sens final rep2
172	Acid	Wk4 Dark	Sens wk4 fresh rep2		172	Neutral	Wk6 Dark	Sens final rep2
173	Acid	Wk4 Dark	Sens wk4 fresh rep2		173	Neutral	Wk6 Dark	Sens final rep2
174	Acid	Wk4 Dark	Sens wk4 fresh rep2		174	Neutral	Wk6 Dark	Sens final rep2
175	Acid	Wk8 Dark	Sens final rep2		175	Neutral	Wk1 Dark	Sens final rep2
176	Acid	Wk8 Dark	Sens final rep2		176	Neutral	Wk1 Dark	Sens final rep2
177	Acid	Wk8 Dark	Sens final rep2		177	Neutral	Wk1 Dark	Sens final rep2
178	Acid	Wk8 Dark	Sens final rep2		178	Neutral	Wk1 Dark	Sens final rep2
179	Acid	Wk6 Dark	Sens final rep2		179	Neutral	Wk4 Light	Sens final rep2
180	Acid	Wk6 Dark	Sens final rep2		180	Neutral	Wk4 Light	Sens final rep2
181	Acid	Wk6 Dark	Sens final rep2		181	Neutral	Wk4 Light	Sens final rep2
182	Acid	Wk6 Dark	Sens final rep2		182	Neutral	Wk4 Light	Sens final rep2
183	Acid	Wk4 Dark	Sens final rep2		183	Neutral	Wk4 Dark	Sens wk4 fresh rep2
184	Acid	Wk4 Dark	Sens final rep2		184	Neutral	Wk4 Dark	Sens wk4 fresh rep2
185	Acid	Wk4 Dark	Sens final rep2		185	Neutral	Wk4 Dark	Sens wk4 fresh rep2
186	Acid	Wk4 Dark	Sens final rep2		186	Neutral	Wk4 Dark	Sens wk4 fresh rep2
187	Acid	Wk8 Light	Sens final rep2		187	Neutral	Wk8 Light	Sens final rep2
188	Acid	Wk8 Light	Sens final rep2		188	Neutral	Wk8 Light	Sens final rep2
189	Acid	Wk8 Light	Sens final rep2		189	Neutral	Wk8 Light	Sens final rep2
190	Acid	Wk8 Light	Sens final rep2		190	Neutral	Wk8 Light	Sens final rep2
191	Acid	Wk4 Light	Sens final rep2		191	Neutral	Wk6 Light	Sens final rep2
192	Acid	Wk4 Light	Sens final rep2		192	Neutral	Wk6 Light	Sens final rep2
193	Acid	Wk4 Light	Sens final rep2		193	Neutral	Wk6 Light	Sens final rep2
194	Acid	Wk4 Light	Sens final rep2		194	Neutral	Wk6 Light	Sens final rep2
195	Acid	Wk4 Light	Sens wk4 fresh rep2		195	Neutral	Wk1 Light	Sens final rep2

No	Acid randomsiation				No	Neutral randomsiation		
196	Acid	Wk4 Light	Sens wk4 fresh rep2		196	Neutral	Wk1 Light	Sens final rep2
197	Acid	Wk4 Light	Sens wk4 fresh rep2		197	Neutral	Wk1 Light	Sens final rep2
198	Acid	Wk4 Light	Sens wk4 fresh rep2		198	Neutral	Wk1 Light	Sens final rep2
199	Acid	Wk0 Light	Sens wk4 froz rep2		199	Neutral	Wk0 Dark	Sens wk4 froz rep2
200	Acid	Wk0 Light	Sens wk4 froz rep2		200	Neutral	Wk0 Dark	Sens wk4 froz rep2
201	Acid	Wk0 Light	Sens wk4 froz rep2		201	Neutral	Wk0 Dark	Sens wk4 froz rep2
202	Acid	Wk0 Light	Sens wk4 froz rep2		202	Neutral	Wk0 Dark	Sens wk4 froz rep2
203	Acid	Wk1 Dark	Sens final rep2		203	Neutral	Wk0 Light	Sens wk4 froz rep2
204	Acid	Wk1 Dark	Sens final rep2		204	Neutral	Wk0 Light	Sens wk4 froz rep2
205	Acid	Wk1 Dark	Sens final rep2		205	Neutral	Wk0 Light	Sens wk4 froz rep2
206	Acid	Wk1 Dark	Sens final rep2		206	Neutral	Wk0 Light	Sens wk4 froz rep2
207	Acid	Wk0 Light	Sens final rep2		207	Neutral	Wk2 Dark	Sens final rep2
208	Acid	Wk0 Light	Sens final rep2		208	Neutral	Wk2 Dark	Sens final rep2
209	Acid	Wk0 Light	Sens final rep2		209	Neutral	Wk2 Dark	Sens final rep2
210	Acid	Wk0 Light	Sens final rep2		210	Neutral	Wk2 Dark	Sens final rep2
211	Acid	Wk1 Light	Sens final rep2		211	Neutral	Wk2 Light	Sens final rep2
212	Acid	Wk1 Light	Sens final rep2		212	Neutral	Wk2 Light	Sens final rep2
213	Acid	Wk1 Light	Sens final rep2		213	Neutral	Wk2 Light	Sens final rep2
214	Acid	Wk1 Light	Sens final rep2		214	Neutral	Wk2 Light	Sens final rep2
215	Acid	Wk0 Dark	Sens final rep2		215	Neutral	Wk0 Dark	Sens final rep2
216	Acid	Wk0 Dark	Sens final rep2		216	Neutral	Wk0 Dark	Sens final rep2
217	Acid	Wk0 Dark	Sens final rep2		217	Neutral	Wk0 Dark	Sens final rep2
218	Acid	Wk0 Dark	Sens final rep2		218	Neutral	Wk0 Dark	Sens final rep2
219	Acid	Wk2 Light	Sens final rep2		219	Neutral	Wk0 Light	Sens final rep2
220	Acid	Wk2 Light	Sens final rep2		220	Neutral	Wk0 Light	Sens final rep2
221	Acid	Wk2 Light	Sens final rep2		221	Neutral	Wk0 Light	Sens final rep2
222	Acid	Wk2 Light	Sens final rep2		222	Neutral	Wk0 Light	Sens final rep2
223	Acid	Wk2 Dark	Phys Chem3		223	Neutral	Wk4 Light	Phys Chem3
224	Acid	Wk2 Dark	Phys Chem3		224	Neutral	Wk4 Light	Phys Chem3
225	Acid	Wk0 Dark	Phys Chem3		225	Neutral	Wk2 Light	Phys Chem3
226	Acid	Wk0 Dark	Phys Chem3		226	Neutral	Wk2 Light	Phys Chem3
227	Acid	Wk1 Light	Phys Chem3		227	Neutral	Wk1 Dark	Phys Chem3
228	Acid	Wk1 Light	Phys Chem3		228	Neutral	Wk1 Dark	Phys Chem3
229	Acid	Wk4 Dark	Phys Chem3		229	Neutral	Wk0 Light	Phys Chem3
230	Acid	Wk4 Dark	Phys Chem3		230	Neutral	Wk0 Light	Phys Chem3
231	Acid	Wk4 Light	Phys Chem3		231	Neutral	Wk8 Dark	Phys Chem3
232	Acid	Wk4 Light	Phys Chem3		232	Neutral	Wk8 Dark	Phys Chem3
233	Acid	Wk8 Dark	Phys Chem3		233	Neutral	Wk6 Dark	Phys Chem3
234	Acid	Wk8 Dark	Phys Chem3		234	Neutral	Wk6 Dark	Phys Chem3
235	Acid	Wk2 Light	Phys Chem3		235	Neutral	Wk8 Light	Phys Chem3
236	Acid	Wk2 Light	Phys Chem3		236	Neutral	Wk8 Light	Phys Chem3
237	Acid	Wk0 Light	Phys Chem3		237	Neutral	Wk4 Dark	Phys Chem3
238	Acid	Wk0 Light	Phys Chem3		238	Neutral	Wk4 Dark	Phys Chem3
239	Acid	Wk8 Light	Phys Chem3		239	Neutral	Wk6 Light	Phys Chem3
240	Acid	Wk8 Light	Phys Chem3		240	Neutral	Wk6 Light	Phys Chem3
241	Acid	Wk6 Light	Phys Chem3		241	Neutral	Wk1 Light	Phys Chem3
242	Acid	Wk6 Light	Phys Chem3		242	Neutral	Wk1 Light	Phys Chem3
243	Acid	Wk6 Dark	Phys Chem3		243	Neutral	Wk2 Dark	Phys Chem3
244	Acid	Wk6 Dark	Phys Chem3		244	Neutral	Wk2 Dark	Phys Chem3
245	Acid	Wk1 Dark	Phys Chem3		245	Neutral	Wk0 Dark	Phys Chem3
246	Acid	Wk1 Dark	Phys Chem3		246	Neutral	Wk0 Dark	Phys Chem3

Appendix 2: Sensory panel assessment instructions and score sheet.

Neutral Broccoli Juice Panel Assessment

Session 1 -16 March 2009

Good afternoon,

Today you will be assessing six juices labelled:

209
827
501
764
649
938

- Each juice will be presented in a random order
- Each sample will be served at **5 minute intervals**
- You will be served three samples then you will have a 15 minute break in the training room before being served the final three samples

For each juice:

1. Stir the juice 5 times with a toothpick
2. Smell the juice and assess the aroma
3. Then assess the flavour , tastes and mouth feel
4. After 30 seconds please assess the after taste
5. Once complete , open your hatch and wait for your next sample

Date: Tues 16 Mar 09 Session 1 Neutral Panellist: Sample:

Scale	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Descriptors	None	Threshold / barely there	Slight / just there		Low			Medium / moderate		Moderately strong		Strong		Very strong	Extreme

AROMA															
Broccoli smell	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Snow Pea Sweet smell	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Peppery / radish aroma	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Chemical aroma	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Metallic aroma	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Other	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Comment															

FLAVOUR & TASTE															
Overall flavour intensity	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Fresh Broccoli flavour	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Peppery / radish flavour	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Sweet taste	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Sour taste	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Salty taste	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Bitter taste	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Chemical flavour	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Other	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Comment															

MOUTHFEEL															
Tingly	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Astringent	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Other	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Comment															

AFTER TASTE															
Peppery - radish	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Sweet aftertaste	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Sour aftertaste	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Bitter aftertaste	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Astringent	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Other	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Comment															

Appendix 3: Broccoli juices definitions for sensory assessments.

Aroma	Description	Scale	Reference
Broccoli smell	Raw broccoli	Intensity: None - Extreme	Raw broccoli
Snow pea sweet	Smells like young green snow pea pods	Intensity: None - Extreme	Snow peas
Peppery - radish	Smells like cut red radish	Intensity: None - Extreme	Grated red radish
Chemical	Artificial, solvent or paint smell. Smells foreign to food.	Intensity: None - Extreme	
Metallic	Smells like the inside of a washed Dole pineapple tin can	Intensity: None - Extreme	A empty Dole pineapple can filled with water and held overnight
Other		Intensity: None - Extreme	
Taste	**Description**	**Scale**	**Reference**
Overall flavour intensity	Overall intensity or strength of flavour of the drink	Intensity: None - Extreme	
Fresh broccoli flavour	Taste associated with raw broccoli and raw green stalky vegetables (cabbage, peas, beans, cauliflower)	Intensity: None – Extreme	Raw broccoli
Peppery – radish flavour	Tastes peppery like cut red radish	Intensity: None - Extreme	Grated red radish
Sweet taste	Taste associated with sugar (sucrose)	Intensity: None - Extreme	Sucrose solution (2%)
Sour taste	Taste associated with lemon juice	Intensity: None - Extreme	Lemon juice
Salty taste	Tastes like table salt (Sodium Chloride)	Intensity: None - Extreme	Salt solutions
Bitter taste	Taste associated with soda water	Intensity: None - Extreme	Schweppes soda water
Chemical flavour	Taste associated with artificial, solvent or paint or tastes foreign to food.	Intensity: None - Extreme	
Other		Intensity: None - Extreme	
Mouth feel	**Description**	**Scale**	**Reference**
Tingly	The effect of fizzy drink or sherbet lollies on the first half of your tongue	Intensity: None - Extreme	Schweppes soda water and fizzy lollies
Astringent	Mouth drying and or puckering	Intensity: None - Extreme	Schweppes tonic water
Other		Intensity: None - Extreme	
After taste	**Description**	**Scale**	**Reference**
Peppery / radish aftertaste	Tastes peppery like cut red radish	Intensity: None - Extreme	Grated red radish
Sweet aftertaste	Taste associated with sugar (sucrose)	Intensity: None - Extreme	Sucrose solution (2%)
Sour aftertaste	Taste associated with lemon juice	Intensity: None - Extreme	Lemon juice
Bitter aftertaste	Taste associated with soda water	Intensity: None - Extreme	Schweppes soda water
Astringent aftertaste	Mouth drying and or puckering	Intensity: None - Extreme	Schweppes tonic water
Other		Intensity: None - Extreme	

Appendix 4: Sensory profiles for neutral and acidified broccoli juice reference samples.

Date: Tues 16 Mar 09 Panellist: Sample: NEUTRAL REF

Scale	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Descriptors	None	Threshold / barely there	Slight / just there		Low			Medium / moderate		Moderately strong		Strong		Very strong	Extreme

AROMA															
Broccoli smell	1	2	3	4	5	6	7	8	9	10	**11**	12	13	14	15
Snow Pea Sweet smell	1	2	3	4	5	6	7	**8**	9	10	11	12	13	14	15
Peppery / radish aroma	1	2	3	4	5	6	**7**	8	9	10	11	12	13	14	15
Chemical aroma	**1**	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Metallic aroma	1	**2**	3	4	5	6	7	8	9	10	11	12	13	14	15
Other	**1**	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Comment															

FLAVOUR & TASTE															
Overall flavour intensity	1	2	3	4	5	6	7	8	9	10	11	**12**	13	14	15
Fresh Broccoli flavour	1	2	3	4	5	6	7	8	9	10	**11**	12	13	14	15
Peppery / radish flavour	1	2	3	4	5	6	7	8	**9**	10	11	12	13	14	15
Sweet taste	1	2	3	4	5	**6**	7	8	9	10	11	12	13	14	15
Sour taste	1	2	**3**	4	5	6	7	8	9	10	11	12	13	14	15
Salty taste	1	**2**	3	4	5	6	7	8	9	10	11	12	13	14	15
Bitter taste	1	2	3	**4**	5	6	7	8	9	10	11	12	13	14	15
Chemical flavour	**1**	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Other	**1**	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Comment															

MOUTHFEEL															
Tingly	1	2	**3**	4	5	6	7	8	9	10	11	12	13	14	15
Astringent	1	2	3	**4**	5	6	7	8	9	10	11	12	13	14	15
Other	**1**	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Comment															

AFTER TASTE															
Peppery - radish	1	2	3	4	5	6	**7**	8	9	10	11	12	13	14	15
Sweet aftertaste	1	2	3	**4**	5	6	7	8	9	10	11	12	13	14	15
Sour aftertaste	1	**2**	3	4	5	6	7	8	9	10	11	12	13	14	15
Bitter aftertaste	1	2	**3**	4	5	6	7	8	9	10	11	12	13	14	15
Astringent	1	2	3	**4**	5	6	7	8	9	10	11	12	13	14	15
Other	**1**	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Comment															

Date: Tues 16 Mar 09 Panellist: Sample: ACID REF

Scale	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Descriptors	None	Threshold / barely there	Slight / just there		Low			Medium / moderate		Moderately strong		Strong		Very strong	Extreme

AROMA															
Broccoli smell	1	2	3	4	5	6	7	**8**	9	10	11	12	13	14	15
Snow Pea Sweet smell	1	2	3	4	**5**	6	7	8	9	10	11	12	13	14	15
Peppery / radish aroma	1	2	3	4	5	**6**	7	8	9	10	11	12	13	14	15
Chemical aroma	**1**	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Metallic aroma	1	**2**	3	4	5	6	7	8	9	10	11	12	13	14	15
Other	**1**	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Comment															

FLAVOUR & TASTE															
Overall flavour intensity	1	2	3	4	5	6	7	8	9	10	11	12	**13**	14	15
Fresh Broccoli flavour	1	2	3	4	**5**	6	7	8	9	10	11	12	13	14	15
Peppery / radish flavour	1	2	3	4	5	6	**7**	8	9	10	11	12	13	14	15
Sweet taste	1	2	3	**4**	5	6	7	8	9	10	11	12	13	14	15
Sour taste	1	2	3	4	5	6	7	8	9	10	**11**	12	13	14	15
Salty taste	1	**2**	3	4	5	6	7	8	9	10	11	12	13	14	15
Bitter taste	1	2	3	**4**	5	6	7	8	9	10	11	12	13	14	15
Chemical flavour	**1**	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Other	**1**	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Comment															

MOUTHFEEL															
Tingly	1	2	3	4	**5**	6	7	8	9	10	11	12	13	14	15
Astringent	1	2	3	4	**5**	6	7	8	9	10	11	12	13	14	15
Other	**1**	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Comment															

AFTER TASTE															
Peppery - radish	1	2	3	4	5	6	**7**	8	9	10	11	12	13	14	15
Sweet aftertaste	1	**2**	3	4	5	6	7	8	9	10	11	12	13	14	15
Sour aftertaste	1	2	3	4	5	6	**7**	8	9	10	11	12	13	14	15
Bitter aftertaste	1	2	**3**	4	5	6	7	8	9	10	11	12	13	14	15
Astringent	1	2	**3**	4	5	6	7	8	9	10	11	12	13	14	15
Other	**1**	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Comment															

Appendix 5: Average sensory attribute scores and ANOVA results for neutral juice.

Storage time (Neutral)	Broccoli smell	Sweet aroma	Peppery – radish aroma	Chemical aroma	Metallic aroma	Other aroma
Light						
0	10.5	7.3	6.9	1.5	1.5	1.8
1	10.8	7.4	7.2	1.2	1.4	1.3
2	10.7	7.5	7.2	1.5	1.3	1.0
4	11.0	7.7	7.0	1.5	1.4	1.4
6	11.0	7.9	7.5	1.5	1.4	1.1
8	11.2	7.6	7.4	1.2	1.6	1.1
Dark						
0	10.7	7.4	7.2	1.3	1.4	1.3
1	10.6	7.5	7.1	1.6	1.4	1.4
2	10.2	7.2	6.8	1.3	1.4	1.4
4	10.8	7.5	7.3	1.4	1.5	1.1
6	10.6	7.2	7.2	1.2	1.3	1.5
8	11.0	8.0	7.1	1.3	1.5	1.5
Least significant differences of means (5% level)						
Light- dark	0.27	0.27	0.26	0.12	0.08	
Store- time	0.47	0.47	0.46	0.21	0.15	
Light- dark. store- time	0.67	0.67	0.65	0.30	0.21	
F test probability values (significance $p<0.05$)						
Light- dark	0.141	0.381	0.463	0.328	0.722	
Store- time	0.092	0.430	0.632	0.798	0.014	
Linear	0.013	0.040	0.163	0.238	0.046	
Nonlinear	0.493	0.963	0.831	0.917	0.031	
Light- dark. store- time	0.799	0.283	0.521	0.023	0.654	
Linear	0.476	0.818	0.331	0.797	0.502	
Nonlinear	0.767	0.187	0.517	0.011	0.585	

Storage time (Neutral)	Overall flavour intensity	Broccoli flavour	Peppery - radish flavour	Sweet taste	Sour taste	Salty taste	Bitter taste	Chemical flavour	Other flavour
Light									
0	12.4	10.9	9.1	6.2	2.8	1.7	4.2	1.1	1.1
1	12.3	10.8	9.5	6.8	2.8	1.9	4.1	1.2	1.5
2	12.2	11.0	9.1	6.3	2.9	1.9	4.1	1.1	1.1
4	12.2	10.7	9.1	7.4	2.8	1.9	4.1	1.2	1.6
6	12.1	10.8	9.0	6.9	2.7	1.8	3.7	1.1	1.5
8	12.2	10.8	9.5	7.0	3.0	1.9	4.1	1.2	1.7
Dark									
0	12.3	10.9	9.8	6.5	2.7	1.9	4.5	1.1	1.1
1	12.4	11.0	9.7	6.8	2.9	1.8	4.1	1.1	1.1
2	12.1	10.6	9.5	6.5	3.1	1.9	4.0	1.1	1.5
4	12.5	10.8	9.9	6.6	2.7	1.9	4.3	1.1	1.4
6	12.1	10.6	9.0	6.7	2.8	2.0	4.5	1.2	1.1
8	12.4	10.7	9.4	6.7	2.7	1.9	4.2	1.1	1.4
Least significant differences of means (5% level)									
Light- dark	0.22	0.26	0.32	0.34	0.20	0.09	0.22	0.07	
Store- time	0.39	0.45	0.55	0.60	0.35	0.15	0.37	0.12	
Light- dark. store- time	0.54	0.63	0.78	0.84	0.49	0.21	0.53	0.18	
F test probability values (significance $p<0.05$)									
Light- dark	0.590	0.642	0.049	0.487	0.881	0.377	0.073	0.534	
Store- time	0.691	0.951	0.431	0.251	0.807	0.618	0.830	0.500	
Linear	0.651	0.464	0.405	0.141	1.000	0.208	0.580	0.223	
Nonlinear	0.584	0.964	0.382	0.345	0.684	0.746	0.768	0.580	
Light- dark. store- time	0.862	0.844	0.574	0.427	0.807	0.519	0.271	0.701	
Linear	0.498	0.660	0.206	0.237	0.659	0.811	0.541	0.861	
Nonlinear	0.838	0.766	0.695	0.476	0.719	0.387	0.199	0.567	

Storage time (Neutral)	Tingly mouth feel	Astringent mouth feel	Other mouth feel	Peppery - radish after taste	Sweet after taste	Sour after taste	Bitter after taste	Astringent after taste	Other after taste
Light									
0	3.8	4.1	1.0	7.5	4.0	2.1	3.4	3.9	1.0
1	3.4	4.0	1.1	7.1	4.0	2.1	3.5	3.6	1.2
2	3.6	4.0	1.0	7.5	4.0	2.0	3.5	3.6	1.0
4	3.3	3.8	1.1	7.0	4.4	2.0	3.4	3.6	1.1
6	3.5	3.7	1.1	7.1	4.3	2.0	3.3	3.5	1.1
8	3.4	4.0	1.2	7.4	3.9	2.2	3.5	3.6	1.3
Dark									
0	3.8	4.2	1.3	7.5	4.1	2.0	3.8	3.7	1.1
1	3.7	4.2	1.2	7.4	4.1	2.2	3.4	3.9	1.1
2	3.7	4.0	1.3	7.5	4.1	2.1	3.2	3.7	1.3
4	3.7	4.0	1.2	7.6	3.8	2.3	3.5	3.6	1.2
6	3.3	4.0	1.0	7.3	4.1	2.0	3.5	3.5	1.1
8	3.5	4.0	1.0	7.4	3.9	2.2	3.6	3.7	1.0
Least significant differences of means (5% level)									
Light- dark	0.21	0.17		0.27	0.24	0.14	0.18	0.16	
Store- time	0.36	0.30		0.47	0.41	0.24	0.31	0.28	
Light- dark. store- time	0.51	0.43		0.66	0.58	0.35	0.44	0.40	
F test probability values (significance $p<0.05$)									
Light- dark	0.220	0.104		0.206	0.614	0.289	0.319	0.782	
Store- time	0.260	0.405		0.723	0.780	0.646	0.699	0.353	
Linear	0.043	0.295		0.531	0.731	0.789	0.834	0.106	
Nonlinear	0.658	0.407		0.654	0.672	0.514	0.566	0.568	
Light- dark. store- time	0.633	0.925		0.873	0.505	0.586	0.241	0.585	
Linear	0.563	0.800		0.981	0.579	0.721	0.889	0.699	
Nonlinear	0.542	0.858		0.769	0.407	0.461	0.153	0.464	

Appendix 6: Average sensory attribute scores and ANOVA results for acidified juice.

Storage time (Acid)	Broccoli smell	Sweet aroma	Peppery – radish aroma	Chemical aroma	Metallic aroma	Other aroma
Light						
0	7.8	4.7	6.4	1.4	1.8	1.0
1	8.0	5.2	6.2	1.4	1.7	1.1
2	6.9	4.5	6.0	1.4	1.9	2.9
4	6.7	4.5	5.7	1.8	1.7	2.6
6	7.4	4.6	5.9	1.5	1.9	1.7
8	6.8	4.3	5.9	1.5	1.7	3.7
Dark						
0	8.1	5.0	6.3	1.3	1.6	1.1
1	8.2	4.9	6.3	1.4	1.7	1.3
2	7.5	5.1	6.1	1.4	1.8	1.3
4	7.4	5.1	6.2	1.4	1.7	1.5
6	7.1	4.5	5.8	1.7	1.6	2.2
8	7.0	4.3	5.9	2.4	1.8	2.6
Least significant differences of means (5% level)						
Light- dark	0.30	0.22	0.23	0.18	0.11	
Store- time	0.53	0.38	0.40	0.31	0.20	
Light- dark. store- time	0.74	0.53	0.57	0.44	0.28	
F test probability values (significance $p<0.05$)						
Light- dark	0.080	**0.045**	0.407	0.592	0.148	
Store- time	**<0.001**	**0.003**	0.114	**0.003**	0.726	
Linear	**<0.001**	**<0.001**	**0.011**	**<0.001**	0.477	
Nonlinear	**0.019**	0.715	0.648	0.730	0.678	
Light- dark. store- time	0.504	0.096	0.555	**0.003**	0.278	
Linear	0.364	0.559	0.863	**0.001**	0.646	
Nonlinear	0.478	0.061	0.417	0.088	0.194	

Storage time (Acid)	Overall flavour intensity	Broccoli flavour	Peppery - radish flavour	Sweet taste	Sour taste	Salty taste	Bitter taste	Chemical flavour	Other flavour
Light									
0	13.0	5.2	7.4	3.6	11.7	2.2	4.2	1.1	1.0
1	13.3	5.3	7.4	3.6	12.0	2.2	3.9	1.2	1.1
2	13.1	5.0	7.3	3.9	11.1	2.2	4.0	1.1	2.2
4	13.1	5.0	7.4	4.3	11.3	2.5	3.9	1.3	1.6
6	13.3	5.4	7.2	4.6	11.5	2.2	4.3	1.2	1.5
8	13.1	5.0	7.0	3.7	11.3	2.4	4.0	1.2	3.2
Dark									
0	13.4	5.1	7.5	3.7	11.8	2.3	4.3	1.1	1.0
1	13.1	5.2	7.4	4.0	11.6	2.1	4.4	1.2	1.2
2	13.7	5.2	7.3	3.7	12.2	2.2	4.0	1.1	1.1
4	13.1	5.5	7.0	3.9	11.3	2.1	4.1	1.2	1.3
6	12.7	5.2	7.2	4.1	11.2	2.3	4.2	1.4	1.6
8	13.3	4.5	7.5	3.9	11.6	2.2	4.5	1.5	3.0
Least significant differences of means (5% level)									
Light- dark	0.22	0.21	0.23	0.25	0.30	0.12	0.21	0.13	
Store- time	0.39	0.37	0.39	0.44	0.52	0.21	0.36	0.22	
Light- dark. store- time	0.54	0.52	0.56	0.62	0.73	0.29	0.52	0.32	
F test probability values (significance $p<0.05$)									
Light- dark	0.538	0.846	0.904	0.649	0.490	0.173	**0.052**	0.206	
Store- time	0.485	**0.038**	0.620	**0.026**	0.166	0.798	0.607	0.238	
Linear	0.501	0.056	0.135	0.062	**0.052**	0.315	0.827	**0.022**	
Nonlinear	0.406	0.082	0.866	0.051	0.393	0.855	0.471	0.824	
Light- dark. store- time	0.064	0.102	0.452	0.338	0.060	0.202	0.459	0.310	
Linear	0.266	0.256	0.275	0.491	1.000	0.738	0.977	**0.031**	
Nonlinear	0.056	0.094	0.475	0.267	**0.032**	0.129	0.326	0.864	

Storage time (Acid)	Tingly mouth feel	Astringent mouth feel	Other after taste	Peppery - radish aftertaste	Sweet after taste	Sour after taste	Bitter after taste	Astringent after taste	Other after taste
Light									
0	4.6	4.8	1.0	6.8	2.2	7.3	3.2	3.2	1.0
1	5.2	5.0	1.0	6.6	2.3	7.3	3.0	3.5	1.0
2	4.9	4.8	1.0	7.0	2.4	7.4	3.1	3.6	1.2
4	5.0	5.3	1.0	6.6	2.5	7.3	2.9	3.6	1.2
6	5.2	5.1	1.0	6.6	2.6	7.2	3.1	3.6	1.0
8	4.6	5.0	1.0	6.3	2.3	6.9	3.0	3.8	1.3
Dark									
0	5.0	5.3	1.0	7.0	2.3	7.8	3.3	3.8	1.0
1	4.8	5.0	1.0	6.8	2.3	7.6	3.1	3.5	1.0
2	5.2	5.4	1.0	6.4	2.2	7.4	3.3	3.7	1.0
4	4.9	5.0	1.1	6.8	2.3	7.0	3.1	3.6	1.0
6	4.7	4.9	1.0	6.7	2.4	7.1	3.0	3.5	1.0
8	5.4	5.2	1.0	6.8	2.1	7.5	3.2	3.7	1.1
Least significant differences of means (5% level)									
Light- dark	0.24	0.21		0.25	0.16	0.21	0.17	0.15	
Store- time	0.42	0.36		0.44	0.27	0.36	0.30	0.26	
Light- dark. store-time	0.59	0.52		0.62	0.39	0.50	0.42	0.36	
F test probability values (significance $p<0.05$)									
Light- dark	0.568	0.268		0.484	0.227	0.074	0.180	0.309	
Store- time	0.825	0.977		0.781	0.270	0.074	0.762	0.443	
Linear	0.533	0.891		0.162	0.537	**0.013**	0.642	0.151	
Nonlinear	0.777	0.942		0.975	0.200	0.414	0.667	0.606	
Light- dark. store-time	**0.041**	0.150		0.162	0.656	0.080	0.821	0.116	
Linear	0.473	0.286		0.309	0.168	0.999	0.974	**0.049**	
Nonlinear	**0.026**	0.136		0.143	0.849	**0.044**	0.701	0.285	