Determination of Individual Sugars and Organic Acids of New Zealand Varietal Apple Juice and Their Use in Evaluating Authenticity.

A THESIS PRESENTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF PHILOSOPHY IN FOOD TECHNOLOGY AT MASSEY UNIVERSITY - NEW ZEALAND.

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1997

ABSTRACT

High pressure liquid chromatography techniques were used to determine sugars and acids in the juice of apples grown in New Zealand. A total of 189 samples were analysed and the results were used to assist with the determination of the authenticity of New Zealand apple juice. The values obtained were compared to other literature values and criteria used to determine authenticity of apple juice. As a number of factors affect the composition of juice, the data was gathered from a number of apple cultivars commonly grown in New Zealand, from different growing regions over two seasons, with the fruit harvested at the three maturities used in juice production. Fruit is also stored for varying lengths of time under different conditions for juice production at a later date, and therefore such samples were included in the testing.

In the apple juices tested the Brix ranged from 8.3 to 15.3 and titratable acidity (calculated as malic acid) from 210 to 1130mg/100ml. Fructose and sorbitol ranged from 4.0 to 8.6g/100ml and 0.13 to 1.4g/100ml respectively. Of the cultivars examined, Granny Smith, Red Delicious, Golden Delicious and Fuji were observed to have sucrose and glucose present at less than 3.5g/100ml which is a commonly reported literature maximum for authentic apple juice. Cox's Orange apple juice was observed to have sucrose levels typically in excess of 3.0 g/100ml for first pick fruit and in excess of 5.0g/100ml, for second and third pick fruit. One sample of this cultivar had the highest sucrose level of 7.5g/100ml seen in the study, and on average was found to have sucrose present at 4.9g/100ml. Cox's Orange apple juice generally had the lowest glucose level with levels typically less than 1.1g/100ml. Braeburn apple juice was observed to have sucrose present at levels frequently in excess of 4.0g/100ml in 1992 and 3.0g/100ml in 1993. Storage trials of this cultivar showed that it was not until the fruit had been stored for prolonged periods (45, 149 and 195 days at ambient, cold and controlled atmosphere conditions respectively) that the sucrose levels of the juice decreased to the 3.5g/100ml referred to above for authentic juice. Royal Gala, Gala, Hillwell, Fiesta, GS330 and GS2850 generally had sucrose levels ranging from 2.0 to 5.0g/100ml.

Malic acid was the most predominate acid present with levels of between 231 and 1067mg/100ml. Quinic and succinic acids were present at levels of 22 to 129mg/100ml and 8 to 41mg/100ml respectively, with succinic acid present at levels four times those that are commonly reported. Citric and shikimic acid levels were typically below 20 and 3.5mg/100ml respectively while fumaric acid never exceeded 0.22mg/100ml.

The juice of cold stored fruit was observed to have succinic and citric acids at levels greater than those observed from ambient and controlled atmosphere storage. The level of fumaric acid in the juice of ambient stored Braeburn fruit showed a marked increase from 0.06mg/100ml to 0.22mg/100ml during storage. Small increases of about 0.03mg/100ml were seen for cold and controlled atmosphere stored Braeburn fruit. Similar trends were observed in the juice of stored Granny Smith fruit.

The application of Brause and Raterman (1982) and the German RSK criteria for authentic apple juice to New Zealand varietal apple juice showed that the cultivars Granny Smith, Red Delicious, Golden Delicious and Fuji produced juice that could be considered authentic. Braeburn, Gala, Royal Gala, Cox's Orange, Hillwell, GS330, GS2850 and Fiesta were observed to have at least one component outside the proposed standard ranges, with some samples exceeding the 95% confidence levels and juices from all would often be considered as "not authentic".

The use of overseas sucrose and glucose levels and their ratios for authentication of juices from all New Zealand apple varieties is inappropriate because values outside of the published guidelines for authenticity were frequently found. The use of criteria for authentication can only be applied to juice from which the standard values are derived. Application of standard values to juices from other regions, cultivars or even years could lead to authentic juice being rejected. While published criteria for authentic juice are a starting point, their application is inappropriate for some cultivars grown New Zealand. If they are applied to New Zealand apple juice the assessment of the juice data needs to be undertaken by an expert or group of experts who have knowledge of juices (rather than the

limited information which is available in the RSK values and commentaries) to be sure that any abnormalities in the data are recognised.

Acknowledgements

I wish to sincerely thank my supervisor Mr. Malcolm Reeves for all valuable advice, guidance and time.

Special thanks go to the people at ENZA Processors in particularly Mr. Jon Marks and Mr. James Wilson (formerly of ENZA) who initiated this project, supplied funding for equipment, as well as arranging for apple samples. Other people who deserve thanking are Miss Anna Parsons, Mr. Russell Murtagh and Mr. Rod Quin who collected the apple samples.

Thanks are extend to the following: Professor Ray Winger who allowed me to carry out the project; Ms Lisa Duizer for her help with use of statistical programs; Ms. June Latham for assistance with HPLC; Mr. Byron McKillop who manufactured specialised equipment; Mr John O'Connor and Mr. Mark Dorsey for helpful advice with computing.

I also appreciate the moral support given by Yuly Indrawati and Bongkot Nippon and other fellow post graduates.

I would also like to thank the academic and technical staff of the Department of Food Technology for encouragement during those difficult times.

Finally I would like to give a big thank-you to my flatmate Miss Fiona Matheson who kindly read this thesis, supplied helpful advice and gave a lot of support and encouragement.

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CHAPTER 1
INTRODUCTION

Apples (genus *Malus*) are known as pome fruits and are members of the family Rosaceae, which also includes pear, quince and strawberry, and are commercially important. During the 1995 season exports of fruit and nuts were worth $876.3 million with the New Zealand Apple and Pear Marketing Board handling 17.2 million carton equivalents of fruit, with offshore sales of $777 million. New Zealand fresh apple exports were worth $475.8 million in the June 1995 year making apples the most popular fruit exported overseas. The main production areas are Hawke's Bay, Nelson and Canterbury where Royal Gala, Braeburn, Fuji, Red Delicious, Granny Smith and Cox's Orange Pippin are the most commonly planted cultivars. In the year ending June 1995 Braeburn accounted for $187.9 million of export sales, up by 56.7%; Cox's Orange $41.0 million, up by 74.7% and Granny Smith $31.1 million, up by 56.7% (Anon, 1996).

The Board is also involved in the processing of fruit and concentrated apple juice exports reached around $40 million in 1994-1995. Significant sales are also made on the local market with Board juice brands accounting for over 60% of the market (Anon, 1996).

Samples of New Zealand apple juice have been examined and the authenticity questioned by customers and research workers, with Brause and Raterman (1982) classifying samples as "authentic, possibly sucrose added". As apple juice and apple juice concentrate is of great value to the New Zealand economy the questions raised over the authenticity is of major concern.

The producer of apple juice and apple juice concentrate who processes exclusively from fresh fruit has no need to worry that the juice might not be authentic. However, many packers never handle the fruit itself, and there is an ever-present possibility for the perpetration of fraud by the unscrupulous. This became particularly topical in 1988 with the conviction and imprisonment in the United States of those involved in the so-called "Beech-nut Scandal", whereby totally artificial mixtures of sugar, acids, water, colouring and

flavourings were sold as apple juice concentrate. (Anon, 1989). However, most frauds are considerably less blatant and involve the "stretching" of concentrate rather than the wholesale substitution with synthetic material.

The detection of fraud is a challenge because of several possibilities available to the fraudsters, such as the addition of other fruit juices, sugars, acids, and the inherent natural variation of the genuine product due to juice provenance, cultivar, production year and processing regime (Lea, 1990).

In order to detect adulteration the composition of the juice is compared to product of known authenticity and if components normally present are unusually high or low then it is possible that the juice has been adulterated, and further investigations by other techniques would be necessary. Also the presence of constituents that are not normally present can indicate adulteration.

The analytical composition of apple and apple juice has been extensively studied with sugars and acids being the major components analysed since they are present in the greatest quantities (Ayres and Fallows, 1951; Burroughs, 1984; Caldwell, 1928; Fuleki *et al.*, 1994, 1995; Lee and Wrolstad, 1988a, 1988b; Mattick and Moyer, 1983; Ryan, 1972).

Early workers used paper and column chromatography, chemical techniques and enzymes to detect sugars and acids. As technology advanced other techniques such as gas liquid chromatography (GLC) and high pressure liquid chromatography (HPLC) became popular. HPLC has become the preferred method as the technique is relatively simple and the samples require very little pretreatment, whereas GLC requires extensive sample pretreatment such as derivatisation.

While developing standards of quality and authenticity Brause and Raterman (1982) reported that " since no sample in the literature search had reported any apples with less than 5% fructose (and in most cases, much higher) nor more than 3.5% glucose or sucrose, a fructose/glucose ratio of 1.6 minimum and a sucrose maximum of 3.5% were chosen as

standards of authenticity". Probably the greatest activity in this area has been in Germany and information in terms of standard values and ranges of variations for apple juice have been published by Bielig *et al.* (1982) and are known as the RSK values. The letters RSK mean **R**ichtwert, **S**chwankungsbreite and **K**ennzahi (standard value, variation-range and reference data). According to the RSK values authentic apple juice should contain total soluble solids of between 11.68g/100ml to 14.81g/100ml; sucrose 0.5g/100ml to 3.0 g/100ml; glucose 1.8g/100ml to 3.5g/100ml; fructose 5.5g/100ml to 8.0g/100ml; sorbitol 0.2g/100ml to 0.7g/100ml and a fructose/glucose ratio of between 2 to 3.3 (glucose/fructose ratio 0.3-0.5).

The composition of fruit are highly specific for that area or cultivar and the standard values used for authenticity may not reflect the composition of fruit from another region, cultivar or a different year. While Brause and Raterman (1982) and the RSK values are a starting point it could well be inappropriate to judge apple juice produced in New Zealand with standards based on European or American juice.

The aim of this study was to examine the sugar and acid composition of juice from apples commonly grown in New Zealand by high pressure liquid chromatography and produce a database that could be used in the authentication of New Zealand produced apple juice and apple juice concentrate and to assess the applicability of the overseas standards for authenticity. As a number of factors affect the composition of apple juice, and in order that the database was valid, samples examined included fruit harvested at maturities commonly used for juice production over two growing seasons from different growing regions. Also as quantities of stored apples are used by juice manufacturers composition data from stored fruit were included in the database. The composition data for New Zealand apple juice was compared with literature values and to the criteria commonly used for authenticating apple juice.

CHAPTER 2
LITERATURE REVIEW

2.1 Composition of Apple and Apple Juice

Apple juice consists of water soluble components of the original fruit, with the main components being carbohydrate 10-12%, organic acids 0.5-1%, pectin 1%, 0.5% of other compounds including amino acids, minerals and phenolics with the remaining 85% being water (Ryan, 1972)

2.1.1 Sugars

The percentage soluble solids or degree Brix is widely used as an index for sugar content with the sugars sucrose, glucose, and fructose constituting about 90% of the carbohydrate material in apple fruit and juice. Other dissolved compounds such as non-volatile acids, the sugar alcohol sorbitol, minerals and amino acids also contribute to this measurement. The composition of apple juice is presented in appendix 1.

Fructose is the major sugar present in apple juice at levels of 5g/100ml to 8g/100ml and accounts for between 45% and 60% of the total sugars. Glucose is present at levels of 2g/100ml to 3g/100ml and accounts for up to 20% of the total sugars (Brause and Raterman, 1982; Hulme, 1958; Lea, 1990; Lee and Wrolstad, 1988b). In cider apples the proportions may be even greater, with glucose comprising 9 to 26% and fructose 74-91% of the reducing sugars. Blanco *et al.* (1992a) report that fructose comprises 68-83% of the reducing sugars for 5 varieties of Asturian (Spain) cider apples.

Sucrose is the least abundant of the three main sugars (Hulme, 1958; Lee and Wrolstad, 1988b; Zyren and Elkins, 1982) at levels of 1g/100ml to 3g/100ml (Brause and Raterman, 1982), and has been reported at levels similar to glucose (Lea, 1990), with Mattick and Moyer (1983) reporting sucrose levels of up to 5.6g/100ml.

Sorbitol, a sugar alcohol, is the fourth major carbohydrate present in apples and is found in quantities ranging from 0.52g/100ml to 1.1g/100ml (appendix 1).

Other sugars that have been reported as being present in apple are galactose in trace amounts (Lee and Wrolstad, 1988b), arabinose (Wali and Hassan, 1965) and xylose being detected by paper chromatography in the skins of apples (Siegelman, 1954) and in cider apple juice (Whiting and Coggins, 1960). Fuleki *et al.*, 1994 and Prabha *et al.*, 1990; report xylose present up to 0.25g/100ml. Also there are reports by Lee and Wrolstad (1988b) and Prabha *et al.*, 1990; of the presence of maltose. Maltose is not normally present in apple and apple juice and its presence is used as an indicator of the addition of high fructose corn syrups (Mattick, 1988). The maltose present may have been generated during rigorous extraction technique, nevertheless, the possibility that it may have originated from the apple fruit should not be overlooked (Lee and Wrolstad, 1988b).

Raffinose was also found to be present at levels below 0.07g/100g during the growth and cold storage of Macintosh Apples (Chan *et al.*, 1972) and below 0.05g/100ml in the juice of fresh and stored fruit from Canada (Fuleki *et al.*, 1994).

2.1.2 Organic Acids

Organic acids make up between 0.25% to 1% (La Belle, 1981) of the soluble solids of apples, with the major organic acid being malic acid, hence titratable acidity is quoted as percentage malic acid. Malic acid accounts for 71 to 94% of the total acids (Lee and Wrolstad, 1988b) with levels of between 270mg/100ml to 1400mg/100ml (Cliff *et al.*, 1991; Evans *et al.*, 1983; Jeuring *et al.*, 1979; Lea, 1990; Morawski, 1984; Ryan, 1972; Ryan and Dupont, 1973) in apple juice and 150 to 1300mg/100g fresh weight of apple fruit reported (Ackermann *et al.*, 1992; Blanco *et al.*, 1988, 1992a; Hulme and Wooltorton, 1957, 1958; Withy *et al.*, 1978).

Quinic acid is also present in substantial concentrations, but the amounts show large variation ranging from 10 to 330mg/100g fresh weight of apple fruit (Blanco *et al.*, 1988,

1992a; Hulme and Wooltorton, 1957, 1958; Withy *et al.*, 1978) and 20 to 400mg/100ml (Coppola and Starr, 1986; Lee and Wrolstad, 1988a, 1988b; Ryan, 1972, Ryan and Dupont, 1973; Wrolstad *et al.*, 1981) in apple juice (appendix 1). In juice quinic acid accounts for less than 23% of the total acids (Lee and Wrolstad, 1988b), although Lee and Wrolstad (1988a) report quinic acid accounting for up to 38% of the total acids.

Citric acid is the third most prevalent acid and usually does not account for more than 2% of the total acids (Lee and Wrolstad, 1988b) with levels typically less than 20mg/100ml in juice (Evans *et al.*, 1983; Lee and Wrolstad, 1988a; Ryan and Dupont, 1973) and 10mg/100g fresh weight in apple fruit (Ackermann *et al.*, 1992; Hulme and Wooltorton, 1957, 1958;). However it has been reported to account for as much as 23% of the total acid of New Zealand Granny Smith apples (Withy *et al.*, 1978) and 9% for Argentine apple juice (Lee and Wrolstad, 1988b). Citric acid levels of 40mg/100ml was found in one sample of American Golden Delicious apple juice (Lee and Wrolstad, 1988a), but the sample history of prolonged post harvest storage may account for the high level, as citric acid accumulates during storage (Hulme and Wooltorton, 1958).

Fumaric acid is present at low levels, less than 3mg/l of apple juice (Evans *et al.*, 1983; Junge and Spadinger, 1982; Lee and Wrolstad, 1988a), although this does not hold true for juice from concentrates. Pasteurisation and concentration can lead to the production of fumaric acid. Mattick (1988) found no more than 4.0g/l in authentic apple juice while as much as 10mg/l for apple juice stored at 100°C for 3 hours has been reported (Evans *et al.*, 1983).

Shikimic acid is present at levels up to 2.0mg/100ml in apple juice (Lee and Wrolstad, 1988a) and 8mg/100g fresh weight in apple fruit (Blanco *et al.*, 1988; Hulme and Wooltorton, 1958).

Succinic acid has been reported at levels of 2mg/100g tissue (Ackermann *et al.*, 1992) and citramalic (25mg/100g tissue) found in the peel of Bramley Seedlings (Hulme and Wooltorton, 1958).

Ascorbic acid is found in small amounts in apple juice and does not contribute much to the acidity (Lee and Wrolstad, 1988a) due to oxidative losses during processing (Lea, 1990). Withy *et al.* (1978) reported apple juice of New Zealand Jonathan apples contained up to 4.1mg/100ml, but in most instances was less than 2mg/100ml.

Phosphoric acid is not detected by high performance liquid chromatography. When gas liquid chromatography is used levels of up to 25mg/100ml of apple juice (Ryan, 1972; Ryan and Dupont, 1973) and 15mg/100g fresh weight (Withy *et al.*, 1978) have been detected.

The presence of lactic acid is usually associated with microbial spoilage (Morvai and Molnar-Perl, 1990; Ulrich, 1970) or reaction with the ion exchange used in the separation of the acids (Ulrich, 1970). Galacturonic acid, up to 280mg/100ml, was present in apple juice obtained by enzyme liquefaction as a result of enzymatic hydrolysis of pectin (Cliff *et al.*, 1991). Cliff *et al.* (1991) also report the presence of sorbic acid at levels up to 210mg/100ml of juice, while sorbic acid at levels of 0.70mg/100g sample was detected by Morvai and Molnar-Perl (1990).

Pyruvic acid (0.15-0.3mg/100g tissue), oxoglutaric acid (0.13-0.33mg/100g tissue) and oxaloacetic acid (0.03-0.06mg/100g tissue) were detected in apples stored at low temperature with oxaloacetic acid increasing to 0.1-0.18mg/100g tissue prior to low temperature breakdown. Low temperature breakdown is a physiological disease which causes browning of the cortical tissue (Hulme *et al.*, 1964).

Tartaric acid (5mg/100g sample), pimelic acid (2.9mg/100g sample) and oxalic acid (0.53mg/100g sample) have been detected in apple juice (Morvai and Molnar-Perl, 1990). Other acids that have been identified in apples by paper chromatography include galacturonic, glycolic, lactic, glyceric, α-ketoglutarate, glyoxylic and isocitrate (Hulme, 1958).

2.1.3 Starch

Starch is a normal constituent of apples. Unripe fruit contains 5-8% starch and as the fruit reaches maturity and ripens the levels fall to less than 1%. The levels continue to decline during the storage of the fruit (Dilley, 1970; Lea, 1990).

2.1.4 Phenolic Compounds

Phenolic compounds (cinnamic acids, benzoic acid and flavonoids) of apple fruit are of particular interest because of their role in taste (bitterness and astringency), colour and appearance due to the ability to form hazes and sediment in the juice (Lee and Wrolstad, 1988b; Spanos and Wrolstad, 1992). Chlorogenic acid and caffeic acid are the two principal cinnamic acids found in apple juice and apple juice concentrate with chlorogenic acid at levels of 18mg/l being present at 10 to 100 times that of caffeic acid (Elkins *et al.*, 1988). Chlorogenic acid (3-O-caffeoyl-D-Quinic acid) is an important cinnamic acid derivative in apples because it contains ortho-phenolic groups which serve as a substrate for polyphenol oxidase in browning reactions (Hulme, 1958; Lea, 1990; Lee and Wrolstad, 1988b; Spanos and Wrolstad, 1992).

Phloretin derivatives, such as phloridzin, are virtually unique to the genus *Malus* in the plant kingdom and are not found in any other Rosaceous fruits (Lea, 1990). By contrast other apple phenolics are also widespread in other fruits such as pears and grapes (Lea, 1990; Spanos and Wrolstad, 1992).

2.1.5 Amino Acids

Apples are low in total amino acids (3 mg/100ml) compared to other fruit such as pears, grapes, plums and strawberries (32, 135, 110, 63 mg/100ml respectively) (Fernandez-Flores *et al.*, 1970). The amino acids that predominate in apples are asparagine, proline, aspartic acid, glutamic acid, and alanine (appendix 1)

2.1.6 Minerals

From appendix 1, it can be seen that potassium is the most abundant mineral present in apples, at levels of 1000mg/l with phosphorous, calcium, sodium, and lead being present at levels typically less than 40mg/l.

2.2 Synthesis of Carbohydrates

Carbohydrates are synthesized by the fixation of carbon dioxide by photosynthesis, with the overall reaction given in equation 1.

$$6\,CO_2 + 6\,H_2O \xrightarrow{\text{light}} C_6H_{12}O_6 + 6O_2 \qquad (1)$$

2.2.1 Photosynthesis

There are three pathways for the photosynthetic fixation of atmospheric carbon dioxide:

a Calvin Cycle or C_3 pathway
b Hatch Slack Cycle or C_4 pathway
c Crassulacum Acid Metabolism Cycle or CAM pathway.

While discussion of the different pathways is outside the scope of the work, a brief account of each pathway is necessary, as the different pathways of carbon dioxide fixation by sugar cane, maize and apples has enabled detection by isotopic analysis for added sugar in apple juice.

(a) C_3 Pathways

The C_3 pathway has been variously called the Calvin Cycle, Reductive Pentose Phosphate Pathway, Photosynthesis Cycle or Photosynthetic Carbon Reduction

Cycle (PCR Cycle) (Lawlor, 1987). Apple and sugar beet are only two of some 300,000 species of plants that utilise the C_3 pathway. The C_3 pathway is the fundamental carbon dioxide assimilating process in all photosynthetic organisms in which carbon dioxide is accumulated and used in the synthesis of starch and carbohydrates.

C_3 plants are predominant in temperate regions that are often well watered, with temperatures below 28°C and have dimly lit environments where light harvesting may be a limiting factor in growth rather than carbon dioxide supply.

(b) C_4 Pathway

Tropical plants such as sorghum, sugar cane and maize not only have a C_3 pathway, but have developed an additional metabolic system (C_4 Pathway) for fixing carbon dioxide and passing the fixed carbon dioxide on to the C_3 cycle. The C_4 cycle increases the efficiency of photosynthesis, with C_4 plants having higher rates of carbon dioxide assimilation than C_3 plants. Therefore C_4 plants are found in environments with high temperature and high illumination, such as those that predominate in the tropics (Stryer, 1981).

(c) CAM Pathway

The other modification to carbon dioxide assimilation is the CAM Pathway which is found in succulent plants from the families cactacea (Cacti) and crassulacea (Stonecrops). Other non succulents such as orchids and pineapple also possess a CAM pathway (Raven *et al.*, 1981).

CAM plants contain both C_4 and C_3 cycles with the accumulation and assimilation of carbon dioxide being separated in time rather than the plants having leaves that are structurally differentiated into tissue with different biochemistry (Lawlor, 1987).

2.2.2 Sorbitol

In many plants, such as grape, sugar beet, sugar cane and tomato (Raven *et al.*, 1981; Walker and Ho, 1978) sucrose is the major product of photosynthesis in the leaves which is then transported via the phloem to the developing fruit (Bieleski, 1969). In plants from the family Rosaceous the above functions are preformed by sorbitol (Bieleski, 1969; Grant and Ap Rees, 1981; Hansen, 1970; Webb and Burley, 1962). However, some plants of the Rosaceous family, such as, rose and strawberry, do not contain sorbitol and use sucrose for these functions (Bieleski, 1977).

The evidence that sorbitol is the major photosynthetic derived carbohydrate in apples is the daily pattern in sorbitol content, namely increases in concentration during the day and a nocturnal decrease (Chan *et al.*, 1972; Chong and Taper, 1971a, 1971b). The sorbitol content was higher during the summer when there was more light available and the levels decreased during the season (Chong and Taper, 1971a, 1971b). The carbohydrate levels of the leaves increased from 4% to 13.7% (dry weight) as photosynthesis increased. The increase was due to sorbitol which accounted for up to 80% of the carbohydrate present (Loescher *et al.*, 1982; Priestley, 1983; Yamaki and Ishikawa, 1986).

The synthesis and metabolism of sorbitol is a complicated process which as yet is not fully understood (Loescher, 1987). Research in other fruits of the family Rosaceae such as plum (Anderson *et al.*, 1961, 1962) pear (Negm and Loescher, 1981; Sawyer, 1963; Yamaki and Moriguchi, 1989), apricot (Bieleski and Redgwell, 1977, 1985; Negm and Loescher, 1981; Redgwell and Bieleski, 1978) peach (Merlo and Passera, 1991; Moing *et al.*, 1992; Negm and Loescher, 1981) and loquat (Hirai, 1979,1981; Yamaki, 1980) has gone someway in eliciting sorbitol synthesis and metabolism pathways. It is thought that sorbitol is synthesized from glucose via glucose-6-phosphate to form sorbitol-6-phosphate (Anderson *et al.*, 1962; Chong and Taper, 1971b; Hirai, 1979; Negm and Loescher, 1979, 1981; Yamaki, 1981), which is hydrolysed to sorbitol (Grant and Ap Rees, 1981; Loescher, 1987; Marlow and Loescher, 1984;Yamaki, 1981). The accumulated sorbitol is translocated to the maturing fruit where it is rapidly converted to other fruit constituents (Yamaki and

Ishikawa, 1986) such as fructose and glucose and ultimately sucrose and starch are produced (Loescher, 1987; Marlow and Loescher, 1984; Yamaki and Moriguchi, 1989).

2.2.3 Sucrose Synthesis

Sucrose is synthesized from glucose-1-phosphate (figure 2.1) with two different pathways possible. Both pathways involve UDP-glucose pyrophosporylase which catalyses the formation of UDP-glucose from glucose-1-phosphate.

(a) The first route involves sucrose synthetase and this route is not thought to be responsible for sucrose synthesis as the reaction is freely reversible. It has been suggested that it is responsible for the synthesis of UDP-glucose from sucrose i.e. sucrose metabolism.

(b) The second route involves the sucrose phosphate synthetase and sucrose phosphatase. This pathway is generally accepted as the major route for the biosynthesis of sucrose as the equilibrium constant for sucrose phosphate synthetase favours sucrose phosphate synthesis. Also the formation of sucrose from sucrose phosphate is irreversible and this pathway can only operate in the direction of sucrose synthesis (Davies, 1974; Hawker, 1985; Lawlor, 1987; Whiting, 1970).

2.2.4 Starch Synthesis

Carbohydrates generated from photosynthesis that are excess to the plant requirements are stored as starch. The starch can be remobilised and consumed in respiration at a later date. (Lawlor, 1987).

The synthesis of starch occurs in the chloroplast of the leaves and fruit, but the rate in each is different. Kidd and West (1947) and Whiting, (1970) found that starch synthesis in fruit is 10% of that which occurs in the leaves.

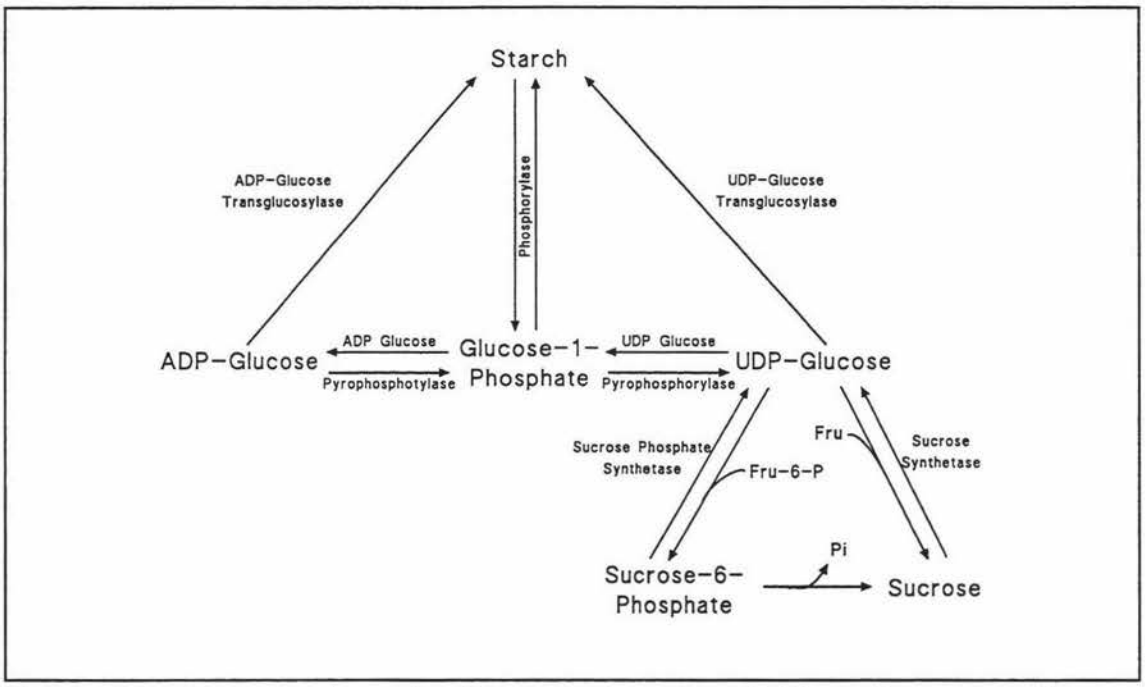

Figure 2.1: General pathway for the synthesis and metabolism of sucrose and starch (Source: Davies, 1974; Lawlor, 1987).

The interconversion of starch and sucrose is possible in many plant tissues (Wills *et al.*, 1989). A general scheme for the synthesis and metabolism of starch and sucrose is shown in figure 2.1.

2.3 Synthesis and Metabolism of Organic Acids

The individual precursors of organic acids are in general other acids or sugars. Many organic acids are present in small quantities (shikimic, fumaric, succinic), while others such as malic, citric and quinic are present in larger amounts. The acids accumulate in the vacuoles of plant tissue when there is a net excess of production over consumption (Beevers *et al.*, 1966).

The citric acid cycle (also known as tricarboxylic acid cycle or Krebs cycle) is the final common pathway for the oxidation of carbohydrates, fatty acids and amino acids and is the principal source of ATP, which represents the energy available for synthesis.

Aromatic acids such as shikimic and quinic acids are synthesized from the carbohydrates phosphoenolpyruvate and D-erythrose-4-phosphate and metabolised via the shikimic acid pathway (Haard and Chism, 1996).

2.4 Physiology and Biochemistry of Fruit

The ripening of the fruit may be defined as a sequence of changes in fruit size, colour, flavour and texture which leads to a state where the fruit is of an acceptable eating quality. Underlying these changes are a series of biochemical changes in composition and metabolism of the fruit. These biochemical changes can be slowed or enhanced by the selection of appropriate harvest dates and storage conditions, thereby reducing or inducing ripening of the fruit.

The life of the fruit can be divided into four physiological stages following initiation or germination (Gortner *et al.*, 1967). These are:

(a) Prematuration -"the developmental period prior to the onset of the maturation processes, and generally including at least half the interval between blossoming and harvest. This stage is characterised by extensive cell enlargement"

(b) Maturation -"the stage of fruit development during which the fruit emerges from the incomplete stage to attain a fullness of growth and maximum edible quality"

(c) Ripening -"the terminal period of maturation during which the fruit attains its full development and its maximum aesthetic and edible quality. Changes taking place during this period are primarily chemical".

(d) Senescence -"the period following fruit development during which growth has ceased and the biochemical processes of ageing replace those of ripening. Senescence may occur either before or after fruit harvest"

However, clear distinction between the various stages is not easily made. The term development is also commonly used and was defined by Gortner *et al.*, 1967 as "the entire period during which new tissue is formed and brought to morphological completion, and perfective chemical changes take place. The period of fruit development covers the stages of prematuration and maturation, the latter of which includes ripening".

2.4.1 Changes During Fruit Development and Ripening

During the first 3 to 4 weeks after pollenation a phase of rapid cell division ensures (Biale, 1964; Hulme and Rhodes, 1971), followed by a period of cell enlargement which accounts for the final size of the fruit (Wills *et al.*, 1989). With cell enlargement vacuoles appear and carbohydrates are transported from the leaves, (although they can be synthesized by the fruit), to the fruit (Biale, 1964; Smock and Neubert, 1950). As the fruit develops and ripens sucrose accumulates (Ackermann *et al.*, 1992; Hulme, 1958; Lea, 1990; Smock and Neubert, 1950) with reports of fructose increasing (Hulme, 1958; Lea, 1990; Smock and Neubert, 1950) and fluctuating around the same value until just before harvest (Ackermann *et al.*, 1992; Blanco *et al.*, 1992a). Glucose begins to increase at the start of the maturation phase (Hulme 1958, Lea, 1990, Smock and Neubert, 1950) before decreasing during growth (Hulme, 1958; Lea, 1990). Hulme (1958) reports that after the initial increase in glucose the levels remain relatively constant for the remainder of the maturation phase.

Sorbitol increases early during fruit growth (Chan *et al.*, 1972) then falls and remains at low levels throughout the growing season (Ackermann *et al.*, 1992; Blanco *et al.*, 1992a; Chan *et al.*, 1972). Starch also begins to accumulate, reaching a peak midway through the season

before decreasing (Hulme, 1958; Lea, 1990). A summary of the changes that occurred are presented in figure 2.2 and 2.3. The titratable acidity increases within the first six weeks of fruit setting and during these early stages of fruit development organic acids other than malic acid predominant. Their content declines rapidly within the first 2-4 weeks and remain low, while the level of malic increases (Hulme, 1958; Krotkov *et al.*, 1951) and after 4 weeks malic acid accounts for up to 80% of the organic acid (Krotkov *et al.*, 1951). At about 6 weeks after petal fall, the malic acid level peaks and begins to decrease (Ackermann *et al.*, 1992; Hulme, 1958; Krotkov *et al.*, 1951) with citric acid mimicking that of malic acid (Ackermann *et al.*, 1992; Hulme, 1958), although a slight increase was observed before a fall was observed (Hulme and Wooltorton, 1957). Quinic acid levels decrease up to harvest with levels most evident during the period of greatest metabolic activity. The respiration rate of peel is 4-5 times that of the pulp and the levels of quinic acid in the peel are twice that observed in the pulp, even at maturity (Hulme, 1958). Shikimic occurs at low levels and was observed by Hulme (1956b) to increase during ripening and senescence, with Ackermann *et al.*, (1992) observing only slight changes and Hulme (1958) not detecting any shikimic acid. The decrease in acid concentration was attributed to a dilution effect caused by a maximum increase during the cell growth phase (Ackermann *et al.*, 1992).

The greatest change in fruit physiology and biochemistry occur just prior to harvest when ripening begins. All sugars suddenly increase, starch decreases and organic acids continue to decrease.

While some of the increase in sugars can be attributed to the breakdown of starch, starch hydrolysis is not directly related to sugar increase as the sugars tend to increase long after starch has disappeared (Hulme, 1958). The increase in glucose just before harvest can be related to starch synthesis and hydrolysis (Ackermann *et al.*, 1992)while Leinbach and Talburt (1953) indicate that the increases in reducing sugars are due to the interconversion of higher sugars and starch in the fruit to reducing sugars. However, Wills *et al.* (1989) suggest that the sugars are derived from the sap imported into the fruit. Of these changes, the change in respiration rate and ethylene production have gained priority in attempts to

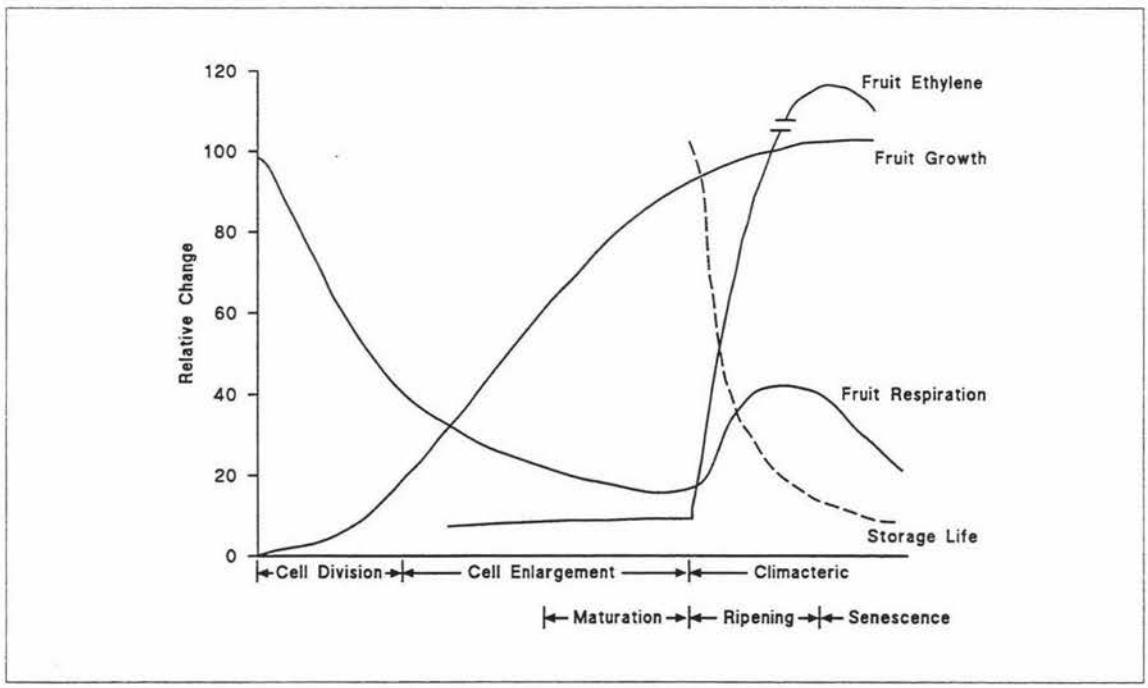

Figure 2.2: Schematic diagram showing the relationship between apple fruit growth and development, respiratory activity, ethylene production, and storage life potential (Source: Massey, 1989).

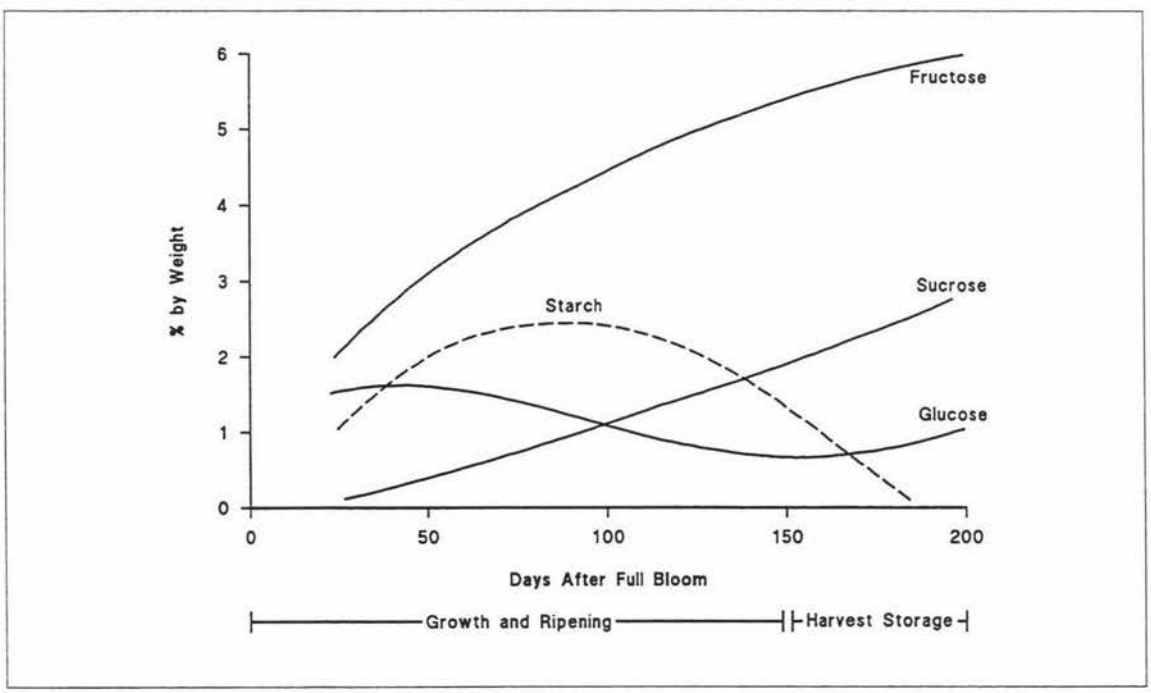

Figure 2.3: Typical changes in levels of fructose, sucrose, glucose and starch during ripening and storage (Source: Lea, 1990).

develop an explanation of the mechanisms of ripening. These are discussed more fully in the following sections.

2.4.2 Respiration

Respiration is the major metabolic process taking place in harvested fruit and can be described as the oxidative breakdown of the more complex materials such as starch, sugars and organic acids, normally present in the cells into simpler molecules such as carbon dioxide and water with the concurrent production of energy and other molecules that can be used by the cell for synthetic reactions (Lee and Wrolstad, 1988b; Wills *et al.*, 1989). The respiratory activity reduces the quantity of food reserve present, and is an irreversible process which cannot be halted once started (Rhodes, 1970).

As the fruit begins to ripen there is an increase in the ethylene concentration and a rise in the respiration rate. The increase in the respiration rate has been termed "the respiratory climacteric". The relationship between the onset of the respiratory climacteric and the ripening hormone ethylene affects the ripening and the storage life of apples.

2.4.2.1 Respiratory Climacteric

The respiratory climacteric is an important phase in the life of the fruit that can be defined as "a period in the ontogeny of certain fruits, during which a series of biochemical changes is initiated by the autocatalytic production of ethylene, marking the change from growth to senescence and involving an increase in respiration and leading to ripening" (Rhodes, 1970).

2.4.2.2 Effects of Ethylene

The presence of ethylene in the intracellular spaces within the fruit at concentrations of 0.1ppm or greater precedes the rise in respiration and ripening (Massey, 1989). An exogenous supply of ethylene from other fruits may trigger off the climacteric in unripe fruit and induce in them the autocatalytic process of ethylene synthesis, a rise in respiration and

ripening. Therefore methods that reduce ethylene concentrations in the storage atmosphere will decrease respiration and fruit ripening.

The sensitivity of the fruit to ethylene can be lessened by lowering the storage temperature and by either raising the level of carbon dioxide or decreasing the level of oxygen. Under these conditions the amount of ethylene required to induce ripening is increased (Smith, 1963).

2.5 Factors Affecting the Composition of Apple Juice

The chemical composition of apple juice varies due to the growing season, region, cultivar, maturity, storage and processing conditions and limited information is available that shows the effect of these variables on the composition of apple juice. In order that valid comparisons are made all fruit should be of an equal maturity. Unfortunately the index to determine maturity is not infallible with a range of different techniques such as colour, ease of separation from the tree, fruit firmness, soluble solids content, starch content (Smock and Neubert, 1950) being used to determine the optimum harvest date.

2.5.1 Growing Season

The climate has a strong influence on the composition of apple cultivars and its effect is often unrecognised (Lea, 1990). Apples grown in warm sunny seasons are as a rule higher in sugar and acid than those grown in a cool cloudy season (Caldwell, 1928; Moyer and Aitken, 1980; Smock and Neubert, 1950), while the juice of apples from a dry season will be higher in soluble solids but less in volume than after a wet season (Moyer and Aitken, 1980).

2.5.1.1 Sugars

In an unpublished study of cider apple juices, made over a ten year period from the same individual trees, in the same orchard, the levels of juice acids and sugars showed a range of

±20% around the mean value with a relative standard deviation of 12% (Lea, 1990). In a study of 216 varieties over a period of four to six years it was found that the sugar content of the juice varied considerable from year to year (Caldwell, 1928).

Fuleki *et al.* (1994) showed that glucose and raffinose concentrations were significantly higher in 1989 (compared to 1990) for six cultivars grown in two regions of Ontario, Canada. Whereas Trautner and Somoygi (1978) reported significant seasonal differences in glucose, sucrose and fructose contents of the ripe fruit of four cultivars in the 3 years studied. This significant variation was explained by Fuleki *et al.* (1994) as being due to the apples being obtained from a commercial supplier and were therefore unlikely that their fruit originated from the same orchard each year.

However, Mattick and Moyer (1983) analysed a total of 98 samples of apples (15 varieties common to 8 growing regions) of the USA, over a period of 3 years and found that the data did not show marked differences in the range of constituents between the years of analysis. "For all practical purposes the mean, coefficient of variation, minimum and maximum for a single attribute are the same for all three years".

2.5.1.2 Organic Acids

While acidity is not as markedly affected by seasonal conditions as sugars, it is affected to some degree. It was noted that following a season of high light intensity and duration (much sunshine) the acidity was high (as well as sugar content) (Smock and Neubert, 1950). The juice from fifteen varieties of apple were examined by Poll (1981) over two years, and had a mean total acidity for all varieties of 0.82g/100ml (range of 0.48g/100ml to 1.25g/100ml), with the total acidity being lower in most varieties in 1978 (mean value of 0.76g/100ml) compared to 1977 (mean value of 0.85 g/100ml). However, Mattick and Moyer (1983) in a large scale 3 year study found no significant differences in total acidity between the different years. The mean value for their study was 0.42g/100ml with a range of 0.15g/100ml to 0.91g/100ml.

There was very little difference in the acidity of juice from Starkling Delicious apples picked from the same orchard at different times during maturation with values ranging from 0.27g/100ml to 0.29g/100ml in one year and 0.24g/100ml to 0.28g/100ml the following year (Kubo and Tamura, 1979). The calculated yearly mean for 5 consecutive years from 1945 to 1949 for the results of Ayres and Fallows (1951) were in the range of 0.75g/100ml to 0.83g/100ml. Fuleki *et al.* (1995) also found no significant difference in the total acids from the juice of fruit grown over two seasons. They did find that the pH was significantly higher in 1989, while quinic acid was significantly higher from the juice of apples grown in Simcoe, Ontario in 1989 and succinic acid from the juice of apples grown in Smithfield, Ontario in 1990.

2.5.2 Growing Region

Sugar and acid levels of apples have been shown to vary from location to location. These differences may also be attributed to soil or climatic variations or both.

2.5.2.1 Sugars

In a study (Smock and Neubert, 1950) it was shown that the sugar content in a given variety varied more from orchard to orchard than it did between varieties in the same orchard.

Withy *et al.*, (1978) showed that on average the juice of apples grown in the Hawke's Bay, New Zealand had a soluble solids content of 11.5%, while the same varieties grown in the Nelson region were observed to have a soluble solids content of 13.3%. The mean value for soluble solids of New Zealand grown apples (both Nelson and Hawke's Bay) was 12.4 °Brix which was similar to that of 12.74°Brix for the juice of American (Mattick and Moyer, 1983) grown fruit but was higher than that of 11.7°Brix for Canadian juice (Ryan, 1972).

A breakdown of Mattick and Moyer (1983) results by geographical region showed a regional variation in which the juice of apples grown in North Carolina and Michigan have

a soluble solids content of 11.3° brix while values of up to 14.9° brix were observed for juice of apples grown in California (Lee and Mattick, 1989; Mattick, 1988). When examining the individual sugars of apple juice from the different regions Fuleki *et al.* (1994) reported that in Lee and Mattick's (1989) study, there were significant differences for Brix, glucose and sorbitol content of the apples grown from the eight regions in the USA. However, the differences observed could be partially attributed to varietal differences as the number of cultivars studied in each state varied from one to seven. In their own study Fuleki *et al.* (1994) found no significant differences in sugar composition of juice except for raffinose, from fresh and stored fruit analysed from the same year in different regions. Raffinose was significantly lower, 0.005-0.01g/100ml, in juice produced from stored fruit compared to 0.005-0.05g/100ml for juice from fresh fruit grown in Simcoe, Ontario for the 2 years of the study.

Golden Delicious apples grown in Hawke's Bay had a calculated sucrose level of 1g/100g fresh weight while similar fruit grown in Nelson had sucrose level of 0.4g/100g fresh weight (Withy *et al.*, 1978). However Golden Delicious fruit from USA and Switzerland had sucrose levels 3.2g/100g fresh weight and 2.7g/100g fresh weight respectively (Wrolstad and Shallenberger, 1981). The sucrose content for Jonathan apples grown in Switzerland was reported to be 1.29g/100g fresh weight (Wrolstad and Shallenberger, 1981). The sucrose content of the same cultivar grown in Nelson and Hawke's Bay, New Zealand was 2.0 g/100g fresh weight and 2.5 g/100g fresh weight respectively (Withy *et al,* 1978).

2.5.2.2 Organic Acids

Moyer and Aitken (1980) report that while the growing conditions and location have an affect on the acidity so to does the variety and condition of the fruit. They further report that in the eastern regions of North America there is a definite increase in the acidity of apples from south to north. In Virigina the acidity tends to be low with a range of 0.25g/100ml to 0.45g/100ml, in Pennsylvania the acidity is usually medium with a range of 0.30g/100ml to 0.55g/100ml, while in Nova Scotia the acidity tends to be high with a range of 0.4g/100ml to 0.85g/100ml.

Mattick (1988) and Lee and Mattick (1989) evaluated Mattick and Moyer (1983) data into 8 regions and found that the mean total acidity ranged from 0.203 in North Carolina to 0.587g/100ml in New York, while Withy *et al.* (1978) report apples grown in Nelson, New Zealand had a acidity of 0.42g/100ml, and Hawke's Bay apples, a mean of 0.55g/100ml. Canadian apple juice had a mean acidity of 0.48g/100ml (Ryan, 1972), while Danish apples had a regional mean of 0.82g/100ml (Poll, 1981) and Chinese Starkling Delicious had a mean acidity of 0.23 g/100ml (Kubo and Tamura, 1979). The acidity of English apple juice ranged up to 1.5g/100ml (Ayres and Fallows, 1951), but their investigation included both "culinary " and "cider" apples which are much higher in acid than the high-sugar, low-acid "dessert" varieties that are the principal source for juice production in most countries (Lee and Wrolstad, 1988b).

2.5.3 Cultivar

2.5.3.1 Sugars

It is well established that the cultivar will affect the amount of total sugars as well as the proportion of individual carbohydrates in apple (Ackermann *et al.*, 1992; Blanco *et al.*, 1992a; Hulme, 1958; Kubo and Tamura, 1979; Wrolstad and Shallenberger, 1981) and apple juice (Ayres and Fallows, 1951; Brause and Raterman, 1982; Fuleki *et al.*, 1994; Lee and Mattick, 1989; Lee and Wrolstad, 1988a; Mattick and Moyer, 1983; Moyer and Aitken, 1980; Poll, 1981; Withy *et al.*, 1978). Table 2.1 gives some cultivars and the levels of sugars reported in the literature.

Table 2.1: Sugar content of different cultivars grown in the same region

Cultivar	Region	Sucrose (g/100ml)	Glucose (g/100ml)	Fructose (g/100ml)	Sorbitol (g/100ml)	Source
Golden Delicious	WA USA, 1981	***0.20***	3.64	8.61	0.28	Lee and Wrolstad, (1988a)
	WA USA, 1982	1.53	2.75	7.88	0.36	Lee and Wrolstad, (1988a)
	Mexico	2.96	2.15	7.26	0.26	Lee and Wrolstad, (1988a)
	Canada, Orchard 1, 1989	2.60	1.95	6.09	0.19	Fuleki *et al.* (1994)
	Canada, Orchard 2, 1989	**4.34**	1.56	6.76	**0.42**	Fuleki *et al.* (1994)
	Canada, Orchard 2, 1990	3.20	1.86	6.76	0.32	Fuleki *et al.* (1994)
	Canada, Orchard 3, 1989	1.72	2.27	5.54	***0.16***	Fuleki *et al.* (1994)
	Canada, Orchard 3, 1990	1.33	1.67	5.01	0.17	Fuleki *et al.* (1994)
	USA	0.7	1.9	5.7	—	Brause and Raterman, (1982)
	South Africa	2.43	2.00	5.18	0.43	Fourie *et al.* (1991)
Granny Smith	New Zealand	1.14	**4.10**	**9.21**	0.38	Lee and Wrolstad, (1988a)
	USA	2.1	3.5	6.0	—	Brause and Raterman, (1982)
	South Africa	2.19	1.75	***3.81***	0.28	Fourie *et al.* (1991)
Mcintosh	USA	1.86	1.76	9.00	0.37	Lee and Wrolstad, (1988a)
	Canada, Orchard 1, 1989	3.12	1.05	5.76	0.20	Fuleki *et al.* (1994)
	Canada, Orchard 1, 1990	3.08	***0.8***	6.14	0.22	Fuleki *et al.* (1994)
	Canada, Orchard 2, 1989	2.36	1.00	5.79	0.20	Fuleki *et al.* (1994)
	Canada, Orchard 2,1990	2.53	1.17	6.04	0.25	Fuleki *et al.* (1994)
	USA	1.0	2.3	8.5	—	Brause and Raterman, (1982)

Bold italic typeface are minimum values

Bold typeface are maximum values

2.5.3.2 Organic Acids

Lee and Mattick's, (1989) breakdown of Mattick and Moyer's (1983) 3 year study by variety reports that the mean acidity varied from 0.20g/100ml for Stayman and Winesap to 0.78g/100ml for Twenty Ounce (range for all samples of 0.15g/100ml to 0.91g/100ml), which was similar to the eight varieties of New Zealand apples (0.19g/100ml-1.01g/100ml) (Withy *et al.*, 1978) and lower than 18 varieties of Danish apples which ranged from 0.48 to 1.25g/100ml (Poll, 1981). Ayres and Fallows (1951) 145 sample investigation of English apples had total acidity ranging from 0.24g/100ml to 1.50g/100ml although some of these samples include the traditionally high acid cider apples

While most reports are of total acidity, there are some reports in the changes of individual acids (malic, quinic, citric). Malic acid ranged from 0.28g/100ml to 0.9g/100ml (Evans *et al.*, 1983), 0.41g/100ml to 0.84g/100ml (Lee and Wrolstad, 1988a), 0.27g/100ml to

1.02g/100ml (Moyer and Aitken, 1980) for different varieties. Citric acid has been found to be quite variable in apple juice from undetectable levels (Evans *et al.*, 1983; Moyer and Aitken, 1980) to levels of 20mg/100ml (Evans *et al.*, 1983; Lee and Wrolstad, 1988a; Moyer and Aitken, 1980). However Lee and Wrolstad (1988a) found one sample that had citric acid at 40mg/100ml but this sample had prolonged post-harvest storage which may account for the high level.

There are few reports in the literature on the quantities of quinic and shikimic acid in authentic apple juice. Quinic acid is more variable than malic acid with levels ranging from 56mg/100ml to 370mg/100ml in the juice of different varieties of apples (Lee and Wrolstad, 1988a), while Ryan, (1972) also found quinic acid levels to be more variable than malic acid and ranged from 20mg/100ml to 41mg/100ml in authentic samples of canned juice.

Shikimic and fumaric acids have been found at levels of between 0.08mg/100ml and 1.92mg/100ml for shikimic acid and at concentrations less than 0.17mg/100ml for fumaric acid (Lee and Wrolstad, 1988a).

2.5.4 Time of Harvest

The optimal date of harvest for that fruit is determined by its use. Both the sugar and organic acid content of the fruit are used as a guide to the readiness of the fruit for harvest (La Belle, 1981) and are related to taste and overall acceptability of the product for consumption (Gorin, 1973). Poll (1981) found that the overall taste was strongly correlated with fruit aroma, although sugar, acid and polyphenol content also played a role in taste. They also found that the sugar:acid ratio rather than total sugar or total acid content were important in determining whether a juice was very sweet or very sour.

For long term storage (cold, controlled atmosphere) the fruit should be harvested prior to the respiratory climacteric. If the fruit are harvested prior to the onset of the respiratory climacteric the respiratory peak is generally lower than if the fruit is allowed to develop on the tree (Hulme and Rhodes, 1971). Once the climacteric rise in respiration is under way

ripening is an irreversible process which can be slowed but not halted by the application of external factors such as storage conditions.

The fruit should be harvested before the climacteric for cold or controlled atmosphere storage to be effective in preserving the quality of the fruit. Fruit that are harvested post climacteric age considerably more rapidly than preclimacteric fruit (Massey, 1989).

2.5.5 Storage of Apples

Apples are stored under a number of different storage regimes such as ambient, cold and controlled atmosphere for utilisation in juice production at a later date. While the fruit is stored, the sugar and acid composition continues to change due to the normal action of respiration, changing the composition of the apple juice or concentrate produced (Hulme and Rhodes, 1971).

2.5.5.1 Ambient Storage

Ambient stored fruit are held at normal atmospheric temperatures in air in palletised bins in the yard close to the processing plant. If the fruit are harvested at the preclimacteric or early on the climacteric rise and held under these conditions the flavour, colour and texture which are associated with the ripe fruit develop quickly as the fruit pass through the climacteric of respiration (Knee, 1971) resulting in a reduced potential storage life. Therefore these conditions are used for relatively short periods prior to processing.

2.5.5.2 Cold Storage

The rates of chemical reactions that occur in living tissue, such as respiration, are influenced by temperature (Wills *et al.*, 1989), thus lowering the temperature of the apple fruit would reduce the respiration rate (Massey, 1989; Wills *et al.*, 1989). Lowering of the temperature causes the oxygen uptake and carbon dioxide production to decrease linearly (Knee, 1971) and thereby reduces the respiration rate and sugar loss.

2.5.5.3 Controlled Atmosphere Storage

Controlled atmosphere storage involves low temperature (as for cold storage) with an atmosphere that has oxygen levels reduced to between 1% and 5% and carbon dioxide levels increased up to 5% compared to ambient air which contains 21% oxygen, 0.01% carbon dioxide and 78% nitrogen (Massey, 1989). As oxygen is required for respiration, reducing it will slow respiration. Increasing the carbon dioxide level will slow down the chemical reaction involved in respiration.

Controlled atmosphere storage reduces the respiration rate and extends the storage life for processing apples to 7-9 months or even more depending on the efficiency of operation of the storage facility, the cultivar and the particular processed product being manufactured (Massey, 1989).

2.5.6 Changes During Storage

2.5.6.1 Sugars

At the time of harvest and during the early stages of storage the sugars continued to increase. After two weeks of storage glucose and fructose concentrations increased (Ackermann *et al.*, 1992; Leinbach and Talburt, 1953) or decreased and levelled off (Ackermann *et al.*, 1992; Blanco *et al.*, 1992a) while sucrose tended to decrease (Ackermann *et al.*, 1992; Blanco *et al.*, 1992a; Hulme, 1958; Leinbach and Talburt, 1953).

The sudden decrease of all three sugars at the beginning of the storage period can be explained by the fact that the apples were harvested just before the climacteric. This phase is characterised by a period of increased respiration during which the sugars and acids are rapidly used as substrates in the metabolic process. Once the cell growth period is terminated the sugar content does not vary by much (Ackermann *et al.*, 1992). The gradual decrease of sugars during the remaining life of the fruit, is due to them serving as respiration substrates (Smock and Neuburt, 1950).

Several authors (Fuleki *et al.*, 1994; Kubo and Tamura, 1979) have studied the composition of apple juice produced from apples stored for up to six months prior to processing. Fuleki *et al.*, 1994 found highly significant differences in all of the identified components except raffinose, sorbitol and total sugars. The sucrose content decreased while both the fructose and glucose concentrations increased on storage, indicating that sucrose was inverted. However they could not account for all the sucrose lost by the increase in fructose and glucose contents, suggesting that some sugar was lost through respiration.

Sorbitol shows a general increasing trend during storage (Ackermann *et al.*, 1992; Blanco *et al.*, 1992a; Chan *et al.*, 1972).

2.5.6.2 Organic Acids

During storage the titratable acidity decreases (Hulme, 1958; Krotkov *et al.*, 1951; Leinbach and Talburt, 1953) but the change in individual acids is interesting. Malic acid suddenly increases just before harvest, and then begins to decrease within 2 weeks of storage (Ackermann *et al.*, 1992; Blanco *et al.*, 1992a; Hulme and Wooltorton, 1958; Krotkov *et al.*, 1951). Citric acid was reported by Blanco *et al.* (1992a) as mimicking malic acid, but Hulme (1958) and Hulme and Wooltorton (1958) report that citric acid increased rapidly during the first 15 days of storage and then more slowly up to 100 days. The production of citric acid may be oxygen dependent, since air stored fruit contains higher levels of citric acid. It has been demonstrated that on oxidation, quinic acid is converted to citric acid but evidence *invivo* is lacking (Kollas, 1964). Blanco *et al.* (1992a) could not determine the presence of citric acid in 5 varieties of cider apples.

Quinic acid rose rapidly from 40mg/100g to 80mg/100g during the first half of storage (40 days) in the pulp but had fallen to 50mg/100g by 100 days. Similar results were seen in the peel, but the levels were 5 x higher than in the pulp (Hulme, 1958). Citramalic was reported by (Hulme, 1958) as not being present at harvest but had increased to 10mg/100g after 25 days of storage and 25mg at the end of storage (100 days).

In the peel shikimic acid appears in appreciable quantities and this acid may arise directly from its closely similar alicyclic analogue quinic acid. If the two acids are normally in equilibrium, then as the fruit "runs down" the equilibrium shifts in favour of shikimic acid. In the pulp shikimic acid only appears at 70 days and rises to 2.1mg/100g at 84 days and then falls and disappears by 98 days (Hulme and Wooltorton, 1958). Hulme (1958) reports that shikimic acid rose steadily, with an increase in rate from 5mg at commencement to 8mg at 100 days, when quinic acid commenced to fall.

The appearance of succinic acid has been related to a physiological disease (carbon dioxide injury) which apple and pears are liable to during storage (Hulme, 1956a, 1958; Hulme and Wooltorton, 1958). Accumulation of succinic acid in apple fruit stored in an atmosphere highly enriched in carbon dioxide has been observed by (Hulme *et al.*,1964). This is probably caused by the suppression of succinate dehydrogenase, which is responsible for the conversion of succinic acid to fumaric acid (Ranson *et al.*, 1957).

The total acid is greater in controlled atmosphere stored fruit than that from air storage, with malic accounting for most of this along with quinic and shikimic. Citric and phosphoric acids were higher in air rather than controlled atmosphere stored fruits. If controlled atmosphere simply slowed down the normal processes that occur in air one would expect malic and quinic acid to be higher in controlled atmosphere fruit and citric and shikimic to be lower (Kollas, 1964). Their results agree with the prediction for malic, quinic and citric but not for shikimic. It is possible that the differences in the "unknown acids" masked the differences in shikimic acid.

The higher malic acid in controlled atmosphere compared to air stored fruit may be due to carbon dioxide fixation; as there is evidence that Macintosh apples in air containing 5% carbon dioxide produce malic acid at significant rates by carbon dioxide fixation (Kollas, 1964). It is unlikely that the higher malic acid content in fruit in a controlled atmosphere could be due to limited oxidation in low oxygen, since its loss in stored apples is the same in nitrogen as in air (Kollas, 1964).

2.5.7 Processing

The processing of the fruit and extraction of the juice affects the analytical and sensory characteristics of the juice. The first procedure in apple juice production is crushing or milling of the apples to a pulp. On milling, many fruit enzymes and substrates are brought into contact and rapid changes can occur in certain juice components, of which the most susceptible are pectin, polyphenols and volatile flavour components. Some aspects of apple juice, such as colour, are entirely a consequence of processing. Different mill types, different press-hall temperatures and different shapes of mash tanks will all play a part in determining the final colour of fresh juice (Lea, 1990).

The juice is extracted from the pulp by pressing and under optimum conditions juice yield is 70% to 80% (w/w), but may be below 65% (w/w) for stored fruit (Cliff *et al.*, 1991). Pectolytic enzymes can be added that break down the cell structure and so allow the juice to flow more easily and increases juice yields. Schols *et al.* (1991) studied the effect of juice manufacturing by straight pressing, pulp enzyming (adding 200mg/kg enzymes to mash pressing), and liquefaction (adding 300mg/kg enzymes a holding prior to at 45°C for 4 hours to solubilise the cell walls) of Golden delicious apples. They found that juice recovery from apples can be increased by enzyme treatment of the apple mash, especially by liquefaction process. Juice from the varying manufacturing processes differed in pH and total acid content depending on enzyme treatment and storage time of apples. When the °Brix of the juice was measured, it was found that liquefaction process gave higher values (12.1 - 13.0°Brix) than pulp enzyming (11.5 - 12.0 °Brix) or straight pressing (8.0 - 11.4 °Brix). Cliff *et al.* (1991) also showed that the juice extraction process as well as apple cultivar affected the juice character, although extraction process had more influence on juice character. For all cultivars diffusion extraction had the lowest total acids and malic acid content and the highest pH. The highest malic acid and soluble solids level was found in juice from enzyme liquefaction, possibly due to the increased extraction of acids and sugars into the juice when the cells were disintegrated (Cliff *et al.*, 1991). With diffusion extraction by manipulating the processing temperature and screw speed (i.e. solids residence time) it is possible to produce products with variations in sugar/acid ratio, pectin content and the

nature of the extracted pectin. However, it is also possible to produce a product with similar characteristics to a pressed juice (Leach *et al.*, 1993).

2.5.8 Natural Variability

2.5.8.1 Sugars

Sucrose and sorbitol are the most variable of the four main carbohydrates with a coefficient of variation typically greater than 35% (Mattick and Moyer, 1983; Ryan, 1972; Wrolstad and Shallenberger, 1981), with a range of 33% to 64% for sucrose and 16% to 64% for sorbitol. (Brause and Raterman, 1982; Lee and Wrolstad, 1988a; Ryan, 1972; Wrolstad and Shallenberger, 1981).

Glucose was reported by (Ryan, 1972) as having a coefficient of variation of 16% but Lee and Wrolstad (1988a) and Mattick and Moyer (1983) reported coefficient of variation for glucose of around 30%. This was confirmed by Wrolstad and Shallenberger (1981) who analysed the mean, standard deviation and coefficient of variation of sucrose, glucose, fructose and sorbitol from the literature, and found that the coefficient of variation for glucose was 35.8%. Of the four main carbohydrates fructose appears to be the least variable, having the lowest coefficient of variation of below 23% (Lee and Wrolstad, 1988a; Mattick and Moyer, 1983; Ryan, 1972; Wrolstad and Shallenberger, 1981).

2.5.8.2 Organic Acids

There is considerable variation both within and among different studies reported in the literature for total and individual acids. Mattick and Moyer (1983) found titratable acidity ranging from 0.15g/100ml to 0.91g/100ml with a coefficient of variation of 41.2%, while Fuleki *et al.* (1995) report values of between, 0.15g/100ml to 1.17g/100ml (coefficient of variation 44.9%0; Lee and Wrolstad (1988a), 0.58g/100ml to 1.2g/100ml (coefficient of variation of 26.5%); Ryan (1972), 0.38g/100ml to 0.58g/100ml (coefficient of variation 9.8%). Of the individual acids malic acid showed the least variation with a coefficient of

variation ranging from 9.3% (Ryan, 1972) to 37.5% (Lee and Wrolstad, 1988a). Quinic was reported to have coefficients of variation ranging from 24.9% to 57.7%; citric, 21.4% to 115%; shikimic, 64.2% to 77.8%. Fumaric and succinic have been reported to show large variation with coefficients of variation of 150% and 73.3% respectively (Fuleki *et al.*, 1994; Lee and Wrolstad, 1988a; Ryan, 1972). There was less variation when the acids were expressed as a percentage of the total acids (Lee and Wrolstad, 1988a).

2.6 Methods and Criteria for the Determination of Adulterated Apple Juice

If in a pure juice the level of the component did not vary greatly it would be possible to specify tight reference values and the potential for adulteration is reduced. As most juice is internationally traded reference values have to be broad enough so as not to reject too many samples, and this could be taken as an incentive for illegal manipulations. Therefore the choice of the "reference" or "base" sample is critical for determining authenticity.

Although there is currently no single method or component of apples for detecting adulteration absolutely, there are a variety of techniques and components available that when considered together can indicate whether or not adulteration may have occurred.

2.6.1 Reference Samples

To detect adulteration it is necessary to have a detailed insight into the composition of authentic juices from various sources, as well as the natural variations and concentrations (reference values).

A number of compilations of analytical data have been published (Brause and Raterman, 1982; Evans *et al.*, 1983; Mattick and Moyer, 1983), along with numerous published accounts of the amounts of various major components present in apples and apple juice (Ayres and Fallows, 1951; Brause and Raterman, 1982; Burroughs, 1984; Evans *et al.* 1983; Fuleki *et al.*, 1994; Lea, 1990; Lee and Wrolstad, 1988a, 1988b; Mattick, 1988; Mattick and

Moyer, 1983; Poll, 1981; Ryan, 1972; Withy *et al.*, 1978; Wrolstad and Shallenberger, 1981). On the basis of these limits, values for authenticity can be derived.

Due to the wide variation in measurements of characteristics due to factors such as growing region, degree of ripening, cultivars and environmental conditions of growing, ripening and storage, the reference set can at most reflect the sources from which the samples were taken. If the base set is collected from juices produced in a certain area or from a specific variety, the averages of the attributes are appropriate only for that area or variety. Similarly, if the samples come from one part of the growing season, they may not reflect concentrations from another part of the season.

2.6.2 RSK Values

The German fruit juice association have taken this concept one step further with the publication of its RSK (**R**ichtwert, **S**chwankungsbreite and **K**ennzahi which means standard value, variation-range and reference data) tables (Bielig *et al.*, 1982, Wallrauch and Faethe, 1988b), which have been produced for many fruit juices including apple. The RSK table contains the medium value, the range, the guide value (appendix 2) and a commentary for these values, for those analytes which are judged to be most relevant (Lea, 1990). The RSK system avoids fixing extremely wide ranges by having a narrow range for some values and those components that are judged most relevant for the juices that legitimately fall outside the indicated range are integrated separately by a commentary (Wallrauch and Faethe, 1988b).

In comparing the RSK criteria to other major compilations of data that have been published (figures 2.4-2.9) it can been seen that not all the values for each component are in complete agreement. Although comparisons with the RSK values can be made, apple juices that differ in a particular parameter are not necessarily adulterated. The RSK values are intended as guidelines with which most pure apple juices will conform, and any departures from this composition should be investigated by further tests to establish if the deviation is due to varietal or climatic factors (specific to the raw material) or to some illegal processing

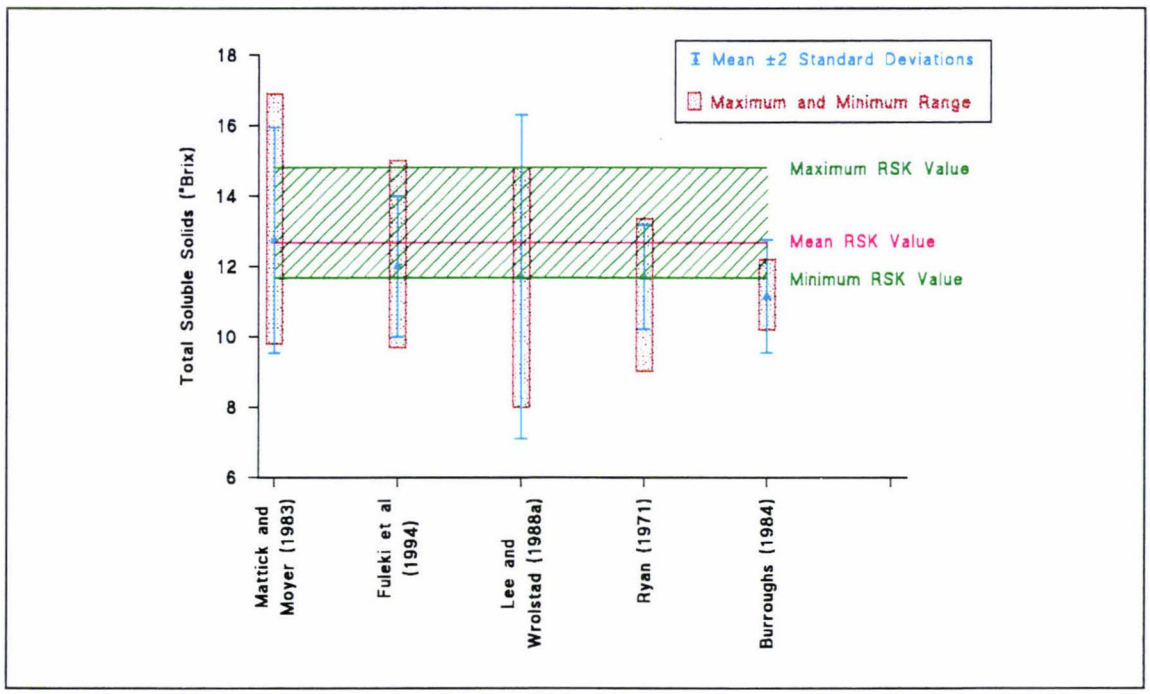

Figure 2.4: Comparison of total soluble solids concentrations from published data with the RSK values for authentic apple juices.

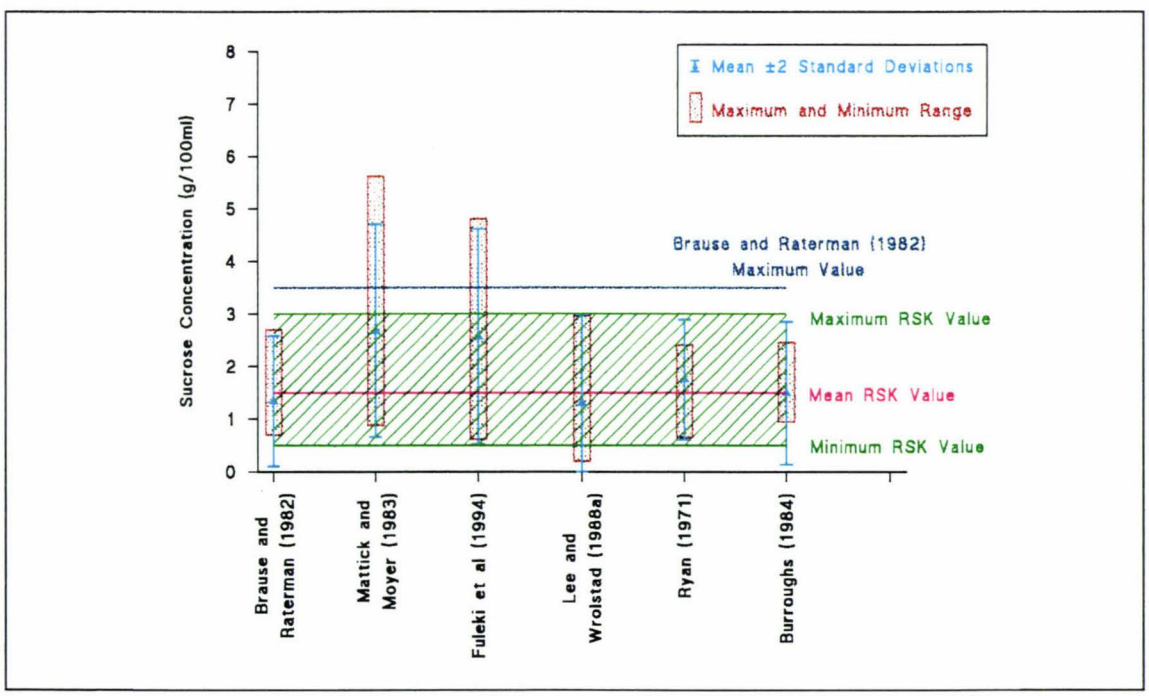

Figure 2.5: Comparison of sucrose concentrations from published data with the RSK and Brause and Raterman (1982) values for authentic apple juices.

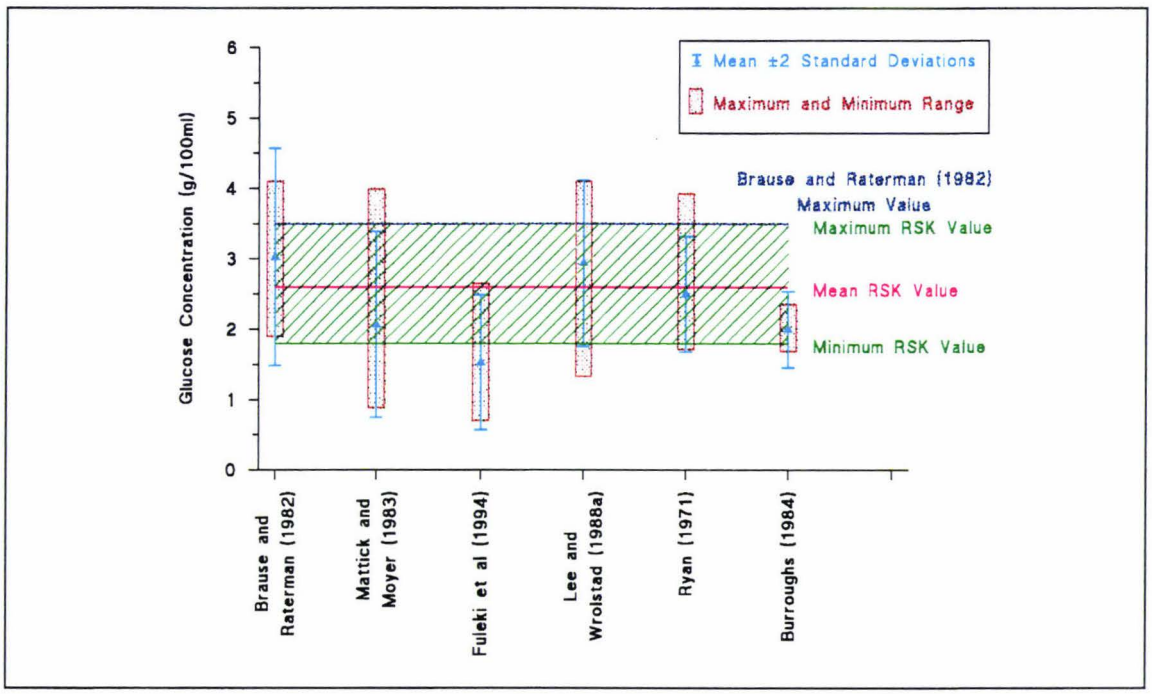

Figure 2.6: Comparison of glucose concentrations from published data with the RSK and Brause and Raterman (1982) values for authentic apple juices.

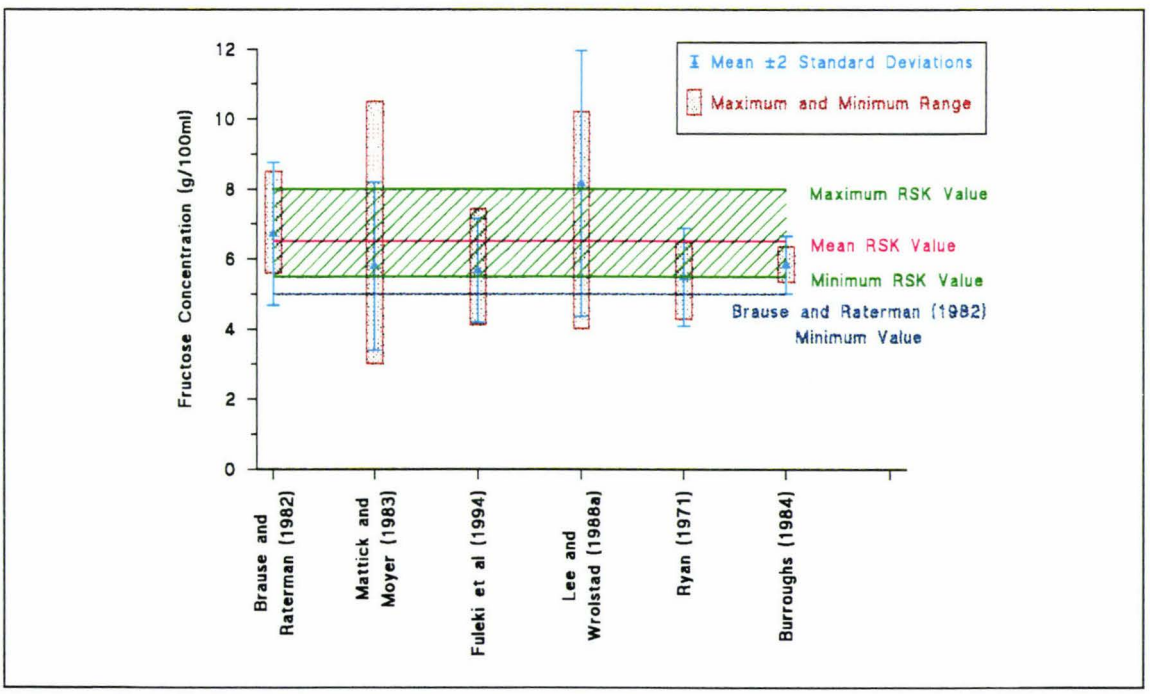

Figure 2.7: Comparison of fructose concentrations from published data with the RSK and Brause and Raterman (1982) values for authentic apple juices.

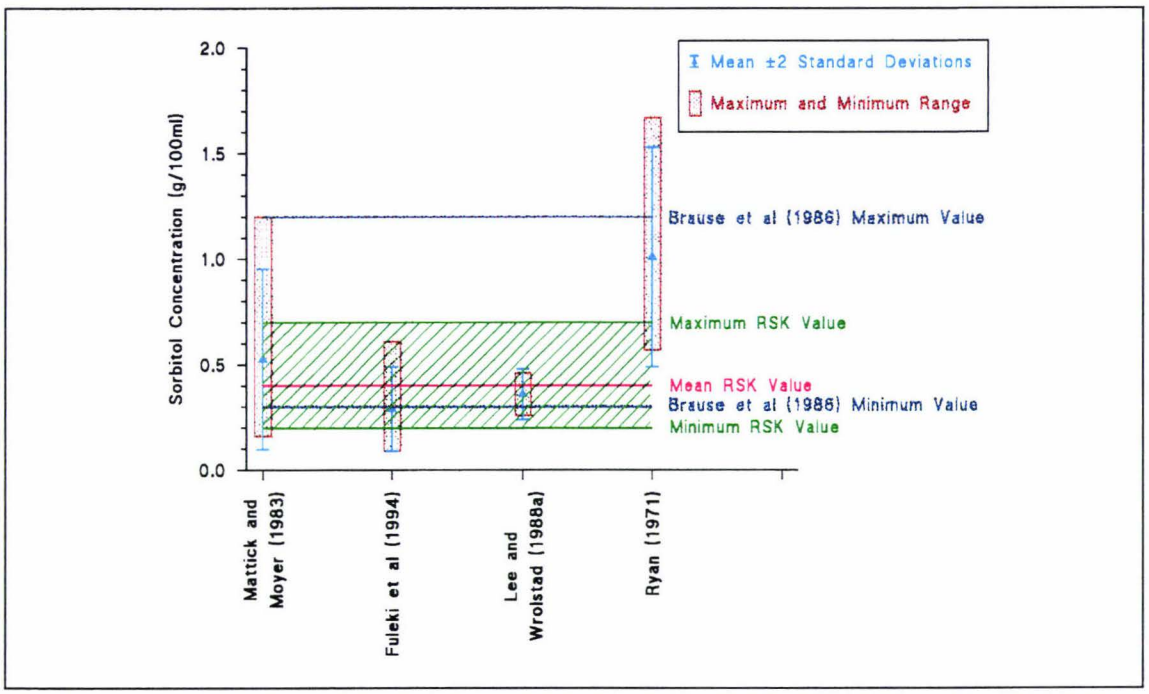

Figure 2.8: Comparison of sorbitol concentrations from published data with the RSK and Brause *et al.* (1986) values for authentic apple juices.

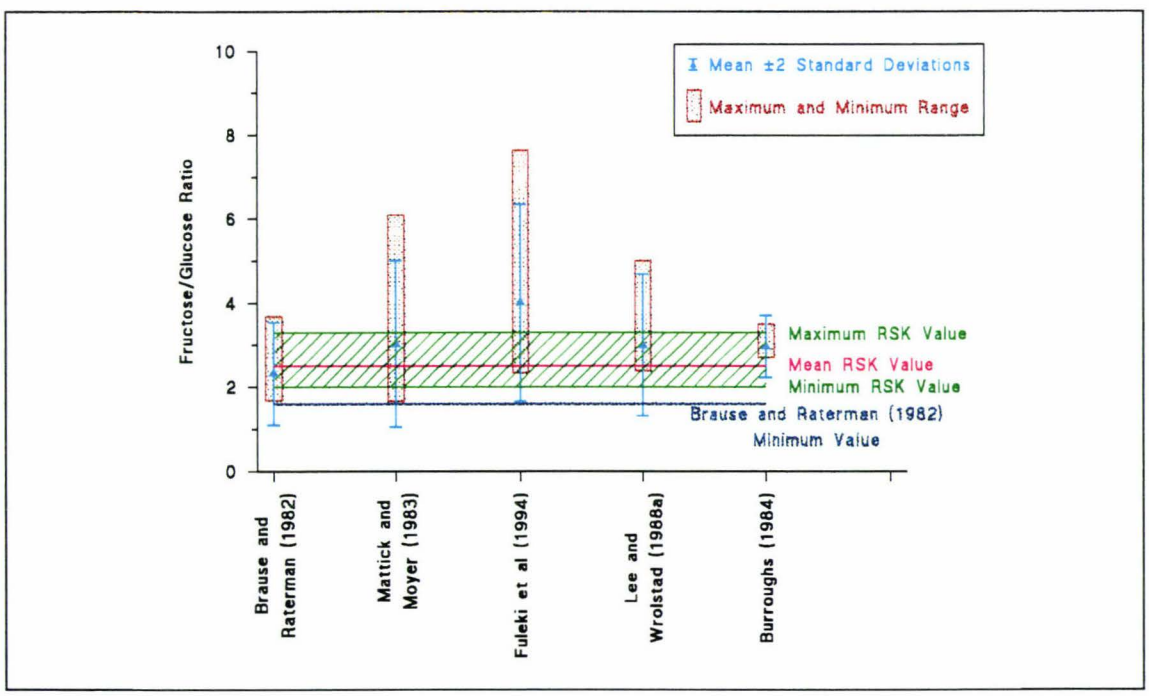

Figure 2.9: Comparison of the fructose/glucose ratios from published data with the RSK and Brause and Raterman (1982) values for authentic apple juices.

(Burroughs, 1984; Lea, 1990). To this end the RSK criteria provide a working basis for the examination of commercial fruit juices including orange, apple, grape and grapefruit.

Countries may modify the the RSK values to suit their region. For example, Dutch authenticity criteria for apple juice were attuned to the RSK values where possible and much attention was paid to the organic acid pattern. A minimum level of 5.2g/l was set for l-malic acid and d-malic acid should be absent. Maximum citric acid levels were set at the same level as RSK values (200mg/l) but the addition of 3g/l is permitted so long as it is stated. It is also a requirement that free asparingine and aspartic acids make up a minimum of 80% of the total amino acids present (Dukel, 1988).

2.6.3 Sugar Profiles

The major justification for using sugar profiles in adulteration investigations is the very large amount of data that is available for glucose, sucrose, fructose and sorbitol concentrations. There is a reasonable maximum for the total sugars of 14 °Brix in apple juice which would tend to limit the levels of individual sugars. Sugars are also relatively stable compounds and little change has been reported as a result of processing (Lee and Wrolstad, 1988b) compared to compounds such as phenolics which can be modified or reduced due to oxidation or fining (Giovanelli and Ravasini, 1993; Schols *et al.*, 1991). However, one must be cautious in establishing minimum and maximum values for sucrose because of the inherent variability. Sharkasi *et al.* (1981) report that sucrose decreased from 2.18g/100ml to 1.72g/100ml after heating apple juice for 20 minutes at 87°C. Over this period the reducing sugars increased from 11.88 to 12.50g/100ml, but the total sugars remained constant at about 14.22g/100ml. The use of sucrose as a respiratory substrate during post harvest storage and the inversion of sucrose in apple juice concentrate during storage will reduce the sucrose content (Lee and Wrolstad, 1988b). Apple juice concentrate that had been stored for 111 days at 37°C was observed to have sucrose decreasing from 13.3 to 5.13g/100g with reducing sugars increasing from 51.5 to 59.2g/100g (Babsky *et al.*, 1986).

The presence of sugars not usually present in apple juice, such as maltose, or abnormal patterns in sucrose, glucose, fructose and sorbitol can be used to detect adulteration. Fitelson (1970) judged some commercial apple concentrates to be adulterated by their abnormal sugar patterns. Lee and Wrolstad, (1988b) report adulteration of apple juice concentrate with starch hydrolysate on the basis of high glucose levels, the presence of maltose, and abnormally high sugar-free extract.

Lee and Wrolstad, (1988b) reported that the detection of trace levels of maltose in apple and the reduced levels of maltose in corn syrup due to technological advances in manufacture made it less effective as a marker for adulteration of apple. Isomaltose has been identified as a by -product of the manufacture of glucose syrups from corn starch and can be easily detected in apple juice adulterated with less than 5% corn syrup (Balmer and McLellan, 1996).

From the literature Brause and Raterman (1982) determined that a true apple juice would "possess the following characteristics: 5%-8% fructose; 2%-3% glucose; 1%-3% sucrose; 90-110mg potassium/100ml; A(365nm) polyphenolics, 0.150 minimum; 2.5meq/l formol index (FI) (minimum); and -25 ±3 ‰ $\delta^{13}C$ (see section 2.6.7). They further decided that up to 3.5% sucrose was possible, that the fructose/glucose ratio would at minimum be 1.6, and the main phenolic present was chlorogenic acid". Therefore Brause and Raterman (1982) report that "since no samples in the literature search had reported any apples with less than 5% fructose (and in most cases, much higher) nor more than 3.5% glucose or sucrose, a fructose/glucose ratio of 1.6 minimum and a sucrose maximum of 3.5% were chosen as standards of authenticity. A maximum of -22.0‰ was chosen from the work of Doner and Phillips, (1981) and Doner *et al.*, 1979".

2.6.4 Component Ratios

A simple method to describe the relationship between two attributes is to derive their ratio (Cohen, 1988).

Ratios have been used (Brause and Raterman, 1982; Sharkasi *et al.*, 1981; Mattick and Moyer, 1983; Wrolstad and Shallenberger, 1981) and can be used for both concentrates and reconstituted juice (Elkins *et al.*, 1988).

The use of data presented as a ratio or percentage of the total sugars by summation appears to be not only more sensitive in detecting adulteration (Elkins *et al.* 1988) but also the standard deviation is lower (Lee and Wrolstad, 1988b) and allows for tighter standard limits. Several factors could account for this.

(a) Eliminating differences caused by expressing concentration in grams per 100g of fruit as compared to grams per 100g of juice.

(b) Eliminating analytical errors in quantification due to percent recovery.

(c) Restricting the variation to the proportions of sugars, in contrast to including the variation of both absolute quantities and the proportions.

However since there is a practical limit to the total sugars both in absolute and percentage terms, then as one component increases the other must decrease and the effect on the ratio is doubled.

2.6.4.1 Fructose/Glucose Ratio

The fructose/glucose ratio has been proposed as a means of detecting adulteration by Brause and Raterman (1982). They recommend a minimum glucose/fructose ratio of 1.6 for authentic juices. However, sucrose is inverted to fructose and glucose in acidic conditions with heat (Sharkasi *et al.*, 1981) and hence the storage and processing conditions would effect sucrose inversion and consequently the fructose/glucose ratio. Mattick and Moyer (1983) report that if apple juice is analysed and the analysis is repeated 6-9 months later the sucrose content decreases due to inversion, resulting in the glucose and fructose content increasing. The increase in fructose and glucose concentration would result in a lower

fructose/glucose ratio. To overcome this variability and negate the effects of the ratio change over a period of time Mattick and Moyer (1983) recommend that sucrose be hydrolysed before the fructose/glucose ratio is determined. This procedure reduces the range of the ratio from 1.67 to 6.09 before inversion to 1.43-3.31 after inversion and the coefficient of variation is reduced by approximately one third (from 32% to 20%).

A low fructose/glucose ratio for apple juice has been used as an indicator of the addition of high fructose corn syrup and inverted beet sugar. Inverted beet sugar would have a fructose/glucose ratio of 1. High fructose corn syrup usually comes in two formulations: one with a 42:58 ratio and the other with a 55:45 ratio. In theory both the inverted beet sugar and either high fructose corn syrup formulations could be detected by a decrease in the fructose/glucose ratio.

Elkins *et al.* (1988) supplied samples of apple juice concentrate, (a pure concentrate, and some samples adulterated with between 25-30% high fructose corn syrup) to other laboratories for analysis. The fructose/glucose ratio had a mean value of 2.19 for the pure sample and a mean value of 1.47 for the adulterated sample. This value clearly falls below the value of 1.6 reported by Brause and Raterman (1982) as the minimum for authentic juices. It also falls outside the 2 standard deviations from the mean (1.76-2.60) reported by Elkins *et al.* (1988) in their work on pure apple concentrate. This indicates that the fructose/glucose ratio can detect the adulteration of apple juice with more than 25% added high fructose corn syrup. However the fructose/glucose ratio does not appear to detect the addition of inverted beet sugar quite as well as the ratio did not fall below 1.6 until the apple concentrate contained more than 40% w/w inverted beet sugar (Elkins *et al.* 1988).

2.6.4.2 Sugars/Total Sugars by Summation

Lee and Wrolstad (1988b) also compared as percentage of the total sugar by summation the data of Mattick and Moyer (1983) and Wrolstad and Shallenberger (1981) and found close agreement indicating that the percentage of total sugars can be used for comparison between different authors. However the coefficient of variation for the glucose/fructose ratio was

found to be greater than the percentage coefficient of variation for glucose or fructose content throughout the literature (Wrolstad and Shallenberger, 1981) because the variability for both of these sugars will be expanded in the ratio calculation.

2.6.4.3 Sugar/(Total Sugars by Summation +Sorbitol)

Elkins *et al.* (1988) investigated the ratio of each individual sugar and sorbitol to the sum of fructose+glucose+sucrose+sorbitol (total sugars + sorbitol) and found that both the fructose/(total sugar + sorbitol) ratio (0.513) and sucrose/(total sugars + sorbitol) ratios (0.257) are outside two standard deviations, 0.514-0.615 (mean 0.565) and 0.219-0.302 (mean 0.261), respectively, from the mean at 20% w/w added invert beet sugar. The glucose/(total sugars + sorbitol) ratio changes very slowly and is of little utility, to detect inverted beet sugar, but could be useful in detecting high fructose corn syrup. The glucose/(total sugars + sorbitol) ratio for the apple concentrate that had 25% to 30% of added high fructose corn syrup had a ratio of 0.357 which was outside 2 standard deviations (0.219 - 0.302) from the mean (0.261).

Addition of beet sugar, which is approximately 100% sucrose would raise the sucrose/(total sugar + sorbitol) ratio to an unacceptable level rapidly and should be easy to detect.

The use of sorbitol/(total sugar + sorbitol) ratio is useful in detecting the addition of pear concentrate (Elkins *et al.*, 1988; Sharkasi *et al.*, 1981). There is a dramatic difference between the sorbitol/(total sugar + sorbitol) ratio of apple concentrate (mean = 0.043) and pear concentrate (mean = 0.17) (Elkins *et al.*, 1988).

2.6.5 Organic Acid Profiles

Non-volatile acids are a class of chemical compounds in second order of importance from a qualitative standpoint in apple juice and standards for total acidity can be established. Mattick and Moyer (1983) found a wide range (0.15 to 0.91% as malic acid) in total acidity. The RSK report a somewhat narrower range (0.45 to 0.76 g malic acid/100ml) and a higher

mean (0.58g/100ml) than Mattick and Moyer (1983) (0.42g/100ml). The RSK recommends that authentic apple juice has a minimum total acidity of 0.45g /100ml as malic acid.

Adulteration with a sweetener will also require the addition of acid to meet company specifications regarding total acidity. If the acid levels fall below the normally expected levels, the fraudster can add small quantities of synthetic food grade acid to make good the deficit. Addition of malic acid to apple juice is less easy to recognise but is chemically distinguishable. Synthetic malic acid is a racemic mixture of d- and l- optical isomers, whereas naturally occurring malic acid is exclusively the l- form. Although these racemates cannot be separated by HPLC, the l- form can be detected by enzymic assays. The l-malic/total malic acid ratio for pure apple juice or concentrate is 1.0. A totally synthetic juice or concentrate would have a ratio of 0.5. There is experimental error in both HPLC and enzymatic methods so it is extremely rare to get a ratio of exactly 1.0. Zyren and Elkins (1985) indicate that l- malic/total malic ratio of less than 0.9 indicates a non authentic juice.

Fumaric acid is the synthetic precursor to malic acid and synthetic malic acid always contains fumaric acid in large quantities as a contaminant. Since fumaric acid is very ultraviolet active (highly absorbing), small quantities can easily be detected by HPLC. Junge and Spadinger (1982) report that fumaric acid levels greater than 3mg/l were not consistent with pure apple juice. However, this does not hold true for juice from concentrate. Evans *et al.* (1983) report that as much as 10mg/l of fumaric acid can be produced by heating the apple juice concentrate at 100°C for three hours. Therefore fumaric determination may not distinguish between an adulterated or over processed juice, but could be an indicator for further examination for adulteration (Evans *et al.*, 1983).

In order to detect adulteration of apple juice concentrate the l-malic /total malic acid ratio along with the fumaric acid and sugar data should be examined (Elkins *et al.*, 1988). They suggested that if the l-malic/total malic ratio is lower (i.e., ≤ 0.90) , and the fumaric acid is high (i.e., >100ppm), the concentrate is adulterated. For ratios less than 0.9, and fumaric acid greater than 100ppm, examine the sucrose level. If the sucrose is high (>10-12%), the concentrate is borderline between authentic and adulterated. To have achieved

concentrations of fumaric acid greater than 100ppm, the concentration step must have exposed the juice to very high temperatures, which would invert the sucrose to fructose and glucose.

The addition of citric acid can be detected by chromatography since genuine apple juice contains virtually no citric acid. Its presence could also mean the addition of pear or citric juices. Elkins *et al.* (1988) found that the level of citric acid in a 50:50 apple-pear concentrate would be acceptable on a single strength basis with the 0.04% limit set by some European countries. (Evans *et al.*, 1983).

The detection of tartaric acid indicates the possible adulteration of apple juice with grape juice while the presence of high levels of quinic and citric acids implies addition of kiwifruit juice.

2.6.6 Statistical Methods

While most work on statistical tests for detection of adulteration have been done on orange juice, the problems and statistical approaches can apply equally well to all fruit juices including apple.

Classifying a juice sample as adulterated is simple if it has one or more components that are not present naturally in the fruit (Brown *et al.*, 1988; Cohen, 1988) such as the presence of tartaric or d-malic acids which indicates addition of grape juice and synthetic malic acid respectively.

The addition of components that are naturally present can be difficult to detect due to the natural variation in sample attributes. Fraudsters take advantage of this to produce adulterated products that may be indistinguishable to the natural product. Therefore the decision on the authenticity can be enhanced by the use of statistical tests.

Many statistical procedures have been proposed to test for adulteration of fruit juice and they fall into several classes (Brown *et al.*, 1988).

(a) Many tests of the constituents or relationships between constituents.

(b) A single test of the relationship of one attribute to other attributes, such as by ratios or regression

(c) A single omnibus test of many attributes such as Chi squared test (multivarient normal test).

All statistical tests require pre-specified standards which are usually derived from a "base or reference population" of previously collected juice samples

The simplest statistical approach in fruit juice quality control is to determine the acceptable range for each attribute and to compare the value of a tested specimen with standards or acceptable limits.

However with multiple modes of adulteration available, multiple testing must be performed to establish authenticity (Brause, 1993; Brause *et al.*, 1986). They chose to call this a matrix method of testing. The matrix proposed for apple juice is shown in table 2.2.

The more samples tested or attributes examined the greater the probability that one will be outside the limits of the standard. The decision to reject a sample as adulterated is then made only if a number of attributes are outside the limits of the standards. The number of attributes that may be outside the limits before the sample is classified as adulterated can be calculated empirically, and is dependent on the number of attributes examined. Using this rule the probability of incorrectly classifying a pure sample as adulterated can be limited to 5% (or to any other level). Dukel (1988) considers that apple juice meets Dutch authenticity criteria if the deviation from the normal is limited to 25% for one or two parameters or 10% for three parameters (however deviation from the minimum °Brix and

the asparagine/aspartic acid norm are not allowed and the deviation is limited to 10% for L-malic acid content). Using data from 236 samples of Israeli orange juice collected during three seasons, Cohen (1988) showed that when 10 juice attributes were examined for juice authenticity, at least three should be outside the standard levels before the sample can be classified as adulterated with at least 95% confidence. If 15 attributes are examined, at least four must be outside the acceptable limits for similar conclusions. Similar results were reported by Vandercook (1988).

Table 2.2: Apple juice matrix for detecting adulteration (Source: Brause, 1993; Brause *et al.*, 1986).

	Authentic Apple Juice	**Pear Added**	**Beet Sucrose Added**	**Beet Invert Added**	**High Fructose Corn Syrup added**	**Cane Invert Added**	**Cane Sucrose Added**	**Totally Synthetic**
% Fructose	5-8	5-8	<5	>5	>5	>5	<5	<6
% Glucose	1-4	1-4	1-3	>4	>4	>4	1-3	>3
% Sucrose	0-5	0-5	could be >5	0-3	0-3	0-3	could be >5	variable
Fruc/Gluc Ratio	>1.6	>1.6	variable	<1.6	<1.6	<1.6	variable	1.00
Chlorogenic Acid (ppm)	>30	>30	lower but variable	lower but variable	lower but variable	lower but variable	lower but variable	none
Malic Acid (mg/100ml)	200-900	200-900	200-700	200-700	200-700	200-700	200-700	200-900
d-Malic (% of total)	>90	>90	>90	>90	>90	>90	>90	50
Proline (ppm)	2-5	>>10	2-5	2-5	2-5	2-5	2-5	none
% Sorbitol	0-1	>>1	0-1	0-1	0-1	0-1	0-1	none
$\delta^{13}C$ (ppt)	-25±1	-25±1	-25±1	-25±1	>-22	>-22	>-22	depends on sugar added

2.6.7 Isotopic Methods

While chemically purified sucrose from sugar cane, high fructose corn syrup, sugar beet and apple juice are all the same and cannot be distinguished from each other using conventional chemical analysis such as HPLC, the use of stable isotope ratio mass spectrometry can provide separation (Krueger, 1988; Moyer and Aitken, 1980). Stable isotope ratios of an element are reported as δ,which are differences in parts per thousand (per mil or ‰) between the isotopic composition of a sample and a standard. There are three isotopic measurements that can be applied to fruit juice authenticity: $^{13}C/^{12}C$ ratio, D/H ratio and $^{18}O/^{16}O$ ratio.

2.6.7.1 Use of $^{13}C/^{12}C$ Ratios

The most important category of isotopic analysis for evaluation of fruit juice authenticity is the ratio of the stable carbon isotopes ^{13}C and ^{12}C, usually expressed as a values of δ, (Krueger, 1988).

Atmospheric carbon dioxide has a $\delta^{13}C$ of -7‰ and this value does not change with topography or geography (Doner, 1988; O'Leary, 1981; Smith and Epstein, 1971). The principle source of variation in $^{13}C/^{12}C$ ratio lies in the different photosynthetic pathways of carbon dioxide fixation. Plants that possess the C_3 pathway have $\delta^{13}C$ value ranging from -22 to -34 ‰ (Bender, 1971; Doner, 1988; Doner and Phillips, 1981; O'Leary, 1981). The deviation in values from the source carbon dioxide is accounted for by the carbon isotope fractionation associated with the carboxylation of ribulose bisphosphate (Doner, 1988). Ribulose bisphosphate carboxylase discriminates against ^{13}C leading to this more negative value (Bender, 1971; Doner *et al.*, 1980; O'Leary, 1981). C_4 plants have $\delta^{13}C$ value of between -9 to -20 ‰ (Bender, 1971; Schmidt *et al.*, 1993; Smith and Epstein, 1971) with CAM plants possessing a wide range of $\delta^{13}C$ values depending on the relative amounts of day and night carbon dioxide fixation (Doner, 1988; O'Leary, 1981).

The $\delta^{13}C$ for apples have been reported as between -22‰ and -31‰ (Brause and Raterman, 1982; Coppola, 1984; Doner *et al.*, 1980; Doner and Phillips, 1981; Krueger, 1988; Lee and Wrolstad, 1988c; Schmidt *et al.*, 1993). There was no significant variation in $\delta^{13}C$ with regard to apple variety or geographical region (Doner *et al.*, 1980; Lee and Wrolstad, 1988c), with the various subfraction of apple (seeds, pulp, sugars, juice, acids and phenolics) also having $\delta^{13}C$ within the range of -22 to -31‰ (Lee and Wrolstad, 1988c). Sugar from sugar cane and high fructose corn syrup, which are derived from C_4 plants, have a $\delta^{13}C$ of around -10‰ (Coppola, 1984; Doner and Phillips, 1981; Krueger, 1988).

Therefore mixtures containing sugars derived from C_3 (apple juice) and C_4 (high fructose corn syrup and cane sugar) plants which would have $^{13}C/^{12}C$ ratios between those of the pure materials (Doner, 1988).

It has been proposed that apple juice with a $\delta^{13}C$ more positive than -22‰ will indicate adulteration at the 99% confidence level. However, because of the small natural variation of $\delta^{13}C$ in apple juice, the addition of small amounts of high fructose corn syrup or cane sugar (up to 10%) cannot be absolutely identified because the resulting $\delta^{13}C$ value shift of the total sugar fraction may be within the limit of natural variation of authentic sugar (Brause and Raterman, 1982; Coppola, 1984; Doner and Phillips, 1981). However, the difference when correlating the $\delta^{13}C$ value of the sugar to that of a second ingredient from the same product, can indicate proof of adulteration, (Schmidt *et al.*, 1993) and be more sensitive to its detection (Lee and Wrolstad, 1988c). For example the addition of 5% of high fructose corn syrup to orange juice was detected through isotope analysis of its juice and pulp. High fructose corn syrup will reside mostly in the soluble fraction (juice), and its presence will lower the negative of the soluble fraction but not the pulp fraction (Lee and Wrolstad, 1988c).

Lee and Wrolstad (1988c) showed that the correlation between $\delta^{13}C$ values for apple juice and its pulp was extremely good (R= 0.93) and suggests a means for more sensitive detection of high fructose corn syrup in products such as "natural" apple juice which contains the pulp.

Schmidt *et al.*, (1993) has taken the comparison of two characteristics from the same product one step further by suggesting using the carbon isotope distribution within a natural compound on the basis that the molecular parts originate from different biosynthetic pathways. Unfortunately, the methods to do this are laborious and cannot be used in routine analysis.

2.6.7.2 Use of D/H and $^{18}O/^{16}O$ Ratios

The major source of hydrogen and oxygen is the local ground water, and the processes of evaporation, condensation and transpiration also cause enrichment in deuterium and the extend of this effect will vary with plant physiology and local climatic conditions (Bricout, 1973; Doner *et al.*,1987; Dubar and Wilson, 1983; Krueger, 1988;).

The D/H and $^{18}O/^{16}O$ ratios can be used to distinguish between natural and reconstituted juice, by calculating the deviation of this isotopic composition from that of rain water (Bricout, 1973). The isotopic composition of apple juice diluted from concentrate would be similar to that of the dilution water. Slower growing fruits such as apples, pears and plums exhibit ^{18}O enrichment than faster growing plants. Typical values of enrichment of water in apples are around 5‰ (Bricout, 1973; Dubar and Wilson, 1983; Krueger, 1988) with sucrose from apple juice and sugar beet both have a $\delta^{18}O$ of +28‰. Therefore oxygen isotopic ratio method has not been a useful technique for the detection of added beet sugar (Doner *et al.*, 1987).

Hydrogen is a labile element and reliable results can be achieved by measuring the non-labile carbon bound hydrogen (Krueger, 1988). The carbon bound hydrogen of apple juice sucrose had a $\delta(D)$ of -27‰ while sucrose derived from beet sugar had a $\delta(D)$ of between -116‰ to -175‰ depending on the source (Doner *et al.*, 1987; Krueger, 1988). Therefore the D/H ratio may be useful to detect sugars from different sources and in particular the addition of beet sugar, which is not detectable by $^{18}O/^{16}O$ or carbon isotope analysis (Doner *et al.*, 1987; Lea, 1990; Krueger, 1988).

2.6.8 Use of Amino Acids

No RSK values are given for amino acids since they are not particularly informative for apples, apart from the fact that proline and arginine should be low (Burroughs, 1984). Proline should be present at less than 15mg/l (mean 8mg/l) and values higher than this indicate adulteration with other juice such as pear (Burroughs, 1984; Wallrauch and Faethe, 1988a, 1988b). No RSK values are given for arginine. However, Blanco *et al.* (1992b) and Wallrauch and Faethe (1988a) showed that the amino acid profile vary from fruit to fruit and can be used to characterise fruit juices. The fraudulent mixing or adulteration of other fruit juice to apple juice can be detected be the presence of foreign amino acids (Blanco *et al.*, 1992b; Wallrauch and Faethe, 1988a).

2.6.9 Use of Absorbance and Fluorescence Spectral Characteristics

Apple juice can be characterised by its absorption and fluorescence spectra, and a change in these curves can indicate adulteration (Petrus, 1988).

2.6.10 Use of Phenolics

As a result of recent advances in chromatography techniques, especially HPLC, phenolics have been individually studied, with some being characteristic of different species as well as varieties within the same species (Fernadez de Simon *et al.*, 1992; Perez-Ilzarbe *et al.*, 1991). The phenolic profile can be used to detect adulteration. For example, phloretin and isorhamnetin derivatives can be used to distinguish between apple and pear juice (Fernadez de Simon *et al.*, 1992).

2.7 Analysis of Sugar and Organic Acids in Apple Juice by HPLC

High pressure liquid chromatography (HPLC) has emerged as the preferred method of separation and quantitative analysis of a wide range of substances such as sugars, acids

phenols and vitamins because it can achieve quick and precise separation of complex mixtures with high resolution.

While HPLC analyses are fast and efficient, many operational parameters must be determined and set before the sample can be analysed. These include the determination of:

(i) techniques to separate the components of interest

(ii) methods to quantify the resulting peaks

(iii) factors affecting precision

In the analysis of foods there are two problems to overcome :

(i) incomplete separation of the components of interest, and

(ii) the removal or separation of interfering substances that could coelute.

Unfortunately there is no ideal method to achieve this and normally methods are modified to give the required separation. Factors that can be modified to achieve this are:

(i) sample pretreatment

(ii) choice of the detector

(iii) column type

(iv) operating conditions such as composition of the mobile phase, flow rate and column temperature.

2.7.1 Sample Pretreatment

Analytically the ideal situation would allow the direct introduction of the sample to be assayed, irrespective of its physical or chemical nature, directly into the chromatographic system, but practically this is not normally possible (Blanco, 1992). The complexity of products such as beverages, fruits, meat products, dairy products and processed foods normally requires extraction of the compounds of interest or removal of interfering compounds by sample pretreatment. The extent of pretreatment will be dictated by the nature of the sample concerned, the chromatographic separation and the detection system. While there are a number of general pretreatments such as the use of ion-exchange columns

to separate the sample into acid and sugar fractions or Sep-Pak®C_{18} cartridges to remove proteins (Morawski, 1984; Shaw, 1988) and anthocyanins (Hong and Wrolstad, 1986a; Wrolstad *et al.*, 1982), many appear to have been developed for a specific application (Giovanelli and Ravasini, 1993; Hulme and Wooltorton, 1958; Schols *et al.*, 1991; Van Buren *et al.*, 1976).

Commonly in the analysis of sugar in fruit juices by HPLC, acids are the major class of interfering substances. Although acids generally elute early from columns selected for sugar separation, sufficient acid can elute at the same time as the first sugars to reduce the quantitative accuracy of the method. Conversely in the analysis of organic acids in fruit juices sugars are the major interfering compounds. The separation of the sample into sugar and acid fractions prior to analysis by the use of an ion exchanger is possible (Coppola *et al.*, 1978; McCord *et al.*, 1984; Palmer and List, 1973). Several ion exchangers have been reported for fruit juices, such as Bio-Rad AG1-X8 (acetate form) (Cliff *et al.*, 1991); Bio-Rad AG1-X8 (hydroxide form) (Mattick and Moyer, 1983); Biorex 5 (chloride form) (Hunter *et al.*, 1991; McCord *et al.*, 1984); Dowex 50 WX-12 (strongly acidic cation) (Ryan and Dupont, 1973); Dowex AG50W-X4 (H^+) coupled with Bio-Rad AG1-X8 (acetate form) (Wrolstad *et al.*, 1980, 1982). Although ion exchangers separate acids and sugars, the procedure to convert the resin into the active form and elute the acids from the column can be lengthy and result in sample dilution.

2.7.1.1 Sample Pretreatment for Sugar Analysis

Sample pretreatment for sugar analysis can be reduced by the use of Waters alumina type A Sep-Pak®C_{18} cartridges which are in effect premade mini columns and only require the application of the sample. Morawski (1984) successfully used these cartridges to remove organic acids in yoghurt without affecting the sugar concentrations. It is possible that these cartridges could be used for the removal of organic acids from apple juice.

2.7.1.2 Sample Pretreatment for Organic Acid Analysis

The analysis of organic acids has been performed without sample pretreatment (apart from filtering and maybe passage through a Sep-Pak®C_{18} cartridge) in juice products (Blanco *et al.*,1988, 1992a; Coppola, 1984; Evans *et al.*, 1983; Frayne, 1986; Hong and Wrolstad, 1986a, 1986b; Jeuring *et al.*, 1979; Lee and Wrolstad, 1988a; Schneider *et al.*, 1987). However, separation of malic, quinic shikimic citric, fumaric and succinic acids from each other and from interferants is difficult. Typically only one acid was being determined and full resolution of all acids was not required. In order to achieve separation of the above acids, sample pretreatment is required with the common treatment being to separate the juice sample into acidic and sugar fractions by the use of ion exchange columns (Coppola *et al.*, 1978; McCord *et al.*, 1984; Palmer and List, 1973). It may be possible to avoid lengthy sample pretreatment by careful choice of the HPLC detector and operating conditions such as columns, flow rate and the temperature.

2.7.2 Detection System in Juice Analysis

While there are a number of different detectors available to quantify sugars and acids in fruit juices, only refractive index and ultraviolet detectors will be discussed here as they are the most common types used.

2.7.2.1 Refractive Index Detectors

Sugars are detected by refractive index detectors as they do not possess chromophores to enable them to be detected by ultraviolet detectors.

There are a number of disadvantages in using refractive index detectors but with careful selection of the operating conditions some of these disadvantages can be minimised or removed.

(i) Refractive index detectors will detect both sugar and acids in apple juice and by careful selection of columns, mobile phase, and temperature or by sample pretreatment these compounds can be separated from one another.

(ii) The refractive index detectors commonly used can detect a difference of 10^{-6} refractive index units (with some detectors detecting differences of up to 10^{-9} refractive index units) between the sample and the mobile phase but they are 2 (Hamilton and Sewell, 1978) to 15 (Bidlingmeyer, 1992) times less sensitive than ultraviolet detectors. Folkes and Taylor (1982) found 40μg of monosaccharide to be near the limit for accurate detection. Generally the level of individual sugars in foods are much greater with minimum levels of 0.1% to 0.5% seen.

(iii) Refractive index detectors are affected by temperature fluctuations and must be thermally insulated to provide a stable baseline.

(iv) Gradient elution is not feasible with refractive index detectors as they respond to any changes in mobile phase composition.

Refractive index detection has been used to detect organic acids (Anderson and Hedlund, 1983; Frayne, 1986; Schneider *et al.*, 1987) but the presence of sugars in the sample causes interference. Organic acids, especially minor acids in juice can be completely masked due to excessive detector response to high sugar concentrations (Frayne, 1986).

2.7.2.2 Ultraviolet Detectors

Ultraviolet detectors utilise absorption of ultraviolet radiation and are more flexible and sensitive than refractive index detection in determining organic acids. While sucrose, glucose and fructose absorb at wavelengths of 190nm to 193nm (Shaw and Wilson, 1983), their quantification is limited as other organic compounds also absorb at these wavelengths

(Scott, 1992). If sugars are quantified by ultraviolet detection then elaborate sample cleanup is necessary (Scott, 1992; Shaw, 1988; Shaw and Wilson, 1983; Woollard, 1983).

Organic acids absorb from 206 to 220nm (Blanco, 1992; Bouzas *et al.*, 1991; Jeuring *et al.*, 1979), with most determinations performed at either 210nm or 214nm (Ackermann *et al.*, 1992; Coppola and Starr, 1986, 1988; Davis and Husband, 1988; Fernandez-Garica and McGregor, 1994; Hunter *et al.*, 1991; Marce *et al.*, 1990; Maxa *et al.*, 1991; Zyren and Elkins, 1985). At these wavelengths fructose absorbs strongly and can mask the detection of organic acids, while no interference from sucrose and glucose occurs.

2.7.3 HPLC Columns for the Separation of Sugars and Organic Acids

There is no column or columns that will give complete separation of sucrose, glucose, fructose, malic, quinic, shikimic, citric, succinic and fumaric in a single sample injection. Some columns such as the Aminex HPX-87H, HPX-87C will give separation of some of these compounds. Sucrose is the most difficult to quantify as it inverts under acid conditions at elevated temperatures used by these columns.

In the determination of sugars four types of columns are commonly used:

(i) Columns using amino or amine bonded silica such as Waters carbohydrate column and Waters μBondapak column

(ii) Styrene divinylbenzene cation exchange resin such as Sugar Pak I, Shodex SC1011 and Aminex HPX range

(iii) Styrene divinylbenzene anion exchange resin such as carbopac PA2

(iv) Reverse phase columns.

Each type of column has slightly different separation abilities. The amino bonded silica and styrene divinylbenzene cation exchange resin columns are most commonly used.

Amino bonded silica columns do not separate glucose from galactose, mannitol, or sorbitol or fructose from xylitol. Raffinose and stachyose have long retention times in these columns.

Amino bonded columns gradually lose their ability to separate sugars with the loss of separation between glucose and fructose particularly noticeable. Styrene divinylbenzene cation exchange columns also have some limitations in their ability to separate some sugars such as sucrose and maltose, xylitol and sorbitol and sucrose and lactose. However, the stationary phase of these columns is not easily subjected to degradation by oxidation, hydrolysis or elevated temperature. Anion exchange columns can use either a silica or divinylbenzene based stationary phase, and separation occurs at alkaline conditions (pH 12-14). Under alkaline conditions silica based columns deteriorate quickly and their life expectancy is short. Divinylbenzene anion exchange columns have the ability to detect sugars down to picomole concentrations and are used mainly for biomedical applications (Scott, 1992). These columns separate most sugars but glucose, galactose, mannose and xylose are poorly resolved (Ball, 1990). Reverse phase columns have very limited use in the separation of sugars because the sugars are only weakly retained on the hydrophobic stationary phase, and the monosaccharides generally elute rapidly as a single unresolved peak (Ball, 1990; Shaw, 1988).

A variety of column types, such as ion-exchange, ion exclusion, ion pair and solvophobic have been used in the HPLC determination of carboxylic acids in foods (Schwarzenbach, 1982).

Some of the columns used for the separation of organic acids in foods and beverages are Aminex HPX87 (Ackermann *et al.*, 1992; Anderson and Hedlund, 1983; Evans *et al.*, 1983; Fernandez-Garica, 1994; Hunter *et al.*, 1991; McCord *et al.*, 1984; Schneider *et al.*, 1987; Schwarzenbach, 1982), Spherisorb ODS2 (Blanco, 1988; Blanco *et al.*,1992a; Marce *et al.*, 1990), Micropak MCH-10 (Hong and Wrolstad, 1986a, 1986b; Lee and Wrolstad, 1988a), Radial Pak (Morawski, 1984; Schwarzenbach, 1982), Reverse phase C_{18} (Jeuring *et al.*, 1979; Schwarzenbach, 1982; Zyren and Elkins, 1985) and Bondapak C_{18} (Coppola *et al.*, 1978).

The use of two columns in series has successfully separated quinic, malic, shikimic, citric and fumaric acids in juices (Coppola, 1984; Coppola and Starr, 1986,1988) and wine (Davis

and Husband, 1988; Frayne, 1986), although some acids do coelute such as ascorbic and shikimic (Coppola and Starr, 1988). The sugars present elute as a single peak with the solvent front.

2.7.4 HPLC Operating Conditions

2.7.4.1 Mobile Phase

The mobile phase is highly influential in accomplishing separation of acids and sugars and must be compatible with the column packing material, sample and equipment.

The separation of sugars using divinylbenzene based resins with water as the mobile phase can be enhanced with the addition of acetonitrile, ethanol or isopropanol. These modifiers will cause shrinkage of the resin bed and reduce column life (Shaw, 1988).

The use of dilute sulphuric acid as the mobile phase in the separation of organic acids is more corrosive on the components of the HPLC system than when dilute phosphoric acid is used (Frayne, 1986). This drawback is more than compensated for by the separation power achieved with fructose and malic being resolved (Schneider *et al.*, 1987). Selection of the mobile phase can also affect the sensitivity of the detector. By changing the mobile phase from 80:20 acetonitrile:water to pure water it is possible to increase the refractive index detector sensitivity 10 fold as the difference in refractive index between water and sugar is greater than the difference between acetonitrile:water and sugar.

2.7.4.2 Flow Rate

By decreasing the flow rates it is possible to improve separation, but the analysis time is increased. If the flow rate is very slow the efficiency of separation decreases as peak broadening occurs (Bidlingmeyer, 1992).

2.7.4.3 Temperature

For optimum sugar separation divinylbenzene based columns should be operated at 80°C to 90°C. An increase in the column temperature by 6°C reduces retention times by about 30% (Hamilton and Sewell, 1978). Elevated temperatures can also be detrimental causing solvent or sample components to decompose and the formation of bubbles in the detector leading to an uneven baseline. Some packings such as silica, deteriorate at higher temperatures.

2.7.5 Quantification of Peaks

The concentration of a specific compound can be determined by using either internal or external standards, with peak areas or peak height used to calibrate the detector response.

2.7.5.1 Calibration Curves

All detector and amplifying systems must be operated in the linear response range. If either the detector or amplifier becomes saturated or overloaded then the response is no longer linear and quantification loses accuracy.

2.7.5.2 Peak Height and Peak Area

The choice of whether to use peak height or area for quantitative analysis depends on the peak shape and separation, and the composition of the mobile phase. Peak heights are subject to less interference from adjacent overlapping peaks (eg tailing and broadening) and less resolution is required when quantifying by peak height. Peak height generally yields more precise quantitative results when flow rate is erratic as it is less dependant on the flow than peak area (Synder and Kirkland, 1979). Peak area values are less influenced by changes in instrumental and chromatographic parameters and are preferred with asymmetrical peaks.

Peak areas are relatively independent of mobile phase composition and are used where the composition of the mobile phase cannot be precisely controlled, such as where highly volatile solvents or gradient elution is used. When frequent standardisation is not feasible or column efficiencies change over time, peak areas are used. However, greater resolution between peaks is required to maintain accuracy when using peak area (Shaw, 1988; Synder and Kirkland, 1979).

2.7.5.3 Internal and External Standards

The use of internal and external standards can increase the precision, however it has been reported (Synder and Kirkland, 1979) that the use of internal standards can actually increase the analysis precision error by a factor of 1.4 times ($\sqrt{2}$) compared to external standards. This is because of the uncertainty of two peak size measurements rather than one. With the use of external standards precise sample injections are required.

2.7.6 Precision

Regardless of other factors and the method of quantifying and measuring the response, sample injection remains a significant source of error with too much sample giving column overload, resulting in peak broadening, loss of separation and decreased precision with which the sugars can be quantified.

When using syringe injection, analysis precision is usually not much better than about ±5% because the results depend greatly on the ability to inject a consistent sample volume (Synder and Kirkland, 1979). However, with an injection loop overall precision of 1% can be obtained (Dolan and Synder, 1989; Synder and Kirkland, 1979). Precision of better than 0.25% can be obtained with automatic sampling systems where operator variability is eliminated, and all analysis and conditions are precisely reproduced (Dolan and Synder, 1989; Synder and Kirkland, 1979).

CHAPTER 3
MATERIALS AND METHODS

3.1 Sampling Procedures

The harvest of apples for export commences and ceases at official picking dates determined by the Ministry of Agriculture and Fisheries in conjunction with the Apple and Pear Marketing Board. Samples of approximately 1kg were obtained from fruit which were harvested one month before the commercial export pick (1st pick), at commercial export maturity (2nd pick), and when the fruit were fully tree ripened (3rd pick). A range of commonly grown apple cultivars was obtained during these picking times and samples were taken from three regions; Hawke's Bay, Nelson and Canterbury in 1992 and 1993. The cultivars sampled were Braeburn, Granny Smith, Cox's Orange, Gala, Royal Gala, Golden Delicious, Red Delicious and Fuji. Not all cultivars were sampled at all three picking dates or from all three growing regions in both years. Single samples of the cultivars Hillwell, Fiesta, GS2850 and GS330 were also examined.

Samples of fruit that had been stored under ambient, cold or controlled atmosphere conditions were obtained from Hastings in both 1992 and 1993. However not all cultivars were stored at all three storage conditions in both years. The combinations that were obtained are presented in table 3.1.

Table 3.1: The sampling regime and the numbers of sample collected for each cultivar.

	Braeburn	Granny Smith	Royal Gala	Gala	Cox's Orange	Red Delicious	Golden Delicious	Fuji	New Cultivars[d]
Hawke's Bay 1992									
1st Pick	1	1	1	1	1	1	1	1	
2nd Pick	1	1	1	1	1	1	1	1	
3rd Pick	1	1	1[b]	1	1	1[c]	1		
Ambient Storage [a]	6	7		5					
Cold Storage	4	4		4					
Controlled Atmosphere Storage	4	6	4						
Nelson 1992									
1st Pick	1	1	1	1	1	1	1	1	
2nd Pick	1	1	1	1	1	1	1	1	
3rd Pick	1	1	1	1	1	1	1	1	
Canterbury 1992									
1st Pick		1		1				1	
2nd Pick		1		1				1	
3rd Pick		1		1				1	
Hawke's Bay 1993									
1st Pick		1	1						
2nd Pick	1	1	1		1	1		1	
3rd Pick		1							
Ambient Storage	10	10							
Cold Storage	7	7							
Controlled Atmosphere Storage	5	5							
Sample Variation	21								
Other									4
Nelson 1993									
1st Pick		1	1						
2nd Pick		1	1						
3rd Pick		1	1						

Table 3.1: continued.

	Braeburn	Granny Smith	Royal Gala	Gala	Cox's Orange	Red Delicious	Golden Delicious	Fuji	New Cultivars[d]
Canterbury 1993									
1st Pick		1	1						
2nd Pick		1	1						
3rd Pick		1	1						
Total Number of Samples	64	57	17	18	7	7	6	9	4

[a] = zero time samples for ambient, cold and controlled atmosphere storage are the same.
[b] = 3rd pick sample same as controlled atmosphere time zero sample.
[c] = The organic acids data for this sample was not analysed.
[d] = One sample of the cultivars Fiesta, Hillwell GS2850 and GS330 were examined.

The conditions of storage for Braeburn, Granny Smith Gala and Royal Gala fruit were:

Ambient: storage at normal ambient conditions

Cold storage: 0.5 °C ± 0.5 °C

Controlled atmosphere storage: 0.5 °C ± 0.5 °C, 2% oxygen, 2% carbon dioxide and the balance nitrogen.

The exception to the above storage conditions were:

Controlled atmosphere storage of Braeburn where conditions of 1% carbon dioxide and 3% oxygen were used and

Controlled atmosphere storage of Gala where conditions of 1.5% carbon dioxide and 1.5% of oxygen were used.

3.2 Apple Juice Extraction

The apples were minced through a 13mm plate in a mincer (Kenwood Chef, Thorn EMI Kenwood Ltd, Hampshire, England). The apple pulp was centrifuged at 1500 rpm for 5 minutes followed by 5 minutes at 3000rpm in a MSE 300 basket centrifuge (MSE Scientific Instruments, Sussex, England) to separate the apple juice and pomace. The juice was frozen

at -30°C for sugar and organic acid analysis at a later date.

3.3 HPLC Analysis of Sugars

The method used to quantify sugars was one recommended by the column manufacturer, although it was found that organic acids interfered with the sugar analysis. To improve sugar and acid separation a method was developed based on a report by Morawski, (1984) that Alumina Type A Sep-Pak® removes acids without affecting the carbohydrate levels. The developmental work included identification of the peaks and improved removal of interfering compounds, and validation of detector response and precision, peak quantification and recoveries. Details are given in appendices 3 to 10.

3.3.1 Liquid Chromatography System

A Waters Associates (Milford, MA, USA) HPLC system was used to separate sugars in apple juice. The system consisted of a Waters Model 6000A solvent delivery system, Valco Instruments (Houston, TX, USA) 6 port Sample Injector, Waters Model R401 differential refractometer operated at 64x attenuation. Sugar separation was achieved using a 8mm x 300mm Shodex SC1011 column packed with sulfonated styrene divinylbenzene cation (Ca^{2+}) exchange resin (Showa Denko K.K., Japan) and a Waters Sugar Pak™ II precolumn insert. The precolumn and analytical column were placed in a stainless steel water jacket connected to a thermostat-controlled water bath maintained at 82°C. A 10μl sample was injected using the fixed volume loop method. The samples were eluted isocratically with Milli-Q water (Milli-Q apparatus, Millipore Corporation, Bedford, MA, USA) containing 50ppm ethylenediamine tetra acetic acid disodium calcium salt (Ca EDTA) (Sigma Chemical Company, St. Louis, MO, USA) at a flow rate of 0.5ml/min. A computer using Peak Simple II software (SRI Instruments, Torrance, CA, USA) was used to record the detector signal and integrate all peaks.

3.3.2 Calibration Curves

A three point external calibration curve was constructed by preparing three external standard solutions each containing sucrose (BDH Chemical Company), glucose (Sigma Chemical Company), fructose (United States Biochemical Corporation, Cleveland, Ohio, USA) and sorbitol (BDH Chemical Company). The final concentration of each of the sugars in the standard was 2%, 4%, 6% and 0.2%, 0.4% and 0.6% for sorbitol. The standards were treated with aluminium oxide (section 3.3.4) and passed through the Sep-Pak® C_{18} (section 3.5) as for the apple juice. The standards were chromatographed at the beginning and end of each day.

3.3.3 Sample Preparation

Apple juice that had previously been extracted and frozen at -30°C was thawed overnight in the refrigerator. A 50ml sample was centrifuged at 3000rpm (600g) for 5 minutes in a MSE Super Minor bench top centrifuge to remove any particulate material. A sample of the apple juice was treated with aluminium oxide, filtered (section 3.3.4) and passed through a Sep-Pak® C_{18} cartridge (section 3.5).

3.3.4 Separation of Organic Acids by the Addition of Aluminium Oxide

Separation of organic acids from apple juice was necessary to enable accurate quantification of the sugars present. Details of the development of this method and its validation are given in appendices 5 and 6.

A 5ml aliquot of apple juice or standard was added to a 25ml beaker containing 0.5g aluminium oxide (Alumina) in the acidic form (M. Woelm, Eschwege, Germany and BDH Chemicals), mixed and allow to stand for 15 minutes. The juice was then decanted into a further 0.5g of aluminium oxide and the process repeated until the juice had been treated with a total of 2.0g of aluminium oxide. The juice was subsequently filtered through a 0.45μm membrane filter (Millipore Corporation, Bedford, MA) to remove all traces of

aluminium oxide. Previously this procedure had been demonstrated to reduce the organic acids to a negligible level, but not affect the sugar levels (appendix 5).

3.3.5 Quantification of Peaks

Quantification of sugar peaks was accomplished by comparing the peak heights and areas to the peak heights and areas of the standards analysed at the beginning and end of each day. It was determined that there was no significant difference between using peak height and areas or the calibration curves constructed at the start and end of the day for sugar quantification (appendices 8 and 9). Duplicate injections were made and the reported result for each apple juice is the average of eight determinations.

For the sugars the recoveries achieved by this method ranged between 94% and 105.4% (appendix 10). Recoveries for sorbitol ranged from 82% to 109% due to the difficulty in quantifying small peaks.

3.4 HPLC Analysis of Organic Acids

As there is no one method available that can simultaneously resolve malic, quinic, shikimic, citric, succinic, fumaric and fructose some method development work was undertaken. Details of the method developed and its validation are given in appendices 11 to 17.

3.4.1 Liquid Chromatography System

A second Waters Associates HPLC system was used to separate the organic acids. The system consisted of a Waters 600 multi-solvent delivery system, a Valco Instruments 6 port Sample Injector and a Lambda-Max 481 LC spectrophotometer. The detector signal was recorded by computer running Peak Simple II software. The column system consisted of Brownlee™ Labs (Applied Biosystems, Santa Clara, CA, USA) 30mm x 4.6mm Reverse Phase C_{18} guard column, 220mm x 4.6mm Spheri-5 Reverse Phase C_{18} and Spheri-5 ODS Reverse Phase C_{18} coupled together in series and all packed with 5μm spherical particles.

The mobile phase was 0.005M sulphuric acid (BDH Chemical Company). The flow rate was 0.4ml/min for 20 minutes before being increased to 0.8ml/min for a further 10 minutes. The columns were maintained at 32°C by placing in a stainless steel water jacket connected to a thermostat-controlled water bath. Detection was carried out at 214nm and samples of 25μl were injected using the fixed volume loop method.

3.4.2 Calibration Curves

A calibration curve was constructed by using two standards solutions. One solution contained malic acid (Sigma Chemical Company) at a final concentration of 12g/l, 0.4g/l quinic (BDH Chemicals Ltd), 0.2g/l shikimic (Sigma Chemical Company), 0.4g/l citric (Ajax Chemicals, Sydney, Australia) and 0.4g/l succinic (BDH Chemicals Ltd). Fumaric acid (0.008g/l) was also present as impurity from malic acid (appendix 16). The second standard contained malic acid at a final concentration of 6g/l, 0.2g/l quinic, 0.1g/l shikimic, 0.2g/l citric, 0.2g/l succinic and 0.004g/l fumaric acid. Both standards also contained fructose at a final concentration of 80g/l. Like the apple juice samples the standards were also passed through Sep-Pak® C_{18} cartridge prior to analysis by HPLC (section 3.5). The standards were chromatographed at the start and end of each day.

3.4.3 Sample Preparation

A 5ml sample of apple juice was thawed and centrifuged (as for sugar analysis) and filtered through a 0.45μm membrane filter (Sartorius AG, Goettingen, Germany) before being passed through a Waters Sep-Pak® C_{18} cartridge (section 3.5).

3.4.4 Quantification of Peaks

Quantification of organic acids was accomplished by comparing peak heights of the sample to peak heights of the standard chromatographed at the start and end of the day. Peak areas were not used as baseline separation of acids was not achieved. As there was no significant difference between standards run at the start and end of the day the final reported result for

the sample is based on an average of two standard determinations. Details of trials to determine this are given in appendices 14 and 15. The accuracy of the method was determined by measuring the recovery of the acids, with recoveries of between 94.6% to 103.3% achieved for quinic, malic, shikimic, fumaric, 119.6% for citric and 81.2% for succinic (appendix 17). Succinic, citric and fumaric acids were difficult to accurately quantify as they were present in small concentrations.

3.5 Sep-Pak®C_{18} Cartridge Activation and Regeneration

The preparation of Sep-Pak®C_{18} cartridge (Waters Associates) for use in sample cleanup was similar to the methods of Quemener and Mercier (1980) and Richmond *et al.* (1981). The Sep-Pak®C_{18} cartridge was placed at the end of a 1ml syringe and the cartridge was prewetted with 3ml of 95% ethanol (BDH Chemical Company) before being flushed with 2 to 3ml of air. The cartridge was then flushed with 5 to 6ml of Milli-Q water followed by 3ml of air to remove as much water as possible. A 3ml aliquot of apple juice or external standard was passed through the cartridge. The first 2ml of sample collected were discarded to avoid dilution affects (appendix 7) with the next 1ml being collected for HPLC analysis. All traces of the sample or standard were removed by flushing the cartridge with 1ml of air. Flushing the cartridge with ethanol after the sample had been collected eluted all the coloured pigments, allowing the cartridge to be reused.

3.6 Soluble Solids, pH, Titratable Acidity

The soluble solids were determined using an Abbe refractometer. The pH was determined using an Orion model SA 520 (Orion Research Incorporated, USA) pH meter. For titratable acidity a 10ml sample of apple juice that had been vacuum degassed, was continuously stirred while being titrated with 0.1M NaoH to an end point of pH 8.2. The titratable acidity was calculated as grams per litre of malic acid.

3.7 Sample Variability

In order to determine the sample variation of the trial, Braeburn fruit was collected from the North, South, East, and West facing sides, as well as from top and bottom of a single tree, from the North facing side of trees from 4 different orchards, from the top corners, bottom corners, centre positions in a bin and from the centre position from 4 bins present in processing yard. Some large and small and fruit were also collected. In all 21 samples were collected on a single day in 1993.

3.8 Statistical Analysis of Data

Statistical Analysis System software (SAS) that uses the method of least squares to fit general linear models (GLM) to unbalanced data sets was used to perform a three way (cultivar, region and year) analysis of variance (ANOVA) of sucrose, glucose, fructose, sorbitol, °Brix, fructose/glucose ratio, malic, quinic, shikimic, citric, succinic and fumaric acids. Data from the study that had information on picking was also analysed using general linear models to determine if significant differences existed in the time of picking. Data from the cultivars Hillwell, Fiesta, GS330 and GS2850 were not included in the statistical analysis as only one sample of each was analysed.

CHAPTER 4
RESULTS AND DISCUSSION
SUGAR COMPOSITION

Commercial apple juice may be produced from fruit that have been harvested at different maturities, contain a range of different cultivars or use fruit that have been stored for varying times under different conditions (ambient, cold or controlled atmosphere storage). Most values reported in the literature are from a range of different cultivars and conditions and it is therefore appropriate to combine all the results for New Zealand apple juice to give an overall mean, standard deviation, maximum and minimum (figures 4.1-4.6, appendix 18) for comparisons with literature values (figures 2.1-2.6). However as the majority (65%) of the data given here is for Granny Smith and Braeburn fruit, single cultivar data is also presented.

4.1 Average Composition

The mean, standard deviation, maximum and minimum values for total soluble solids, sucrose, fructose, glucose, sorbitol and fructose/glucose ratio (figure 4.1-4.6, appendix 18) for average composition of apple juice from New Zealand grown fruit was similar to that which are presented in the literature (figures 2.1-2.6)

The soluble solids of authentic apple juice was reported as consisting of 12 ±1.0°Brix by Brause and Raterman (1982), 12.74°Brix with a standard deviation of ±1.6°Brix by Mattick and Moyer (1983), 12°Brix with a standard deviation of ±1.0°Brix by Fuleki *et al.* (1994) and 11.7°Brix with a standard deviation of 2.33°Brix (Lee and Wrolstad, 1988a). Similar levels (mean of 11.5°Brix with a standard deviation of ±1.4°Brix) were observed in the samples of New Zealand apple juice that were examined (figure 4.1)

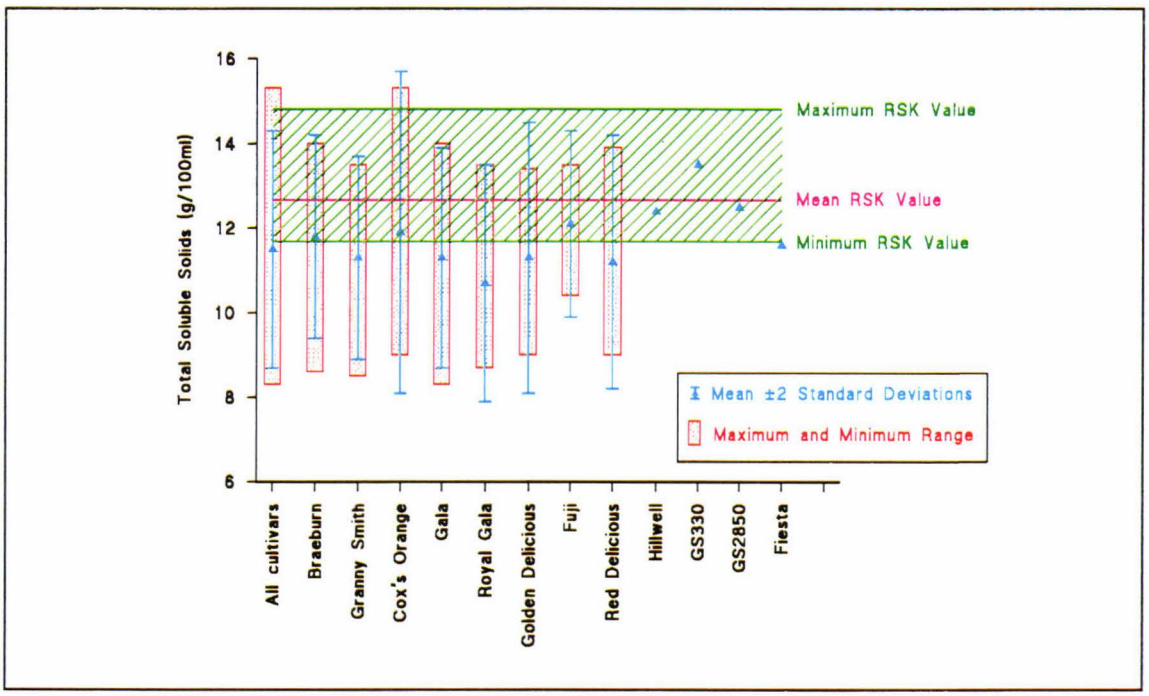

Figure 4.1: Comparison of total soluble solids concentrations for New Zealand varietal apple juices with the RSK values.

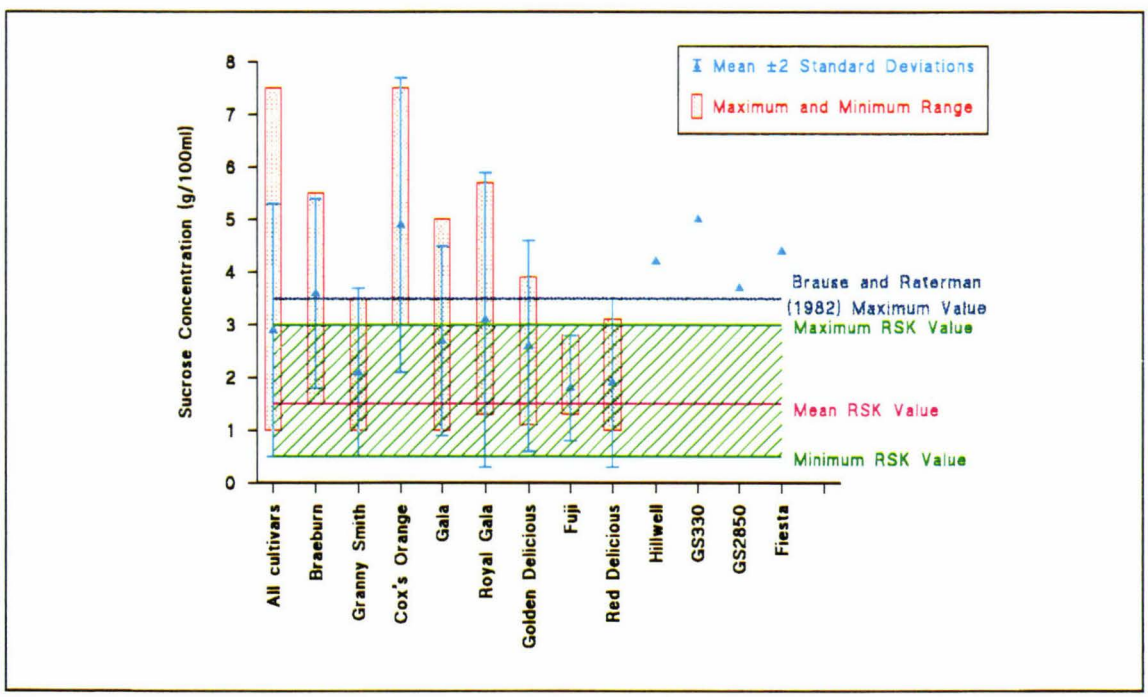

Figure 4.2: Comparison of sucrose concentrations for New Zealand varietal apple juices with the RSK and Brause and Raterman (1982) values.

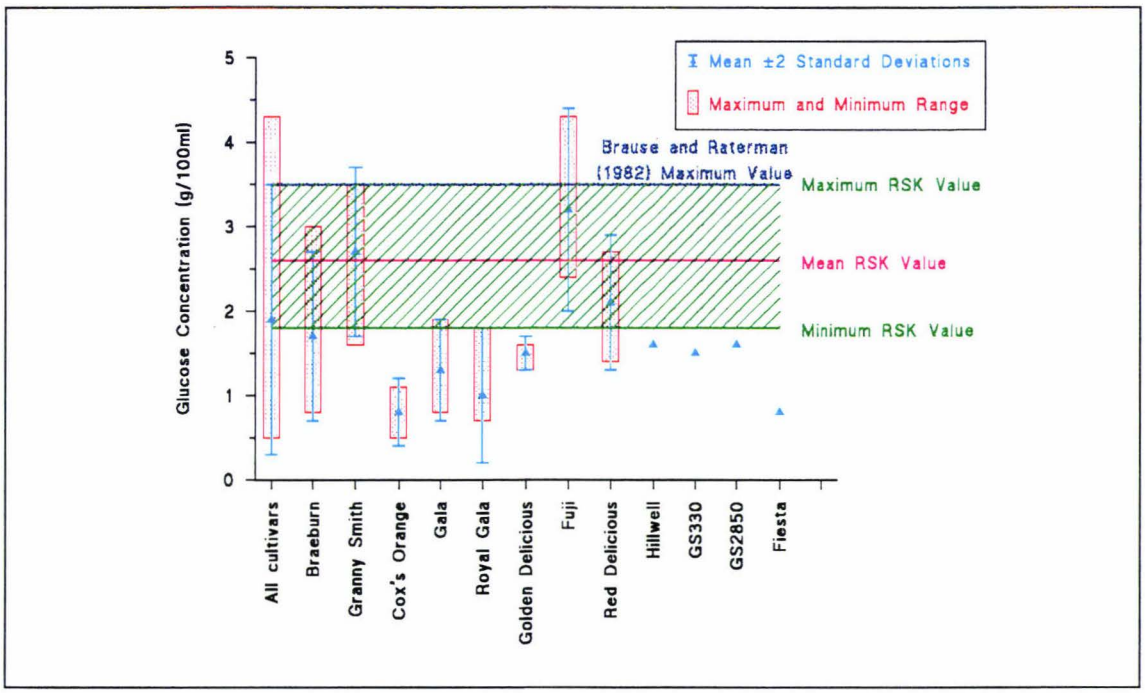

Figure 4.3: Comparison of glucose concentrations for New Zealand varietal apple juices with the RSK and Brause and Raterman (1982) values.

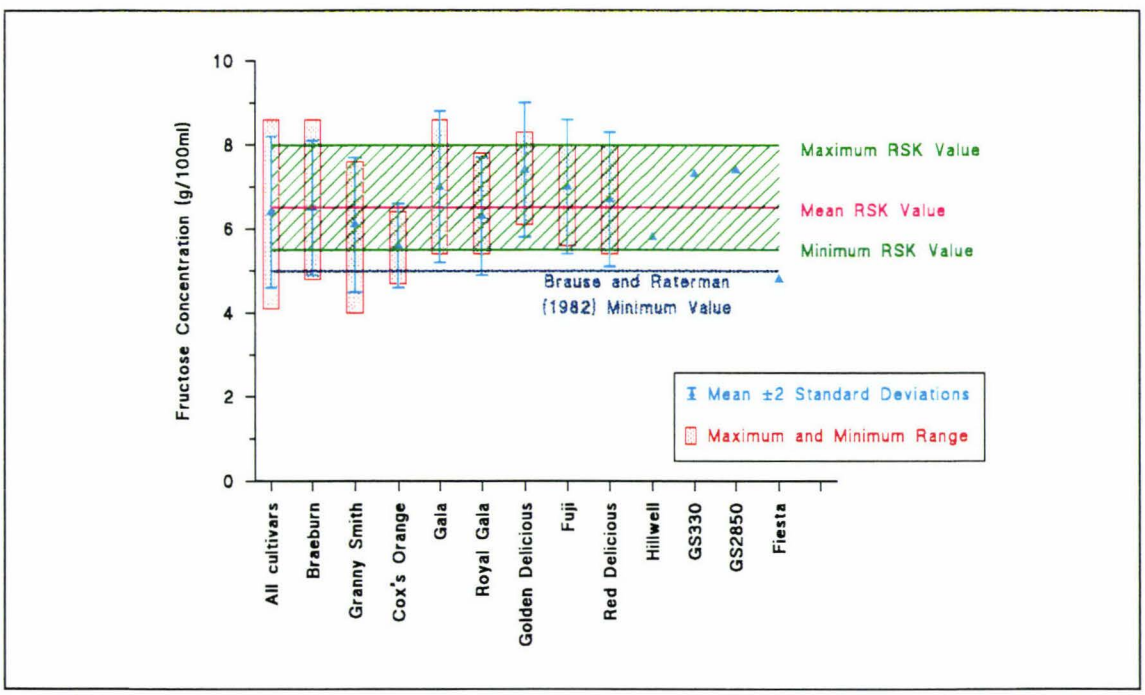

Figure 4.4: Comparison of fructose concentrations for New Zealand varietal apple juices with the RSK and Brause and Raterman (1982) values.

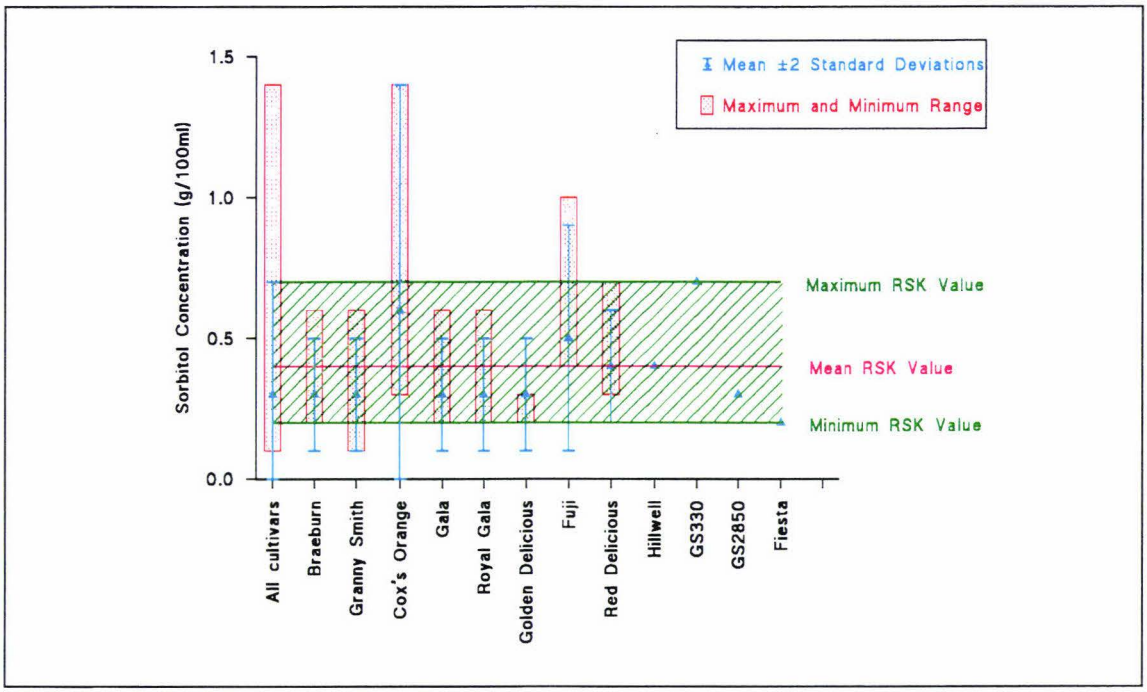

Figure 4.5: Comparison of sorbitol concentrations for New Zealand varietal apple juices with the RSK and Brause *et al.* (1986) values.

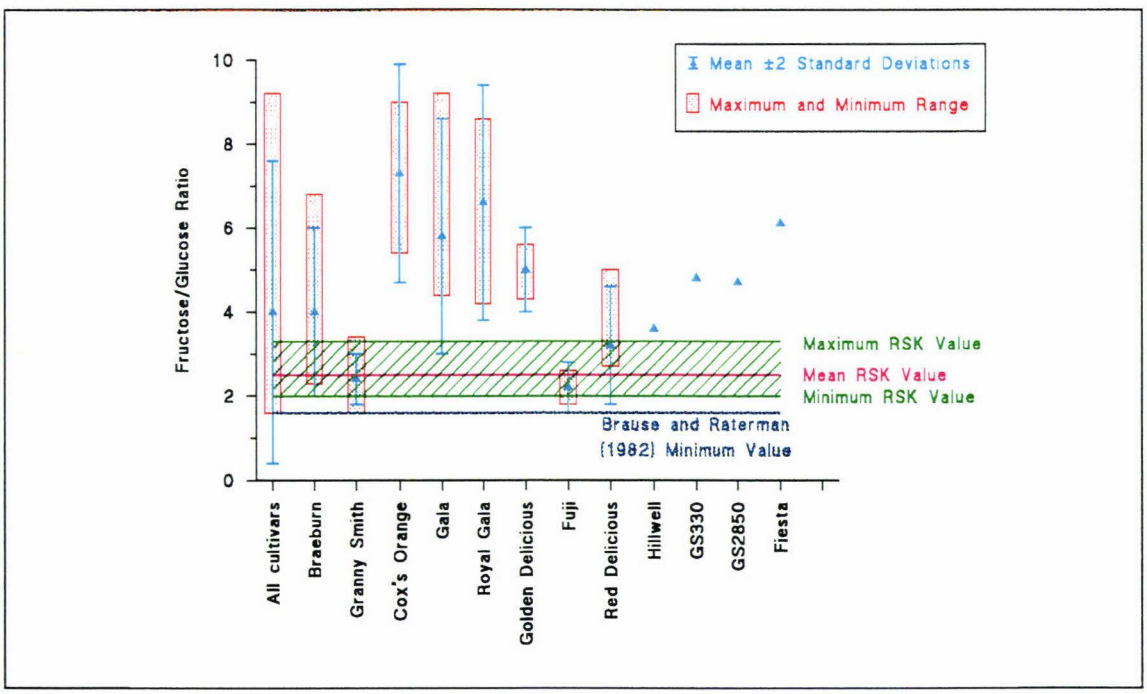

Figure 4.6: Comparison of fructose/glucose ratios for New Zealand varietal apple juices with the RSK and Brause and Raterman (1982) values.

The mean sucrose level of 2.9g/100ml (standard deviation ±1.2g/100ml, n=189) for New Zealand apple juice was close to the maximum value of 3.0g/100ml proposed by the German RSK value for authentic juice and below the maximum value of 3.5g/100ml suggested by Brause and Raterman (1982) for authentic juice (figure 4.2). Mattick and Moyer (1983) and Fuleki *et al.* (1994) reported mean sucrose levels of 2.68g/100ml (standard deviation of ±1.01g/100ml, n=93) and 2.57g/100ml (standard deviation of ±1.02g/100ml, n=77) respectively which are similar to that which was found for the juice of New Zealand grown fruit. However New Zealand fruit were observed to have mean sucrose levels greater than those reported for the databases of Lee and Wrolstad (1988a) and Ryan (1972). When Brause and Raterman (1982) analyzed 9 authentic apple juices they found a mean sucrose level of 1.34g/100ml (standard deviation of ±0.62g/100ml) which is much lower than what was observed for New Zealand apple juice.

The mean glucose level found in the juice of New Zealand grown fruit was 1.9g/100ml (standard deviation ±0.8g/100ml), which was within the limits proposed by the German RSK values but close to their proposed minimum value of 1.8g/100ml (figure 4.3). Brause and Raterman (1982) state that authentic juice should have no more than 3.5g/100ml of glucose and report a mean value of 3.03g/100ml (standard deviation ±0.80g/100ml) with values as low as 1.9g/100ml for the authentic juices that they analysed. Ryan (1972) and Lee and Wrolstad (1988a) found mean glucose levels of 2.50g/100ml (standard deviation ±0.42g/100ml, n=20) and 2.94g/100ml (standard deviation ±1.09g/100ml, n=8) respectively. These values are greater than mean glucose level which was seen for New Zealand fruit. However the mean glucose level of New Zealand juice was similar to the value of 2.07g/100ml (standard deviation ±0.67g/100ml) which was reported by Mattick and Moyer (1983).

The mean fructose level of 6.4g/100ml (standard deviation ±0.9) for New Zealand apple juice exceeded the minimum level of 5.0g/100ml proposed by Brause and Raterman (1982) and was within the German RSK limits of 5.5g/100ml to 8.0g/100ml (figure 4.4). Brause and Raterman (1982) do not give an upper limit although they report that "examination of the literature revealed that fructose was typically present up to a level of 8.0g/100ml". For

authentic juices that they analysed the mean fructose level was 6.7g/100ml (standard deviation ±1.02g/100ml) with two of the nine samples exceeding 8.0g/100ml with values of 8.1g/100ml and 8.6g/100ml for Red Delicious and Mcintosh respectively. Fuleki *et al.* (1994), Mattick and Moyer (1983), and Ryan (1972) report mean fructose levels of between 5.41g/100ml and 5.79g/100ml which are lower than that observed for New Zealand apple juice. However, the mean fructose level observed for New Zealand apple juice was lower than the mean of 8.16g/100ml (standard deviation ±1.90g/100ml) reported by Lee and Wrolstad (1988a). Their high fructose and glucose and low sucrose level are probably due to the samples being stored for prolonged periods (Lee and Wrolstad, 1988a). The maximum fructose level for New Zealand juice was 8.6g/100ml and was generally within the range of the reported databases, and much less than the maximum of 10.50g/100ml given by Mattick and Moyers (1983) database.

The mean sorbitol present in New Zealand produced apple juice was 0.34g/100ml with a standard deviation of ±0.15g/100ml (figure 4.5). This was similar to the that which was found by Fuleki *et al.* (1994) (mean 0.29 ±0.1g/100ml), Lee and Wrolstad (1988a) (mean 0.36 ±0.06g/100ml) and was within the range of 0.2 to 0.7g/100ml suggested by the German RSK values for authentic apple juice. The sorbitol level found in New Zealand apple juice was lower than the mean value of 0.52g/100ml (standard deviation ±0.21g/100ml) and 1.01g/100ml (standard deviation ±0.26g/100ml) for Mattick and Moyer (1983) and Ryan (1972) respectively, databases.

A minimum fructose/glucose ratio of 1.6 has been suggested by Brause and Raterman (1982) and for the German RSK assessment the reciprocal, glucose/fructose ratio a value between 0.3 to 0.5 for authentic apple juice. For ease of comparisons, the German RSK glucose/fructose ratio can be converted to a fructose/glucose ratio giving a range for authentic apple juice of 2.0 to 3.3. Brause and Raterman (1982) do not give an upper limit. The fructose/glucose ratio was always greater than the minimum value suggested by Brause and Raterman (1982) for authentic juice with a mean ratio of 4.0 and a standard deviation of ±1.8 (figure 4.6). The mean ratio was greater than the maximum of 3.3 given by the German RSK values. The ratio was similar to that which was found by Fuleki *et al.* (1994)

but less than those given for Brause and Raterman (1982) Lee and Wrolstad (1988a), Mattick and Moyer (1983) and databases. The maximum ratio found in the literature was 7.64 (Fuleki *et al.*, 1994), which was less than the maximum value of 9.2 seen for New Zealand apple juice. This difference is due to the low glucose present in some New Zealand juice which causes the fructose/glucose ratio to be elevated.

4.2 Braeburn

The mean, standard deviation, maximum and minimum for sucrose, glucose, fructose, sorbitol, fructose/glucose ratio and total soluble solids for the juice of Braeburn apples are shown in figures 4.1 to 4.6 with the individual data in appendix 19. While the total soluble solids for Braeburn apple juice ranged from 8.6°Brix to 14.0°Brix and was similar to literature reports and other cultivars examined, the individual sugar composition was interesting.

Fructose was present at levels of between 4.6g/100ml and 8.6g/100ml which was similar to those found by Brause and Raterman (1982), Fuleki *et al.* (1994), Lee and Wrolstad (1988a), Mattick and Moyer (1983), Ryan (1972) and Wrolstad and Shallenberger (1981). Using the criteria of Brause and Raterman (1982), 95.3% of Braeburn apple juice samples analysed had fructose levels within limits for authentic apple juice while 86% of the samples were within the limits suggested by the German RSK Values (Bielig *et al.*, 1982).

Glucose was present at levels of between 0.8g/100ml and 3.0g/100ml with the maximum value of 3.5g/100ml suggested by Brause and Raterman (1982) and German RSK never being exceeded. However the glucose level for 60% of the samples fell below the suggested German RSK minimum value of 1.8g/100ml for authentic apple juice. While the glucose levels were generally below the German RSK values, they are similar to the range obtained by Fuleki *et al.* (1994) and Mattick and Moyer (1983).

At no stage did Braeburn apple juice have a fructose/glucose ratio of less than 2.0 and 69% of all samples exceeded the German RSK maximum value of 3.3 with a maximum ratio of

6.8 observed. While the fructose/glucose ratio exceeds the RSK maximum value it can be integrated into the RSK guidelines by use of a commentary. For example the RSK guidelines state " in exceptional cases, the glucose/fructose ratio may be slightly lower than 0.3" (Wallrauch and Faethe, 1988b) (In terms of the fructose/glucose ratio used here for comparisons, the ratio can be slightly higher than 3.3), but they do not give a minimum value. The mean fructose/glucose ratio for Braeburn apple juice was 3.9 and a 95% confidence limit of 3.6 to 4.1, which exceeded the maximum RSK value of 3.3 (Lee and Wrolstad, 1988b). Wrolstad and Shallenberger (1981) established that apple has a fructose/glucose ratio of well over two (average value 2.7). Among other fruits only pear is similar (average value of 3.8) and pear has a much higher sorbitol and proline level, making its use as an adulterant easy to detect.

The situation for sucrose was somewhat different.

Juice of Braeburn apples contained sucrose at levels of between 1.5g/100ml and 5.5g/100ml over the two years in which samples were collected. In 1992 the sucrose content always exceeded the maximum level of 3.5g/100ml reported by Brause and Raterman (1982) and 24% of all samples exceeded this limit in 1993 (appendix 19). The application of 95% confidence limits to Mattick and Moyer (1983) database (Lee and Wrolstad, 1988b) yields a maximum value of 4.7g/100ml for sucrose content. This figure was exceeded by 35% of the Braeburn juice samples in 1992 and 2.2% (one sample) in 1993. The maximum level of 3.0g/100ml recommended by the German RSK values for authentic juice was always exceeded in 1992 and by 55% of the samples in 1993.

4.2.1 Storage

While fruit is held under different storage conditions for juice processing at a later date, the sugars continue to change and affect the composition of the resulting juice.

During storage in 1993 the total soluble solids remained between 12.0°Brix and 14.0°Brix (figure 4.7) with sucrose levels decreasing (figure 4.8) and fructose, glucose and sorbitol

showing slight increases (figures 4.9, 4.10 and 4.11). The fructose/glucose ratio also showed a decrease during storage with Brause and Raterman (1982) minimum value of 1.6 always exceeded (figure 4.12). Similar trends were seen in 1992 and as the storage time was shorter than that of 1993 the changes in sugar with time are given in appendix 24.

The sucrose level for the juice of stored fruit exceeded the German RSK and Brause and Raterman (1982) maximum limits in 1992. Values typically in excess of 4g/100ml were observed even at the end the of storage period, that is, after fruit had been stored for 42 days at ambient, 112 days at cold and 156 days at controlled atmosphere conditions. Unfortunately no fruit was analysed for sugars after these times in 1992.

In 1993 the fruit was variously stored for 64 days at ambient, 206 days at cold and 206 days at controlled atmosphere conditions. Sucrose was present at levels of 4.1g/100ml at the start of storage and did not fall to levels below the RSK maximum until the fruit had been stored for 45, 149 and 195 days for ambient, cold and controlled atmosphere storage respectively. By the end of the storage periods examined the sucrose level had decreased by between 34% to 48% to be present at levels of between 2.1g/100ml and 2.7g/100ml. These values are below the maximum level proposed by Brause and Raterman (1982) and within the ranged suggested by the German RSK values. This decrease is due to sucrose being inverted (Lee and Wrolstad, 1988b) and its use as a respiratory substrate (Fuleki *et al.*, 1994).

Sucrose concentrations increase during fruit ripening and first pick fruit generally had sucrose present at levels less than that seen for second and third pick fruit. Levels of 2.6g/100ml were observed for first pick fruit and at this level was below the German RSK maximum value. The only other time that sucrose levels were below the RSK maximum value was at the end of storage.

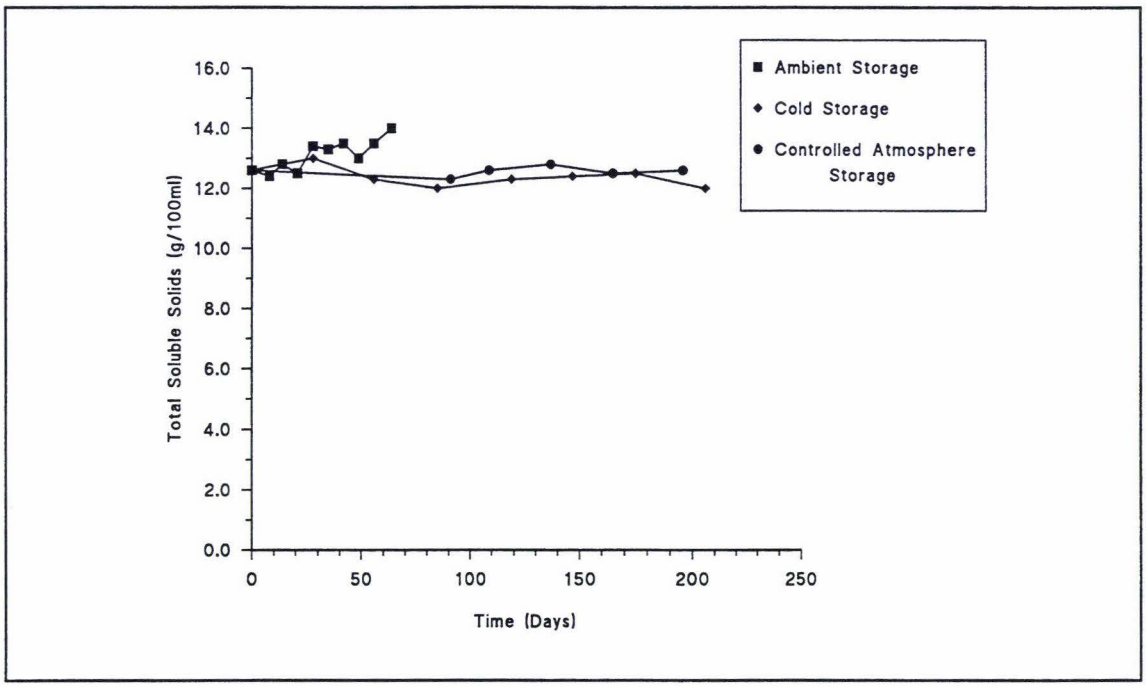

Figure 4.7: Effect on juice soluble solids concentrations of different storage regimes for Braeburn apples in 1993.

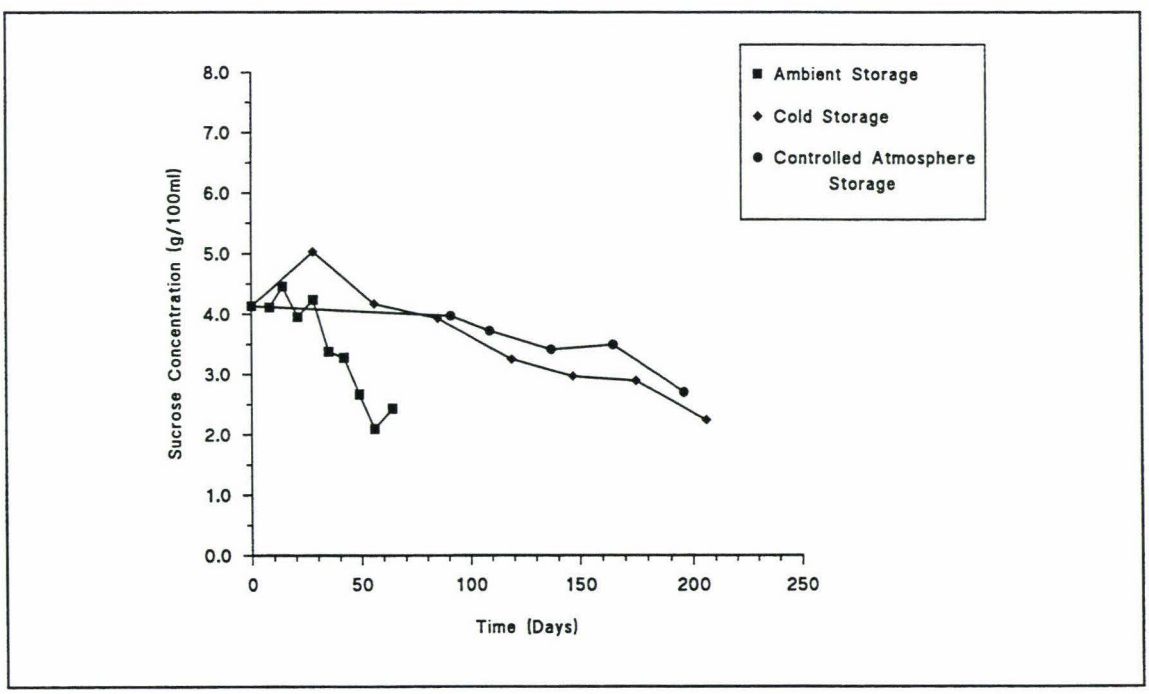

Figure 4.8: Effect on juice sucrose concentrations of different storage regimes for Braeburn apples in 1993.

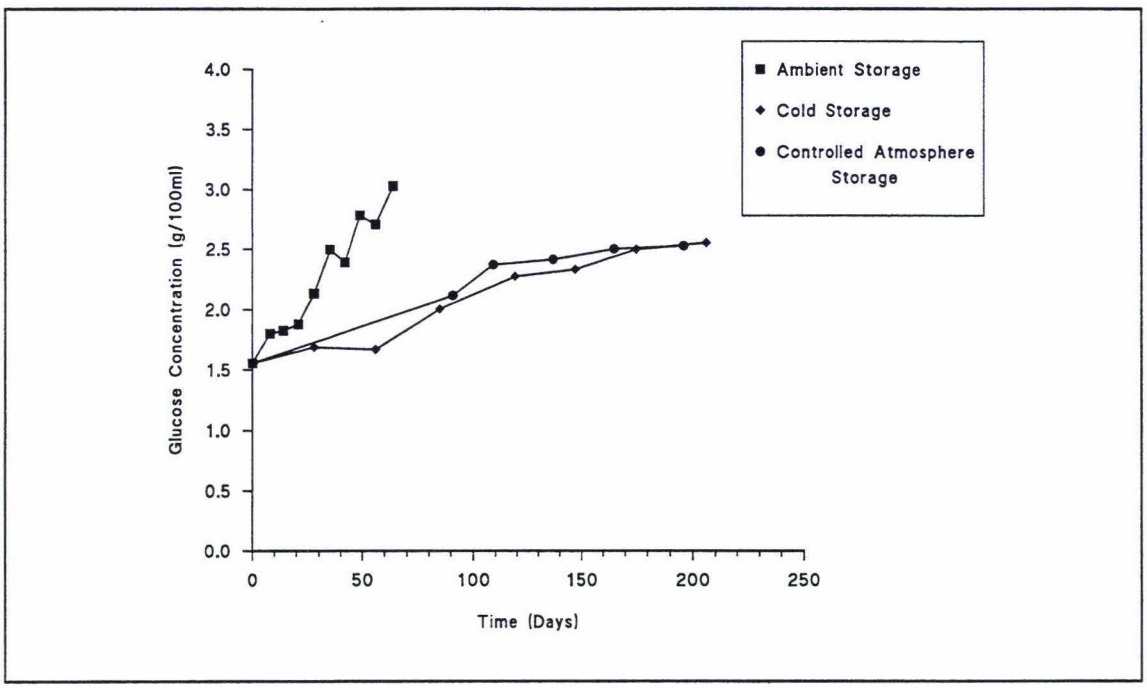

Figure 4.9: Effect on juice glucose concentrations of different storage regimes for Braeburn apples in 1993.

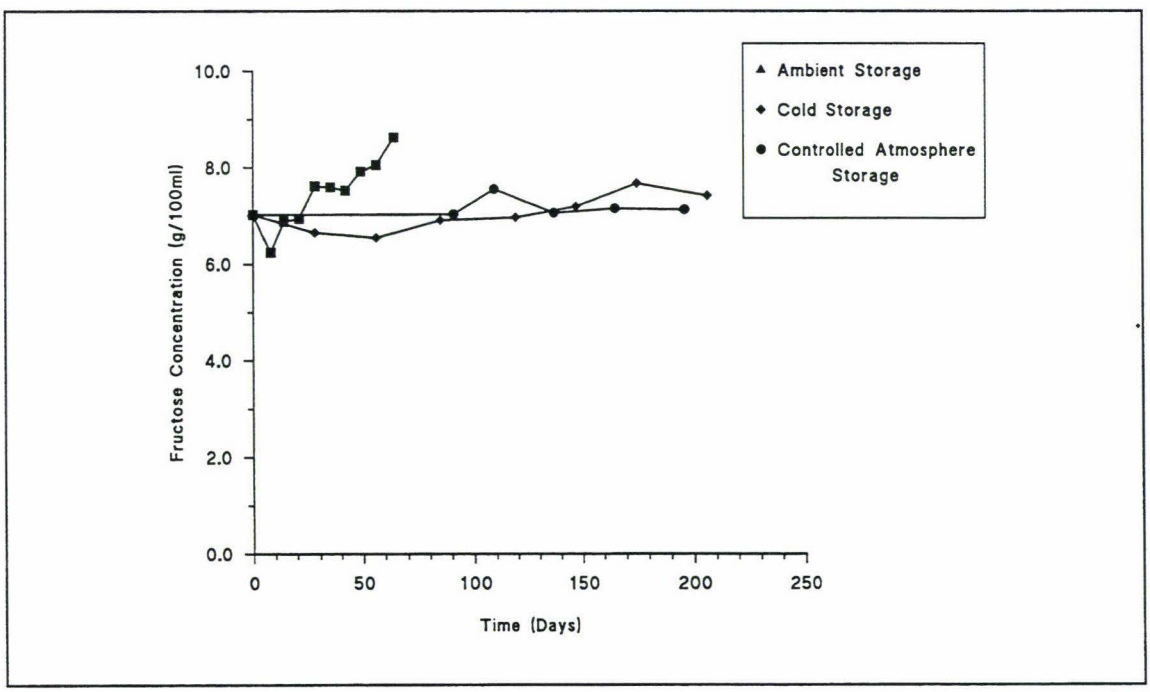

Figure 4.10: Effect on juice fructose concentrations of different storage regimes for Braeburn apples in 1993.

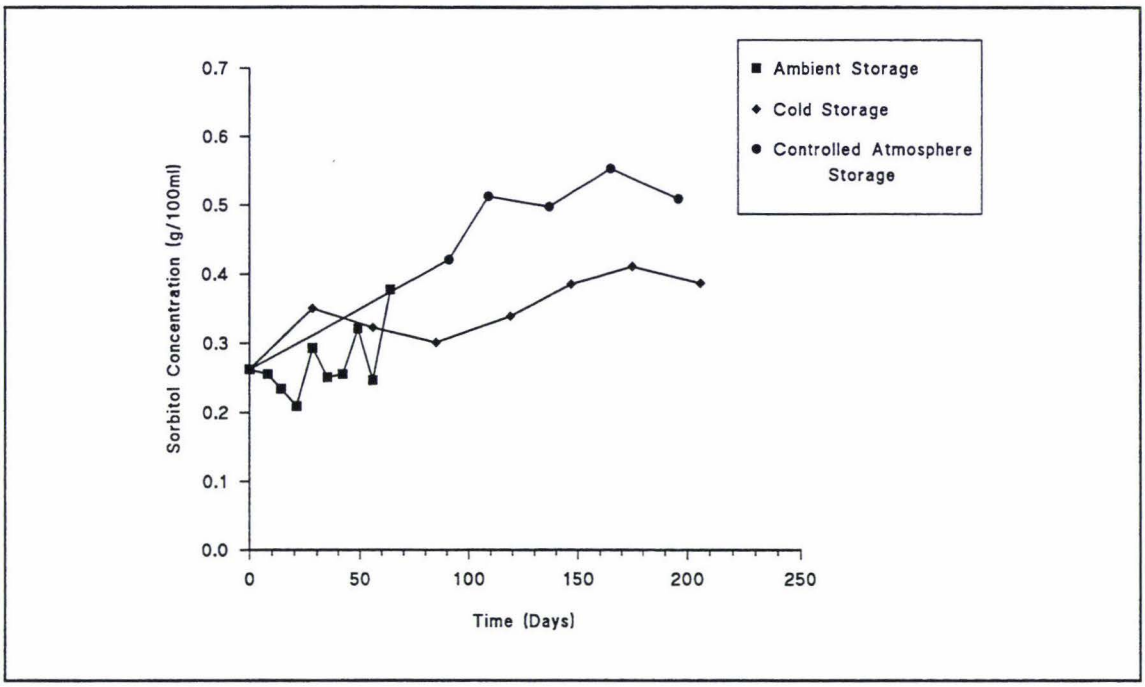

Figure 4.11: Effect on juice sorbitol concentrations of different storage regimes for Braeburn apples in 1993.

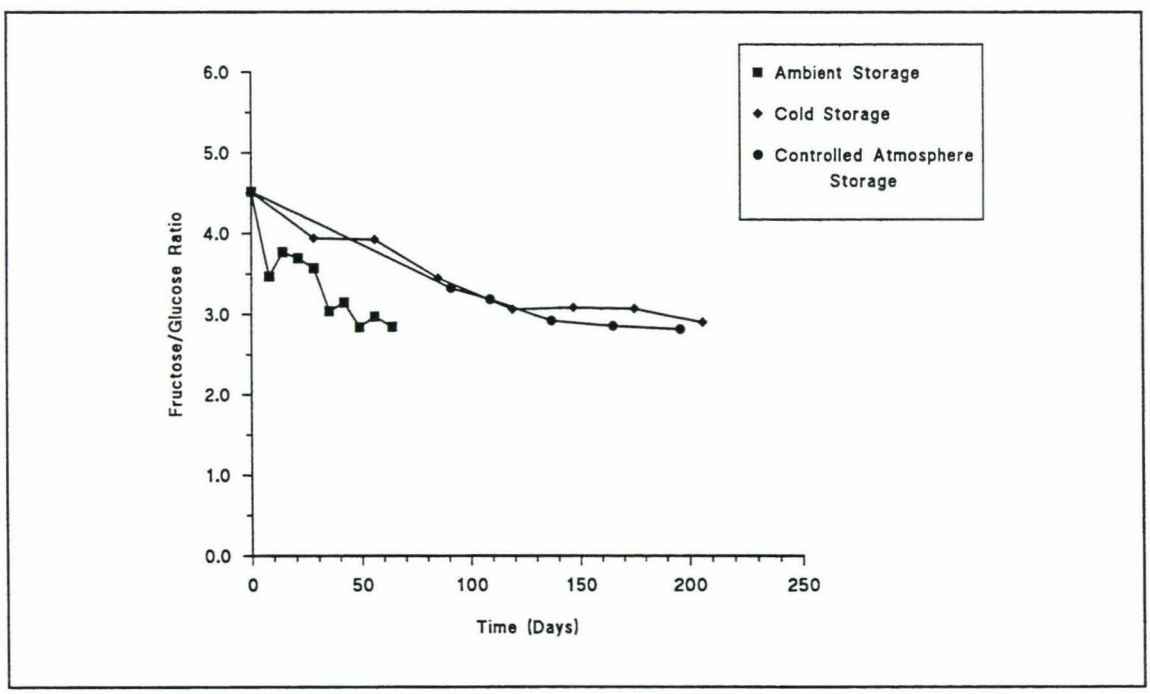

Figure 4.12: Effect on juice fructose/glucose ratios of different storage regimes for Braeburn apples in 1993.

At the start of storage in 1992 glucose was present at levels of 1.0g/100ml with slightly higher levels of 1.6g/100ml seen in 1993. By the end of fruit storage in 1992 the glucose level in the juice had increased by between 32% to 88% to reach levels of between 1.6g/100ml and 1.9g/100ml. After a similar period of fruit storage in 1993 the glucose concentration of the juice showed increases of between 42% to 56% to levels of between 2.2g/100ml and 2.4g/100ml.

The production of juice from Braeburn apples that had been stored for short periods (8 days at ambient, 45 days at cold and 51 days at controlled atmosphere conditions in 1993) were observed to have glucose levels that were below the minimum RSK values for authentic juice. However by the end of ambient, cold and some controlled atmosphere storage in 1993, glucose had increased by between 62% and 94% to reach a maximum level of 2.5g/100ml to 3.0g/100ml. The juice from stored fruit in 1992 was generally less than the German RSK minimum value, but the storage period was not as long as that of 1993.

Both Brause and Raterman (1982) and the German RSK proposed maximum values for glucose in authentic juice were not exceeded by any stored fruit in 1992 or 1993.

Fructose increased by between 1.5% to 22.8% from between 6.5g/100ml and 7.0 at the start of storage to between 7.1g/100ml and 8.6g/100ml by the end of storage in both years. Fructose levels seen during storage were always within Brause and Raterman (1982) and the German RSK with limits.

The maximum levels of glucose and fructose corresponded to a minimum level of sucrose and are probably due to inversion of sucrose (Lee and Wrolstad, 1988b).

While it is generally recognised that during storage the sucrose level decreases and the fructose and glucose levels increase, it is highly likely that the juice from stored Braeburn fruit would have sucrose levels that exceed the RSK and Brause and Raterman (1982) maximum values and glucose levels lower than the minimum RSK value.

4.2.2 Data as Percentage of the Total Sugars by Summation

The presentation of the individual sugars as a percentage of the total sugars by summation makes it possible to compare data from a number of different sources (Elkins *et al.*, 1988; Lee and Wrolstad, 1988b). Sucrose has been reported to account for up to 30% of the total sugars, although sucrose was reported by Lee and Wrolstad (1988a) to comprise up to 46% of the total sugars for Mattick and Moyer (1983) results. In the samples of Braeburn apple juice that were examined it was found that sucrose accounted for up to 44% of the total sugars (appendix 19). Using the Lee and Wrolstad (1988b) suggested value of 42.3% for sucrose at the 95% confidence level for detecting adulterated samples, all but one (1.4%) of the samples of Braeburn apple juice would be considered as authentic. However using the data of Elkins *et al.* (1988) and assuming a normal distribution it is possible to calculate a 95% confidence range of 6.8% to 20.5% (±2 standard deviations) for sucrose as a percentage of the total sugars. These values are about 50% of those suggested by Lee and Wrolstad (1988b) and of those observed here. The difference could be due to Elkins *et al.* (1988) using reconstituted apple juice for their determinations. As stated by Shaw (1988) "there is more variability in composition of fresh fruit than there is in processed fruit products due to several factors, including degree of ripeness, source, cultivar, sample size, and the fact that there is no blending ....".

The implication of this is that if solely Braeburn apples are used in the production of apple juice concentrate and assuming 22% inversion of sucrose due to the heating process (Sharkasi *et al.*, 1981), the resulting concentrate would exceed the 95% confidence limit calculated for Elkins *et al.* (1988) data. The concentrate would be incorrectly identified as adulterated with sucrose.

Glucose accounted for 7% to 25% (mean 14.5%) of the total sugars which is slightly lower than that which was given by Lee and Wrolstad (1988a), Wrolstad and Shallenberger (1981).

Fructose made up between 48% and 62% of the total sugars which is in agreement with Wrolstad and Shallenberger (1981) and Mattick and Moyer (1983), but lower than that

which Lee and Wrolstad (1988a) observed for apple juices.

Sorbitol ranged from 0.2 to 0.6g/100ml, which corresponds to other reported values (figure 2.8) and was within the limits of 0.2 to 0.7g/100ml as proposed by the German RSK values and similar to Brause *et al.* (1986) limits of 0.3 to 1.2g/l. The sorbitol concentration was variable, perhaps due to errors in analysing small peaks from the HPLC, and no discernable trends were observed.

By the RSK guidelines and Brause and Raterman (1982) suggested limits for fructose, °Brix, sorbitol levels and fructose/glucose ratio Braeburn apple juice can be considered authentic. Examination of the sucrose and glucose levels shows that up to 60% of the juice would be considered as not authentic.

4.3 Granny Smith

Granny Smith juice had a total soluble solids of between 8.5°Brix and 13.5°Brix, (figure 4.1, appendix 20) which was similar to the levels observed for Braeburn apple juice (8.3°Brix to 14.1°Brix). However unlike Braeburn juice the glucose, fructose and sucrose levels were similar to those values reported in the databases of Burroughs (1984), Fuleki *et al.* (1994), Lee and Wrolstad (1988a), Mattick and Moyer (1983), Ryan (1972) and Wrolstad and Shallenberger (1981).

The mean fructose level of Granny Smith juice was 6.1g/100ml with a range of 4.0g/100ml to 7.6g/100ml (figure 4.4). This is similar to the fructose level of 6.0g/100ml reported by Brause and Raterman (1982) for the one sample of this cultivar that they examined. It is also similar to the mean of 6.5g/100ml suggested by the German RSK for authentic apple juice. While no samples exceeded the maximum limit of 8.0g/100ml proposed by the German RSK, 24.5% of the samples had fructose present at levels less than the German RSK proposed minimum of 5.5g/100ml and 12.3% below Brause and Raterman (1982) minimum value of 5.0g/100ml. These samples were generally first or second pick fruit which were also observed to have the lowest total soluble solids.

The mean sucrose and glucose levels were 2.7g/100ml and 2.1g/100ml respectively with ranges of 1.0g/100ml to 3.5g/100ml for sucrose and 1.6g/100ml to 3.5g/100ml for glucose. These are similar to that which were found by Brause and Raterman (1982) for Granny Smith. Glucose levels never exceeded Brause and Raterman (1982) or the German RSK maximum value for authentic juice. Only one sample, first pick from Canterbury was found to have glucose at a level (1.6g/100ml) which was below the minimum RSK value.

Sucrose was generally present within the range of the German RSK recommended values with only 17.5% of the Granny Smith samples exceeding the maximum value and no samples falling below their suggested minimum value. No samples exceeded Brause and Raterman (1982) maximum value and all samples fell within the 95% confidence value suggested by Lee and Wrolstad (1988b).

The fructose/glucose ratio always exceeded the minimum value of 1.6 suggested by Brause and Raterman (1982) for authentic juice with 91.3% of all samples falling within the German RSK limits.

4.3.1 Storage

As was observed in the juice of stored Braeburn fruit the sucrose level decreased and fructose and glucose for Granny Smith increased during storage with the total soluble solid remaining between 10.5°Brix and 13.5°Brix. Sorbitol levels showed an increase during cold and controlled atmosphere storage and a decrease when stored at ambient conditions. A fructose/glucose ratio of 2.0 was observed during the storage period. The changes in sugars with time for fruit stored at either ambient, cold or controlled atmosphere conditions in 1993 are presented in figures 4.13-4.18 and for 1992 in appendix 25.

Sucrose was present at 2.1g/100ml at the start of storage in 1993 and had decreased by 47% to 1.1g/100ml after 64 days at ambient, 206 days at cold and 206 days at controlled atmosphere conditions. Over the same storage period glucose increased by up to 34% from 2.5g/100ml to between 2.6g/100ml and 3.3g/100ml, with fructose increasing by as much as

20% from 6.1g/100ml to between 6.3g/100ml and 7.3g/100ml.

Sorbitol ranged from 0.1g/100ml to 0.6g/100ml which corresponds to the reported values found in the literature. These levels were usually within the limits proposed by the German RSK values.

Unlike that which was seen in stored Braeburn fruit the sucrose, glucose and fructose levels were always within the limits of the German RSK values and never exceeded the maximum limit proposed by Brause and Raterman (1982). Overall the effect of storage was unimportant with regard to the proposed limits for authentic juice.

4.3.2 Data as Percentage of the Total Sugars by Summation

As a percentage of the total sugars, the maximum sucrose level observed was 30.8% with a mean of 18.7% (appendix 20) which is similar to the level seen in the databases of Lee and Wrolstad (1988a) and Mattick and Moyer (1983) as detailed by Lee and Wrolstad (1988a), Wrolstad and Shallenberger (1981). No samples exceeded the 95% confidence limit of 42.3% for Mattick and Moyer (1983) database (Lee and Wrolstad, 1988b). The mean level is similar to the that proposed by Elkins *et al.* (1988), although 37% of the samples analysed had levels in excess of their calculated 95% confidence limit of 20.8%, but their database is based on reconstituted juice and the results from this study were from fresh fruit. Both glucose and fructose were present at levels similar to that reported in the above databases. Glucose typically accounted for between 16% and 32% of the total sugars, while fructose accounted for 50% to 65% of the total sugars.

Therefore by the criteria established by both Brause and Raterman (1982) the German RSK values, and by comparison to other published databases the juice from Granny Smith apples grown in New Zealand would be considered authentic.

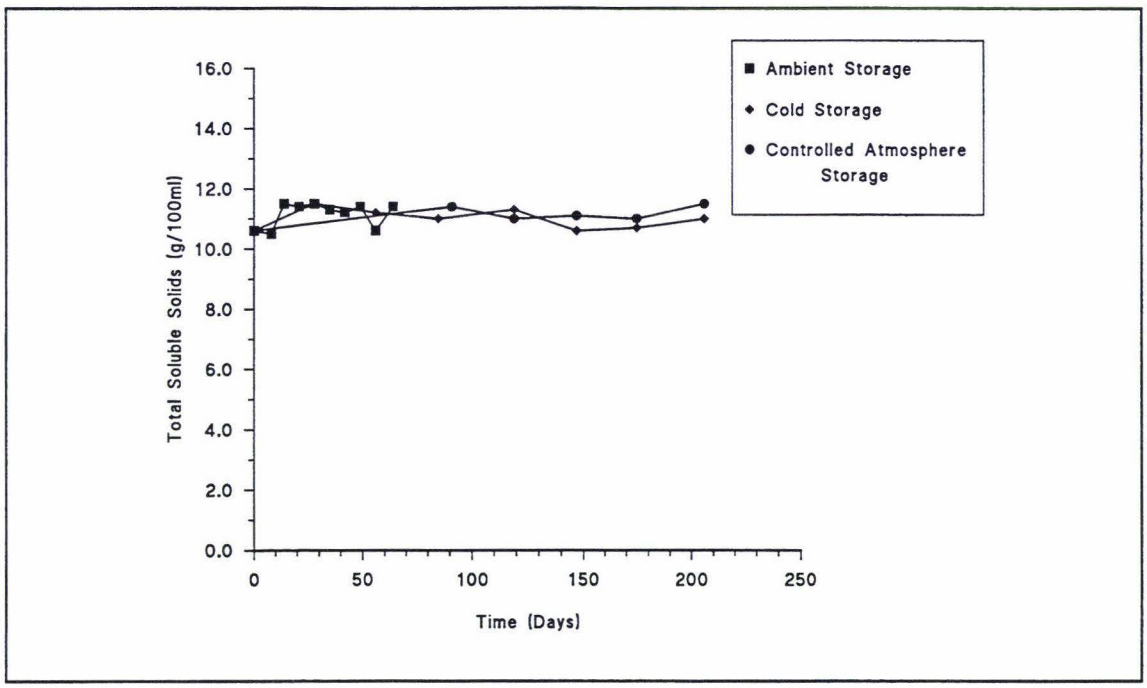

Figure 4.13: Effect on juice soluble solids concentrations of different storage regimes for Granny Smith apples in 1993.

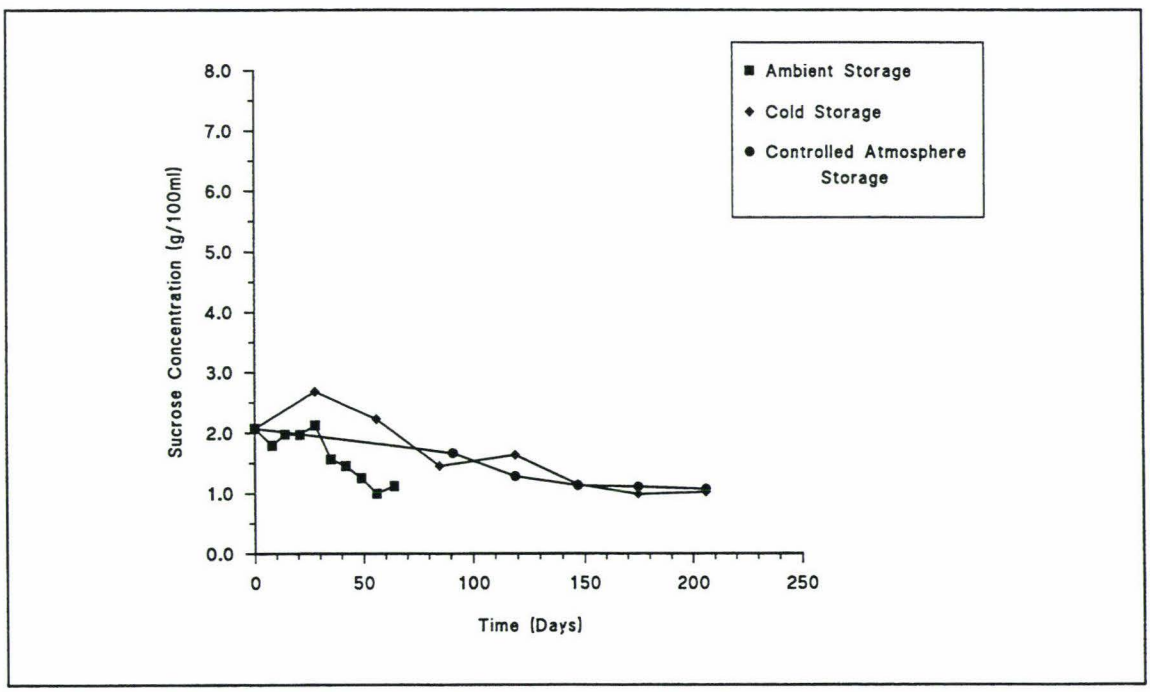

Figure 4.14: Effect on juice sucrose concentrations of different storage regimes for Granny Smith apples in 1993.

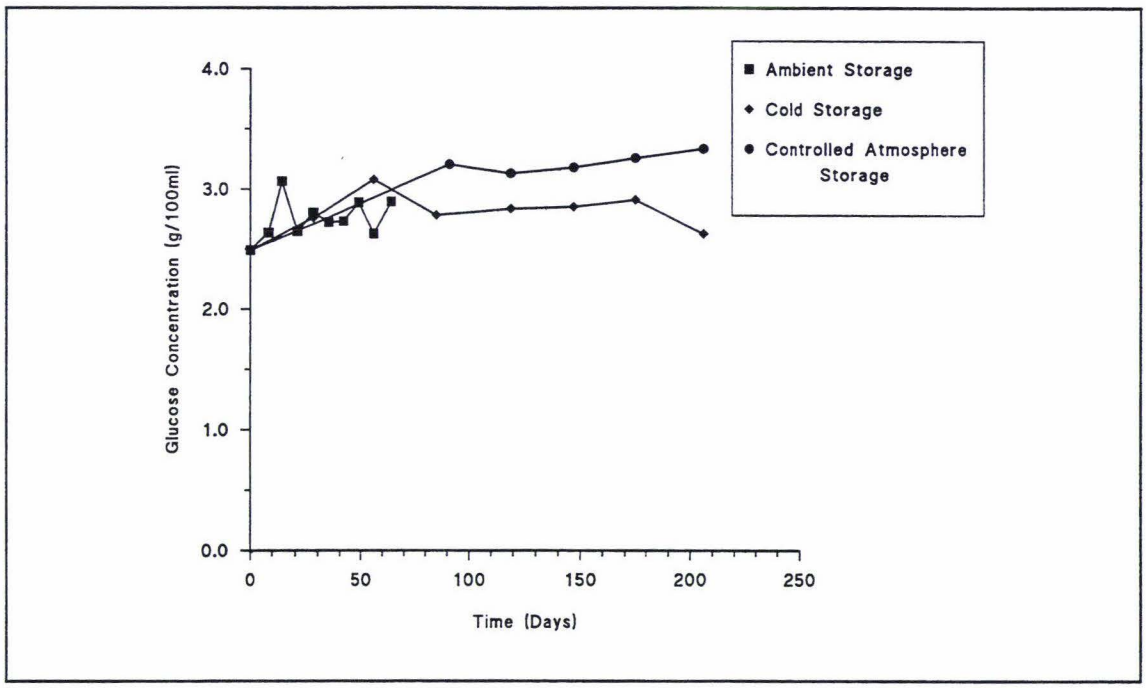

Figure 4.15: Effect on juice glucose concentrations of different storage regimes for Granny Smith apples in 1993.

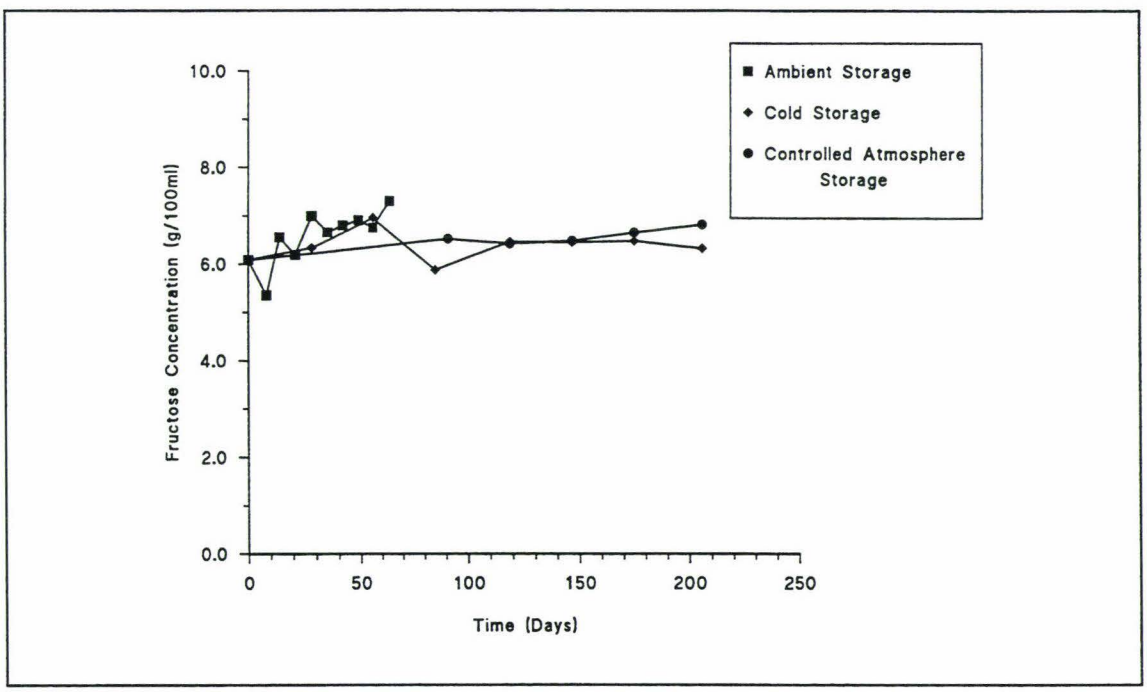

Figure 4.16: Effect on juice fructose concentrations of different storage regimes for Granny Smith apples in 1993.

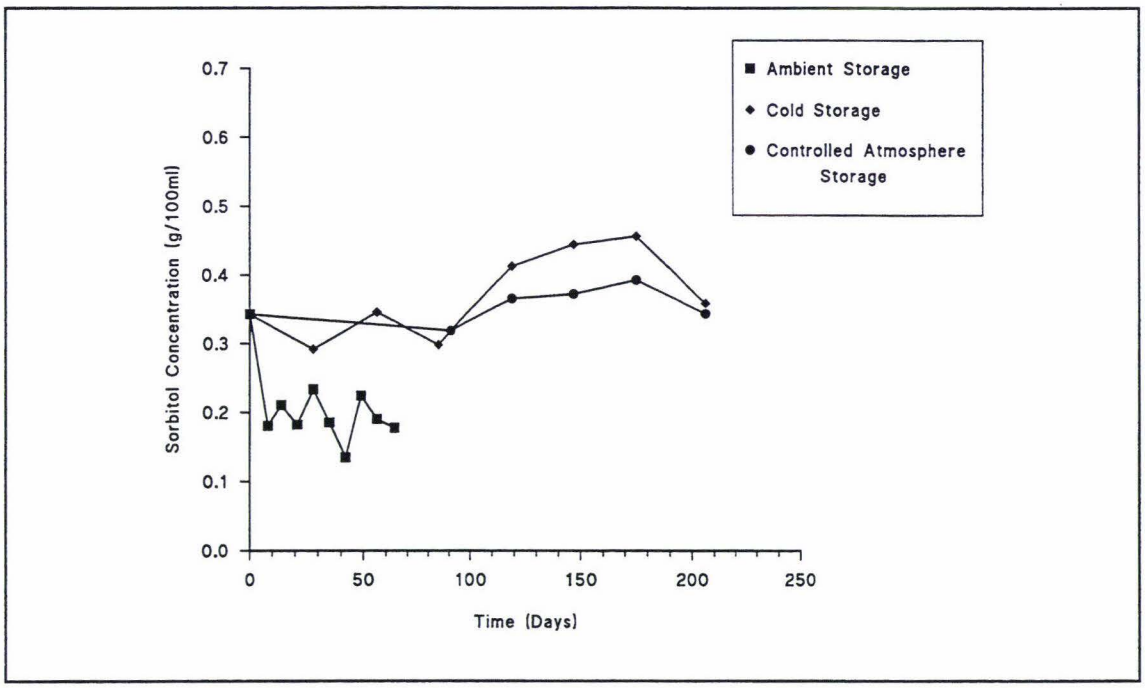

Figure 4.17: Effect on juice sorbitol concentrations of different storage regimes for Granny Smith apples in 1993.

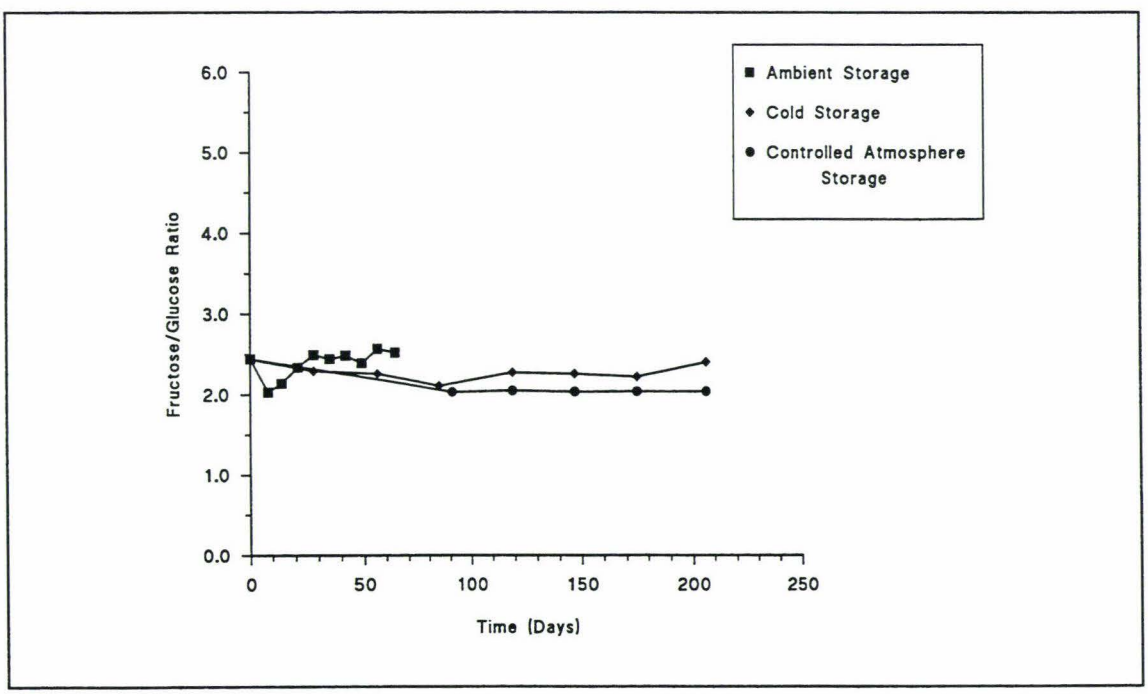

Figure 4.18: Effect on juice fructose/glucose ratios of different storage regimes for Granny Smith apples in 1993.

4.4 Cox's Orange

Only first, second and third pick fruit from Nelson and Hawke's Bay in 1992 and second pick from Hawke's Bay in 1993 were analysed with the mean and standard deviation for each of the individual sugars, total soluble solids and fructose/glucose ratio presented in figures 4.1 to 4.6 and appendix 23-table A23.2.

It was found that the total soluble solids for Cox's Orange juice ranged from 9.0°Brix to 12.7°Brix. This is similar to values reported in the literature (figure 2.1) and other cultivars analysed in this study (figure 4.1). However a third pick sample from Nelson in 1993 reached 15.3°Brix which was the highest level of all cultivars analysed. While this level may seem high it is still less than the maximum total soluble solids of 16.1g/100ml reported by Ayres and Fallows (1951) for Cox's Orange and less than the maximum of 16.9g/100ml reported by Mattick and Moyer (1983), although the cultivar that this occurred in is not stated.

Fructose was present between 4.7g/100ml and 6.4g/100ml (mean 5.6g/100ml with a standard deviation of ±0.5g/100ml) and accounted for between 42.7% and 57.4% of the total sugars. These results are similar to that which was found with other cultivars analysed (figure 4.4) and commonly reported in the literature (figure 2.4). Only one sample (a first pick sample from Hawke's Bay) had fructose present at a level less than the Brause and Raterman (1982) minimum value of 5g/100ml, with 42% of the samples having fructose present at a level less than the minimum value of 5.5g/100ml proposed by the German RSK. No samples exceeded the German RSK maximum value of 8g/100ml.

Cox's Orange apple juice was observed to have the lowest glucose level of all cultivars analysed (figure 4.3). The mean glucose level for Cox's Orange apple juice was 0.8g/100ml (standard deviation ±0.2g/100ml) with a range of 0.5g/100ml to 1.1g/100ml typically being observed. This corresponded to less than 8.6% of the total sugars. At this level, glucose was less than the minimum value suggested by the German RSK.

The fructose/glucose ratio ranged from 5.4 to 9.0 which was always in excess of the Brause and Raterman (1982) minimum value, and exceeded the maximum German RSK limit of 3.3. The low glucose content of Cox's Orange apple juice has an influence on the fructose/ glucose ratio and causes the large ratio.

The sucrose level observed in Cox's Orange apple juice presented interesting and somewhat unexpected results.

A mean sucrose level of 4.9g/100ml (standard deviation of ±1.4) was observed with a range of 3.0g/100ml to 7.5g/100ml (figure 4.2). At these levels sucrose accounted for between 36.2% and 50.0% of the total sugars. The lowest sucrose level observed occurred in fruit harvested at the start of the season (1st pick) and by the start of the commercial export pick (2nd pick) the sucrose level had increased to over 4.8g/100ml and accounted for up to 42% of the total sugars. By the time the fruit was fully tree ripened, the sucrose content was generally in excess of 5.0g/100ml. This trend occurred in fruit harvested from both Nelson and Hawke's Bay in 1992, and even though only one sample was analysed in 1993 (second pick from Hawke's Bay), it to was observed to have sucrose present in excess of 5.0g/100ml.

One sample of fully tree ripened fruit was observed to have sucrose present at 7.5g/100ml, although this sample had a total soluble solids of 15.3°Brix. In this sample sucrose accounted for 50% of the total sugars a little above the range of 36% to 45% that which was observed for other Cox's Orange samples. Ayres and Fallows (1951) report the sucrose level for Cox's Orange apple juice from fruit harvested over 4 years and found that sucrose was present at levels between 3.2g/100ml and 6.0g/100ml with only 23% of the samples having sucrose present at less than 3.0g/100ml. In a study of New Zealand cultivars Withy *et al.* (1978) found the highest sucrose occurred in Cox's Orange with levels of 3.9g/100g to 4.1g/100g fresh weight. All other cultivars that they examined had sucrose present at less than 3.2g/100g fresh weight.

The amount of sucrose present in New Zealand grown Cox's Orange was consistently equal

to or greater than the maximum levels reported in the literature. The highest sucrose level reported in the literature was 7.0g/100ml by Ayres and Fallows (1951) for the cultivar Brownlee Russet with Mattick and Moyer (1983), Fuleki *et al.* (1994), Wrolstad and Shallenberger (1981), Lee and Wrolstad (1988a) and Ryan (1972) reporting maximum levels of 5.6g/100ml, 4.8g/100ml, 3.8g/100ml, 3.0g/100ml and 2.4g/100ml respectively although the cultivars involved are not given.

Even at the early stage of the season (1st pick) the sucrose level in Cox's Orange apple juice exceeded the German RSK maximum value but were within the limits proposed by Brause and Raterman (1982). The second and third pick fruit not only exceeded the maximum limit suggested by Brause and Raterman (1982), but also exceeded the 95% confidence limit of 4.7g/100ml for Mattick and Moyer (1983) database (Lee and Wrolstad, 1988b). Even when sucrose is presented as a percentage of the total sugars it generally exceeded the 95% confidence limit of 42.3% proposed by Lee and Wrolstad (1988b) for Mattick and Moyer (1983) database.

Sorbitol levels were not unusual with values ranging from 0.3 to 0.6g/100ml, although one sample reached 1.4g/100ml. This sample of Cox's Orange also had the highest total soluble solids, sucrose, glucose and fructose levels.

The use of Cox's orange in the production of single varietal apple juice or apple juice concentrate or in blended juices (depending on the quantities of Cox's Orange added) will almost certainly result in the sucrose levels exceeding and glucose levels below the commonly used criteria of Brause and Raterman (1982), German RSK, and Lee and Wrolstad (1988b) for authentic juice resulting in the juices being rejected as adulterated with sugar. However the high levels of sucrose and low glucose levels appear to be a characteristic of this cultivar.

4.5 Gala

The total soluble solids of Gala apple juice generally ranged from 8.3°Brix to 12.4°Brix

although a 3rd pick sample from Canterbury reached 14.0°Brix (figure 4.1, appendix 21).

The mean fructose level was 6.9g/100ml (range 5.4g/100ml to 8.6g/100ml) and accounted for 64% of the total sugars (appendix 21), which was similar to those which were proposed by Brause and Raterman (1982) and the German RSK values for authentic juice. Glucose was present at levels of 0.8g/100ml to 1.9g/100ml (mean 1.3g/100ml) and was below German RSK minimum value (figures 4.3 and 4.4). Glucose accounted for less than 16% of the total sugars present and was lower than that which was reported in the literature by Wrolstad and Shallenberger (1981). The low glucose level caused the fructose/glucose ratio to be elevated with the ratio ranging from 4.4 to 9.2. This is in excess of the German RSK maximum value of 3.3.

Sucrose levels ranged from 1g/100ml to 5g/100ml (mean 2.8g/100ml) with the lowest level observed in a sample of first pick fruit from the Nelson region and the maximum level occurring in a sample of fully tree ripened fruit grown in Canterbury (figure 4.2). Sucrose generally accounted for less than 30% of the total sugars Of Gala apple juice samples analysed 32% were observed to have sucrose content greater than the German RSK maximum value, and these samples were generally second and third pick fruits grown in Hawke's Bay, Nelson and Canterbury and fruit at the start of storage. No samples had sucrose present at levels less than the German RSK minimum of 0.5g/100ml. Only 13% (3 samples) of the Gala apple samples had sucrose present in excess of Brause and Raterman (1982) maximum value with one sample (third pick from Canterbury) exceeding the 95% confidence limit for Mattick and Moyer (1983) data (Lee and Wrolstad (1988b). These samples were generally fully tree ripened fruit.

Gala is a result of a cross between Kidds Orange Red (Cox's Orange Pippin x Delicious) and Golden Delicious and originated in New Zealand in 1934 (Plotto *et al.*, 1995). The levels of sucrose seen may be due to the Cox's Orange parentage.

Like other cultivars analysed the sucrose level decreased during storage, with an increase in fructose and glucose being observed (appendix 26). A the start of storage sucrose was

present at 3.1g/100ml and after the fruit was stored for 7 days at ambient and 37 days at cold storage conditions the level decreased to less than 3.0g/100ml. By the end of storage, that is, 40 days at ambient and 119 days at cold storage sucrose had decreased to 1.9g/100ml and 2.0g/100ml respectively.

During storage glucose increased from 1.4g/100ml at the start of storage to between 1.6g/100ml and 1.9g/100ml by the end. Fructose also increased during storage from 6.5g/100ml start and reached levels of 7.3g/100ml to 8.6g/100ml by the end. Glucose was below the German RSK minimum value in all samples except for fruit that was stored under controlled atmosphere conditions for longer than 80 days. During storage fructose had reached levels that were in excess of the German RSK maximum but were within the limits proposed by Brause and Raterman (1982).

Sorbitol generally ranged from 0.16 to 0.58g/100ml which are within the German RSK limits of 0.2g/100ml to 0.7g/100ml (figure 4.5).

4.6 Royal Gala

Royal Gala apple juice had levels of 1.3g/100ml to 5.7g/100ml, 0.7g/100ml to 1.8g/100ml and 5.4g/100ml to 7.8g/100ml for sucrose, glucose and fructose respectively, with the total soluble solids ranging from 8.7°Brix to 13.5°Brix (figures 4.1 to 4.6, appendix 22).

Fructose was present at concentrations similar to that observed in other cultivars and to those which have been reported in the literature and accounted for between 50% to 70% of the total sugars. Glucose accounted for 6% to 17% of the total sugars which like Gala apple juice was lower than that which was reported in the literature by Wrolstad and Shallenberger (1981).

As was seen in other cultivars that had high sucrose, the glucose level of Royal Gala apple juice was less than the minimum German RSK values, leading to a fructose/glucose ratio of 4.1 to 8.5 that exceeded the German RSK maximum value.

The highest sucrose levels for Royal Gala of 4.7g/100ml to 5.7g/100ml occurred in the juice of fully tree ripened fruit grown in Nelson in 1992 and 1993 and in Canterbury in 1993. These samples also exceeded the 95% confidence level for Mattick and Moyer (1983) database (Lee and Wrolstad, 1988b). While the sucrose level (3.6g/100ml to 3.7g/100ml) for fruit harvested at a similar maturity in Hawke's Bay was lower, Brause and Raterman (1982) maximum was still exceeded.

The levels of individual sugars seen in Royal Gala was similar to that which was observed for Gala apple. This is not totally unexpected as Royal Gala is a descendant of Gala.

When the data is expressed as a percentage of the total sugars only first pick fruit and fruit that were stored under controlled atmosphere conditions were observed to have sucrose accounting for less than 30% of the total sugars, with values of 19 to 26% and 12 to 18% respectively. In all other samples sucrose accounted for 30% to 43% of the total sugars.

Sorbitol levels ranged from 0.2 to 0.6g/100ml with no discernable trends being apparent, although fully tree ripened fruit usually had the highest levels of sorbitol.

Based on the German RSK and Brause and Raterman (1982) criteria for sucrose, between 44% and 50% of Royal Gala apple juice would be deemed as adulterated with sucrose because they exceed the proposed maximum limits with 16% of the samples exceed the 95% confidence limit for Mattick and Moyer (1983) database (Lee and Wrolstad, 1988b). No samples were observed to have sucrose present at levels below the German RSK suggested minimum limits.

4.7 Golden Delicious

Juice from Golden Delicious apples had total soluble solids from 9.0°Brix to 13.4°Brix. The individual make up of sugars consisted of sucrose; 1.1g/100ml to 3.9g/100ml, glucose; 1.3g/100ml to 1.6g/100ml and fructose 6.1g/100ml to 8.3g/100ml (figures 4.1 to 4.6, appendix 23-table A23.3).

Fructose was present at levels similar to those which were observed in other cultivars and to that which was reported in the literature (appendix 1).

In contrast glucose was lower than commonly reported values and was always below the minimum proposed the German RSK for authentic apple juice. This in turn affects the fructose/glucose ratio which exceeded the maximum suggested by German RSK with a range of between 4.3 to 5.6 being observed.

Sucrose was present at levels similar to glucose in fruit harvested at the start of the season (1st pick), but during ripening on the tree (2nd and 3rd pick) sucrose was accumulated in greater quantities and was usually present at twice the level of glucose. Glucose and fructose remained relatively unchanged during this time. While the levels of sucrose increased during ripening, they were usually below the maximum value proposed by Brause and Raterman (1982). Only one sample, third pick from Nelson, exceeded their maximum value, but this sample did not exceed the 95% confidence limit of the Mattick and Moyer (1983) database (Lee and Wrolstad, 1988b).

4.8 Fuji and Red Delicious

Fuji and Red Delicious apple juice were observed to have total soluble solids ranging from 10.4°Brix to 13.5°Brix and 9.0°Brix to 13.9°Brix respectively (figures 4.1 to 4.6, appendix 23-tables A23.4 and A23.1).

Fructose levels in both cultivars ranged from 5.4g/100ml to 8.0g/100ml and was similar to those of the other cultivars analysed. These levels are also within the limits suggested by Brause and Raterman (1982) and German RSK.

The juice from Red Delicious apples had glucose present at between 1.4g/100ml to 2.7g/100ml which was similar to the range of 1.0g/100ml to 3.1g/100ml observed for sucrose. However the glucose content of Fuji apples was observed to be at twice that of sucrose, with glucose typically ranging from 2.4g/100ml to 4.3g/100ml and sucrose from

1.3g/100ml to 2.8g/100ml.

The fructose/glucose ratio for Red Delicious and Fuji always exceeded the minimum value of 1.6 suggested by Brause and Raterman (1982) and was below the maximum of 3.3 given by the German RSK.

Juice from both Red Delicious and Fuji apples would be classed as authentic using the criteria of both Brause and Raterman (1982) and the German RSK as fructose, glucose, sucrose and the fructose/glucose ratio are all within their suggested limits.

Sorbitol levels for both Red Delicious and Fuji were within the limits of 0.2 to 0.7g/100ml proposed by the German RSK, however one sample of fully tree ripened Fuji reached 1.0g/100ml.

4.9 Hillwell, GS330, GS2850 and Fiesta

The sugar profile of four new cultivars (Hillwell, GS330, GS2850 and Fiesta) were determined although only one sample of each cultivar was analysed. The total soluble solids for these cultivars ranged from 11.6°Brix to 13.5°Brix, which were similar to other cultivars examined (figure 4.1-4.6, appendix 23-table 23.5).

The sucrose present in these cultivars was between 3.7g/100ml to 5.0g/100ml. At these levels both Brause and Raterman (1982) and the German RSK maximum values are exceeded, with GS330 exceeding the 95% confidence limit of the Mattick and Moyer (1983) database (Lee and Wrolstad, 1988b).

As with other cultivars with high sucrose, these cultivars had low glucose. Typical glucose levels were between 0.8g/100ml and 1.6g/100ml which were less than the German RSK minimum value.

Fructose was present at 4.8g/100ml to 7.5g/100ml which was similar to other samples

examined. The low glucose cause the fructose/glucose ratio to be elevated, and in these cultivars the ratio always exceeded the German RSK maximum value.

Sorbitol was present at levels of 0.21g/100ml to 0.74g/100ml.

4.10 Statistical Analysis of Data

4.10.1 Sample Variation

The results for the determination of sucrose, glucose, fructose, sorbitol and °Brix for the juice of Braeburn apples collected on a single day in 1993 show small variations as can be seen from the data in table 4.1 and appendix 19-table A19.4.

The coefficient of variation for the results of °Brix was 6.3% while those for sucrose, glucose and fructose ranged from 9.7% to 15.2%. Sucrose, glucose and fructose were observed to have standard deviations from 0.3g/100ml to 0.6g/100ml which were slightly less than the value of 0.7 seen for °Brix. The larger coefficients of variation seen for the sucrose, glucose and fructose is probably due to the lower individual sugars present in the juice (table 4.1). Sorbitol was present at much lower levels (0.16g/100ml to 0.40g/100ml) than the other sugars, resulting in small peaks which were difficult to integrate causing the large coefficient of variation.

Table 4.1: Minimum, maximum, mean, standard deviation and coefficient of variation in the individual sugar concentrations of Braeburn apple juices sampled on the same day from Hawke's Bay in 1993.

	Sucrose (g/100ml)	Glucose (g/100ml)	Fructose (g/100ml)	Sorbitol (g/100ml)	Fruc/gluc Ratio
Mean	2.88	1.61	5.90	0.26	10.49
Standard Deviation	0.45	0.29	0.57	0.06	0.66
Minimum	1.48	1.19	4.75	0.16	8.6
Maximum	3.53	2.32	6.84	0.40	11.4
Number of Samples	21	21	21	21	21
Coefficient of Variation (%)	15.58	18.27	9.70	21.40	6.33

Table 4.1: Continued

	Sucrose as Percentage of Total Sugars	Glucose as Percentage of Total Sugars	Fructose as Percentage of Total Sugars	Sorbitol as Percentage of Total Sugars + Sorbitol	°Brix	Total Sugars (g/100ml)
Mean	27.60	15.61	56.79	2.41	10.49	10.34
Standard Deviation	3.16	3.21	2.61	0.36	0.66	0.87
Minimum	17.75	11.69	52.30	1.88	8.6	8.32
Maximum	33.66	25.19	61.65	3.32	11.4	11.76
Number of Samples	21	21	21	21	21	21
Coefficient of Variation (%)	11.44	20.59	4.60	14.86	6.33	8.40

4.10.2 Cultivar

Braeburn was found to have sucrose levels that were significantly higher than Fuji, Gala, Red Delicious, Golden Delicious, Granny Smith and Royal Gala and significantly lower than

Cox's Orange (table 4.2). There was no significant difference in the sucrose level of Gala, Golden Delicious, Granny Smith and Red Delicious or Red Delicious, Golden Delicious and Fuji.

While the sucrose levels of Cox's Orange was significantly higher than other cultivars, the glucose level was significantly lower. Whereas Fuji apple juice had glucose levels that were significantly higher than any other cultivars. Fuji also had the lowest sucrose level. Gala and Royal Gala had glucose levels that were significantly lower than Braeburn, Fuji, Golden Delicious, Granny Smith and Red Delicious. There was no significant difference in glucose levels for Braeburn, Gala and Golden Delicious.

The fructose levels of Cox's Orange and Granny Smith were not significantly different, but these cultivars were significantly lower than other cultivars examined. Braeburn, Fuji, Golden Delicious and Red Delicious were observed to have no significant difference in fructose levels.

Golden Delicious had the lowest sorbitol level with the level being significantly lower than Cox's Orange, Fuji, Red Delicious and not significantly different from Braeburn, Gala, Granny Smith and Royal Gala. Cox's Orange had the highest mean sorbitol level and was not significantly different to Fuji but these cultivars were significantly higher than the other cultivars examined.

The Brix level of Braeburn apple juice was significantly higher than Granny Smith, Royal Gala and Gala but not significantly different to Cox's Orange, Fuji, Golden Delicious and Red Delicious. There was no significant difference in the Brix level of Royal Gala, Red Delicious, Granny Smith, Golden Delicious, Gala and Cox's Orange.

Cox's Orange had the highest fructose/glucose ratio and was not significantly different to Royal Gala, but these cultivars were significantly higher than the other cultivars examined. Braeburn was significantly higher than Granny Smith and not significantly different to Golden Delicious. Fuji was observed to have the lowest mean fructose/glucose ratio and

was significantly lower than the other cultivars, except Granny Smith which was not significantly different.

Table 4.2: Least-squared means (lsmeans) of individual sugars and related components in the juice of eight apple cultivars.

Cultivar (S.E.)	Number of Samples	Sucrose (g/100ml)	Glucose (g/100ml)	Fructose (g/100ml)	Sorbitol (g/100ml)	°Brix	Fruc/Gluc Ratio
Braeburn	64	3.94 c[1] (0.16)	1.50 c (0.08)	6.22 b (0.14)	0.35 ad (0.02)	11.79 c (0.23)	4.43 c (0.16)
Cox's Orange	7	4.73 d (0.35)	0.77 a (0.17)	5.47 a (0.31)	0.57 e (0.05)	11.49 ac (0.51)	7.24 e (0.34)
Fuji	9	1.50 a (0.30)	3.25 d (0.15)	6.94 c (0.27)	0.50 de (0.04)	11.78 bc (0.44)	2.03 a (0.29)
Gala	18	2.51 b (0.23)	1.26 cb (0.11)	6.84 c (0.21)	0.27 b (0.03)	10.89 ab (0.33)	5.79 d (0.22)
Golden Delicious	6	2.28 ab (0.38)	1.49 c (0.19)	7.24 c (0.34)	0.25 ab (0.06)	10.84 abc (0.55)	4.83 c (0.37)
Granny Smith	57	2.26 b (0.14)	2.49 e (0.07)	5.93 ab (0.13)	0.35 ad (0.02)	11.17 ab (0.21)	2.64 ab (0.14)
Red Delicious	7	1.79 ab (0.35)	2.10 f (0.17)	6.54 bc (0.31)	0.41 dc (0.05)	10.87 abc (0.51)	3.22 b (0.34)
Royal Gala	17	3.12 e (0.22)	0.97 ab (0.11)	6.21 b (0.20)	0.31 abc (0.03)	10.67 a (0.32)	6.64 e (0.21)

[1] Means within the same column followed by the same letter are not significantly different ($P \leq 0.05$), S.E. = Standard Error of lsmeans

4.10.3 Region

The glucose and fructose levels of Hawke's Bay grown fruit were significantly higher than that of fruit from Canterbury and Nelson regions while the fructose/glucose ratio was significantly lower. Fruit from Canterbury and Nelson were observed to have no significant difference in glucose and fructose levels or in the fructose/glucose ratio (table 4.3).

There was no significant difference in sucrose, sorbitol and °Brix for the three apple growing regions.

Table 4.3: Least-squared means of individual sugars and related components in the juice of apple cultivars grown in three regions of New Zealand.

Region (S.E.)	Number of Samples	Sucrose (g/100ml)	Glucose (g/100ml)	Fructose (g/100ml)	Sorbitol (g/100ml)	°Brix	Fruc/Gluc Ratio
Canterbury	15	2.96 a[1] (0.25)	1.54 a (0.12)	6.26 a (0.22)	0.41a (0.04)	11.04 a (0.36)	4.85 a (0.24)
Hawke's Bay	140	2.50 a (0.12)	1.94 b (0.06)	6.74 b (0.11)	0.35 a (0.02)	11.35 a (0.17)	4.23 b (0.12)
Nelson	30	2.84 a (0.17)	1.71 a (0.09)	6.28 a (0.16)	0.37 a (0.03)	11.17 a (0.25)	4.73 a (0.107)

[1] Means within the same column followed by the same letter are not significantly different ($P \leq 0.05$), S.E. = Standard Error of lsmeans

4.10.4 Year

Sucrose and sorbitol levels, °Brix and fructose/glucose ratio in 1992 were significantly higher ($p \leq 0.05$) than that which was observed 1993, while glucose was significantly lower. These difference could be due the warmer summer of 1991-1992 that was experienced. (Anon 1991b, 1992, 1993) There was no significant difference in fructose levels between the two years (table 4.4).

Table 4.4: Least-squared means of individual sugars and related components in the juice of apple cultivars sampled over two growing seasons.

Year (S.E.)	Number of Samples	Sucrose (g/100ml)	Glucose (g/100ml)	Fructose (g/100ml)	Sorbitol (g/100ml)	°Brix	Fruc/Gluc Ratio
1992	98	3.13 a[1] (0.12)	1.63 a (0.06)	6.47 a (0.11)	0.41 a (0.02)	11.59 a (0.17)	4.86 a (0.12)
1993	87	2.40 b (0.16)	1.83 b (0.08)	6.38 a (0.14)	0.34 b (0.02)	10.79 b (0.23)	4.34 b (0.23)

[1] Means within the same column followed by the same letter are not significantly different ($P \leq 0.05$) S.E. = Standard Error of lsmeans

4.10.5 Stage of Harvest

A three way ANOVA was performed and found that there was a significant difference between cultivar, region and year. However when a four way ANOVA (cultivar, region, year and time of picking) was carried out with data that was available for all variables, the stage of picking was significant while year became non significant.

Third pick fruit were observed to have °Brix levels that were significantly higher and significantly lower than second pick fruit (table 4.5). The sucrose and fructose levels of first pick fruit was significantly lower than second and third pick fruit, while there was no significant difference between second and third picks.

There was no significant difference in sorbitol level between first and second or second and third pick fruit but third pick fruit was significantly higher than first pick fruit. The precision for the determination of sorbitol was variable (recoveries from 82 to 109%) and may mask some of the differences.

There were no significant difference in glucose levels or the fructose/glucose ratio.

Table 4.5: Least-squared means of individual sugars and related components in the juice of apple cultivars harvested at different maturities.

Stage of Maturity (S.E.)	Number of Samples	Sucrose (g/100ml)	Glucose (g/100ml)	Fructose (g/100ml)	Sorbitol (g/100ml)	°Brix	Fruc/Gluc Ratio
1st Pick	26	1.83 a[1] (0.16)	1.50 a (0.07)	5.69 a (0.11)	0.32 a (0.03)	9.66 a (0.19)	4.71 a (0.20)
2nd Pick	29	3.10 b (0.15)	1.65 a (0.06)	6.45 b (0.10)	0.38 ac (0.03)	11.35 b (0.17)	4.81 a (0.19)
3rd Pick	23	3.59 b (0.17)	1.76 a (0.07)	6.82 b (0.12)	0.48 bc (0.03)	12.46 c (0.20)	4.78 a (0.22)

[1] Means within the same column followed by the same letter are not significantly different ($P \leq 0.05$), S.E. = Standard Error of lsmeans

4.11 Conclusion

The analytical composition of apple and apple juice has been extensively studied (Ayres and Fallows, 1951; Brause and Raterman, 1982; Burroughs, 1984; Caldwell, 1928; Fuleki *et al.*, 1994; Lee and Wrolstad, 1988a; Mattick and Moyer, 1983; Ryan, 1972) in order to develop standards of quality and authenticity. Probably the greatest activity in this area has been in Germany and information in terms of standard values and ranges of variations (RSK values) for apple juice have been published by Bielig *et al.* (1982). They propose that authentic apple juice would contain total soluble solids of between 11.68g/100ml to 14.81g/100ml; sucrose 0.5g/100ml to 3.0 g/100ml; glucose 1.8g/100ml to 3.5g/100ml; fructose 5.5g/100ml to 8.0g/100ml; sorbitol 0.2g/100ml to 0.7g/100ml and a fructose /glucose ratio of between 2 to 3.3.

When a composite analysis of all samples of New Zealand apple juice is carried out the mean, standard deviation, maximum and minimum for total soluble solids, sucrose, glucose, fructose, sorbitol and fructose/glucose ratio are similar to that which is given in Burroughs (1984), Fuleki *et al.* (1994), Lee and Wrolstad (1988a), Mattick and Moyer (1983), Ryan (1972) databases. On average New Zealand composite apple juice fall within the proposed limits of Brause and Raterman (1982) and the German RSK for authentic juice.

Total soluble solids and fructose levels were similar for all cultivars throughout the study. Typical values for total soluble solids were from 8.3 °Brix for first pick Gala from Nelson to 15.3 °Brix for a third pick Cox's Orange from Hawke's Bay, with fructose ranging from 4.0g/100ml for a first pick Granny Smith sample from Canterbury to 8.6g/100ml for Gala fruit that had been stored under controlled atmosphere conditions for 119 days.

While the Brix level for each cultivar was similar, the individual sugars, especially sucrose and glucose were found to be different. For example, first pick Cox's Orange from Hawke's Bay were observed to have the sucrose present at 3.0g/100ml, glucose at 0.5g/100ml, fructose at 4.7g/100ml and a total soluble solids of 9.0 °Brix. First pick Red Delicious were observed to have the same Brix level, but sucrose was present at 1.0g/100ml, glucose at

1.8g/100ml and fructose at 5.4g/100ml.

Sucrose ranged from a low of 1.0g/100ml in first pick Red Delicious to 7.5g/100ml in third pick Cox's Orange with glucose ranging from 0.5g/100ml in first pick Cox's Orange to 4.2g/100ml in third pick Fuji. Sorbitol was present at levels between 0.13g/100ml and 1.40g/100ml while the fructose/glucose ratio always exceeded the minium value of 1.6 proposed by German RSK, with a maximum of 9.1 seen in a sample of first pick Gala from Canterbury in 1993.

Importantly, when each cultivar is examined individually, it was found that the composition of some cultivars exceeded the limits for the individual components proposed by Brause and Raterman (1982) and the German RSK values for authentic juice, and by their criteria would be considered adulterated. As most apple juice concentrate is produced from single cultivars this could lead to individual batches having compositions which may imply fraudulent practices had been used during production.

The most notable of these were Cox's Orange where the sucrose reached a high of 7.5g/100ml, and was consistently above 5.0g/100ml for second and third pick fruit. At these levels sucrose not only exceeded Brause and Raterman (1982) and the German RSK maximum limit, but also the 95% confidence level of 4.7g/100ml for Mattick and Moyer (1983) results (Lee and Wrolstad, 1988b) and almost certainly any juice produced from Cox Orange would be rejected as adulterated with sucrose. As Cox's Orange is becoming more commonly grown in New Zealand its use in juice production will increase and the high sucrose levels will be of major concern to the juice processing industry when the product is tested by regulatory authorities for authenticity.

Other techniques such as stable isotope analysis may be necessary to prove authenticity but this technique has limitations. Stable isotope analysis may show that high fructose corn syrup or cane sugar has not be added but cannot show that the addition of beet sugar has not occurred.

In contrast the glucose level for Cox's Orange was present up to a maximum of 1.1g/100ml which is below the minimum value proposed by the German RSK. Brause and Raterman (1982) do not give a minimum level. Naturally if one index was not met then others may also not be met since glucose, fructose and sucrose have levels that are not totally independent.

Braeburn fruit were also observed to have sucrose level that exceeded the maximum limits proposed by Brause and Raterman (1982) and the German RSK. The sucrose level of all Braeburn apple juices examined always exceeded Brause and Raterman (1982) maximum value of 3.5g/100ml in 1992, with sucrose levels in excess of 4.0g/100ml. The 95% confidence limit of 4.7g/100ml for Mattick and Moyer (1983) database (Lee and Wrolstad, 1988b) was exceeded by 12% of the Braeburn juice samples. In 1993 only 24% of Braeburn apple juices had sucrose levels that exceeded Brause and Raterman (1982) maximum value of 3.5g/100ml with one sample exceeding Lee and Wrolstad (1988b) 95% confidence limit. The German RSK maximum value of 3.0g/100ml was exceeded by all Braeburn juices in 1992 and by 55% of samples in 1993. Commercially this is particularly important because of the low cost of sucrose and thus the incentive for addition is high.

The glucose level of all Braeburn juices was below the maximum value of 3.5g/100ml proposed by Brause and Raterman (1982) and the German RSK, with 60% of the samples falling below the minimum value of 1.8g/100ml suggested by the German RSK values. However the level of glucose was within the range which was observed by Fuleki *et al.* (1994) and Mattick and Moyer (1983).

Gala and Royal Gala fruit exceeded the maximum RSK value for sucrose in 32% and 50% of the samples respectively. However only 13% of the Gala samples and 44% of the Royal Gala samples exceeded Brause and Raterman (1982) maximum level. One sample of Gala and four of Royal Gala exceeded the 95% confidence level for Mattick and Moyer (1983) database (Lee and Wrolstad (1988b)), and these samples were usually fully tree ripened.

Like that which was seen for Braeburn and Cox's Orange, Gala and Royal Gala fruit usually

had glucose levels below the German RSK minimum value of 1.8g/100ml.

Hillwell, GS330, GS2850 and Fiesta are recently developed cultivars and even though only one sample of each was analysed, all indications are that they too will be high sucrose producing cultivars. Levels of sucrose seen in these cultivars were between 3.7g/100ml and 5.0g/100ml, thereby exceeding Brause and Raterman (1982) maximum limit. The glucose level of these cultivars followed earlier trends and was below the minimum German RSK value with levels of 0.8g/100ml to 1.6g/100ml seen.

There is no evidence available as to why some cultivars have high levels of sucrose and low glucose and further work in this area is neccessary. It is possible that the enzymes involved in the synthesis and metabolism of sucrose and glucose are highly active or are being suppressed.

Golden Delicious, Granny Smith, Fuji and Red Delicious generally had sucrose present at levels which were within the German RSK and Brause and Raterman (1982) proposed limits. However while the glucose level for Granny Smith and Red Delicious were within the RSK standard limits, Golden Delicious had glucose present below the German RSK minimum value. Fuji was seen to have the highest glucose level of all cultivars and was consistently above 3.0g/100ml and even exceeded Brause and Raterman (1982) and the RSK maximum value of 3.5g/100ml with levels of up to 4.3g/100ml seen.

The fructose level of all juice examined generally fell within the limits proposed by Brause and Raterman (1982) and the German RSK values.

Wrolstad and Shallenberger (1981) established that apple has a high fructose/glucose ratio of well over 2.00 (mean 2.7) while Brause and Raterman (1982) decided that a minimum ratio of 1.6 was possible. The results found for New Zealand apple juice always exceeded their minimum value and had a range of 1.6 to 9.2 with a mean of 4.0. The German RSK give a range of 2.0 to 3.3 for the fructose/glucose ratio and 55% of all samples analysed fell outside this range.

Granny Smith and Braeburn fruit are commonly stored under ambient, cold and controlled atmosphere conditions for juice production at a later date and the changes in individual sugars were monitored during the storage period. As the fruit was stored sucrose levels decreased while glucose and fructose increased. Through out the storage periods the individual sugars for Granny Smith were within the limits suggested by Brause and Raterman (1982) and the German RSK.

However, with Braeburn apple juice the sucrose level did not fall below Brause and Raterman (1982) maximum level until the fruit had been stored for longer than 45 days at ambient, 149 days at cold or 195 days at controlled atmosphere conditions. Glucose was below the RSK minimum value and it was not until the fruit had been stored for longer than 8 days at ambient, 45 days at cold or 51 days at controlled atmosphere conditions that levels were within the German RSK standard range. Fructose over the storage period showed slight increases but was within the proposed limits.

Since there are multiple modes of adulteration, i.e. addition of sugar to cover the Brix, alternate juices, beet sugar, minerals and natural flavourings, multiple components must be looked at to establish authenticity. Based on Brause and Raterman (1982) criteria for sucrose, glucose, fructose and fructose/glucose ratio Granny Smith, Fuji, Red Delicious and Golden Delicious would be classified as authentic as they rarely exceed their proposed limits. The situation for Braeburn, Gala, Royal Gala and Cox's Orange is not so clear cut. While these cultivars had glucose and fructose levels and fructose/glucose ratios within Brause and Raterman (1982) limits they frequently exceeded the proposed maximum of 3.5g/100ml for sucrose. Therefore using Brause and Raterman (1982) criteria it is possible to wrongly reject these cultivars as adulterated with sucrose.

The German RSK system gives information in terms of standard values as well as ranges of variation for a number of components found in apple juice. Using their criteria for sucrose, glucose, fructose, sorbitol and fructose/glucose ratio only Granny Smith and Red Delicious would be considered authentic as each component usually fell within their proposed ranges. Fuji had glucose present at levels of up to 4.3g/100ml, which exceeded criteria for authentic

apple juice. Cox's Orange, Braeburn, Gala and Royal Gala generally fell outside their limits for at least two components (glucose and sucrose) and the juices from these cultivars could be classified as not authentic. However the two components are not totally independent. and when sucrose was high glucose was low.

CHAPTER 5
RESULTS AND DISCUSSION
ORGANIC ACID COMPOSITION

5.1 Total Acids, Titratable Acidity and pH

Acidity of juice is characterised by pH, titratable acidity and total acids (Ulrich,1970) and as well as sugar influences the flavour and whether or not the juice is acceptable to the consumer.

The pH of the juices examined ranged from 3.16 to 4.15 with a mean of 3.47 (figure 5.1) which was similar to that which has been reported elsewhere (Lee and Wrolstad, 1988b). The pH of Cox's Orange and Granny Smith were significantly lower than other cultivars with Red Delicious having the highest pH, which was not significantly different to Gala and Royal Gala (table 5.1).

The mean titratable acidity, calculated as malic acid, was 520mg/100ml with a range of 210 to 1130mg/100ml (figure 5.2, appendix 27) and was similar to the results reported by Lee and Wrolstad, 1988a, 1988b; Ryan, 1972; Withy *et al.*, 1978. Ayres and Fallows (1951) have reported a mean titratable acidity of 780mg/100ml which is higher than reports elsewhere, but they included data from high acid "cider" apple cultivars.

Of the cultivars examined it was found that Cox's Orange and Granny Smith had the highest mean titratable acidity of 884 and 636mg/100ml respectively, with Fuji and Red Delicious having the lowest mean titratable acidity of 287 and 247mg/100ml respectively (figure 5.2, appendices 28 to 32). The total acids (quinic + malic + shikimic + citric + succinic + fumaric) ranged from 304mg/100ml to 1208mg/100ml (figure 5.3) and was similar to that which was observed with titratable acidity. The titratable acidity and total acids of Cox's Orange were significantly higher than the other cultivars examined, while titratable acidity and total acids of Red Delicious were significantly lower than other cultivars except Gala and Fuji which were not significantly different (table 5.2).

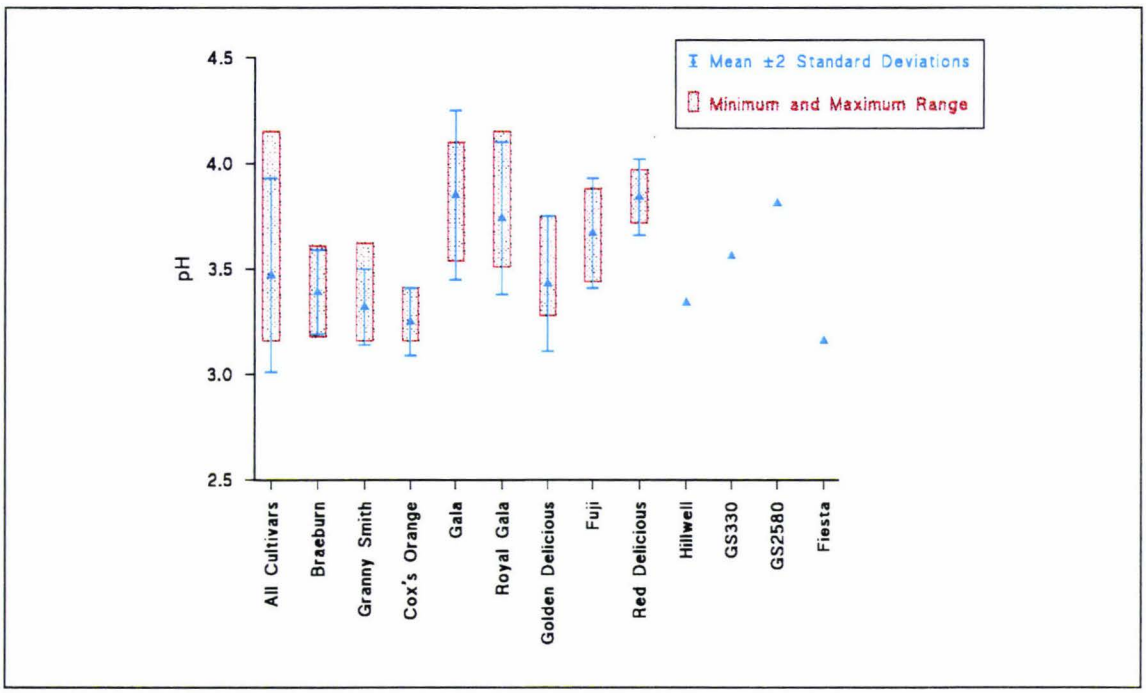

Figure 5.1: pH levels for New Zealand varietal apple juices.

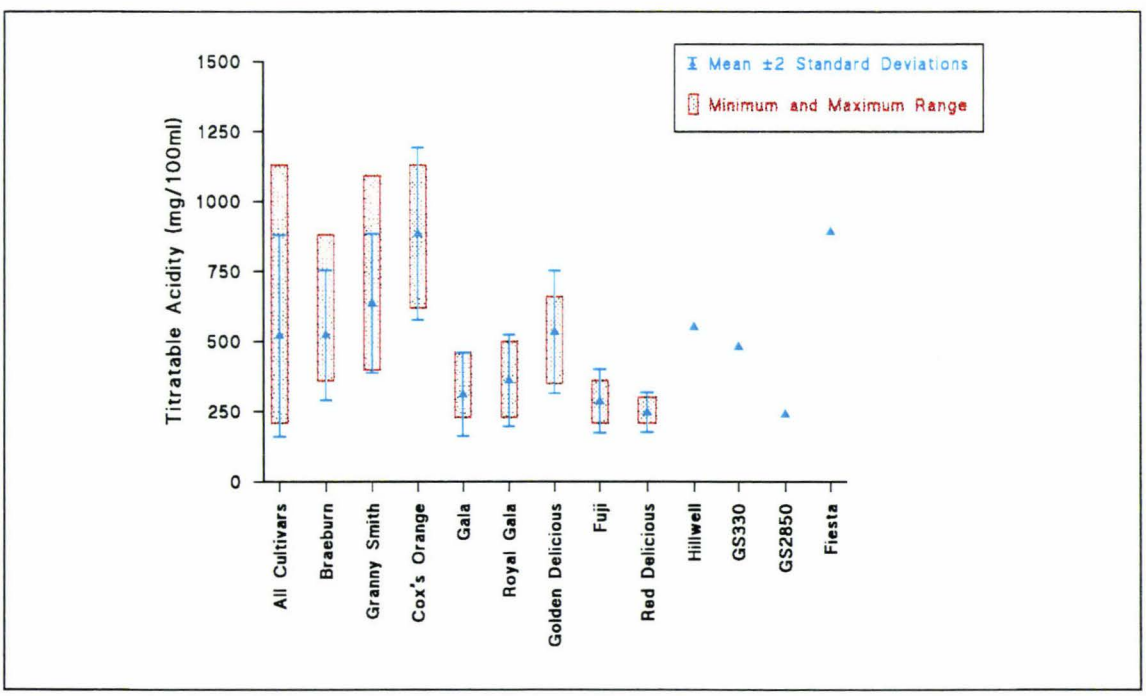

Figure 5.2: Titratable acidity concentrations for New Zealand varietal apple juices.

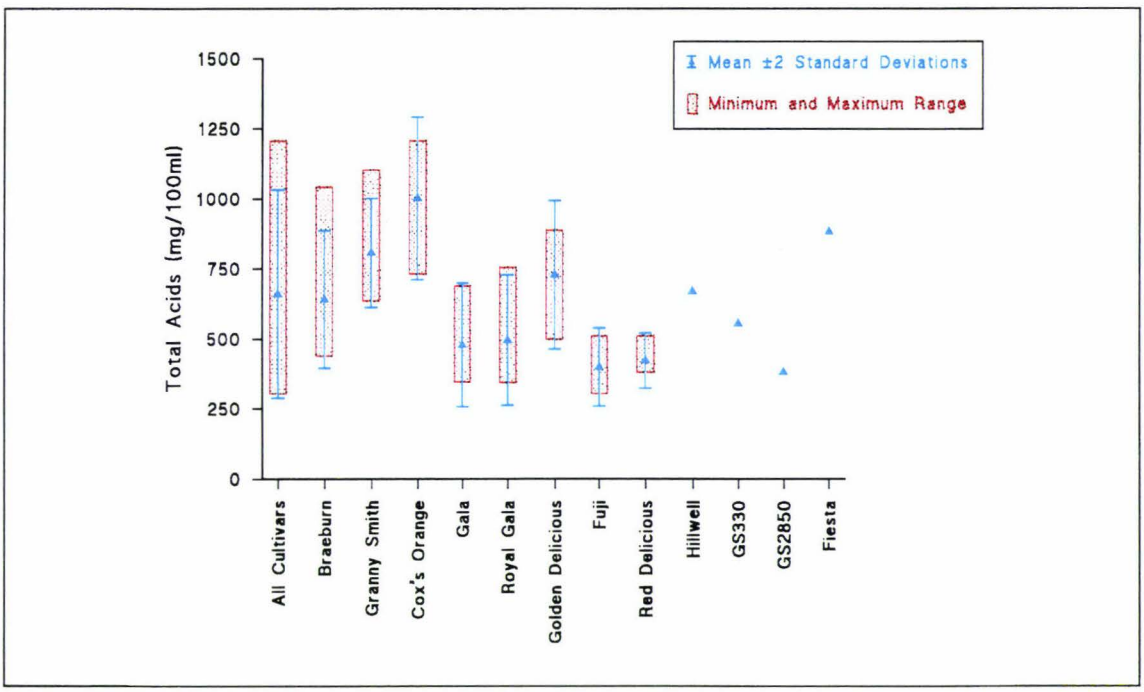

Figure 5.3: Total acid concentrations for New Zealand varietal apple juices.

The total acids and titratable acidity of Braeburn were not significantly different to Golden Delicious, but were significantly lower than Granny Smith and Cox's Orange, with pH being significantly higher in Granny Smith and not significantly different to Golden Delicious. Since titratable acidity measures only the free carboxylic groups while HPLC will account for the free as well as the bound acids (acid salts, salts), the values for titratable acidity will be lower than total acids (Bollard, 1970; Fuleki *et al.*, 1995). A regression equation (equation 2) was established for the calculation of the total acid content in apple juice from titratable acidity measurements.

$$\text{Total acids (mg/100ml)} = \text{titratable acidity} \times 0.973 + 158.23 \qquad (2)$$

In juice of fruit harvested from Hawke's Bay the titratable acidity was significantly lower and pH significantly higher than those from Nelson or Canterbury. The total acids of juice produced from Hawke's Bay was significantly lower than that from Nelson but there was no significant difference between Hawke's Bay and Canterbury or Canterbury and Nelson (table 5.2). There was no significant difference in titratable acidity and total acids between 1992 and 1993 seasons, but pH was significantly higher in 1992 (table 5.3).

Of the samples that could be statistically analysed by picks, it was found that first pick samples had total acids that were significantly higher than second and third picks, with no significant difference between second and third picks. The titratable acidity and pH of first pick samples were significantly higher and lower respectively than third pick, with no significant difference between first and second or second and third picks (table 5.4).

At the start of ambient, cold and controlled atmosphere storage in 1992 and 1993 the juice of Braeburn fruit were observed to have total acid of 770mg/100ml (appendix 28-tables A28.1 and A28.2 and appendix 33) and 753mg/100ml (figure 5.4) respectively. By the end of storage the total acids had decreased to levels of between 439mg/100ml and 545mg/100ml.

The total acid observed at the start of storage for Granny Smith in 1992 was 753mg/100ml

decreasing to between 636 and 725mg/100ml by the end of storage (appendices 29-table A29.1 and appendix 34). Although the level of total acid at the start of storage was 912mg/100ml in 1993 (appendix 29-table A29.1), by the end of storage similar levels of acids were present as that which was observed in 1992 (figure 5.5).

The titratable acidity for Braeburn and Granny Smith apple juices were observed to have similar trends and levels as total acid (figures 5.6 and 5.7)

The pH increased from between 3.20 and 3.36 at the start of storage to between 3.53 and 3.61 at the end of storage for Braeburn apple (figure 5.8), with the pH for Granny Smith apple increasing from between 3.16 and 3.27 at the start of storage to between 3.38 and 3.62 by the end (figure 5.9).

Table 5.1: Least-squared means (lsmeans) for pH, total acids and titratable acidity in the juice of eight apple cultivars.

Cultivar (S.E.)	Number of Samples	pH	Total Acids (mg/100ml)	Titratable Acidity (mg/100ml)
Braeburn	64	3.35 d[1] (0.02)	677.3 c (19.8)	576.9 c (19.1)
Cox's Orange	7	3.22 a (0.05)	1015.1 d (43.6)	907.9 e (41.9)
Fuji	9	3.65 c (0.04)	405.0 a (37.6)	292.6 ab (36.2)
Gala	18	3.80 b (0.03)	506.0 b (28.5)	349.4 ab (27.4)
Golden Delicious	6	3.39 de (0.05)	738.1 c (47.3)	551.6 c (45.4)
Granny Smith	57	3.29 ae (0.02)	836.1 e (17.8)	678.3 d (17.1)
Red Delicious	6	3..81 b (0.05)	429.6 ab (46.8)	262.4 a (45.0)
Royal Gala	17	3.72 bc (0.03)	501.9 b (27.3)	372.2 b (26.3)

[1] Means within the same column followed by the same letter are not significantly different ($P \leq 0.05$), S.E. = Standard Error of lsmeans

Table 5.2: Least-squared means for pH, total acids and titratable acidity in the juice of apple cultivars grown in three regions of New Zealand.

Region (S.E.)	Number of Samples	pH	Total Acids (mg/100ml)	Titratable Acidity (mg/100ml)
Canterbury	15	3.51 a[1] (0.03)	643.5 ab (31.0)	520.3 b (29.8)
Hawke's Bay	139	3.58 b (0.02)	596.2 a (15.1)	435.8 a (14.5)
Nelson	30	3.49 a (0.02)	676.2 b (21.5)	540.6 b (20.6)

[1] Means within the same column followed by the same letter are not significantly different ($P \leq 0.05$), S.E. = Standard Error of lsmeans

Table 5.3: Least-squared means for pH, total acids and titratable acidity in the juice of apple cultivars sampled over two growing seasons.

Year (S.E.)	Number of Samples	pH	Total Acids (mg/100ml)	Titratable Acidity (mg/100ml)
1992	97	3.56 b[1] (0.02)	631.8 a (15.1)	491.3 a (14.5)
1993	87	3.50 a (0.02)	645.5 a (19.7)	506.6 a (18.9)

[1] Means within the same column followed by the same letter are not significantly different ($P \leq 0.05$) S.E. = Standard Error of lsmeans

Table 5.4: Least-squared means for pH, total acids and titratable acidity in the juice of apple cultivars harvested at different maturities.

Stage of Maturity (S.E.)	Number of Samples	pH	Total Acids (mg/100ml)	Titratable Acidity (mg/100ml)
1st Pick	26	3.42 a[1] (0.05)	778.1 b (43.7)	613.5 a (45.5)
2nd Pick	29	3.51 ab (0.04)	638.1 a (41.4)	495.2 ab (43.0)
3rd Pick	22	3.63 b (0.05)	578.6 a (47.5)	450.9 b (49.4)

[1] Means within the same column followed by the same letter are not significantly different ($P \leq 0.05$), S.E. = Standard Error of lsmeans

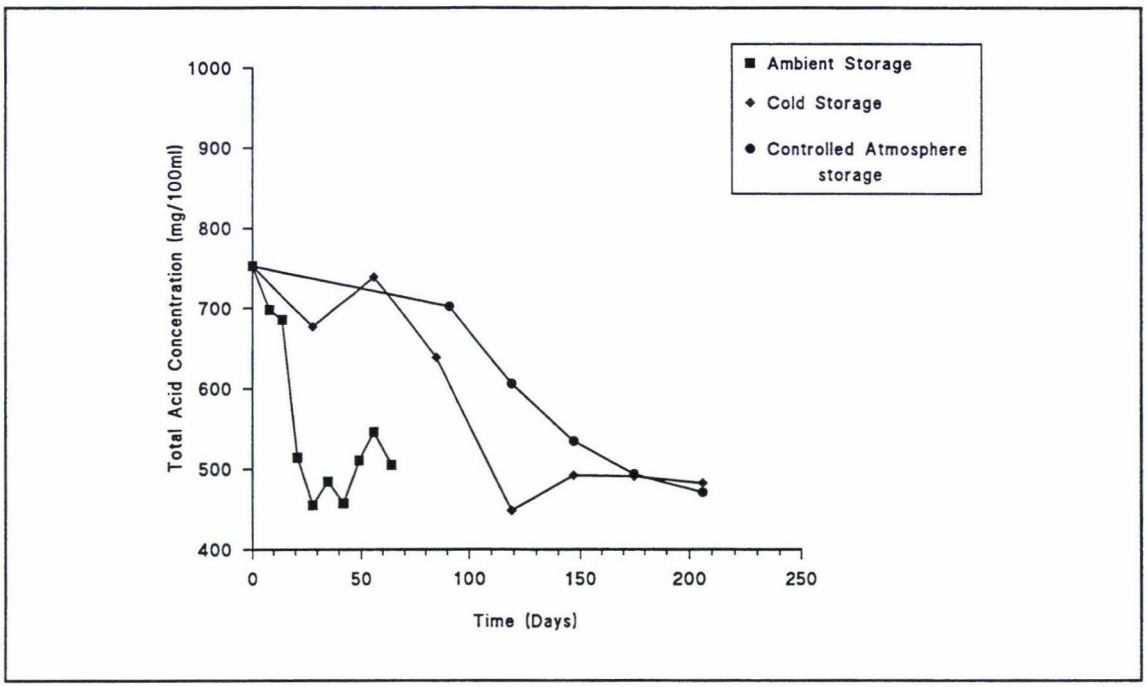

Figure 5.4: Effect on juice total acid concentrations of different storage regimes for Braeburn apples in 1993.

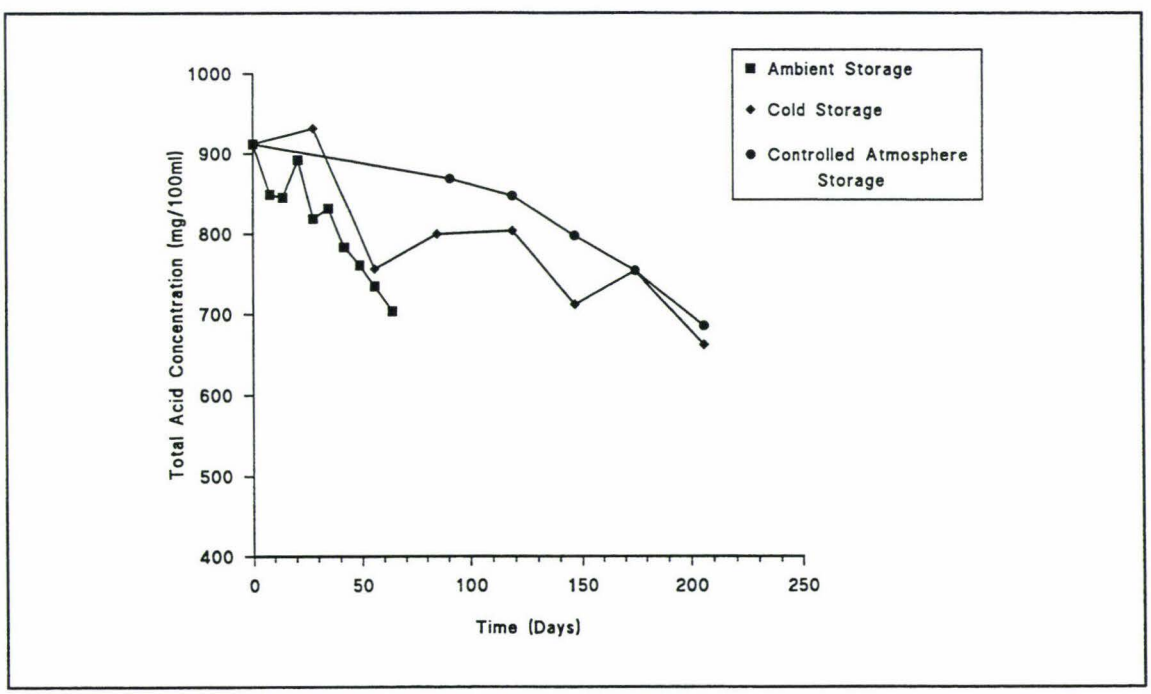

Figure 5.5: Effect on juice total acid concentrations of different storage regimes for Granny Smith apples in 1993.

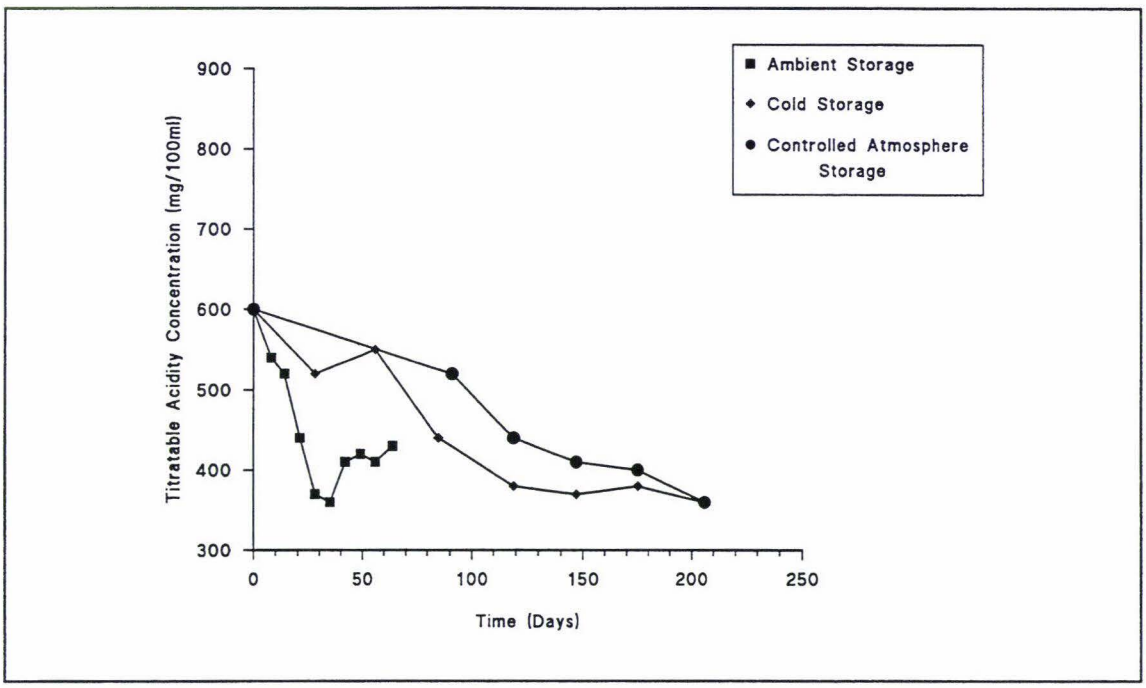

Figure 5.6: Effect on juice titratable acidity concentrations of different storage regimes for Braeburn apples in 1993.

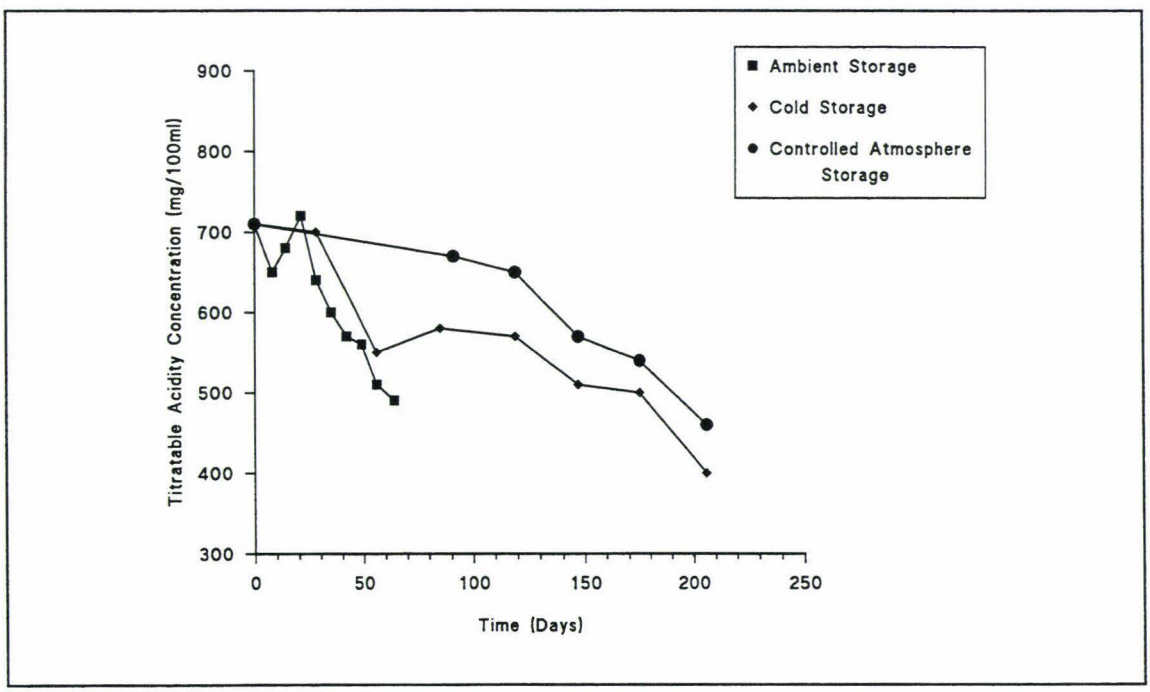

Figure 5.7: Effect on juice titratable acidity concentrations of different storage regimes for Granny Smith apples in 1993.

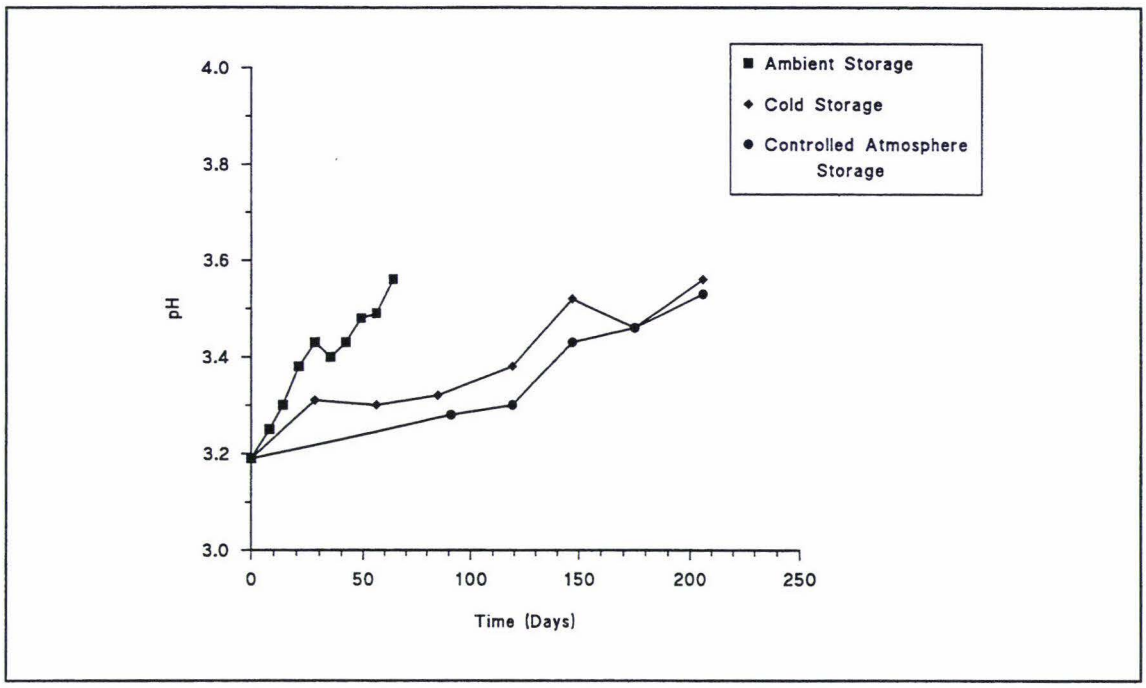

Figure 5.8: Effect on juice pH of different storage regimes for Braeburn apples in 1993.

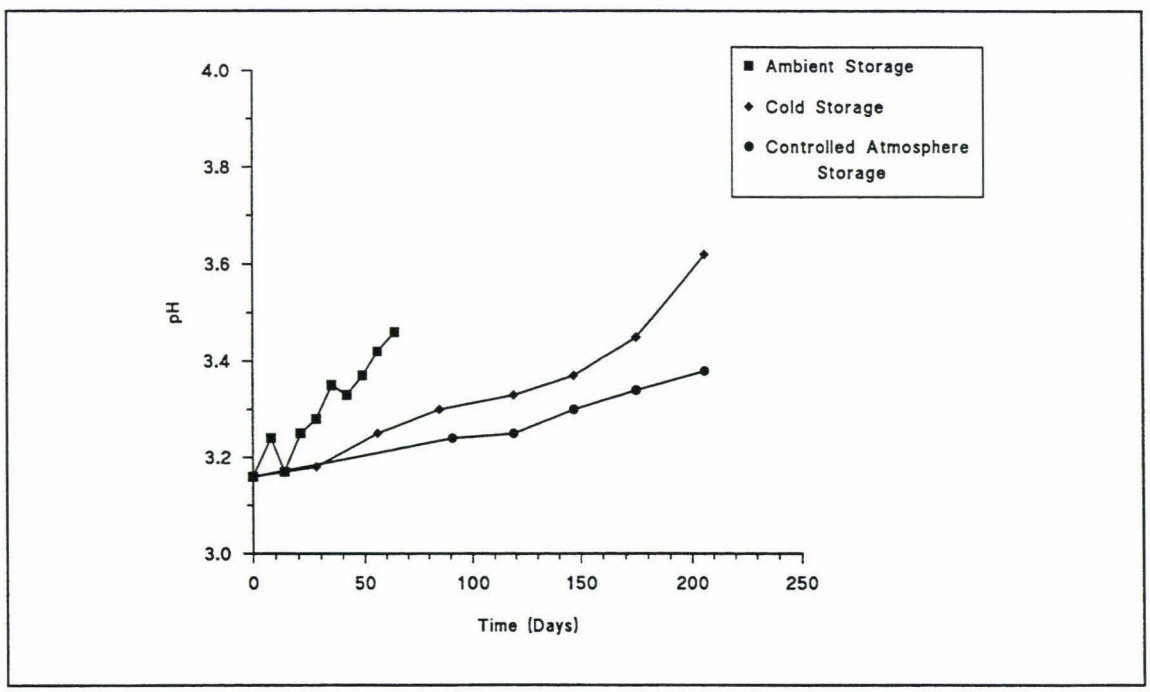

Figure 5.9: Effect on juice pH of different storage regimes for Granny Smith apples in 1993.

5.2 Malic Acid

Malic acid was found at levels between 231 and 1067mg/100ml (figure 5.10) and in all cultivars, except Red Delicious, accounted for 84 to 91% of the total acids (appendices 28 to 32). In Red Delicious malic acid accounted for 71% of the total acids (appendix 32-table A32.1). The low malic acid (mean 302mg/100ml) seen in Red Delicious was observed in fruit collected from both Hawke's Bay and Nelson. Although the malic acid level in Red Delicious was lower than other cultivars examined, malic acid as a percentage of the total acids for all cultivars was similar to those reported by Lee and Wrolstad (1988b). They report that malic acid comprises 71 to 94% of the total acids.

The actual concentrations of malic acid observed showed varietial differences. The highest mean malic acid levels of 906mg/100ml occurred in Cox's Orange with Granny Smith, Golden Delicious, Braeburn, Royal Gala, and Gala Fuji and Red Delicious observed to have the levels of 739, 661, 580, 422, 408, 338 and 302mg/100ml respectively (figure 5.10). The malic acid level of Cox's Orange was significantly higher than all other cultivars, with Red Delicious being significantly lower than the rest except for that which was seen in Fuji. Braeburn and Golden Delicious were not significantly different, but were significantly lower than Granny Smith and higher than Royal Gala, Gala and Fuji (table 5.5).

The juices from Nelson fruit were significantly higher in malic acid content than samples from Hawke's Bay, but were not significantly different to Canterbury. There was no significant difference in malic acid content in samples between Canterbury and Hawke's Bay (table 5.6).

There was no significant difference in malic acid levels between the two years fruit were harvested or between first and second picks or second and third picks, but first pick samples were significantly higher than third pick samples (tables 5.7 and 5.8).

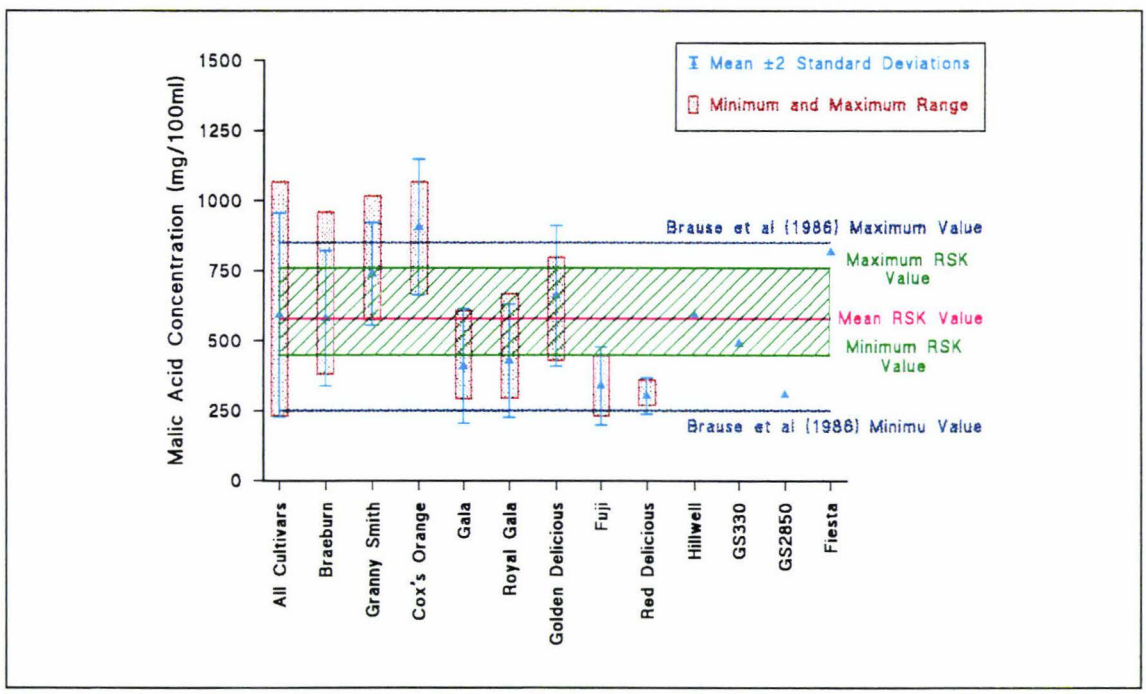

Figure 5.10: Comparison of malic acid concentrations for New Zealand varietal apple juices with the RSK and Brause *et al.* (1986) values.

Table 5.5: Least-squared means (lsmeans) for malic, quinic and succinic acids in the juice of eight apple cultivars.

Cultivar (S.E.)	Number of Samples	Malic Acid (mg/100ml)	Quinic Acid (mg/100ml)	Succinic Acid (mg/100ml)
Braeburn	64	611.6 c[1] (18.8)	37.7 a (1.9)	18.4 ac (1.0)
Cox's Orange	7	914.4 d (41.4)	51.9 c (4.1)	34.1 d (2.2)
Fuji	9	341.8 a (35.7)	41.6 abc (3.6)	15.6 a (1.9)
Gala	18	431.1 b (27.0)	44.1 bc (2.7)	20.5 cb (1.4)
Golden Delicious	6	665.4 c (44.9)	40.0 ab (4.5)	24.4 b (2.3)
Granny Smith	57	763.2 e (16.9)	43.2 b (1.7)	21.3 b (0.9)
Red Delicious	6	305.3 a (44.4)	99.1 d (4.4)	18.5 ab (2.3)
Royal Gala	17	432.7 b (25.9)	41.5 ab (2.6)	19.3 ab (1.4)

[1] Means within the same column followed by the same letter are not significantly different ($P \leq 0.05$), S.E. = Standard Error of lsmeans

Table 5.6: Least-squared means for malic, quinic and succinic acids in the juice of apple cultivars grown in three regions of New Zealand.

Region (S.E.)	Number of Samples	Malic acid (mg/100ml)	Quinic Acid (mg/100ml)	Succinic Acid (mg/100ml)
Canterbury	15	558.9 ab[1] (29.4)	52.3 b (2.9)	23.3 b (1.5)
Hawke's Bay	139	521.7 a (14.3)	45.3 a (1.4)	19.8 a (0.7)
Nelson	30	593.9 b (20.4)	52.1 b (2.0)	21.4 ab (1.1)

[1] Means within the same column followed by the same letter are not significantly different ($P \leq 0.05$), S.E. = Standard Error of lsmeans

Table 5.7: Least-squared means for malic, quinic and succinic acids in the juice of apple cultivars sampled over two growing seasons.

Year (S.E.)	Number of Samples	Malic Acid (mg/100ml)	Quinic Acid (mg/100ml)	Succinic Acid (mg/100ml)
1992	97	553.7 a[1] (14.3)	46.3 a (1.4)	22.8 b (0.7)
1993	87	562.7 a (18.6)	53.4 b (1.9)	20.1 a (1.0)

[1] Means within the same column followed by the same letter are not significantly different ($P \leq 0.05$) S.E. = Standard Error of lsmeans

Table 5.8: Least-squared means for malic, quinic and succinic acids in the juice of apple cultivars harvested at different maturities.

Stage of Maturity (S.E.)	Number of Samples	Malic Acid (mg/100ml)	Quinic Acid (mg/100ml)	Succinic Acid (mg/100ml)
1st Pick	26	685.1 a[1] (43.1)	62.2 b (3.5)	21.6 a (1.2)
2nd Pick	29	569.4 ab (40.8)	40.0 a (3.3)	20.7 a (1.1)
3rd Pick	22	508.0 b (46.9)	36.7 a (3.8)	25.5 b (1.3)

[1] Means within the same column followed by the same letter are not significantly different ($P \leq 0.05$), S.E. = Standard Error of lsmeans

Besides sugars, malic acid, the dominant acid in apples, is the main substrate for respiration in apples via the TCA cycle (Hulme and Rhodes, 1971; Ulrich, 1970; Wills *et al.*,1989), and the decrease in malic acid content during storage has also been observed by Hulme (1958), Hulme and Wooltorton (1957), Krotkov *et al.* (1951) and Ulrich (1970).

At the start of storage, malic acid levels of between 713 and 691mg/100ml were observed for Braeburn apples. During storage in 1993 the malic acid level decreased and by the end of storage levels of between 382 and 451mg/100ml were observed (figure 5.11). This was a decrease of between 36 to 46% (table 5.9). Malic acid levels for Granny Smith apple also decreased during storage (figure 5.12) and at the start of storage in 1993 levels of 849mg/100ml were seen. By the end of the storage period, the malic acid had decreased by

24 to 32% to reach levels of 575 to 640mg/100ml (table 5.9)

The RSK values proposed by Bielig *et al.*, (1982) did not give limits for malic acid, but did state that "the quanity of l-malic acid which can be determined enzymatically is higher than the numeric value of the titratable total acid expressed as tartaric acid. If the content of l-malic acid (enzymatic determination) is below the value as to titratable total acid (expressed as tartaric acid) then it has to be analysed whether other fruit acids have been added." However Wrolstad (1985) state RSK values for malic acid of 450 to 760mg/100ml (mean 580mg/100ml). Intially Brause and Raterman (1982) did not include limits for malic acid in their criteria for authentic apple juice, but later they concluded (Brause *et al.*, 1986) that authentic apple juice would contain between 250 and 850mg/100ml l-malic acid.

Except for the juice from Cox's Orange and first pick Granny Smith all other New Zealand apple juice examined generally had malic acid levels within this proposed range. First pick Granny Smith fruit from the three regions of New Zealand studied were observed to have malic acid present at levels in excess of 850mg/100ml. As the fruit ripened the malic acid levels decreased and the juice from second and third pick fruit were within Brause *et al.*, (1986) limits for authentic juice. However the juice from first, second and third pick Cox's Orange fruit were always in excess of the proposed limits for authentic juice.

Table 5.9: The percentage decrease of malic acid in the juice or stored of Braeburn and Granny Smith apples.

Sample	Braeburn			Granny Smith		
	Start of Storage (mg/100ml)	End of Storage (mg/100ml)	% Decrease	Start of Storage (mg/100ml)	End of Storage (mg/100ml)	% Decrease
Ambient 1992	713.8	451.9	36.7	698.5	666.3	4.6
Cold 1992	713.8	382.3	46.4	698.5	622.0	10.9
Controlled Atmosphere 1992	713.8	411.3	42.4	698.5	589.2	15.6
Ambient 1993	691.2	442.6	36.0	849.5	640.4	24.6
Cold 1993	691.2	403.5	41.6	849.5	575.5	32.3
Controlled Atmosphere 1993	691.2	408.1	41.0	849.5	622.9	26.7

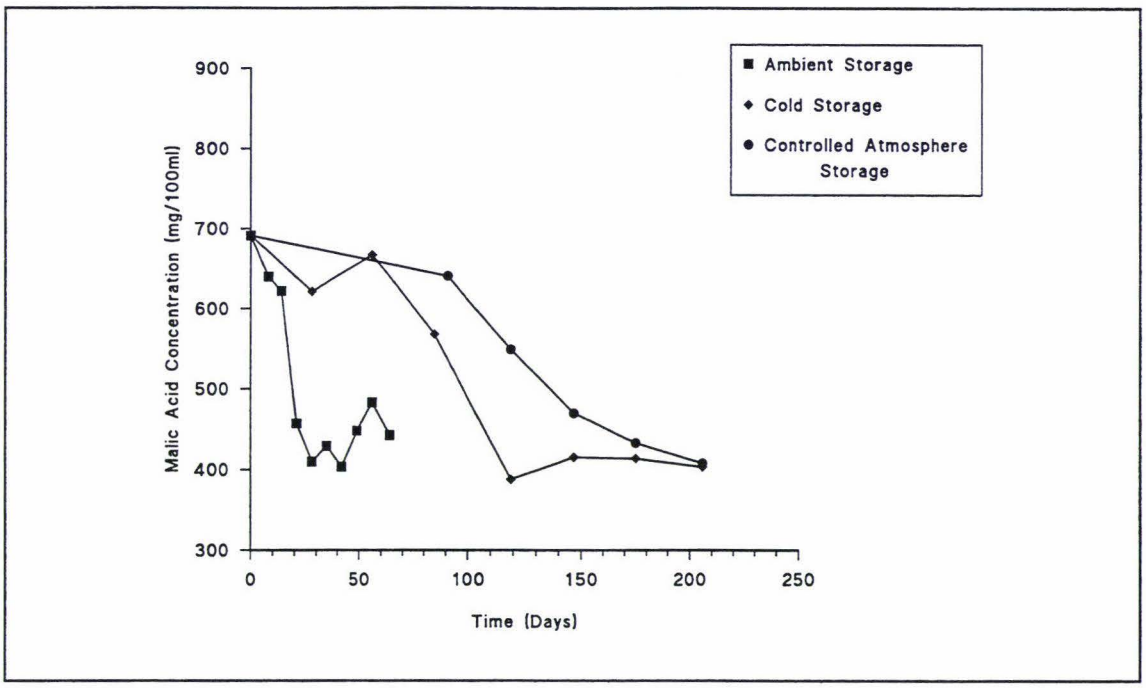

Figure 5.11: Effect on juice malic acid concentrations of different storage regimes for Braeburn apples in 1993.

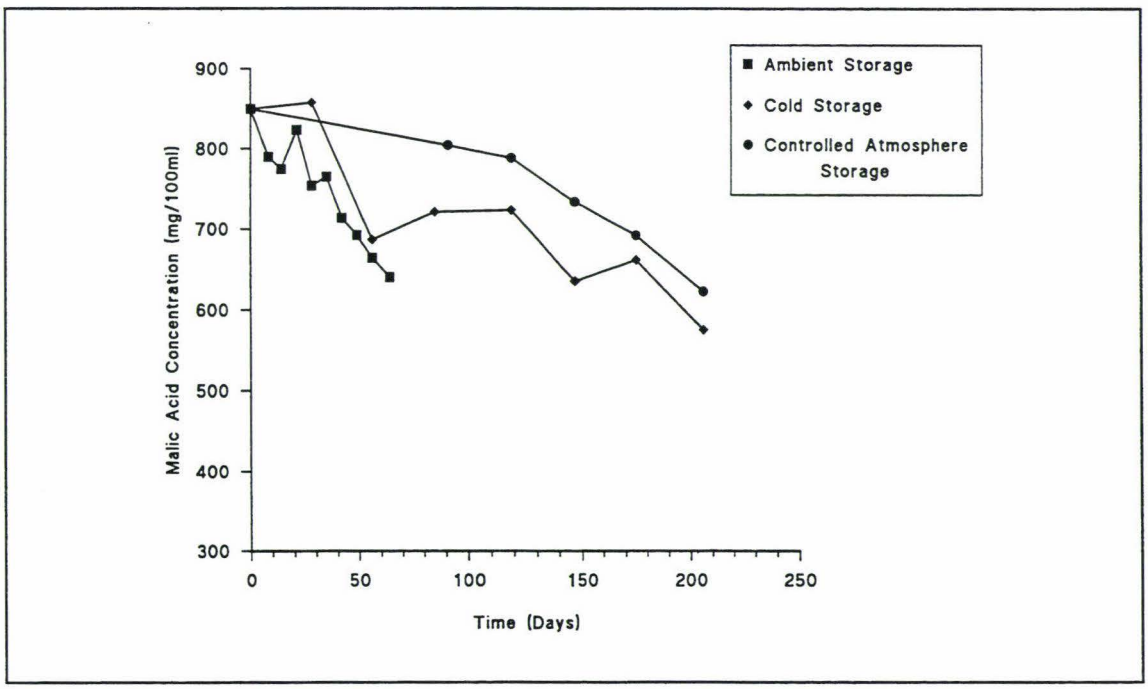

Figure 5.12: Effect on juice malic acid concentrations of different storage regimes for Granny Smith apples in 1993.

5.3 Quinic Acid

The mean level of quinic acid for most cultivars ranged from 35mg/100ml (for Braeburn) to 48mg/100ml (for Cox's Orange) and accounted for between 2.9 and 10% of the total acid (figure 5.13). Red Delicious was an exception to this, with a mean level of 95.5mg/100ml accounting for 22.5% of the total acid present. At this level it was similar to the 100mg/100ml, (23% of the total acid) reported by Coppola and Starr (1986), Lee and Wrolstad, (1988b), Ryan (1972) and Wrolstad *et al.*, (1981) but not as unusual as the 199 to 370mg/100ml (38% of the total acid) reported by Lee and Wrolstad, (1988a, 1988b).

Hulme (1958) reported that in immature fruits quinic acid levels were greater than malic acid and while no very early fruit was examined, it was observed that first pick fruit had highest levels of quinic acid present and the levels decreased as the fruit matured. First pick Red Delicious was observed to have the highest level of quinic acid. However in this cultivar, even fully tree ripened fruit up to 20% of the total acids as quinic acid. The nature of the acid composition of Red Delicious is such that it has low levels of malic acid (section 5.2) and high levels of quinic but the total acidity is similar to the other cultivars examined. Similar levels of quinic and malic acids were observed in both 1992 and 1993 from fruit grown from both Hawke's Bay and Nelson and are probably a characteristic of this cultivar.

Red Delicious had quinic acid present at levels that were significantly higher than other cultivars. Golden Delicious had the lowest quinic acid level but was not significantly different to Braeburn, Fuji, Gala, Royal Gala or Granny Smith. Fuji was significantly lower than Red Delicious and not significantly different to the other cultivars, while Granny Smith was only significant different (lower) to Red Delicious and Cox's Orange (table 5.5).

As for total acidity apple juices from Hawke's Bay fruit had quinic acid present at significantly lower levels than that which was seen from Nelson and Canterbury, with there being no difference in samples between Canterbury and Nelson (table 5.6).

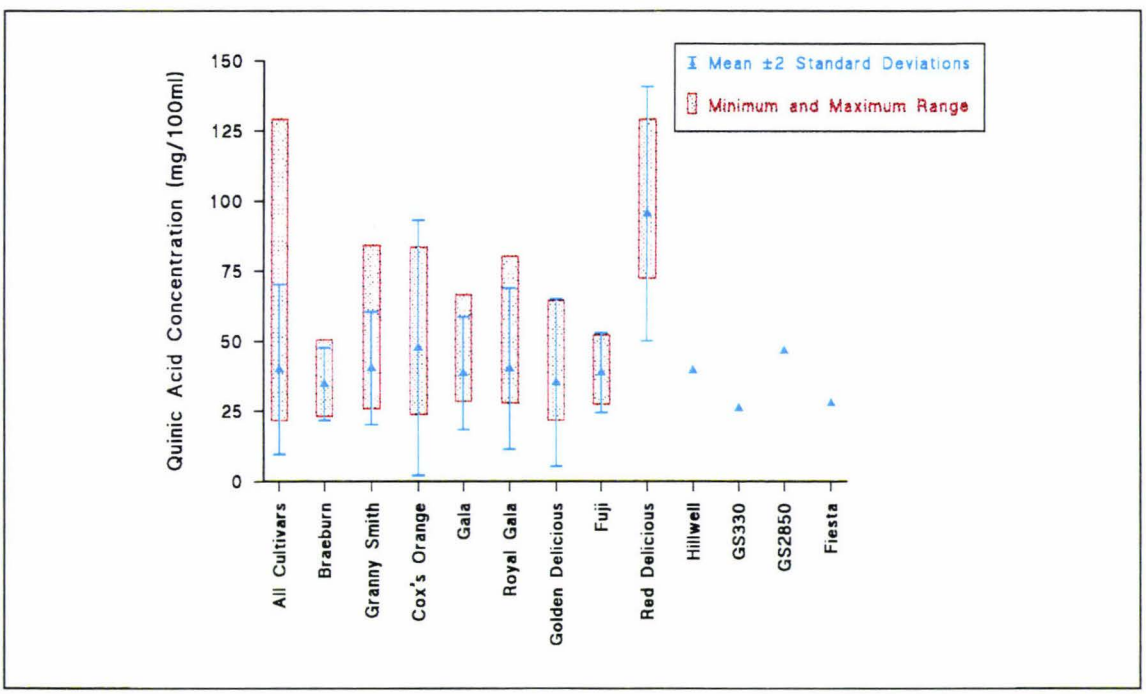

Figure 5.13: Quinic acid concentrations for New Zealand varietal apple juices.

Levels of quinic acid in the juice of fruit harvested in 1993 were significantly higher than that which was observed for 1992 (table 5.7). There was no significant difference in quinic acid levels in the juice of second or third pick apples, however the levels in first pick fruit were significantly higher than that seen in second or third pick samples (table 5.8).

When the Braeburn and Granny Smith fruit were stored at either ambient, cold or controlled atmosphere conditions the quinic acid levels in the resulting juice were variable and fluctuated from 23.3mg/100ml to 47.6mg/100ml (figures 5.14 and 5.15, appendices 33 and 34).

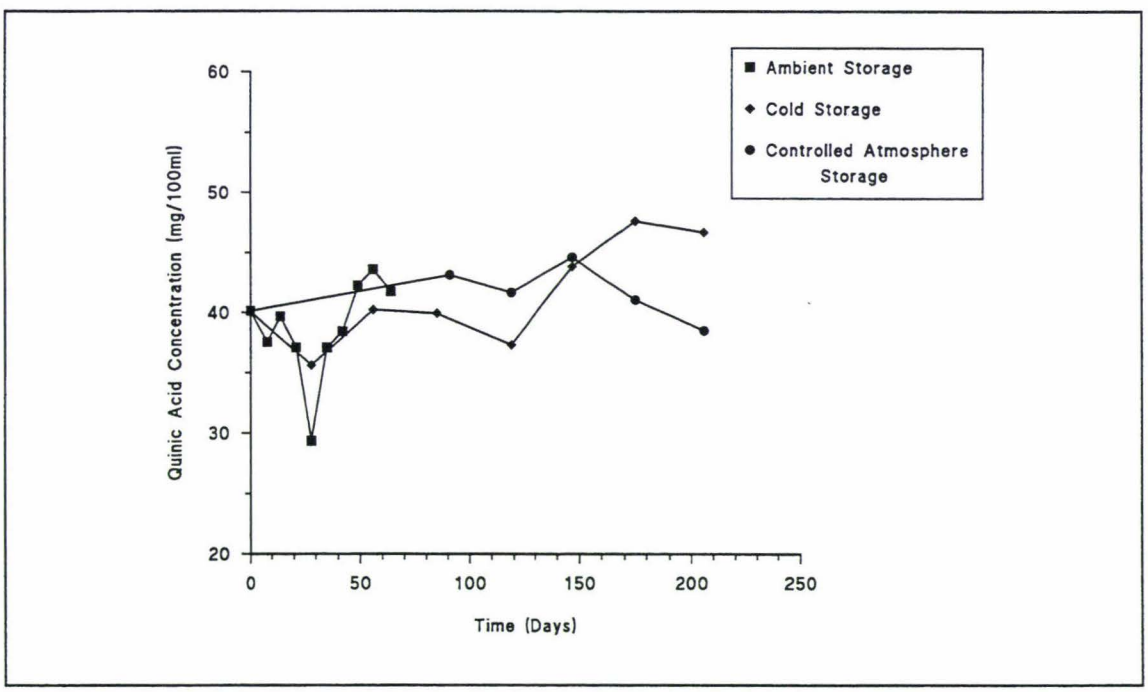

Figure 5.14: Effect on juice quinic acid concentrations of different storage regimes for Braeburn apples in 1993.

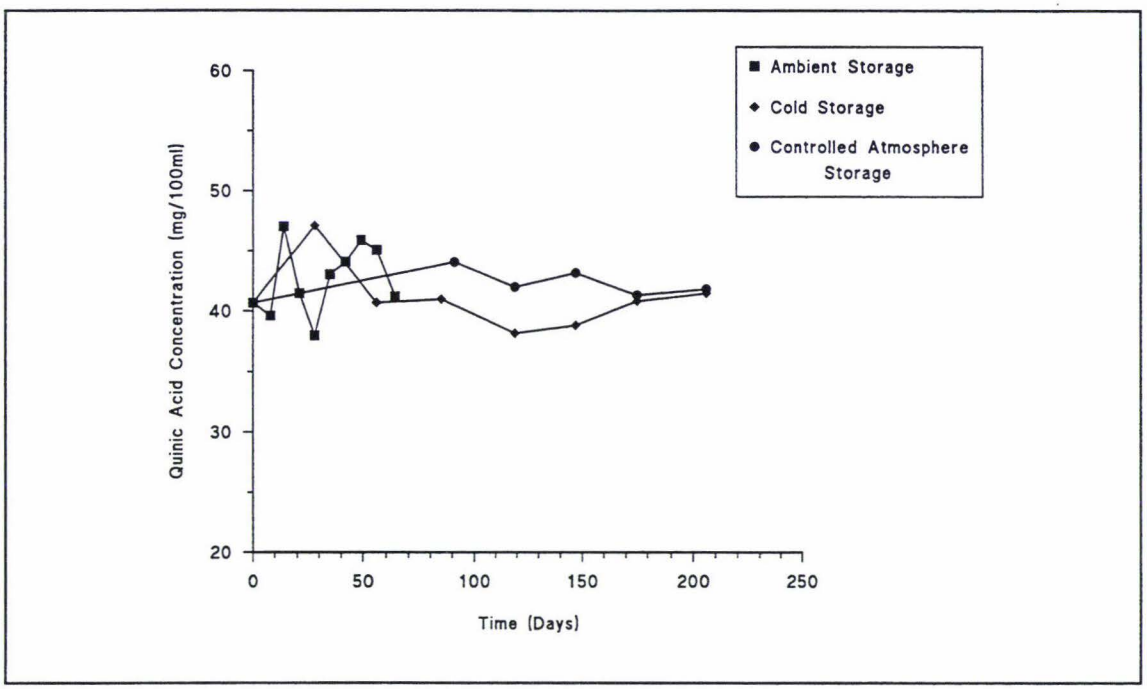

Figure 5.15: Effect on juice quinic acid concentrations of different storage regimes for Granny Smith apples in 1993.

5.4 Succinic Acid

Succinic acid was the third most prevalent acid observed in apple juice at 8 to 42mg/100ml (figure 5.16) which accounted for between 1 and 10% of the total acid (appendix 27). These levels are much greater than the maximum levels of 10mg/100ml published (Ackermann *et al.*, 1992; Fuleki *et al.*, 1995; Hulme, 1956a). Generally the appearance of succinic acid is associated with physiological diseases of apples in storage such as carbon dioxide injury and low temperature breakdown (Hulme, 1956a; Hulme,1958; Hulme and Wooltorton, 1958; Hulme *et al.*, 1964). The high levels of succinic acid seen in New Zealand apples were unlikely due to physiological diseases as freshly picked fruit were observed to have levels greater than 10mg/100ml. However, samples were not examined for signs of disorders. The high and fluctuating level of succinic acid could be due to other acids coeluting and masking the detector response. Also the succinic acid peaks were small and difficult to resolve from baseline noise for accurate quantification.

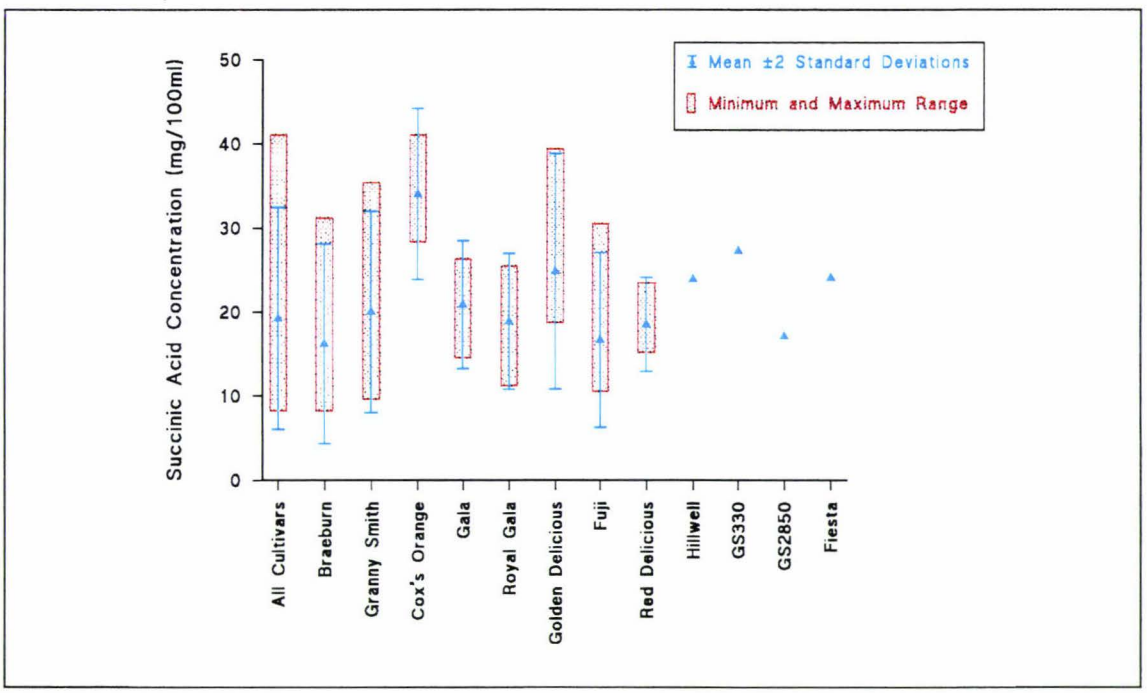

Figure 5.16: Succinic acid concentrations for New Zealand varietal apple juices.

Cox's Orange was significantly higher in succinic acid than the other cultivars, while Fuji was observed to have the lowest mean level but was not significantly different to Braeburn, Red Delicious or Royal Gala (table 5.5). Braeburn was significantly lower than Granny Smith and Golden Delicious while Granny Smith was not significantly different to Red Delicious, Royal Gala, Golden Delicious or Gala.

The juice of fruit from Hawke's Bay had succinic acid present at significantly lower levels than Nelson or Canterbury with no significant differences seen in juices of apples from Canterbury and Nelson or Nelson and Hawke's Bay (table 5.6).

Succinic acid levels in 1993 and first and second pick samples were significantly lower than in 1992 and third pick samples respectively (table 5.7 and 5.8).

When Braeburn and Granny Smith fruit were stored at either ambient, cold or controlled atmosphere conditions in 1993 only cold stored fruit showed any noticeable changes in the succinic acid levels (figures 5.17 and 5.18). In 1993 it was observed that in cold stored Braeburn and Granny Smith fruit, the succinic acid level increased from 13.7mg/100ml and 13.4mg/100ml respectively at the start of storage to 21.1mg/100ml and 30.7mg/100ml respectively after 206 days of storage. Similar trends were observed in 1992 (appendices 33 and 34). Low temperature injury is preceded by the accumulation of oxaloacetate, which in small amounts inhibits the oxidation of succinate to fumarate by succinate dehydrogenase, resulting in an increase in succinic acid and other metabolites before this stage in the TCA cycle (Hulme *et al.*, 1964; Pardee and Potter, 1948; Tyler, 1960).

The presence of high levels of carbon dioxide can aggravate the occurance of low temperature breakdown. Hulme (1958) showed that the storage of apples in hypernormal concentrations of carbon dioxide resulted in the physiological disease carbon dioxide injury which was preceded by the appearance of succinic acid. Ranson *et al.*, (1960) found that the activity of succinic dehydrogenase was inhibited by carbon dioxide and that succinic acid appears to be toxic to apples even at low concentration (Neal and Hulme, 1958).

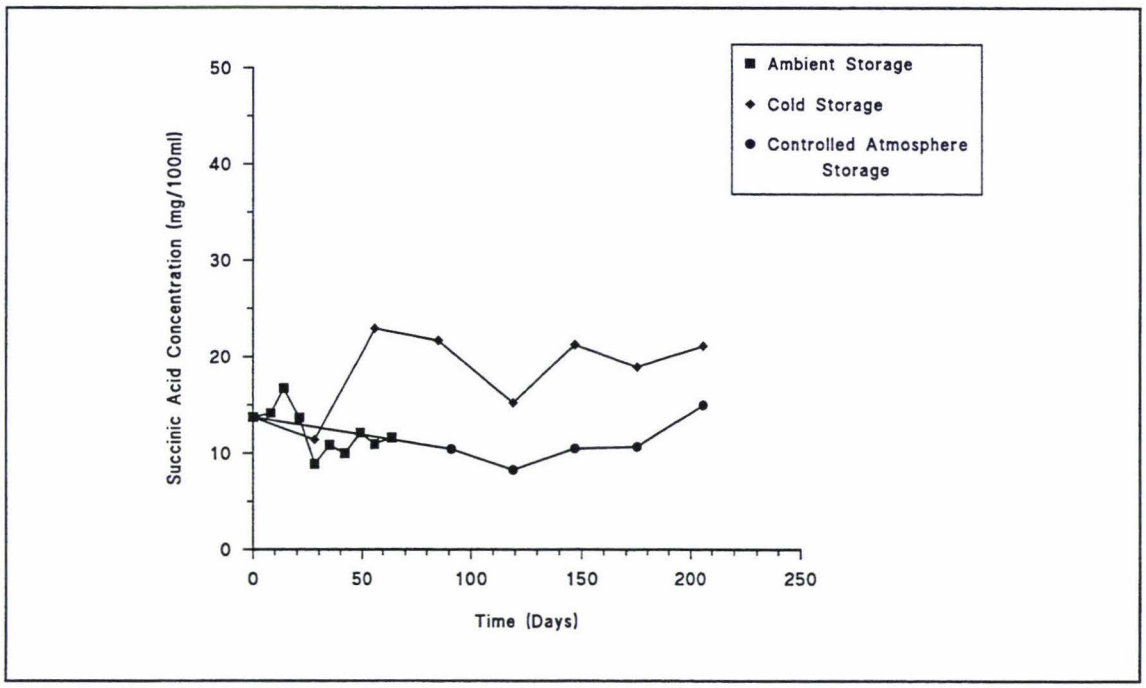

Figure 5.17: Effect on juice succinic acid concentrations of different storage regimes for Braeburn apples in 1993.

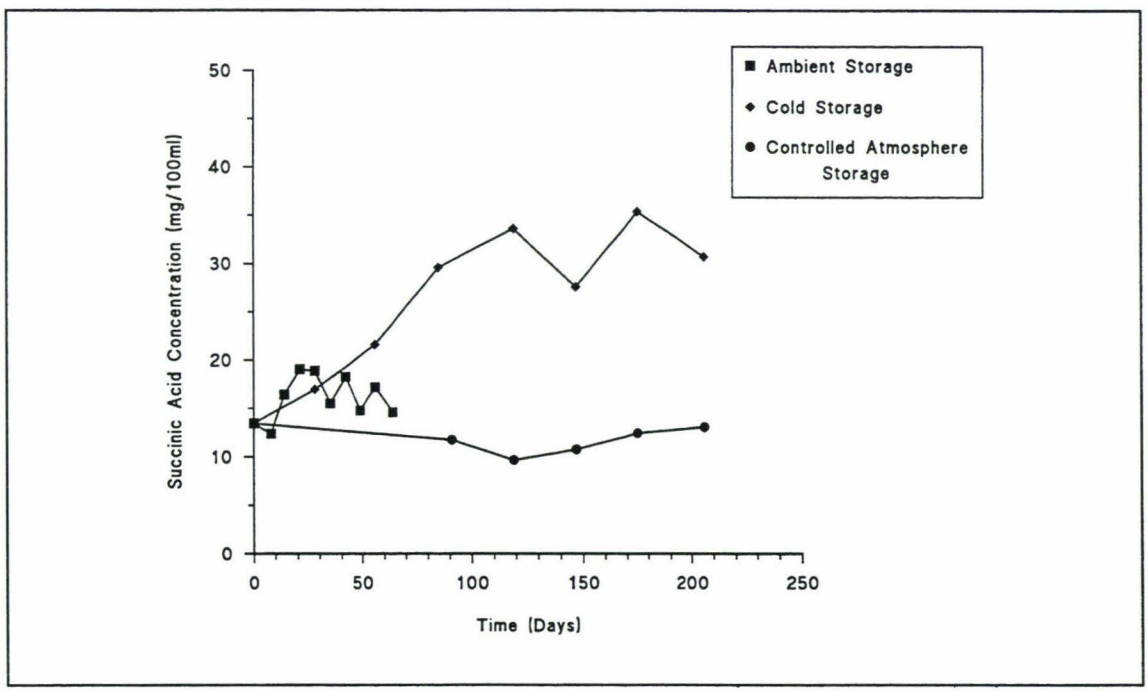

Figure 5.18: Effect on juice succinic acid concentrations of different storage regimes for Granny Smith apples in 1993.

During the first half of controlled atmosphere storage, the juice of Braeburn and Granny Smith apples showed slight decreases in succinic acid levels from 13mg/100ml to 8.2mg/100ml and 9.2mg/100ml respectively. While the temperature for controlled atmosphere storage was the same as that for cold storage conditions (0.5°C) an increase in succinic acid levels was not observed until the end of the storage period where levels of 15mg/100ml in Braeburn juice and 13mg/100ml in Granny Smith juice were seen (figures 5.17 and 5.18). The lower levels of succinic acid seen in controlled atmosphere stored fruit is probably due to the level of carbon dioxide (< 2%) and oxygen (< 3%) used. Levels of greater than 3% carbon dioxide have been reported to acheive carbon dioxide injury (Wilkinson and Fidler, 1973). It has also been claimed that the storage in less than 3% oxygen (in the absence of carbon dioxide) reduces the susceptibility of the fruit low temperature breakdown and allows lower temperatures to be used. However this was not consistent from one year to the next for English apples (Wilkinson and Fidler, 1973).

With most disorders the metabolic events leading to the manifestation of symptoms are not fully understood

5.5 Citric Acid

Citric acid was present at levels of 2 to 21mg/100ml (figure 5.19) and accounted for between 0.5% to 4.8% of the total acids (appendix 27). These levels were similar to that which has been reported in the literature, where it is found to be the third most prevalent acid (Ackermann *et al.*, 1992; Coppola and Starr, 1986; Dupont, 1973; Evans *et al.*, 1983; Hulme and Wooltorton 1957, 1958; Jeuring *et al.*, 1979; Lea, 1990; Lee and Wrolstad, 1988a, 1988b; Ryan, 1972; Wrolstad *et al.*, 1981). In the samples examined citric acid was found to be the fourth most common acid present. Citric acid levels were within the range of 5 to 20 mg/100ml proposed by the German RSK values (Bielig *et al.*, 1982) and never exceeded the maximum of 40mg/100ml allowed by some European countries (Evans *et al.*, 1983).

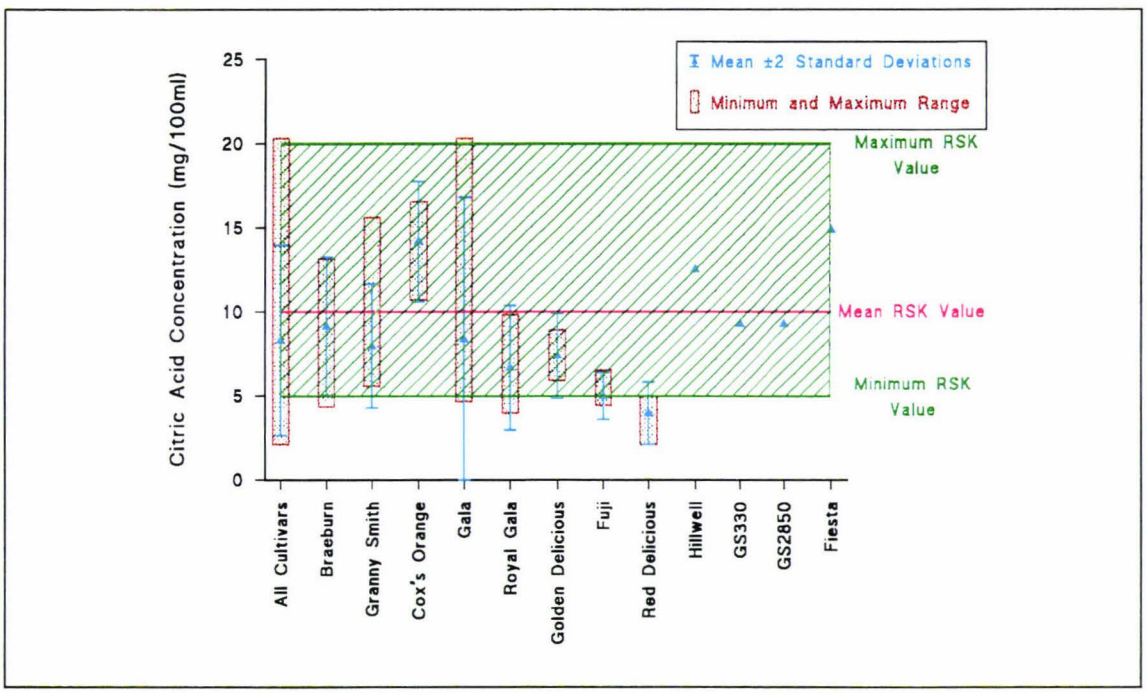

Figure 5.19: Comparison of citric acid concentrations for New Zealand varietal apple juices with the RSK values.

There have been reports of Granny Smith apples having levels of citric acid up to levels of 90mg/100ml (Turner, 1949), with Withy *et al.* (1978) finding levels of citric acid of up to 120mg/100g fresh weight and accounting for 22% of the total acids for fruit grown in Nelson, New Zealand. They also found high levels of citric acid in Red Delicious (75mg/100g fresh weight) which accounted for 14% of the total acids. During this study the level of citric acid found in Granny Smith apple juice was between 5.5 to 15.6mg/100ml (0.72 to 2.15% of the total acids, (appendix 29-table A29.4)) while Red Delicious had levels of between 2.10 to 4.98mg/100ml (0.54 to 1.21% of the total acids, appendix 32-table A32.1). For all cultivars examined the citric acid level never exceeded 5% of the total acids which is similar to reports by Krotkov *et al.* (1951).

Citric acid content of Cox's Orange apple juice was significantly the highest of the cultivars examined, with Red Delicious having citric acid levels that were significantly lower than other cultivars except Fuji. Granny Smith had levels of citric acid that were not significantly different from Royal Gala, Golden Delicious or Gala but was significantly lower than the level found in Braeburn juice (table 5.10).

There was no significant difference in citric acid levels in the juice of fruit due to regions, growing season or picking dates that the fruit are harvested for juice production (table 5.11, 5.12 and 5.13).

It has been reported (Hulme and Wooltorton, 1958) that citric acid levels increase from around 6.5mg/100g to 9.5mg /100g fresh weight in the pulp during storage at 15°C. The citric acid levels observed showed slight but variable increases for Braeburn and Granny Smith fruit (figures 5.20 and 5.21, appendices 33 and 34). The level of citric acid in juice from Braeburn fruit at the end of storage was generally 3 to 4mg/100ml higher than the level of 8.6mg/100ml and 7.0mg/100ml for 1992 and 1993 respectively seen at the start of storage. The level of citric acid in the juice of Granny Smith fruit at the start of storage in 1992 was 6.0mg/100ml, and as with Braeburn, by the end of the fruit storage period the levels of citric acid were observed to increase by 3 to 4mg/100ml. Granny Smith fruit in 1993 that were stored under cold storage conditions showed marked increases in citric acid

levels of the resulting juice, increasing from 7.69mg/100ml to 14.25mg/100ml (figure 5.21).

Table 5.10: Least-squared means (lsmeans) for citric, shikimic and fumaric acids in the juice of eight apple cultivars.

Cultivar (S.E.)	Number of Samples	Citric Acid (mg/100ml)	Shikimic Acid (mg/100ml)	Fumaric Acid (mg/100ml)
Braeburn	64	8.9 c[1] (0.4)	0.69 ab (0.7)	0.07 a (0.006)
Cox's Orange	7	14.1 f (0.9)	0.40 ad (0.15)	0.10 c (0.012)
Fuji	9	5.0 ae 0.8)	0.91 b (0.13)	0.07 ab (0.011)
Gala	18	8.3 cd (0.6)	2.00 c (0.10)	0.09 bc (0.008)
Golden Delicious	6	7.4 bcd (1.0)	0.89 b (0.16)	0.06 ab (0.013)
Granny Smith	57	7.8 bd (0.4)	0.45 d (0.06)	0.07 a (0.005)
Red Delicious	6	4.0 a (1.0)	2.74 e (0.16)	0.07 ab (0.013)
Royal Gala	17	6.6 be (0.6)	1.76 c (0.09)	0.08 ac (0.008)

[1] Means within the same column followed by the same letter are not significantly different ($P \leq 0.05$), S.E. = Standard Error of lsmeans

Table 5.11: Least-squared means for citric, shikimic and fumaric acids in the juice of apple cultivars grown in three regions of New Zealand.

Region (S.E.)	Number of Samples	Citric acid (mg/100ml)	Shikimic Acid (mg/100ml)	Fumaric Acid (mg/100ml)
Canterbury	15	7.7 a[1] (0.6)	1.21 a (0.11)	0.075 a (0.009)
Hawke's Bay	139	8.0 a (0.3)	1.23 a (0.05)	0.084 a (0.004)
Nelson	30	7.5 a (0.4)	1.25 a (0.07)	0.071 a (0.06)

[1] Means within the same column followed by the same letter are not significantly different ($P \leq 0.05$), S.E. = Standard Error of lsmeans

Table 5.12: Least-squared means for citric, shikimic and fumaric acids in the juice of apple cultivars sampled over two growing seasons.

Year (S.E.)	Number of Samples	Citric Acid (mg/100ml)	Shikimic Acid (mg/100ml)	Fumaric Acid (mg/100ml)
1992	97	7.7 a[1] (0.3)	1.09 a (0.05)	0.079 a (0.004)
1993	87	7.8 a (0.4)	1.37 b (0.07)	0.074 a (0.006)

[1] Means within the same column followed by the same letter are not significantly different ($P \leq 0.05$) S.E. = Standard Error of lsmeans

Table 5.13: Least-squared means for citric, shikimic and fumaric acids in the juice of apple cultivars harvested at different maturities.

Stage of Maturity (S.E.)	Number of Samples	Citric Acid (mg/100ml)	Shikimic Acid (mg/100ml)	Fumaric Acid (mg/100ml)
1st Pick	26	7.6 a[1] (0.6)	1.59 b (0.16)	0.080 b (0.005)
2nd Pick	29	7.0 a (0.6)	1.02 a (0.15)	0.065 a (0.004)
3rd Pick	22	7.5 a (0.7)	0.81 a (0.17)	0.078 ab (0.005)

[1] Means within the same column followed by the same letter are not significantly different ($P \leq 0.05$), S.E. = Standard Error of lsmeans

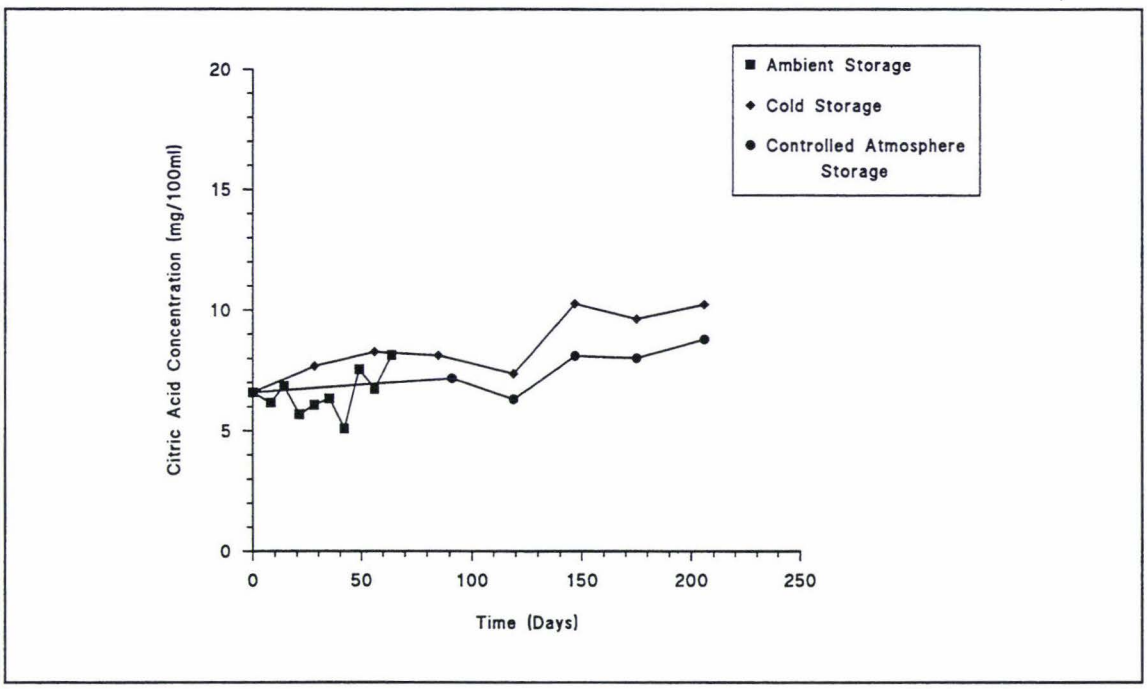

Figure 5.20: Effect on juice citric acid concentrations of different storage regimes for Braeburn apples in 1993.

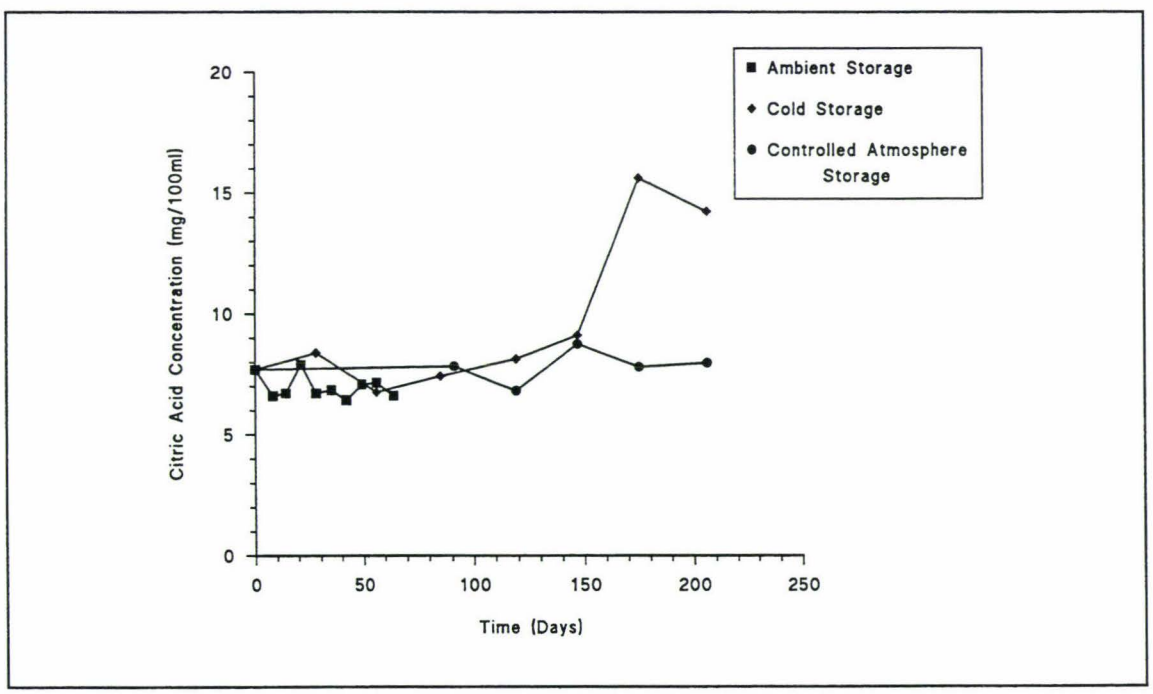

Figure 5.21: Effect on juice citric acid concentrations of different storage regimes for Granny Smith apples in 1993.

5.6 Shikimic Acid

Shikimic acid was present at low and variable levels with values ranging from 0.01 to 3.59mg/100ml (figure 5.22), accounting for between 0.47 to 2.7% of the total acids (appendix 27). The literature reports variable levels of shikimic acid with levels typically below 8mg/100ml (Blanco *et al.*, 1992a; Hulme and Wooltorton, 1958; Kollas, 1964; Lee and Wrolstad, 1988a; Ryan, 1972).

Red Delicious was observed to have shikimic acid at levels that were significantly higher than other cultivars examined, with Cox's Orange having the lowest level (table 5.10). The level seen in Cox's Orange was not significantly different to Braeburn or Granny Smith, but Braeburn had significantly higher levels than Granny Smith.

There was no significant difference in acid levels due to region but levels from 1992 growing season were significantly lower than 1993 (table 5.11 and 5.12).

The stage of maturity at which the fruit is harvested showed some differences with first pick fruit being significantly higher than second or third picks, but there were no significant differences between second and third picks (table 5.13).

In the present study the coefficient of variation was 70% (appendix 27) for the determination of shikimic acid which was similar to the value of 64% reported by Lee and Wrolstad, 1988a. Due to the fluctuations seen in shikimic acid levels, it was not possible to observe any distinctive trends during storage of the fruit (figures 5.23 and 5.24, appendices 33 and 34). It is difficult to determine the cause of these variations.

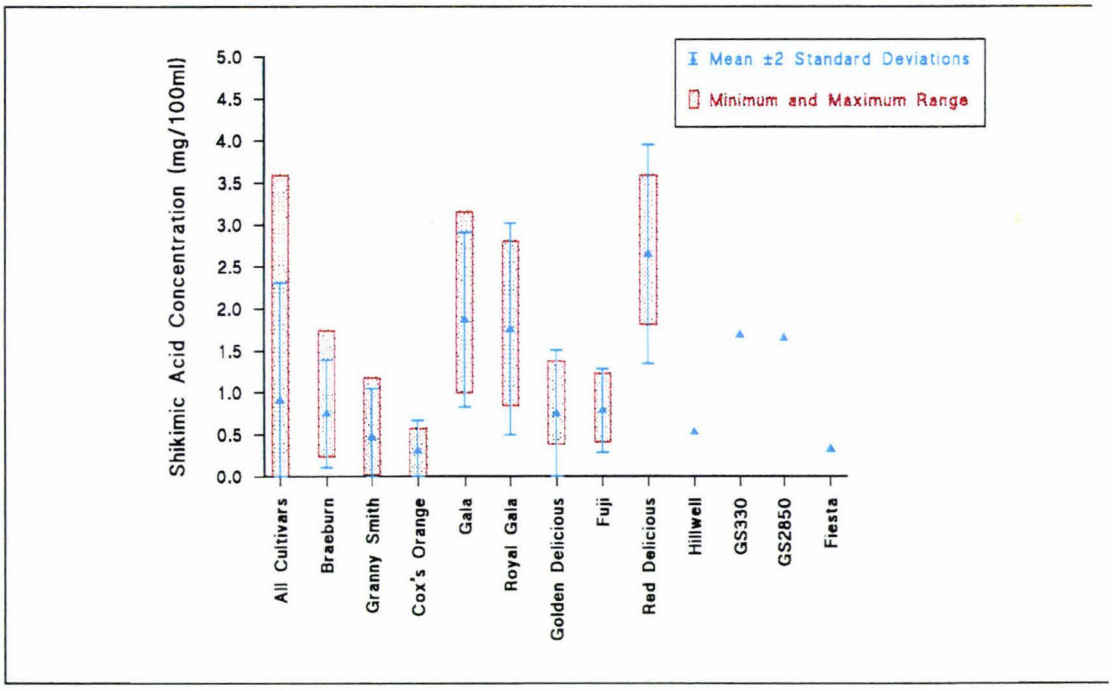

Figure 5.22: Shikimic acid concentrations for New Zealand varietal apple juices.

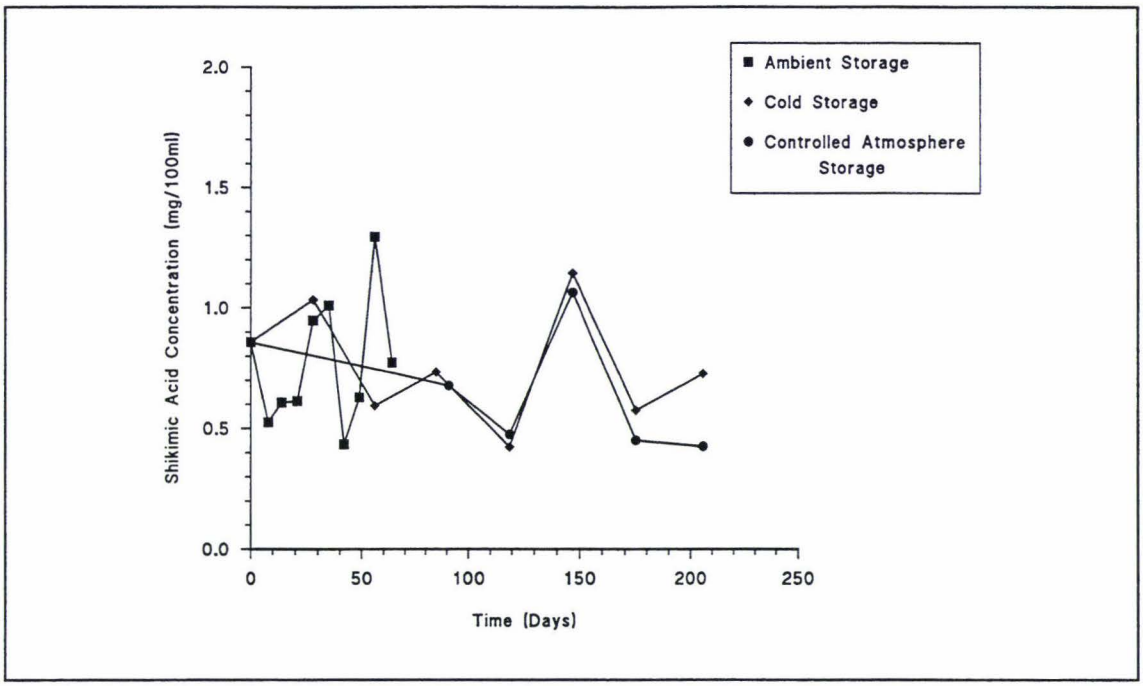

Figure 5.23: Effect on juice shikimic acid concentrations of different storage regimes for Braeburn apples in 1993.

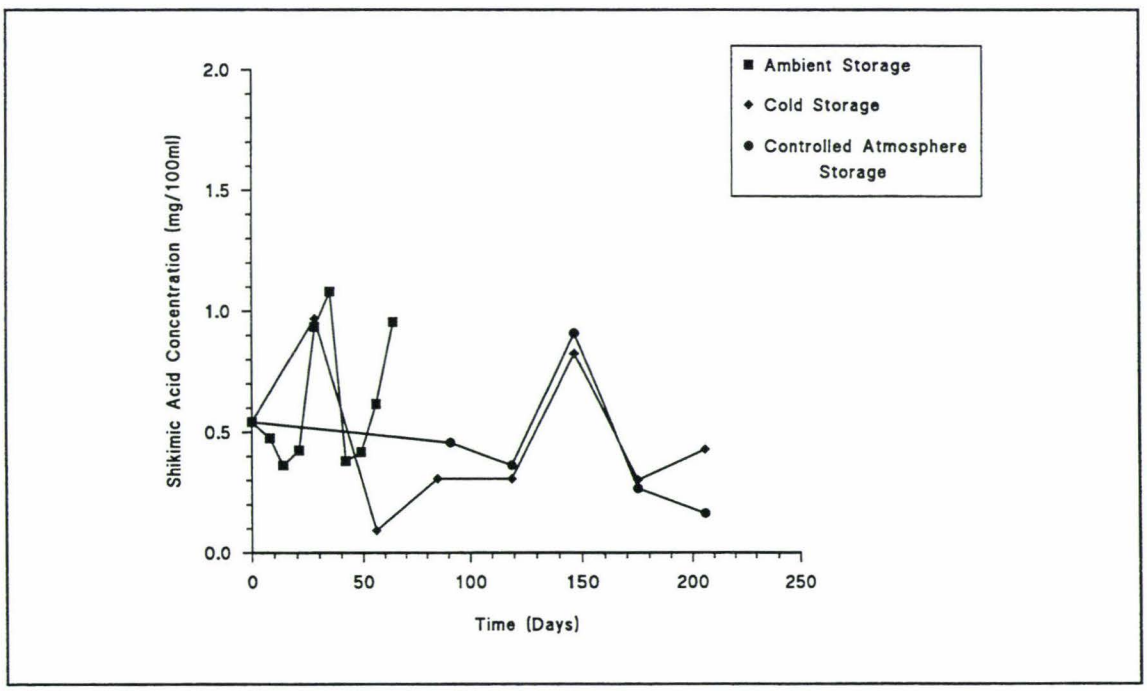

Figure 5.24: Effect on juice shikimic acid concentrations of different storage regimes for Granny Smith apples in 1993.

5.7 Fumaric Acid

Fumaric acid was present at low levels with values typically less than 0.22mg/100ml (figure 5.25) and accounted for less than 0.02% of the total acids (appendix 27). These results are consistent with Lee and Wrolstad, 1988a and Evans *et al.*, 1983 who also found levels below the maximum level of 0.3mg/100ml proposed by Junge and Spadinger (1982) for authentic apple juice. It is however, not surprising that the samples in this study had low levels of fumaric acid as they were not subjected to heat processes of pasteurisation or concentration which will produce fumaric acid from malic acid dehydration.

Gala, Royal Gala and Cox Orange had fumaric acid present at significantly higher levels than the other cultivars, with Cox's Orange have the highest level. Golden Delicious was observed to have the lowest fumaric acid content and was not significantly different to the other cultivars except Cox's Orange (table 5.10).

There was no significant difference between growing regions or year of harvest for fumaric acid content (tables 5.11 and 5.12). Second pick fruit was significantly lower than first pick, but not significantly different to third pick fruit, while there was no difference between first pick and third pick samples (table 5.13).

During storage the fumaric acid level of ambient stored fruit increased rapidly from 0.06mg/100ml for both Braeburn and Granny Smith to reach levels of 0.20 and 0.13mg/100ml respectively. Cold and controlled atmosphere stored Braeburn fruit and controlled atmosphere stored Granny Smith showed slight increases of 0.02mg/100ml during the storage period (figure 5.26). However cold stored Granny Smith fruit showed relatively little change in the amounts of fumaric acid up to 120 days of storage. At this stage fumaric acid levels began to increase and continued to increase quite rapidly right up to the end of the storage period where concentration of 0.13mg/100ml was seen (figure 5.27)

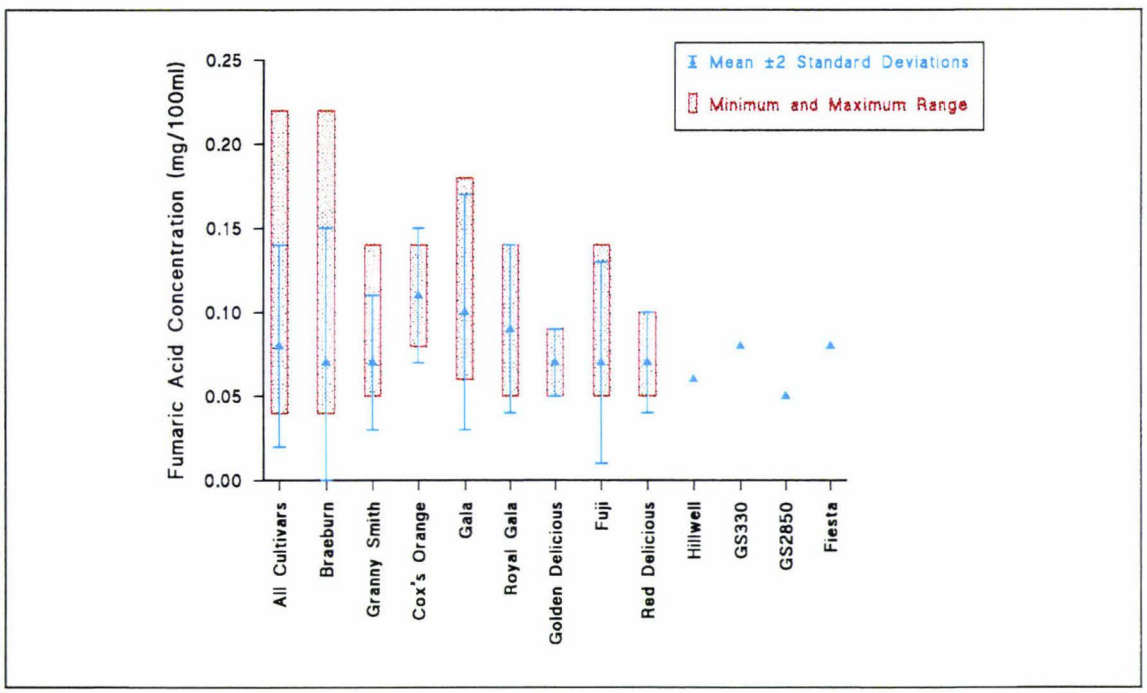

Figure 5.25: Fumaric acid concentrations for New Zealand varietal apple juice.

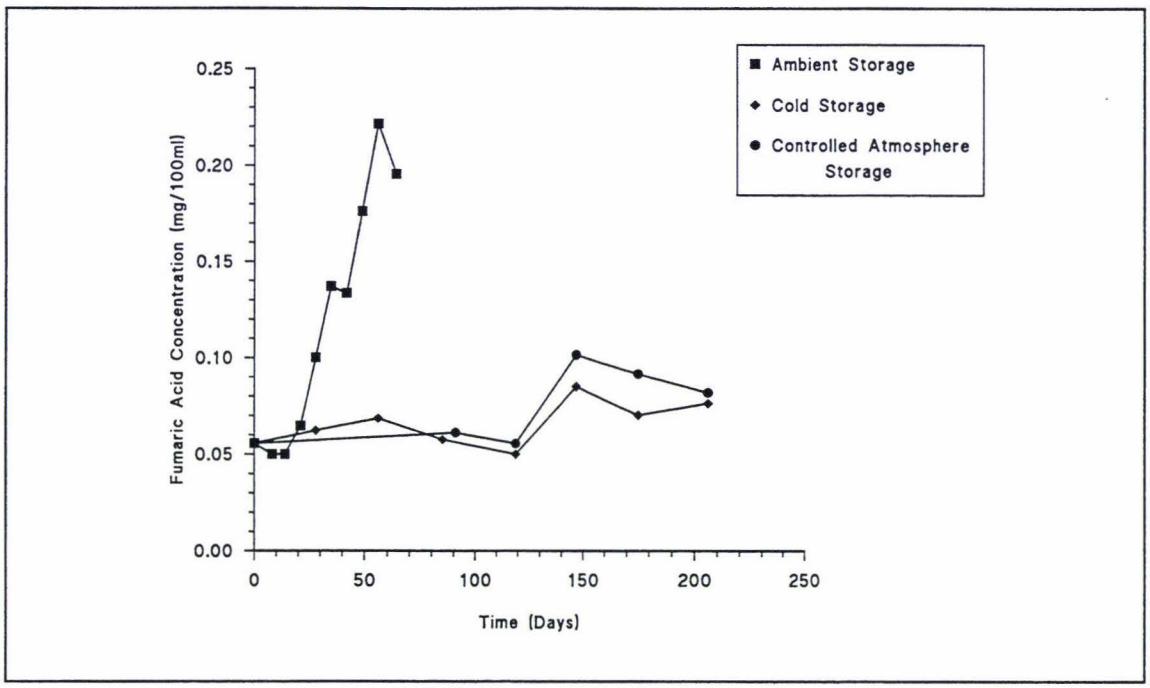

Figure 5.26: Effect on juice fumaric acid concentrations of different storage regimes for Braeburn apples in 1993.

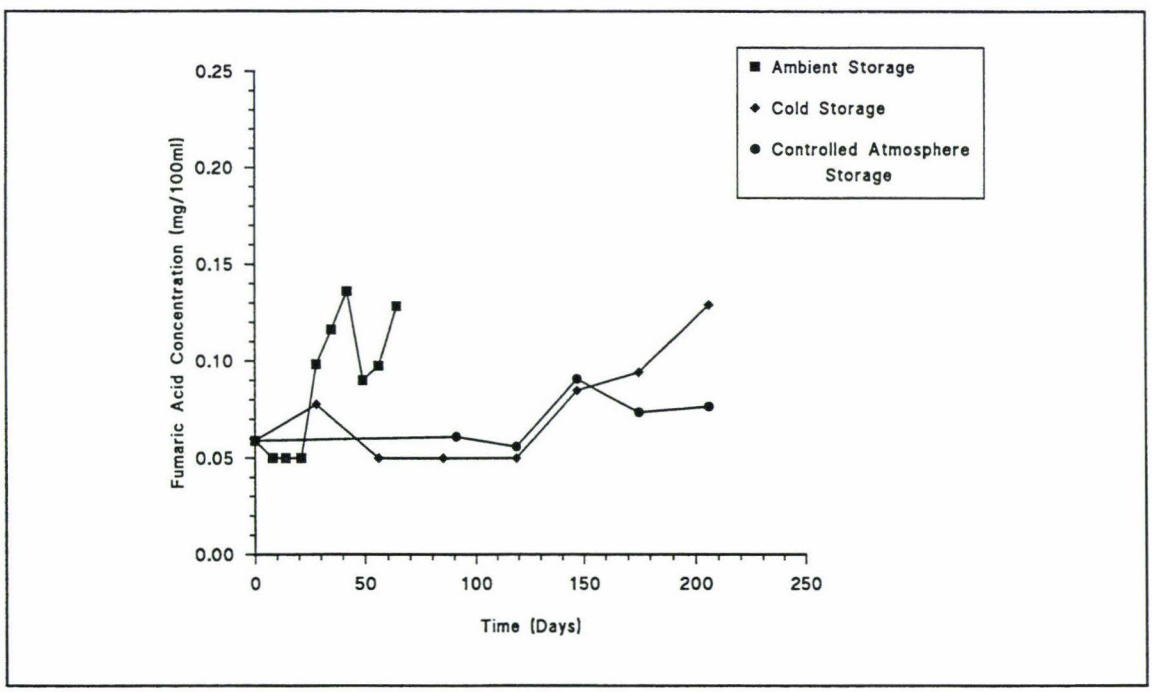

Figure 5.27: Effect on juice fumaric acid concentrations of different storage regimes for Granny Smith apples in 1993.

5.8 Hillwell, GS330, GS2850 and Fiesta

One sample of each of the cultivars, Hillwell, GS330, GS2850 and Fiesta were examined and found to have individual acids, total acids, titratable acidity and pH levels that were similar to those of the other cultivars (appendix 32-table A32.5).

5.9 Sample Variation

The results for the determination of individual acids, total acids, titratable acidity and pH levels from the juice of Braeburn apples collected on the same day from Hawke's Bay in 1993 showed small variations in the components as can be seen from appendix 28-table A28.4. The coefficient of variation was generally less than 20% for all components except for succinic acid, which had a coefficient of variation of 32%.

5.10 Conclusion

The cultivars examined were Royal Gala, Gala, Braeburn, Granny Smith, Golden Delicious, Fuji, Cox's Orange and Red Delicious, with one sample each of Hillwell, GS330, GS2850 and Fiesta also analysed. The pH ranged from 3.16 to 4.15, with titratable acidity and total acids present at levels of 210 to 1130mg/100ml and 304 to 1208mg/100ml respectively. Malic acid was the predominate acid present at levels of between 231 and 1068mg/100ml with the levels of the other acids being, quinic, 21 to 129mg/100ml; succinic, 8 to 41mg/100ml; citric, 2 to 20mg/100ml; shikimic, 0.01 to 3.6mg/100ml and fumaric, 0.04 to 0.22mg/100ml. Generally it was found that there were significant differences between cultivars for all components of the apple, while the regional effect was only significant for malic acid, quinic acid, pH, titratable acidity and total acids. Significant differences between years were found for malic acid, quinic acid, shikimic acid, succinic acid and pH.

Malic acid has been reported as comprising between 70 to 94% of the total acids and in all cultivars examined, except for Red Delicious, malic acid accounted for between 84 to 94% of the total acids. In Red Delicious malic acid accounted for 71% of the total acids, but at

this level it was still within the range of values commonly reported in the literature. This cultivar also had high levels of quinic acid, which accounted for up to 27.9% of the total acids, whereas the other cultivars examined were observed to have quinic acid accounting for between 2.95 and 10% of the total acids. Quinic acid levels can show large fluctuations with it being reported to comprise up to 38% of the total acids (Lee and Wrolstad, 1988b). Shikimic, citric, succinic and fumaric acids in Red Delicious apple juice were all present at levels similar to those which was observed in other cultivars examined.

While citric acid is usually the third most prevalent acid present in apple juice, and even though it was found at concentrations similar to those which are reported in the literature and fell within the RSK limits for authentic apple juice, it was the fourth acid in the juice analysed here. Succinic acid was found to be the third most common acid, being present up to four times published levels, and accounted for up to 4.5% of the total acids. The appearance of succinic acid is an indication of physiological storage disorders, low temperature breakdown and carbon dioxide injury. Since samples were analysed as soon as possible after harvest and still found to contain high levels of succinic acid, it is unlikely that succinic acid arose from physiological disorders. It is possible that during HPLC analysis other compounds coeluted, but this was not investigated.

Shikimic and fumaric acids made up a minor proportion of the total acids with shikimic accounting for less than 2% and fumaric less than 0.02%.

Titratable acidity, total acids and malic acid decreased during ripening of the fruit and this decrease continued once the fruit were harvested and during storage. Quinic acid was observed to fluctuate during all forms of storage. Increases in succinic and citric acid levels during cold storage of both Braeburn and Granny Smith were observed, while controlled atmosphere stored fruit only showed increases in these acids levels during the second half of storage. These changes are possibly due to disorders such as low temperature breakdown and carbon dioxide injury as the highest levels of acids were seen at the end of the storage periods. The juice of Braeburn and Granny Smith fruit that were stored under ambient conditions showed rapid increases in fumaric acid levels during storage, while under

controlled atmosphere conditions slight increases occurred after the fruit were stored for longer than 120 days. Cold stored Granny Smith showed rapid increases in fumaric acid levels during the second half of storage to reach levels of 0.13mg/100ml but it is not known why this increase occurred.

Non volatile acids are the class of chemical compounds in second order of importance from a quantitative standpoint in apple juice. Brause *et al.* (1986) proposed limits of 250 to 850mg/100ml, while the RSK values show a somewhat narrower range of 0.45 to 0.76 (Wrolstad, 1985).

Except for the juice of Cox's Orange and first pick Granny Smith the malic acid levels of all samples examined were within Brause *et al.* (1986) proposed limit for authentic juice. Therefore examination of malic acid levels alone is not sufficient to determine the authenticity of juice from Cox's Orange or early picked Granny Smith fruit. These samples were observed to have levels generally in the range of 837 to 1067mg/100ml which exceeded Brause *et al.* (1986) maximum level. Fumaric acid levels in these samples was less than 0.14mg/100ml which is less than the 0.3mg/100ml that Junge and Spadinger (1982) reported for authentic. However extremes in processing can result in the formation of fumaric acid and before any conclusion about the authenticity of the juice from Cox's Orange and early pick Granny Smith fruit can be made the processing history should be examined. Alternatively test for the presence of d-malic acid which does not occur naturally in apple juice should be performed.

CHAPTER 6.0
GENERAL CONCLUSION

6.1 General

The price of apple juice and apple juice concentrate varies according to supply and demand, which can encourage unscrupulous suppliers to adulterate juice giving them an unfair advantage and excessive returns. It is therefore of great importance for both the consumer and honest supplier that appropriate and validated compositional standards are established. However, as shown in this study, to set standards based on compositional data for a particular range of apple varieties and then to apply this standard to the juice from different varieties, grown in different countries and in different years is fraught with danger.

6.2 Sugar Standards and New Zealand Apple Juices

In the scientific literature there is a considerable amount of data for the composition of apple juice. Various attempts have been made to derive, from such data, standards that can be used to evaluate the authenticity of apple juice in commercial trade. The German RSK values and the values proposed by Brause and Raterman (1982) are two sets of standards based on sugar and organic acid levels. No one set of standards is, as yet, universally accepted. However, some of these standards such as a maximum sucrose level of 3.5g/100ml, minimum fructose/glucose ratio of 1.6 and the presence of glucose at levels of between 1.8 and 3.5g/100ml, are being used to evaluate juice in the trade. By these standards some commercial juices, including some from New Zealand have been judged to be of doubtful authenticity for they have failed to meet the criteria in one or more ways. In turn this has led to the questioning by the suppliers of the validity of standards.

At the same time it is possible to appreciate that in setting standards, such as the German RSK values, it is important that the criteria are not to loose. If the standards are set too wide there is an incentive and opportunity for adulteration, while standards that are too narrow will exclude juice from cultivars or whole geographic regions. Thus the values have

to be a compromise, set to balance between the normal range and maximum variation that can be expected.

This study has clearly demonstrated that many apples grown in New Zealand can produce juices which will not meet published authenticity standards. The authenticity of juices from New Zealand grown Braeburn, Cox's Orange, Gala, Royal Gala, Hillwell, GS330, GS2850 and Fiesta are likely to be questioned due to the high levels of sucrose. Levels were consistently above 3.5g/100ml and regularly above 4.0g/100ml for these cultivars, with one juice sample from Cox's Orange reaching 7.5g/100ml. The sucrose level of Cox's Orange, GS330 and some juices from Braeburn, Royal Gala and Gala not only exceeded the standard values but also exceeded the 95% confidence level of 4.7g/100ml proposed by Lee and Wrolstad (1988b) based on Mattick and Moyer (1983) results. Granny Smith, Golden Delicious, Red Delicious and Fuji were observed to have sucrose levels generally below 3.5g/100ml which are within the published standards.

As would be expected the total sugars of ripe apples are relatively constant, typically falling into the range of 8.1 to 14.4 at the 95% confidence level. Consequently when one particular sugar is depressed then one or more of the others are likely to be present at elevated levels. Juices from Braeburn apples were observed to have glucose levels as low as 0.8g/100ml while the sucrose and total sugar levels for the same sample was 5.0g/100ml and 11.2g/100ml respectively. Cox's Orange apple juice also had high levels of sucrose with levels consistently above 5g/100ml with the glucose level always being less than 1.1g/100ml with most samples observed to have total sugars ranging from 9 to 12.3g/100ml. Both of these components are outside the German RSK proposed standard values for authentic juice. Similar trends were seen for other cultivars that were observed to have high sucrose levels.

Besides varietal differences there are changes in individual sugars during storage, stage of maturity at which the fruit are harvested and seasonal differences that will affect the composition of the apple juice and need to be considered when determining the authenticity. Sucrose values were observed to decrease while fructose and glucose levels increased during

storage. Application of the standard values for sucrose in the juice from stored Braeburn apples in 1993 would lead to questioning the authenticity as the maximum RSK value of 3.0g/100ml was exceeded. It was only after Braeburn apples were stored for longer than 45, 149 and 195 days under ambient, cold and controlled atmosphere conditions respectively that the sucrose levels decreased to levels within the RSK standard values. While the glucose and fructose levels increased during storage they were within the RSK values for authentic apple juice. Similar trends were seen in 1992 except that the maximum value of 3.5g/100ml proposed by Brause and Raterman (1982) was exceeded and the glucose level was below the minimum RSK value of 1.8g/100ml. This difference is probably a seasonal effect as the summer of 1991-1992 was warmer than that of 1992-1993. The juice of all cultivars in 1992 was observed to have sucrose significantly higher and glucose significant lower than that which was observed in 1993. The mean sucrose level in the juices of all samples analysed in 1992 was 3.1g/100ml and 2.4g/100ml in 1993, while the mean glucose levels of 1.6 and 1.8 g/100ml were observed in 1992 and 1993 respectively.

Although there was no significant differences in juice sucrose levels of fruit from Hawke's Bay, Canterbury or Nelson, the mean levels of between 2.50 and 2.96g/100ml were close to the maximum RSK value.

As fruit ripen the sucrose, glucose and fructose levels of the juice all were observed to increase. The sucrose and fructose content in the juice of first pick fruit were significantly lower than second and third pick fruit, while there was no significant differences for these sugars in the juices of second and third pick fruit. However the mean sucrose levels of between 3.1 and 3.6g/100ml in the juice of these samples exceeded the maximum RSK value of 3.0g/100ml. There were no significant differences in the juices of first, second or third pick fruit for glucose, however the mean levels of between 1.5 and 1.8g/100ml were generally less than the minimum value proposed by the German RSK values for authentic juice.

6.3 Required Sugar Standards for New Zealand Juices

In order for RSK and Brause and Raterman (1982) standards to include 95% of New Zealand apple juices from the varieties in this study the maximum limit for sucrose needs to be extended to 5.3g/100ml. The maximum limit of and 3.5g/100ml for glucose proposed by the RSK and Brause and Raterman (1982) is sufficient for New Zealand apple juice but the RSK minimum value of 1.8g/100ml needs to be reduced to 0.3g/100ml to include 95% of New Zealand apple juice in their standards for authenticity. However these new limits are large and will offer the opportunity for the unscrupulous to adulterate the juice from suitable varieties. Therefore alternative approaches other than solely examining the sugar profiles are necessary to detect fraudulent practises or else have country specific standards.

6.4 Organic Acid Standards and New Zealand Apple Juices

Organic acids are the second largest class of compounds found in apple juice and their use in the authentication of apple juice has received some attention. To this end some standards have been published for authentic juice.

The RSK set limits of 450 to 760mg/100ml for the total acidity (calculated as malic acid) with the revised matrix of Brause *et al.* (1986) recommending that l-malic acid (the predominate acid) be present at levels of 250 to 850mg/100ml in authentic apple juice. While the levels of malic acid decreased in the juice of stored Braeburn and Granny Smith fruit, the levels observed were within the Brause *et al.*, (1986) limits for authentic juice.

Most of the New Zealand apple juices analysed were observed to have malic acid compositions similar to the published values, although there were some exceptions. The most notable of these was Cox's Orange where malic acid levels in excess of 850mg/100ml were common.

Of the minor acids only citric and fumaric acids have limits proposed for authentic juices. The RSK values state that citric acid should be present at levels of 5 to 20mg/100ml, with

Junge and Spadinger (1982) recommending that authentic apple juice should have no more than 0.3mg/100ml fumaric acid present. These limits for citric and fumaric acids were never exceeded even though the acids were observed to increase during storage.

Quinic acid is the second most predominate acid present in apple juice at levels of 23 to 84mg/100ml, accounting for between 3 and 10% of the total acids. Red Delicious apple juice was the exception to this and was observed to have quinic acid accounting for up to 28% of the total acids, at levels of between 72 and 129mg/100ml. However the total acidity in Red Delicious apple juice was similar to other cultivars as the malic acid level was observed to account for only 71% of the total acids, whereas in other cultivars malic acid accounted for between 84 and 92% of the total acids.

Succinic acid was observed to be present at levels of 8 to 41mg/100ml which is higher than the maximum value of 10mg/100ml reported elsewhere. The presence of high levels of succinic acid is usually associated with disorders such as low temperature breakdown and carbon dioxide injury but the observed levels were found in fresh fruit. The reason for the high levels could be due to other components coeluting during analysis giving false high reading, but this is unclear.

While there were differences in the individual acids due to factors such as growing season, region, the levels seen were generally similar to published values. For example, malic acid in the juice of fruit from Hawke's Bay was not significantly different to that which was seen in the juice of Canterbury fruit but was significantly lower than levels in Nelson apples. There were no significant differences between the two growing seasons in malic acid levels, between first and second pick or between second and third picks, but first pick samples were significantly lower than third pick.

Organic acid standards also have important limitations. It is possible to manipulate acid levels by the of addition malic acid, although the cost of pure l-malic acid is prohibitively expensive for it to be used as an adulterant. The use of cheaper racemic malic acid, which contains both l- and d- forms can be detected by the total malic/l-malic acid ratio. A total

malic/l-malic acid ratio of 0.9 or less would indicate a non-authentic sample. Also synthetic malic acid has fumaric acid present as an impurity which can easily be detected by HPLC. Fumaric acid at concentrations of greater than 0.3mg/100ml would be evidence that malic acid has been added, although levels of up to 10mg/100ml can be produced when the juice is subjected to heat.

6.5 Future Methods

From the present work it is apparent that standards based on sugars, with or without organic acids will need to be set very wide to include all authentic juices that are produced locally and internationally.

While the German RSK values and those proposed by Brause *et al.* (1986) Brause and Raterman (1982) and Wrolstad (1985) for sugars are a useful starting point, due to the natural variation in sugars, the application of these standard values to New Zealand apple juices is not appropriate, will give erroneous results and is apparently based on inappropriate data and should not be used by themselves. The organic acid composition should also be examined when determining the authenticity of New Zealand apple juice.

Further techniques such as examination of multiple components (matrix method) or stable isotope ratios, while not examined in this study, could yield more information on the authenticity of New Zealand apple juice, but limitations in using these techniques exist.

When assessing multiple components the chances of detecting adulteration are increased because the probability that more than one component falls outside the standard values by chance in authentic juices is reduced. Also it is costly for the unscrupulous supplier to ensure that the overall balance of parameters matches the values that would be expected for a particular country of origin or fruit variety. However, in order for the matrix method to be applied the components examined must be independent.

Stable isotope ratio techniques are gaining more use in establishing authenticity of apple

juice and have received much attention over the years. Stable isotope ratios are useful in detecting the addition of some types of sugars and are now being included in standards for authenticity but this technique requires the use of sophisticated and expensive equipment.

While it is understandable that the detection of fraud is an objective in commerce care is necessary to ensure that legitimate products are not unduly penalised. In order to effectively use published standard values as much information as possible as to the sample's origin and processing is required and the standard values may need to be complimented by other techniques. If New Zealand apple juice is to be internationally traded then standards used to assess the authenticity of New Zealand juice should be developed from the many cultivars that are commonly grown in New Zealand. Alternatively New Zealand processors should carefully select cultivars or blend any juices and concentrates to meet standards used by international traders.

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APPENDICES

APPENDIX 1

COMPOSITIONAL DATA FOR APPLE JUICE

Table A1.1: Literature data for the composition of apple juice (Source: modified from Lee and Wrolstad, 1988b).

Parameter	Mean	Range	Number of Samples	Source
Specific gravity	1.0511	1.0372 - 1.0705	93	Mattick and Moyer (1983)
	1.0456	1.0409 - 1.0503	9	Ryan (1972)
	1.0510	1.0330 - 1.0520	29	Withy *et al.* (1978)
	1.0440	1.0330 - 1.0520	20	Rotstein *et al.* (1969)
	1.0488	1.0450 - 1.0570		Bielig *et al.* (1982)
Total Sugars (g/100ml)[a]	10.57	6.08 - 16.87	93	Mattick and Moyer (1983)
	10.74	9.02 - 13.34	20	Ryan (1972)
	11.0	9.04 - 13.98	12	Wrolstad and Shallenberger (1981)
	9.87	7.52 - 11.36	20	Rotstein *et al.* (1969)
	10.17	7.79 - 13.21	77	Fuleki *et al.* (1994)
Soluble solids (%)	12.74	9.80 - 16.90	93	Mattick and Moyer (1983)
	11.71	10.54 - 13.26	20	Ryan (1972)
	13.50	10.70 - 15.60	6	Blumenthal and Helbling (1977)
	10.04	8.18 - 12.01	20	Rotstein *et al.* (1969)
	12.08	11.18 - 14.01		Bielig *et al.* (1982)
	12.00	9.7 - 15.0	77	Fuleki *et al.* (1994)
Sucrose (g/100ml)	2.68	0.88 - 5.62	93	Mattick and Moyer (1983)
	1.75	0.65 - 2.40	20	Ryan (1972)
	1.33	0.70 - 2.70	8	Brause and Raterman (1982)
	1.50	0.50 - 3.00		Bielig *et al.* (1982)
	1.02	0.62 - 4.80	77	Fuleki *et al.* (1994)
Glucose (g/100ml)	2.07	0.89 - 3.99	93	Mattick and Moyer (1983)
	2.50	1.72 - 3.93	20	Ryan (1972)
	3.04	1.90 - 4.10	8	Brause and Raterman (1982)
	2.60	1.80 - 3.50		Bielig *et al.* (1982)
	1.53	0.70 - 2.65	77	Fuleki *et al.* (1994)

Table A1.1: Continued

Parameter	Mean	Range	Number of Samples	Source
Fructose (g/100ml)	5.79	3.00 - 10.5	93	Mattick and Moyer (1983)
	5.48	4.29 - 6.48	20	Ryan (1972)
	6.83	5.60 - 8.50	8	Brause and Raterman (1982)
	6.50	5.50 - 8.00		Bielig *et al.* (1982)
	5.67	4.12 - 7.43	77	Fuleki *et al.* (1994)
Sorbitol (g/100ml)	0.52	0.16 - 1.20	93	Mattick and Moyer (1983)
	1.01	0.57 - 1.67	20	Ryan (1972)
	0.39	0.17 - 0.63	6	Blumenthal and Helbling (1977)
	0.51	0.20 - 1.01	6	Wrolstad and Shallenberger (1981)
	0.40	0.20 - 0.70		Bielig *et al.* (1982)
	0.29	0.09 - 0.61	77	Fuleki *et al.* (1994)
Xylose (g/100ml)	0.09	tr[b] - 0.17	77	Fuleki *et al.* (1994)
	0.25	0.22 - 0.28	3	Prabha *et al.* (1990)
Raffinose (g/100ml)	0.01	tr - 0.05	77	Fuleki *et al.* (1994)
Fructose/Glucose Ratio	3.03	1.67 - 6.09	93	Mattick and Moyer (1983)
	2.37	1.68 - 3.68	8	Brause and Raterman (1982)
	2.70	1.53 - 5.26	13	Wrolstad and Shallenberger (1981)
	2.50	2.00 - 3.33		Bielig *et al.* (1982)
	4.01	2.34 - 7.64	77	Fuleki *et al.* (1994)
Titratable Acidity (% malic)	0.42	0.15 - 0.91	93	Mattick and Moyer (1983)
	0.49	0.38 - 0.58	20	Ryan (1972)
	0.48	0.19 - 1.01	29	Withy *et al.* (1978)
	0.40	0.19 - 0.85	20	Rotstein *et al.* (1969)
	0.78	0.24 - 1.50	145	Ayres and Fallows (1951)
	0.58	0.45 - 0.76		Bielig *et al.* (1982)
	0.50	0.15 - 1.17	77	Fuleki *et al.* (1994)

Table A1.1: Continued

Parameter	Mean	Range	Number of Samples	Source
pH	3.69	3.23 - 6.54	93	Mattick and Moyer (1983)
	3.45	3.38 - 3.52	9	Ryan (1972)
	3.69	3.28 - 4.10	29	Withy *et al.* (1978)
	3.44	3.20 - 4.00	20	Rotstein *et al.* (1969)
	3.62	3.22 - 4.41	77	Fuleki *et al.* (1994)
Malic Acid (mg/100ml)	628	545 - 760	20	Ryan (1972)
	508	220 - 900	30	Evans *et al.* (1983)
	1000	710 - 1330	6	Blumenthal and Helbling (1977)
	753	247 - 1573	77	Fuleki *et al.* (1994)
Citric Acid (mg/100ml)	20	10 - 40	12	Evans *et al.* (1983)
	7.5	5 - 10	6	Blumenthal and Helbling (1977)
	10	5 - 20		Bielig *et al.* (1982)
	38.7	26.3 - 71.4	77	Fuleki *et al.* (1994)
Quinic Acid (mg/100ml)	29.9	20.4 - 41.5	20	Ryan (1972)
	30.7	1 - 75.4	77	Fuleki *et al.* (1994)
Phosphoric Acid (mg/100ml)	15.1	10.5 - 25.5	20	Ryan (1972)
Fumaric Acid (mg/100ml)	0.26	0.09 - 0.43	14	Evans *et al.* (1983)
Isocitric Acid (mg/100ml)	3.2	0.5 - 8.0	6	Blumenthal and Helbling (1977)
Ascorbic Acid (mg/100ml)	0.74	0.1 - 4.1	29	Withy *et al.* (1978)
Lactic Acid (mg/100ml)	7.3	1.0 - 21.9	77	Fuleki *et al.* (1994)
Succinic Acid (mg/100ml)	1.5	0.1 - 5.1	77	Fuleki *et al.* (1994)
Shikimic Acid (mg/100ml)	0.9	0.1 - 2.6	77	Fuleki *et al.* (1994)
Polyphenolics ($A_{325\ nm}$)	0.47	0.308 - 0.651	7	Brause and Raterman (1982)

Table A1.1: Continued

Parameter	Mean	Range	Number of Samples	Source
Chlorogenic Acid (mg/100ml)	17.0	9.30 - 23.2	8	Brause and Raterman (1982)
	12.6	<1 - 32	8	Van Buren *et al.* (1976)
	7.3	1 - 8.5	77	Fuleki *et al.* (1994)
Caffeic Acid (ppm)	3.46	1.40 - 6.40	7	Brause and Raterman (1982)
Potassium (ppm)	1073	685 - 1510	93	Mattick and Moyer (1983)
	1300	1140 - 1490	21	Ryan (1972)
	1005	680 - 1280	6	Brause and Raterman (1982)
	1200	900 - 1500		Bielig *et al.* (1982)
Cadmium (ppb)	6.22	1.10 - 29.1	93	Mattick and Moyer (1983)
Calcium (ppm)	38.61	19.7 - 63.4	93	Mattick and Moyer (1983)
	59	30 - 120		Bielig *et al.* (1982)
Iron (ppm)	1.10	0.28 - 3.72	93	Mattick and Moyer (1983)
Lead (ppb)	33.6	11.3 - 163	93	Mattick and Moyer (1983)
Phosphorus (ppm)	125	29.2 - 290	93	Mattick and Moyer (1983)
Zinc (ppm)	0.37	0.15 - 1.06	93	Mattick and Moyer (1983)
Sodium (ppm)	20.8	13.5 - 53.3	93	Mattick and Moyer (1983)
	28.1	9.0 - 49.0	23	Bielig *et al.* (1982)
	35.06	4.0 - 176	18	Gortges (1981)
Ash (%)	0.21	0.11 - 0.30	93	Mattick and Moyer (1983)
	0.24	0.21 - 0.26	21	Ryan (1972)
	0.24	0.14 - 0.33	29	Withy *et al.* (1978)
	0.25	0.19 - 0.35		Bielig *et al.* (1982)
Total amino acids (meq/100ml)	0.308	0.211 - 0.470	21	Ryan (1972)
Formol number	3.19	1.67 - 7.40	7	Brause and Raterman (1982)
	4.5	2.5 - 10	7	Bielig *et al.* (1982)
Proline (ppm)	5.47	1.27 - 13.8	93	Mattick and Moyer (1983)
	3.70	2.0 - 4.4	7	Brause and Raterman (1982)
	8			Bielig *et al.* (1982)
Aspartic acid (mmol/l)	0.93	0.62 - 1.45	12	Bielig and Hofsommer (1982)

Table A1.1: Continued

Parameter	Mean	Range	Number of Samples	Source
Threonine (mmol/l)	0.05	0.02 - 0.08	12	Bielig and Hofsommer (1982)
Serine (mmol/l)	0.19	0.07 - 0.36	12	Bielig and Hofsommer (1982)
Asparagine (mmol/l)	5.25	1.63 - 12.88	12	Bielig and Hofsommer (1982)
Glutamic acid (mmol/l)	0.35	0.17 - 0.62	12	Bielig and Hofsommer (1982)
Glutamine (mmol/l)	0.05	0.01 - 0.08	12	Bielig and Hofsommer (1982)
Glycine (mmol/l)	0.02	0.01 - 0.03	12	Bielig and Hofsommer (1982)
Alanine (mmol/l)	0.13	0.08 - 0.26	12	Bielig and Hofsommer (1982)
Valine (mmol/l)	0.07	0.03 - 0.17	12	Bielig and Hofsommer (1982)
Isoleucine (mmol/l)	0.06	0.02 - 0.10	10	Bielig and Hofsommer (1982)
Leucine (mmol/l)	0.05	0.02 - 0.11	10	Bielig and Hofsommer (1982)
Phenylalanine (mmol/l)	0.04	0.01 - 0.15	10	Bielig and Hofsommer (1982)
γ- Aminobutyric acid (mmol/l)	0.09	0.03 - 0.13	12	Bielig and Hofsommer (1982)
Ammonia (mmol/l)	0.26	0.06 - 0.28	12	Bielig and Hofsommer (1982)
Lysine (mmol/l)	0.02	0.01 - 0.04	10	Bielig and Hofsommer (1982)
Histidine (mmol/l)	0.02	0.01 - 0.04	10	Bielig and Hofsommer (1982)

[a] Sum of glucose, fructose, and sucrose except for Ryan (1972), which also includes sorbitol.

[b] tr = trace

APPENDIX 2

RSK VALUES FOR AUTHENTIC APPLE JUICE

Table A2.1: RSK values for authentic apple juice (Source: Wallrauch and Faethe, 1988b).

	Units	Standard	Range	Mean
Relative density, 20°C/20°C		min 1.0450	1.0450 - 1.057	1.0488
°Brix, Ref. corrected		min 11.18	11.18 - 14.01	12.08
Total soluble solids	g/l	min 116.8	116.8 - 148.1	126.7
Titratable acids (pH 7.0) expressed as tartaric acid	g/l	min 5.0	5.0 - 8.5	6.5
Titratable acids (pH 7.0) expressed as	meq/l	min 66.7	66.7 - 113.3	86.7
Total sulphuric acid	mg/l	max 10		
Ethanol	g/l	max 3.0		
Volatile acid expressed as acetic acid	g/l	max 0.4		
Lactic acid	g/l	max 0.5		
Citric acid	mg/l		50 - 200	100
Tartaric acid	g/l		not detectable	
Glucose	g/l		18 - 35	26
Fructose	g/l		55 - 80	65
Glucose/fructose ratio		max 0.5	0.3 - 0.5	0.40
Sucrose	g/l		5 - 30	15
D-Sorbitol		min 2.5	2 - 7	4
Reduction-free extract	g/l	min 18	18 -29	22
Ash	g/l	min 2.1	1.9 - 3.5	2.55
Alkalinity number		min 11	11 - 14	13
Potassium	mg/l	min 1000	900 - 1500	1200
Sodium	mg/l	max 30		
Magnesium	mg/l		40 - 70	52
Calcium	mg/l		30 - 120	59

Table A2.1: Continued

	Units	Standard	Range	Mean
Chloride	mg/l	max 50		
Nitrate	mg/l	max 10		
Phosphate	mg/l	min 150	130 - 300	220
Sulphate	mg/l	max 150		
Formol number	0.1N NaOH/100ml		2.5 - 10	4.5
Proline	mg/l	max 15		8

For commentary on these values see Bielig *et al.* (1982); Wallrauch and Faethe, (1988b).

APPENDIX 3
SEPARATION OF SUGARS

A3.1 Separation of Sugars in a Standard and in Apple Juice

A3.1.1 Materials and Methods

The separation of glucose, fructose, sucrose each at a final concentration of 6% (w/v) and sorbitol at 0.6% (w/v) in a standard was performed on the Shodex column using the conditions recommended by the manufacturer (section 3.3.1).

A sample of Cox's Orange apples was juiced according to the procedure in section 3.2. Two 5ml aliquots of the juice were taken for further analysis. One sample was treated with 8mg of invertase and incubated at 30°C for 12 hours while the other sample was left untreated. Both samples were filtered though a 0.45μm filter before being passed through a Sep-Pak®C_{18} cartridge (section 3.5) and analysed by HPLC.

A3.1.2 Results and Discussion

The identity of each peak was established by comparing the retention times to those of individually chromatographed sugars (table A3.1). The peaks were further confirmed by observing an increase in peak size of standards that had been spiked with the individual sugars.

Table A3.1: Retention times of carbohydrates separated on a Shodex SC1011 column.

Carbohydrate	Retention Time (minutes)	Carbohydrate	Retention Time (minutes)
Sucrose	11.42	**Glucose**	13.45
Fructose	16.27	**Sorbitol**	24.62

While baseline separation of the standard containing glucose, sucrose, fructose and sorbitol was achieved (figure A3.1), the method was not suitable for the separation of these carbohydrates in apple juice as a peak (peak A), was observed to coelute with glucose (figure A3.2). In the enzyme treated sample peak A was found to also coelute with sucrose (figure A3.3). Peak A affects the size of both sucrose and glucose peaks, making accurate quantification of these sugars difficult.

It has been reported that organic acids interfere with the quantification of sugars (Anderson and Hedlund, 1983; Blanco, 1992; Frayne, 1986; Morawski, 1984: Schneider *et al.*, 1987; Schwarzenbach, 1982). Further investigations later confirmed that peak A was malic acid (appendix 4) and a technique was developed to remove it (appendices 5 and 6).

A3.2 Conclusion

The method recommended by the column manufacturer separated sucrose, glucose, fructose and sorbitol, and once the organic acids were removed baseline separation of all sugars was achieved (figure A6.2). This method was therefore used for all subsequent sugar analysis.

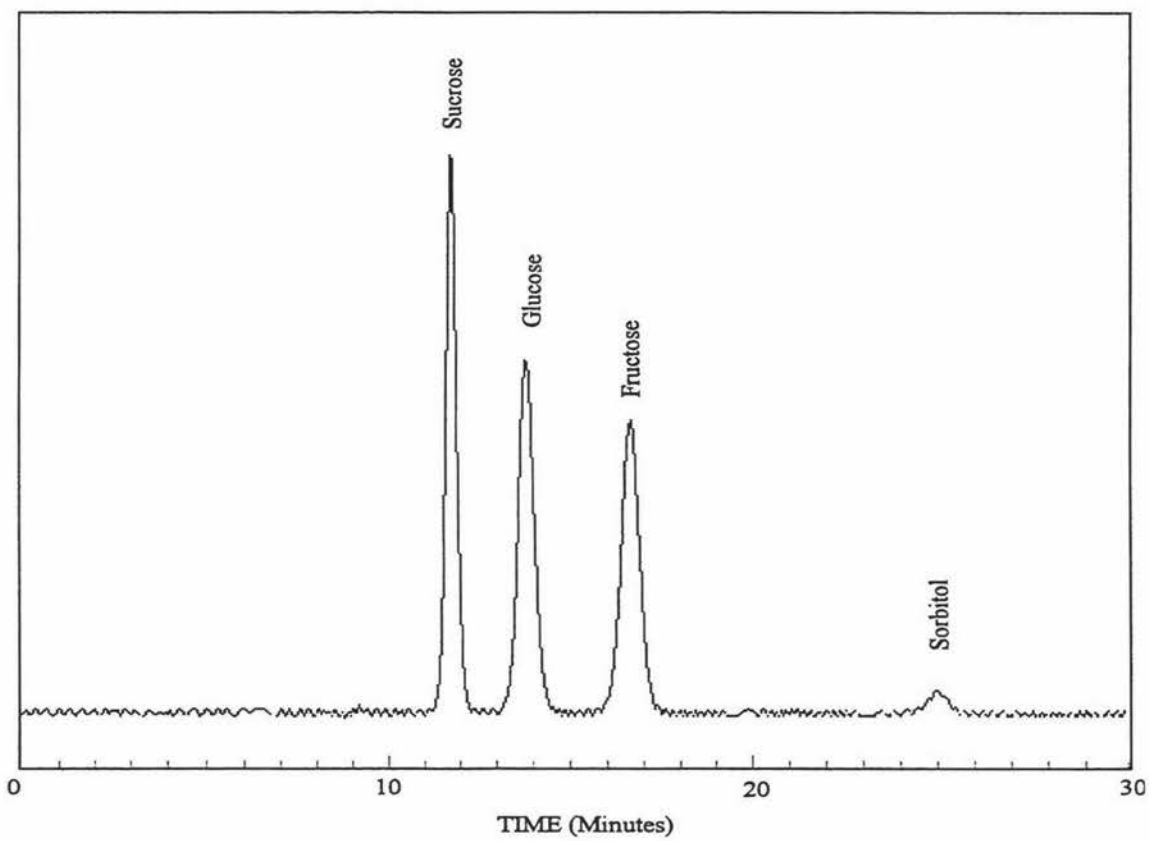

Figure A3.1: Separation of a standard sugar solution containing sucrose, glucose, fructose each at 6%(w/v) and sorbitol at 0.6%(w/v).

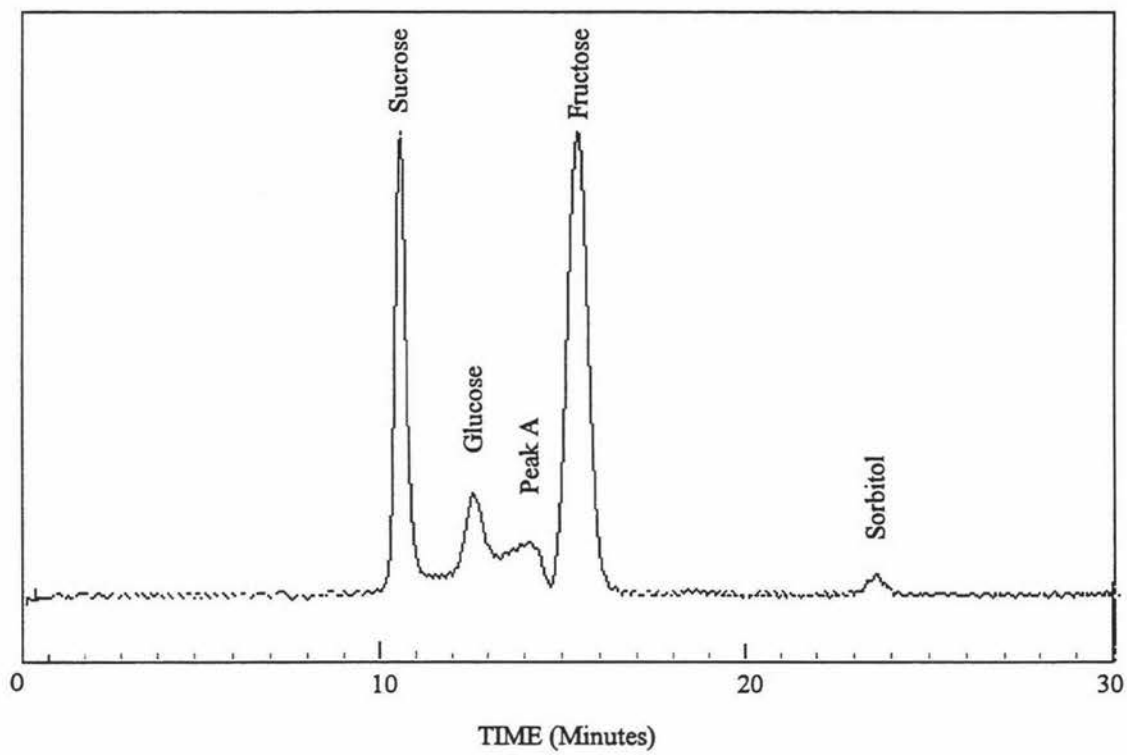

Figure A3.2: Separation of sugars in an untreated apple juice.

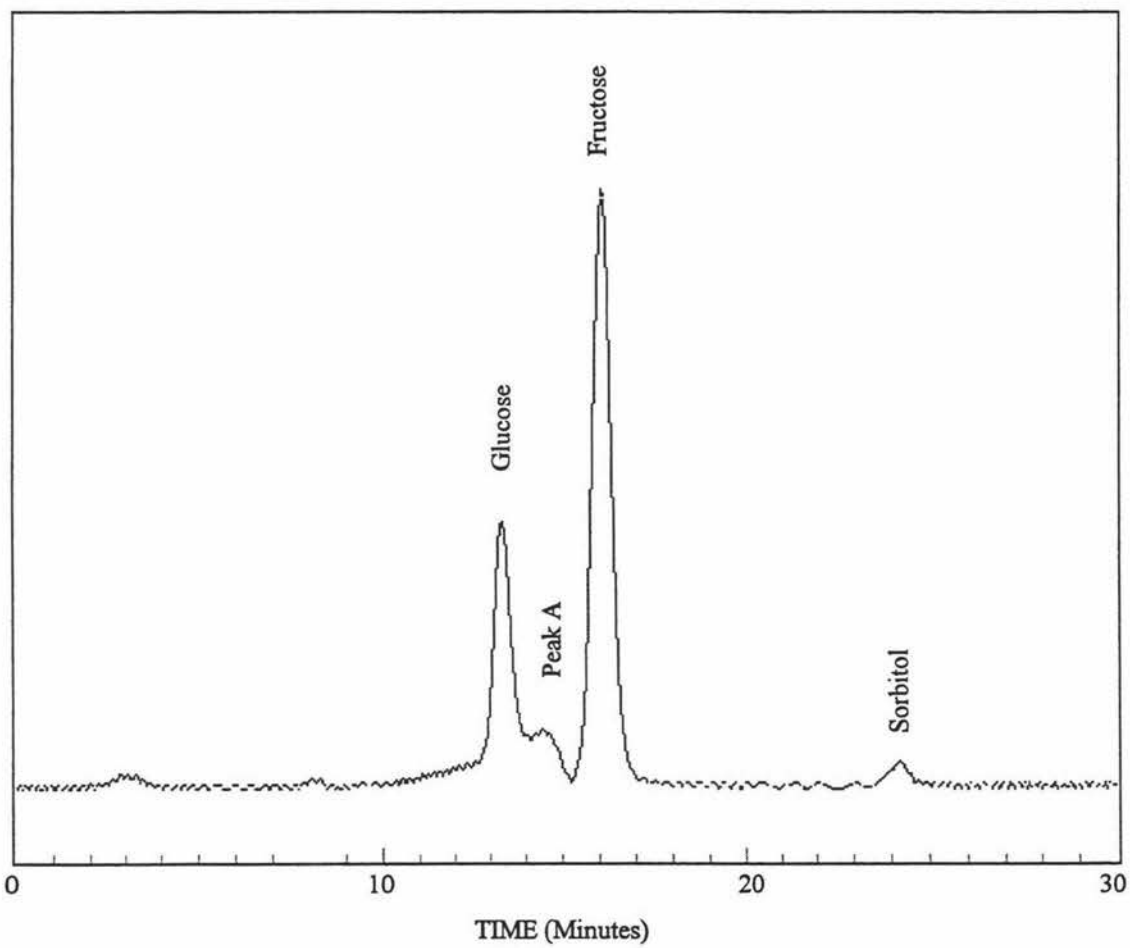

Figure A3.3: Separation of sugars in an apple juice treated with invertase.

APPENDIX 4
IDENTIFICATION OF INTERFERING PEAK IN THE QUANTIFICATION OF SUGARS IN APPLE JUICE

A4.1 Materials and Methods

An aqueous 15g/l malic acid solution was prepared and analysed by HPLC to identify peak A in apple juice.

A4.2 Results and Discussion

Malic acid peak was found to have a retention time of about 13 minutes, taking 4 minutes to completely elute (figure A4.1). Peak A in apple juice (figure A3.2) was also observed to have a similar properties and was concluded to probably be malic acid. This is in agreement with a number of reports where organic acids have been found to interfere with the quantification of sugars in foods (Anderson and Hedlund, 1983; Frayne, 1986; Morawski, 1984; Schneider *et al.*, 1987; Schwarzenbach, 1982).

A4.3 Conclusion

Malic acid was identified as coeluting with both sucrose and glucose and a method was developed to remove it so that accurate quantification of the sugars could be achieved.

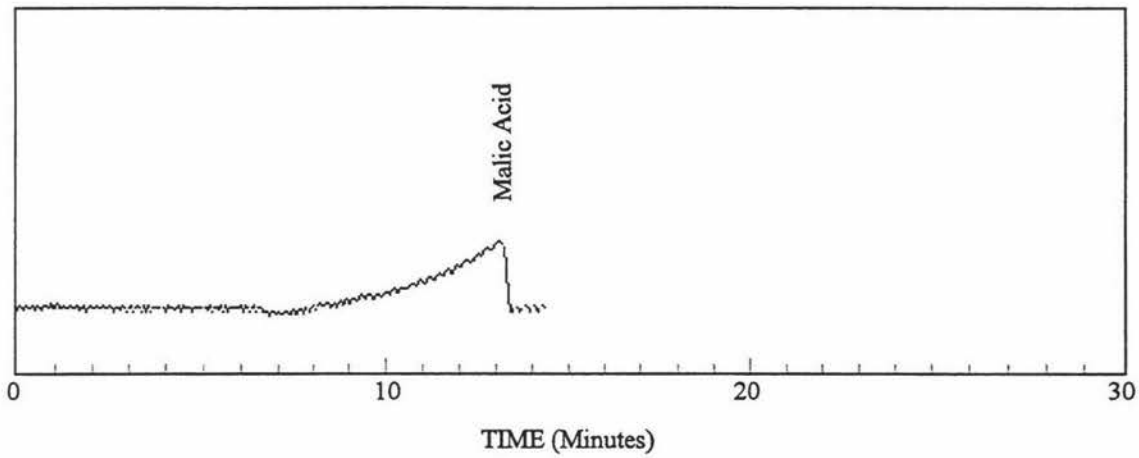

Figure A4.1: Chromatogram of a 15g/l malic acid solution.

APPENDIX 5
REMOVAL OF ORGANIC ACIDS FOR SUGAR QUANTIFICATION

A5.1 Materials and Methods

A sugar-acid standard was prepared that contained sucrose, glucose, fructose each at a final concentration of 4% (w/v), malic, quinic, citric each at 2% (w/v) and sorbitol with a final concentration of 0.4% (w/v).

A sugar standard was prepared that contained glucose, fructose, sucrose each at a concentration of 4% (w/v) and sorbitol at a concentration of 0.4% (w/v).

To 10ml of the sugar-acid standard and sugar standard, 1g of the ion exchanger aluminium oxide (alumina) was added and allowed to stand for 15 minutes with occasional stirring. After this time the ion exchanger was removed by filtering through a 0.45μm filter and the samples chromatographed (section 3.3.1). Peak heights and areas were determined and the reduction in peak size was calculated (equation 3) by comparison with a control sample that had not been treated. The result was the average by the two methods of peak quantification.

$$\%\ CHANGE\ IN\ PEAK\ SIZE = 100 - \frac{SAMPLE\ PEAK\ AREA\ (OR\ PEAK\ HEIGHT)}{CONTROL\ PEAK\ AREA\ (OR\ PEAK\ HEIGHT)} X\ 100 \qquad (3)$$

A5.2 Results and Discussion

Aluminium oxide is the packing material used in Waters Alumina Type A Sep-Pak® cartridges which are used as an alternative to acidic alumina open column and thin layer chromatography cleanup (Anon, 1991a). These cartridges were used to remove organic acids in the quantification of sugars in yoghurt without affecting the sugar levels (Morawski, 1984) and it may be possible to use these cartridges to remove organic acids from apple juice. However these cartridges were not evaluated as they are expensive.

Using the sugar-acid standard mixture, aluminium oxide was found to reduce the size of the sorbitol, glucose, fructose and sucrose peaks by 5.6, 4.2, 2.7 and 1.3% respectively. While the reduction in peak size is probably due to the absorbtion of acids on to the ion exchanger, it is also probable that some of the sugars are being retained and further work was undertaken to assess the effect of aluminium oxide on the sugar levels.

Using the sugar standard it was observed that aluminium oxide reduced the size of the fructose, glucose and sucrose peaks by 1.5, 1.1 and 0.3% respectively. Aluminium oxide has no affect on the sugar concentrations and can be used for sample clean up. Use of aluminium oxide appears to produced a slight increase (4.6%) in the size of the sorbitol peak but this increase was probably due to analytical errors in measuring the small sorbitol peak.

A method was developed to remove organic acids from apple juice using aluminium oxide that is available for the preparation of chromatography columns.

APPENDIX 6
MODE OF ADDITION OF ALUMINIUM OXIDE

A6.1 Materials and Methods

A6.1.1 Sequential Addition

A 5ml aliquot of malic acid standard (15g/l) was treated by the addition of 0.5g of aluminum oxide and allowed to stand for 15 minutes with occasional stirring. After this time the supernatant was decanted off and retreated with aluminium oxide until the sample had been treated with a total of 2.0g of aluminium oxide. The aluminum oxide was removed by filtering through a 0.45μm membrane filter. The sample was then ready for analysis by HPLC. The peak height of the treated sample was compared with an untreated sample.

An apple juice sample was also treated as described above. After removing the aluminium oxide by filtration the sample was passed through a Sep-Pak®C_{18} cartridge (section 3.5) to remove coloured pigments prior to HPLC analysis.

A6.1.2 Single Addition

A single addition of 1.0g and 2.0g of aluminium oxide was added to 5ml of malic acid standard (15g/l), allowed to stand for 15 minutes before the aluminium oxide was removed by filtering through a 0.45μm filter prior to HPLC analysis. The peak height of the treated sample was compared with an untreated sample.

A6.2 Results and Discussion

Alumina Type A Sep-Pak® cartridges contain 1.88g of aluminium oxide (Anon, 1991a) and a similar quantity (2.0g) was tested to see if removal of acid could be achieved. The addition of aluminum oxide by the sequential and single additions resulted in a reduction in the peak size by 53% to 57%, when compared to an untreated sample (table A6.1).

Table A6.1: Amount of malic acid removed by sequential and single addition of aluminium oxide

	Sequential		Single Step	
Amount Alumina Added to 5ml of Sample (g)	**Reduction in Peak Height (%)**	**Amount of Malic Acid Removed (g/l)**	**Reduction in Peak Height (%)**	**Amount of Malic Acid Removed (g/l)**
0	0	0	0	0
0.5	21	3.2		
1.0	32	4.8	31	4.7
1.5	43	6.5		
2.0	57	8.6	53	8.0

As the presence of acids in the sample reduce the sugar column's life, the acid samples analysed by HPLC were kept to a minimum, and therefore no standard curves were prepared to quantify the amount of acid removed. However as the standard contained 15g/l acid, and the peak size was reduced by around 53%, it was assumed that 53% of the acid was removed. This corresponds to over 8g/l acid being removed.

Of the two techniques examined the sequential method of addition of aluminium oxide was preferred. This was because the removal of 0.5g of aluminium oxide from the sample by filtration was easier than filtering the sample that contained 2.0g of aluminium oxide that was used in the single step method.

When the sequential mode of addition of aluminium oxide was applied to an apple juice sample, it was observed that the complete removal of the acid peak was achieved (figure A6.1), allowing accurate quantification of sucrose, glucose, fructose and sorbitol.

A6.3 Conclusion

The unknown peak that eluted between glucose and fructose was identified as the organic acid. This acid was removed by treating the apple juice with aluminium oxide. The addition of aluminium oxide in 4 sequential steps of 0.5g/5ml removed over 8g/l malic acid and resulted in baseline separation of sucrose, glucose, fructose and sorbitol (figure A6.1).

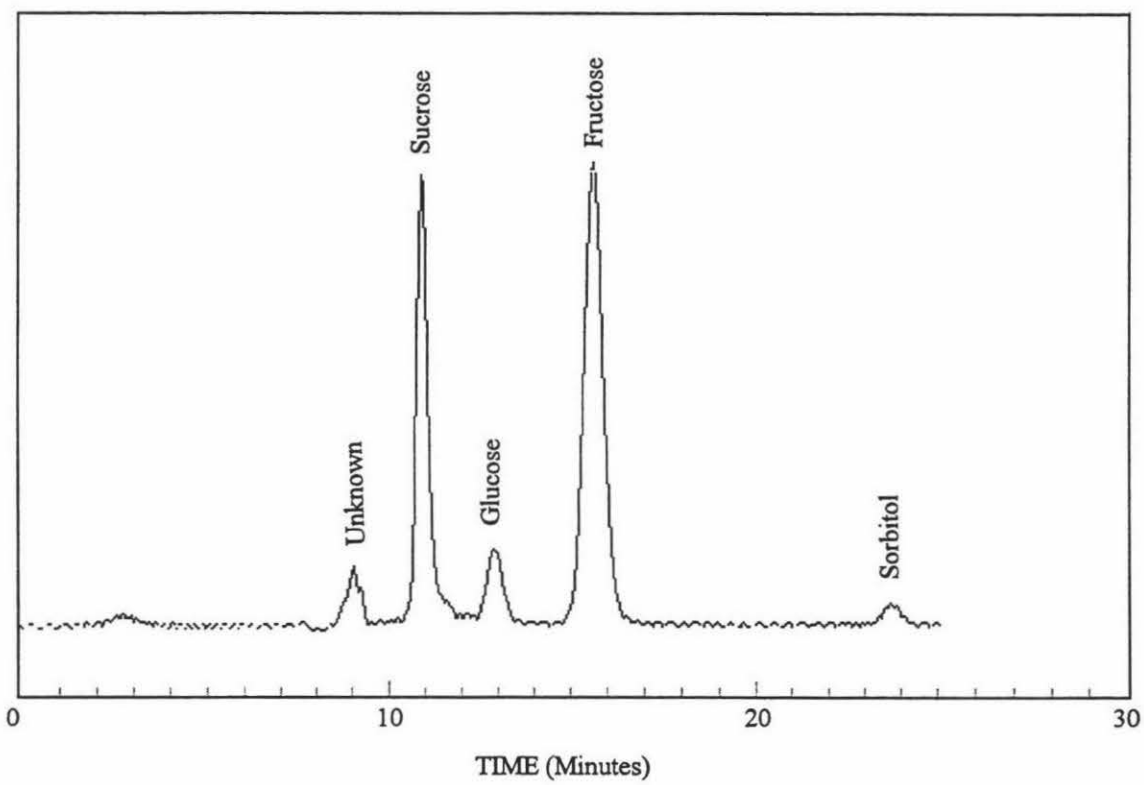

Figure A6.1: Separation of sugars in an apple juice after treatment with aluminium oxide.

APPENDIX 7

REMOVAL OF COLOURED PIGMENTS FROM APPLE JUICE

A7.1 Materials and Methods

A range of sugar standards consisting of sucrose, glucose, fructose each at a final concentration of 2, 4, 6% (w/v)and sorbitol at 0.2, 0.4 and 0.6% (w/v) was prepared. These solutions were passed through the Sep-Pak®C_{18} cartridges and 1ml aliquots sequentially collected and analysed by HPLC. The volume required to be passed through the Sep-Pak®C_{18} cartridges to avoid any dilution associated with their use was calculated.

A7.2 Results and Discussion

In order to prolong the life of the column it was necessary to remove all coloured pigments from the sample before analysis by HPLC could proceed. Before the Sep-Pak®C_{18} cartridges can be used it is recommended that they be preconditioned with an organic solvent (Anon, 1991a) and this leads to some dilution affects with the initial samples collected. From the results (figures A7.1 to A7.4) it was established that the first 2ml of sample passed through the Sep-Pak®C_{18} cartridges should be discarded to avoid dilution effects. Following sample volumes collected will be representative of the original sample.

When a heavily pigment apple juice sample is applied to a Sep-Pak®C_{18} cartridge, a total of 3ml can be applied before the cartridge becomes overloaded and the pigments pass through.

A7.3 Conclusion

The coloured pigments were removed by passing the apple juice through Sep-Pak®C_{18} cartridges. The first 2ml were discarded due to dilution effects with the next 1ml collected for HPLC analysis.

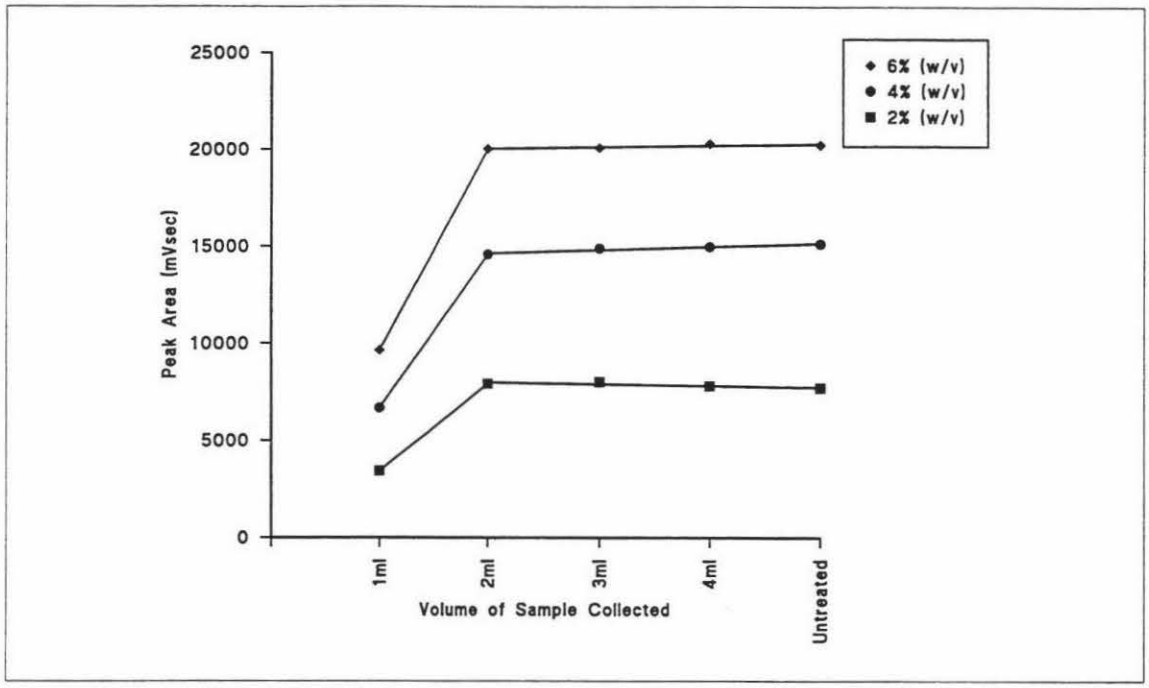

Figure A7.1: Effect of passing varying volumes of sucrose solutions through a Sep-Pak®C_{18} cartridge on peak area.

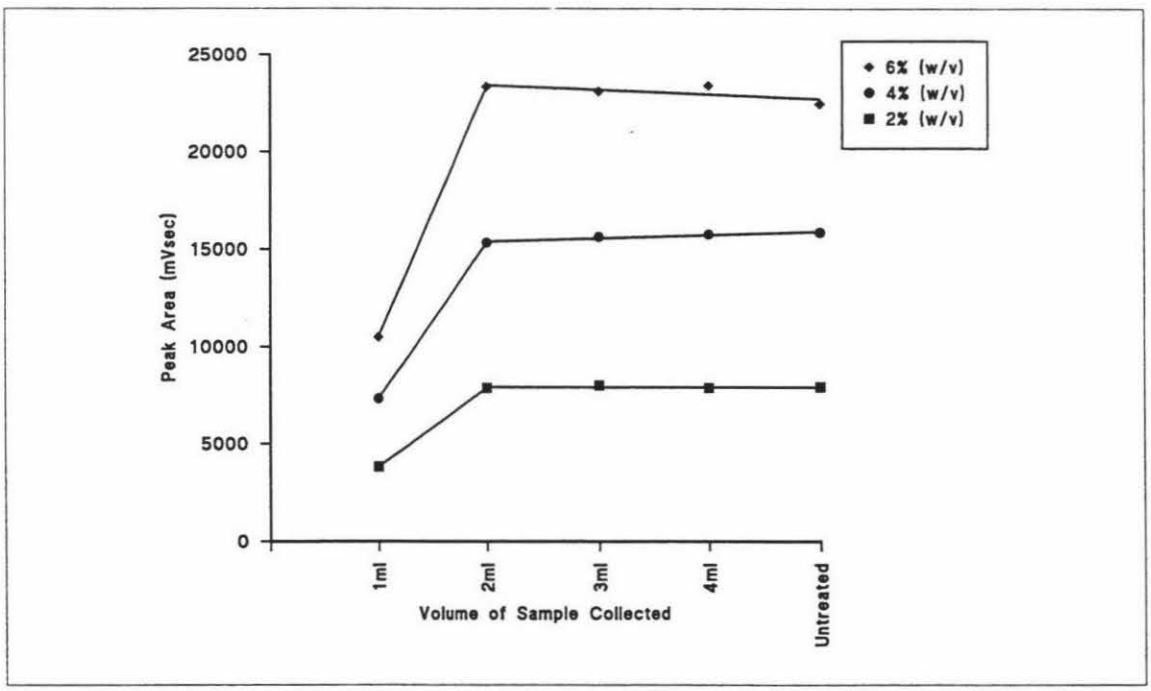

Figure A7.2: Effect of passing varying volumes of glucose solutions through a Sep-Pak®C_{18} cartridge on peak area.

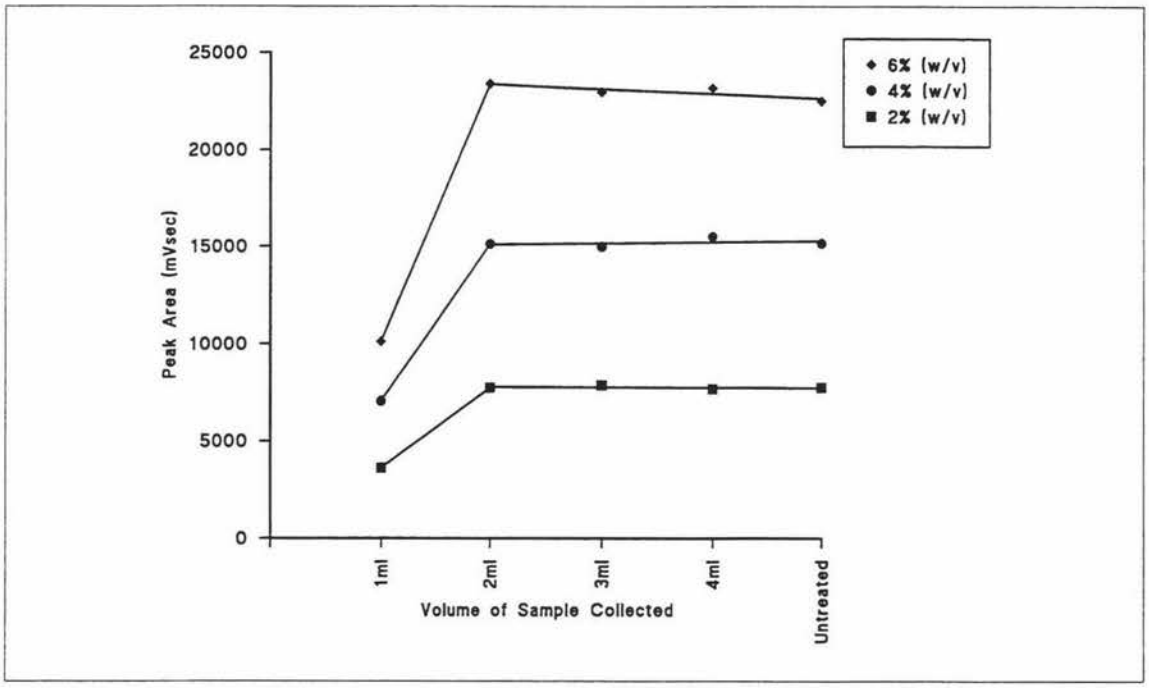

Figure A7.3: Effect of passing varying volumes of fructose solutions through a Sep-Pak®C_{18} cartridge on peak area.

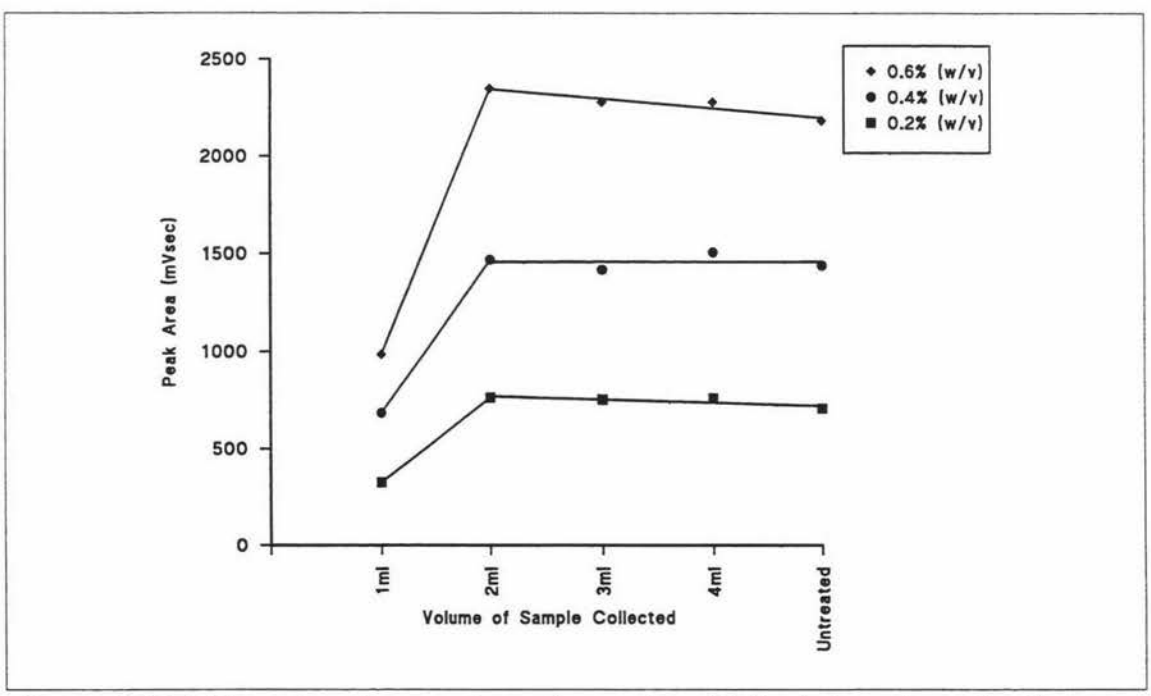

Figure A7.4: Effect of passing varying volumes of sorbitol solutions through a Sep-Pak®C_{18} cartridge on peak area.

APPENDIX 8
DETECTOR RESPONSE AND PRECISION FOR SUGAR QUANTIFICATION

The linearity of the system needed to be verified/determined over a range of sugar concentrations because of the wide range of sugar concentrations that were likely to be encountered in the juices.

A8.1 Materials and Methods

Standard solutions were prepared so that the final concentration of each individual sugar (glucose, sucrose, fructose) covered the range 1 to 10% (w/v), with sorbitol covering the range 0.1 to 1% (w/v). These solutions were made up in duplicate and replicate analysis of each solution was performed. Both peak heights and area were used to determine the detector response and the precision of each quantification method compared.

A8.2 Results and Discussion

The detector response to glucose, sucrose, fructose and sorbitol was linear over several orders of concentrations when determined by peak height and area. The sugar concentration at which the maximum linear regression coefficient (R^2) was obtained was determined to be the maximum linear range for detector response.

The detector response for glucose and fructose when determined by peak height or area, and sucrose when determined by peak area were linear up to a concentration of 9% (w/v) (figures A8.1 to A8.3). At this concentration R^2 was generally in excess of 0.9995 (table A8.1). However when sucrose was determined by peak height it was linear up to a concentration of 7% (w/v) (figure A8.1) with a maximum linear regression coefficient of 0.9975 being observed (table A8.1).

The precision or reproducibility of the method had a coefficient of variation with values typically below 2% for sucrose, glucose, and fructose when their concentration was below

9% (w/v) (tables A8.2 to A8.4), while at concentrations greater than 9% (w/v) the coefficient of variation increased and ranged from 2.6% to 4.2%. The decrease in the linear regression coefficient and the increase in coefficient of variation at high sugar levels was due to the detector response becoming non linear.

To quantify the sugars accurately, glucose and fructose concentration in apple juice must be below 9% (w/v) and sucrose below 7% (w/v), or when related to an injection volume of 10µl, 0.9mg and 0.7mg respectively. Juice which exceeded these concentrations must be diluted for accurate testing.

Sorbitol was linear up to a concentration of 1% (w/v) (figure A8.4) with R^2 increasing as the concentration of sorbitol increased (table A8.1). At a concentration of 1% (w/v) sorbitol was observed to have a R^2 of about 0.997 when determined by either peak height or area. The precision of the method for determining sorbitol was observed to have a coefficient of variation of between 1.7% and 13.5%. An increase in the coefficient of variation being observed as the sorbitol concentration decreased (table A8.5). This increase was due to the small peaks obtained at low sorbitol concentrations, making accurate integration difficult. Quantification is made even more difficult when the peaks are broad due to late elution. The integration of small broad peaks affects peak area analysis more than peak heights. Coefficient of variation of between 6.5% and 13.5% were observed when sorbitol was determined by peak area at concentration less than 0.6% (w/v). However when sorbitol was determined by peak height over the same concentration range the coefficient of variation decreased to between 2% and 8.6%.

A8.3 Conclusion

The maximum level of sucrose, glucose, fructose and sorbitol reported by Lee and Wrolstad (1988b) and Mattick and Moyer (1983) were 5.6, 4.1, 8.0 and 1.7% (w/v) respectively. Over this range and with a sample injection volume of 10µl the detector response was linear enabling the direct injection of undiluted apple juice samples.

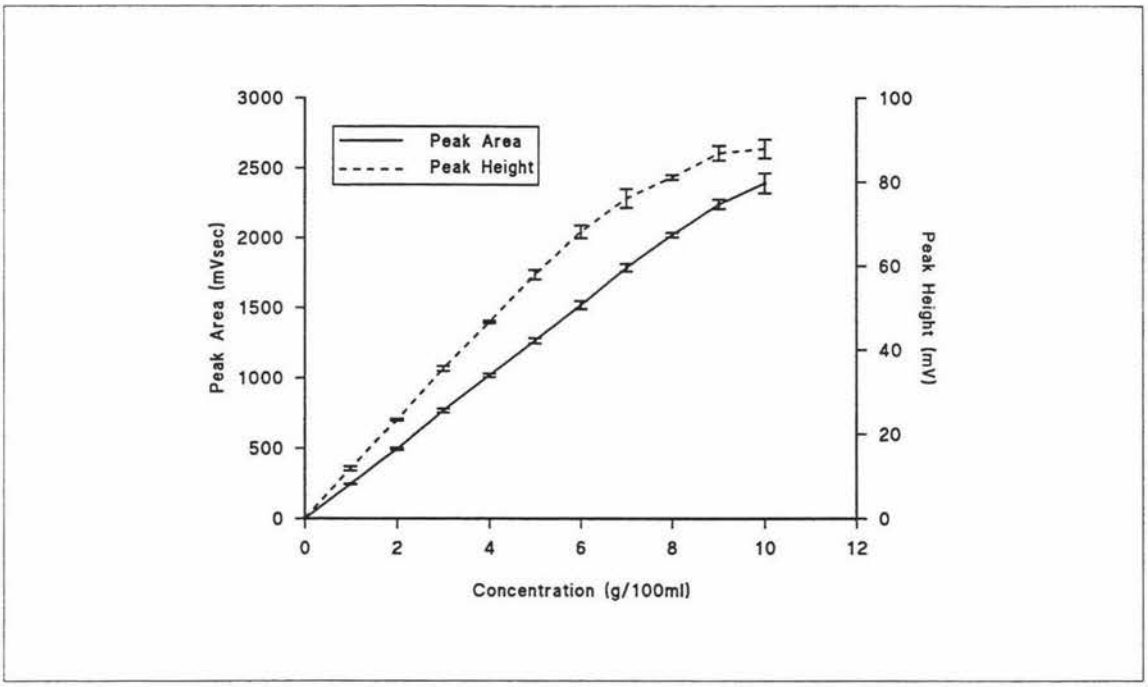

Figure A8.1: Effect of sucrose concentrations on detector response.

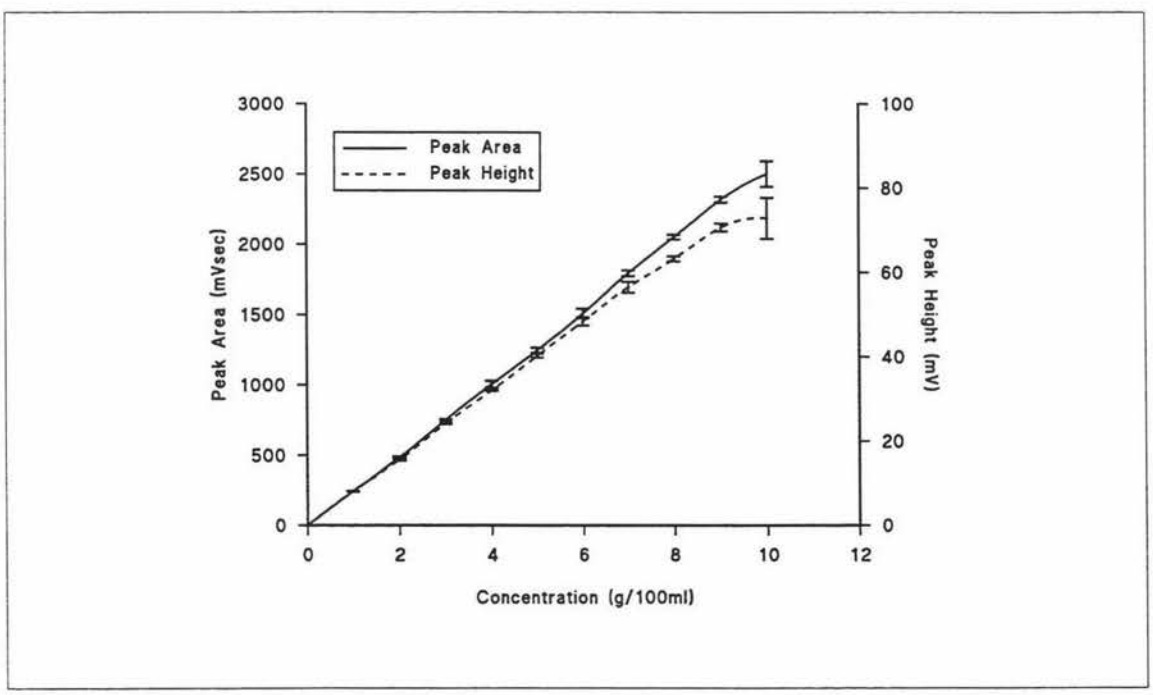

Figure A8.2: Effect of glucose concentrations on detector response.

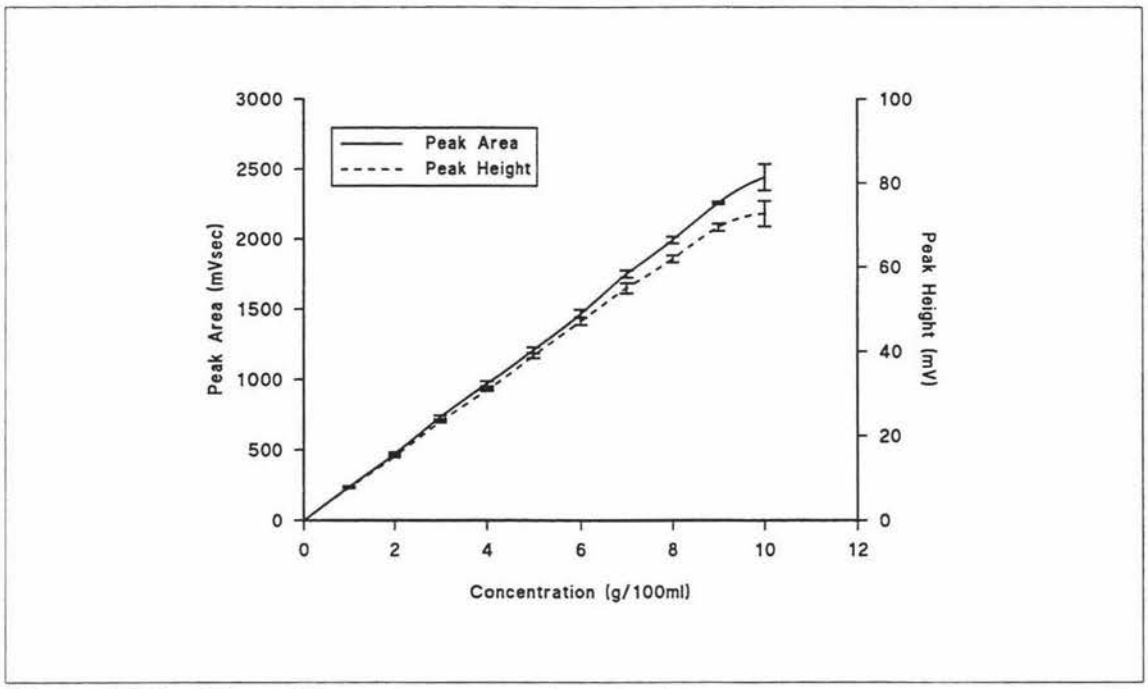

Figure A8.3: Effect of fructose concentrations on detector response.

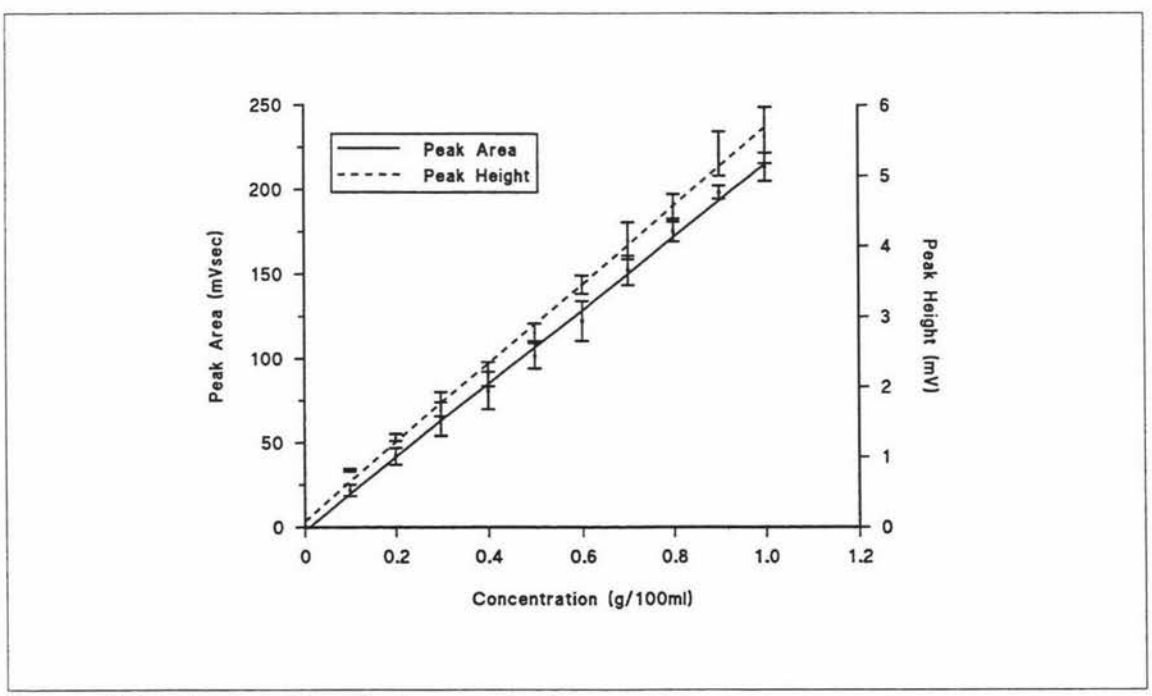

Figure A8.4: Effect of sorbitol concentrations on detector response.

Table A8.1: Linear regression coefficients for sucrose, glucose, fructose and sorbitol at varying concentrations.

	Concentration (% w/v)	Peak Area	Peak Height		Concentration (% w/v)	Peak Area	Peak Height
Sucrose	10	0.9980	0.9701	Glucose	10	0.9994	0.9950
	9	0.9997	0.9848		9	0.9996	0.9995
	8	0.9999	0.9911		8	0.9996	0.9997
	7	0.9999	0.9975		7	0.9996	0.9996
Fructose	10	0.9994	0.9971	Sorbitol	1.0	0.9976	0.9964
	9	0.9995	0.9998		0.9	0.9969	0.9960
	8	0.9995	0.9998		0.8	0.9965	0.9940
	7	0.9994	0.9998		0.7	0.9967	0.9924

Table A8.2: Comparison of peak heights with peak areas for sucrose.

	Sucrose Peak Area			Sucrose Peak Height		
Concentration (% w/v)	Mean (mv min)	Standard Deviation (mv min)	Coefficient of Variation (%)	Mean (mv)	Standard Deviation (mv)	Coefficient of Variation (%)
1	243.7	2.3	1.0	11.8	0.4	3.6
2	492.7	7.6	1.6	23.4	0.2	0.9
3	767.1	11.2	1.4	35.6	0.5	1.4
4	1017.5	10.9	2.1	46.6	0.3	0.6
5	1264.7	15.6	1.2	59.0	1.0	1.7
6	1518.6	25.1	1.7	68.1	1.3	1.9
7	1787.6	23.6	1.3	76.1	1.9	2.6
8	2020.4	13.5	0.7	81.1	0.5	0.6
9	2242.2	29.3	1.3	86.9	1.5	1.7
10	2392.6	61.6	2.6	87.9	1.9	2.2

Table A8.3: Comparison of peak heights with peak areas for glucose.

	Glucose Peak Area			Glucose Peak Height		
Concentration (%w/v)	Mean (mv min)	Standard Deviation (mv min)	Coefficient of Variation (%)	Mean (mv)	Standard Deviation (mv)	Coefficient of Variation (%)
1	242.8	1.1	0.5	8.1	1.0	1.2
2	483.4	5.8	1.2	15.6	0.2	1.2
3	750.3	5.8	0.8	24.2	0.2	0.7
4	1003.8	20.4	2.0	32.0	0.1	0.4
5	1245.2	15.9	1.3	40.2	0.4	0.9
6	1508.4	30.4	2.0	48.4	0.8	1.7
7	1794.5	18.1	1.0	56.5	1.1	2.0
8	2051.4	14.0	0.7	63.2	0.5	0.8
9	2318.1	17.8	0.8	70.7	0.8	1.2
10	2500.8	78.8	3.2	72.8	4.2	5.8

Table A8.4: Comparison of peak heights with peak areas for fructose.

	Fructose Peak Area			Fructose Peak Height		
Concentration (%w/v)	Mean (mv min)	Standard Deviation (mv min)	Coefficient of Variation (%)	Mean (mv)	Standard Deviation (mv)	Coefficient of Variation (%)
1	236.6	4.4	1.9	7.7	0.1	1.1
2	470.2	9.4	2.0	15.1	0.2	1.3
3	730.4	10.6	1.5	23.3	0.2	0.8
4	969.5	16.0	1.7	30.8	0.2	0.7
5	1208.7	16.7	1.4	39.0	0.5	1.3
6	1465.7	25.4	1.7	47.1	0.8	1.7
7	1749.6	22.4	1.3	55.0	1.1	1.9
8	1991.0	20.5	1.0	61.9	0.7	1.1
9	2257.7	8.7	0.4	69.4	0.8	1.1
10	2442.3	81.4	3.3	72.7	2.7	3.7

Table A8.5: Comparison of peak heights with peak areas for sorbitol.

	Sorbitol Peak Area			Sorbitol Peak Height		
Concentration (%w/v)	**Mean (mv min)**	**Standard Deviation (mv min)**	**Coefficient of Variation (%)**	**Mean (mv)**	**Standard Deviation (mv)**	**Coefficient of Variation (%)**
0.1	21.7	2.9	13.2	0.8	0.1	2.1
0.2	41.9	4.3	10.3	1.3	0.1	3.4
0.3	64.1	8.7	13.5	1.8	0.2	8.6
0.4	81.0	9.7	11.9	2.2	0.2	6.8
0.5	101.7	6.6	6.5	2.8	0.1	3.9
0.6	122.3	10.2	8.3	3.5	0.1	3.2
0.7	152.2	7.5	4.9	4.1	0.2	5.5
0.8	175.1	5.1	2.9	4.6	0.2	3.3
0.9	198.4	3.4	1.7	5.3	0.3	5.1
1.0	213.6	7.2	3.4	5.6	0.3	6.2

APPENDIX 9
QUANTIFICATION OF SUGAR PEAKS

A9.1 Materials and Methods

A mixed sugar standard containing glucose, fructose, sucrose, each at either 2, 4 or 6% (w/v) and sorbitol at either 0.2, 0.4 or 0.6% (w/v) was used to establish calibration curves. These standards were run at the start and end of each day to determine the stability and precision of the HPLC system. Both peak height and area were used to quantify the peaks and the values normalised to remove day to day variation. Normalisation of each individual sugar was performed by taking the ratio of peak height or area at each concentration to the peak height or area obtained at a sugar concentration of 4% (w/v).

Two way analysis of variance was performed on the normalised calibration data for each sugar (glucose, sucrose, fructose and sorbitol) in the standards to assess the within day variation (difference between calibration curves constructed at the start and end of the day) and the peak quantification method (peak area or height) (Two way analysis of variance was not performed on sugars at a concentration of 4% (w/v) as they always had a value of 1 after normalisation).

The within day variation and peak quantification method for apple juice samples were also compared using two way analysis of variance. These samples were not normalised and the analysis carried out on the actual sugar concentrations obtained for each sample.

A9.2 Results and Discussion

There was no significant difference at the 99% confidence level for the quantification of glucose and fructose at concentrations of 2% (w/v) and 6% (w/v) by either peak height or area. There was a significant difference at the 99% confidence level when determining the sucrose concentrations of 6% (w/v) by peak height and area (table A9.1). This is probably due to the detector response becoming non-linear. The determination of sorbitol by peak

height or area over the range 0.2% (w/v) to 0.6% (w/v) showed significant difference at the 99% confidence level, due to the peaks being small and broad, making quantification difficult.

There was no significant difference for sucrose, glucose, fructose or sorbitol when determined using either beginning or end of the day calibration curves, indicating that the HPLC conditions remained stable throughout the day.

Two way analysis of variance of all apple juice samples in this study showed there was no significant difference in using peak height or area to quantify sucrose, glucose, fructose or sorbitol (table A9.2). The use of calibration curves constructed at the start and end of the day to quantify sugars in apple juice also showed no significant differences. Therefore glucose, sucrose, fructose and sorbitol in apple juice samples were determined by averaging the 4 values calculated from peak area and height using calibration plots constructed at the start and end of the day. As replicate injection of each juice sample occurred, the final reported result for each sample is an average of 8 calculations.

Table A9.1: Summary of two way analysis of variance on normalised data for sucrose, glucose, fructose and sorbitol standards.

Compound	Concentration	Factor	F-ratio	Significance
Sucrose	2	start day/end day	0.634	ns
	2	peak area/peak height	0.285	ns
	2	startend*areaheight	0.382	ns
	6	start day/end day	0.959	ns
	6	peak area/peak height	25.82	*
	6	startend*areaheight	$5.1x10^{-6}$	ns
Glucose	2	start day/end day	0.431	ns
	2	peak area/peak height	0.068	ns
	2	startend*areaheight	0.464	ns
	6	start day/end day	2.313	ns
	6	peak area/peak height	0.276	ns
	6	startend*areaheight	0.246	ns
Fructose	2	start day/end day	0.155	ns
	2	peak area/peak height	$7.1x10^{-3}$	ns
	2	startend*areaheight	0.100	ns
	6	start day/end day	1.760	ns
	6	peak area/peak height	0.049	ns
	6	startend*areaheight	0.283	ns
Sorbitol	0.2	start day/end day	2.653	ns
	0.2	peak area/peak height	44.82	*
	0.2	startend*areaheight	0.915	ns
	0.6	start day/end day	1.214	ns
	0.6	peak area/peak height	14.07	*
	0.6	startend*areaheight	$1.7x10^{-3}$	ns

ns, not significant; *, significance at the $P \leq 0.01$
start day/end day = calibration curves constructed at the start and end of each day
peak area/peak height = quantification of standards by peak area and height
startend*areaheight = Interaction term

Table A9.2: Summary of two way analysis of sucrose, glucose, fructose and sorbitol for apple juice samples.

Compound	Factor	F-ratio	Significance
Sucrose	start day/end day	0.116	ns
	peak area/peak height	0.263	ns
	startend*areaheight	0.024	ns
Glucose	start day/end day	6.2×10^{-3}	ns
	peak area/peak height	0.033	ns
	startend*areaheight	8.0×10^{-4}	ns
Fructose	start day/end day	0.018	ns
	peak area/peak height	0.204	ns
	startend*areaheight	0.077	ns
Sorbitol	start day/end day	0.067	ns
	peak area/peak height	0.293	ns
	startend*areaheight	0.154	ns

ns, not significant
start day/end day = calibration curves constructed at the start and end of each day
peak area/peak height = quantification of standards by peak area and height
startend*areaheight = Interaction term

APPENDIX 10
RECOVERY OF SUGARS

A10.1 Materials and Methods

The recoveries of glucose, sucrose fructose and sorbitol were determined by:

(i) diluting an apple juice by 20%, 40%, 60% and 80% and analysing the sample by HPLC

(ii) dilution of a apple juice by 80% and adding various levels of sucrose, glucose, fructose and sorbitol and analysing the sample by HPLC

The recoveries for glucose, fructose, sucrose and sorbitol were based on the difference between the level observed in the spiked or diluted samples and the amount of the individual sugars expected to be present from known additions.

A10.2 Results and Discussion

The recoveries for sucrose, glucose, fructose were between 94% and 105.4% and sorbitol ranging from 82% to 109% (tables A10.1 and A10.2). Overall the recoveries testify to the accuracy of the method used to quantify glucose, fructose and sucrose. Sorbitol had the largest variation due to the difficulties in quantifying the small peaks.

Table A10.1: Recoveries of sucrose, glucose, fructose and sorbitol in diluted apple juice.

Compound	% Dilution	Calculated Concentration (g/100ml)	Measured concentration (g/100ml)	% Recovery
Sucrose	Undiluted	2.649	2.649	100.0
	20	2.119	2.158	101.8
	40	1.589	1.622	102.0
	60	1.060	1.117	105.4
	80	0.530	0.546	103.0
Glucose	Undiluted	2.299	2.299	100.0
	20	1.840	1.823	99.1
	40	1.380	1.298	94.0
	60	0.920	0.927	100.7
	80	0.460	0.447	96.0
Fructose	Undiluted	6.867	6.867	100.0
	20	5.494	5.543	100.9
	40	4.120	3.973	96.4
	60	2.747	2.700	98.3
	80	1.373	1.317	95.9
Sorbitol	Undiluted	0.868	0.868	100.0
	20	0.694	0.685	98.6
	40	0.521	0.500	96.0
	60	0.347	0.352	101.5
	80	0.174	0.162	95.0

Table A10.2: Recoveries for sucrose, glucose, fructose and sorbitol in diluted apple juice spiked with varying concentrations of individual sugars.

Compound	Amount of Added Sugar (g/100ml)	Calculated Concentration (g/100ml)	Measured Concentration (g/100ml)	% Recovery
Sucrose	0.0	0.523	0.523	100.0
	1.0	1.523	1.522	100.0
	1.5	2.023	1.981	98.0
	2.0	2.523	2.465	97.7
	2.5	3.023	3.047	100.8
	3.0	3.523	3.655	103.8
	3.5	4.023	4.107	102.1
Glucose	0.0	0.377	0.377	100.0
	1.0	1.377	1.371	99.5
	1.5	1.877	1.795	95.6
	2.0	2.377	2.265	95.3
	2.5	2.877	2.845	98.9
	3.0	3.377	3.419	101.2
	3.5	3.877	3.842	99.1
Fructose	0.0	1.174	1.174	100.0
	1.0	2.174	2.215	101.8
	1.5	2.674	2.584	96.6
	2.0	3.174	3.085	97.2
	2.5	3.674	3.706	100.9
	3.0	1.474	4.282	102.6
	3.5	4.674	4.717	100.9
Sorbitol	0.00	0.145	0.145	100.0
	0.10	0.245	0.260	106.4
	0.15	0.295	0.242	82.0
	0.20	0.345	0.290	84.1
	0.25	0.395	0.422	106.9
	0.30	0.445	0.485	109.1
	0.35	0.465	0.539	109.0

APPENDIX 11
SEPARATION OF ORGANIC ACIDS

A11.1 Materials and Methods

The determination of organic acids in apple juice was based on the high pressure liquid chromatography procedure developed in this laboratory (Davis and Husbands, 1988). This procedure uses a reverse phase C_{18} guard column (30mm x 4.6mm) and two Brownlee™ Labs analytical columns (220mm x 4.6mm Spheri-5 reverse phase C_{18} and 220mm x 4.6mm Polypore-H cation exchange) in series. A mobile phase of 0.01M phosphoric acid at a flow rate of 0.25ml/min was used. Detection was by absorbance at 210nm. The best separation was achieved by maintaining the column temperature at 31°C to 32°C.

Individual standards of malic, quinic, citric, succinic all at 1g/l, shikimic and fumaric at 0.1g/l, fructose, sucrose and glucose at 5g/l were prepared and chromatographed to obtain the retention times of each compound.

An apple juice was also chromatographed to determine if resolution of these acids could be achieved.

A11.2 Results and Discussion

The retention time of each component was determined and is shown in table A11.1. All acids had different retention times and are able to be resolved, but the times indicate that incomplete resolution of sugars would occur. In apple juice there was incomplete separation of fructose and quinic acid with quinic acid occurring as a shoulder on the fructose peak. This is because fructose is present at much greater concentrations than quinic and the detector response to fructose masks the quinic acid present. Further development work to the procedure of Davis and Husbands (1988) is required.

Table A11.1: Retention times of organic acids and sugars using Spheri-5 reverse phase C_{18} and Polypore-H columns in series maintained at 31 to 32°C with a mobile phase of 0.01M phosphoric acid at a flow rate of 0.25ml/min and detection at 210nm.

Compound	Retention Time (minutes)	Compound	Retention Time (minutes)
Glucose	14.8	**Sucrose**	15.1
Fructose	15.4	**Quinic**	16.7
Malic	18.5	**Shikimic**	19.6
Citric	24.9	**Succinic**	28.3
Fumaric	34.3		

APPENDIX 12

METHOD DEVELOPMENT FOR THE SEPARATION OF ORGANIC ACIDS

Rather than using lengthy clean up procedures (such as the separation of the apple juice into acidic and sugar fractions with ion exchange columns) to reduce the interference of sugars in the detection of organic acids other techniques were investigated. These included varying the detector wavelength, column type and changing the operating conditions such as mobile phase, flow rate and column temperature.

A12.1 Detector Wavelength

A12.1.1 Materials and Methods

Three standard sugar solutions that contain 10% (w/v) of either glucose, fructose and sucrose were prepared. Each solution was analysed at 210nm and 214nm using an ultraviolet-visible spectrophotometer (Ultrospec II from Pharmacia LKB Biochrom Ltd).

A12.1.2 Results and Discussion

It was found that all three sugars had a lower absorbance at 214nm than at 210nm (table A12.1), and that fructose was the strongest being 50 times that of glucose. At this wavelength any interference by sugars could be lessened and hence all subsequent organic acid analyses were carried out at 214nm.

Table A12.1: Absorbance of a 10% (w/v) glucose, sucrose and fructose solution at 210nm and 214nm.

Sugar	A_{210}	A_{214}
Glucose	0.084	0.022
Fructose	1.724	1.080
Sucrose	0.173	0.085

A12.2 HPLC Column and Mobile Phase

As Coppola and Starr (1988) recommend that two HPLC columns (reverse phase C_{18} and Radial-Pak C_{18} or a second reverse phase C_{18} columns if a Radial-Pak C_{18} is unavailable) be used, the Polypore-H column was replaced with a Spheri-5-ODS reverse phase C_{18} column and the effects of this change investigated. At the same time the mobile phase was changed from phosphoric acid to 0.005M sulphuric acid because of the superior separation power reportedly achieved (Schwarzenbach, 1982).

A12.2.1 Materials and Methods

Individual organic acid standards of quinic, malic, citric, succinic all at 1g/l, shikimic and fumaric at 0.1g/l and fructose at 5g/l were prepared in Milli-Q water. These acids were combined to prepare a mixture so that the final concentration of each acid was the same as the individual standards. This mixture also contained fructose at a final concentration of 80g/l.

The standards were analysed using the following HPLC conditions: Brownlee™ Labs 30mm x 4.6mm Reverse Phase C_{18} guard column, 220mm x 4.6mm Spheri-5 Reverse Phase C_{18} and Spheri-5 ODS Reverse Phase C_{18} coupled together in series, detection wavelength of 214nm, mobile phase of 0.005M sulphuric acid at a flow rate of 0.5ml/min and the column maintained at a temperature of 22°C.

A12.2.2 Results and Discussion

The identity of each peak in the standard mixture was determined by comparing the retention times to those of organic acids chromatographed individually (table A12.2).

By substituting the Polypore-H column with a second reverse phase C_{18} column, as recommended by Coppola and Starr (1988), and changing the mobile phase from phosphoric to dilute sulphuric acid, separation of fructose from quinic acid was achieved (figure A12.1).

Table A12.2: Retention times of organic acids and sugars using a mobile phase of 0.005M sulphuric acid at a flow rate of 0.5ml/min and Spheri-5 reverse phase C_{18} and Spheri-5 ODS reverse phase C_{18} columns in series maintained at 22°C with detection at 214nm.

Compound	Retention Time (minutes)	Compound	Retention Time (minutes)
Fructose	19.0	**Quinic**	20.9
Malic	24.4	**Shikimic**	26.4
Citric	43.9	**Succinic**	45.4
Fumaric	50+		

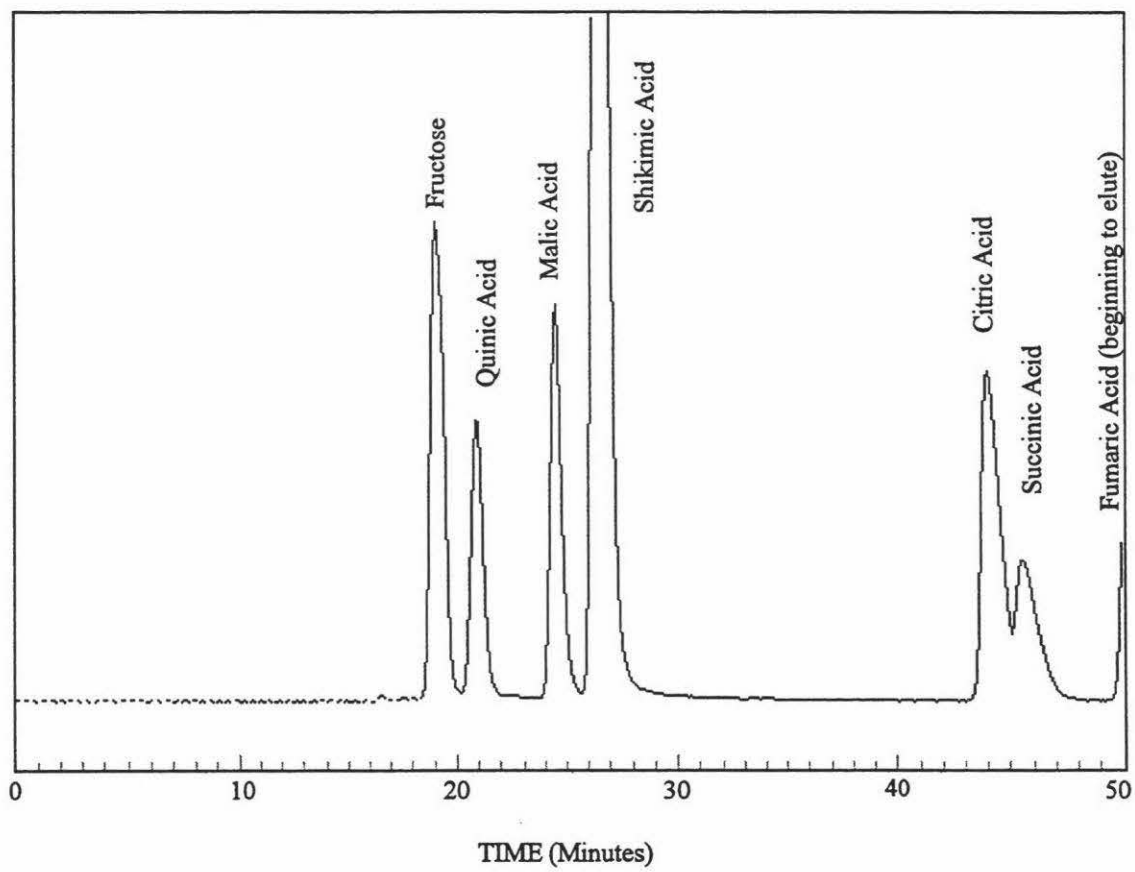

Figure A12.1: Separation of a standard solution containing fructose, quinic, malic, shikimic, citric, succinic and fumaric acids using a mobile phase of 0.005M sulphuric acid at a flow rate of 0.5ml/min and Spheri-5 reverse phase C_{18} and Spheri-5 ODS reverse phase C_{18} columns in series maintained at 22°C with detection at 214nm.

This change also maintained the separation of malic, shikimic, citric, succinic and fumaric acids. Unfortunately the analysis time was considered excessive with fumaric acid eluting after 50 minutes. Further work is required to reduce the analysis time.

A12.3 Reduction of Analysis Time

A12.3.1 Materials and Methods

An organic acid standard mixture that contained final concentration of 1g/l of each of the acids malic, quinic, citric, succinic, 0.1g/l of shikimic and fumaric acids and 80g/l fructose was prepared. A number of mobile phase flow rates and column temperatures were examined to reduce the analysis time and maximise the separation of organic acids and fructose.

The applicability of changes in temperature and flow rate during the analysis of apple juice was confirmed.

A12.3.2 Results and Discussion

The developed method enabled separation of fructose and quinic acid to be achieved, but the analysis time was in excess of 50 minutes (section A12.2). Increasing the flow rate from 0.25ml/min to 0.8ml/min reduced the analysis time to 20 minutes but citric and succinic acids coeluted (figure A12.2). A summary of changes to flow rate and column temperatures examined are given in table A12.3. Increasing the column temperature from 22°C to 32°C resulted in the separation of these acids but the increase in temperature also reduced resolution between fructose, quinic, malic and shikimic acids.

It was observed that a low flow rate was required to achieve separation of fructose, quinic, malic and shikimic acids. A high flow rate reduced the analysis time but also reduced the resolution of fructose and quinic, malic and shikimic acids however adequate separation of the late eluting acids (citric, succinic and fumaric) was maintained. Therefore the final

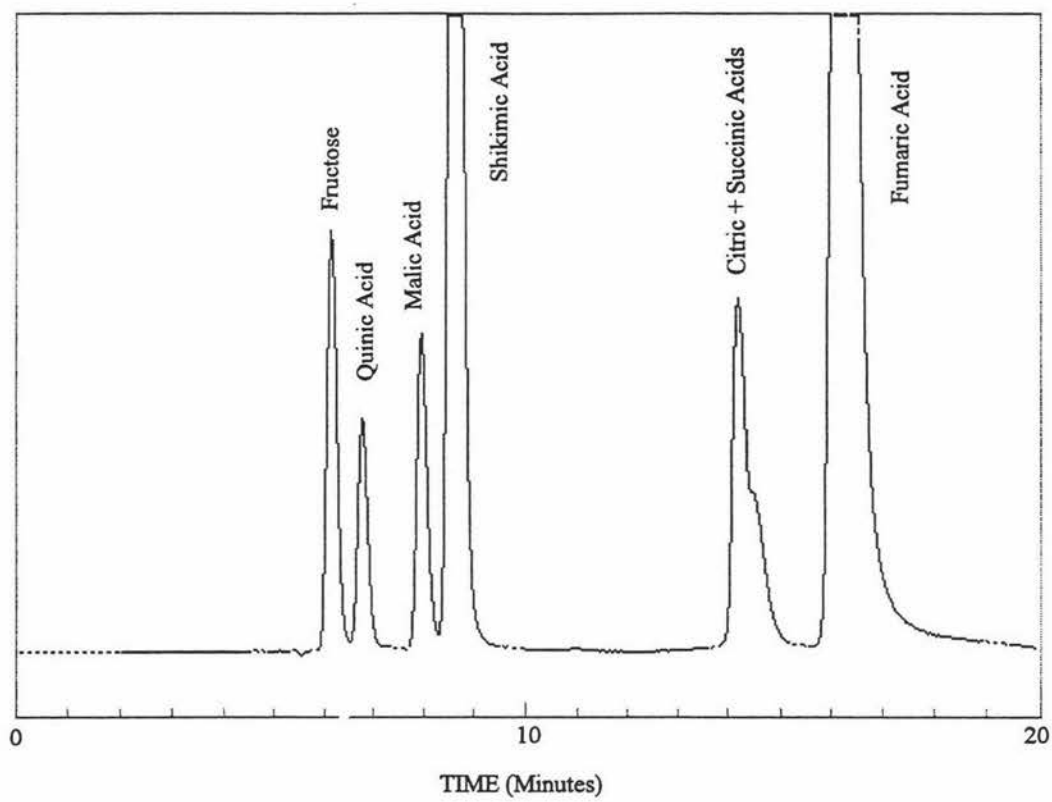

Figure A12.2: Separation of a standard solution containing fructose, quinic, malic, shikimic, citric, succinic and fumaric acids using a mobile phase of 0.005M sulphuric acid at a flow rate of 0.8ml/min and Spheri-5 reverse phase C_{18} and Spheri-5 ODS reverse phase C_{18} columns in series maintained at 22°C with detection at 214nm.

Table A12.3: Effect of changes in mobile phase flow rates and column temperature on separation of organic acids and fructose.

Flow Rate (ml/min)	Temperature (°C)	Analysis Time (minute)	Comments
0.25	22	50	Partial separation of succinic and citric. long analysis time
0.80	22	20	Unable to resolve succinic and citric
0.80	32	16	Reduced resolution of fructose, quinic, malic and shikimic. Complete separation of succinic and citric
0.60	32	24	Reduced resolution of fructose, quinic, malic and shikimic. Complete separation of succinic and citric
0.40 ml/min for 20 min +0.8 ml/min for 10 min	32	30	Resolution of all acids and fructose with reasonable analysis time

analysis method consisted of a flow programme where the flow rate was 0.4ml/min for 20 minutes so fructose, quinic, malic and shikimic acid could be separated, followed by 10 minutes at a flow rate of 0.8ml/min. The column temperature was maintained at 32°C enabling the separation of citric, succinic and fumaric acids (figure A12.3). This method gave analysis times of 30 minutes and was used for all subsequent work.

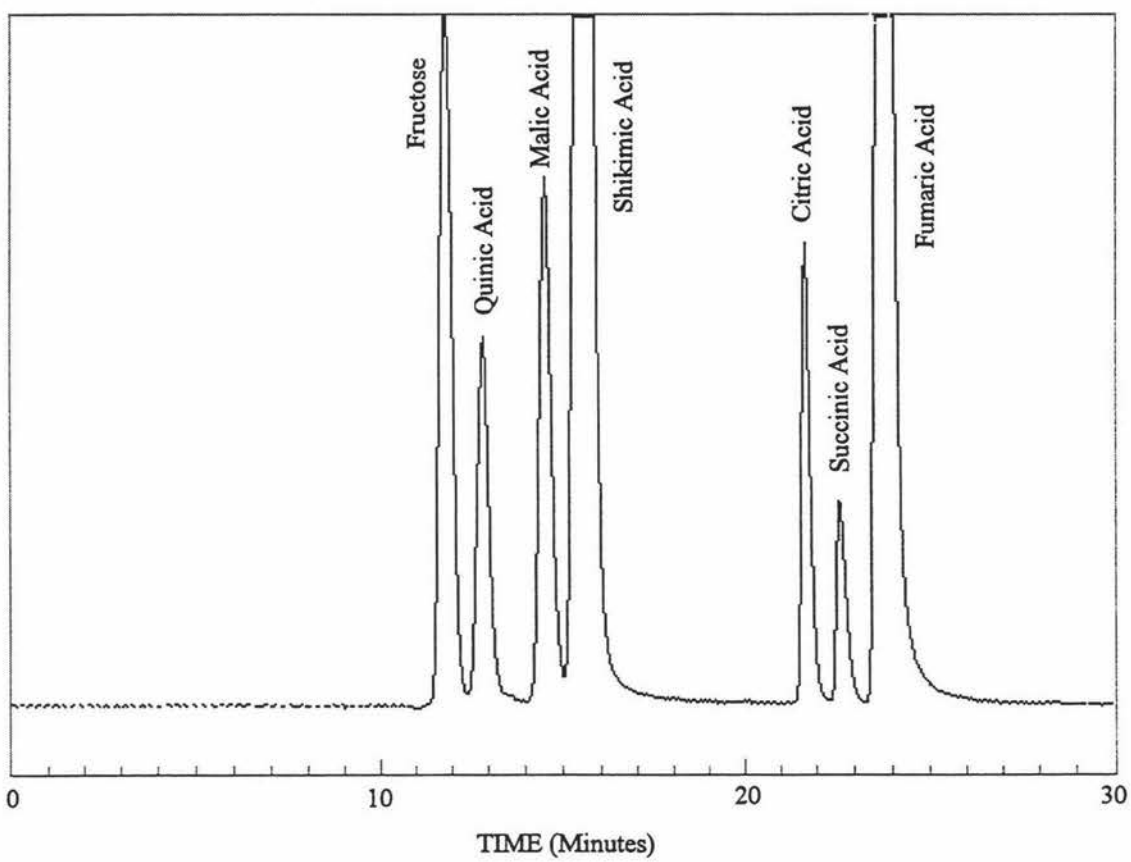

Figure A12.3: Separation of a standard solution containing fructose, quinic, malic, shikimic, citric, succinic and fumaric acids using a mobile phase of 0.005M sulphuric acid at a flow rate of 0.4ml/min for 20 minutes followed by 0.8ml/min for 10 minutes using Spheri-5 reverse phase C_{18} and Spheri-5 ODS reverse phase C_{18} columns in series maintained at 32°C with detection at 214nm.

APPENDIX 13
METHOD VALIDATION OF ORGANIC ACID ANALYSIS

As a new method (section 3.4.1) was developed it was necessary to validate the method before the quantification of organic acids in apple juice could proceed. The confirmation of peak identity, detector response, quantification by both peak height and area and recoveries were investigated.

A13.1 Identification of Organic Acids

A13.1.1 Materials and Methods

An organic acid standard was prepared in Milli-Q water that contained a final concentration of 3.75g/l malic, 0.125g/l quinic, 0.05g/l shikimic, 0.125g/l citric, 0.125g/l succinic, 0.0013g/l fumaric and 20g/l fructose.

The identification of each peak was confirmed by spiking the standard mixture and apple juice with individual acids and sugars at concentrations of 6g/l malic, 0.2g/l quinic, 0.1g/l shikimic, 0.3g/l citric, 0.2g/l succinic, 2.0×10^{-3}g/l fumaric acid, 10g/l fructose, 20g/l sucrose, 20g/l glucose and 0.3g/l ascorbic acid.

A13.1.2 Results and Discussion

The retention times of the compounds (table A13.1) present in apple juice were identified by spiking the working standard and apple juice with the various amounts of individual acids and observing an increase in the peak size. A typical chromatography for an apple juice is shown in figure A13.1.

Some apple juice samples had an peak occurring after shikimic acid (figure A13.1). By comparing retention times of this peak to those of the standards it was thought that the peak was ascorbic acid (table A13.1). Ascorbic acid is usually present in low levels in apple juice,

with Withy *et al.*, (1978) reporting values typically less than 2mg/100ml. Therefore it was not quantified during this study.

Table A13.1: Retention times of organic acids and sugars using a mobile phase of 0.005M sulphuric acid at a flow rate of 0.4ml/min for 20 minutes followed by 0.8ml/min for 10 minutes and Speri-5 reverse phase C_{18} and Spheri-5 ODS reverse phase C_{18} columns in series maintained at 32°C with detection at 214nm.

Compound	Retention Time (minutes)	Compound	Retention Time (minutes)
Glucose	11.79	**Fructose**	11.67
Quinic	12.61	**Sucrose**	13.73
Malic	13.92	**Shikimic**	14.87
Ascorbic Acid	15.50	**Citric**	20.43
Succinic	21.53	**Fumaric**	22.53

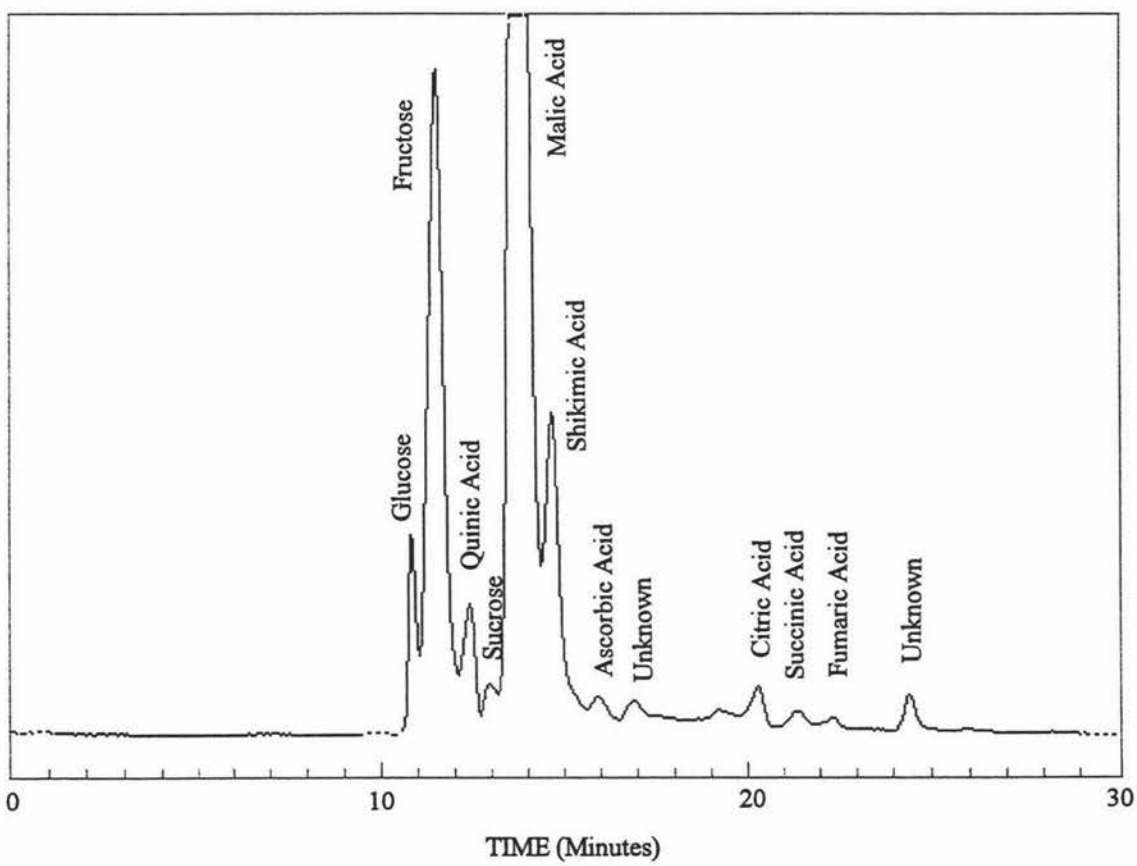

Figure A13.1: Separation of sugars and organic acids in an apple juice using a mobile phase of 0.005M sulphuric acid at a flow rate of 0.4ml/min for 20 minutes followed by 0.8ml/min for 10 minutes and Spheri-5 reverse phase C_{18} and Spheri-5 ODS reverse phase C_{18} columns in series maintained at 32°C with detection at 214nm.

APPENDIX 14

DETECTOR RESPONSE FOR ORGANIC ACID QUANTIFICATION

The detector response to quinic, malic, shikimic, citric, succinic and fumaric acids was determined to ensure that linearity was maintained over the range of concentrations expected in apple juice.

A14.1 Materials and Methods

Standards covering the range 3g/l to 15g/l malic, 0.1g/l to 0.5g/l quinic, 0.05g/l to 0.25g/l shikimic, 0.1g/l to 0.5g/l citric, 0.1g/l to 0.5g/l succinic and 0.002g/l to 0.01g/l fumaric were prepared and chromatographed to establish the detector response. Peak height and area were used to quantify the detector response.

A14.2 Results and Discussion

The detector response was generally linear over the range of concentrations tested, although the response was different for each individual acid. The linear regression coefficient (R^2) for each acid was generally in excess of 0.9938 (table A14.1). At concentration greater than 12g/l, 0.4g/l and 0.20g/l for malic, quinic and shikimic acids respectively the detector response became non-linear, and the regression coefficient decreased. This non-linearity is due to column overload and detector saturation. Citric, succinic and fumaric acids maintained linearity up to concentrations of 0.5g/l, 0.5g/l and 0.02g/l respectively (figures A14.1 to A14.6).

Table A14.1: Linear regression coefficients (R^2) for malic, quinic, shikimic, citric, succinic and fumaric acids.

	Conc. (g/l)	Peak Area	Peak Height		Conc. (g/l)	Peak Area	Peak Height
Malic acid	15	0.9961	0.9836	Quinic Acid	0.5	0.9892	0.9968
	12	0.9994	0.9938		0.4	0.9955	0.9998
	9	0.9989	0.9945		0.3	0.9979	0.9996
	6	0.9972	0.9950		0.2	0.9999	0.9998
Shikimic Acid	0.25	0.9959	0.9881	Citric Acid	0.5	0.9990	0.9987
	0.20	0.9993	0.9950		0.4	0.9999	0.9997
	0.15	0.9988	0.9957		0.3	0.9997	0.9998
	0.10	0.9971	0.9961		0.2	0.9994	0.9995
Succinic Acid	0.5	0.9982	0.9974	Fumaric Acid	0.01	0.9986	0.9989
	0.4	0.9985	0.9997		0.008	0.9993	0.9998
	0.3	0.9982	0.9996		0.006	0.9988	0.9997
	0.2	0.9990	0.9997		0.004	0.9990	0.9998

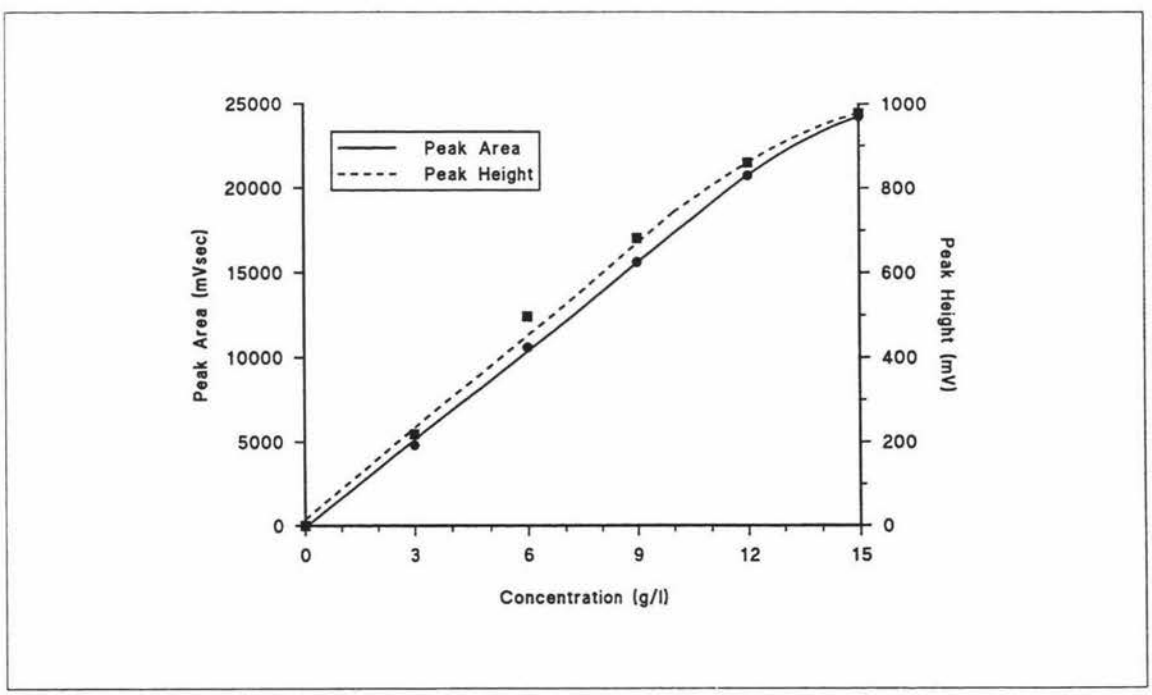

Figure A14.1: Effect of malic acid concentrations on detector response.

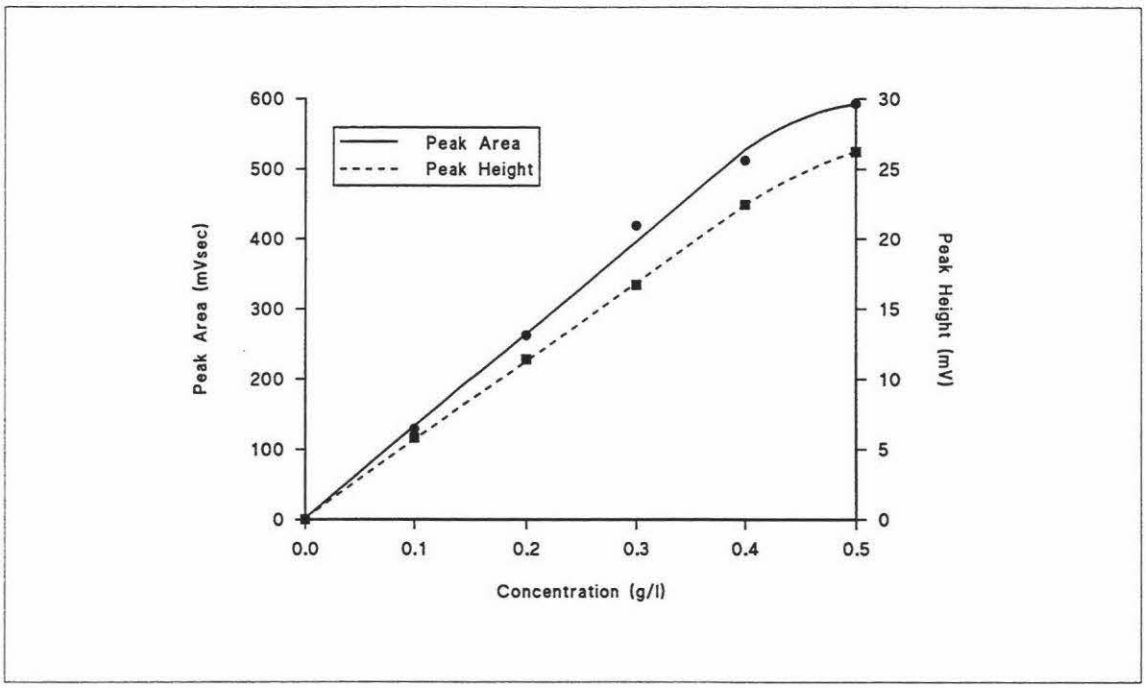

Figure A14.2: Effect of quinic acid concentrations on detector response.

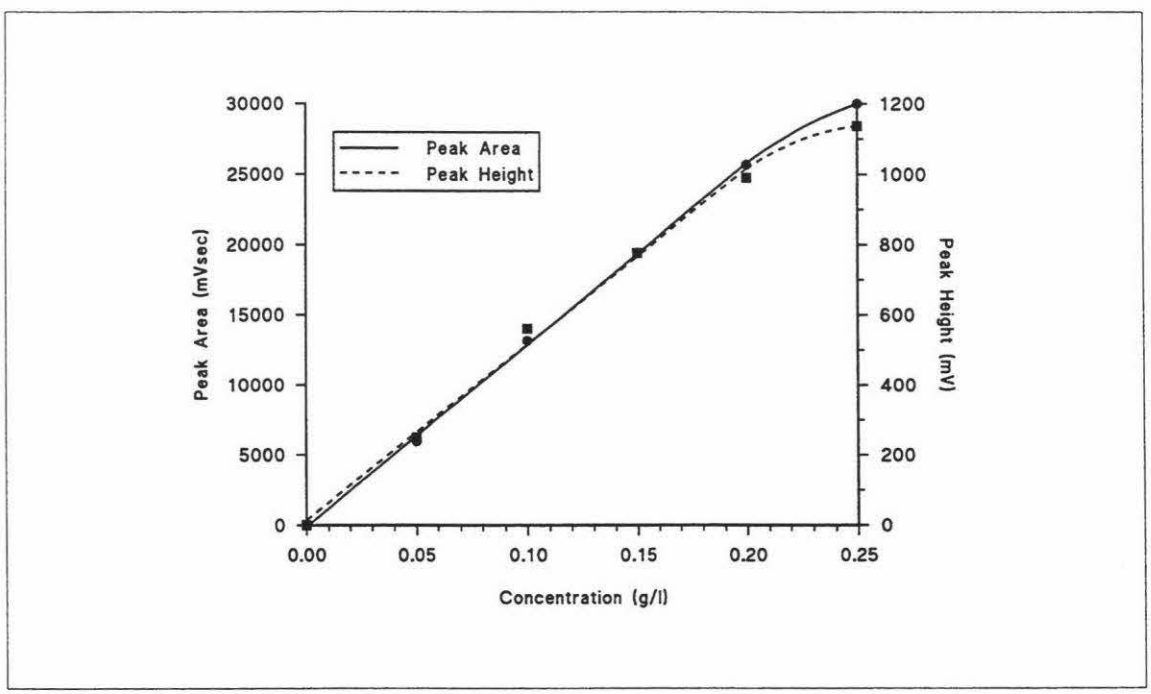

Figure A14.3: Effect of shikimic acid concentrations on detector response.

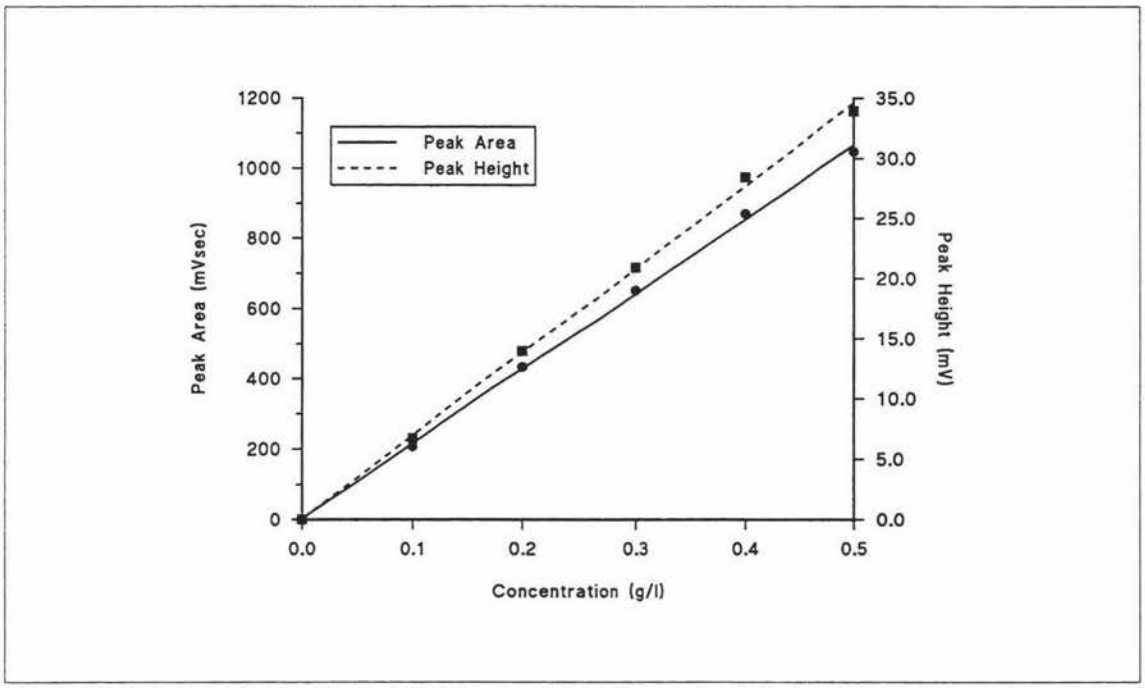

Figure A14.4: Effect of citric acid concentrations on detector response.

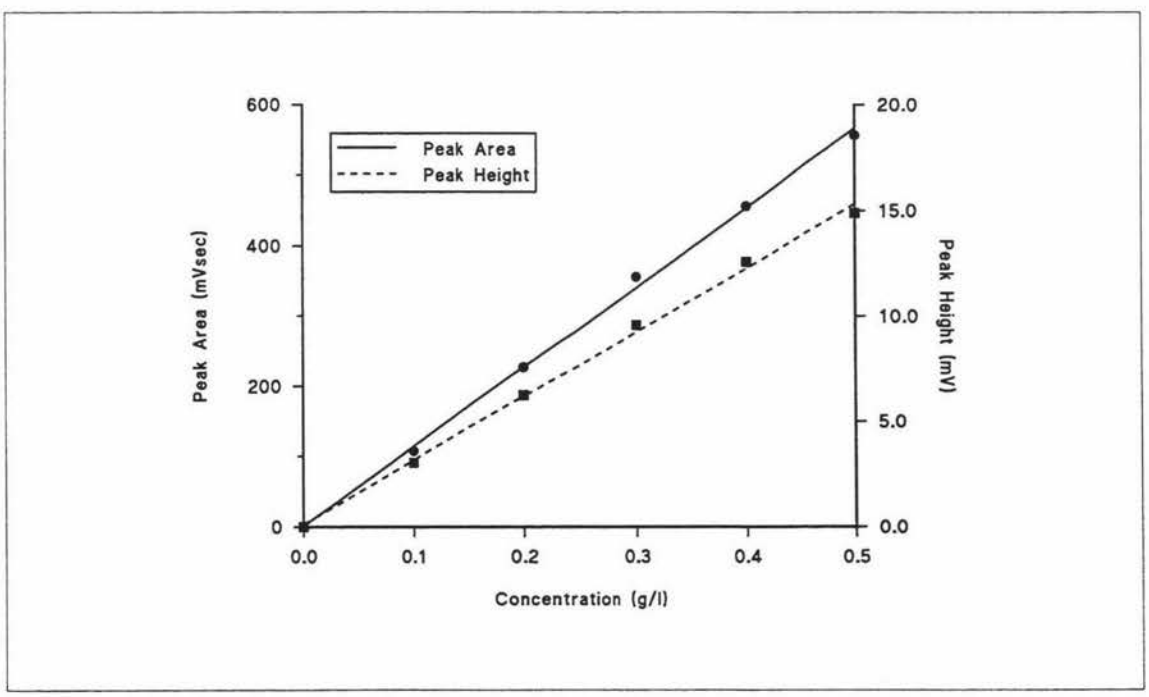

Figure A14.5: Effect of succinic acid concentrations on detector response.

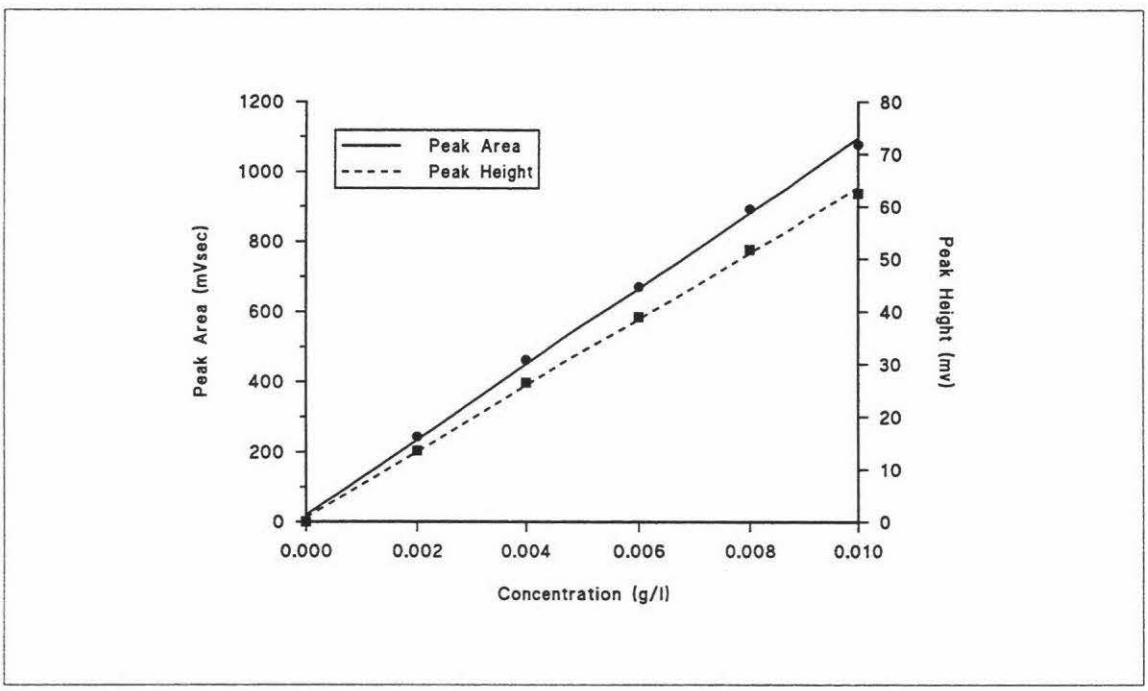

Figure A14.6: Effect of fumaric acid concentrations on detector response.

APPENDIX 15
QUANTIFICATION OF ORGANIC ACID PEAKS

A15.1 Materials and Methods

The same apple juice was analysed 10 times over a period of two days and the acid concentration determined by both peak height and area. The mean, standard deviation and coefficient of variation were calculated.

A15.2 Results and Discussion

The determination of organic acids by peak height generally had a lower coefficient of variation than when the corresponding peaks were determined by peak area (table A15.1). Malic acid is the predominant acid present in apple juice and as such gives the largest response. Shikimic and fumaric are present in much smaller quantities but are highly absorbing and also produce large detector response. These peaks are less affected by the adjacent overlapping peaks and baseline noise when determined by peak area with coefficients of variation of less than 4.4%. Quinic, citric, and succinic are present at low concentrations and are difficult to distinguish from baseline noise and adjacent peaks resulting in coefficients of variation of between 12 and 22% when determined by peak area.

When the six organic acids were quantified by peak height the coefficient of variation was typically less than 8.0%. Peak height was less affected by adjacent overlapping peaks and as such peak heights were used to quantify all organic acids in this study.

Table A15.1: Comparison of peak heights with peak areas for individual organic acids.

Organic acid	Peak Height (mv)			Peak Area (mvsec)		
	Mean	Standard Deviation	Coefficient of Variation (%)	Mean	Standard Deviation	Coefficient of Variation (%)
Quinic	7.8	0.20	2.6	162.14	19.36	11.9
Malic	291.5	11.60	3.9	6220.35	272.44	4.4
Shikimic	239.9	7.72	3.2	5590.85	78.89	1.4
Citric	8.8	0.37	4.2	237.57	50.89	21.4
Succinic	3.8	0.31	7.9	59.96	7.23	12.1
Fumaric	22.6	0.50	2.2	405.98	10.45	2.6

APPENDIX 16
QUANTIFICATION OF FUMARIC ACID IN SYNTHETIC MALIC ACID

A synthetic malic acid cannot be produced without the presence of fumaric acid (Coppola and Starr, 1986; Elkins *et al.*, 1988; Lee and Wrolstad, 1988b; Mattick, 1988) Fumaric acid is cosynthesized by the dehydration of malic acid (Elkins *et al.*, 1988; Mattick, 1988). It was therefore necessary to determine the amount of fumaric acid in the commercial malic acid that was used in the preparation of calibration curves.

A16.1 Materials and Methods

Fumaric acid (BDH Chemical Company) standards covering the range 0.001g/l to 0.005g/l were prepared, and chromatographed to obtain a standard curve. Malic acid standards covering the range 3g/l to 15g/l were prepared and chromatographed to determined the concentration of fumaric acid present. Using peak height, the fumaric acid concentration in malic acid standard was quantified by comparing it with that of a fumaric acid standard.

A16.2 Results and Discussion

The amount of fumaric acid found in the malic acid standard was 0.067% (w/v), which is similar to that reported by Mattick (1988). It was reported that the fumaric acid impurity present in the malic acid does not affect the quantification of malic acid in apple or cranberry juice. As the fumaric acid content of the malic acid standard is known, it is possible to construct calibration curves for fumaric acid by weighing out varying amounts of malic acid. A two point calibration curve for fumaric acid was constructed by preparing malic acid standards containing 6g/l and 12g/l malic acid. These standards would also contain 0.004 and 0.008g/l of fumaric acid.

APPENDIX 17

RECOVERY OF ORGANIC ACIDS

A17.1 Materials and Methods

Apple juice samples were spiked with individual organic acids (table A17.1) and the recoveries determined by the difference between the level observed in the spiked samples and the amount of the individual sugars calculated to be present. Quantification was by peak height.

A17.2 Results and Discussion

The recoveries of the acids generally ranged from between 94.6 to 103.3% (table A17.1). The exception to this was succinic and citric acids which were observed to have recoveries of 81.2% and 120.1% respectively. The recoveries seen for these acids was due to the difficulties in resolving and quantifying the small peaks from baseline noise.

Table A17.1: Recoveries of added quinic, malic, shikimic, citric, succinic and fumaric acids in apple juice spiked with known concentrations of individual organic acids.

Sample	Untreated Sample (g/l)	Amount of Added Acid (g/l)	Calculated Concentration (g/l)	Measured Concentration (g/l)	% Recovery
Quinic	0.479	0.2	0.679	0.701	103.3
Malic	3.103	6.0	9.103	9.296	102.1
Shikimic	0.010	0.1	0.110	0.104	94.6
Citric	0.068	0.3	0.368	0.440	119.6
Succinic	0.182	0.2	0.382	0.310	81.2
Fumaric	0.0009	0.004	0.0049	0.0047	96.9

APPENDIX 18

INDIVIDUAL SUGARS AND RELATED COMPONENTS OF ALL CULTIVARS COMBINED TO GIVE THE "AVERAGE COMPOSITION OF NATURAL APPLE JUICE"

Table A18.1: Minimum, maximum, mean, standard deviation, and coefficient of variation in the sugar concentrations of all cultivars combined to give the "average composition of natural apple juice".

	Sucrose (g/100ml)	Glucose (g/100ml)	Fructose (g/100ml)	Sorbitol (g/100ml)	Total Sugars	Sucrose as % Total Sugars	Glucose as % Total Sugars	Fructose as % Total Sugars	Sorbitol as % Total Sugars+ Sorbitol	Fruc/Gluc Ratio	°Brix
Minimum	0.98	0.52	4.03	0.13	7.42	9.54	5.89	42.66	1.21	1.62	8.3
Maximum	7.50	4.25	8.62	1.40	15.01	49.98	32.44	73.28	8.53	9.19	15.3
Number of Samples	189	189	189	189	189	189	189	189	189	189	189
Mean	2.86	1.94	6.44	0.34	11.24	25.07	17.32	57.61	2.89	3.96	11.49
Standard Deviation	1.23	0.79	0.88	0.15	1.60	9.06	6.85	5.31	0.97	1.77	1.35
Coefficient of variation (%)	42.96	40.57	13.68	43.84	14.19	36.12	39.53	9.21	33.54	44.72	11.72

APPENDIX 19
INDIVIDUAL SUGARS AND RELATED COMPONENTS FOR BRAEBURN APPLE JUICES

Table A19.1: Individual sugar concentrations in the juice of Hawke's Bay Braeburn apples that were stored at different conditions in 1992.

Storage Time (Days)	Sucrose (g/100ml)	Glucose (g/100ml)	Fructose (g/100ml)	Sorbitol (g/100ml)	Total Sugars (g/100ml)	Sucrose as % of Total Sugars	Glucose as % of Total Sugars	Fructose as % of Total Sugars	Sorbitol As % of Total Sugars + Sorbitol	Fruc/Gluc Ratio	°Brix
Ambient Storage											
0	4.58	1.04	6.49	0.32	12.11	37.85	8.56	53.59	2.60	6.26	11.8
14	**5.51**	1.11	6.80	0.30	13.41	41.07	8.25	50.68	2.18	6.14	12.8
20	4.03	1.38	5.88	0.21	11.29	35.68	12.22	52.11	1.83	4.26	11.5
27	5.10	1.18	6.06	0.25	12.34	41.31	9.55	49.14	1.96	5.15	12.6
36	4.46	1.47	6.00	0.21	11.92	37.38	12.32	50.30	1.72	4.08	12.5
42	5.45	1.56	6.80	0.29	13.81	39.46	11.32	49.22	2.06	4.35	13.4
Cold Storage											
0	4.58	1.04	6.49	0.32	12.11	37.85	8.56	53.59	2.60	6.26	11.8
27	4.60	1.15	6.22	0.30	11.97	38.44	9.59	51.97	2.45	5.42	12.0
56	5.07	1.21	6.48	0.39	12.76	39.72	9.48	50.80	2.95	5.36	12.6
85	4.75	1.37	6.31	0.29	12.43	38.20	11.06	50.75	2.31	4.59	12.9
112	4.73	1.37	6.18	0.34	12.28	38.51	11.16	50.33	2.73	4.51	13.0
Controlled Atmosphere											
0	4.58	1.04	6.49	0.32	12.11	37.85	8.56	53.59	2.60	6.26	11.8
57	4.51	1.45	6.83	0.40	12.78	35.27	11.32	53.41	3.04	4.72	12.9
91	4.50	1.50	6.70	0.33	12.70	35.42	11.84	52.74	2.56	4.46	13.2
138	4.46	1.91	6.78	0.46	13.14	33.92	14.53	51.55	3.39	3.55	13.3
156	4.82	1.95	7.32	0.48	14.09	34.19	13.82	51.99	3.31	3.76	13.5

Table A19.2: Individual sugar concentrations in the juice of Hawke's Bay Braeburn apples that were stored at different conditions in 1993.

Storage Time (Days)	Sucrose (g/100ml)	Glucose (g/100ml)	Fructose (g/100ml)	Sorbitol (g/100ml)	Total Sugars (g/100ml)	Sucrose as % of Total Sugars	Glucose as % of Total Sugars	Fructose as % of Total Sugars	Sorbitol as % of Total Sugars + Sorbitol	Fruc/Gluc Ratio	°Brix
Ambient Storage											
0	4.13	1.56	7.02	0.26	12.71	32.53	12.23	55.24	2.02	4.52	12.6
8	4.11	1.80	6.24	0.26	12.15	33.85	14.81	51.34	2.06	3.47	12.4
14	4.46	1.83	6.88	0.23	13.17	33.87	13.87	52.26	1.75	3.77	12.8
21	3.96	1.88	6.94	0.21	12.77	30.96	14.71	54.33	***1.61***	3.69	12.5
28	4.24	2.13	7.61	0.29	13.98	30.32	15.24	54.44	2.05	3.57	13.4
35	3.38	2.50	7.59	0.25	13.47	25.10	18.54	56.36	1.83	3.04	13.3
42	3.28	2.39	7.52	0.26	13.19	24.85	18.13	57.02	1.90	3.15	13.5
49	2.67	2.78	7.91	0.32	13.37	19.97	20.82	59.21	2.35	2.84	13.0
56	2.10	2.71	8.05	0.25	12.85	***16.30***	21.06	**62.64**	1.88	2.97	13.5
64	2.43	**3.030**	**8.62**	0.38	**14.08**	17.28	21.51	61.22	2.61	2.85	**14.0**
Cold Storage											
0	4.13	1.56	7.02	0.26	12.71	32.53	12.23	55.24	2.02	4.52	12.6
28	5.03	1.69	6.65	0.35	13.37	37.61	12.63	49.76	2.55	3.94	13.0
56	4.17	1.67	6.55	0.32	12.38	33.66	13.48	52.86	2.54	3.92	12.3
85	3.93	2.00	6.91	0.30	12.84	30.60	15.60	53.80	2.29	3.45	12.0
119	3.25	2.27	6.97	0.34	12.49	26.05	18.19	55.76	2.64	3.07	12.3
147	2.97	2.33	7.19	0.39	12.49	23.79	18.65	57.56	2.99	3.09	12.4
175	2.90	2.50	7.67	0.41	13.07	22.16	19.11	58.73	3.05	3.07	12.5
206	2.25	2.55	7.42	0.39	12.22	18.39	20.89	60.71	3.07	2.91	12.0
Controlled Atmosphere Storage											
0	4.13	1.56	7.02	0.26	12.71	32.53	12.23	55.24	2.02	4.52	12.6
91	3.97	2.12	7.03	0.42	13.12	30.28	16.12	53.60	3.11	3.33	12.3
119	3.73	2.37	7.55	0.51	13.65	27.30	17.37	55.34	3.62	3.19	12.6
147	3.42	2.42	7.06	0.50	12.89	26.52	18.73	54.76	3.72	2.92	12.8
175	3.50	2.50	7.15	**0.55**	13.15	26.60	19.02	54.38	4.04	2.86	12.5
206	2.71	2.53	7.13	0.51	12.37	21.90	20.44	57.66	3.95	2.82	12.6

Table A19.3: Individual sugar concentrations in the juice of Braeburn apples that were harvested at commerical maturity.

Region	Sample Date	Stage of Picking	Sucrose (g/100ml)	Glucose (g/100ml)	Fructose (g/100ml)	Sorbitol (g/100ml)	Total Sugars (g/100ml)	Sucrose as % of Total Sugars	Glucose as % of Total Sugars	Fructose as % of Total Sugars	Sorbitol as % of Total Sugars + Sorbitol	Fruc/Gluc Ratio	°Brix
Hawke's Bay	20/2/92	1st Pick	2.55	1.28	5.54	0.30	9.37	27.25	13.61	59.14	3.07	4.35	9.5
Hawke's Bay	17/3/92	2nd Pick	3.57	1.09	6.04	0.31	10.71	33.38	10.21	56.41	2.80	5.52	10.8
Hawke's Bay	5/5/92	3rd Pick	3.10	1.31	6.48	0.30	10.88	28.45	12.02	59.53	2.66	4.95	11.0
Hawke's Bay	15/4/93	2nd Pick	3.12	1.56	6.44	0.35	11.12	28.02	14.05	57.93	3.08	4.12	11.0
Nelson	11/3/92	1st Pick	2.56	0.93	5.51	0.37	9.00	28.42	10.34	61.25	3.94	5.93	10.1
Nelson	14/4/92	2nd Pick	4.96	***0.80***	5.44	0.53	11.20	**44.25**	***7.17***	***48.58***	**4.50**	**6.77**	12.0
Nelson	14/5/92	3rd Pick	3.53	1.30	5.88	0.44	10.71	32.92	12.13	54.95	3.93	4.53	12.0

Table A19.4: Variation in the individual sugar concentrations in the juice of Braeburn apples harvested from different positions on a tree, different trees and orchards, different bins present in the processing yard and different positions within a bin. All samples were collected on the same day from Hawke's Bay in 1993.

Sample	Sucrose (g/100ml)	Glucose (g/100ml)	Fructose (g/100ml)	Sorbitol (g/100ml)	Total Sugars (g/100ml)	Sucrose as % of Total Sugars	Glucose as % of Total Sugars	Fructose as % of Total Sugars	Sorbitol as % of Total Sugars + Sorbitol	Fruc/Gluc Ratio	°Brix
North	3.04	1.62	5.27	0.23	9.93	30.62	16.29	53.10	2.24	3.26	10.0
South	2.77	1.48	5.46	0.19	9.70	28.53	15.23	56.24	1.91	3.69	10.0
East	2.36	1.42	5.14	0.23	8.95	26.70	15.86	57.44	2.49	3.62	9.5
West	2.82	1.43	5.27	0.23	9.53	29.60	15.05	55.34	2.32	3.68	10.4
Top	2.88	1.85	5.52	0.27	10.25	28.12	18.07	53.82	2.61	2.98	10.6
Bottom	***1.48***	2.10	***4.75***	***0.16***	***8.32***	17.75	**25.19**	57.06	1.89	***2.27***	***8.6***
Orchard 2	2.99	2.10	6.32	0.32	11.41	26.20	18.40	55.40	2.75	3.01	11.3
Orchard 2	3.42	1.66	6.68	0.32	11.76	29.11	14.08	56.82	2.68	4.04	11.4
Orchard 3	3.53	1.70	6.33	0.40	11.56	30.52	14.73	54.75	3.32	3.72	11.3
Orchard 4	3.44	1.19	5.59	0.31	10.22	33.66	11.69	54.65	2.90	4.68	10.4

Table A19.4: continued

Sample	Sucrose (g/100ml)	Glucose (g/100ml)	Fructose (g/100ml)	Sorbitol (g/100ml)	Total Sugars (g/100ml)	Sucrose as % of Total Sugars	Glucose as % of Total Sugars	Fructose as % of Total Sugars	Sorbitol as % of Total Sugars + Sorbitol	Fruc/Gluc Ratio	°Brix
Centre	2.80	1.73	6.21	0.29	10.74	26.09	16.12	57.79	2.59	3.59	11.0
Top Corner 1	3.19	1.34	6.43	0.24	10.96	29.10	12.22	58.68	2.11	4.80	10.7
Bottom corner 1	2.66	1.30	6.17	0.24	10.13	26.28	12.83	60.89	2.27	4.75	10.1
Bottom corner 2	2.69	1.30	6.05	0.19	10.05	26.79	12.98	60.23	1.88	4.64	10.6
Top corner 2	2.90	1.33	6.79	0.28	11.02	26.32	12.03	61.65	2.47	5.12	10.5
Bin 2	3.40	1.64	5.52	0.29	10.56	32.22	15.48	52.30	2.63	3.38	10.6
Bin 3	2.85	2.32	6.03	0.29	11.20	25.44	20.75	53.81	2.50	2.60	11.0
Bin 4	2.34	1.57	5.56	0.20	9.47	24.69	16.60	58.72	2.05	3.54	10.0
Bin 5	2.62	1.88	5.58	0.21	10.09	26.01	18.65	55.34	1.99	2.97	10.0
Large Fruit	3.05	1.44	6.84	0.31	11.33	26.89	12.72	60.40	2.62	4.75	11.3
Small Fruit	3.17	1.41	6.38	0.28	10.95	28.90	12.85	58.26	2.50	4.54	10.9
Minimum	1.48	1.19	4.75	0.16	8.32	17.75	11.69	52.30	1.88	2.27	8.6
Maximum	3.53	2.32	6.84	0.40	11.76	33.66	25.19	61.65	3.32	5.12	11.4
Number of Samples	21	21	21	21	21	21	21	21	21	21	21
Mean	2.89	1.61	5.90	0.26	10.34	27.60	15.61	56.79	2.41	3.79	10.49
Standard Deviation	0.45	0.29	0.57	0.06	0.87	3.16	3.21	2.61	0.36	0.79	0.66
Coefficient of Variation (%)	15.58	18.27	9.70	21.40	8.40	11.44	20.59	4.60	14.86	20.81	6.33

Table A19.5: Minimum, maximum, mean, standard deviation and coefficient of variation in the individual sugar concentrations of Braeburn apple juice.

	Sucrose (g/100ml)	Glucose (g/100ml)	Fructose (g/100ml)	Sorbitol (g/100ml)	Total Sugars (g/100ml)	Sucrose as % of Total Sugars	Glucose as % of Total Sugars	Fructose as % of Total Sugars	Sorbitol as % of Total Sugars + Sorbitol	Fruc/Gluc Ratio	°Brix
Minimum	1.48	0.80	4.75	0.16	8.32	16.30	7.17	48.58	1.61	2.27	8.6
Maximum	5.51	3.03	8.62	0.55	14.09	44.25	25.19	62.64	4.50	6.77	14.0
Nunber of Samples	64	64	64	64	64	64	64	64	64	64	64
Mean	3.55	1.74	6.50	0.32	11.78	29.98	14.74	55.28	2.62	3.98	11.8
Standard Deviation	0.91	0.51	0.78	0.09	1.43	6.14	3.75	3.51	0.64	0.98	1.24
Coefficient of Variation (%)	25.68	29.07	12.00	28.59	12.13	20.47	25.45	6.34	24.51	24.52	10.5

Bold italics typeface = minimum values
Bold typeface = maximum values

APPENDIX 20

INDIVIDUAL SUGARS AND RELATED COMPONENTS FOR GRANNY SMITH APPLE JUICES

Table A20.1: Individual sugar concentrations in the juice of Hawke's Bay Granny Smith apples that were stored at different conditions in 1992.

Storage Time (Days)	Sucrose (g/100ml)	Glucose (g/100ml)	Fructose (g/100ml)	Sorbitol (g/100ml)	Total Sugars (g/100ml)	Sucrose as % of Total Sugars	Glucose as % of Total Sugars	Fructose as % of Total Sugars	Sorbitol as % of Total Sugars + Sorbitol	Fruc/Gluc Ratio	°Brix
Ambient Storage											
0	3.25	2.88	6.89	0.37	13.03	24.98	22.10	52.93	2.73	2.40	13.0
7	3.07	3.07	6.84	0.42	12.98	23.62	23.66	52.72	3.14	2.23	13.0
14	3.44	2.83	7.04	0.48	**13.31**	25.87	21.23	52.90	3.49	2.49	13.1
23	3.14	2.66	6.58	0.40	12.38	25.34	21.51	53.15	3.09	2.47	13.1
28	3.17	2.44	6.65	0.39	12.26	25.88	19.90	54.22	3.07	2.73	12.7
35	2.94	2.54	6.80	0.42	12.28	23.94	20.71	55.35	3.28	2.67	12.6
42	3.11	2.53	**7.57**	0.41	13.21	23.57	19.12	57.31	3.02	3.00	**13.5**
Cold Storage											
0	3.25	2.88	6.89	0.37	13.03	24.98	22.10	52.93	2.73	2.40	13.0
28	3.35	2.62	6.01	0.36	12.00	27.94	21.86	***50.20***	2.89	2.30	12.0
56	2.58	2.78	6.30	0.47	11.66	22.16	23.80	54.04	3.84	2.27	12.0
77	2.80	2.60	6.68	0.45	12.07	23.17	21.50	55.33	3.63	2.57	12.5
106	2.40	3.09	7.50	0.56	13.00	18.48	23.81	57.72	4.15	2.42	**13.5**
Controlled Atmosphere											
0	3.25	2.88	6.89	0.37	13.03	24.98	22.10	52.93	2.73	2.40	13.0
35	2.57	2.98	6.25	0.40	11.80	21.76	25.23	53.01	3.29	2.10	12.5
63	2.47	3.10	6.53	0.49	12.10	20.40	25.65	53.95	3.86	2.10	12.3
97	1.69	**3.52**	6.60	0.52	11.81	14.32	29.83	55.85	4.21	1.87	12.0
131	1.06	3.21	5.91	0.47	10.18	10.43	31.50	58.07	4.40	1.84	11.0
161	1.43	3.51	7.14	**0.58**	12.08	11.85	29.02	59.12	4.55	2.04	12.2
196	1.30	3.13	6.75	0.57	11.18	11.61	28.00	60.39	**4.87**	2.16	12.0

Table A20.2: Individual sugar concentrations in the juice of Hawke's Bay Granny Smith apples that were stored at different conditions in 1993.

Storage Time (days)	Sucrose (g/100ml)	Glucose (g/100ml)	Fructose (g/100ml)	Sorbitol (g/100ml)	Total Sugars (g/100ml)	Sucrose as % of Total Sugars	Glucose as % of Total Sugars	Fructose as % of Total Sugars	Sorbitol as % of Total Sugars + Sorbitol	Fruc/Gluc Ratio	°Brix
Ambient Storage											
0	2.08	2.49	6.08	0.34	10.65	19.51	23.38	57.12	3.12	2.44	10.6
8	1.79	2.64	5.35	0.18	9.78	18.32	26.95	54.73	1.81	2.03	10.5
14	1.98	3.06	6.55	0.21	11.59	17.05	26.43	56.52	1.78	2.14	11.5
21	1.97	2.65	6.19	0.18	10.81	18.23	24.50	57.27	1.66	2.34	11.4
28	2.13	2.80	6.99	0.23	11.93	17.86	23.51	58.64	1.92	2.50	11.5
35	1.57	2.72	6.65	0.19	10.94	14.30	24.90	60.80	1.66	2.44	11.3
42	1.45	2.73	6.79	0.14	10.98	13.23	24.90	61.87	***1.21***	2.49	11.2
49	1.25	2.89	6.90	0.22	11.04	11.33	26.15	62.52	1.99	2.39	11.4
56	1.00	2.63	6.75	0.19	10.38	9.59	25.34	**65.07**	1.80	2.57	10.6
64	1.12	2.90	7.31	0.18	11.32	9.89	25.58	64.53	1.54	2.52	11.4
Cold Storage											
0	2.08	2.49	6.08	0.34	10.65	19.51	23.38	57.12	3.12	2.44	10.6
28	2.68	2.76	6.33	0.29	11.77	22.76	23.46	53.79	2.42	2.29	11.5
56	2.23	3.08	6.95	0.35	12.26	18.17	25.11	56.72	2.74	2.26	11.2
85	1.45	2.79	5.88	0.30	10.11	14.35	27.54	58.11	2.86	2.11	11.0
119	1.64	2.84	6.46	0.41	10.94	14.97	25.95	59.08	3.63	2.28	11.3
147	1.15	2.85	6.45	0.44	10.45	10.97	27.30	61.73	4.07	2.26	10.6
175	***0.99***	2.91	6.48	0.46	10.38	***9.54***	28.05	62.42	4.21	2.23	10.7
206	1.03	2.63	6.33	0.36	9.98	10.27	26.34	63.40	3.46	2.41	11.0
Controlled Atmosphere											
0	2.08	2.49	6.08	0.34	10.65	19.51	23.38	57.12	3.12	2.44	10.6
91	1.67	3.21	6.52	0.32	11.39	14.62	28.14	57.24	2.72	2.03	11.4
119	1.28	3.13	6.42	0.37	10.84	11.85	28.89	59.26	3.26	2.05	11.0
147	1.13	3.18	6.48	0.37	10.80	10.48	29.47	60.05	3.33	2.04	11.1
175	1.11	3.26	6.65	0.39	11.02	10.07	29.57	60.36	3.44	2.04	11.0
206	1.07	3.34	6.82	0.34	11.23	9.55	29.72	60.73	2.97	2.04	11.5

Table A20.3: Individual sugar concentrations in the juice of Granny Smith apples that were harvested at commericial maturity.

Region	Sample Date	Stage of Picking	Sucrose (g/100ml)	Glucose (g/100ml)	Fructose (g/100ml)	Sorbitol (g/100ml)	Total Sugars (g/100ml)	Sucrose as % of Total Sugars	Glucose as % of Total Sugars	Fructose as % of Total Sugars	Sorbitol as % of Total Sugars + Sorbitol	Fruc/Gluc Ratio	°Brix
Hawke's Bay	6/3/92	1st Pick	1.59	1.91	4.78	0.18	8.29	19.24	23.06	57.70	2.15	2.50	9.4
Hawke's Bay	7/4/92	2nd Pick	2.90	1.91	5.36	0.19	10.16	28.50	18.75	52.75	1.83	2.81	10.5
Hawke's Bay	29/4/92	3rd Pick	2.51	2.31	5.40	0.24	10.21	24.53	22.58	52.89	2.28	2.34	10.8
Hawke's Bay	25/2/93	1st Pick	1.42	1.88	4.24	0.19	***7.54***	18.77	24.94	56.29	2.39	2.26	***8.5***
Hawke's Bay	25/3/93	2nd Pick	1.56	1.85	4.38	0.17	7.79	19.98	23.80	56.23	2.16	2.36	***8.5***
Hawke's Bay	9/6/93	3rd Pick	1.78	2.10	5.12	0.14	9.00	19.77	23.36	56.87	1.55	2.43	9.5
Nelson	9/3/92	1st Pick	1.19	2.02	4.41	***0.13***	7.62	15.67	26.49	57.85	1.66	2.18	8.8
Nelson	20/4/92	2nd Pick	2.87	2.16	5.26	0.22	10.28	27.87	20.98	51.15	2.13	2.44	11.0
Nelson	17/5/92	3rd Pick	3.22	2.06	5.66	0.24	10.93	29.43	18.84	51.74	2.17	2.75	11.0
Nelson	8/3/93	1st Pick	1.67	2.07	5.26	0.23	9.00	18.55	22.99	58.46	2.45	2.54	9.8
Nelson	18/4/93	2nd Pick	3.12	2.08	5.96	0.33	11.16	27.92	18.66	53.48	2.87	2.86	11.5
Nelson	6/5/93	3rd Pick	**3.51**	2.09	5.79	0.39	11.39	**30.85**	18.34	50.80	3.31	2.77	12.7
Canterbury	11/3/92	1st Pick	1.47	***1.61***	5.05	0.20	8.12	18.09	19.78	62.14	2.44	3.14	9.5
Canterbury	24/4/92	2nd Pick	2.89	1.85	6.30	0.34	11.04	26.17	***16.73***	57.09	2.97	**3.41**	11.5
Canterbury	29/5/92	3rd Pick	2.99	2.32	6.06	0.43	11.37	26.27	20.42	53.30	3.61	2.61	11.8
Canterbury	16/3/93	1st Pick	1.43	2.14	***4.03***	0.27	7.60	18.86	28.11	53.03	3.42	1.89	8.6
Canterbury	23/3/93	2nd Pick	1.70	2.45	4.80	0.34	8.95	18.96	27.36	53.69	3.62	1.96	9.9
Canterbury	21/5/93	3rd Pick	1.41	3.02	4.87	0.18	9.30	15.14	**32.44**	52.42	1.88	***1.62***	10.0

Table A20.4: Minimum, maximum, mean, standard deviation and coefficient of variation in the individual sugar concentrations of Granny Smith apple juice.

	Sucrose (g/100ml)	Glucose (g/100ml)	Fructose (g/100ml)	Sorbitol (g/100ml)	Total Sugars (g/100ml)	Sucrose as % of Total Sugars	Glucose as % of Total Sugars	Fructose as % of Total Sugars	Sorbitol as % of Total Sugars + Sorbitol	Fruc/Gluc Ratio	°Brix
Minimum	0.99	1.61	4.03	0.13	7.54	9.54	16.73	50.20	1.21	1.62	8.5
Maximum	3.51	3.52	7.57	0.58	13.31	30.85	32.44	65.07	4.87	3.41	13.5
Number of Samples	57	57	57	57	57	57	57	57	57	57	57
Mean	2.05	2.65	6.14	0.33	10.84	18.72	24.51	56.77	2.88	2.36	11.3
Standard Deviation	0.79	0.46	0.84	0.12	1.45	6.07	3.58	3.66	0.88	0.33	1.2
Coefficient of Variation (%)	38.51	17.54	13.68	37.02	13.34	32.43	14.60	6.45	30.44	13.79	10.8

Bold italic typeface = minimum values
Bold typeface = maximum values

APPENDIX 21

INDIVIDUAL SUGARS AND RELATED COMPONENTS FOR GALA APPLE JUICES

Table A21.1: Individual sugar concentrations in the juice of Hawke's Bay Gala apples that were stored at different conditions in 1992.

Storage Time (Days)	Sucrose (g/100ml)	Glucose (g/100ml)	Fructose (g/100ml)	Sorbitol (g/100ml)	Total Sugars (g/100ml)	Sucrose as % of Total Sugars	Glucose as % of Total Sugars	Fructose as % of Total Sugars	Sorbitol as % of Total Sugars + Sorbitol	Fruc/Gluc Ratio	°Brix
Ambient Storage											
0	3.14	1.37	6.51	0.25	11.03	28.52	12.40	59.09	2.24	4.77	12.2
6	2.98	1.26	6.65	0.22	10.89	27.39	11.58	61.03	2.00	5.27	11.4
20	2.42	1.32	7.23	***0.16***	10.97	22.02	12.05	65.93	***1.42***	5.47	11.5
34	1.98	1.63	7.38	0.22	10.99	18.05	14.83	67.12	1.96	4.53	12.4
40	1.94	1.59	7.27	0.18	10.80	18.00	14.68	67.32	1.68	4.59	11.4
Cold Storage											
0	3.14	1.37	6.51	0.25	11.03	28.52	12.40	59.09	2.24	4.77	12.2
34	3.06	1.64	7.69	0.36	12.39	24.71	13.21	62.08	2.82	4.70	12.0
56	2.48	1.44	6.96	0.30	10.89	22.77	13.25	63.98	2.67	4.83	11.9
83	1.85	1.85	8.04	0.29	11.74	15.76	**15.74**	68.50	2.44	***4.35***	12.0
119	2.04	**1.92**	**8.62**	0.53	12.58	16.19	15.27	68.54	**4.05**	4.49	11.9

Table A21.2: Individual sugar concentrations in the juice of Gala apples that were harvested at commerical maturity.

Region	Sample Date	Stage of Picking	Sucrose (g/100ml)	Glucose (g/100ml)	Fructose (g/100ml)	Sorbitol (g/100ml)	Total Sugars (g/100ml)	Sucrose as % of Total Sugars	Glucose as % of Total Sugars	Fructose as % of Total Sugars	Sorbitol as % of Total Sugars + Sorbitol	Fruc/Gluc Ratio	°Brix
Hawke's Bay	28/1/92	1st Pick	1.82	0.95	5.60	0.26	8.37	21.72	11.36	66.91	2.96	5.89	8.6
Hawke's Bay	13/2/92	2nd Pick	2.75	1.01	5.99	0.25	9.74	28.21	10.32	61.48	2.52	5.96	10.2
Hawke's Bay	18/3/92	3rd Pick	3.14	1.37	6.51	0.25	11.03	28.52	12.40	59.09	2.24	4.77	12.2
Hawke's Bay	26/2/93	2nd Pick	2.94	1.23	7.19	0.32	11.37	25.90	10.84	63.27	2.76	5.84	11.2
Nelson	27/1/92	1st Pick	***1.00***	0.98	***5.43***	0.17	***7.42***	***13.50***	13.22	**73.28**	2.30	5.54	***8.3***
Nelson	26/2/92	2nd Pick	2.67	0.89	6.13	0.18	9.70	27.54	9.20	63.26	1.78	6.88	9.8
Nelson	6/4/92	3rd Pick	4.12	0.85	7.05	0.20	12.02	34.25	***7.08***	58.68	1.67	8.30	12.0
Canterbury	13/2/92	1st Pick	2.72	***0.78***	7.19	0.39	10.69	25.43	7.32	67.25	3.50	**9.19**	11.0
Canterbury	2/3/92	2nd Pick	3.77	1.01	7.48	0.31	12.26	30.74	8.24	61.02	2.48	7.40	12.0
Canterbury	6/4/92	3rd Pick	**4.97**	1.16	8.20	**0.55**	**14.33**	**34.69**	8.11	***57.20***	3.67	7.05	**14.0**

Table A21.3: Minimum, maximum, mean, standard deviation and coefficient of variation in the individual sugar concentrations of Gala apple juice.

	Sucrose (g/100ml)	Glucose (g/100ml)	Fructose (g/100ml)	Sorbitol (g/100ml)	Total Sugars (g/100ml)	Sucrose as % of Total Sugars	Glucose as % of Total Sugars	Fructose as % of Total Sugars	Sorbitol as % of Total Sugars + Sorbitol	Fruc/Gluc Ratio	°Brix
Minimum	1.00	0.78	5.43	0.16	7.42	13.50	7.07	57.20	1.42	4.35	8.3
Maximum	4.97	1.92	8.62	0.55	14.33	34.69	15.74	73.28	4.05	9.19	14.0
Number of Samples	18	18	18	18	18	18	18	18	18	18	18
Mean	2.70	1.27	7.04	0.29	11.01	24.19	11.60	64.22	2.50	5.84	11.3
Standard Deviation	0.91	0.34	0.84	0.11	1.53	6.03	2.68	4.06	0.70	1.37	1.3
Coefficient of Variation (%)	33.56	26.60	11.96	38.23	13.88	24.91	23.14	6.33	28.15	23.42	11.8

Bold italic typeface = minimum value
Bold typeface =maximum value

APPENDIX 22

INDIVIDUAL SUGARS AND RELATED COMPONENTS FOR ROYAL GALA APPLE JUICES.

Table A22.1: Individual sugar concentrations in the juice of Hawke's Bay Royal Gala apples that were stored at different conditions in 1992.

Storage Time (Days)	Sucrose (g/100ml)	Glucose (g/100ml)	Fructose (g/100ml)	Sorbitol (g/100ml)	Total Sugars (g/100ml)	Sucrose as % of Total Sugars	Glucose as % of Total Sugars	Fructose as % of Total Sugars	Sorbitol as % of Total Sugars + Sorbitol	Fruc/Gluc Ratio	°Brix
Controlled Atmosphere											
0	3.71	0.80	5.97	0.23	10.49	35.41	7.64	56.94	2.16	7.45	11.4
30	1.62	**1.84**	**7.83**	0.42	11.29	14.31	16.29	69.39	3.57	4.26	11.1
65	2.03	1.64	7.30	0.33	10.97	18.53	14.99	66.49	2.90	4.44	12.1
91	***1.32***	1.79	7.49	0.37	10.60	***12.42***	**16.90**	**70.68**	3.34	**4.18**	11.5

Table A22.2: Individual sugar concentrations in the juice of Royal Gala apples that were harvested at commerical maturity.

Region	Sample Date	Stage of Picking	Sucrose (g/100ml)	Glucose (g/100ml)	Fructose (g/100ml)	Sorbitol (g/100ml)	Total Sugars (g/100ml)	Sucrose as % of Total Sugars	Glucose as % of Total Sugars	Fructose as % of Total Sugars	Sorbitol as % of Total Sugars + Sorbitol	Fruc/Gluc Ratio	°Brix
Hawke's Bay	28/1/92	1st Pick	2.40	***0.73***	6.00	0.28	9.13	26.31	7.96	65.73	2.97	8.26	9.6
Hawke's Bay	17/2/92	2nd Pick	3.71	0.77	6.11	0.26	10.59	35.03	7.30	57.67	2.40	7.90	10.5
Hawke's Bay	4/5/92	3rd Pick	3.71	0.80	5.97	0.23	10.49	35.41	7.64	56.94	2.16	7.45	11.4
Hawke's Bay	19/1/93	1st Pick	1.61	0.95	5.65	0.22	8.21	19.63	11.57	68.80	2.59	5.95	9.0
Hawke's Bay	26/2/93	2nd Pick	3.58	0.89	6.58	0.20	11.04	32.39	8.03	59.58	***1.82***	7.42	10.5
Nelson	5/2/92	1st Pick	1.90	0.81	***5.38***	0.27	***8.09***	23.51	9.98	66.51	3.25	6.66	9.0
Nelson	9/3/92	2nd Pick	4.66	0.97	6.21	0.27	11.84	39.35	8.22	52.42	2.25	6.37	12.0
Nelson	18/4/92	3rd Pick	**5.74**	0.77	6.60	0.37	13.11	**43.78**	***5.89***	***50.33***	2.76	**8.55**	12.6
Nelson	20/1/93	1st Pick	1.99	0.78	5.50	0.31	8.27	24.08	9.41	66.51	3.60	7.07	8.8
Nelson	24/2/93	2nd Pick	3.29	0.73	5.85	0.24	9.87	33.35	7.39	59.26	2.39	8.02	10.5
Nelson	1/4/93	3rd Pick	5.63	1.03	7.04	**0.56**	**13.70**	41.09	7.48	51.43	**3.91**	6.87	**13.5**
Canterbury	16/2/93	1st Pick	1.72	1.08	5.45	0.19	8.25	20.89	13.14	65.98	2.29	5.02	***8.7***
Canterbury	16/3/93	2nd Pick	2.68	0.87	5.50	***0.19***	9.05	29.58	9.66	60.76	2.04	6.29	9.6
Canterbury	24/4/93	3rd Pick	4.78	0.92	6.31	0.47	12.01	39.77	7.65	52.58	3.73	6.87	12.2

Table A22.3: Minimum, maximum, mean, standard deviation and coefficient of variation in the individual sugar concentrations of Royal Gala apple juice.

	Sucrose (g/100ml)	Glucose (g/100ml)	Fructose (g/100ml)	Sorbitol (g/100ml)	Total Sugars (g/100ml)	Sucrose as % of Total Sugars	Glucose as % of Total Sugars	Fructose as % of Total Sugars	Sorbitol as % of Total Sugars + Sorbitol	Fruc/Gluc Ratio	°Brix
Minimum	1.32	0.73	5.38	0.19	8.09	12.42	5.89	50.33	1.82	4.18	8.7
Maximum	5.74	1.84	7.83	0.56	13.69	43.78	16.90	70.68	3.91	8.55	13.5
Number of Samples	17	17	17	17	17	17	17	17	17	17	17
Mean	3.08	1.02	6.28	0.31	10.38	28.79	9.97	61.24	2.82	6.56	10.7
Standard Deviation	1.41	0.36	0.74	0.10	1.67	9.25	3.29	6.62	0.63	1.35	1.4
Coefficient of Variation (%)	45.61	34.88	11.72	32.73	16.10	32.74	33.02	10.81	22.32	20.59	13.3

Bold italic typeface = minimum value
Bold typeface = maximum value

APPENDIX 23

INDIVIDUAL SUGARS AND RELATED COMPONENTS FOR RED DELICIOUS, COX'S ORANGE, GOLDEN DELICIOUS, FUJI, HILLWELL, GS330,GS2850 AND FIESTA APPLE JUICES.

Table A23.1: Individual sugar concentrations in the juice of Red Delicious apples that were harvested at commerical maturity.

Region	Sample Date	Stage of Picking	Sucrose (g/100ml)	Glucose (g/100ml)	Fructose (g/100ml)	Sorbitol (g/100ml)	Total Sugars (g/100ml)	Sucrose as % of Total Sugars	Glucose as % of Total Sugars	Fructose as % of Total Sugars	Sorbitol as % of Total Sugars + Sorbitol	Fruc/Gluc Ratio	°Brix
Hawke's Bay	5/2/92	1st Pick	1.02	1.83	***5.35***	0.33	***8.20***	12.45	22.35	**65.20**	3.89	2.92	***9.0***
Hawke's Bay	6/3/92	2nd Pick	2.06	2.34	6.57	0.41	10.98	18.78	21.35	59.87	3.59	2.80	11.5
Hawke's Bay	7/4/92	3rd Pick	**3.07**	***1.39***	6.89	0.49	11.36	**27.04**	***12.27***	60.69	4.16	**4.95**	12.2
Hawke's Bay	25/3/93	2nd Pick	1.72	1.88	6.41	***0.30***	10.01	17.18	18.82	64.01	***2.91***	3.40	10.3
Nelson	23/2/92	1st Pick	***0.98***	2.37	6.29	0.31	9.64	***10.16***	**24.60**	65.24	3.06	***2.65***	10.2
Nelson	26/3/92	2nd Pick	1.73	2.46	7.35	0.40	11.55	15.00	21.33	63.67	3.35	2.99	11.6
Nelson	27/4/92	3rd Pick	2.91	**2.68**	**7.97**	**0.69**	**13.56**	21.45	19.80	***58.75***	**4.83**	2.97	**13.9**
Minimum			0.98	1.39	5.35	0.30	8.20	10.16	12.27	58.75	2.91	2.65	9.0
Maximum			3.07	2.68	7.97	0.69	13.56	27.04	24.60	65.24	4.83	4.95	13.9
Number of Samples			7	7	7	7	7	7	7	7	7	7	7
Mean			1.93	2.14	6.69	0.42	10.76	17.44	20.07	62.49	3.68	3.24	11.2
Standard Deviation			0.76	0.42	0.77	0.13	1.57	5.26	3.61	2.47	0.62	0.73	1.4
Coefficient of Variation (%)			39.64	19.46	11.52	30.43	14.57	30.18	18.01	3.95	16.84	22.50	13.1

Bold italic typeface = minimum value
Bold typeface = maximum values

Table A23.2: Individual sugar concentrations in the juice of Cox's Orange apples that were harvested at commerical maturity.

Region	Sample Date	Stage of Picking	Sucrose (g/100ml)	Glucose (g/100ml)	Fructose (g/100ml)	Sorbitol (g/100ml)	Total Sugars (g/100ml)	Sucrose as % of Total Sugars	Glucose as % of Total Sugars	Fructose as % of Total Sugars	Sorbitol as % of Total Sugars + Sorbitol	Fruc/Gluc Ratio	°Brix
Hawke's Bay	24/1/92	1st Pick	***3.03***	***0.53***	***4.70***	0.30	***8.26***	36.68	6.40	56.92	3.46	8.90	***9.0***
Hawke's Bay	13/2/92	2nd Pick	4.81	0.88	5.72	0.63	11.42	42.14	7.73	50.13	5.26	6.49	12.0
Hawke's Bay	4/3/92	3rd Pick	4.91	0.80	6.09	0.48	11.79	41.63	6.75	51.62	3.89	7.64	12.7
Hawke's Bay	1/3/93	2nd Pick	5.08	0.71	5.43	***0.26***	11.23	45.28	***6.34***	48.38	***2.29***	7.63	11.1
Nelson	24/1/92	1st Pick	3.25	0.58	5.16	0.33	8.99	***36.16***	6.41	**57.43**	3.57	**8.96**	10.4
Nelson	24/2/92	2nd Pick	5.51	1.07	5.80	0.62	12.37	44.52	**8.63**	46.85	4.77	***5.43***	12.5
Nelson	23/3/92	3rd Pick	**7.50**	**1.11**	**6.40**	**1.40**	**15.01**	**49.98**	7.36	***42.66***	**8.53**	5.80	**15.3**
Minimum			3.03	0.53	4.70	0.26	8.26	36.16	6.34	42.66	2.29	5.43	9.0
Maximum			7.50	1.11	6.40	1.40	15.01	49.98	8.63	57.43	8.53	8.96	15.3
Number of Samples			7	7	7	7	7	7	7	7	7	7	7
Mean			4.87	0.81	5.61	0.57	11.30	42.34	7.09	50.57	4.54	7.26	`11.9
Standard Deviation			1.38	0.21	0.53	0.36	2.06	4.51	0.80	4.93	1.86	1.31	1.9
Coefficient of Variation (%)			28.41	25.68	9.44	63.43	18.26	10.66	11.28	9.74	40.88	17.99	15.57

Bold italic typeface = minimum values

Bold typeface = maximum values

Table A23.3: Individual sugar concentrations in the juice of Golden Delicious apples that were harvested at commerical maturity.

Region	Sample Date	Stage of Picking	Sucrose (g/100ml)	Glucose (g/100ml)	Fructose (g/100ml)	Sorbitol (g/100ml)	Total Sugars (g/100ml)	Sucrose as % of Total Sugars	Glucose as % of Total Sugars	Fructose as % of Total Sugars	Sorbitol as % of Total Sugars + Sorbitol	Fruc/Gluc Ratio	°Brix
Hawke's Bay	5/2/92	1st Pick	1.45	1.58	6.75	0.33	9.78	14.77	16.17	69.06	**3.21**	4.27	9.6
Hawke's Bay	6/3/92	2nd Pick	3.10	1.49	7.95	**0.33**	12.55	24.75	11.90	63.35	2.59	5.32	12.1
Hawke's Bay	29/4/92	3rd Pick	3.13	**1.60**	7.91	0.27	12.64	24.75	12.68	62.58	2.07	4.94	12.5
Nelson	27/1/92	1st Pick	***1.12***	1.42	***6.06***	0.23	***8.60***	***12.99***	**16.55**	**70.46**	2.56	***4.26***	***9.0***
Nelson	5/3/92	2nd Pick	2.58	***1.33***	7.23	***0.20***	11.13	23.16	11.95	64.89	***1.74***	5.43	11.3
Nelson	6/4/92	3rd Pick	**3.92**	1.50	**8.33**	0.27	**13.75**	**28.48**	***10.91***	***60.61***	1.92	**5.56**	**13.4**
Minimum			1.12	1.33	6.06	0.20	8.60	12.99	10.91	60.61	1.74	4.26	9.0
Maximum			3.92	1.60	8.33	0.33	13.75	28.48	16.55	70.46	3.21	5.56	13.4
Number of Samples			6	6	6	6	6	6	6	6	6	6	6
Mean			2.55	1.49	7.37	0.27	11.41	21.48	13.36	65.16	2.35	4.96	11.3
Standard Deviation			0.98	0.09	0.78	0.05	1.78	5.63	2.19	3.51	0.50	0.53	1.6
Coefficient of Variation (%)			38.53	6.19	10.61	18.13	15.56	26.21	16.36	5.39	21.20	10.65	13.8

Bold italic typeface = minimum values
Bold typeface = maximum values

Table A23.4: Individual sugar concentrations in the juice of Fuji apples that were harvested at commerical maturity.

Region	Sample Date	Stage of Picking	Sucrose (g/100ml)	Glucose (g/100ml)	Fructose (g/100ml)	Sorbitol (g/100ml)	Total Sugars (g/100ml)	Sucrose as % of Total Sugars	Glucose as % of Total Sugars	Fructose as % of Total Sugars	Sorbitol as % of Total Sugars + Sorbitol	Fruc/Gluc Ratio	°Brix
Hawke's Bay	4/3/92	1st Pick	1.46	***2.40***	***5.62***	***0.35***	***9.48***	15.41	25.31	59.28	3.58	2.34	***10.4***
Hawke's Bay	7/4/92	2nd Pick	2.11	2.92	7.04	0.47	12.07	17.49	24.17	58.35	3.72	2.41	12.1
Hawke's Bay	1/4/93	2nd Pick	1.78	2.56	6.23	0.40	10.57	16.87	24.25	58.88	3.67	2.43	11.4
Nelson	16/3/92	1st Pick	***1.28***	3.45	6.22	0.39	10.94	11.69	**31.52**	56.79	***3.43***	***1.80***	10.9
Nelson	22/4/92	2nd Pick	1.62	4.00	7.86	0.58	13.48	12.03	29.67	58.30	4.09	1.97	13.3
Nelson	22/5/92	3rd Pick	1.34	**4.25**	7.90	0.51	**13.49**	***9.97***	31.49	58.55	3.63	1.86	**13.5**
Canterbury	18/3/92	1st Pick	1.53	2.91	6.54	0.40	10.98	13.90	26.53	59.58	3.49	2.25	11.2
Canterbury	23/4/92	2nd Pick	**2.82**	3.02	7.41	0.73	13.25	**21.28**	***22.79***	***55.93***	5.22	2.45	12.8
Canterbury	29/5/92	3rd Pick	2.15	3.04	**7.97**	**0.96**	13.16	16.32	23.12	**60.56**	**6.80**	**2.62**	13.2
Minimum			1.28	2.40	5.62	0.35	9.48	9.97	22.79	55.93	3.43	1.80	10.4
Maximum			2.82	4.25	7.97	0.96	13.49	21.28	31.52	60.56	6.80	2.62	13.5
Number of Samples			9	9	9	9	9	9	9	9	9	9	9
Mean			1.79	3.17	6.98	0.53	11.94	15.00	26.54	58.47	4.18	2.24	12.1
Standard Deviation			0.47	0.58	0.82	0.19	1.41	3.29	3.29	1.32	1.06	0.28	1.1
Coefficient of Variation (%)			26.08	18.37	11.68	35.32	11.79	21.94	12.39	2.26	25.35	12.28	9.0

Bold italic typeface = minimum values
Bold typeface = maximum values

Table A23.5: Individual sugar concentrations in the juice of Hillwell, GS330, GS2850 and Fiesta apples that were harvested at commerical maturity from Hawke's Bay.

Cultivar	Sucrose (g/100ml)	Glucose (g/100ml)	Fructose (g/100ml)	Sorbitol (g/100ml)	Total Sugars (g/100ml)	Sucrose as % of Total Sugars	Glucose as % of Total Sugars	Fructose as % of Total Sugars	Sorbitol as % of Total Sugars + Sorbitol	Fruc/Gluc Ratio	°Brix
Hillwell	4.16	1.61	5.82	0.40	11.60	35.89	13.92	50.20	3.37	3.61	12.4
GS330	4.96	1.51	7.29	0.74	13.76	36.08	10.98	52.95	5.08	4.82	13.5
GS2850	3.73	1.60	7.44	0.33	12.77	29.23	12.50	58.28	2.53	4.66	12.5
Fiesta	4.41	0.79	4.77	0.21	9.98	44.23	7.91	47.86	2.03	6.06	11.6

APPENDIX 24

CHANGES IN THE INDIVIDUAL SUGAR CONCENTRATIONS IN THE JUICE OF BRAEBURN APPLES STORED AT DIFFERENT CONDITIONS IN 1992

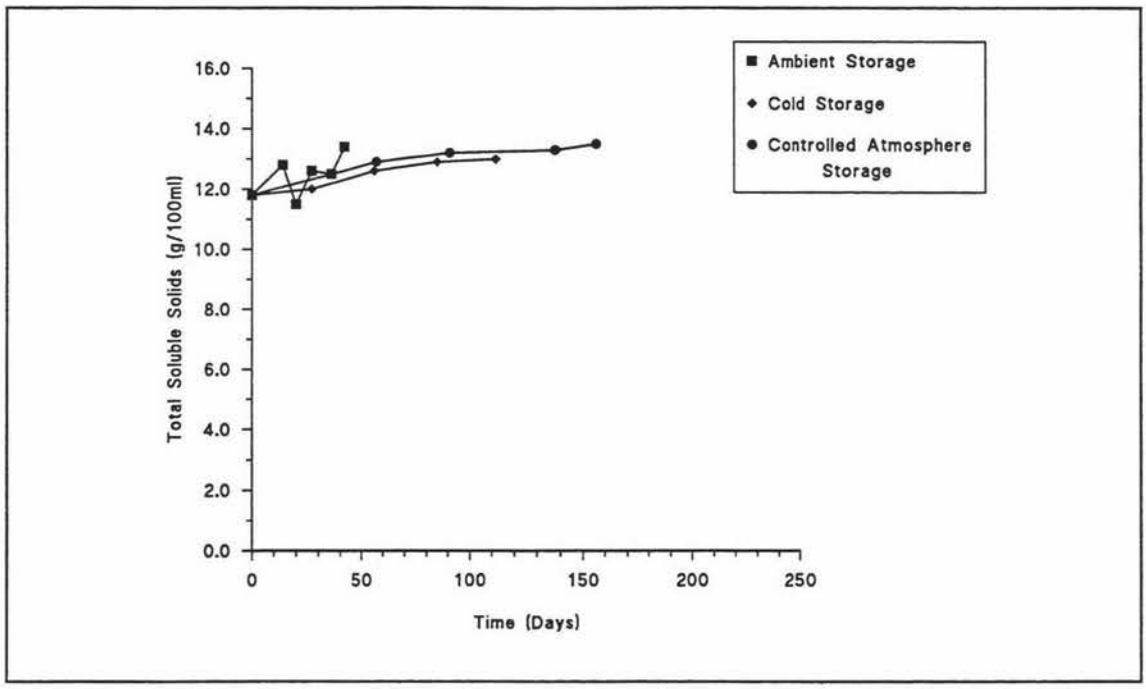

Figure A24.1: Effect on juice total soluble solids concentrations of different storage regimes for Braeburn apples in 1992.

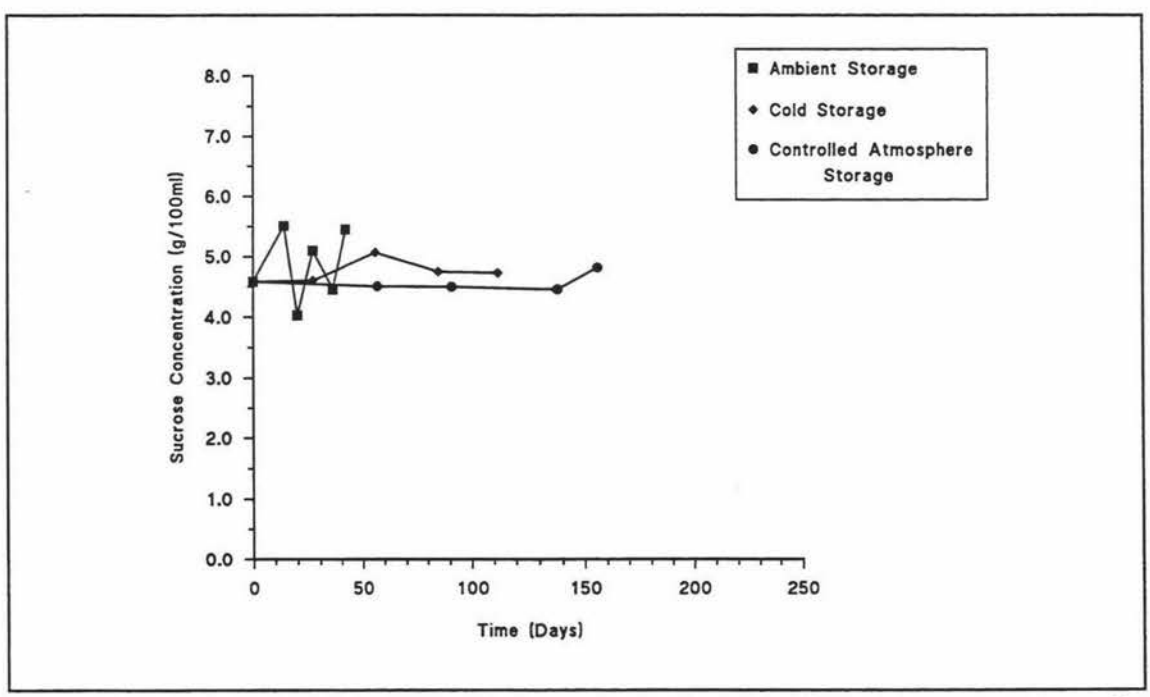

Figure A24.2: Effect on juice sucrose concentrations of different storage regimes for Braeburn apples in 1992.

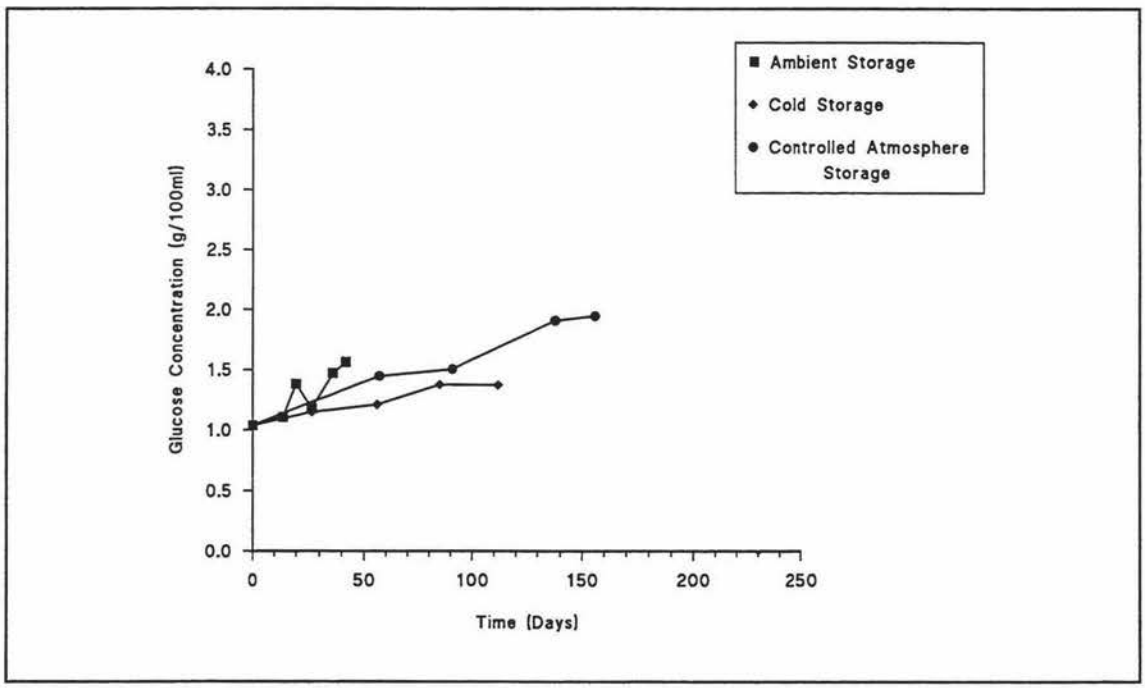

Figure A24.3: Effect on juice glucose concentrations of different storage regimes for Braeburn apples in 1992.

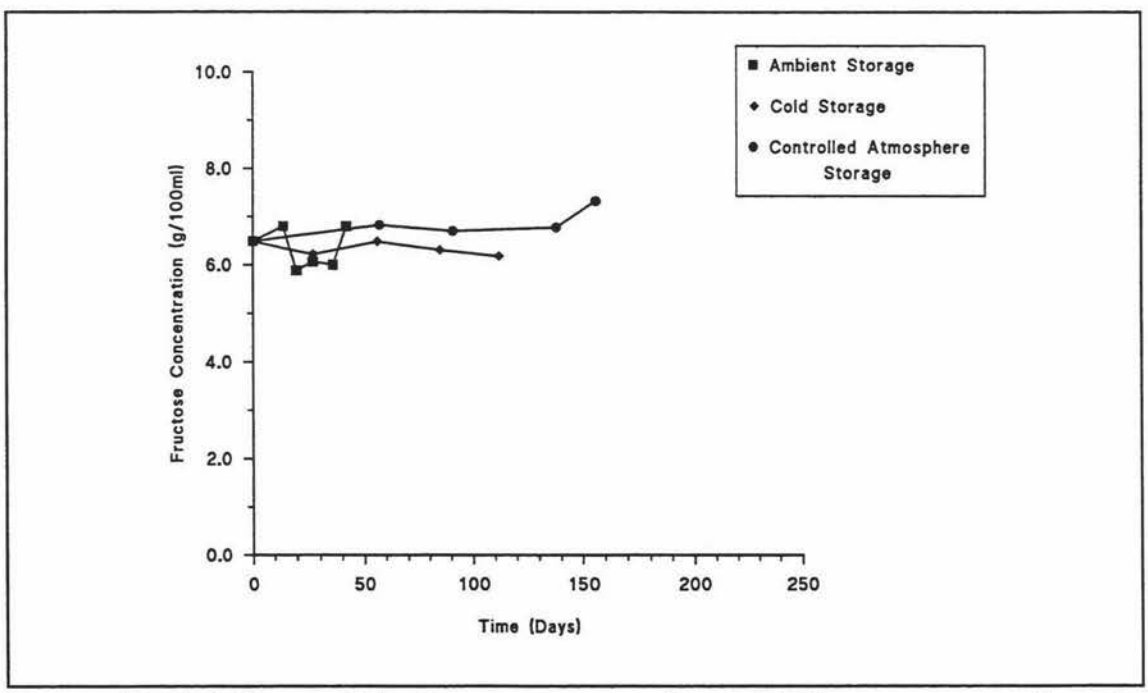

Figure A24.4: Effect on juice fructose concentrations of different storage regimes for Braeburn apples in 1992.

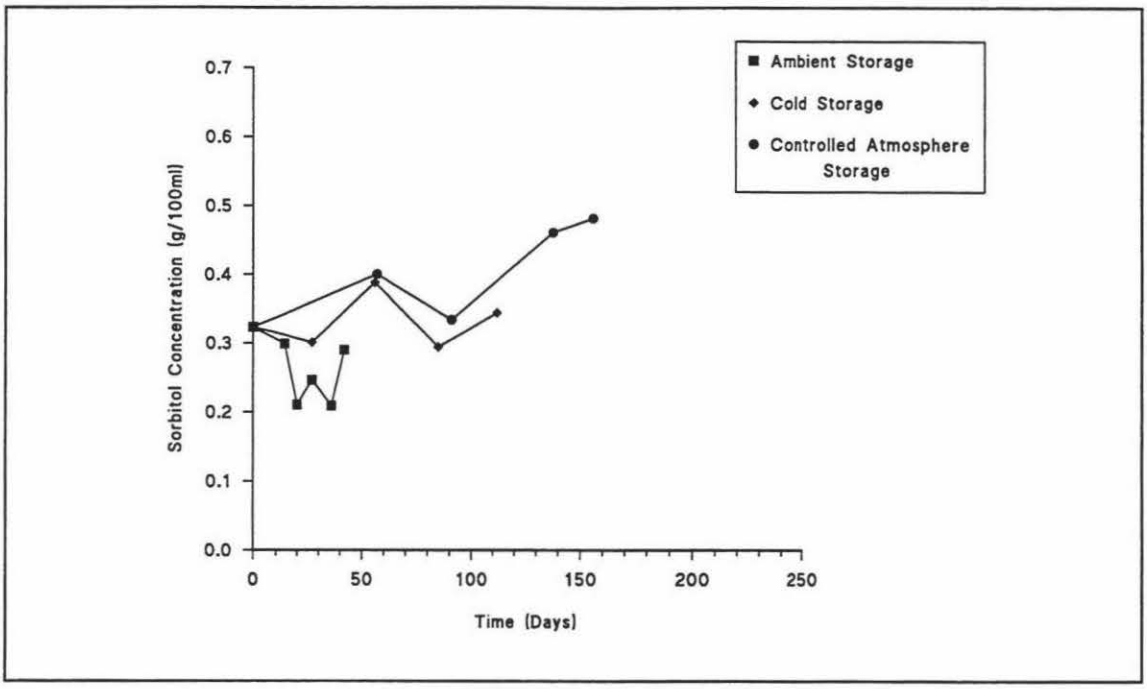

Figure A24.5: Effect on juice sorbitol concentrations of different storage regimes for Braeburn apples in 1992.

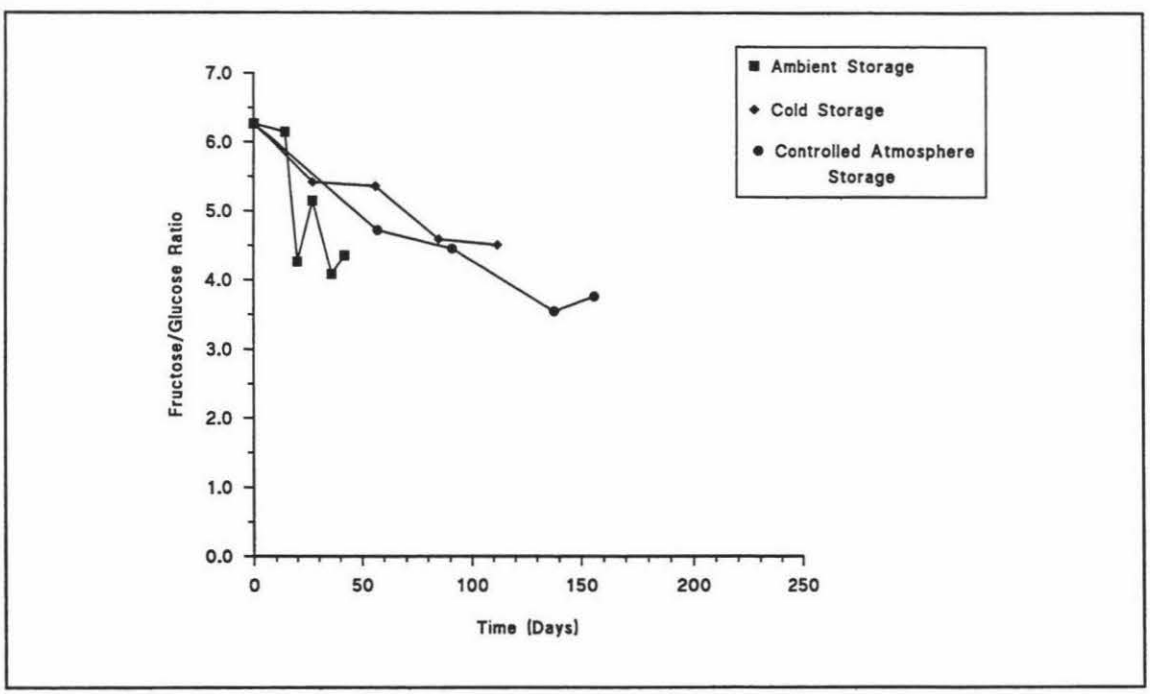

Figure A24.6: Effect on juice fructose/glucose ratios of different storage regimes for Braeburn apples in 1992.

APPENDIX 25

CHANGES IN THE INDIVIDUAL SUGAR CONCENTRATIONS IN THE JUICE OF GRANNY SMITH APPLES STORED AT DIFFERENT CONDITIONS IN 1992

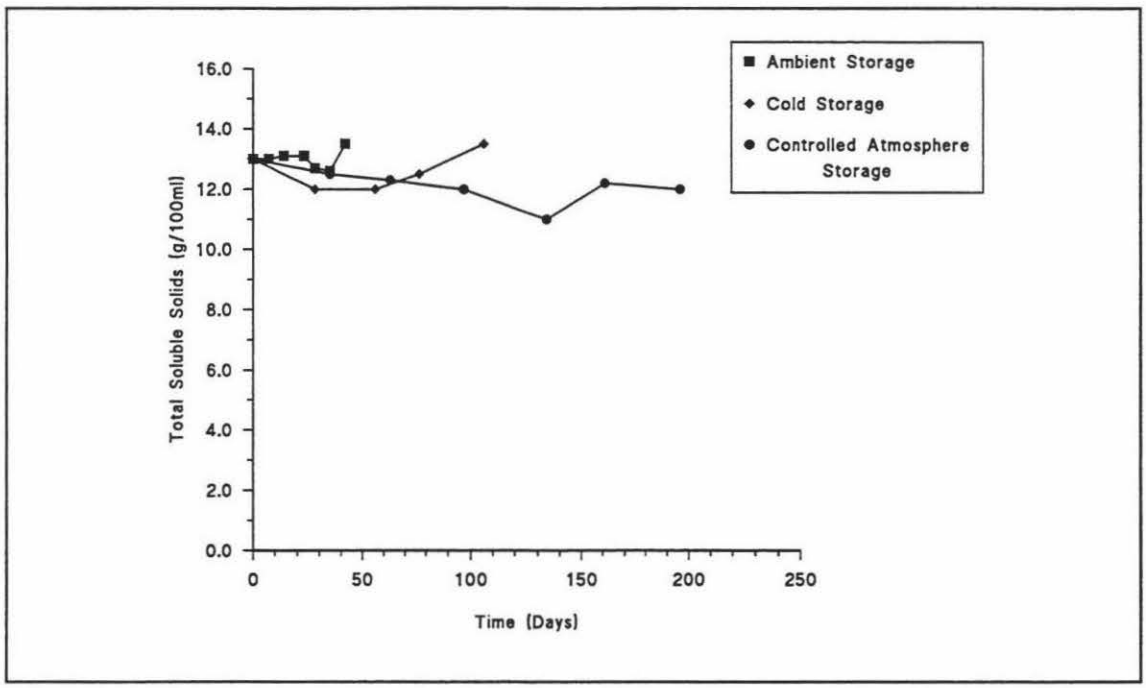

Figure A25.1: Effect on juice total soluble solids concentrations of different storage regimes for Granny Smith apples in 1992.

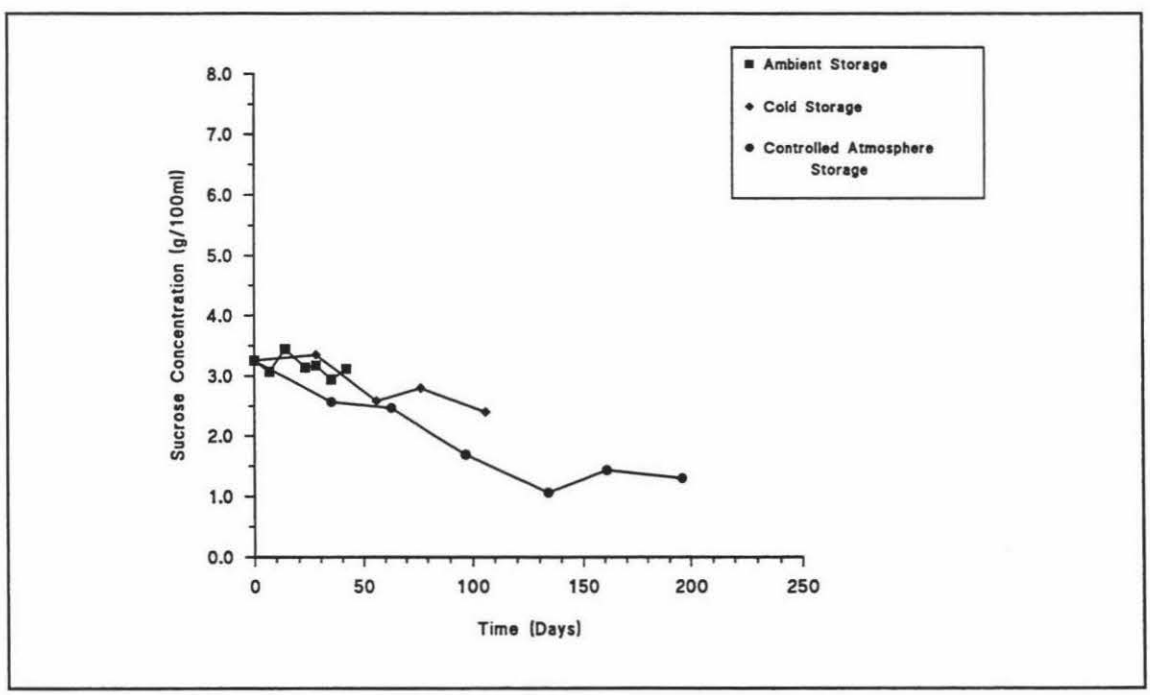

Figure A25.2: Effect on juice sucrose concentrations of different storage regimes for Granny Smith apples in 1992.

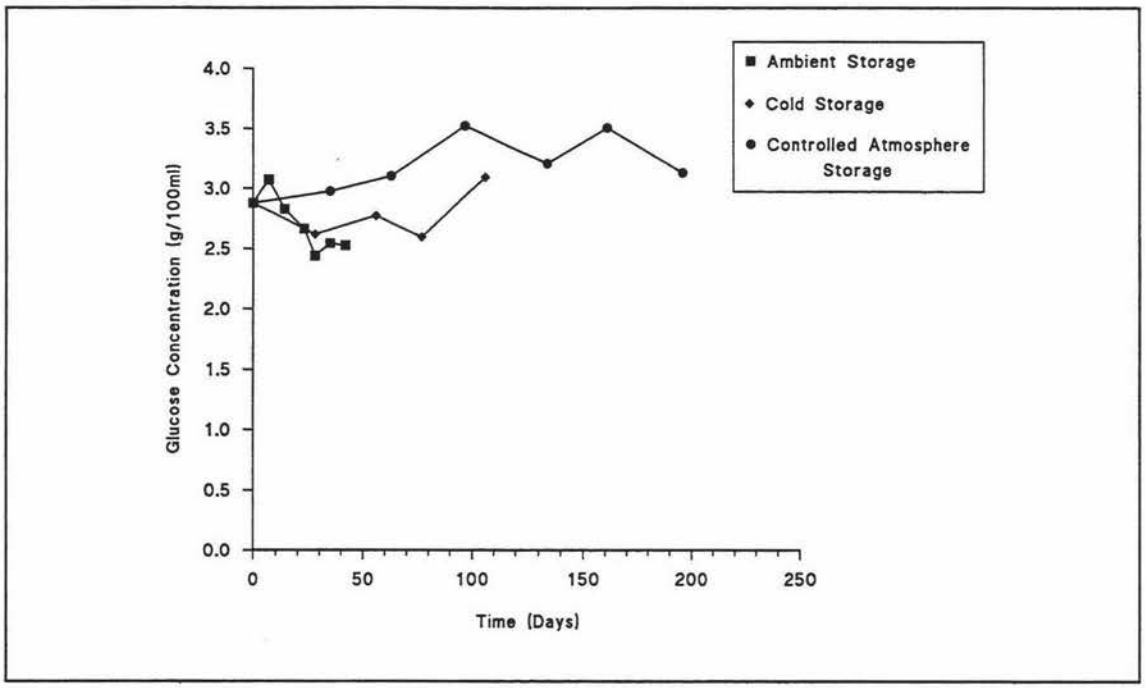

Figure A25.3: Effect on juice glucose concentrations of different storage regimes for Granny Smith apples in 1992.

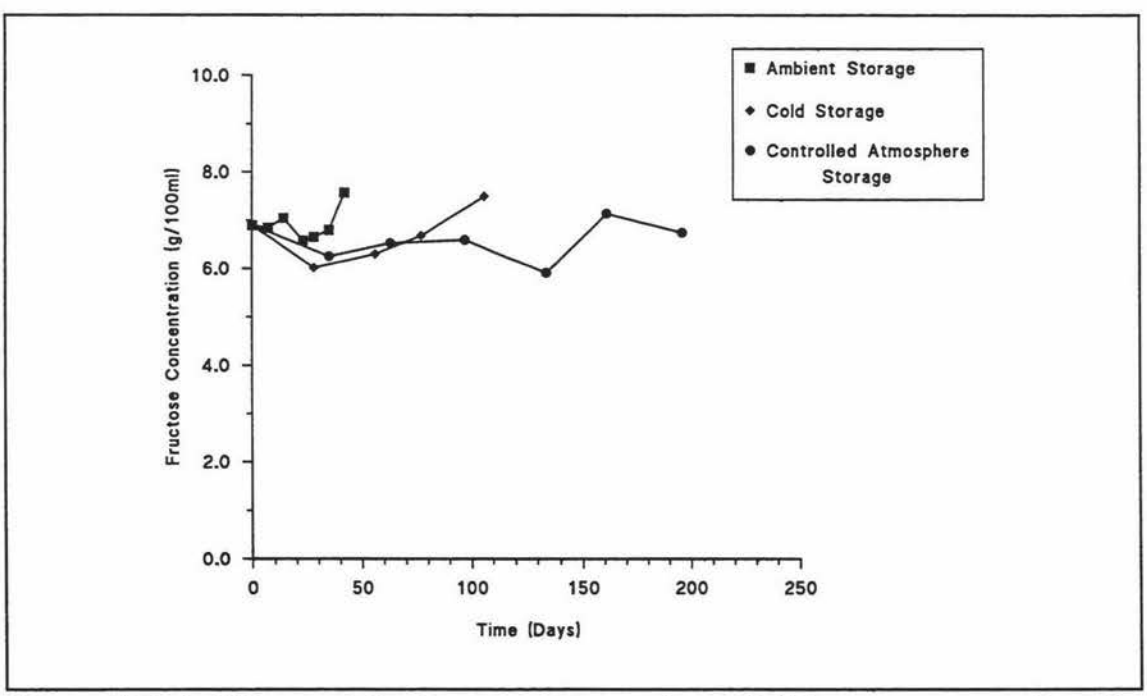

Figure A25.4: Effect on juice fructose concentrations of different storage regimes for Granny Smith apples in 1992.

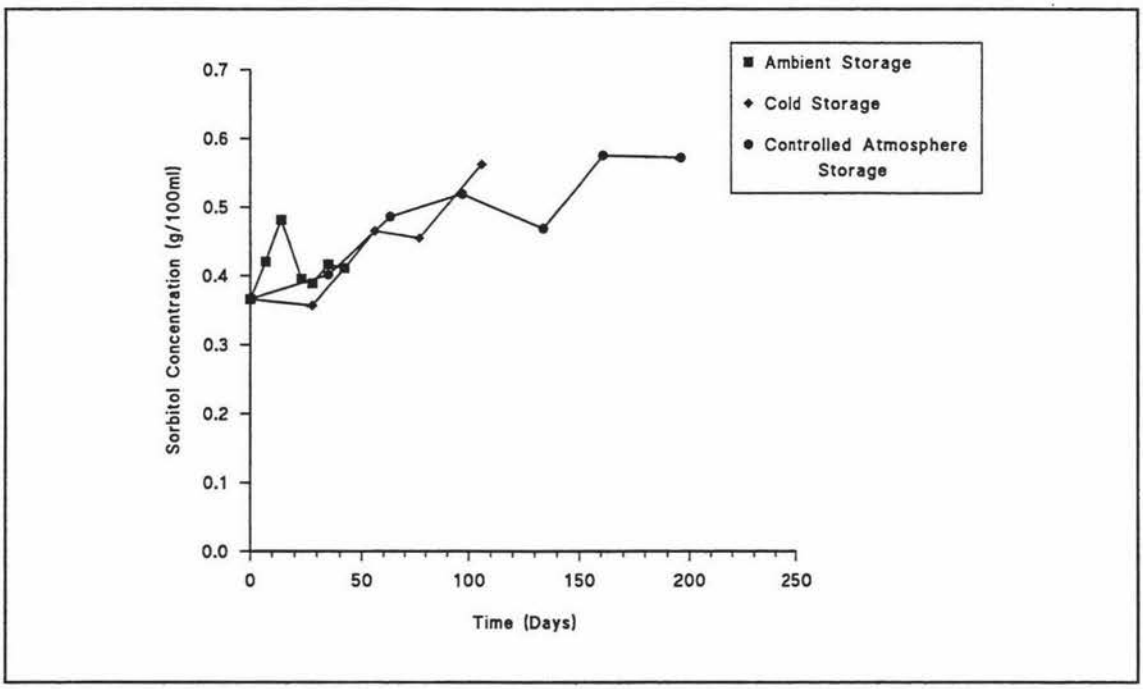

Figure A25.5: Effect on juice sorbitol concentrations of different storage regimes for Granny Smith apples in 1992.

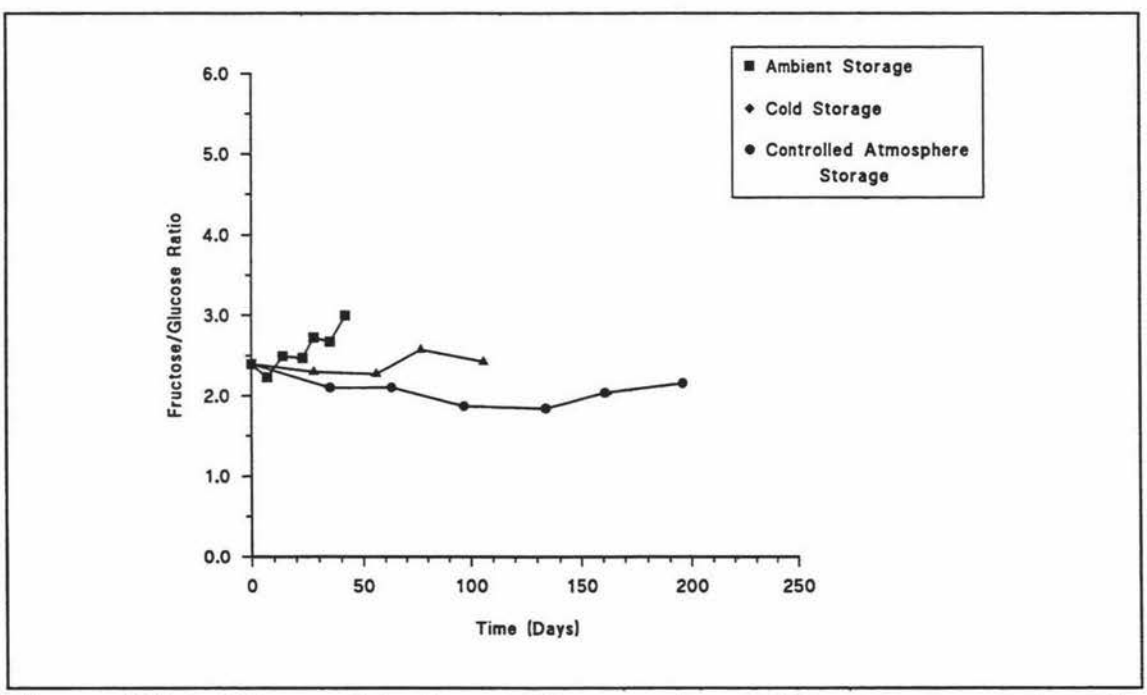

Figure A25.6: Effect on juice fructose/glucose ratios of different storage regimes for Granny Smith apples in 1992.

APPENDIX 26

CHANGES IN THE INDIVIDUAL SUGAR CONCENTRATIONS IN THE JUICE OF ROYAL GALA AND GALA APPLES STORED AT DIFFERENT CONDITIONS IN 1992

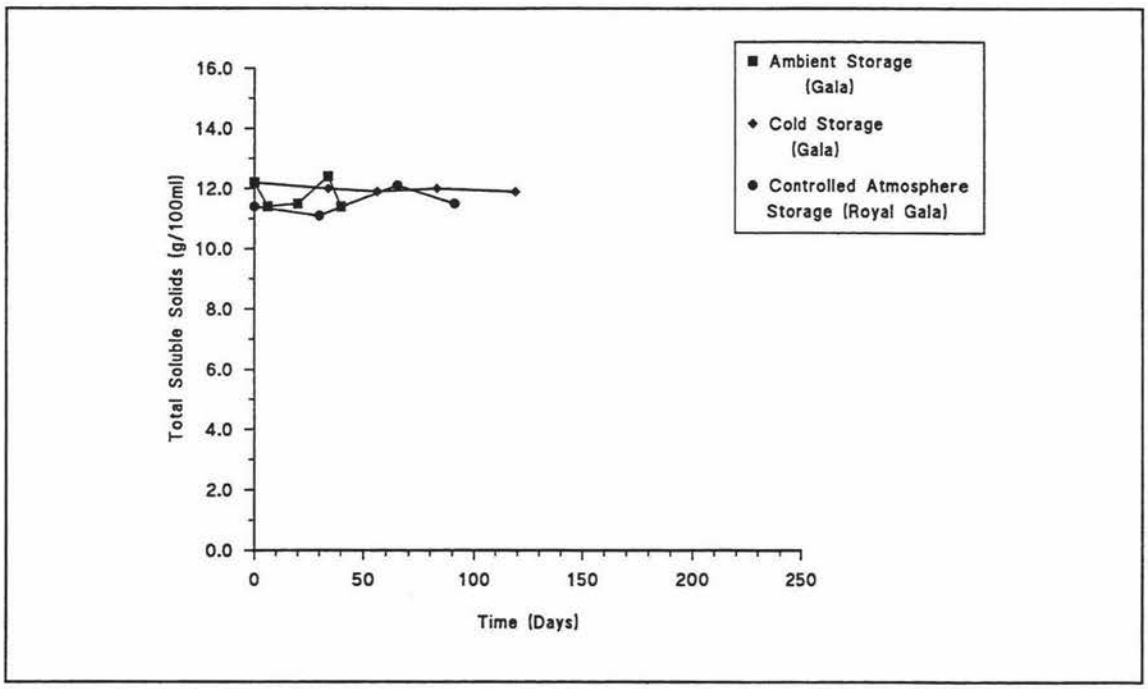

Figure A26.1: Effect on juice total soluble solids concentrations of different storage regimes for Gala and Royal Gala apples in 1992.

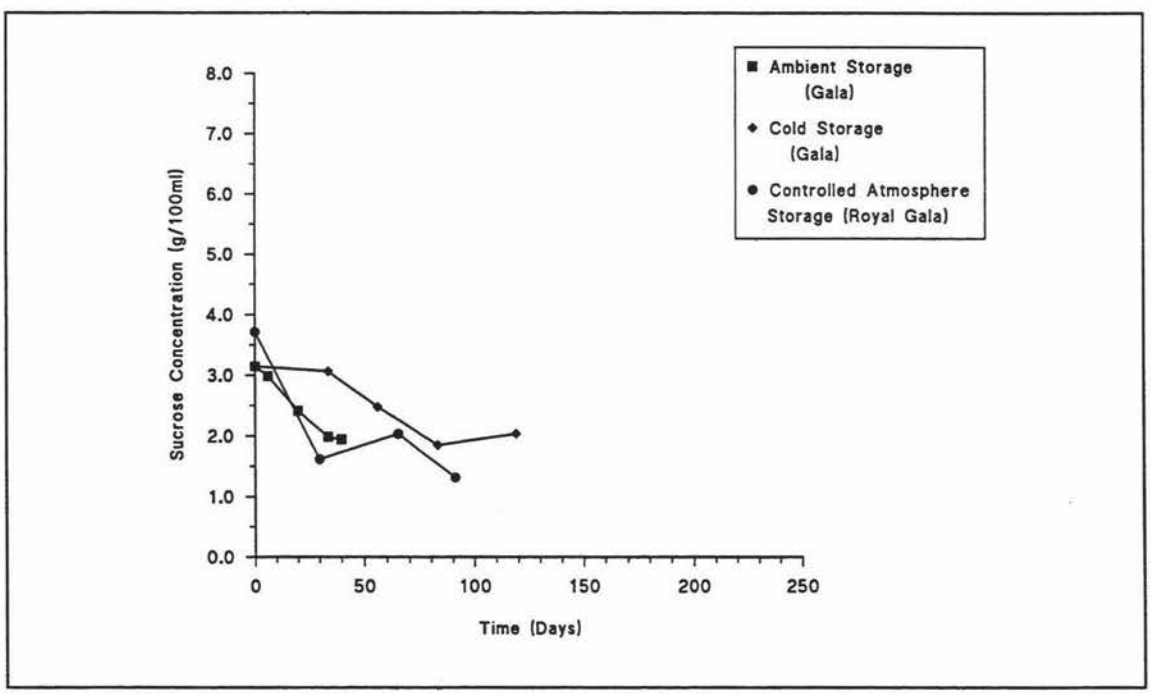

Figure A26.2: Effect on juice sucrose concentrations of different storage regimes for Gala and Royal Gala apples in 1992.

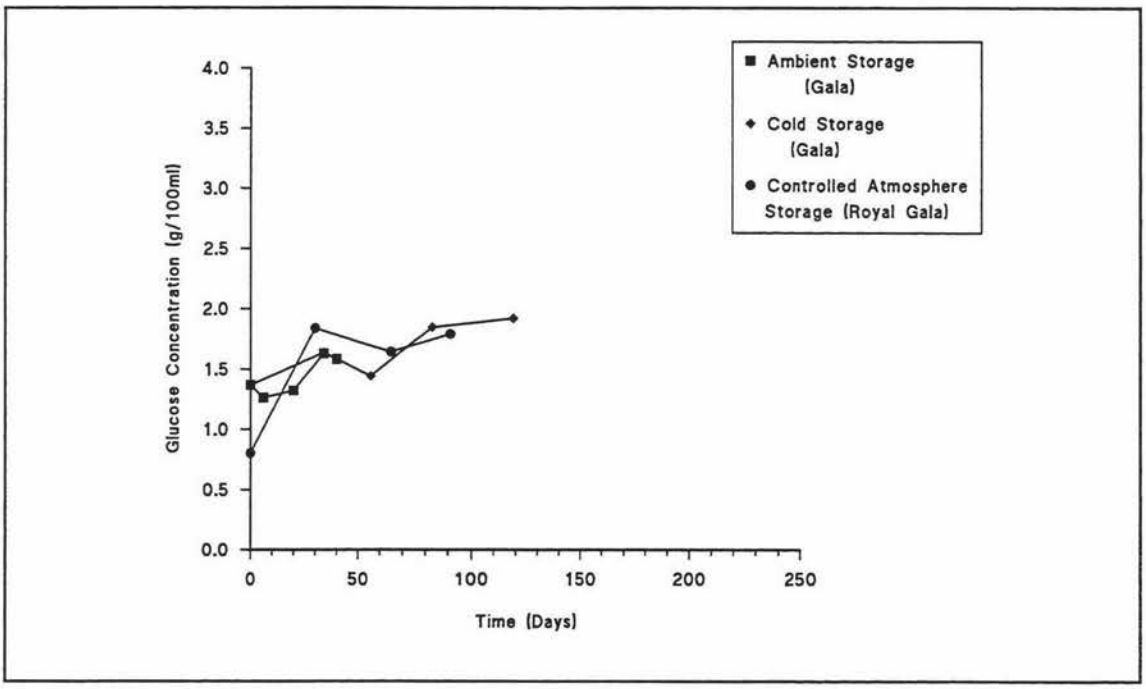

Figure A26.3: Effect on juice glucose concentrations of different storage regimes for Gala and Royal Gala apples in 1992.

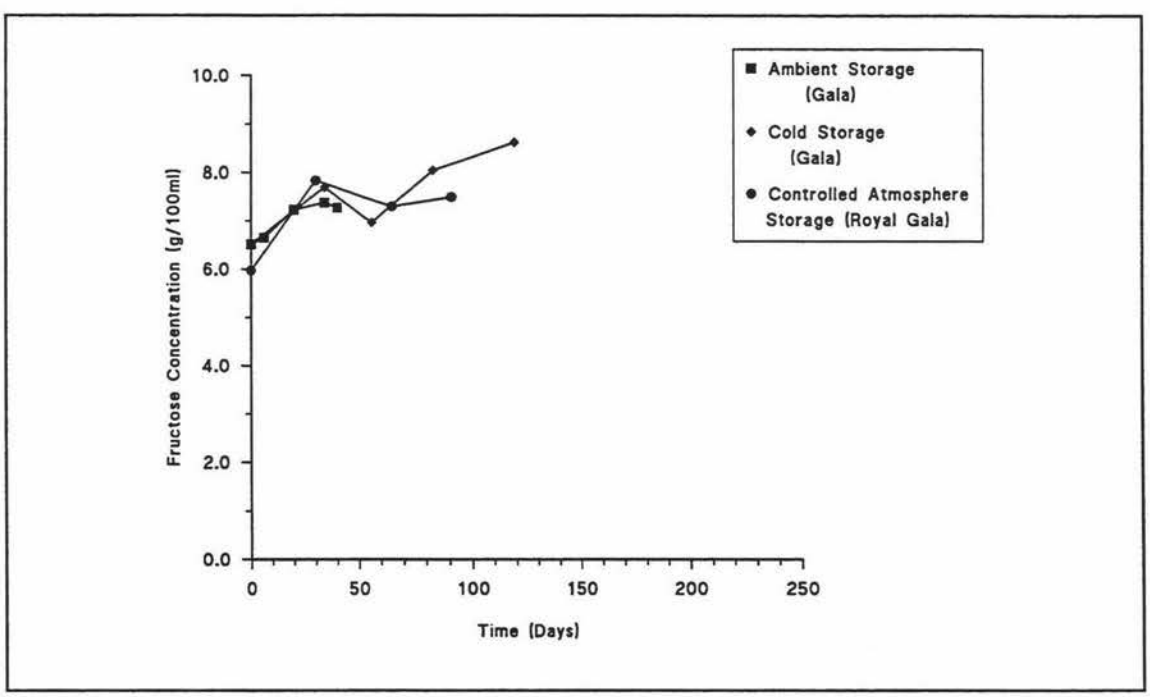

Figure A26.4: Effect on juice fructose concentrations of different storage regimes for Gala and Royal Gala apples in 1992.

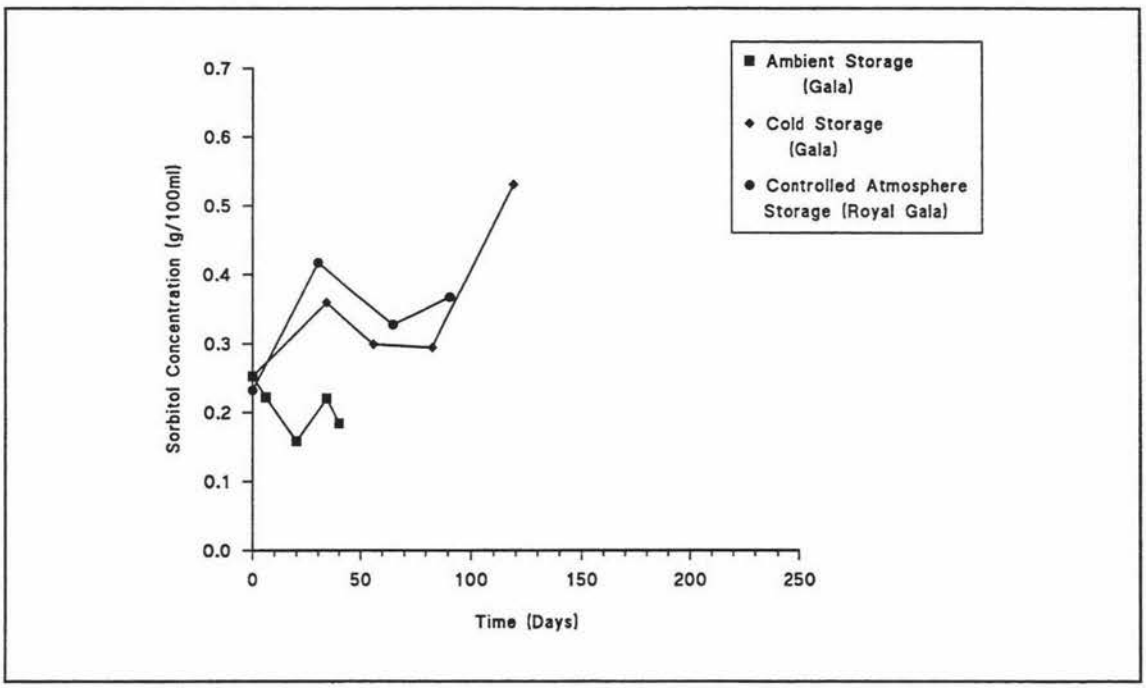

Figure A26.5: Effect on juice sorbitol concentrations of different storage regimes for Gala and Royal Gala apples in 1992.

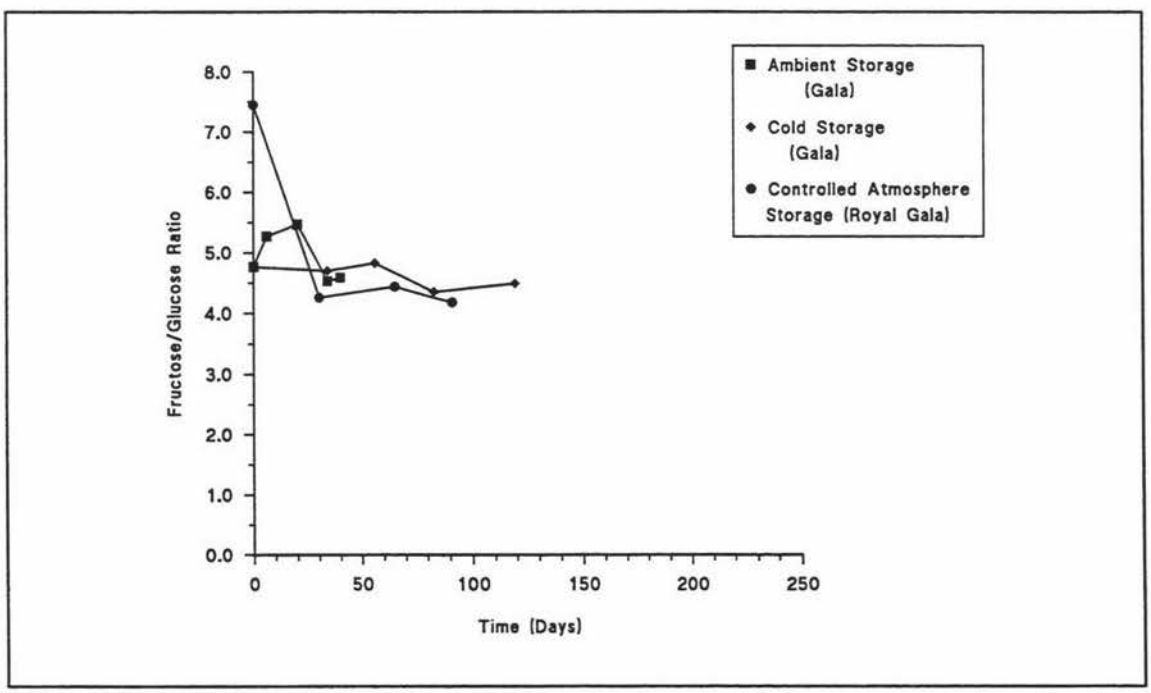

Figure A26.6: Effect on juice fructose/glucose ratios of different storage regimes for Gala and Royal Gala apples in 1992.

APPENDIX 27

INDIVIDUAL ORGANIC ACIDS AND RELATED COMPONENTS OF ALL CULTIVARS COMBINED TO GIVE THE "AVERAGE COMPOSITION OF NATURAL APPLE JUICE"

Table A27.1: Minimum, maximum, mean, standard deviation and coefficient of variation in the individual organic acid concentrations of all cultivars combined to give the "average composition of natural apple juice".

Sample	Quinic Acid (mg/100ml)	Malic Acid (mg/100ml)	Shikimic Acid (mg/100ml)	Citric Acid (mg/100ml)	Succinic Acid (mg/100ml)	Fumaric Acid (mg/100ml)	Total Acids (mg/100ml)	pH	Titratable Acidity (mg/100ml)	Quinic Acid as % of Total Acids	Malic Acid as % of Total Acids	Shikimic Acid as % of Total Acids	Citric Acid as % of Total Acids	Succinic Acid as % of Total Acids	Fumaric Acid as % of Total acids
Minimum	21.75	231.24	0.01	2.10	8.28	0.040	304.35	3.16	210.00	2.91	66.74	0.00	0.54	1.14	0.005
Maximum	129.26	1067.59	3.59	20.28	41.11	0.222	1208.12	4.15	1130.00	27.95	93.25	0.74	4.74	10.02	0.046
Number of Samples	188	188	188	188	188	188	188	188	188	188	188	188	188	188	188
Mean	39.97	591.69	0.91	8.30	19.23	0.077	660.18	3.47	515.74	6.58	88.81	0.17	1.33	3.11	0.013
Standard Deviation	15.14	182.48	0.70	2.83	6.60	0.032	186.14	0.23	182.92	3.56	4.48	0.15	0.56	1.30	0.008
Coefficient of variation (%)	37.88	30.84	76.30	34.08	34.35	41.33	28.19	6.67	35.47	54.06	5.05	92.82	41.83	41.78	63.58

APPENDIX 28

INDIVIDUAL ORGANIC ACIDS AND RELATED COMPONENTS FOR BRAEBURN APPLE JUICES.

Table A28.1: Individual organic acid concentrations in the juice of Hawke's Bay Braeburn apples that were stored at different conditions in 1992.

Storage Time (Days)	Quinic Acid (mg/100ml)	Malic Acid (mg/100ml)	Shikimic Acid (mg/100ml)	Citric Acid (mg/100ml)	Succinic Acid (mg/100ml)	Fumaric Acid (mg/100ml)	Total Acids (mg/100ml)	pH	Titratable Acidity (mg/100ml)	Quinic Acid as % of Total Acids	Malic Acid as % of Total Acids	Shikimic Acid as % of Total Acids	Citric Acid as % of Total Acids	Succinic Acid as % of Total Acids	Fumaric Acid as % of Total acids
Ambient Storage															
0	26.20	713.75	0.43	8.60	20.93	0.050	769.96	3.36	600	3.40	92.70	0.06	1.12	2.72	***0.006***
14	25.32	601.75	0.31	8.39	28.27	0.079	664.12	3.48	500	3.81	90.61	0.05	1.26	4.26	0.012
20	29.77	639.09	0.55	8.68	23.11	0.056	701.26	3.46	500	4.25	91.13	0.08	1.24	3.30	0.008
27	24.61	567.40	0.35	8.20	20.43	0.124	621.12	3.50	480	3.96	91.35	0.06	1.32	3.29	0.020
36	27.09	589.87	0.56	9.18	21.81	0.112	648.62	3.54	460	4.18	90.94	0.09	1.41	3.36	0.017
42	27.47	451.90	0.29	9.26	17.88	0.161	506.95	3.58	440	5.42	89.14	0.06	1.83	3.53	0.032
Cold Storage															
0	26.20	713.75	0.43	8.60	20.93	0.050	769.96	3.36	600	3.40	92.70	0.06	1.12	2.72	0.006
27	27.81	632.90	0.43	9.79	23.66	0.050	694.64	3.39	510	4.00	91.11	0.06	1.41	3.41	0.007
56	***23.29***	612.34	0.45	11.04	30.15	0.076	677.34	3.41	500	3.44	90.40	0.07	1.63	4.45	0.011
85	28.28	426.06	0.32	9.23	18.51	0.122	482.52	3.56	400	5.86	88.30	0.07	1.91	3.84	0.025
112	26.92	***382.28***	***0.24***	11.29	18.15	0.076	***438.96***	**3.61**	370	6.13	87.09	0.05	**2.57**	4.14	0.017
Controlled Atmosphere Storage															
0	26.20	713.75	0.43	8.60	20.93	0.050	769.96	3.36	600	3.40	92.70	0.06	1.12	2.72	***0.006***
57	25.58	603.34	0.29	9.47	17.83	0.052	656.56	3.39	480	3.90	91.89	***0.04***	1.44	2.72	0.008
91	27.74	599.17	0.31	12.20	16.72	0.074	656.22	3.48	470	4.23	91.31	0.05	1.86	2.55	0.011
138	30.68	592.01	0.54	10.64	14.95	0.080	648.90	3.49	480	4.73	91.23	0.08	1.64	2.30	0.012
156	29.93	411.33	0.37	10.40	14.19	0.114	466.35	3.58	380	6.42	88.20	0.08	2.23	3.04	0.025

Table A28.2: Individual organic acid concentrations in the juice of Hawke's Bay Braeburn apples that were stored at different conditions in 1993.

Storage Time (Days)	Quinic Acid (mg/100ml)	Malic Acid (mg/100ml)	Shikimic Acid (mg/100ml)	Citric Acid (mg/100ml)	Succinic Acid (mg/100ml)	Fumaric Acid (mg/100ml)	Total Acids (mg/100ml)	pH	Titratable Acidity (mg/100ml)	Quinic Acid as % of Total Acids	Malic Acid as % of Total Acids	Shikimic Acid as % of Total Acids	Citric Acid as % of Total Acids	Succinic Acid as % of Total Acids	Fumaric Acid as % of Total acids
Ambient Storage															
0	40.13	691.23	0.86	6.59	13.73	0.056	752.60	3.19	600	5.33	91.85	0.11	0.88	1.82	0.007
8	37.52	639.70	0.53	6.17	14.14	0.050	698.11	3.25	540	5.37	91.63	0.08	0.88	2.03	0.007
14	39.69	621.77	0.61	6.87	16.72	0.050	685.70	3.30	520	5.79	90.68	0.09	1.00	2.44	0.007
21	37.09	457.03	0.61	5.69	13.66	0.065	514.15	3.38	440	7.21	88.89	0.12	1.11	2.66	0.013
28	29.38	409.82	0.95	6.07	8.88	0.100	455.21	3.43	370	6.45	90.03	0.21	1.33	1.95	0.022
35	37.06	429.16	1.01	6.33	10.85	0.137	484.56	3.40	***360***	7.65	88.57	0.21	1.31	2.24	0.028
42	38.41	403.55	0.43	5.09	10.00	0.134	457.62	3.43	410	8.39	88.18	0.09	1.11	2.19	0.029
49	42.25	447.94	0.63	7.55	12.13	0.176	510.67	3.48	420	8.27	87.72	0.12	1.48	2.38	0.034
56	43.60	482.99	1.29	6.73	10.97	**0.222**	545.81	3.49	410	7.99	88.49	**0.24**	1.23	2.01	**0.041**
64	41.76	442.58	0.77	8.15	11.62	0.196	505.07	3.56	430	8.27	87.63	0.15	1.61	2.30	0.039
Cold Storage															
0	40.13	691.23	0.86	6.59	13.73	0.056	752.60	3.19	600	5.33	91.85	0.11	0.88	1.82	0.007
28	35.59	621.19	1.03	7.68	11.40	0.062	676.96	3.31	520	5.26	91.76	0.15	1.13	1.68	0.009
56	40.24	666.91	0.59	8.27	22.95	0.069	739.03	3.30	550	5.45	90.24	0.08	1.12	3.10	0.009
85	39.95	568.34	0.73	8.13	21.69	0.058	638.91	3.32	440	6.25	88.96	0.11	1.27	3.40	0.009
119	37.31	388.12	0.42	7.38	15.24	0.050	448.52	3.38	380	8.32	86.53	0.09	1.65	3.40	0.011
147	43.84	415.41	1.14	10.27	21.27	0.085	492.02	3.52	370	8.91	84.43	0.23	2.09	4.32	0.017
175	47.62	413.93	0.58	9.63	18.97	0.070	490.80	3.46	380	**9.70**	84.34	0.12	1.96	3.86	0.014
206	46.69	403.49	0.73	10.23	21.14	0.076	482.36	3.56	360	9.68	***83.65***	0.15	2.12	4.38	0.016
Controlled Atmosphere Storage															
0	40.13	691.23	0.86	6.59	13.73	0.056	752.60	3.19	600	5.33	91.85	0.11	0.88	1.82	0.007
91	43.15	640.72	0.68	7.18	10.45	0.061	702.23	3.28	520	6.14	91.24	0.10	1.02	1.49	0.009
119	41.68	549.35	0.48	6.31	***8.28***	0.056	606.16	3.30	440	6.88	90.63	0.08	1.04	1.37	0.009
147	44.60	470.01	1.06	8.11	10.51	0.102	534.40	3.43	410	8.35	87.95	0.20	1.52	1.97	0.019
175	41.07	433.18	0.45	8.02	10.69	0.092	493.50	3.46	400	8.32	87.78	0.09	1.62	2.17	0.019
206	38.50	408.11	0.43	8.79	15.01	0.082	470.91	3.53	360	8.18	86.66	0.09	1.87	3.19	0.017

Table A28.3: Variation in the individual organic acid concentrations in the juice of Braeburn apples harvested from different positions on a tree, different trees and orchards, different bins present in the processing yard and different positions within a bin. All samples were collected on the same day from Hawke's Bay in 1993.

Sample	Quinic Acid (mg/100ml)	Malic Acid (mg/100ml)	Shikimic Acid (mg/100ml)	Citric Acid (mg/100ml)	Succinic Acid (mg/100ml)	Fumaric Acid (mg/100ml)	Total Acids (mg/100ml)	pH	Titratable Acidity (mg/100ml)	Quinic Acid as % of Total Acids	Malic Acid as % of Total Acids	Shikimic Acid as % of Total Acids	Citric Acid as % of Total Acids	Succinic Acid as % of Total Acids	Fumaric Acid as % of Total acids
North Side	31.29	619.41	0.87	10.35	11.51	0.050	673.48	3.38	580	4.65	91.97	0.13	1.54	1.71	0.007
South Side	35.90	703.54	0.93	11.49	8.98	0.046	760.88	3.33	620	4.72	92.46	0.12	1.51	***1.18***	***0.006***
East Side	37.28	790.56	1.30	12.90	13.44	0.050	855.53	3.28	760	4.36	92.41	0.15	1.51	1.57	***0.006***
West Side	31.54	692.17	1.22	11.67	13.14	0.050	749.78	3.32	700	4.21	92.32	0.16	1.56	1.75	0.007
Top of Tree	35.68	679.29	0.82	5.80	22.94	0.062	744.60	3.37	640	4.79	91.23	0.11	0.78	3.08	0.008
Bottom of Tree	43.74	706.63	**1.74**	11.23	12.95	0.050	776.34	3.33	640	5.63	91.02	0.22	1.45	1.67	***0.006***
Orchard 2	30.42	516.37	1.10	9.46	11.81	0.043	569.21	3.37	500	5.35	90.72	0.19	1.66	2.07	0.008
Orchard 2	28.01	597.91	1.02	11.73	11.96	0.050	650.68	3.31	580	4.31	91.89	0.16	1.80	1.84	0.008
Orchard 3	32.96	592.01	0.60	9.63	14.48	0.050	649.72	3.22	560	5.07	91.12	0.09	1.48	2.23	0.008
Orchard 4	33.61	665.53	1.00	12.74	12.36	0.050	725.29	3.33	680	4.63	91.76	0.14	1.76	1.70	0.007
Centre of Bin	35.30	582.67	1.11	10.63	12.28	0.050	642.04	3.31	550	5.50	90.75	0.17	1.66	1.91	0.008
Bottom Corner of Bin 1	31.68	591.62	1.04	10.15	10.35	0.047	644.89	3.32	580	4.91	91.74	0.16	1.57	1.60	0.007
Top Corner of Bin 1	31.34	618.54	0.99	11.84	11.01	0.050	673.78	3.34	600	4.65	91.80	0.15	1.76	1.63	0.007
Bottom Corner of Bin 2	31.12	564.35	1.18	8.90	8.41	***0.040***	613.99	3.37	520	5.07	91.91	0.19	1.45	1.37	0.007
Top Corner of Bin 2	32.02	623.44	1.02	9.94	9.36	0.046	675.82	3.31	620	4.74	92.25	0.15	1.47	1.38	0.007

Table A28.3: Continued

Sample	Quinic Acid (mg/100ml)	Malic Acid (mg/100ml)	Shikimic Acid (mg/100ml)	Citric Acid (mg/100ml)	Succinic Acid (mg/100ml)	Fumaric Acid (mg/100ml)	Total Acids (mg/100ml)	pH	Titratable Acidity (mg/100ml)	Quinic Acid as % of Total Acids	Malic Acid as % of Total Acids	Shikimic Acid as % of Total Acids	Citric Acid as % of Total Acids	Succinic Acid as % of Total Acids	Fumaric Acid as % of Total acids
Bin 2	26.87	678.94	1.06	11.84	12.21	0.051	730.98	3.35	680	3.68	92.88	0.14	1.62	1.67	0.007
Bin 3	36.86	609.92	0.97	9.22	24.91	0.051	681.94	3.29	560	5.41	89.44	0.14	1.35	3.65	0.007
Bin 4	34.16	658.12	1.23	**13.15**	18.10	0.050	724.80	3.29	650	4.71	90.80	0.17	1.81	2.50	0.007
Bin 5	31.77	577.89	1.03	10.82	10.85	0.050	632.42	3.41	560	5.02	91.38	0.16	1.71	1.72	0.008
Large Apple	37.64	594.34	0.93	10.64	10.53	0.050	654.12	3.45	520	5.75	90.86	0.14	1.63	1.61	0.008
Small Apple	33.05	630.13	0.79	10.49	10.55	0.050	685.06	3.38	600	4.82	91.98	0.12	1.53	1.54	0.007
Minimum	26.87	516.37	0.60	5.80	8.41	0.040	569.21	3.22	500.00	3.68	89.44	0.09	0.78	1.18	0.006
Maximum	43.74	790.56	1.74	13.15	24.91	0.062	855.53	3.45	760.00	5.75	92.88	0.22	1.81	3.65	0.008
Number of Samples	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
Mean	33.44	633.02	1.04	10.69	12.96	0.049	691.21	3.34	604.76	4.86	91.56	0.15	1.55	1.88	0.007
Standard Deviation	3.61	60.04	0.22	1.60	4.10	0.004	63.19	0.05	63.89	0.49	0.77	0.03	0.21	0.57	0.001
Coefficient of Variation (%)	10.79	9.48	21.16	14.95	31.66	8.06	9.14	1.46	10.56	10.10	0.85	19.51	13.69	30.19	8.67

Table A28.4: Individual organic acid concentations in the juice of Braeburn apples that were harvested at commercial maturity.

Region	Sample Date	Stage of Picking	Quinic Acid (mg/100ml)	Malic Acid (mg/100ml)	Shikimic Acid (mg/100ml)	Citric Acid (mg/100ml)	Succinic Acid (mg/100ml)	Fumaric Acid (mg/100ml)	Total Acids (mg/100ml)	pH	Titratable Acidity (mg/100ml)	Quinic Acid as % of Total Acids	Malic Acid as % of Total Acids	Shikimic Acid as % of Total Acids	Citric Acid as % of Total Acids	Succinic Acid as % of Total Acids	Fumaric Acid as % of Total acids
Hawke's Bay	20/2/92	1st Pick	39.68	828.72	0.60	10.84	29.48	0.080	909.40	3.22	710	4.36	91.13	0.07	1.19	3.24	0.009
Hawke's Bay	17/3/92	2nd Pick	31.54	682.21	0.39	7.90	20.34	0.054	742.42	3.29	560	4.25	91.89	0.05	1.06	2.74	0.007
Hawke's Bay	5/5/92	3rd Pick	35.29	452.81	0.78	6.35	27.99	0.067	523.27	3.45	420	6.74	86.53	0.15	1.21	**5.35**	0.013
Hawke's Bay	15/4/93	2nd Pick	28.76	577.68	0.79	***4.33***	15.20	0.050	626.81	3.27	440	4.59	92.16	0.13	***0.69***	2.43	0.008
Nelson	11/3/92	1st Pick	**50.53**	**960.49**	0.76	10.39	21.73	0.080	**1043.97**	***3.18***	**880**	4.84	92.00	0.07	1.00	2.08	0.008
Nelson	14/4/92	2nd Pick	25.22	808.17	0.37	10.72	22.18	0.050	866.71	3.28	820	***2.91***	**93.25**	0.04	1.24	2.56	***0.006***
Nelson	14/5/92	3rd Pick	44.13	699.89	0.85	7.67	**31.21**	0.050	783.80	3.42	650	5.63	89.29	0.11	0.98	3.98	***0.006***

Table A28.5: Minimum, maximum, mean, standard deviation and coefficient of variation in the individual organic acid concentrations of Braeburn apple juice.

Sample	Quinic Acid (mg/100ml)	Malic Acid (mg/100ml)	Shikimic Acid (mg/100ml)	Citric Acid (mg/100ml)	Succinic Acid (mg/100ml)	Fumaric Acid (mg/100ml)	Total Acids (mg/100ml)	pH	Titratable Acidity (mg/100ml)	Quinic Acid as % of Total Acids	Malic Acid as % of Total Acids	Shikimic Acid as % of Total Acids	Citric Acid as % of Total Acids	Succinic Acid as % of Total Acids	Fumaric Acid as % of Total acids
Minimum	23.29	382.28	0.24	4.33	8.28	0.040	438.96	3.18	360.00	2.91	83.65	0.04	0.69	1.18	0.006
Maximum	50.53	960.49	1.74	13.15	31.21	0.222	1043.97	3.61	880.00	9.70	93.25	0.24	2.57	5.35	0.041
Number of Samples	64	64	64	64	64	64	64	64	64	64	64	64	64	64	64
Mean	34.77	580.17	0.75	9.13	16.21	0.074	641.10	3.39	521.56	5.64	90.19	0.12	1.46	2.58	0.013
Standard Deviation	6.48	120.57	0.32	2.05	5.94	0.038	122.67	0.10	116.27	1.63	2.17	0.05	0.36	0.95	0.008
Coefficient of variation (%)	18.64	20.78	42.66	22.47	36.63	51.30	19.13	3.03	22.29	28.86	2.40	42.74	24.74	36.70	66.70

Bold italics typeface = minimum values
Bold typeface = maximum values

APPENDIX 29

INDIVIDUAL ORGANIC ACIDS AND RELATED COMPONENTS FOR GRANNY SMITH APPLE JUICES

Table A29.1: Individual organic acid concentrations in the juice of Hawke's Bay Granny Smith apples that were stored at different conditions in 1992.

Storage Time (Days)	Quinic Acid (mg/100ml)	Malic Acid (mg/100ml)	Shikimic Acid (mg/100ml)	Citric Acid (mg/100ml)	Succinic Acid (mg/100ml)	Fumaric Acid (mg/100ml)	Total Acids (mg/100ml)	pH	Titratable Acidity (mg/100ml)	Quinic Acid as % of Total Acids	Malic Acid as % of Total Acids	Shikimic Acid as % of Total Acids	Citric Acid as % of Total Acids	Succinic Acid as % of Total Acids	Fumaric Acid as % of Total acids
Ambient Storage															
0	31.08	698.47	0.59	5.99	16.57	0.056	752.75	3.27	620	4.13	92.79	0.08	0.80	2.20	0.007
7	32.45	710.07	0.16	6.99	17.73	0.051	767.45	3.25	640	4.23	92.52	0.02	0.91	2.31	0.007
14	***26.09***	651.86	0.11	5.82	15.54	0.053	699.48	3.33	640	3.73	**93.19**	0.02	0.83	2.22	0.008
23	27.34	688.00	0.58	6.82	19.49	0.086	742.32	3.32	600	***3.68***	92.68	0.08	0.92	2.62	0.012
28	28.33	681.16	0.16	6.21	20.88	0.067	736.80	3.29	580	3.84	92.45	0.02	0.84	2.83	0.009
35	28.62	674.05	0.60	7.03	22.25	0.088	732.63	3.37	560	3.91	92.00	0.08	0.96	3.04	0.012
42	28.93	666.32	0.59	7.31	22.60	0.117	725.85	3.42	540	3.98	91.80	0.08	1.01	3.11	0.016
Cold Storage															
0	31.08	698.47	0.59	5.99	16.57	0.056	752.75	3.27	620	4.13	92.79	0.08	0.80	2.20	0.007
28	32.62	747.24	0.23	6.80	20.62	***0.050***	807.55	3.27	660	4.04	92.53	0.03	0.84	2.55	0.006
56	30.49	753.30	0.28	8.28	27.55	0.062	819.96	3.32	640	3.72	91.87	0.03	1.01	3.36	0.008
77	31.84	731.77	0.72	8.77	25.84	0.091	799.04	3.38	590	3.98	91.58	0.09	1.10	3.23	0.011
106	29.05	622.04	0.13	7.31	18.63	0.062	677.22	3.39	520	4.29	91.85	0.02	1.08	2.75	0.009
Controlled Atmosphere Storage															
0	31.08	698.47	0.59	5.99	16.57	0.056	752.75	3.27	620	4.13	92.79	0.08	0.80	2.20	0.007
35	32.23	749.41	0.14	8.09	15.18	***0.050***	805.10	3.29	660	4.00	93.08	0.02	1.00	1.89	0.006
63	33.82	743.58	0.06	7.79	15.97	***0.050***	801.27	3.33	620	4.22	92.80	0.01	0.97	1.99	0.006
97	38.24	736.01	0.13	7.97	15.87	***0.050***	798.28	3.34	600	4.79	92.20	0.02	1.00	1.99	0.006
131	36.48	703.92	0.28	7.36	13.90	0.057	762.00	3.41	580	4.79	92.38	0.04	0.97	1.82	0.007
161	35.59	658.61	0.06	9.19	16.21	0.054	719.72	3.48	520	4.95	91.51	0.01	1.28	2.25	0.008
196	28.92	589.21	***0.03***	7.22	11.08	0.061	***636.53***	3.42	520	4.54	92.57	***0.00***	1.14	1.74	0.010

Table A29.2: Individual organic acid concentrations in the juice of Hawke's Bay Granny Smith apples that were stored at different conditions in 1993.

Storage Time (Days)	Quinic Acid (mg/100ml)l	Malic Acid (mg/100ml)	Shikimic Acid (mg/100ml)	Citric Acid (mg/100ml)	Succinic Acid (mg/100ml)	Fumaric Acid (mg/100ml)	Total Acids (mg/100ml)	pH	Titratable Acidity (mg/100ml)	Quinic Acid as % of Total Acids	Malic Acid as % of Total Acids	Shikimic Acid as % of Total Acids	Citric Acid as % of Total Acids	Succinic Acid as % of Total Acids	Fumaric Acid as % of Total acids
Ambient Storage															
0	40.68	849.54	0.54	7.69	13.43	0.059	911.94	***3.16***	710	4.46	93.16	0.06	0.84	1.47	0.006
8	39.61	789.76	0.48	6.60	12.38	***0.050***	848.87	3.24	650	4.67	93.04	0.06	0.78	1.46	0.006
14	47.01	774.67	0.36	6.71	16.39	***0.050***	845.19	3.17	680	5.56	91.66	0.04	0.79	1.94	0.006
21	41.45	823.18	0.42	7.91	19.03	***0.050***	892.04	3.25	720	4.65	92.28	0.05	0.89	2.13	0.006
28	37.97	754.05	0.94	6.71	18.87	0.098	818.63	3.28	640	4.64	92.11	0.11	0.82	2.30	0.012
35	43.02	765.08	**1.08**	6.84	15.49	0.116	831.63	3.35	600	5.17	92.00	0.13	0.82	1.86	0.014
42	44.06	713.74	0.38	6.42	18.23	**0.136**	782.97	3.33	570	5.63	91.16	0.05	0.82	2.33	0.017
49	45.87	692.26	0.42	7.08	14.77	0.090	760.47	3.37	560	6.03	91.03	0.05	0.93	1.94	0.012
56	45.05	664.34	0.62	7.14	17.17	0.097	734.42	3.42	510	6.13	90.46	0.08	0.97	2.34	0.013
64	41.19	640.35	0.96	6.61	14.57	0.128	703.81	3.46	490	5.85	90.98	**0.14**	0.94	2.07	0.018
Cold Storage															
0	40.68	849.54	0.54	7.69	13.43	0.059	911.94	3.16	710	4.46	93.16	0.06	0.84	1.47	0.006
28	47.07	857.60	0.97	8.39	16.94	0.078	931.05	3.18	700	5.06	92.11	0.10	0.90	1.82	0.008
56	40.69	686.91	0.09	6.75	21.61	***0.050***	756.11	3.25	550	5.38	90.85	0.01	0.89	2.86	0.007
85	40.98	721.40	0.31	7.42	29.60	***0.050***	799.75	3.30	580	5.12	90.20	0.04	0.93	3.70	0.006
119	38.16	723.52	0.31	8.15	33.62	***0.050***	803.80	3.33	570	4.75	90.01	0.04	1.01	4.18	0.006
147	38.81	635.54	0.83	9.12	27.61	0.085	711.99	3.37	510	5.45	89.26	0.12	1.28	3.88	0.012
175	40.82	661.87	0.30	**15.62**	**35.41**	0.094	754.11	3.45	500	5.41	87.77	0.04	2.07	**4.69**	0.012
206	41.48	***575.50***	0.43	14.25	30.73	0.129	662.52	**3.62**	***400***	6.26	86.86	0.06	**2.15**	4.64	**0.019**
Controlled Atmosphere Storage															
0	40.68	849.54	0.54	7.69	13.43	0.059	911.94	3.16	710	4.46	93.16	0.06	0.84	1.47	0.006
91	44.05	804.45	0.46	7.83	11.76	0.061	868.61	3.24	670	5.07	92.61	0.05	0.90	1.35	0.007
119	41.98	788.65	0.36	6.82	***9.67***	0.056	847.53	3.25	650	4.95	93.05	0.04	0.80	***1.14***	0.007
147	43.15	734.08	0.91	8.76	10.76	0.091	797.75	3.30	570	5.41	92.02	0.11	1.10	1.35	0.011
175	41.31	692.33	0.27	7.81	12.45	0.074	754.25	3.34	540	5.48	91.79	0.04	1.04	1.65	0.010
206	41.81	622.90	0.16	7.99	13.08	0.077	686.02	3.38	460	6.10	90.80	0.02	1.16	1.91	0.011

Table A29.3: Individual organic acid concentrations in the juice of Granny Smith apples that were harvested at commercial maturity.

Region	Sample Date	Stage of Picking	Quinic Acid (mg/100ml)	Malic Acid (mg/100ml)	Shikimic Acid (mg/100ml)	Citric Acid (mg/100ml)	Succinic Acid (mg/100ml)	Fumaric Acid (mg/100ml)	Total Acids (mg/100ml)	pH	Titratable Acidity (mg/100ml)	Quinic Acid % of Total Acids	Malic Acid % of Total Acids	Shikimic Acid % of Total Acids	Citric Acid % of Total Acids	Succinic Acid % of Total Acids	Fumaric Acid % of Total acids
Hawke's Bay	6/3/92	1st Pick	52.61	837.99	0.50	8.08	18.84	0.080	918.11	3.21	810	5.73	91.27	0.05	0.88	2.05	0.009
Hawke's Bay	7/4/92	2nd Pick	38.35	792.82	0.79	7.36	19.58	0.059	858.95	3.26	660	4.46	92.30	0.09	0.86	2.28	0.007
Hawke's Bay	29/4/92	3rd Pick	39.08	764.59	0.56	7.39	27.21	0.054	838.90	3.36	660	4.66	91.14	0.07	0.88	3.24	0.006
Hawke's Bay	25/2/93	1st Pick	53.69	885.74	0.87	6.95	20.23	0.069	967.55	3.26	780	5.55	91.54	0.09	***0.72***	2.09	0.007
Hawke's Bay	25/3/93	2nd Pick	46.28	779.32	1.01	8.06	18.99	0.066	853.73	3.31	760	5.42	91.28	0.12	0.94	2.22	0.008
Hawke's Bay	9/6/93	3rd Pick	34.89	675.54	0.57	5.82	25.25	0.065	742.13	3.47	520	4.70	91.03	0.08	0.78	3.40	0.009
Nelson	9/3/92	1st Pick	61.80	965.59	0.70	8.45	19.86	0.078	1056.47	***3.16***	910	5.85	91.40	0.07	0.80	1.88	0.007
Nelson	20/4/92	2nd Pick	36.37	780.88	0.26	7.05	25.55	***0.050***	850.15	3.27	650	4.28	91.85	0.03	0.83	3.01	0.006
Nelson	17/5/92	3rd Pick	29.30	665.09	0.21	5.97	21.53	0.059	722.18	3.32	540	4.06	92.10	0.03	0.83	2.98	0.008
Nelson	8/3/93	1st Pick	55.63	**1016.14**	0.53	11.99	19.17	0.053	**1103.51**	3.19	**1090**	5.04	92.08	0.05	1.09	1.74	***0.005***
Nelson	18/4/93	2nd Pick	36.81	826.75	0.43	10.74	26.01	0.053	900.79	3.29	840	4.09	91.78	0.05	1.19	2.89	0.006
Nelson	6/5/93	3rd Pick	37.03	749.80	0.22	11.24	31.64	0.056	829.98	3.36	780	4.46	90.34	0.03	1.35	3.81	0.007
Canterbury	11/3/92	1st Pick	52.68	992.44	0.53	9.04	21.64	0.078	1076.41	3.19	970	4.89	92.20	0.05	0.84	2.01	0.007
Canterbury	24/4/92	2nd Pick	33.19	682.49	0.14	***5.56***	18.78	0.051	740.22	3.32	600	4.48	92.20	0.02	0.75	2.54	0.007
Canterbury	29/5/92	3rd Pick	36.82	711.26	0.25	8.36	29.08	0.096	785.85	3.38	640	4.68	90.51	0.03	1.06	3.70	0.012
Canterbury	16/3/93	1st Pick	**84.23**	867.49	1.18	9.30	18.73	0.086	981.01	3.21	840	**8.59**	88.43	0.12	0.95	1.91	0.009
Canterbury	23/3/93	2nd Pick	58.17	709.53	0.74	8.75	19.87	0.058	797.12	3.39	620	7.30	89.01	0.09	1.10	2.49	0.007
Canterbury	21/5/93	3rd Pick	57.51	624.89	0.64	9.62	28.60	0.053	721.31	3.44	650	7.97	***86.63***	0.09	1.33	3.96	0.007

Table A29.4: Minimum, maximum, mean, standard deviation and coefficient of variation in the individual organic acid concentrations of Granny Smith apple juice.

Storage Time (Days)	Quinic Acid (mg/100ml)	Malic Acid (mg/100ml)	Shikimic Acid (mg/100ml)	Citric Acid (mg/100ml)	Succinic Acid (mg/100ml)	Fumaric Acid (mg/100ml)	Total Acids (mg/100ml)	pH	Titratable Acidity (mg/100ml)	Quinic Acid as % of Total Acids	Malic Acid as % of Total Acids	Shikimic Acid as % of Total Acids	Citric Acid as % of Total Acids	Succinic Acid as % of Total Acids	Fumaric Acid as % of Total acids
Minimum	26.09	575.50	0.03	5.56	9.67	0.050	636.53	3.16	400.00	3.68	86.63	0.00	0.72	1.14	0.005
Maximum	84.23	1016.14	1.18	15.62	35.41	0.136	1103.51	3.62	1090.00	8.59	93.19	0.14	2.15	4.69	0.019
Number of Samples	57	57	57	57	57	57	57	57	57	57	57	57	57	57	57
Mean	40.40	738.69	0.47	7.95	20.00	0.071	807.57	3.32	635.79	4.99	91.44	0.06	0.99	2.51	0.009
Standard Deviation	10.08	91.52	0.29	1.84	6.00	0.023	97.30	0.09	123.91	0.98	1.44	0.03	0.26	0.83	0.003
Coefficient of Variation (%)	24.94	12.39	62.50	23.18	29.98	32.11	12.05	2.72	19.49	19.63	1.58	61.08	25.99	32.93	37.19

Bold italic typeface = minimum values
Bold typeface = maximum values

APPENDIX 30
INDIVIDUAL ORGANIC ACIDS AND RELATED COMPONENTS FOR GALA APPLE JUICES

Table A30.1: Individual organic acid concentrations in the juice of Hawke's Bay Gala apples that were stored at different conditions in 1992.

Storage Time (Days)	Quinic Acid (mg/100ml)	Malic Acid (mg/100ml)	Shikimic Acid (mg/100ml)	Citric Acid (mg/100ml)	Succinic Acid (mg/100ml)	Fumaric Acid (mg/100ml)	Total Acids (mg/100ml)	pH	Titratable Acidity (mg/100ml)	Quinic Acid as % of Total Acids	Malic Acid as % of Total Acids	Shikimic Acid as % of Total Acids	Citric Acid as % of Total Acids	Succinic Acid as % of Total Acids	Fumaric Acid as % of Total acids
Ambient Storage															
0	34.94	398.38	2.00	7.99	22.19	0.066	465.57	3.86	320	7.51	85.57	0.43	1.72	4.77	0.014
6	29.26	299.01	1.57	6.34	24.17	0.073	360.43	4.04	250	8.12	82.96	0.44	1.76	**6.71**	0.020
20	34.79	341.60	1.82	7.76	19.84	0.107	405.92	4.00	250	8.57	84.15	0.45	1.91	4.89	0.026
34	35.87	330.16	1.81	9.28	16.81	0.142	394.07	4.07	230	9.10	83.78	0.46	2.35	4.27	0.036
40	31.04	318.13	1.66	9.82	14.78	0.120	375.56	**4.10**	250	8.27	84.71	0.44	2.62	3.93	0.032
Cold Storage															
0	34.94	398.38	2.00	7.99	22.19	0.066	465.57	3.86	320	7.51	85.57	0.43	1.72	4.77	0.014
34	34.89	345.26	1.89	10.83	19.18	0.097	412.15	4.08	240	8.47	83.77	0.46	2.63	4.65	0.023
56	33.65	364.73	1.96	12.12	19.15	0.104	431.71	3.97	270	7.79	84.48	0.45	2.81	4.44	0.024
83	34.28	333.15	1.92	16.92	25.46	0.169	411.89	4.03	250	8.32	80.88	0.47	4.11	6.18	0.041
119	36.75	342.03	1.98	**20.28**	**26.35**	**0.178**	427.57	4.10	240	8.59	***79.99***	0.46	**4.74**	6.16	**0.042**

Table A30.2: Individual organic acid concentrations in the juice of Gala apples that were harvested at commercial maturity.

Region	Sample Date	Stage of Picking	Quinic Acid (mg/100ml)	Malic Acid (mg/100ml)	Shikimic Acid (mg/100ml)	Citric Acid (mg/100ml)	Succinic Acid (mg/100ml)	Fumaric Acid (mg/100ml)	Total Acids (mg/100ml)	pH	Titratable Acidity (mg/100ml)	Quinic Acid as % of Total Acids	Malic Acid as % of Total Acids	Shikimic Acid as % of Total Acids	Citric Acid as % of Total Acids	Succinic Acid as % of Total Acids	Fumaric Acid as % of Total acids
Hawke's Bay	28/1/92	1st Pick	59.88	576.78	2.82	5.17	24.08	0.101	668.82	3.64	420	8.95	86.24	0.42	0.77	3.60	0.015
Hawke's Bay	13/2/92	2nd Pick	44.21	470.03	2.27	6.42	25.18	0.090	548.20	3.68	380	8.07	85.74	0.41	1.17	4.59	0.016
Hawke's Bay	18/3/92	3rd Pick	34.94	398.38	2.00	7.99	22.19	0.066	465.57	3.86	320	7.51	85.57	0.43	1.72	4.77	0.014
Hawke's Bay	26/2/93	2nd Pick	41.08	574.08	1.47	5.37	25.18	0.062	647.25	3.62	410	6.35	88.70	***0.23***	0.83	3.89	***0.010***
Nelson	27/1/92	1st Pick	**66.64**	572.24	**3.16**	5.40	16.73	0.070	664.24	3.61	400	**10.03**	86.15	**0.48**	0.81	***2.52***	0.011
Nelson	26/2/92	2nd Pick	36.58	404.81	1.77	***4.67***	16.95	0.085	464.87	3.63	330	7.87	87.08	0.38	1.00	3.65	0.018
Nelson	6/4/92	3rd Pick	***28.61***	***292.18***	1.09	4.86	19.83	***0.061***	***346.64***	3.89	***230***	8.25	84.29	0.31	1.40	5.72	0.018
Canterbury	13/2/92	1st Pick	48.95	**608.60**	2.20	5.00	24.71	0.070	**689.53**	***3.54***	**460**	7.10	88.26	0.32	***0.73***	3.58	***0.010***
Canterbury	2/3/92	2nd Pick	29.80	429.46	1.33	6.50	***14.60***	0.085	481.78	3.66	360	***6.19***	**89.14**	0.28	1.35	3.03	0.018
Canterbury	6/4/92	3rd Pick	33.82	358.84	***1.00***	5.73	20.72	0.064	420.18	3.80	310	8.05	85.40	0.24	1.36	4.93	0.015

Table A30.3: Minimum, maximum, mean, standard deviation and coefficient of variation in the individual organic acid concentrations of Gala apple juice.

Sample	Quinic Acid (mg/100ml)	Malic Acid (mg/100ml)	Shikimic Acid (mg/100ml)	Citric Acid (mg/100ml)	Succinic Acid (mg/100ml)	Fumaric Acid (mg/100ml)	Total Acids (mg/100ml)	pH	Titratable Acidity (mg/100ml)	Quinic Acid as % of Total Acids	Malic Acid as % of Total Acids	Shikimic Acid as % of Total Acids	Citric Acid as % of Total Acids	Succinic Acid as % of Total Acids	Fumaric Acid as % of Total acids
Minimum	28.61	292.18	1.00	4.67	14.60	0.061	346.64	3.54	230.00	6.19	79.99	0.23	0.73	2.52	0.010
Maximum	66.64	608.60	3.16	20.28	26.35	0.178	689.53	4.10	460.00	10.03	89.14	0.48	4.74	6.71	0.042
Number of Samples	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18
Mean	38.61	408.86	1.87	8.36	20.88	0.097	478.69	3.85	311.11	8.09	85.07	0.40	1.89	4.53	0.022
Standard Deviation	10.08	102.66	0.52	4.23	3.80	0.035	110.63	0.20	74.23	0.89	2.36	0.08	1.11	1.10	0.010
Coefficient of variation (%)	26.10	25.11	27.65	50.63	18.18	35.70	23.11	5.12	23.86	11.06	2.77	20.33	58.71	24.18	45.43

Bold italic typeface = minimum values

Bold typeface = maximum values

APPENDIX 31
INDIVIDUAL ORGANIC ACIDS AND RELATED COMPONENTS FOR ROYAL GALA APPLE JUICES

Table A31.1: Individual organic acid concentrations in the juice of Hawke's Bay Royal Gala apples that were stored at different conditions in 1992.

Storage Time (Days)	Quinic Acid (mg/100ml)	Malic Acid (mg/100ml)	Shikimic Acid (mg/100ml)	Citric Acid (mg/100ml)	Succinic Acid (mg/100ml)	Fumaric Acid (mg/100ml)	Total Acids (mg/100ml)	pH	Titratable Acidity (mg/100ml)	Quinic Acid as % of Total Acids	Malic Acid as % of Total Acids	Shikimic Acid as % of Total Acids	Citric Acid as % of Total Acids	Succinic Acid as % of Total Acids	Fumaric Acid as % of Total acids
Controlled Atmosphere Storage															
0	35.79	312.36	1.45	7.37	22.90	0.140	380.01	4.15	230	9.42	***82.20***	0.38	1.94	6.03	0.037
30	33.67	340.26	1.81	8.51	13.01	0.113	397.38	3.97	280	8.47	85.63	0.46	2.14	3.27	0.028
65	28.48	***294.49***	1.43	8.31	***11.23***	0.114	***344.05***	3.99	240	8.28	85.60	0.41	2.42	3.26	0.033
91	29.47	325.47	1.56	**9.84**	12.74	0.124	379.21	3.96	250	7.77	85.83	0.41	**2.59**	3.36	0.033

Table A31.2: Individual organic acid concentrations in the juice of Royal Gala apples that were harvested at commercial maturity.

Region	Sample Date	Stage of Picking	Quinic Acid (mg/100ml)	Malic Acid (mg/100ml)	Shikimic Acid (mg/100ml)	Citric Acid (mg/100ml)	Succinic Acid (mg/100ml)	Fumaric Acid (mg/100ml)	Total Acids (mg/100ml)	pH	Titratable Acidity (mg/100ml)	Quinic Acid as % of Total Acids	Malic Acid as % of Total Acids	Shikimic Acid as % of Total Acids	Citric Acid as % of Total Acids	Succinic Acid as % of Total Acids	Fumaric Acid as % of Total acids
Hawke's Bay	28/1/92	1st Pick	40.65	427.67	1.82	5.01	15.04	0.083	490.26	3.57	400	8.29	87.23	0.37	1.02	3.07	0.017
Hawke's Bay	17/2/92	2nd Pick	31.49	429.50	1.56	4.69	17.05	0.073	484.37	3.60	360	6.50	**88.67**	0.32	0.97	3.52	0.015
Hawke's Bay	4/5/92	3rd Pick	35.79	312.36	1.45	7.37	22.90	**0.140**	380.01	**4.15**	***230***	9.42	82.20	0.38	**1.94**	**6.03**	**0.037**
Hawke's Bay	19/1/93	1st Pick	**80.25**	618.81	2.78	4.75	21.02	0.087	727.69	***3.51***	450	**11.03**	85.04	0.38	0.65	2.89	0.012
Hawke's Bay	26/2/93	2nd Pick	35.78	426.09	1.25	4.15	18.08	0.055	485.41	3.56	350	7.37	87.78	0.26	0.85	3.72	***0.011***
Nelson	5/2/92	1st Pick	59.99	**667.09**	2.67	4.86	21.09	0.099	**755.80**	3.55	**500**	7.94	88.26	0.35	0.64	***2.79***	0.013
Nelson	9/3/92	2nd Pick	32.41	424.09	1.07	5.27	21.19	***0.051***	484.09	3.67	370	6.70	87.61	0.22	1.09	4.38	0.010
Nelson	18/4/92	3rd Pick	28.24	311.74	***0.85***	***3.95***	15.44	***0.051***	360.28	3.77	280	7.84	86.53	0.24	1.10	4.29	0.014
Nelson	20/1/93	1st Pick	61.46	533.89	**2.81**	7.62	**25.49**	0.118	631.39	3.63	500	9.73	84.56	0.45	1.21	4.04	0.019
Nelson	24/2/93	2nd Pick	39.30	477.80	1.71	7.52	23.51	0.061	549.90	3.70	420	7.15	86.89	0.31	1.37	4.28	***0.011***
Nelson	1/4/93	3rd Pick	**27.97**	387.98	0.94	7.44	18.65	0.070	443.04	3.85	350	***6.31***	87.57	***0.21***	1.68	4.21	0.016
Canterbury	16/2/93	1st Pick	54.14	473.61	2.79	9.76	21.56	0.066	561.92	3.66	420	9.63	84.28	**0.50**	1.74	3.84	0.012
Canterbury	16/3/93	2nd Pick	34.33	434.94	1.97	7.63	21.13	0.064	500.07	3.66	380	6.87	86.98	0.39	1.53	4.23	0.013
Canterbury	24/4/93	3rd Pick	31.67	390.13	1.38	6.71	21.64	0.082	451.62	3.71	350	7.01	86.39	0.31	1.49	4.79	0.018

Table A31.3: Minimum, maximum, mean, standard deviation and coefficient of variation in the individual organic acid concentrations of Royal Gala apple juice.

Sample	Quinic Acid (mg/100ml)	Malic Acid (mg/100ml)	Shikimic Acid (mg/100ml)	Citric Acid (mg/100ml)	Succinic Acid (mg/100ml)	Fumaric Acid (mg/100ml)	Total Acids (mg/100ml)	pH	Titratable Acidity (mg/100ml)	Quinic Acid as % of Total Acids	Malic Acid as % of Total Acids	Shikimic Acid as % of Total Acids	Citric Acid as % of Total Acids	Succinic Acid as % of Total Acids	Fumaric Acid as % of Total acids
Minimum	27.97	294.49	0.85	3.95	11.23	0.051	344.05	3.51	230.00	6.31	82.20	0.21	0.64	2.79	0.011
Maximum	80.25	667.09	2.81	9.84	25.49	0.140	755.80	4.15	500.00	11.03	88.67	0.50	2.59	6.03	0.037
Number of Samples	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17
Mean	40.30	428.00	1.76	6.67	18.87	0.085	495.68	3.74	360.59	8.02	86.30	0.35	1.44	3.88	0.18
Standard Deviation	14.38	101.57	0.63	1.86	4.04	0.027	116.39	0.18	81.56	1.27	1.60	0.08	0.57	0.78	0.008
Coefficient of variation (%)	35.67	23.73	35.89	27.85	21.40	31.663	23.48	4.80	22.62	15.86	1.85	23.50	39.52	20.03	45.92

Bold italic typeface = minimum values
Bold typeface = maximum values

APPENDIX 32

INDIVIDUAL ORGANIC ACIDS AND RELATED COMPONENTS FOR RED DELICIOUS, COX'S ORANGE, GOLDEN DELICIOUS, FUJI, HILLWELL, GS2850, GS330 AND FIESTA APPLE JUICES

Table A32.1: Individual organic acid concentrations in the juice of Red Delicious apples that were harvested at commercial maturity.

Region	Sample Date	Stage of Picking	Quinic Acid (mg/100ml)	Malic Acid (mg/100ml)	Shikimic Acid (mg/100ml)	Citric Acid (mg/100ml)	Succinic Acid (mg/100ml)	Fumaric Acid (mg/100ml)	Total Acids (mg/100ml)	pH	Titratable Acidity (mg/100ml)	Quinic Acid as % of Total Acids	Malic Acid as % of Total Acids	Shikimic Acid as % of Total Acids	Citric Acid as % of Total Acids	Succinic Acid as % of Total Acids	Fumaric Acid as % of Total acids
Hawke's Bay	5/2/92	1st Pick	123.04	**360.05**	**3.59**	**4.98**	20.13	0.080	**511.87**	***3.72***	**300**	24.04	70.34	0.70	0.97	3.93	0.016
Hawke's Bay	6/3/92	2nd Pick	74.69	279.88	***1.82***	4.39	19.14	0.073	***379.99***	**3.97**	***210***	19.66	73.65	***0.48***	1.16	5.04	0.019
Hawke's Bay	25/3/93	3rd Pick	***72.52***	321.92	2.08	3.94	***15.18***	***0.050***	415.69	3.82	280	***17.45***	**77.44**	0.50	0.95	***3.65***	***0.012***
Hawke's Bay	23/2/92	2nd Pick	**129.26**	308.70	3.44	3.75	17.29	0.074	462.51	3.76	260	**27.95**	***66.74***	**0.74**	0.81	3.74	0.016
Nelson	26/3/92	1st Pick	92.95	271.95	2.59	***2.10***	15.86	0.053	385.50	3.82	210	24.11	70.55	0.67	***0.54***	4.12	0.014
Nelson	27/4/92	2nd Pick	80.63	***268.88***	2.40	4.62	**23.46**	**0.102**	380.09	3.94	220	21.21	70.74	0.63	**1.21**	**6.17**	**0.027**
Minimum			72.52	268.88	1.82	2.10	15.18	0.050	379.99	3.72	210.00	17.45	66.74	0.48	0.54	3.65	0.012
Maximum			129.26	360.05	3.59	4.98	23.46	0.102	511.87	3.97	300.00	27.95	77.44	0.74	1.21	6.17	0.027
Number of Samples			6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
Mean			95.51	301.90	2.65	3.96	18.51	0.072	422.61	3.84	246.67	22.40	71.58	0.62	0.94	4.44	0.017
Standard Deviation			22.68	32.41	0.65	0.93	2.80	0.017	49.34	0.09	35.43	3.41	3.30	0.10	0.22	0.90	0.005
Coefficient of variation (%)			23.75	10.73	24.69	23.43	15.14	24.19	11.68	2.34	14.37	15.23	4.61	15.90	23.63	20.22	28.06

Bold italic typeface = minimum values

Bold typeface = maximum values

Table A32.2: Individual organic acid concentrations in the juice of Cox's Orange apples that were harvested at commercial maturity.

Region	Sample Date	Stage of Picking	Quinic Acid (mg/100ml)	Malic Acid (mg/100ml)	Shikimic Acid (mg/100ml)	Citric Acid (mg/100ml)	Succinic Acid (mg/100ml)	Fumaric Acid (mg/100ml)	Total Acids (mg/100ml)	pH	Titratable Acidity (mg/100ml)	Quinic Acid as % of Total Acids	Malic Acid as % of Total Acids	Shikimic Acid as % of Total Acids	Citric Acid as % of Total Acids	Succinic Acid as % of Total Acids	Fumaric Acid as % of Total acids
Hawke's Bay	24/1/92	1st Pick	76.98	1035.96	**0.58**	14.61	36.85	**0.140**	1165.12	***3.16***	1030	6.61	88.91	**0.05**	1.25	3.16	0.012
Hawke's Bay	13/2/92	2nd Pick	41.70	891.33	0.34	14.38	29.93	0.097	977.78	3.26	850	4.26	91.16	0.03	1.47	***3.06***	0.010
Hawke's Bay	4/3/92	3rd Pick	37.25	921.17	0.45	**16.55**	**41.11**	0.103	1016.64	3.32	920	3.66	90.61	0.04	1.63	**4.04**	0.010
Hawke's Bay	1/3/93	2nd Pick	37.24	871.21	0.28	***10.71***	29.61	***0.076***	949.12	3.21	780	3.92	**91.79**	0.03	***1.13***	3.12	***0.008***
Nelson	24/1/92	1st Pick	**83.43**	**1067.59**	0.39	15.62	40.97	0.126	**1208.12**	3.17	**1130**	**6.91**	***88.37***	0.03	1.29	3.39	0.010
Nelson	24/2/92	2nd Pick	33.12	886.33	0.11	14.63	31.47	0.098	965.76	3.25	860	3.43	91.78	0.01	1.52	3.26	0.010
Nelson	23/3/92	3rd Pick	***23.91***	***666.35***	***0.01***	12.71	***28.38***	0.097	***731.46***	**3.41**	***620***	***3.27***	91.10	***0.00***	**1.74**	3.88	**0.013**
Minimum			23.91	666.35	0.01	10.71	28.38	0.076	731.46	3.16	620.00	3.27	88.37	0.00	1.13	3.06	0.008
Maximum			83.43	1067.59	0.58	16.55	41.11	0.140	1208.12	3.41	1130.00	6.91	91.79	0.05	1.74	4.04	0.013
Number of Samples			7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
Mean			47.66	905.71	0.31	14.17	34.04	0.105	1002.00	3.25	884.29	4.58	90.53	0.03	1.43	3.42	0.011
Standard Deviation			21.27	120.99	0.18	1.79	5.08	0.020	145.01	0.08	153.70	1.41	1.26	0.02	0.20	0.36	0.002
Coefficient of variation (%)			44.63	13.36	59.02	12.61	14.94	18.66	14.47	2.51	17.38	30.80	1.39	55.14	14.06	10.57	14.62

Bold italic typeface = minimum values

Bold typeface = maximum values

Table A32.3: Individual organic acid concentrations in the juice of Golden Delicious apples that were harvested at commercial maturity.

Region	Sample Date	Stage of Picking	Quinic Acid (mg/100ml)	Malic Acid (mg/100ml)	Shikimic Acid (mg/100ml)	Citric Acid (mg/100ml)	Succinic Acid (mg/100ml)	Fumaric Acid (mg/100ml)	Total Acids (mg/100ml)	pH	Titratable Acidity (mg/100ml)	Quinic Acid as % of Total Acids	Malic Acid as % of Total Acids	Shikimic Acid as % of Total Acids	Citric Acid as % of Total Acids	Succinic Acid as % of Total Acids	Fumaric Acid as % of Total acids
Hawke's Bay	5/2/92	1st Pick	44.15	794.63	**1.38**	8.55	24.82	**0.091**	873.63	3.30	**660**	5.05	90.96	**0.16**	0.98	2.84	0.010
Hawke's Bay	6/3/92	2nd Pick	25.12	697.99	0.54	8.42	25.65	0.066	757.78	3.41	580	***3.31***	92.11	0.07	1.11	3.38	0.009
Hawke's Bay	29/4/92	3rd Pick	***21.75***	***428.82***	0.48	**8.93**	**39.42**	0.059	***499.46***	**3.75**	***350***	4.36	***85.86***	0.10	**1.79**	**7.89**	**0.012**
Nelson	27/1/92	1st Pick	**64.66**	**796.93**	1.17	6.15	19.49	0.064	**888.46**	**3.28**	650	**7.28**	89.70	0.13	***0.69***	***2.19***	***0.007***
Nelson	5/3/92	2nd Pick	28.70	634.07	0.57	***5.91***	***18.80***	0.065	688.12	3.36	500	4.17	**92.15**	0.08	0.86	2.73	0.009
Nelson	6/4/92	3rd Pick	26.92	611.01	***0.39***	6.38	20.78	***0.050***	665.52	3.45	460	4.04	91.81	***0.06***	0.96	3.12	0.008
Minimum			21.75	428.82	0.39	5.91	18.80	0.050	499.46	3.28	350.00	3.31	85.86	0.06	0.69	2.19	0.007
Maximum			64.66	796.93	1.38	8.93	39.42	0.091	888.46	3.75	660.00	7.28	92.15	0.16	1.79	7.89	0.012
Number of Samples			6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
Mean			35.22	660.58	0.75	7.39	24.83	0.066	728.83	3.43	533.33	4.70	90.43	0.10	1.06	3.69	0.009
Standard Deviation			14.95	125.72	0.38	1.26	7.01	0.012	132.64	0.16	109.49	1.26	2.21	0.03	0.35	1.91	0.002
Coefficient of variation (%)			42.45	19.03	50.05	17.08	28.23	18.95	18.20	4.58	20.53	26.78	2.45	34.90	32.68	51.77	17.52

Bold italic typeface = minimum values
Bold typeface = maximum values

Table A32.4: Individual organic acid concentrations in the juice of Fuji apples that were harvested at commercial maturity.

Region	Sample Date	Stage of Picking	Quinic Acid (mg/100ml)	Malic Acid (mg/100ml)	Shikimic Acid (mg/100ml)	Citric Acid (mg/100ml)	Succinic Acid (mg/100ml)	Fumaric Acid (mg/100ml)	Total Acids (mg/100ml)	pH	Titratable Acidity (mg/100ml)	Quinic Acid as % of Total Acids	Malic Acid as % of Total Acids	Shikimic Acid as % of Total Acids	Citric Acid as % of Total Acids	Succinic Acid as % of Total Acids	Fumaric Acid as % of Total acids
Hawke's Bay	4/3/92	1st Pick	42.72	**444.86**	0.82	5.63	17.44	0.052	**511.52**	3.63	**360**	8.35	86.97	0.16	1.10	3.41	***0.010***
Hawke's Bay	7/4/92	2nd Pick	***27.65***	366.26	0.66	5.58	***10.60***	0.051	410.80	3.78	280	***6.73***	**89.16**	0.16	1.36	***2.58***	0.012
Hawke's Bay	1/4/93	2nd Pick	36.14	405.48	**1.23**	**6.52**	12.49	***0.050***	461.91	3.70	340	7.82	87.78	**0.27**	1.41	2.70	0.011
Nelson	16/3/92	1st Pick	**52.26**	383.30	1.09	***4.42***	16.18	***0.050***	457.30	***3.44***	340	11.43	83.82	0.24	***0.97***	3.54	0.011
Nelson	22/4/92	2nd Pick	35.67	282.72	0.75	4.61	14.78	0.068	338.59	3.70	240	10.54	83.50	0.22	1.36	4.36	0.020
Nelson	22/5/92	3rd Pick	44.25	252.37	0.74	4.67	14.19	0.118	316.35	3.77	210	**13.99**	79.78	0.23	**1.48**	4.49	0.037
Canterbury	18/3/92	1st Pick	42.66	380.91	0.93	4.44	15.13	***0.050***	444.12	3.51	340	9.61	85.77	0.21	1.00	3.41	0.011
Canterbury	23/4/92	2nd Pick	30.19	297.94	***0.42***	4.60	18.79	0.066	352.00	3.63	260	8.58	84.64	***0.12***	1.31	5.34	0.019
Canterbury	29/5/92	3rd Pick	37.53	***231.24***	0.50	4.43	**30.51**	**0.141**	***304.35***	**3.88**	***210***	12.33	***75.98***	0.16	1.46	**10.02**	**0.046**
Minimum			27.65	231.24	0.42	4.42	10.60	0.050	304.35	3.44	210.00	6.73	75.98	0.12	0.97	2.58	0.010
Maximum			52.26	444.86	1.23	6.52	30.51	0.141	511.52	3.88	360.00	13.99	89.16	0.27	1.48	10.02	0.046
Number of Samples			9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
Mean			38.79	338.34	0.79	4.99	16.68	0.072	399.66	3.67	286.67	9.93	84.15	0.20	1.27	4.43	0.020
Standard Deviation			7.13	69.96	0.25	0.70	5.41	0.032	69.79	0.13	56.37	2.21	3.88	0.05	0.18	2.14	0.012
Coefficient of variation (%)			18.40	20.68	31.04	14.08	32.42	44.76	17.46	3.51	19.66	22.25	4.61	23.03	14.55	48.44	63.00

Bold italic typeface = minimum values

Bold typeface = maximum values

Table A32.5: Individual organic acid concentrations in the juice of Hillwell, GS330, GS2850 and Fiesta apples that were harvested at commercial maturity from Hawke's Bay.

Cultivar	Quinic Acid (mg/100ml)	Malic Acid (mg/100ml)	Shikimic Acid (mg/100ml)	Citric Acid (mg/100ml)	Succinic Acid (mg/100ml)	Fumaric Acid (mg/100ml)	Total Acids (mg/100ml)	pH	Titratable Acidity (mg/100ml)	Quinic Acid as % of Total Acids	Malic Acid as % of Total Acids	Shikimic Acid as % of Total Acids	Citric Acid as % of Total Acids	Succinic Acid as % of Total Acids	Fumaric Acid as % of Total acids
Hillwell	39.53	593.50	0.53	12.51	23.91	0.056	670.03	3.34	550	5.90	88.58	0.08	1.87	3.57	0.008
GS330	26.05	490.88	1.69	9.28	27.27	0.083	555.25	3.56	480	4.69	88.41	0.30	1.67	4.91	0.015
GS2850	46.58	306.08	1.65	9.25	17.12	0.050	380.73	3.81	240	12.23	80.39	0.43	2.43	4.50	0.013
Fiesta	27.75	815.68	0.33	14.88	24.08	0.075	882.79	3.16	890	3.14	92.40	0.04	1.69	2.73	0.008

APPENDIX 33

CHANGES IN THE INDIVIDUAL ORGANIC ACID CONCENTRATIONS IN THE JUICE OF BRAEBURN APPLES STORED AT DIFFERENT CONDITIONS IN 1992

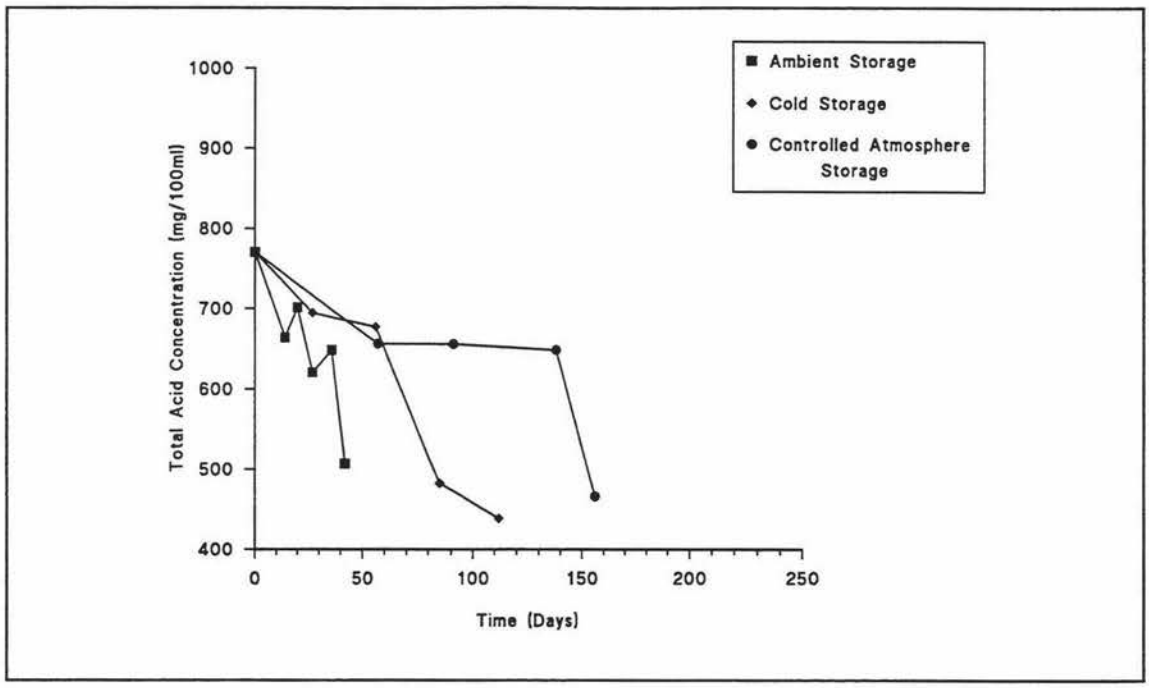

Figure A33.1: Effect on juice total acid concentrations of different storage regimes for Braeburn apples in 1992.

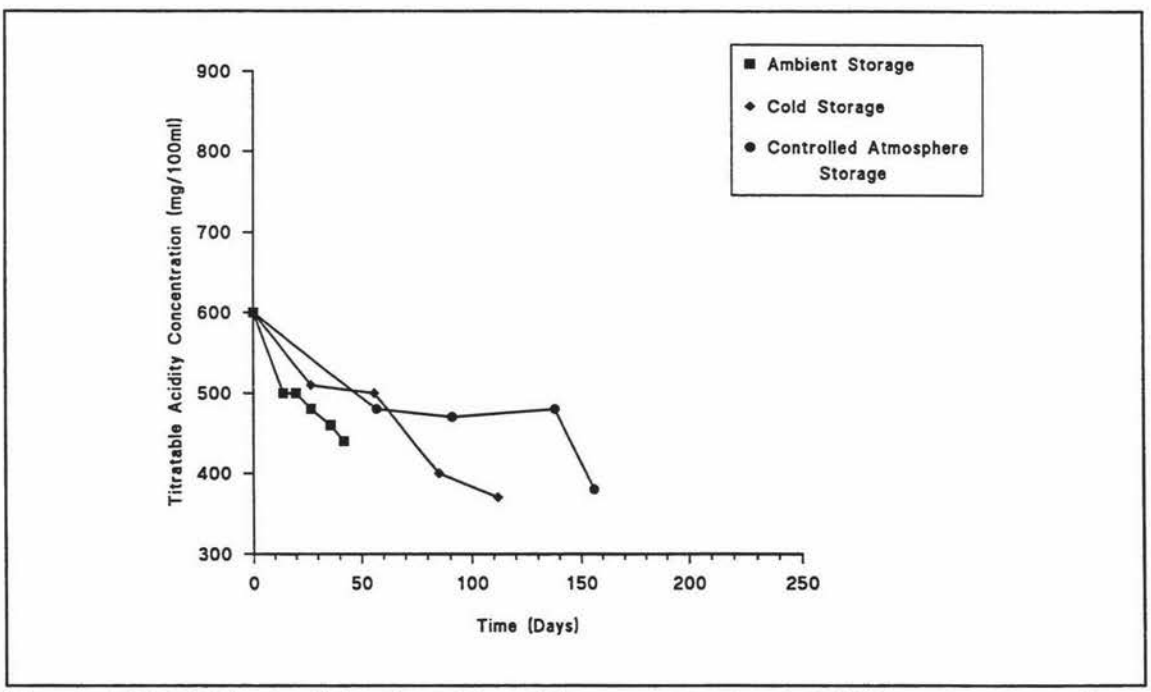

Figure A33.2: Effect on juice titratable acidity concentrations of different storage regimes for Braeburn apples in 1992.

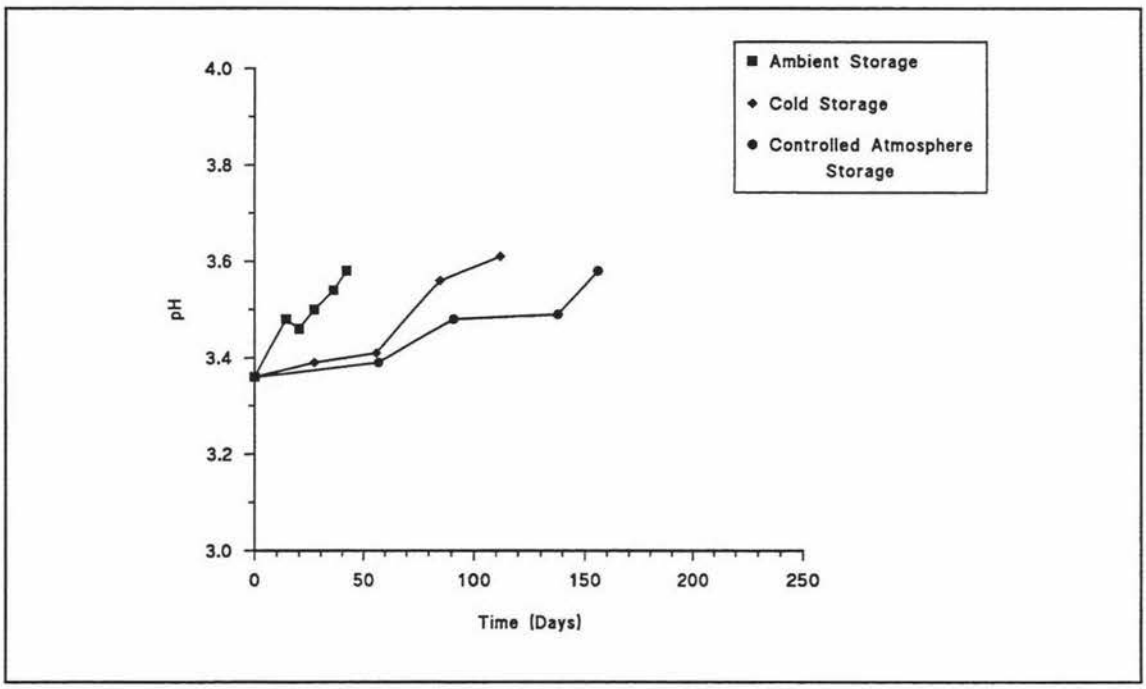

Figure A33.3: Effect on juice pH of different storage regimes for Braeburn apples in 1992.

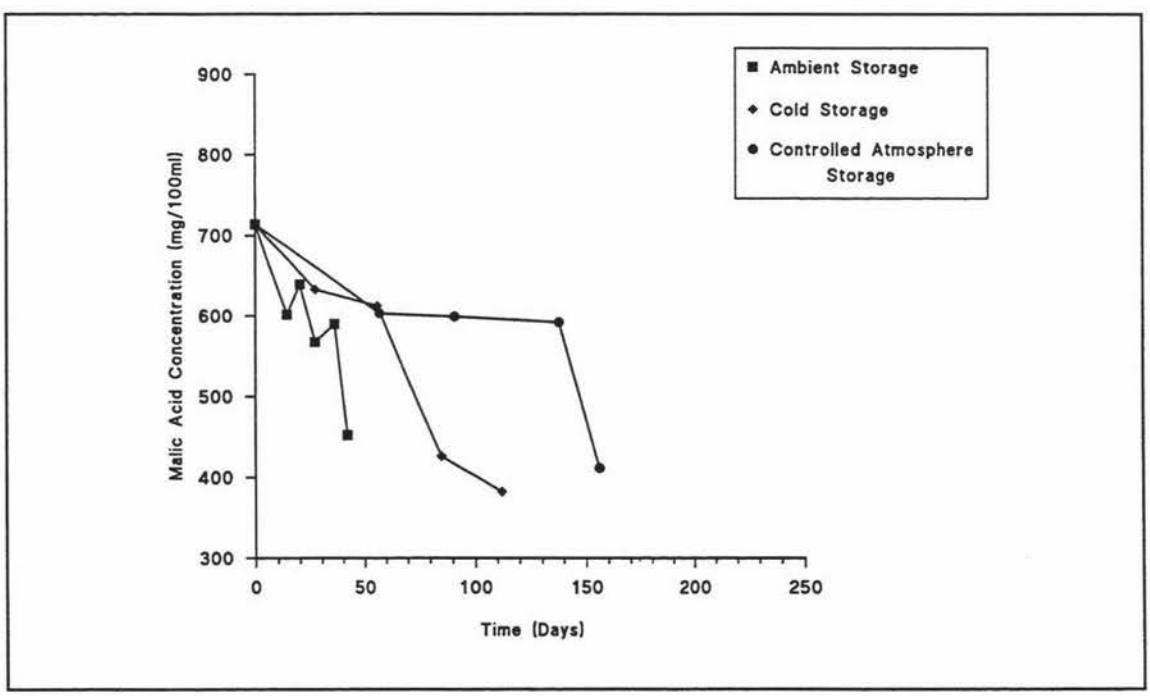

Figure A33.4: Effect on juice malic acid concentrations of different storage regimes for Braeburn apples in 1992.

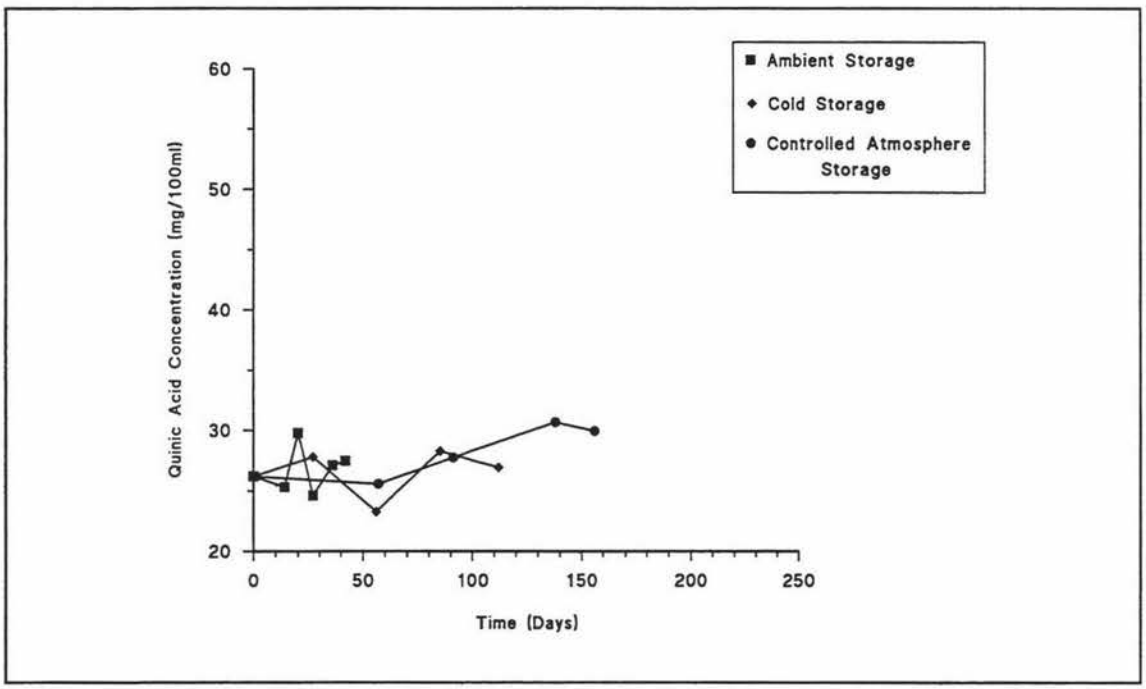

Figure A33.5: Effect on juice quinic acid concentrations of different storage regimes for Braeburn apples in 1992.

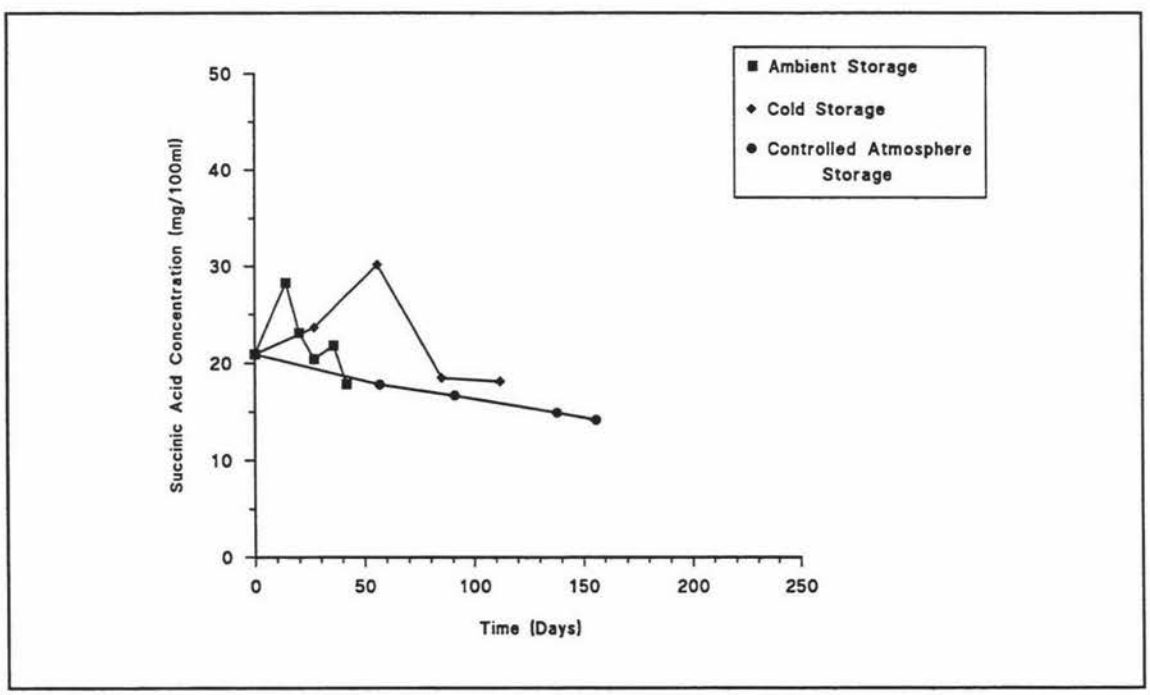

Figure A33.6: Effect on juice succinic acid concentrations of different storage regimes for Braeburn apples in 1992.

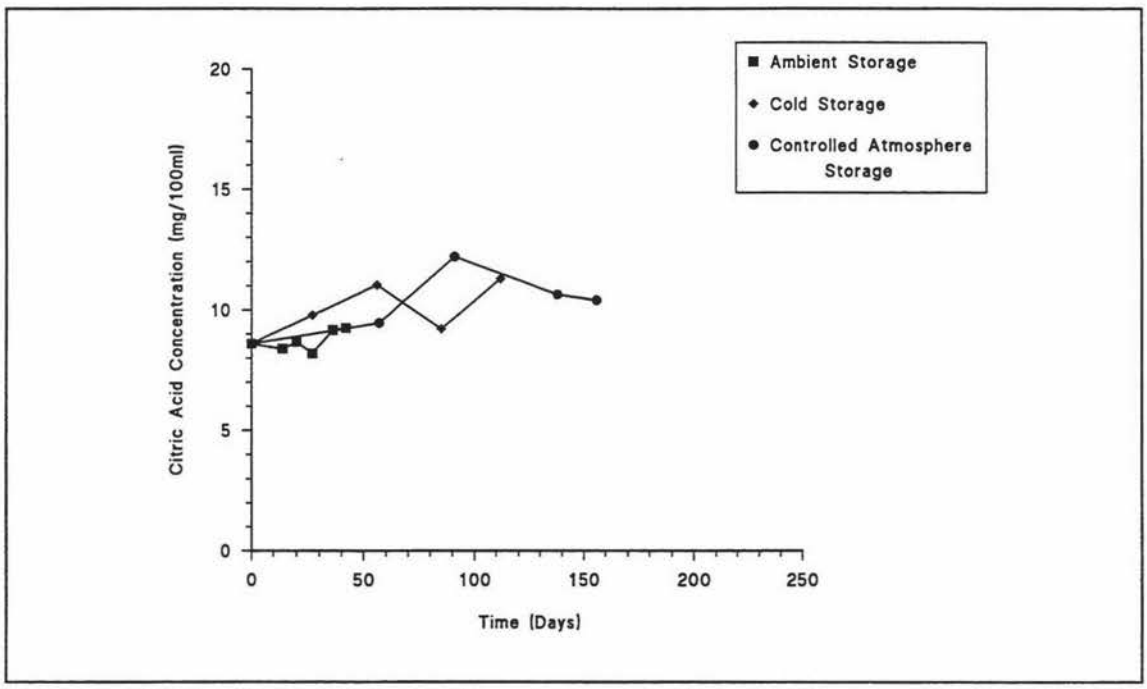

Figure A33.7: Effect on juice citric acid concentrations of different storage regimes for Braeburn apples in 1992.

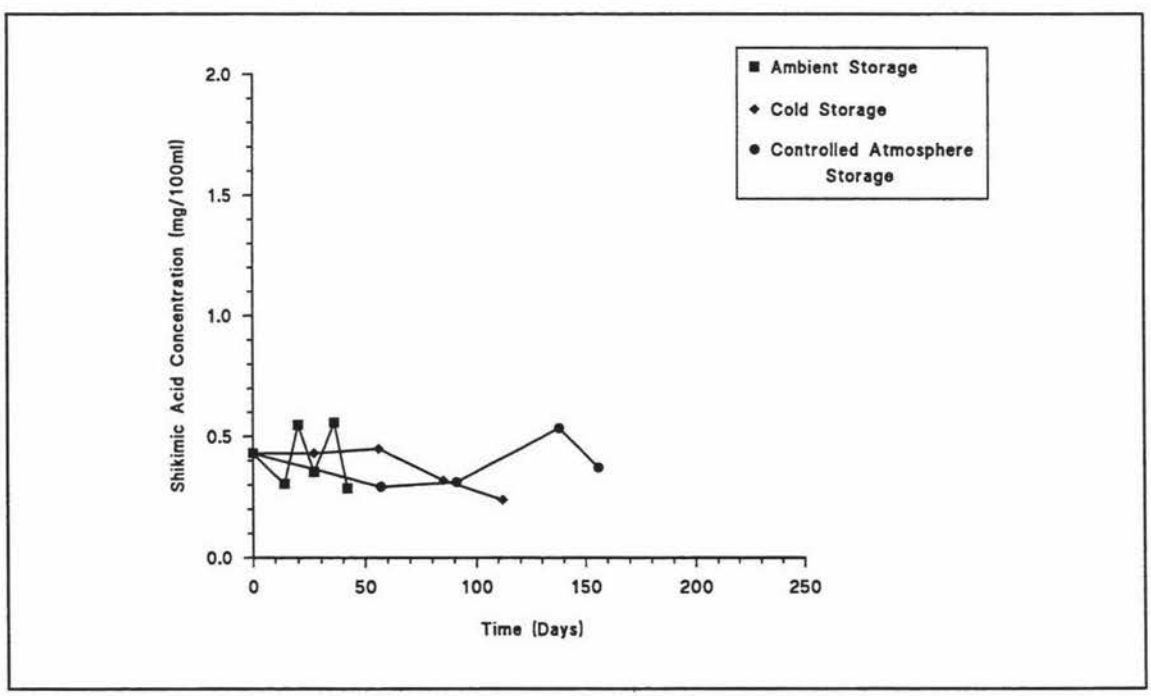

Figure A33.8: Effect on juice shikimic acid concentrations of different storage regimes for Braeburn apples in 1992.

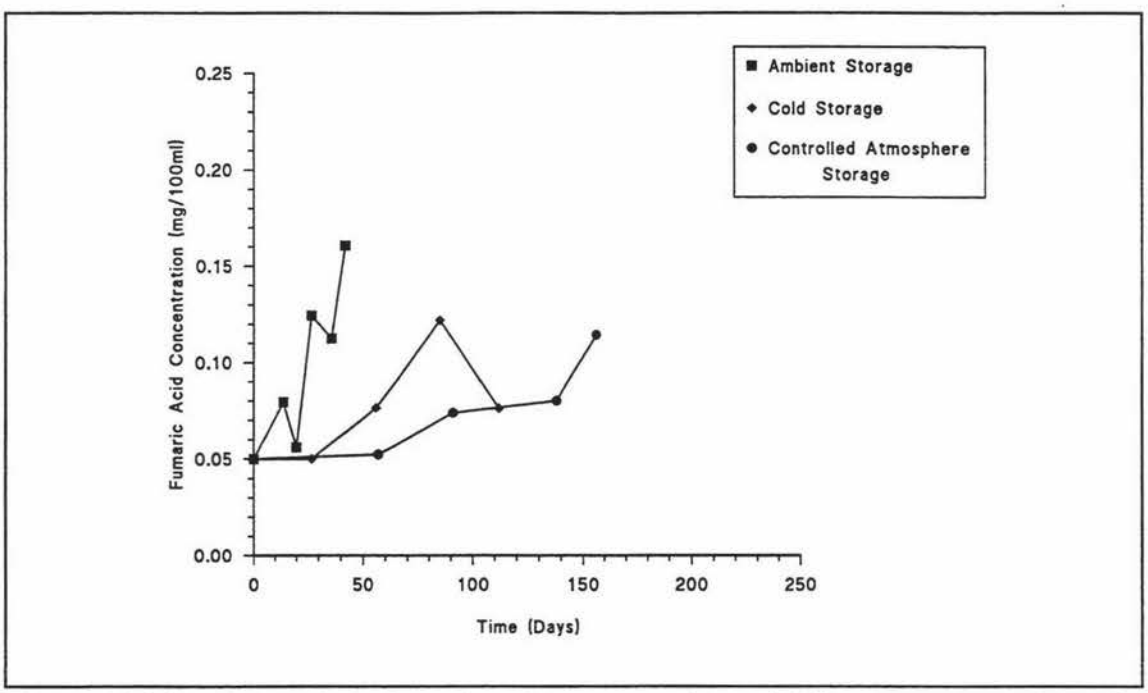

Figure A33.9: Effect on juice fumaric acid concentrations of different storage regimes for Braeburn apples in 1992.

APPENDIX 34

CHANGES IN THE INDIVIDUAL ORGANIC ACID CONCENTRATIONS IN THE JUICE OF GRANNY SMITH APPLES STORED AT DIFFERENT CONDITIONS IN 1992

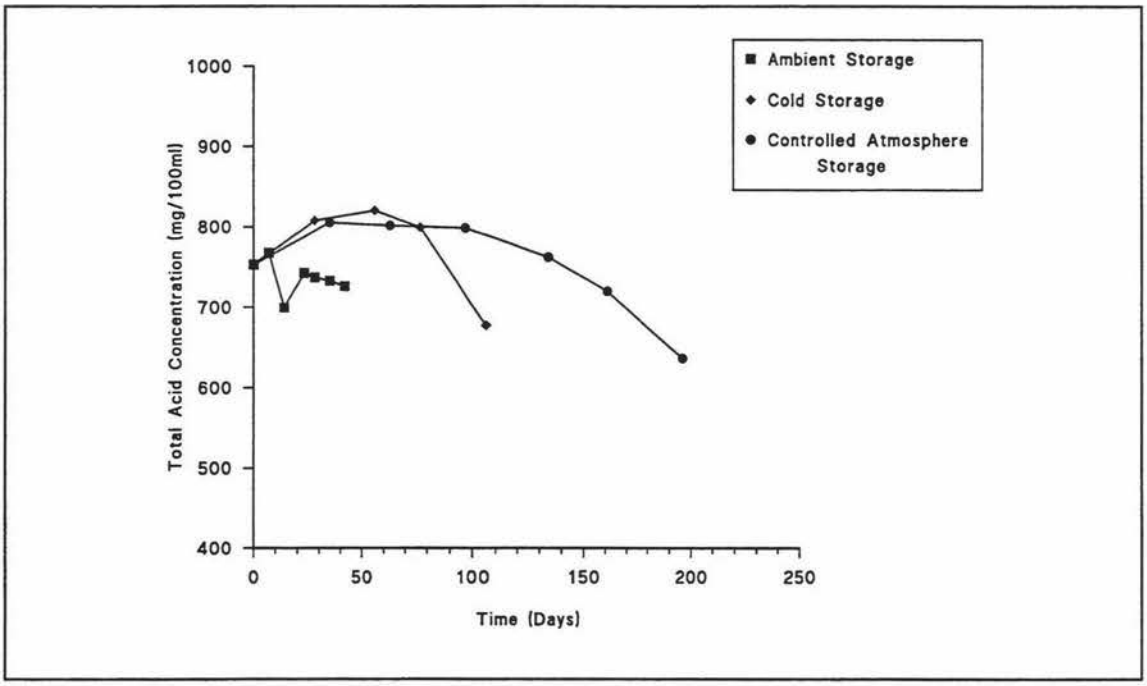

Figure A34.1: Effect on juice total acid concentrations of different storage regimes for Granny Smith apples in 1992.

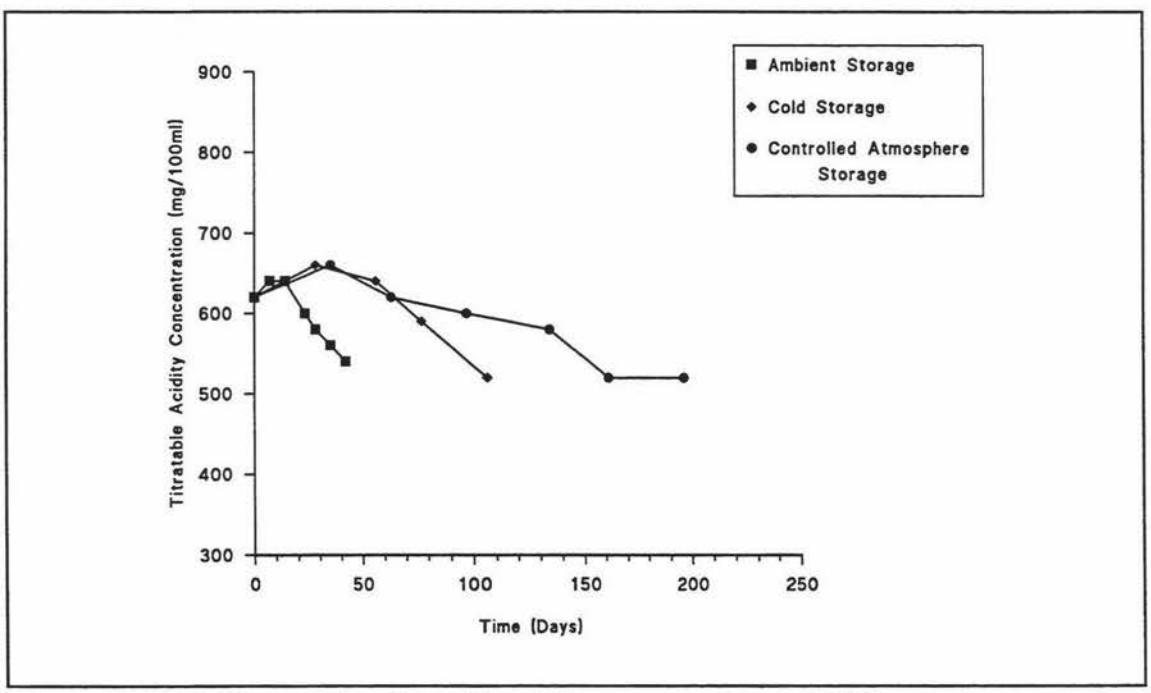

Figure A34.2: Effect on juice titratable acidity concentrations of different storage regimes for Granny Smith apples in 1992.

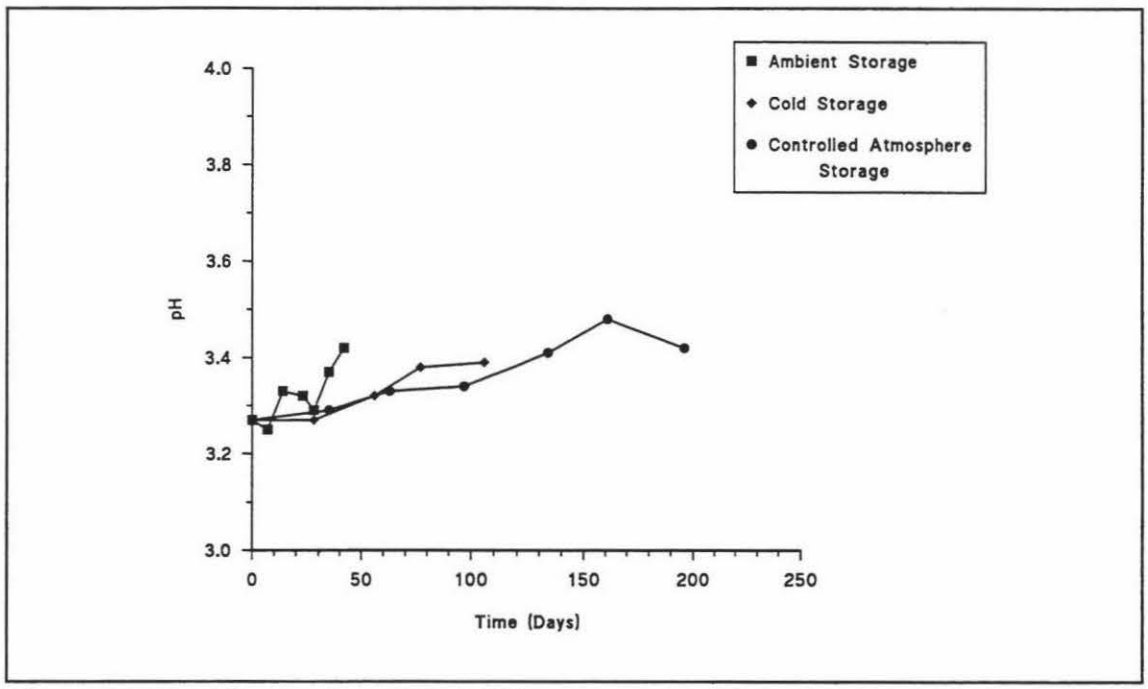

Figure A34.3: Effect on juice pH of different storage regimes for Granny Smith apples in 1992.

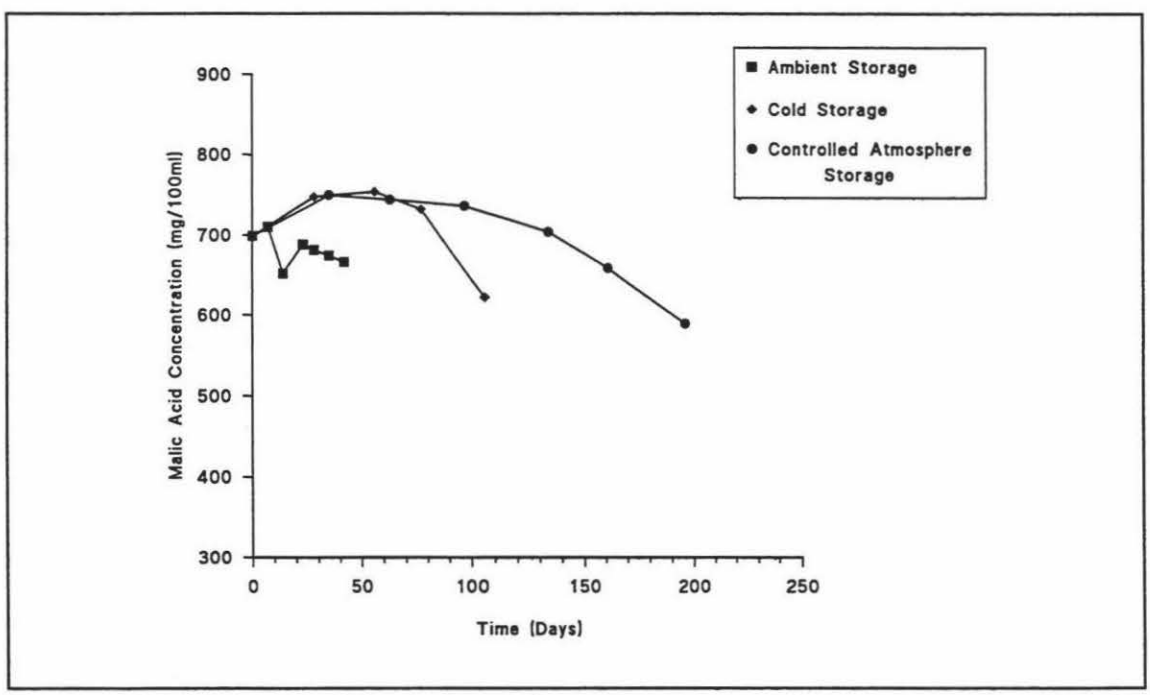

Figure A34.4: Effect on juice malic acid concentrations of different storage regimes for Granny Smith apples in 1992.

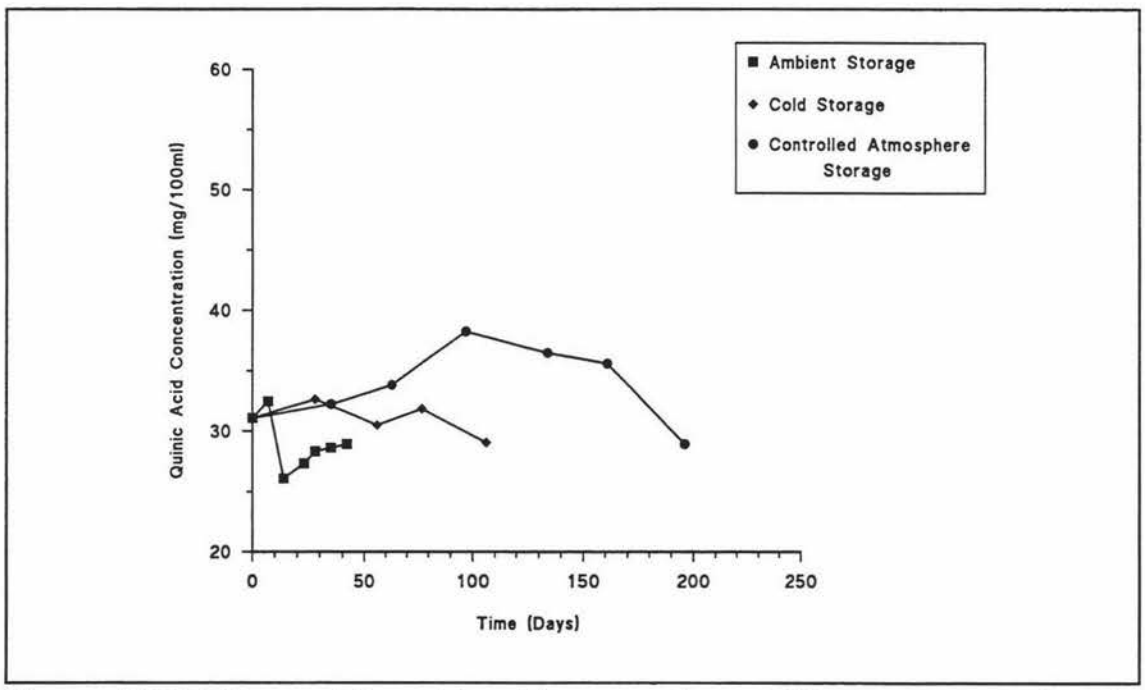

Figure A34.5: Effect on juice quinic acid concentrations of different storage regimes for Granny Smith apples in 1992.

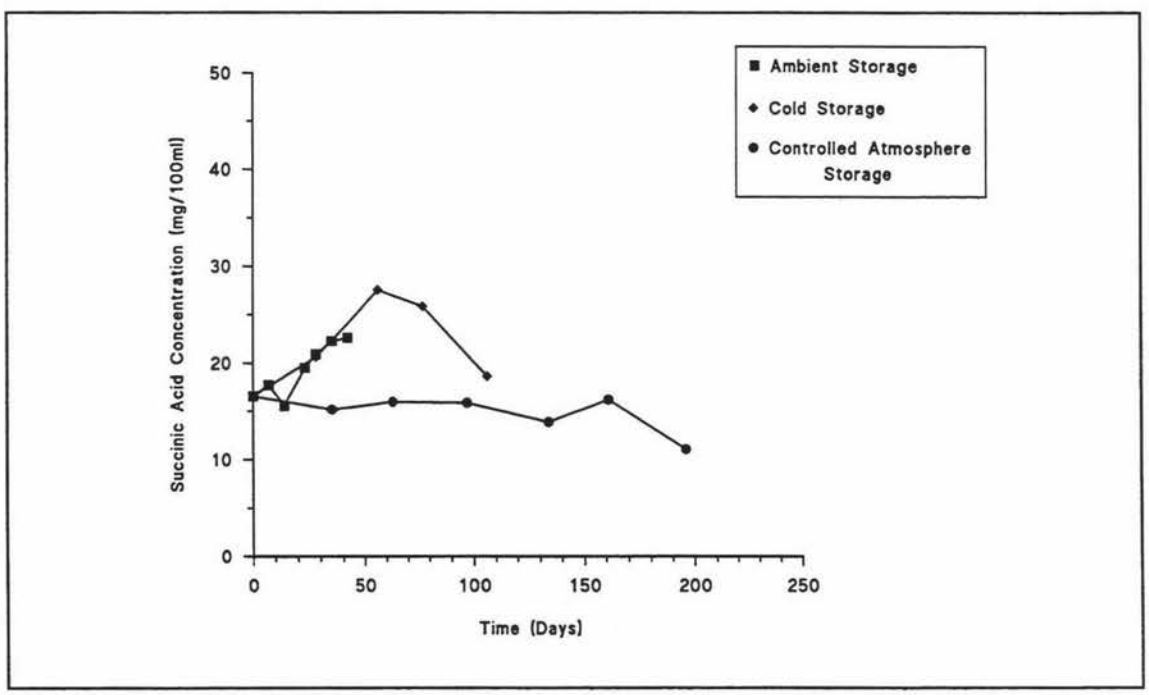

Figure A34.6: Effect on juice succinic acid concentrations of different storage regimes for Granny Smith apples in 1992.

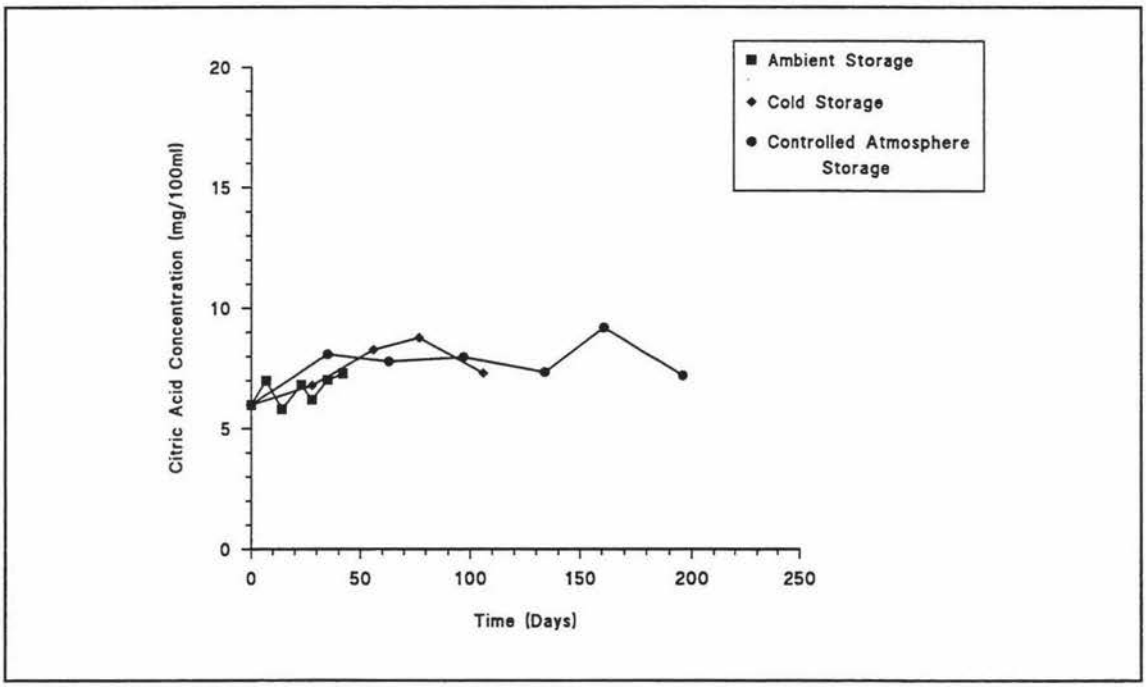

Figure A34.7: Effect on juice citric acid concentrations of different storage regimes for Granny Smith apples in 1992.

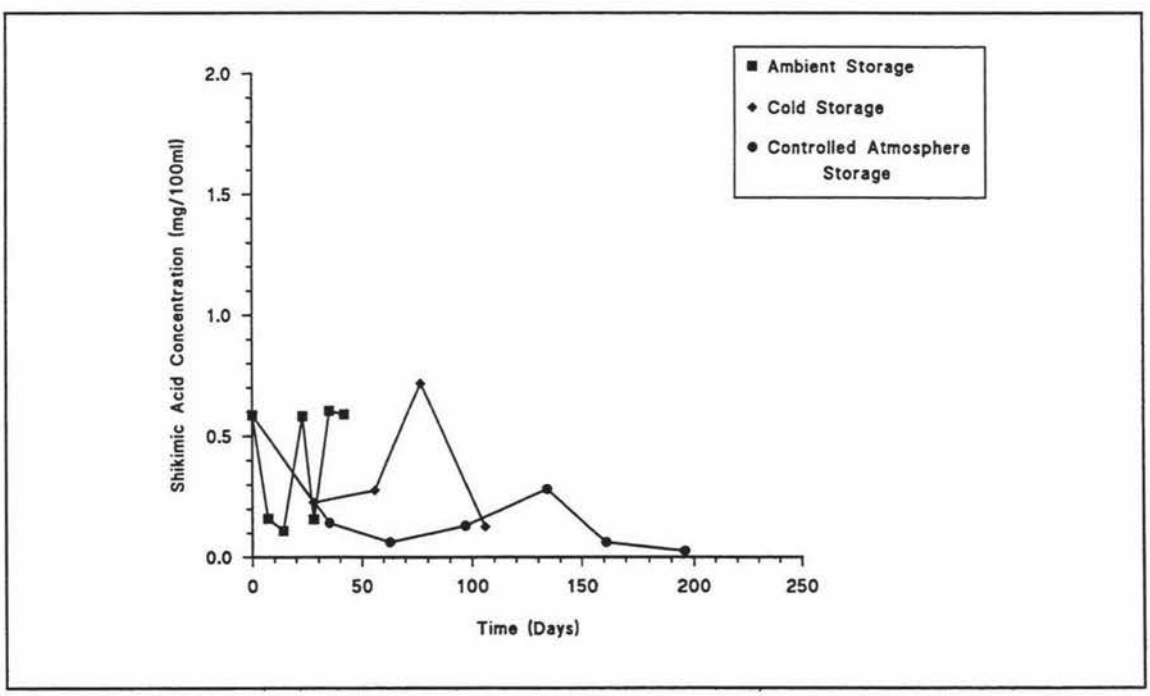

Figure A34.8: Effect on juice shikimic acid concentrations of different storage regimes for Granny Smith apples in 1992.

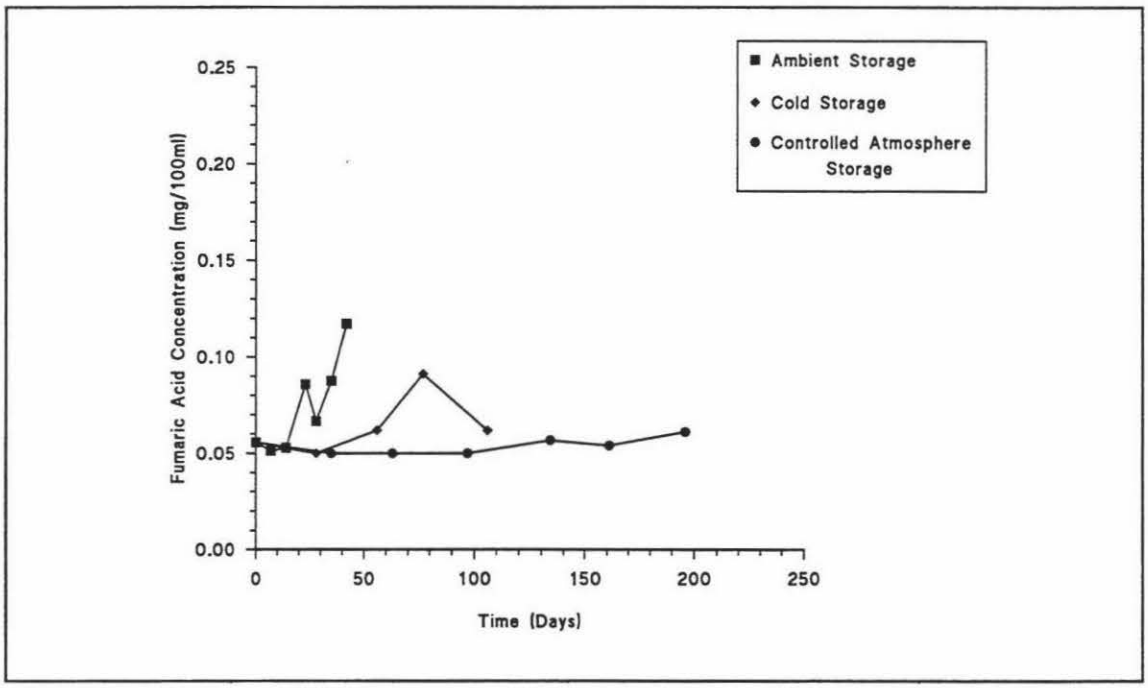

Figure A34.9: Effect on juice fumaric acid concentrations of different storage regimes for Granny Smith apples in 1992.

APPENDIX 35

CHANGES IN THE INDIVIDUAL ORGANIC ACID CONCENTRATIONS IN THE JUICE OF ROYAL GALA AND GALA APPLES STORED AT DIFFERENT CONDITIONS IN 1992

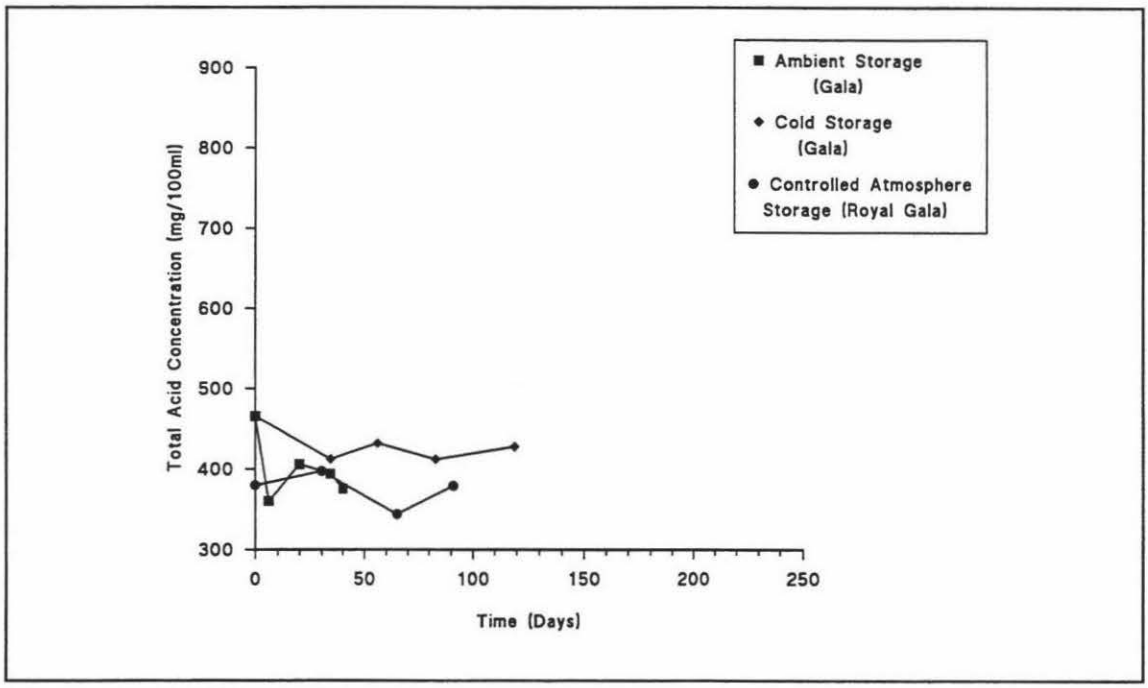

Figure A35.1: Effect on juice total acid concentrations of different storage regimes for Gala and Royal Gala apples in 1992.

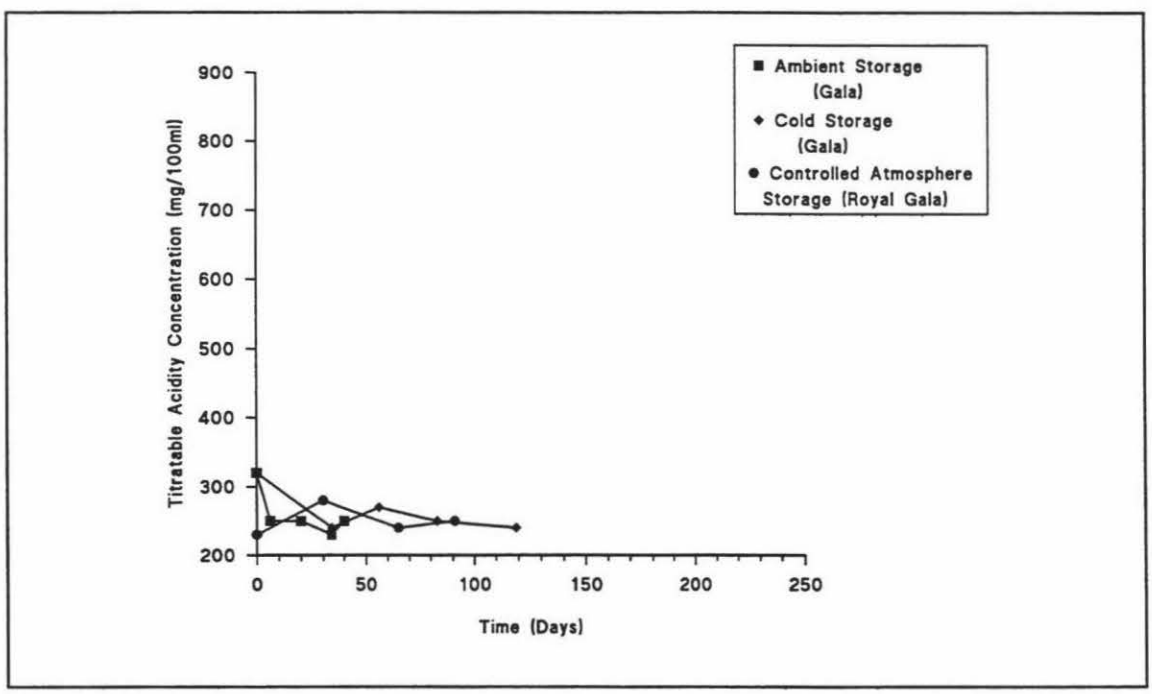

Figure A35.2: Effect on juice titratable acidity concentrations of different storage regimes for Gala and Royal Gala apples in 1992.

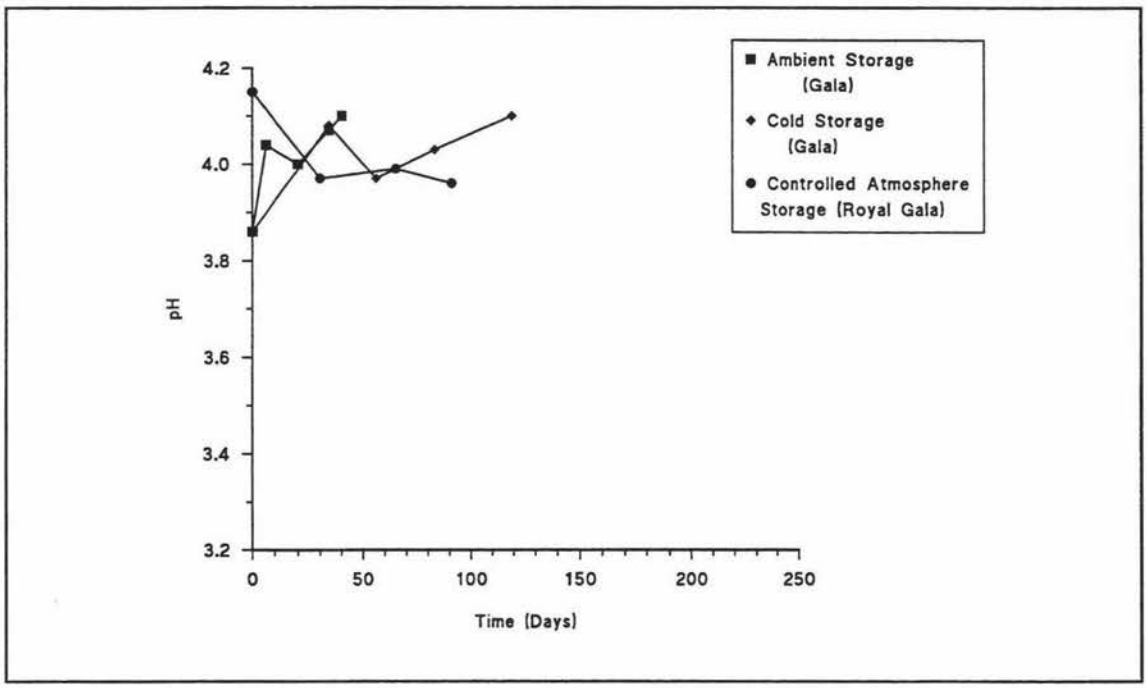

Figure A35.3: Effect on juice pH of different storage regimes for Gala and Royal Gala apples in 1992.

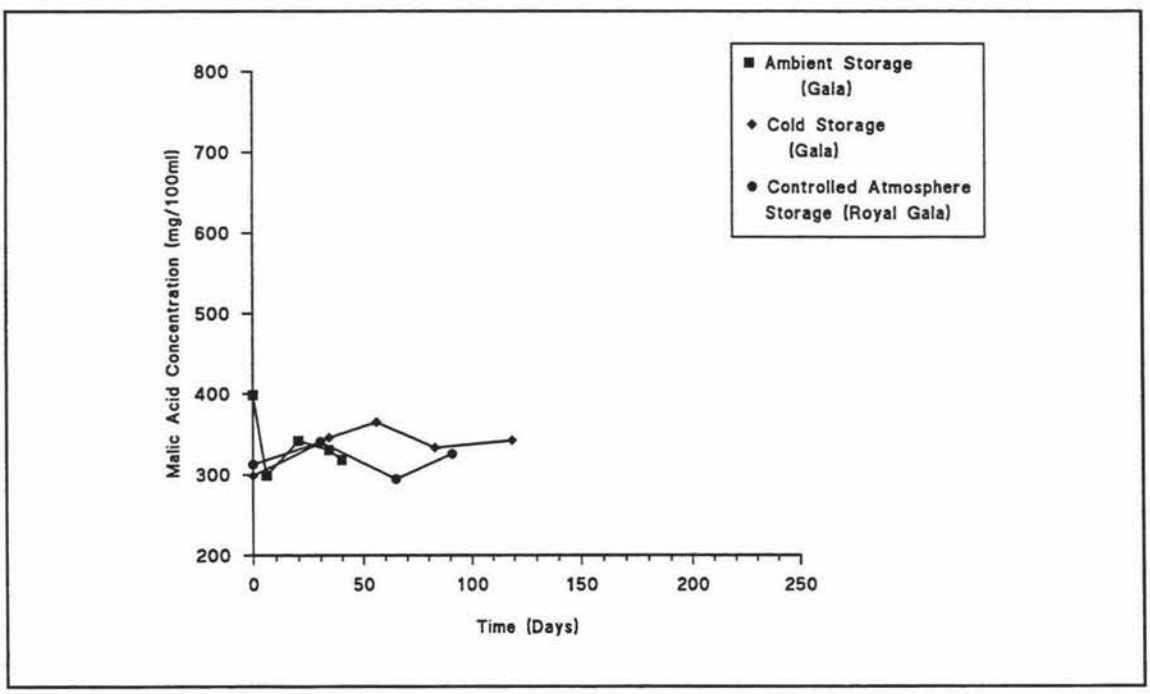

Figure A35.4: Effect on juice malic acid concentrations of different storage regimes for Gala and Royal Gala apples in 1992.

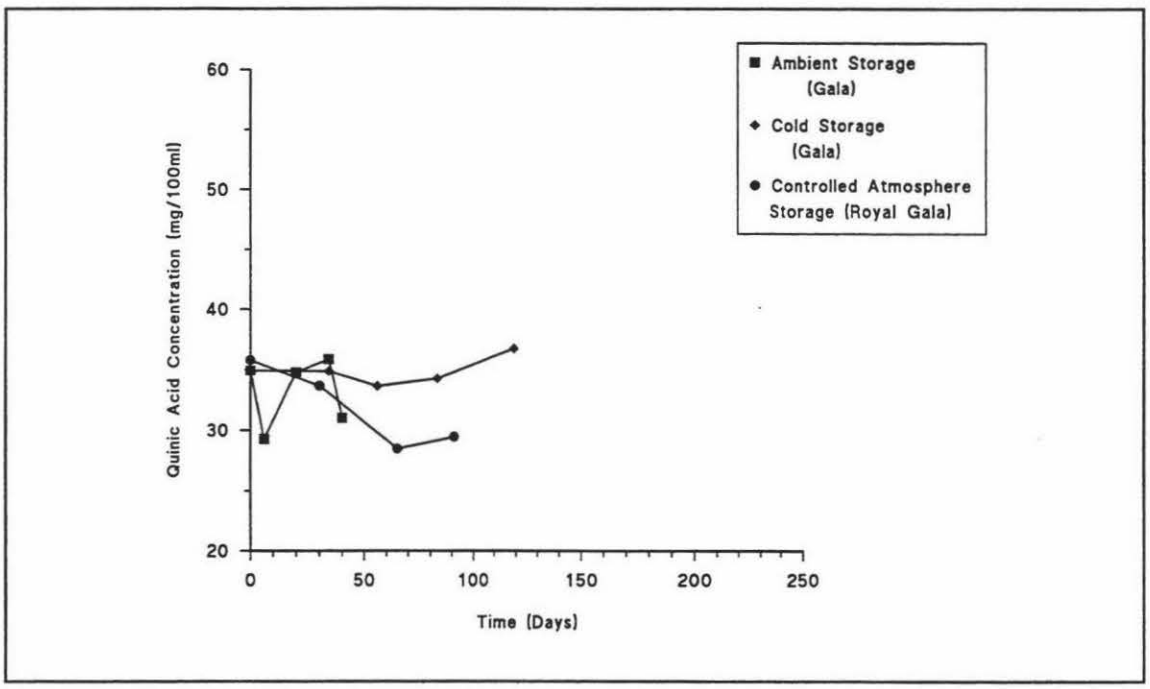

Figure A35.5: Effect on juice quinic acid concentrations of different storage regimes for Gala and Royal Gala apples in 1992.

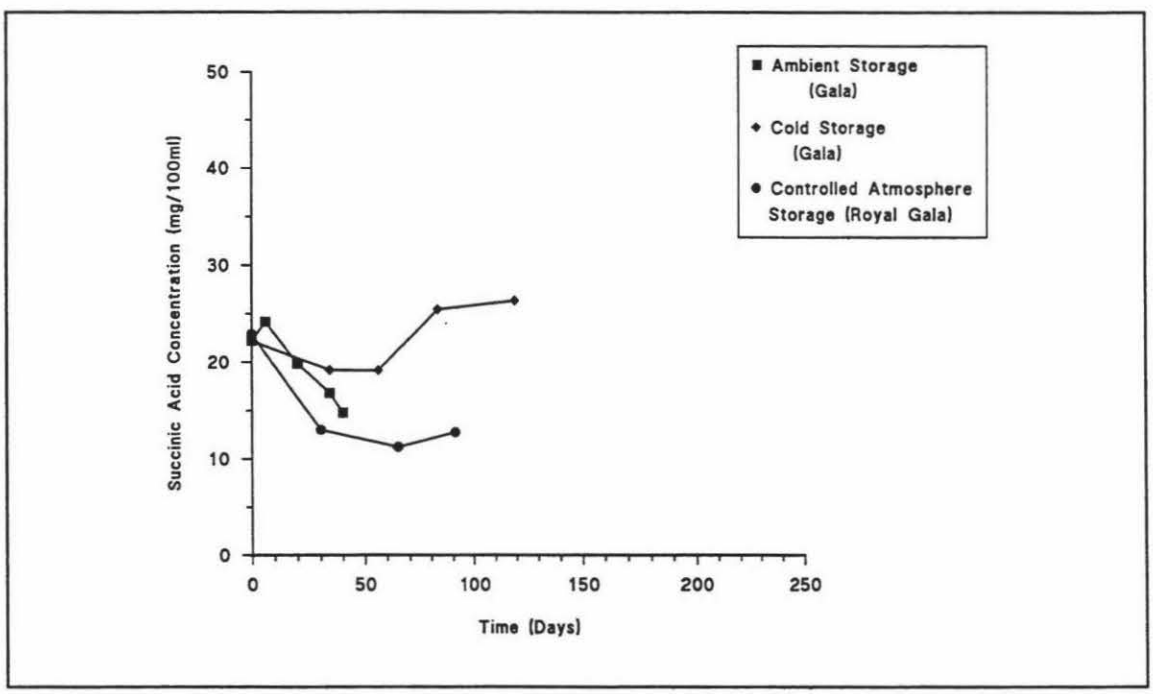

Figure A35.6: Effect on juice succinic acid concentrations of different storage regimes for Gala and Royal Gala apples in 1992.

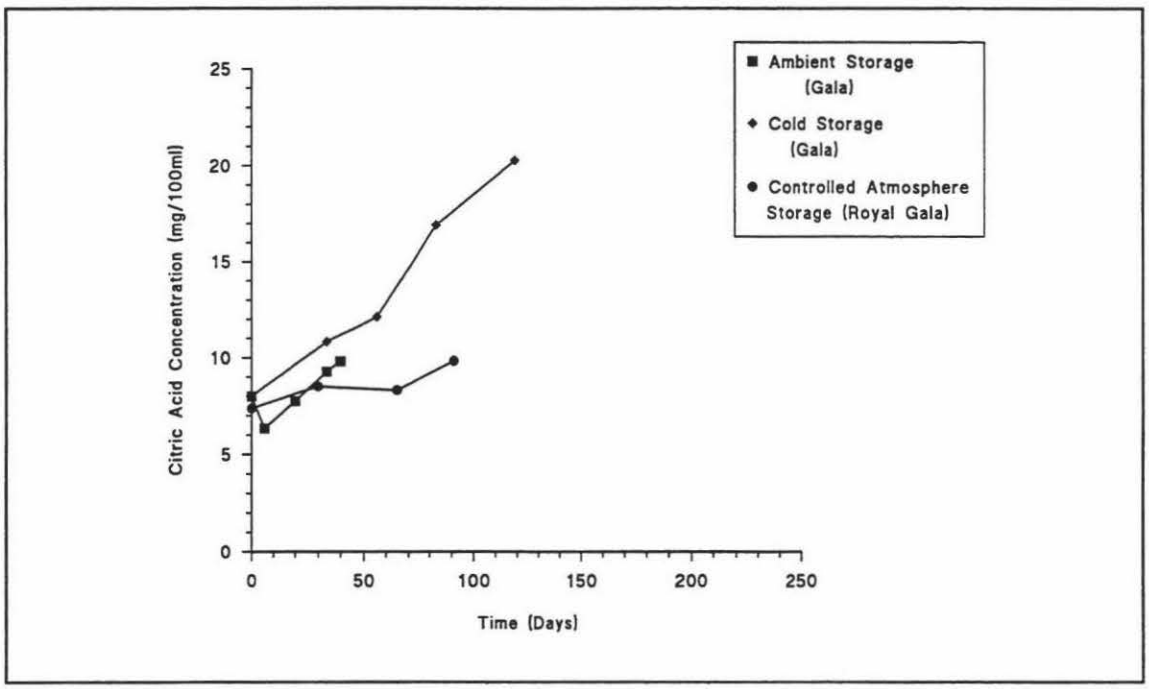

Figure A35.7: Effect on juice citric acid concentrations of different storage regimes for Gala and Royal Gala apples in 1992.

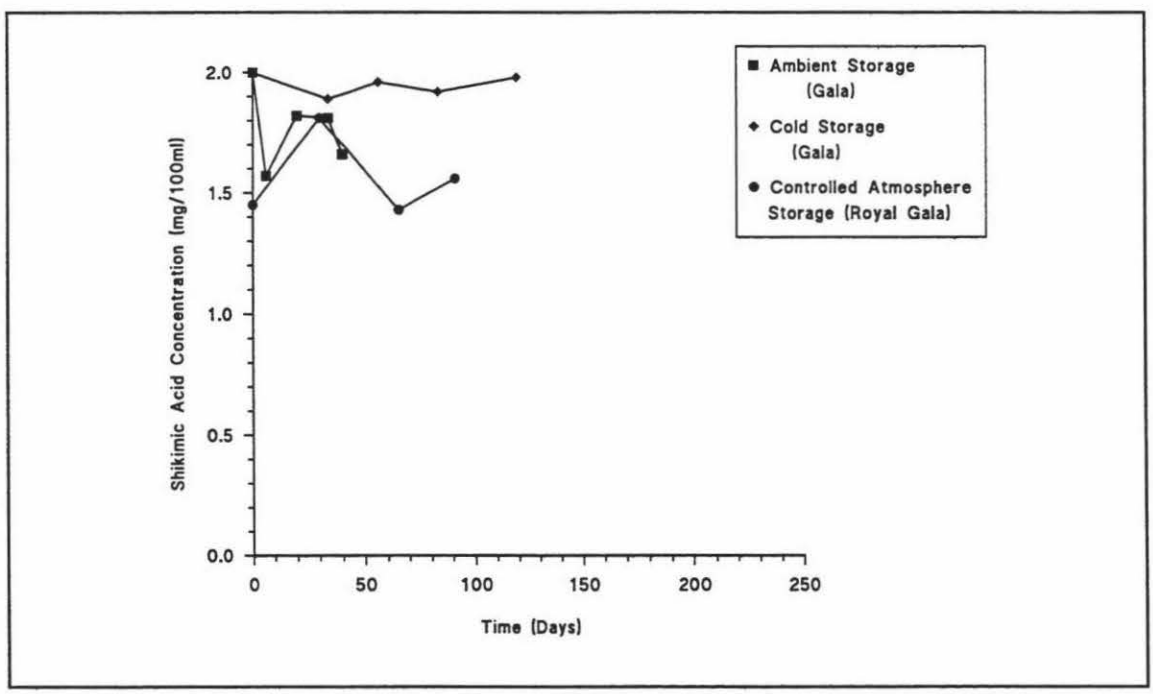

Figure A35.8: Effect on juice shikimic acid concentrations of different storage regimes for Gala and Royal Gala apples in 1992.

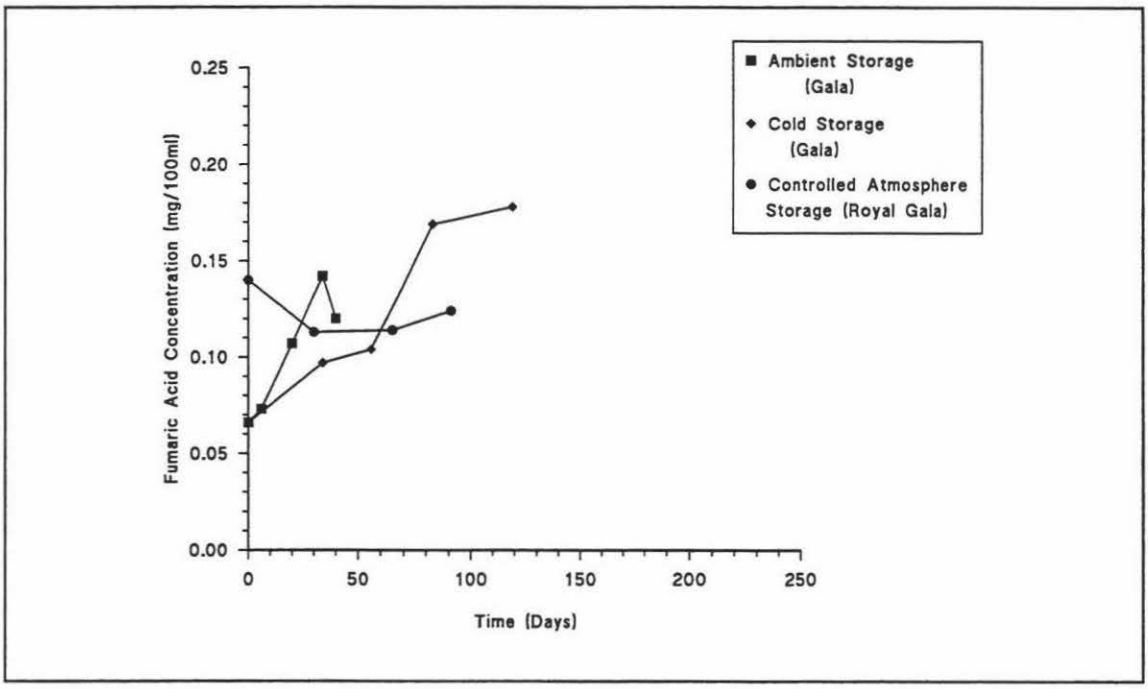

Figure A35.9: Effect on juice fumaric acid concentrations of different storage regimes for Gala and Royal Gala apples in 1992.