THE INTERACTION BETWEEN SUGARS AND ACIDS AND THEIR EFFECTS ON CONSUMER ACCEPTANCE OF KIWIFRUIT PULP

Presented by

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ABSTRACT

A model system using kiwifruit (*Actinidia deliciosa* (A. Chev) Liang *et* Ferguson var *deliciosa* cv Hayward) pulp has been developed so that consumer perceptions of sugar and acid can be explored in a realistic, homogenous product where natural variation between fruit and within fruit is eliminated. Use of a pulp model system enabled the sugar and acid level in kiwifruit to be manipulated using sugar and acid stock solutions. Fruit from an early harvest were selected to suppress the development of esters in the fruit at 'eating ripeness' so that sugar and acid relationships could be assessed without the influence of ester odour compounds. To compare and contrast sugar and acid relationships in kiwifruit with ester levels typical of fruit harvested at the recommended harvest maturity, odour compounds were incorporated into a portion of the pulp. Consumer's 'overall liking' ratings of the pulp increased with rising Brix. Increasing Brix level was also shown to increase 'sweetness liking', 'acidity liking', and perception of 'sweetness intensity'. Variations in Brix and acid level elicited the same consumer response to pulp with added odour compounds as to pulp without added odour compounds.

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ABBREVIATIONS

APC	Aerobic Plate Count
CFU	Colony Forming Unit
°C	Degrees Celsius
g	Gram
GC	Gas Chromatography
GC-FID	Gas Chromatography - Flame Ionisation Detection
hr	Hour
kgf	Kilogramforce
L	Litre
N	Molar
min	Minute
mg	Milligram
mm	Millimetre
mL	Millilitre
mol/L	Molar Litres
%	Percent
RI	Refractive Index
RO	Reverse Osmosis
TA	Titratable Acidity
µL	Microlitre
sec	Second

CHAPTER ONE

Introduction

As recently as 1970, kiwifruit was virtually an unknown commodity on international markets (Earp, 1990). Throughout the 1980's it was introduced as an exotic fruit in many European countries (Ferguson and Bollard, 1990). By the mid 1990's kiwifruit had moved from being an exotic item sold by the piece to a mass market item sold in bulk. New Zealand's kiwifruit exporting is now a business worth over $442 million in export sales (Belrose Inc., 1999).

Among the many attributes of kiwifruit contributing to its popularity is the unique flavour. An important aspect of the eating experience of a whole, fresh kiwifruit is the perception of sweetness, acidity and odour in the mouth during mastication. The contribution of each of these components and the way in which they interact, largely influence the overall acceptability of the ripe fruit. As kiwifruit ripen, the sugar, acid and odour compound composition of the fruit changes (MacRae *et al.*, 1989a) and consequently the flavour profile of the fruit is affected (Paterson *et al.*, 1991). During ripening, starch is hydrolysed to fruit sugars (Reid *et al.*, 1982), and organic acids are metabolised (Matsumoto *et al.*, 1983). It is the accumulation of fruit sugars and concomitant decrease in organic acids that are responsible for the increasing sweetness and decreasing acidity perceived as the kiwifruit progresses through the ripening process. The odour compound composition of kiwifruit is very sensitive to the degree of fruit ripeness (Bartley and Schwede, 1989). Progressing ripeness is associated with a rapid increase in the level of ester odour compounds that are responsible for the 'fruity' odours characteristic of ripe fruit (Paterson *et al.*, 1991). Accompanying this is a decrease in aldehydes, resulting in a loss of 'green' odour associated with unripe fruit (Young and Paterson, 1985). It has been suggested that generally, New Zealand consumers prefer soft (0.45 – 0.65 kgf) kiwifruit (Stec *et al.*, 1989). The characteristics of soft kiwifruit enjoyed by New Zealanders have been reported to include intense fruity odours and flavours and a balance between perceived sweetness and acidity, favouring sweetness (Stec *et al.*, 1989).

The contribution of sugars, acids and odour compounds to the sensory attributes of kiwifruit has been investigated by Paterson *et al.*, (1991) using whole, fresh fruit. The use of whole, fresh fruit allowed no control over differences between individual fruit. To overcome the confounding influences introduced by the use of whole, fresh fruit, Gilbert *et al.*, (1996) explored consumer perception of kiwifruit odour compounds using a model solution system. While this study has provided valuable insights into kiwifruit flavour, it provided information on major odour compounds in model solutions, not in kiwifruit.

To overcome the limitations of a model solution system and the natural variation of whole fruit, I propose to develop a model system using 'pulped' whole fruit. This will enable the relationships between sugars, acids and odour compounds in kiwifruit to be investigated in a homogenous kiwifruit based product.

1.1 Harvest Maturity of Kiwifruit

The quality of ripe kiwifruit is directly related to the maturity of the fruit at harvest (Reid and Harris, 1977). Soluble solids concentration is the measurement used to indicate crop maturity, and hence determine the date a crop should be harvested. In New Zealand, the Ministry of Agriculture and Fisheries (MAF) prohibit the harvesting of export kiwifruit before the crop has reached a maturity index of 6.2 % soluble solids (MAF publication, 1980). Below this point the fruit is considered to be physiologically immature, still relying on the vine for nutrients and unable to ripen independently with acceptable flavour characteristics (Harman, 1981). The soluble solids concentration is usually based on the refractive index (RI) and is measured on a refractometer (MAF publication, 1980). The RI of a liquid is greatly affected by the presence of dissolved substances, such as salts, sugars and organic acids. A liquid with high concentrations of sugars and acids and other dissolved substances will have a high refractive index, and therefore will give a high % soluble solids measurement (Bieleski and Clark, 1995). In kiwifruit juice, % soluble solids is closely related to sugar concentration, so that the RI of the juice reflects the amount of sugar in the juice

(Given, 1993). Another term for % soluble solids is Brix level. From this point on in this thesis, % soluble solids will be referred to as Brix level.

Acceptability testing of kiwifruit harvested at a range of maturities has established the importance of a minimum harvest maturity standard. The results obtained by Harman (1981) from a taste panel consisting of 60 consumers assessing the flavour quality of stored kiwifruit harvested at either below 6.2° Brix or at 6.2° Brix or above, demonstrated that fruit harvested below the minimum maturity standard had low intensities of sweetness, acidity and odour. Overall the flavour of these fruit was considered to be poor and hence unacceptable. Fruit harvested below the minimum harvest maturity have low levels of ester odour compounds at 'eating ripeness' (Young and Paterson, 1985). 'Eating ripeness' is considered to be the point at which kiwifruit are ready-to-eat and is dependant on fruit firmness and Brix level. These 'eating ripeness' parameters will be discussed in section 1.3.

Flavour is not the only attribute of kiwifruit to be affected by a premature harvest. After storage, the texture of kiwifruit harvested below 6.2° Brix, tends to break down and deteriorate at a faster rate than fruit harvested at or above the minimum harvest maturity standard (Harman, 1981). The texture of the fruit at 'eating ripeness' is not important in this study as the fruit is to be pulped. Fruit in the proposed study will be harvested at 5.0° Brix, earlier than the recommended harvest maturity of 6.2° Brix. An early harvest will ensure that at 'eating ripeness' the fruit will have low levels of ester odour compounds (Young and Paterson, 1985). It is desirable that at 'eating ripeness' the fruit in the proposed study have low levels of esters, as the pulp will be adulterated with additional odour compounds to simulate kiwifruit with ester levels typical of fruit harvested at the recommended harvest maturity of 6.2° Brix.

Although Brix level at harvest is an appropriate maturity index for kiwifruit grown in New Zealand, it is recognised that it may not be the best predictor of fruit quality for kiwifruit grown in other countries. For example, based on the results of a study of kiwifruit grown in California, Crisoto *et al.*, (1984) argue that due to the differences in Brix level between different growing locations, Brix level used in combination with a measure of fruit firmness at the time of harvest would be more satisfactory as a crop

maturity index. In Australia, a study involving sensory evaluation of the kiwifruit variety 'Dexter', found that the Brix level of ripe fruit is believed to be a better predictor of flavour quality than the Brix level at harvest (Scott *et al.*, 1986). Although this measure is not much use as an indicator for harvest.

1.2 Postharvest Kiwifruit Ripening

Ripening of fruit involves a series of physico-chemical changes, both degradative and synthetic, which transform a fruit from physiologically mature but inedible, to edible. Hydrolysis of starch occurs during the ripening process resulting in increased levels of sucrose, fructose and glucose, hence an increase in Brix level is observed (Reid *et al.*, 1982). The accumulation of fruit sugars produced from the hydrolysis of starch, affects the taste and texture of the fruit. The increased concentrations of sugars sweetens fruit, and the loss of starch affects texture as the starch granules are degraded (MacRae, 1988). A concomitant decrease in acidity and consequent rise in pH result from the metabolism of organic acids during the ripening process (Matsumoto *et al.*, 1983). These changes result in a ripe fruit that is now palatable.

Ethylene, an unsaturated hydrocarbon gas, is produced naturally by fruits during ripening, senescence and in response to stress and wounding. In concert with other plant hormones, ethylene probably acts to exercise control over the fruit ripening process. Due to the simultaneous increase in both respiration rate and ethylene production, kiwifruit are generally considered to be climacteric (Given, 1993). Exposure to very low concentrations of exogenous ethylene will cause an acceleration in the ripening process of climacteric fruits (Reid and Harris, 1977). The process of exposing kiwifruit to low concentrations of ethylene to induce the onset of ripening is a common commercial practice. Ripening fruit with ethylene is advantageous not only because it promotes rapid softening, but because the rate in which the different areas within the edible portion of the fruit soften is more uniform when fruit are exposed to ethylene (Lallu *et al.*, 1989). Fruit in the proposed study will be ethylene treated to guarantee that fruit will be at 'eating ripeness' on consumer testing days.

The use of ethylene as a postharvest treatment of kiwifruit, combined with specific storage conditions can affect the chemical and physical properties of kiwifruit at eating ripeness. For example, Lallu *et al.*, (1989) identified that a storage period of less than six weeks at 0° C combined with ethylene treatment, produces kiwifruit that soften to eating ripeness more quickly than untreated fruit. Young and Paterson, (1985) have noted the effect of postharvest handling on the odour volatile composition of kiwifruit. Fruit from a range of harvest maturities were stored for varying lengths of time prior to receiving ethylene treatment. It was found that increasing the storage time of fruit prior to treating with ethylene will result in a decreased ester concentration in the fruit at 'eating ripeness' especially in fruit harvested below the minimum harvest maturity. Therefore fruit in the proposed study will be stored for eight weeks at 0° C prior to ethylene ripening to minimise the presence of ester odour compounds in the ripe fruit.

1.3 'Eating Ripeness' Parameters

As discussed in section 1.1, 'eating ripeness' is the point at which fruit are ready for consumption. 'Eating ripeness' is determined by measuring the firmness and the Brix level of the ripened fruit.

Firmness of kiwifruit may be measured using a penetrometer. A penetrometer measures the force required to penetrate fruit flesh. Kiwifruit firmness is usually measured using a penetrometer with a probe of 7.9 mm in diameter (Harker *et al.*, 1996). The penetrometer measures firmness as kgf, which may be readily converted to Newtons. Assessment of fruit firmness should be carried out when the internal temperature of the fruit is 20° C (Lallu *et al.*, 1989). Kiwifruit considered to be 'eating-ripe' have a firmness within the range 0.4 - 0.8 kgf (Lallu *et al.*, 1989). Fruit in the proposed study will be considered to be 'eating ripe' when the fruit has a firmness of 0.7 kgf.

At 'eating ripeness', kiwifruit, irrespective of postharvest handling treatment, have a Brix level of between 13.0 - 14.5° (Lallu *et al.*, 1989). However, there may be

considerable variations in Brix between different fruit of the same crop, due largely to the position of the fruit on the vine (Ford, 1984). Furthermore, Brix level is not the same throughout an individual fruit. Brix level increases along the longitudinal axis of the fruit, such that the blossom end of the fruit has a consistently higher Brix level than the stem end of the same fruit (Hopkirk *et al.*, 1986). Pulping the experimental fruit will avoid Brix level variation within fruit and between fruit.

1.4 Kiwifruit Physiology

The edible portion of a kiwifruit is made up of three different parts, which are very different, visually and chemically. The physiological characteristics of each tissue zone within the edible portion of kiwifruit has been described by MacRae, (1988). The cells in the core region are regularly shaped and closely packed with little air space in between. The inner pericarp contains the seeds and is comprised of large, oblong cells that closely resemble the flesh cells of an orange. The fleshy outer pericarp is made up of cells irregular in shape, size and alignment. It is the size, shape and alignment of cells in these different tissues that give each zone its characteristic texture. Each of the tissue zones have different sugar, acid and starch compositions. It is the ratio of these chemical components that give rise to the distinct taste qualities of each zone of tissue. For example, the inner core is relatively sweet, due to a higher sugar:acid ratio and the inner pericarp tastes sour due to the low level of sugar present. Each of these tissue zones undergo the changes associated with the ripening process at a different rate (Redgwell *et al.*, 1990). Therefore the proportions of each tissue present and its stage of ripeness will inevitably have an influence on the overall taste perceptions experienced during consumption of the fruit. As kiwifruit are not harvested 'ready-to-eat' the postharvest handling of the fruit will vastly influence the relative amounts of sugars and starch within these tissue zones, thereby influencing the acceptability of the ripe fruit (MacRae *et al.*, 1989a). Pulping the fruit will produce a homogenous blend of all the edible areas of the fruit.

1.4.1 Composition of sugars and acids in kiwifruit

The major organic acids in kiwifruit are citric, quinic and malic acids (Heatherbell, 1975). Citric acid is the most abundant acid in kiwifruit, followed closely by quinic acid (MacRae *et al.*, 1989b). MacRae *et al.*, (1989b) found that fruit harvested at 4.8° Brix and ripened with ethylene after six weeks storage at 0° C typically had 1.3 mg/g malic acid, 8.4 mg/g citric acid and 7.5 mg/g quinic acid. Of the three major organic acids present in kiwifruit, malic acid has been found to have the lowest sensory threshold. This is based on the findings of Amerine *et al.*, (1965) who showed that malic acid has a lower recognition threshold (0.0016N) than citric acid (0.0023N). The low threshold of malic acid, in relation to the other major organic acids was noted by McMath *et al.*, (1991a) (unpublished) in a study to explore acid perception using a kiwifruit juice base with varying amounts of quinic, malic and citric acid added. Although not the dominant acid quantitatively, malic acid may be the most appropriate acid to manipulate the acidity of the pulp, due to the lower threshold level of this acid.

At 'eating ripeness', kiwifruit contain little or no starch (Wright and Heatherbell, 1967). The starch present in the kiwifruit prior to 'eating ripeness' is converted to sugar during the ripening process (Okuse and Ryugo, 1981). The accumulation of sugars in the fruit as the starch is hydrolysed is reflected in the increasing Brix level of the fruit during the ripening process. The major sugars present in kiwifruit at 'eating ripeness', are fructose, glucose and sucrose (Beever and Hopkirk, 1990). In fruit harvested at 4.8° Brix (below the recommended harvest maturity standard) and stored for six weeks at 0° C, MacRae *et al.*, (1989b) found that there was 26.2 mg/g of fructose, 30.3 mg/g of glucose and 1.8 mg/g of sucrose present in the fruit at 'eating ripeness'. In fruit harvested above the recommended harvest maturity of 6.2° Brix, the composition of major fruit sugars was considerably different. MacRae *et al.*, (1989b) found 33.5 mg/g of fructose, 33.1 mg/g of glucose and 9.6 mg/g of sucrose present in fruit at 'eating ripeness' harvested at 6.4° Brix and stored for six weeks at 0° C.

1.5 The effect of odour, sweetness, and acidity perception on kiwifruit flavour

The perception of odour compounds, sugars and acids in the mouth during mastication is a major part of the kiwifruit eating experience. The vacuole is the single largest component of a fruit cell (John and Yamaki, 1994). Contained in the vacuole are the organic acids and fruit sugars along with salts and other organic material (Canel *et al.*, 1995). During mastication, the vacuolar membrane, the tonoplast, is ruptured and the vacuole contents are released into the mouth. It is this release of organic acids and sugars into the mouth during mastication that gives rise to the perception of sweetness and acidity in kiwifruit.

In order to detect both sweetness and acidity, molecules must come in contact with the receptor organs, located on the tongue and soft palate (Schiffman, 1996). These are known as taste buds. A taste bud is a specialised organ consisting of a cluster of epithelial cells from which nerve fibres lead to the brain (Lawless and Heymann, 1998). Tastants must be in solution so that they can come in contact with the taste bud (Schallenberger, 1993). The taste bud is capable of recognising five basic tastes; sweet, sour, salty and bitter and umami.

Also responsible for flavour and odour perception, are olfactory organs. The olfactory organs are clusters of epithelial cells located high in the nasal cavity and are responsible for detecting odours (Schiffman, 1996). During swallowing, a small vacuum is created in the cavity at the back of the nose, and a small gust of odour laden air from the food is drawn over the olfactory receptors (Lawless and Heymann, 1998). The trigeminal nerve is responsible for detecting chemical feeling factors (Schiffman, 1996). The combination of taste, odour and feel contribute to flavour perception.

In a study to assess the contribution of odour compounds and other chemicals to the sensory perception of kiwifruit, Paterson *et al.*, (1991) showed flavour to be the most important factor in consumer liking of kiwifruit. Flavour is a complex attribute made up of odour, taste and chemical feeling factors (Lawless and Heymann, 1998).

1.5.1 Effect of odour perception on kiwifruit flavour

Gas Chromatographic-Olfactory (odour-port) techniques have enabled several groups of researchers to investigate the odour compounds present in kiwifruit (Young *et al.*, 1983; Takeoka *et al.*, 1986; Bartley and Schwede, 1989 and Young *et al.*, 1995). The main volatile compounds found in kiwifruit include methyl and ethyl butanoate, Z- and E-2-hexenal, hexanol, Z- and E-3-hexenol and methyl benzoate. It is well established that aldehydes impart a 'green, vegetative' aroma to the fruit and esters are responsible for the 'fruity' aromas prevalent in the ripe kiwifruit (Young and Paterson, 1985). GC analysis of the odour compound composition of the pulp in the proposed study will confirm the presence of esters at low levels as a result of the early harvest date. Comparisons and contrasts will be made between the odour compound profile of fruit harvested at 5.0° Brix in the current (proposed) research and fruit harvested at the recommended harvest maturity of 6.2° Brix in previous published research.

Of all the components of flavour, volatile odour compounds sensed by the olfactory receptors make the largest contribution to the perception of flavour (Lawless and Heymann, 1998). The effect of odour on consumer liking of kiwifruit, was investigated by Gilbert *et al.*, (1996) using a New Zealand consumer panel to assess an aqueous model kiwifruit system. They demonstrated that increasing levels of ethyl butanoate increased 'overall liking', 'liking of aroma' and 'liking of flavour', and also increased perceived intensities of 'kiwifruit aroma' and 'kiwifruit flavour'. In contrast, increasing levels of E-2-hexenal decreased the 'degree of liking' of aroma, flavour and 'overall liking', but increased the perceived intensities of 'kiwifruit aroma' and 'kiwifruit flavour', similar to ethyl butanoate. Increasing levels of hexenal increased the perceived intensity of 'kiwifruit aroma', but did not affect the degree of liking attributes. In the Gilbert *et al.*, (1996) study, ethyl butanoate and E-2-hexenal had the most prominent effects on perceived intensity and acceptability of kiwifruit flavour. Therefore, these odour compounds were selected to incorporate into the kiwifruit pulp in the current study to simulate kiwifruit with odour compound levels typical of fruit harvested at 6.2° Brix.

1.5.2 The effect of tastant perception on kiwifruit flavour

The effect of tastants on consumer acceptance of fruit was investigated by Valdes *et al.*, (1956) who showed increasing sweetness in fruit products increased consumer liking. Increasing sugar concentration in fresh tomatoes has been reported to increase tomato flavour acceptance (Malundo *et al.*, 1995). Barnes *et al.*, (1991) have demonstrated the importance of sweetness to consumer acceptance of fruit flavoured yoghurts. This effect of increased sweetness increasing consumer acceptance was also demonstrated in kiwifruit by Jordan *et al.*, (1996) (unpublished). It was found that Brix level was positively correlated with consumer's 'overall liking' of kiwifruit. However, in a study using fresh, whole kiwifruit, Stec *et al.*, (1989) found that there was no correlation between Brix level at 'eating ripeness' and fruit acceptability. Esti *et al.*, (1998) also failed to find a relationship between Brix level and acceptability. The fruit used in both of these studies; Stec *et al.*, (1989) and Esti *et al.*, (1998), were whole, unprocessed kiwifruit. There is tremendous variation between individual kiwifruit (Young *et al.*, 1995). Use of whole fruit would have introduced confounding factors such as variation within fruit (Hopkirk *et al.*, 1986), and variation between fruit, in particular amongst odour compound components (Paterson *et al.*, 1991). It is hypothesised that the use of a model kiwifruit pulp system where variation between fruit and within fruit is eliminated, will confirm the findings of Jordan *et al.*, (1996) and demonstrate that increasing Brix level in kiwifruit at 'eating ripeness' will increase consumers 'overall liking' of ripe kiwifruit.

1.5.3 The effect of chemical feeling factors on kiwifruit flavour

The effect of chemical feeling factors on consumer response to kiwifruit has yet to be investigated, although the mechanism of irritation by calcium oxalate crystals present in kiwifruit has been described (Perera *et al.*, 1990). The chemical feeling factors associated with fruit is a separate area of research and will not be covered in this thesis.

1.5.4 The effect of interactions between sweetness, acidity and odour compounds on kiwifruit flavour

As discussed previously, the perception of sweetness, acidity and flavour in kiwifruit is very important in consumer acceptance of kiwifruit. It is well established that sweetness and acidity interact together in solution and influence the perception of

individual tastants (Pangborn, 1960; Schifferstein and Fritjers, 1990; Bonnans and Noble, 1993; Stampanoni, 1993). It has been suggested that kiwifruit odour compounds and tastants interact together to influence overall flavour perception (McMath *et al.*, 1991b). Due to the major contribution of sweetness, acidity and odour to kiwifruit acceptability, the psychophysics of tastants and odour compounds in kiwifruit is of considerable interest.

In general, the presence of acid suppresses sweetness perception while the presence of sugar suppresses acidity perception (Pangborn, 1960; Schifferstein and Fritjers, 1990; Bonnans and Noble, 1993 and Stampanoni, 1993). McBride and Johnson, (1987) demonstrated suppression of sweetness by acids and some suppression of acidity by sweeteners using a lemon juice drink. The use of a kiwifruit pulp to evaluate sweetness and acid perception in kiwifruit will enable conclusions to be drawn with regard to sugar and acid interactions in kiwifruit in the absence of confounding factors such as fruit to fruit variability.

Studies have confirmed that sweetness intensity increases as sugar concentration increases (Moskowitz, 1970a; Moskowitz, 1970b). In an experiment to assess the relationship between sweetness, pleasantness and concentration of 43 different sugars, Moskowitz, (1971) reported that pleasantness of sweetness does not increase endlessly with increasing sugar concentration. Rather, the perceived pleasantness of sweetness decreases at a certain concentration specific for each sugar and eventually becomes unpleasant. The pleasantness of the sweetness resulting from glucose, fructose and sucrose decreased at 1.0 mol/L, 0.3 mol/L and 1.0 mol/L respectively. In a study involving whole, fresh fruit of which Brix level ranged from 9.0 - 19.5°, Pringle *et al.*, (1991) found that the most acceptable fruit were those at the upper quartile of this range, though the fruit with the highest Brix level within this range were not the most acceptable. It is interesting that Pringle *et al.*, (1991) comment on kiwifruit acceptability, as these researchers made no mention of any form of sensory evaluation being carried out on their kiwifruit.

In a study using fresh, whole kiwifruit, MacRae *et al.*, (1990) found a lack of correlation between perceived sweetness intensity and measured sugar content, and

suggested that sweetness perception may be enhanced by the presence of esters. McMath *et al.*, (1991b) have also suggested that perceived sweetness intensity of kiwifruit is associated with high ester levels. Comparing the ratings of sweetness intensity in pulp with low esters and pulp with high esters will establish the nature of the relationship between perceived sweetness intensity and esters in a homogenous kiwifruit pulp model system.

Selected odour compounds are known to have causative effects on acid perception. Gilbert *et al.*, (1996) demonstrated that increasing levels of E-2-hexenal in an aqueous kiwifruit model system increased the perceived intensity of acidity. The esters, methyl propanoate and methyl butanoate have been noted by McMath *et al.*, (1991b) as increasing acid perception in kiwifruit.

1.6 Factors affecting kiwifruit flavour acceptance

There are several discrepancies in previous research into kiwifruit flavour and acceptability. Two factors which are largely responsible for these discrepancies are; the variability of the fruit during the ripening process, and the effect of confounding factors introduced through the use of whole, fresh fruit, such as between and within fruit variation. These factors are discussed in detail below.

1.6.1 Whole fruit versus a model system

While previous studies of kiwifruit flavour have provided insights into consumer acceptance, (MacRae *et al.*, 1990; McMath *et al.*, 1991b; Paterson *et al.*, 1991; Gilbert *et al.*, 1996) these experiments have been subject to confounding influences due to their use of whole fruit or, have relied on unrealistic model systems to examine acceptance of kiwifruit. MacRae *et al.*, (1990) and Paterson *et al.*, (1991) examined whole, fresh kiwifruit in an attempt to correlate physicochemical attributes of the fruit with taste and odour attributes respectively. MacRae *et al.*, (1990) found no correlation between soluble solids, sugar content, sugar:acid ratios and consumer sweetness perception. Paterson *et al.*, (1991) were unable to establish strong relationships between the odour compound profile of fruit harvested at different

maturities, receiving a variety of storage regimes, and consumer acceptability. In both of these studies, the use of whole fruit prevented the experimenters from controlling differences between individual fruit. To overcome the confounding influences introduced by the use of whole, fresh fruit, Gilbert *et al.*, (1996) used an aqueous model solution system. The model system was a solution of sugars (fructose, glucose and sucrose) and acids (citric, malic and quinic) at levels which approximated those found in a kiwifruit at 'eating ripeness' harvested at the recommended harvest maturity. Use of a model system allowed levels of volatiles identified as contributing to kiwifruit flavour by Young *et al.*, (1983) to be assessed for their effects on flavour intensity and acceptability. While this study provided valuable insights into kiwifruit flavour in a model system, it did not provide information on key odour compounds in actual kiwifruit because it was an aqueous model system.

1.6.2 Physicochemical composition at consumption

The study of kiwifruit flavour is further complicated by the changing physiochemical composition of the fruit between harvest and consumption (MacRae *et al.*, 1989b). These changes alter the flavour profile of kiwifruit (Paterson *et al.*, 1991). Factors influencing the degree of physicochemical change which occurs include, the maturity of fruit at harvest and the conditions (temperature, atmosphere and packaging) under which the fruit is stored (Stec *et al.*, 1989). Furthermore, the softness of the fruit at the time of consumption also influences flavour due to the increasing ester component as the fruit softens (Paterson *et al.*, 1991). With increasing ripeness, there is a decrease in aldehydes resulting in a loss of the 'green' flavour character associated with aldehydes, and a dramatic increase in esters which impart 'fruity' notes in the fruit (Young and Paterson, 1985). Bartley and Schwede, (1989) identified that ethyl butanoate was almost solely responsible for this dramatic rise in ester concentration.

As ripening is associated with an increase in Brix level and volatile ester concentration, ripe, soft fruit are perceived to be considerably sweeter and more intensely flavoured than firm fruit (Esti *et al.*, 1998). The relationship between fruit firmness and flavour acceptability has been examined in kiwifruit (Stec *et al.*, 1989). A significant relationship between fruit firmness and overall fruit acceptability was found, with soft fruit (0.45 - 0.65 kgf) being more acceptable than firm (0.6 - 0.8 kgf) or hard (0.8 - 1.0 kgf) fruit. In a study involving sensory evaluation of a range of

kiwifruit varieties by a trained panel, Cotter *et al.*, (1991) found that fruit firmness and its effects on overall acceptability was a matter of personal preference. In this study, Cotter *et al.*, (1991) used a trained panel to rate the 'overall acceptability' of a range of different kiwifruit varieties. The practice of using a trained panel to assign hedonic ratings should be avoided (Lawless and Heymann, 1998) as once a panel has been trained, individuals are no longer representative of consumers in the marketplace (Meilgaard *et al.*, 1991). Consumer response to the adulterated kiwifruit pulp in the proposed study will be averaged to generate mean scores, thus allowing no differentiation between groups of consumers preferring either soft or firm fruit.

1.7 Proposed Study

The proposed study has three main objectives;

1. To develop a kiwifruit pulp model system that best mimics the experience of eating a whole, fresh kiwifruit.

A model system will eliminate the effects of the natural variation that exists between fruit and within fruit that have been confounding factors in previous research carried out by MacRae *et al.*, (1990); McMath *et al.*, (1991b) and Paterson *et al.*, (1991) using whole, fresh kiwifruit. A kiwifruit pulp based model system will provide a more realistic substrate than the aqueous kiwifruit model solutions used by Gilbert *et al.*, (1996).

2. To investigate the interaction between sugars and acid in a kiwifruit pulp model system with low ester levels and to determine the effect of these tastants on consumer acceptability of kiwifruit pulp.

To achieve this objective, varying amounts of a sugar stock solution will be incorporated into the kiwifruit pulp model system to attain kiwifruit pulp treatments with Brix levels ranging from 11 to 16°. Within each of these Brix levels, three

distinct levels of acidity will be achieved through the addition of an acid stock solution.

3. To identify the effect of ester concentration on consumer acceptability of kiwifruit pulp with varying sugar:acid ratios.

This objective will be accomplished through the addition of an odour compound stock solution to a portion of the base pulp to achieve a separate kiwifruit pulp model system with ester levels typical of kiwifruit harvested at the recommended harvest maturity of 6.2° Brix. Consumer response to this kiwifruit pulp model system will be compared and contrasted to consumer response to the kiwifruit pulp model system with low ester levels.

A New Zealand consumer panel will evaluate the adulterated kiwifruit pulp for 'overall liking', 'sweetness liking', 'acid liking' and perception of flavour, sweetness and acid intensity so that the effect of sugars, acid and esters on consumer acceptance of kiwifruit can be investigated and the interactions between these tastants and odour compounds can be identified.

CHAPTER TWO

Frequently Used Methods

The instrumental and chemical methods described in this chapter are techniques used on more than one occasion in this thesis. To be concise, they are included as a separate chapter.

2.1 Assessment of Titratable Acidity

The TA was assessed by diluting 10 g of kiwifruit juice to 100 mL with 'Microlene™' filtered water. The diluted juice was titrated against a 0.1 N solution of sodium hydroxide, using 0.3 mL of phenylphthalein indicator. The dilute juice was titrated to a pink colour that persisted for 30 secs (AOAC, 1996a). Results are given as citric acid equivalent as citric acid is the most abundant organic acid present in kiwifruit (MacRae *et al.*, 1990). Titrations were carried out in triplicate.

2.2 Assessment of Brix level

The Brix level of kiwifruit was assessed using an 'Atago™' digital refractometer. The refractometer was first adjusted to zero with distilled water and the surface of the well thoroughly dried with a clean tissue. Juice was collected from both the stem and blossom ends of the fruit after removal of 1.5 cm of kiwifruit tissue. The juice was collected into the well of the digital refractometer and the Brix level was determined (MAF publication, 1980).

2.3 Assessment of Flesh Firmness

Flesh firmness was determined using a hand held 'Effegi' penetrometer fitted with a 7.9 mm plunger (Watkins and Harman, 1981). Two firmness measurements were carried out on each fruit. Assessments were carried out at the fruit equator, 90° apart after the removal of 2.0 mm of skin. The fruit was placed on a hard, stationary surface and held firmly. The tip of the plunger was pushed into the fruit at a constant speed until the inscribed line was reached.

2.4 Microbiological Examination

Aerobic plate count (APC) was determined using the AOAC, (1996b) methods. Fruit pulp was sampled immediately after pulping (T_0) and after 24 hrs of storage at 4° C (T_{24}). At both sampling times, (T_0 and T_{24}) an APC was carried out to quantify micro organisms and establish pulp safety for consumer sensory testing.

10 g of pulp was weighed into a sterile stomacher bag and 90 mL of 0.1 % peptone was added. This is a 1:10 dilution. The sample was macerated by hand from the outside of the bag for 2 min. Petrie dishes were labelled 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}. 1 mL of the 1:10 dilution was added to the petrie dish labelled 10^{-1}. Further serial dilutions were made by combining 9 mL of sterile 0.1 % buffered peptone and 1 mL of the initial 1:10 dilution of sample, resulting in a 1:100 dilution. 1 mL of the 1:100 dilution was added to the petrie dish labelled 10^{-2}, and 1 mL of the 1:100 dilution was added to 9 mL of sterile 0.1 % buffered peptone, resulting in a 1:1000 dilution. The same serial dilution process was continued until the sample was a 10^{-6} dilution. Approximately 15 mL of standard methods agar that had been tempered to 45° C was poured onto the petrie dishes. Dishes were gently swirled to distribute bacteria and allowed to set. Petrie dishes were inverted and incubated at 30° C for 72 hrs. Colonies on the plates were counted and expressed as Colony Forming Units (CFU)/g.

2.5 Gas Chromatography Analysis

2.5.1 Analysis of odour compounds

Instrumental assessment of the odour compounds associated with the kiwifruit pulp was conducted, using Gas Chromatography with Flame Ionisation Detection (GC-FID). Pulp (1.0 g) was weighed into 50 mL Quickfit (Jobling, England) test tubes fitted with gas inlet and outlet lines. The headspace was flushed with air at 20 mL/min for 20 min at 20° C into stainless steel cartridges packed with 100 mg of Chromosorb '105'. The collected volatiles were analysed by gas chromatography (Young and Paterson, 1985) using a ZBWax (Phenomenix, Torrane, CA, USA) capillary column (30 m x 0.32 mm). The temperature program used was: 30° C, held for 6 min, 3° C/min to 102° C, 5° C/min to 190° C, hold 10 min. The carrier gas was H_2 at 30 cm/sec. For quantitation purposes, an average response factor for the flame ionisation detector was calculated using ethyl butanoate, methyl butanoate, hexanal, butanol, pent-1-en-3-ol, methyl hexanoate, E-hex-2-enal, ethyl hexanoate, hexanol, Z-hex-3-enol, E-hex-2-enol, methyl benzoate and ethyl benzoate. Testing of the kiwifruit pulp was carried out in triplicate on each day of sensory testing.

2.5.2 Analysis of sugars and acids

Fresh kiwifruit pulp (1 g) was weighed into 15 mL of chilled MeOH:$CHCl_3$:H_2O (12:5:3 v/v) (MCW) and stored overnight at -15° C. The supernatant was transferred to a 50 mL test tube. The residue was crushed with a glass rod with a further 10 mL of MCW. The MCW layer was decanted and combined with the original extract. Chloroform ($CHCl_3$) (7 mL) and reverse osmosis (RO) purified water (10 mL) were added to the combined MCW extract and held at 4° C overnight. The MCW extract had separated into an aqueous and an organic phase. The lower (organic) layer was removed with a pasteur pipette and discarded. The aqueous layer was transferred to a tared flask and taken to dryness on a rotary evaporator (40° C, 1.33 - 2.66 kPa). The dried sample was made up to 10 g with RO water, 5 mL transferred to a plastic vial and kept at -15° C until required. A 2 mL portion of this solution was passed down Sephadex™ SP25-H^+ and QAE25-formate ion exchange columns in series according to the method of Bieleski, (1994) in order to separate the acids and sugars. Each

fraction was made up to 8 mL with water and kept frozen until required for GC analysis.

An aliquot of the sugar fraction (30 μL) and aribitol (20 μL) (internal standard) was transferred into a 300 μL insert contained in an auto-sampler vial and freeze dried. N-trimethylsilyl imidazole (1.5 mEq/mL) in pyridine (100 μL) was added to the freeze dried residue. The vial was capped and heated to 70° C for 30 mins.

An aliquot of the acid fraction (30 µL) and tartaric acid (20 μL) (internal standard) was transferred into a 300 µL insert contained in an auto-sampler vial and freeze dried. N-Methyl-N-(trimethylsilyl) tri-fluoroacetamide (MFSTA) (100 µL) was added to the residue. The vial was capped and heated to 70° C for 30 mins.

GC conditions for sugar analysis were: J & W Scientific DB 1701 (15 M x 0.32 mm i.d.); carrier gas was H_2 at 40 cm/s; injector temperature - 250° C, column temperature - 170° C, hold 2 min, 1° C/min to 183° C, 15° C/min to 250° C, hold 12.5 min.

GC conditions for acid analysis were: J & W Scientific DB 1701 (15 M x 0.32 mm i.d.); carrier gas was H_2 at 40 cm/s; injector temperature - 250° C, column temperature - 150° C, hold 1 min, 3° C/min to 195° C, hold 12.5 min.

CHAPTER THREE

The development of a model system for determining tastant relationships in kiwifruit.

3.1 INTRODUCTION

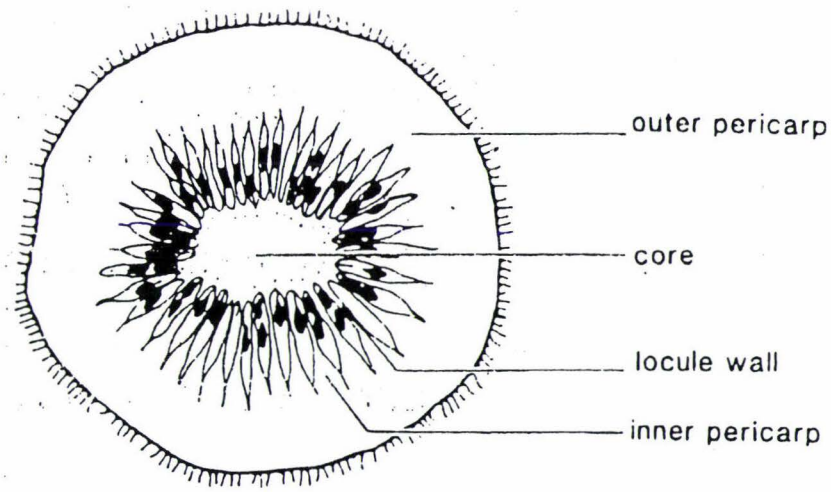
Flavour is a complex attribute made up of odour, taste and chemical feeling factors (Lawless and Heymann, 1998). The characteristic flavour of kiwifruit is the product of the interaction between odour compounds, sugars and acids. During fruit ripening, acid levels decrease (Matsumoto *et al.,* 1983), sugar levels increase (MacRae, 1988) and a concomitant increase in odour compounds is observed (Bartley and Schwede, 1989). With progressing ripeness, there is a dramatic increase in esters, resulting in the characteristic 'fruity' flavour notes prevalent in a kiwifruit at 'eating ripeness' (Young and Paterson, 1985).

In a study using fresh, whole kiwifruit, harvested at different maturities and ripened either immediately after harvest or after six weeks storage at 0° C, Paterson *et al.,* (1991) investigated the contribution of odour compounds, sugars and acids to the sensory attributes of kiwifruit. In this study, the use of whole, fresh fruit allowed no control over differences between individual fruit. To overcome the confounding influences introduced by the use of whole, fresh fruit, Gilbert *et al.,* (1996) experimented with an aqueous model solution system. Use of model solutions enabled Gilbert *et al.,* (1996) to explore perception and acceptability of selected kiwifruit odour compounds in a controlled system. While this study has provided a valuable insight into kiwifruit flavour, in reality, it provided information on key odour compounds in solution and not in a kiwifruit.

Most fruit are comprised of an exterior skin and an edible inner portion e.g. bananas, stonefruit and apples. A typical kiwifruit is ovoid in shape, weighs approximately 100 g, and has a tough, brown hairy exterior with a luminous green, melting flesh interior when ripe. Kiwifruit differ from other fruit in that the edible portion is not

homogenous but is made up of three different parts (Figure 3.1), all three are very different visually and chemically.

Figure 3.1. Cross section of a kiwifruit



MacRae, (1988)

Each region is considered to have significantly different taste qualities. Differences in taste perception are the result of differences in the sugar, acid and starch composition in each region (MacRae *et al.,* 1989a). The core region has a higher sugar:acid ratio (1.93) than the outer pericarp (1.14) and the inner pericarp (0.86) (MacRae, 1988). Thus the core region would be perceived as sweeter than the other regions and the inner pericarp would be perceived as the most acidic region. It follows then, that the amount of each type of tissue within an individual fruit will influence the overall eating experience of that fruit.

The aim of this work was to create a kiwifruit pulp model system that best mimics the experience of eating a whole, fresh kiwifruit to explore tastant relationships in kiwifruit. A model pulp system provides a homogenous product where natural variation between fruit and within fruit is eliminated. The additional advantage of a pulp system is that sugar, acid and odour compound composition can be easily manipulated through the incorporation of known stock solutions.

3.2 METHODS AND MATERIALS

3.2.1 Fruit

The kiwifruit used in this study were obtained from a local supermarket. Limited fruit history was available. It is assumed, based on MAF regulations (MAF publication, 1980) that the fruit were harvested at 6.2° Brix, and stored until transported to the retail outlet. As this is a preliminary investigation into the feasibility of using kiwifruit pulp as a model system, the history of the fruit is not important. Once it has been established that kiwifruit pulp is appropriate for use as a model system, future work will involve fruit that has been selected and controlled.

3.2.2 Preparation of the fruit for pulping

The pulp was prepared by peeling 20 kg (approximately 200 fruit) of kiwifruit using a small, sharp kitchen knife, to a depth of approximately 2 mm. The peeled fruit, including the core and seed region were chopped into slices 1 cm thick and placed in a 'Breville™' domestic food processor with a bowl capacity of 2 L and fitted with a metal chopping blade. On each day of consumer testing, pulping was carried out in 1kg batches which were subsequently combined and mixed thoroughly. Pulp (1 kg) was weighed into 12 separate, 2 L glass beakers. Each beaker was covered with plastic wrap, and stored at 4° C for up to nine hrs. Pulp not used within the nine hrs was discarded.

3.2.3 Establishment of processing variables for pulp

In order to mimic the experience of eating a whole, fresh kiwifruit it was important to ensure that there were as many intact cells present in the pulp as possible. The effect of processing time on the pulp was examined to determine the most appropriate length of processing so that a minimum number of cells were ruptured, and the product was homogenous. Pulp from a 1 kg batch was examined under a light microscope after 30, 60 and 90 secs processing on high power. After processing the kiwifruit for 30, 60 or 90 secs, 1 mL of the processed pulp was placed into a micro tube and diluted 1:1 with a 0.6 molar solution of sucrose to maintain cellular osmolarity. The mixture was inverted to ensure even mixing and a drop placed on a glass slide and covered

with a cover slip. The samples were viewed under a light microscope at x31.25 magnification. Trials were carried out in triplicate for each processing time.

3.2.4 Establishment of pulp stability for consumer testing

To ensure pulp stability over the period of consumer testing, pulp was examined for APC and colour stability.

3.2.4.1 APC for microbiological stability

As the pulp was consumed by a large group of people, it was critical that the pulp was free from harmful micro-organisms for the duration of the testing day and would not cause food borne illness. An APC is the method used to quantify micro-organisms in products. APC was determined using the AOAC, (1996b) method. Pulp was sampled immediately after pulping (T_0) and after 24 hrs (T_{24}) of storage at 4° C following the methods outlined in section 2.4.

3.2.4.2 L*a*b* recordings of colour stability

Colour measurements were conducted to ensure that the colour of the samples did not change between the consumer testing sessions. From a fresh batch of pulp, 100 g was measured into three separate beakers and was stored at 4° C for nine hrs. Using a 'Minolta™' colorimeter, L*a*b* colour values of the pulp were determined at the time of pulp preparation (T_0) and three, six and nine hrs after pulping, T_3, T_6, and T_9 respectively. The spectral sensor of the 'Minolta™' colourimeter was held on the surface of the pulp and L*a*b* colour values were measured. Readings were taken on triplicate samples from each sampling time. The 'Minolta™' registered five readings at each measurement and calculated the mean value for each of the L*a*b* colour measurements. Results were analysed by ANOVA ('Minitab 12.1©', 1998) to determine if a significant difference existed between the L*a*b* values over the storage period.

3.2.5 The incorporation of sugar stock solution into the pulp

The sugar stock solution used in this experiment consisted of a mixture of glucose (509.1 g/L), fructose (455.7 g/L) and sucrose (35.6 g/L) dissolved in 'Microlene™' filtered water at the ratio of 14.3:12.8:1.0 of glucose, fructose and sucrose. The composition of the sugar stock solution was based on the sugar ratio naturally occurring in eating ripe (0.7 kgf) kiwifruit reported by MacRae *et al.,* (1989b). Preliminary laboratory work was carried out to determine levels of sugar stock solution to be added to pulp to achieve the desired Brix levels (Appendix 1).

3.2.6 The incorporation of acid stock solution to the pulp

To establish a method for altering acid levels in the kiwifruit pulp, 7.5 molar solutions of both citric and malic acid were prepared by dissolving 144 g and 100 g of citric acid monohydrate and D-L-malic acid respectively in 100 mL of 'Microlene™' filtered water. Preliminary laboratory work, trialed the incorporation of each of these acid stock solutions into the pulp to achieve three levels of acidity (Appendix 2).

3.2.7 Differences in viscosity between the pulp treatment with the greatest amount of sugar stock solution added and the treatment with the least amount added

To ensure that panellists would not detect any differences in viscosity among treatments as a result of the addition of the sugar stock solution, a benchtop triangle test was conducted. Water, in volumes equivalent to the amount of sugar stock solution in the high and low Brix treatments, was added to 100 g of kiwifruit pulp. Three 30 mL samples of pulp were presented to a total of 15 panellists simultaneously. Two of the samples were taken from one of the pulp treatments, and were therefore identical. The other sample was taken from the other of the pulp treatments. The samples were coded with a three-digit random number and were presented in lidded, plastic crème cups. Panellists were asked to identify the odd sample by circling the corresponding code on a paper ballot. The total number of correct responses were collated and compared to 'The table of minimum number of correct assessments to establish significant differentiation' (Poste *et al.,* 1991) to establish if a significant difference existed between the samples.

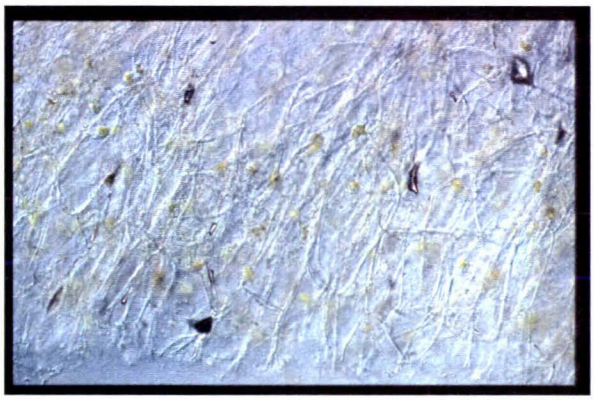
3.3 RESULTS

3.3.1 Establishment of processing variables for pulp

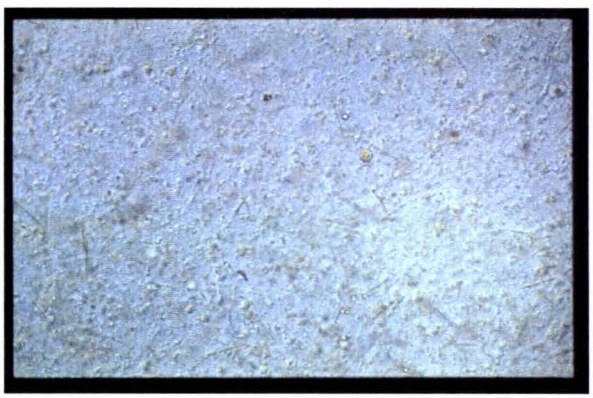
After processing the kiwifruit for 30 secs, the majority of the fruit cells remained intact (Figure 3.2a). Processing the fruit for 60 secs ruptured considerably more fruit cells (Figure 3.2b). Kiwifruit pulp that was processed for 90 secs had extensively ruptured cells (Figure 3.2c).

Figure 3.2. The effect of processing time on kiwifruit pulp cellular integrity as viewed under a light microscope (x 31.25) after a) 30, b) 60 and c) 90 secs of processing.

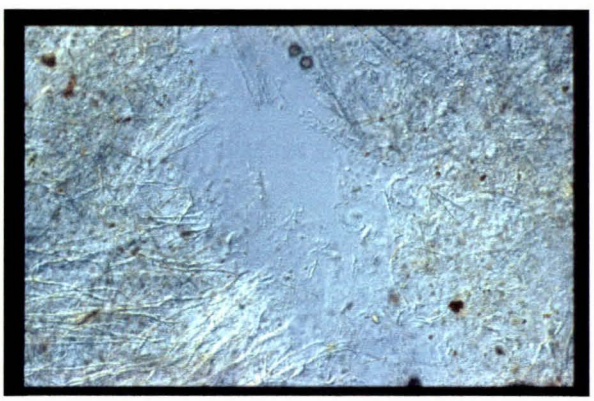
a)



b)



c)

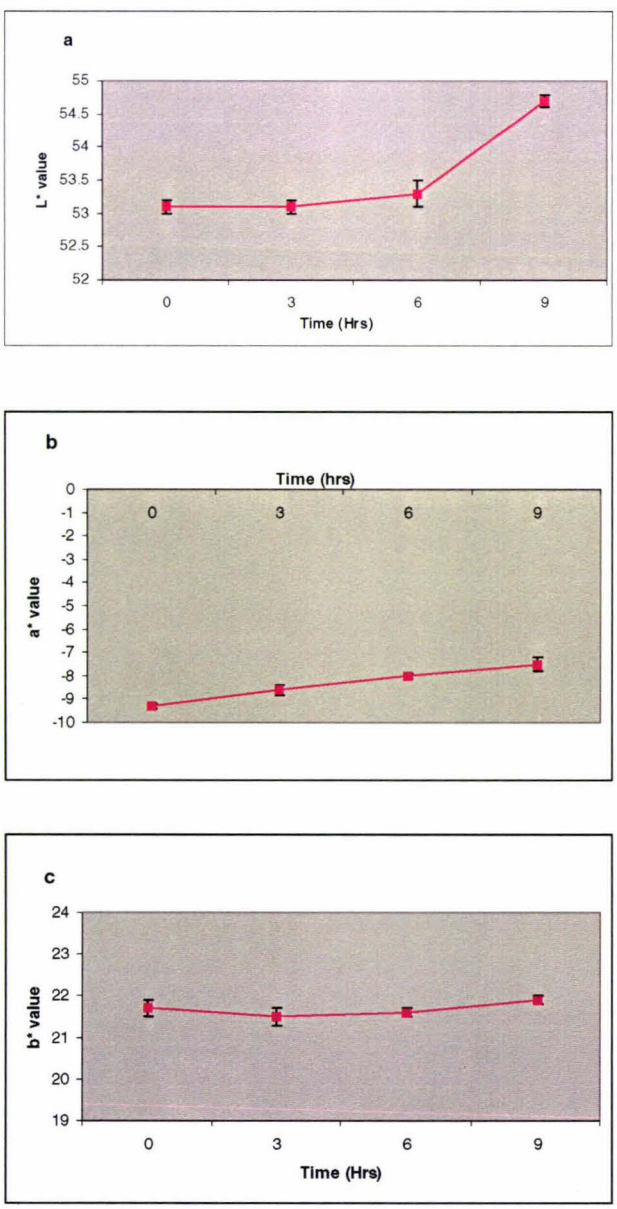


3.3.2 Establishment of pulp stability for consumer testing

Results from the APC of pulp at T_0 and T_{24} found an average of 720 CFU/g present in kiwifruit pulp stored at 4° C for 24 hr (T_{24}) and an average of 1935 CFU/g in freshly pulped samples (T_0)

L*a*b* colour values of the pulp were recorded over a nine hr period. After nine hrs of storage at 4° C, significant colour changes were recorded in kiwifruit pulp for L* and a* values, no significant differences were noted for b* values over the storage period (Figure 3.3).

Figure 3.3. L*a*b* colour values of kiwifruit pulp stored at 4° C over a nine hour time period. a) L* value b) a* value c) b* value.



In the L*a*b* colour space, L* indicates lightness, and a* and b* indicate colour directions. A significant increase ($F_{(3,8)} = 145.7$, p value = <0.001) in L* value was observed over the storage period, meaning that the pulp became lighter. There was also a significant increase ($F_{(3,8)} = 55.7$, p value = <0.001) in a* value over the storage period towards a less negative value, therefore the pulp became less green. There was no significant change in b* value over the storage period.

3.3.3 The incorporation of sugar stock solution to the pulp

In general, with the addition of 2 mL of the sugar stock solution to 100 g of pulp, there was an approximate increase in the Brix level by 1° (Appendix 1, Table A1.2). This occurred regardless of the amount of sugar stock solution already present in the pulp. This will be used as a guide to achieving desired Brix levels within the pulp in future research.

3.3.4 The incorporation of acid stock solution to the pulp

The addition of a citric acid stock solution into the pulp did not provide ideal results in terms of achieving a model system where sugar and acid relationships could be investigated (Appendix 2). Through the incorporation of malic acid stock solution into the pulp, three distinct levels of acidity were produced. The base pulp was selected as the low acid treatment, addition of 3 mL and 5 mL of malic acid stock solution gave rise to the medium and high acid treatments respectively. Constant viscosities were maintained across all treatments with the addition of water to ensure each treatment received equivalent volumes of fluid

3.3.5 Difference in viscosity between the pulp treatment with the greatest amount of sugar stock solution added and the treatment with the least amount added.

The benchtop triangle test was conducted to determine if there was a significant difference in the viscosity of the pulp as a result of the sugar stock solution addition. Results of the triangle test showed that seven out of the 15 panellists correctly identified the odd sample. A comparison of these results to table values shows that no significant difference ($p > 0.05$) exists between the sample representative of the highest Brix level and the sample representative of the lowest Brix level. As there

was no perceived differences between the two samples, it was assumed that the viscosity of the two samples was the same, and will be assumed to be the same in future research.

3.4 DISCUSSION

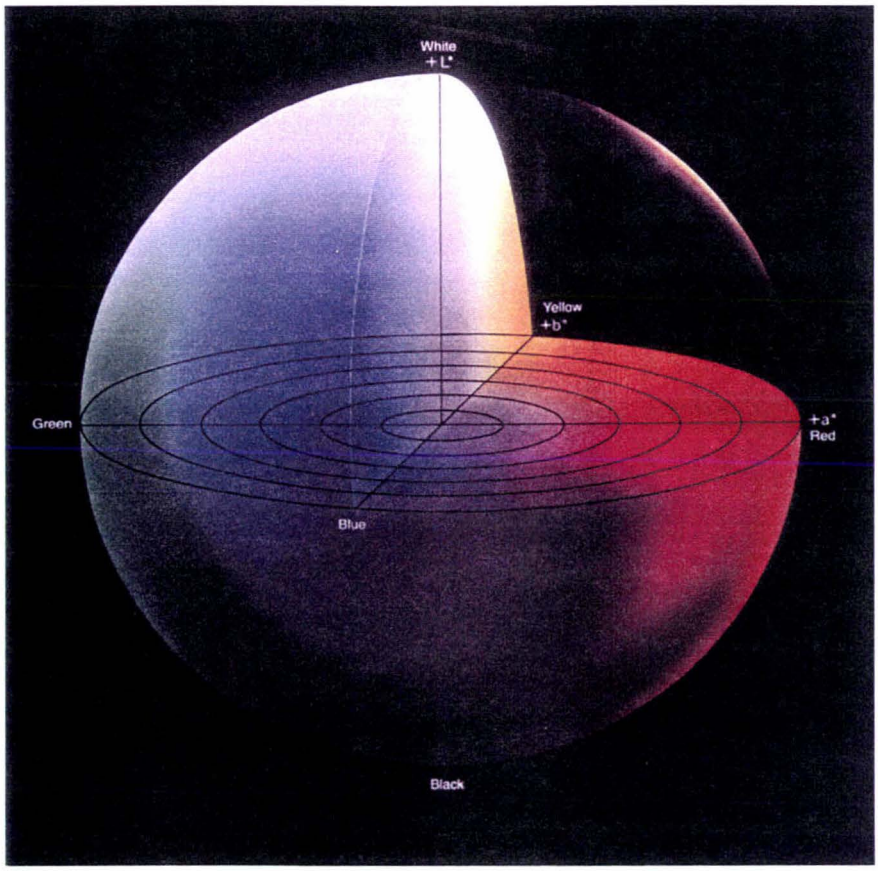
A model kiwifruit pulp system was required that would best mimic the experience of consuming a kiwifruit at 'eating ripeness'. An important aspect of the kiwifruit eating experience is the sensation of individual cells rupturing in the mouth during mastication. The challenge in developing a kiwifruit model system was to produce a pulp with a maximal number of intact cells so that the experience of eating a whole, fresh kiwifruit would be simulated as closely as possible, whilst ensuring a sufficiently uniform product with respect to taste and texture. The vacuole is the single largest component of a fruit cell (John and Yamaki, 1994). During mastication the vacuolar membrane, the tonoplast, is ruptured and vacuolar contents are released into the mouth. The vacuole is the storage site of organic acids (Canel *et al.,* 1995), and the release of organic acids as the vacuole ruptures might be expected to influence the perception of acidity during mastication. The aim was to mimic the experience of eating a whole, fresh kiwifruit where intact fruit cells were introduced into the oral cavity. To ensure that cell damage was minimal, processing time trials were carried out, and the resulting pulp was examined under a light microscope. After assessing the pulp under a light microscope, a processing time of 30 secs was selected for the experimental pulp. After this length of processing, the pulped product was homogenous with a minimum number of cells ruptured.

Vacuoles rupture gradually during mastication, and therefore the release of organic acids into the mouth during mastication is a gradual process. A major difference between the fresh, whole kiwifruit eating experience and the experience of consuming kiwifruit pulp, is that the pulp has been adulterated with acid. Therefore the consumer instantly experiences the perception of the total acid of the fruit, whereas as a consequence of the gradual rupture of vacuoles during mastication of a whole, fresh fruit, the consumer experiences a prolonged, lower intensity perception of acid. A time-intensity study comparing acid perception in whole, fresh kiwifruit to acid perception in an adulterated kiwifruit pulp model system would be a further step towards understanding acid perception in kiwifruit and is recommended for future research.

Consumer evaluations were carried out over an entire day. Pulp stability therefore became critical, especially with regard to panellist safety. According to the Ministry of Health's microbiological standards for food, the APC of 'ready-to-eat' fruit must not exceed 10^6 CFU/g (Ministry of Health, 1994). The APC of kiwifruit pulp was well below these limits, therefore the pulp was safe and acceptable for consumption for up to 24 hrs of storage at 4° C. Fresh pulp was produced in batches on each day of consumer testing, and was discarded after nine hrs of storage at 4° C.

Another stability factor of concern was pulp colour. To ensure that colour did not influence consumer response to the adulterated pulp, it was paramount that the colour of the pulp was consistent throughout the entire day of consumer testing. The colour stability of the pulp was established by monitoring colour using co-ordinates on the L*a*b* colour space (Weatherall, 1989). In the L*a*b* colour space, colour may be considered as existing at a unique point in a three dimensional colour space with axes of L*, a* and b* (Figure 3.4).

Figure 3.4. L*a*b* colour solid



(Minolta, 1994)

Figure 3.4 shows that +a* is the red direction, -a* is the green direction, +b* is the yellow direction, –b* is the blue direction and L* measures the perception of lightness. A shift in either L*, a* or b* values represents a shift in colour towards the direction that each value represents. The increase in the L* value of the pulp over the storage time represents a gradual increase in lightness. The increase in the a* value represents a gradual shift to a less intense green colour. Chlorophyll is responsible for the green colour in kiwifruit and has been described by Coultate, (1993). The structure of chlorophyll is based on a series of porphyrin rings, linked to a Mg^{2+} ion. At low pH, the Mg^{2+} ion is displaced from the porphyrin ring structure and a phaeophytin is formed. Phaeophytins are dirty brown in colour. The processing of fresh kiwifruit into a pulp has ruptured a number of vacuoles causing the release of organic acids into the pulped product. The acidic pulp has led to chlorophyll degradation. The b* value remained constant at approximately 21 at each time interval. Translating this value onto the colour space showed that the pulp remained

constant in the yellow colour range. It was not determined whether a panel could register these colour changes, therefore it was decided to conduct the consumer evaluations of the pulp under red light. Colour differences can be masked by coloured light (Jellinek, 1985).

An important feature of the pulp was the need to be able to manipulate sugar and acid levels. Gilbert *et al.*, (1996) successfully incorporated odour compounds into a sugar and acid model base solution. A pulp model system presented a few more challenges than a simple aqueous solution system as sugars and acids are naturally occuring at all stages of ripening in kiwifruit tissue. The aim was to modify the levels naturally present. The concentrations of sugars and acids naturally present in the pulp were increased through the incorporation of sugar and acid stock solutions into the pulp. A sugar stock solution was made based on the composition of sugars naturally occurring in 'eating ripe' kiwifruit reported by MacRae *et al.*, (1989b). Extensive laboratory work trialed the incorporation of the sugar stock solution into the pulp at varying amounts to increase the Brix level of the pulp in increments of 1°. It was found that adding 2.0 mL of sugar stock solution to 100 g of pulp raised the Brix level of the pulp by approximately 1°. A benchtop triangle test determined that consumers could not detect a difference between pulp with a viscosity representative of the highest Brix level treatment and pulp with a viscosity representative of the lowest Brix level treatment in the study.

Malic and citric acids were trialed to determine which acid would be more appropriate to incorporate into the pulp to achieve three distinct levels of acidity. Citric acid is six times more abundant in 'eating ripe' kiwifruit than malic acid (MacRae *et al.*, 1989b). However, Amerine *et al.*, (1965) have shown that malic acid has a lower recognition threshold (0.0016N) than citric acid (0.0023N). Taking into account the relative sensory thresholds of the two organic acids, and considering the quantities of each naturally present in 'eating ripe' kiwifruit, it is clear that citric acid contributes more to the perceived sourness of kiwifruit than malic acid. However, due to the higher threshold of citric acid, in order to get a sensory effect greater quantities of citric acid stock solution was required that the pulp became very watery. A slight salty taste was also observed when citric acid was incorporated into the pulp treatments. For these

reasons it was not practical to use citric acid, instead malic acid was used to adjust sourness perception. Three distinct levels of acidity were produced in the pulp. The base pulp was selected as the low acid treatment, addition of 3 mL and 5 mL of malic acid stock solution gave rise to the medium and high acid treatments respectively. Constant viscosities were maintained across all treatments with the addition of water to ensure each treatment received equivalent volumes of fluid.

3.5 CONCLUSION

A kiwifruit pulp model system has been developed that best mimics the experience of eating a fresh, whole kiwifruit. A processing method was selected that produced a homogenous product, yet allowed as many whole fruit cells as possible to enter the mouth intact for mastication. The kiwifruit pulp model system was microbiologically safe for the duration of the consumer testing day. A sugar stock solution based on the levels occurring naturally in 'eating ripe' kiwifruit, was incorporated into the pulp to manipulate Brix level of the model system. Acidity was successfully altered through the addition of a malic acid stock solution.

CHAPTER FOUR

An investigation into the interaction between sugars and acids and their effect on consumer acceptance of kiwifruit pulp

The substantial contribution of this chapter is published in Journal of Sensory Studies, Rossiter et al., (in press).

4.1 INTRODUCTION

The kiwifruit industry uses a minimum harvest maturity standard of 6.2° Brix (Harman, 1981). Fruit harvested below this Brix level have low levels of ester odour compounds at 'eating ripeness' (Young and Paterson, 1985). Therefore an early harvest would ensure that when ripened, fruit would have low concentrations of esters.

Consumer acceptance of ripe kiwifruit is strongly influenced by sweetness and acid perception (McMath *et al.,* 1991b). The interrelationship between sweet and acid taste has previously been explored using model solutions (Schifferstein and Fritjers, 1990; Bonnans and Noble, 1993; Stampononi, 1993 and Cardello, 1996). Typically, the phenomenon of sweetness suppression by acids and acidity suppression by sweeteners has been demonstrated. Valdes *et al.,* (1956), using whole, fresh apricots, pears and peaches, demonstrated that increasing sweetness in fruit increased consumer liking. This effect of increased sweetness increasing consumer liking was demonstrated in kiwifruit by Jordan *et al.,* (1996) (unpublished). The objective of the current study is to explore the interaction between tastants (sweetness and acidity) and their effect on consumer acceptability by using a kiwifruit pulp model system to which tastants were added.

In this study, kiwifruit pulp was adulterated using a sugar stock solution to achieve Brix levels, ranging from 11 - 16°. Kiwifruit at 'eating ripeness' are characterised by a Brix level of between 13.0 – 14.5°, and a firmness within the range of 0.4 – 0.8 kgf

(Lallu *et al.*, 1989). The range of Brix in the current study was chosen to encompass 'eating ripe' fruit that have low Brix (i.e. 11 and 12°), typical Brix (i.e. 13 and 14°) and high Brix (i.e. 15 and 16°). Three distinct levels of acidity; (Low, Medium and High) were created in the pulp treatments through the incorporation of a malic acid stock solution. TA in kiwifruit ranges between 10.0 and 16.0 mg citric acid /g FW (Beever and Hopkirk, 1990). The acid levels created in the pulp were selected to fall within this range.

4.2 METHOD AND MATERIALS

4.2.1 Fruit

Kiwifruit, free from bruises and blemishes were harvested from 'Punchbowl Coolstores' orchard, South Auckland, New Zealand, on April 9th, 1998 and on April 13th, 1999. Fruit from both harvests, 1998 and 1999, were harvested at 5.0° Brix, stored for eight weeks at 0° C, and were then treated with ethylene to induce ripening (1000 ppm, 12 hrs, 20° C). Subsequent storage of fruit harvested in Year One (1998) was at 20° C for four days to 11° Brix. Year Two (1999) fruit were allowed to ripen further to 13° Brix and were ripened for five days at 20° C. During the storage period, fruit firmness, Brix level and titratable acidity of fruit from both years of the study were measured weekly on a sample of 20 fruit following the methods outlined in sections 2.1, 2.2 and 2.3. The same measurements were taken daily during the ripening period.

4.2.2 Sample Preparation

Pulp was made according to the method developed in section 3.2.2. On each day of consumer testing, fruit was processed for 30 secs on high power in a 'Breville™' food processor. Pulping was carried out in 1 kg batches, which were combined and mixed thoroughly.

To examine the effects of increasing sugar concentration on consumer acceptance attributes, the pulp was adulterated using a sugar stock solution comprising of glucose 509.1 g/L, fructose 455.7 g/L and sucrose 35.6 g/L dissolved in 'Microlene™' filtered water at the ratio of 14.3:12.8:1.0 of glucose, fructose and sucrose. Following the methods developed in section 3.2.5, sugar stock solution was added to 1 kg treatments of pulp to achieve final levels of 11, 12, 13 and 14° Brix in Year One of the study and 13, 14, 15 and 16° Brix in Year Two (Table 4.1). Brix was measured at room temperature on an 'Atago™' digital refractometer according to the method outlined in section 2.2.

Table 4.1. The volume of sugar and acid stock solutions added to kiwifruit pulp (1.0 kg) for consumer evaluation of tastant relationships in kiwifruit.

Acid	Brix					
	11	**12**	**13**	**14**	**15**	**16**
Low Year 1	0mL sugar[1] 0mL acid[2] 5mL water	15mL sugar 0mL acid 5mL water	37.5mL sugar 0mL acid 5mL water	52.5mL sugar 0mL acid 5mL water		
Medium Year 1	0mL sugar 3mL acid 2mL water	15mL sugar 3mL acid 2mL water	37.5mL sugar 3mL acid 2mL water	52.5mL sugar 3mL acid 2mL water		
High Year 1	0mL sugar 5mL acid 0mL water	15mL sugar 5mL acid 0mL water	37.5mL sugar 5mL acid 0mL water	52.5mL sugar 5mL acid 0mL water		
Low Year 2			0mL sugar 0mL acid 5mL water	22mL sugar 0mL acid 5mL water	43mL sugar 0mL acid 5mL water	62mL sugar 0mL acid 5mL water
Medium Year 2			0mL sugar 3mL acid 2mL water	22mL sugar 3mL acid 2mL water	43mL sugar 3mL acid 2mL water	62mL sugar 3mL acid 2mL water
High Year 2			0mL sugar 5mL acid 0mL water	22mL sugar 5mL acid 0mL water	43mL sugar 5mL acid 0mL water	62mL sugar 5mL acid 0mL water

[1] Sugar = Sugar stock solution: 14.3:12.8:1.0 of glucose, fructose and sucrose
[2] Acid = Acid stock solution: 1000.0 g/L malic acid

To investigate the effect of increasing acid level on consumer acceptance attributes of kiwifruit, the pulp was further adulterated using a 7.5 molar malic acid stock solution, prepared by dissolving 100 g of D-L-malic acid in 100 mL of 'Microlene™' filtered water. The malic acid stock solution was added to each of the Brix treatments, to achieve three distinct acid levels; Low, Medium and High (Table 4.1). Water was added to maintain constant volumes across all treatments. The TA of each acid level was measured following the method described in section 2.1. Due to the buffering effect of saliva, Nybom, (1963) demonstrated using apples that there was a better correlation between perceived sourness and TA than between perceived sourness and pH. Based on this observation, the acidity of the adulterated pulp was measured using TA rather than pH.

4.2.3 Instrumental analysis using GC of sugars, acids and odour compounds in unadulterated pulp

On each day of consumer testing prior to adulteration, a sample (10 g) of pulp was collected for odour compound analysis by GC-FID according to the methods outlined in section 2.5.1. An additional sample was taken and frozen with liquid nitrogen and stored at -15° C for subsequent analysis of sugars and acids by GC following the methods outlined in section 2.5.2. Unfortunately the sample for instrumental analysis on Day Two of consumer testing in Year Two (1999) was inadvertently lost and resampling was not possible.

4.2.4 Consumer evaluation of pulp adulterated with sugars and acids

The kiwifruit pulp was evaluated by a total of 120 New Zealand consumers. Consumers were selected according to their prior purchase, consumption and liking of fresh kiwifruit. Testing was carried out in eight sessions over two days.

Consumers were presented with 30 mL of the adulterated kiwifruit pulp treatments in lidded, plastic crème cups on a white tray. A white plastic teaspoon was provided as an eating utensil. Each of the 120 consumers was presented with four samples monadically (one sample presented at a time and all attributes rated for that sample). This resulted in 40 assessments of each sample. Consumers were required to assess

each sample for 'overall liking', 'sweetness liking' and 'acidity liking' and also their perceived intensity of 'overall flavour', 'sweetness' and 'acidity'. Intensities for all attributes were scored on 150 mm unstructured line scales (Meilgaard *et al.,* 1988). Descriptive anchors for the acceptability attributes were 'dislike very much' and 'like very much' at 0 mm and 150 mm respectively. Anchors for the intensity attributes were 'very weak' to 'very strong' at 10 mm and 140 mm respectively. Assessments were recorded on separate paper ballots. A copy of the questionnaire is located in Appendix 3.

Samples were evaluated in individual tasting booths at HortResearch's Sensory Science Facility at the Mt Albert Research Centre, Auckland, New Zealand. The sensory evaluation booth area was held at 20° C, red lighting was used to obscure any colour differences between samples and a positive airflow was maintained to remove any odours from the testing area. 'Microlene™' filtered water and plain water crackers were provided as palate cleansers.

Samples were allocated to panellists according to a split plot design that was completely balanced to minimise order and carry-over effects (Ball, 1997).

4.2.5 Data Analysis

The consumer data were analysed by Residual Maximum Likelihood (REML), using the GENSTAT Statistical Package. REML is a generalisation of analysis of variance that is suitable for use with moderately unbalanced designs. The REML analysis weights each response value in a manner that properly reflects estimates of the variance contributions of the different sources of variability e.g. between sessions, and between panellists within a session (Payne *et al.*, 1993). It also provides estimates of these variance contributions. Because the REML means are weighted means, they may differ slightly from simple arithmetic means of the data values. To compare statistical differences between means, Tukey's Least Significant Difference (LSD) values were calculated, at the 5 % level, from the standard errors of difference.

The instrumental data was analysed using a one-way Analysis of Variance (ANOVA) ('Minitab 12.1©', 1998).

4.3 RESULTS

4.3.1 Fruit

In Year One (1998), kiwifruit ripened for four days at 20° C had a mean flesh firmness of 0.7 kgf and were therefore considered to be 'eating ripe' (Lallu *et al.*, 1989). The average Brix level of the fruit was 11°. The fruit had a TA of 11.0 mg citric acid/g fresh weight. Year Two (1999) fruit were allowed to ripen further to a higher Brix level so that the relationships found in the first year of the study could be extended at higher Brix levels. After five days storage at 20° C, fruit had an average Brix of 13°, and a firmness of 0.7 kgf. The fruit had a TA of 11.8 mg citric acid/g fresh weight.

4.3.2 Instrumental analysis using GC of sugars, acids and odour compounds in unadulterated pulp

The major sugars in ripe kiwifruit are fructose, glucose and sucrose (Beever and Hopkirk, 1990). Fructose, glucose and sucrose were present in similar concentrations on each day of consumer testing (Table 4.2).

Table 4.2. The concentration of major sugars in unadulterated kiwifruit pulp obtained by GC analysis in Year One (1998) and Year Two (1999)

		Sugar (mg/g)		
		Fructose	**Glucose**	**Sucrose**
Year 1	**Day 1**	25.6+/- 0.8[1]	23.8 +/- 0.4	14.2 +/- 5.0
Year 1	**Day 2**	29.3 +/- 5.8	27.1 +/- 5.6	18.1 +/- 3.2
Year 2	**Day 1**	32.1 +/- 1.7	27.9 +/- 1.0	15.9 +/- 0.8
Year 2	**Day 2**[2]	-	-	-

[1]n = 3
[2]samples not tested

In Year One of the study, there was no significant difference in the concentrations of fructose, glucose and sucrose on each day of consumer testing. This result gives confidence that there was no day to day variability in sugar concentration over the two days of consumer testing. In Year Two, the concentrations of fructose and glucose were significantly higher ($F_{(1,4)}$ = 34.73, p value = 0.004 and $F_{(1,4)}$ = 44.94, p value = 0.003 respectively) on Day One of consumer testing than on Day One of consumer testing in Year One.

The major organic acids present in the kiwifruit pulp were malic, citric and quinic acids (Table 4.3).

Table 4.3. The concentration of major acids in unadulterated kiwifruit pulp obtained by GC analysis, in Year One (1998) and Year Two (1999)

		Acid (mg/g)		
		Malic	**Quinic**	**Citric**
Year 1	**Day 1**	2.1 +/- 0.3[1]	11.6 +/- 0.8	9.1 +/- 1.0
Year 1	**Day 2**	1.9 +/- 0.4	8.2 +/- 1.0	11.4 +/- 1.5
Year 2	**Day 1**	1.1 +/- 0.1	8.8 +/- 0.2	10.9 +/- 0.7
Year 2	**Day 2**[2]	-	-	-

[1]n = 3
[2]samples not tested

In Year One of the study there was significantly more ($F_{(1,4)}$ = 21.33, p value = 0.010) quinic acid in the pulp used on Day One of consumer testing than on Day Two. The concentration of citric and malic acids were not significantly different across the two days of consumer testing in Year One. Data is missing for Day Two of the second year of the study, therefore for Year Two (1999) comparisons can not be made across the two days of consumer testing. There was significantly less ($F_{(1,4)}$ = 30.49, p value = 0.005) malic acid in the pulp used on the first day of consumer testing in Year Two than on consumer testing days in Year One. In both years of the study, TA of the pulp

was the same on consecutive consumer testing days, therefore the total amount of acids in the pulp was the same on each day of consumer testing (Table 4.4).

Table 4.4. Titratable acidity of unadulterated kiwifruit pulp on each day of consumer testing in Year 1 (1998) and Year 2 (1999)

		TA (mg citric acid /g)
Year 1	**Day 1**	11.0 +/- 0.1[1]
Year 1	**Day 2**	11.0 +/- 0.1
Year 2	**Day 1**	11.8 +/- 0.2
Year 2	**Day 2**	11.8 +/- 0.1

[1] n = 3

The TA of the pulp was significantly lower ($F_{(1,4)} = 56.69$, p value = <0.001) in Year One than in Year Two of the study.

4.3.3 Odour compound composition of unadulterated kiwifruit pulp

The composition of the major odour compounds in the pulp in Year One (1998) and Year Two (1999) is shown in Table 4.5.

Table 4.5. Odour compound composition (ng/g) obtained by GC analysis, of unadulterated kiwifruit pulp in Year One (1998) and Year Two (1999)

	Year 1	Year 1	Year 2	Year 2
	Day 1	Day 2	Day 1	Day 2[2]
Ethanol	151 +/- 87	229 +/- 145	3037 +/- 175	-
Hexanol	166 +/- 76	198 +/- 86	330 +/- 13	-
E-2-hexanol	157 +/- 68	300 +/- 119	413 +/-18	-
Hexanal	1356 +/- 173	405 +/- 282	103 +/- 1	-
E-2-hexenal	11740 +/- 1603	7573 +/- 1929	3870 +/- 88	-
Ethyl butanoate	79 +/- 14	72 +/- 32	352 +/-18	-
Methyl butanoate	0 +/- 0	0 +/- 0	946 +/- 63	-

[1]n = 3
[2]sample not collected

In Year One (1998), the odour compound profile is dominated by aldehydes, in particular, E-2-hexenal and hexanal. The concentration of hexenal was significantly higher ($F_{(2,7)}$ = 30.62, p value = < 0.001) on the first day of consumer testing. All other major kiwifruit odour compounds were consistent between the two days of testing. Due to missing data for Day Two of Year Two (1999), comparisons can not be made across the two days of consumer testing in the second year of the study. In response to the longer period of fruit ripening in Year Two, the pulp had a greater presence of the esters, ethyl and methyl butanoate and less aldehydes than in Year One. Ethanol was present in Year Two pulp at significantly higher ($F_{(2,7)}$ = 428.32, p value = <0.001) concentrations than in Year One. One example of each of the sugar, acid and odour compound GC traces are located in Appendix 4 (Figures A4.1 - A4.3).

4.3.4 Titratable acidity of adulterated kiwifruit pulp treatments

Malic acid stock solution was added to the pulp to achieve treatments with three distinct levels of acidity; low, medium and high according to Table 4.1. The TA of the pulp at each of these levels of acidity is shown in Table 4.6.

Table 4.6. Titratable acidity of adulterated kiwifruit pulp treatments in Year One (1998) and Year Two (1999)

	Year 1		Year 2	
	Day 1	**Day 2**	**Day 1**	**Day 2**
Acid Level	**TA (mg citric acid/g)**			
Low	11.0 +/- 0.1[1]	11.0 +/-0.1	11.8 +/-0.2	11.8 +/- 0.1
Medium	12.6 +/- 0.2	12.6 +/-0.1	12.8 +/ 0.2	12.8 +/- 0.2
High	13.5 +/- 0.0	13.5 +/-0.6	14.4 +/-0.1	14.4 +/- 0.5

[1]n = 3

4.3.5 Consumer Demographics

The demographics of the consumers participating in this study are shown in Table 4.7.

Table 4.7. Demographics of Consumer Panel in Year One (1998) and Year Two (1999) used to evaluate adulterated kiwifruit pulp treatments

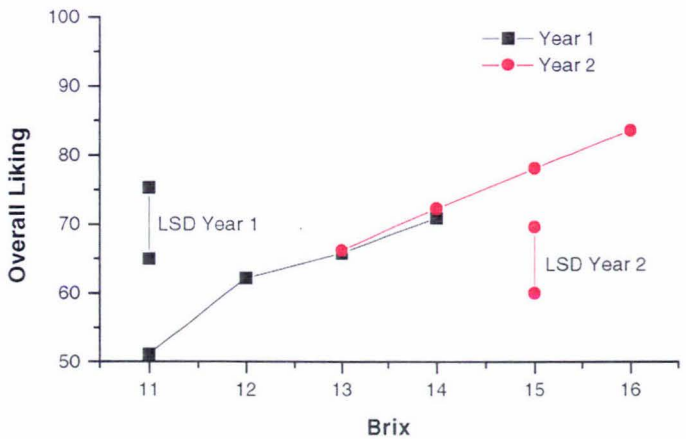
		Year 1	Year 2
		Percentage (%)	
Gender	Male	34	33
	Female	66	67
Age Group (Yrs)	18-30	23	19
	31-45	36	33
	46-60	17	17
	60+	24	31
	Total	120	120

There was a considerably higher proportion of females than males participating in both years of the study. Each age category from 18 - 60 + years was well represented. The consumer panel was different over the two years of the study, although every effort was made in Year Two (1999) to mimic the Year One (1998) consumer demographics.

4.3.6 Consumer response to pulp adulterated with sugars and acid

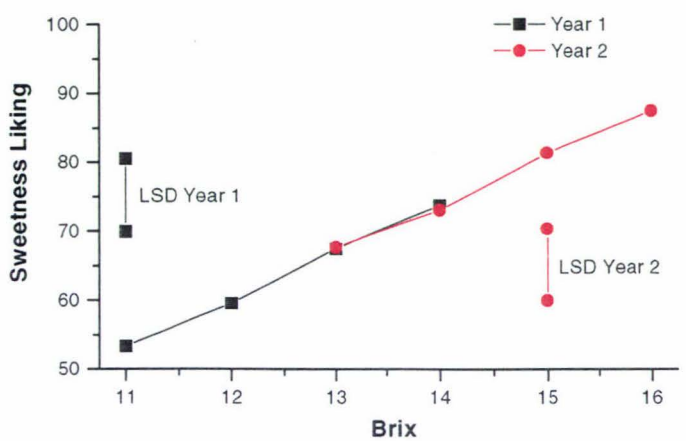
Consumer response to increasing Brix levels in the pulp by adulteration with a sugar stock solution is shown in Figures 4.1 – 4.6. Increasing Brix level was shown to increase 'overall liking', 'sweetness liking', 'acidity liking', and perception of 'sweetness intensity'. For 'overall liking', the 11° Brix treatment was liked significantly less ($F_{(3,117)} = 8.81$, p value = <0.001) than all other treatments in the first year of the study (Figure 4.1).

Figure 4.1. 'Overall liking' of kiwifruit pulp adulterated with a sugar stock solution to achieve specific final Brix levels



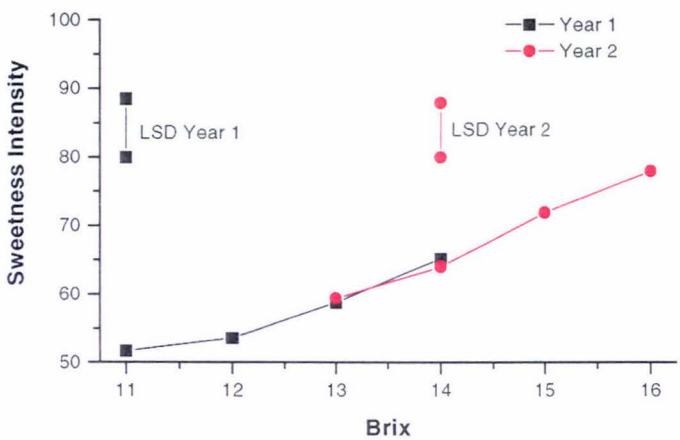
There were no significant differences in liking between the 12, 13 and 14° Brix treatments. In Year Two, the 15 and 16° Brix treatments were liked significantly more ($F_{(3,117)}$ = 8.22, p value = <0.001) than the 13° Brix treatments. 'Sweetness liking' (Figure 4.2) was found to increase linearly as the Brix level increased from 11 – 16°.

Figure 4.2. 'Sweetness liking' of kiwifruit pulp adulterated with a sugar stock solution to achieve specific final Brix levels



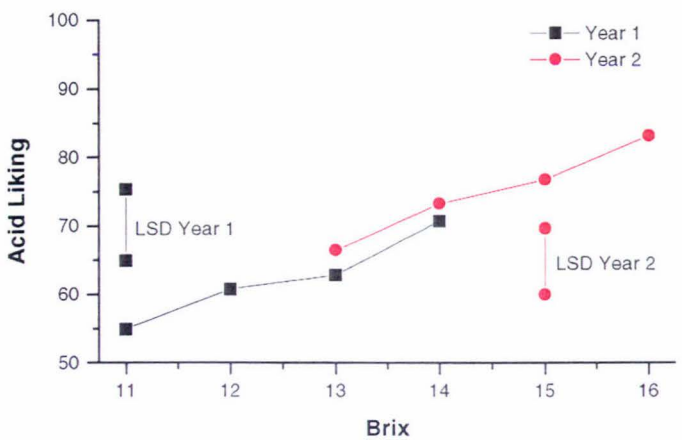
Perception of 'sweetness intensity' (Figure 4.3) was shown to significantly increase in both Year 1 ($F_{(3,117)} = 6.74$, p value = <0.001) and Year Two ($F_{(3,117)} = 14.46$, p value = <0.001) with increasing Brix level.

Figure 4.3. Sweetness intensity perception of kiwifruit pulp adulterated with a sugar stock solution to achieve specific final Brix levels



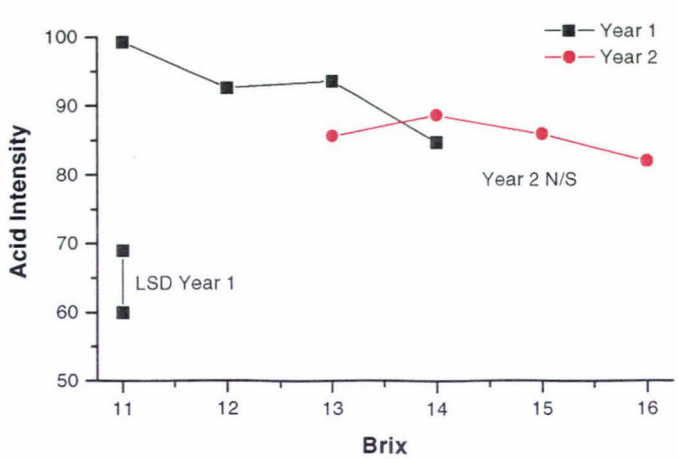
With increasing Brix levels, in the range 11 - 16°, 'acidity liking' increased significantly in Year 1 ($F_{(3,117)} = 5.24$, p value = <0.001) and Year 2 ($F_{(3,117)} = 6.90$, p value = <0.001) (Figure 4.4).

Figure 4.4. 'Acid liking' of kiwifruit pulp adulterated with a sugar stock solution to achieve specific final Brix levels



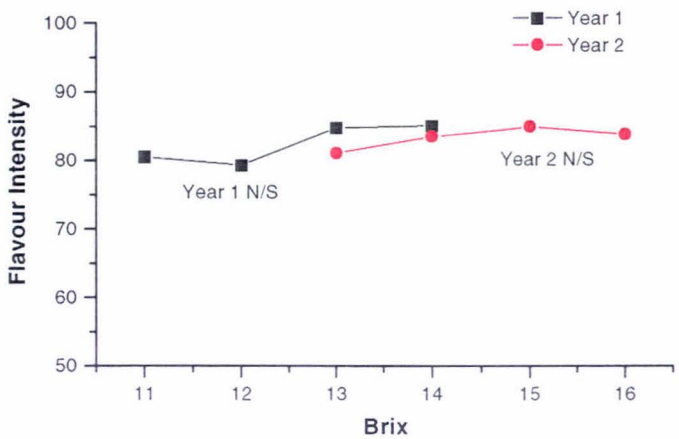
Conversely, perceived 'acid intensity' decreased significantly ($F_{(3,117)} = 5.95$, p value = <0.001) as Brix increased from 11° Brix to 14° Brix (Figure 4.5).

Figure 4.5. Acid intensity perception of kiwifruit pulp adulterated with a sugar stock solution to achieve specific final Brix levels



Increasing Brix above 14° had no effect on the perception of 'acid intensity'. There were no changes in perceived 'flavour intensity' with increasing Brix (Figure 4.6).

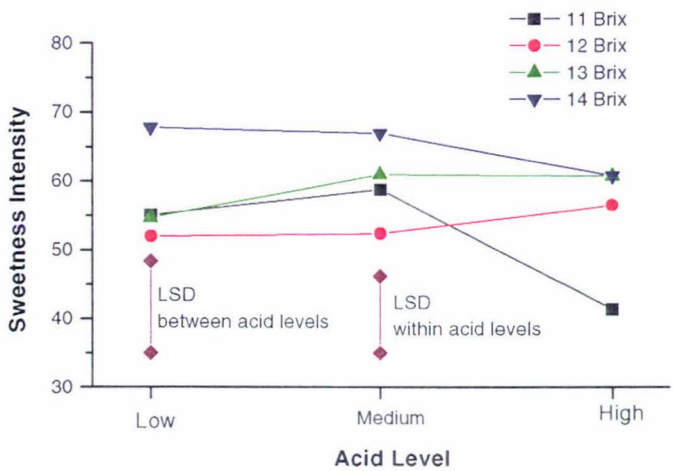
Figure 4.6. Flavour intensity perception of kiwifruit pulp adulterated with a sugar stock solution to achieve specific final Brix levels



Changes in acid level had no effect on consumer response for the attributes tested (Appendix 5, Figures A5.1a - A5.1f).

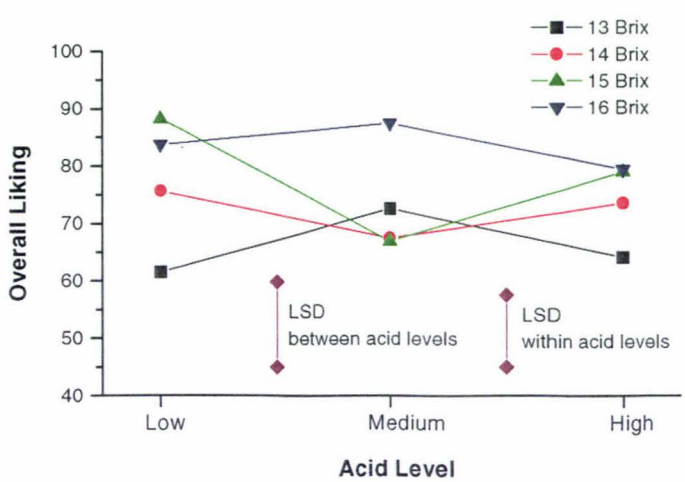
Analysis of the interactions between Brix and acid level on the attributes tested was examined. There was an interaction between Brix and acid level on perceived 'sweetness intensity' in Year One of the study (Figure 4.7).

Figure 4.7. Interaction between Brix and acid level on perceived sweetness intensity of kiwifruit pulp adulterated with sugars and acid in Year One (1998)



At the lowest Brix level of the study (11°), perception of 'sweetness intensity' appeared to decrease in the presence of increasing acid. This trend did not apply to treatments with a Brix of 12° or greater. An interaction between Brix and acidity was observed in Year Two of the study for 'overall liking' (Figure 4.8).

Figure 4.8. Interaction between Brix and acid level on 'overall liking' of kiwifruit pulp adulterated with sugars and acid in Year Two (1999)



The 15° Brix treatment at the medium level of acidity was liked significantly less ($F_{(6,351)}$ = 2.69, p value = 0.015) than at the low acid level. There were no significant differences in 'overall liking' as acid level increased at any of the other Brix levels. No other interactions between Brix and acid level were observed (Appendix 6, Figure A6.1a-e and Appendix 7, Figures A7.1a-e).

4.4 DISCUSSION

Ripening of kiwifruit results in flesh softening, increases in sugars and odour compounds, and a concomitant decrease in acidity (Redgwell *et al.,* 1990). In order to obtain kiwifruit that was soft enough to pulp, yet relatively low in Brix, odour compounds and organic acids, fruit were harvested early (5.0° Brix) in relation to the minimum harvest maturity standard of 6.2° Brix used commercially. Young and Paterson, (1985) have shown that fruit harvested at low Brix levels ripen with low levels of esters, the predominant odour compounds in ripe kiwifruit. It was desirable to evaluate the sugar and acid relationship in kiwifruit in the presence of minimal esters so that esters would not influence the consumer evaluations. In Year Two of the study, the fruit were allowed to ripen further to 13° Brix so that relationships observed in Year One could be investigated at higher Brix levels. Accompanying the increasing Brix level of fruit during the ripening process is an increase in volatile esters and a concomitant decrease in organic acids and aldehyde odour compounds (Young and Paterson, 1985).

A comparison of the main odour producing compounds from the current study with those found by McMath *et al.*, (1991b) in fruit harvested at 6.2° Brix and ethylene ripened, showed that the ester, ethyl butanoate was lower in the current fruit (an average of 76 ng/g and 352 ng/g in Years One and Two respectively versus 4248 ng/g), while E-2-hexenal, an aldehyde was higher in the current fruit (an average of 9657 ng/g and 3870 ng/g in Years One and Two respectively versus 189 ng/g). Using Gas Chromatography Olfaction (GCO) techniques, Young *et al.,* (1983) found that esters such as ethyl butanoate impart a 'fruity' odour and aldehydes impart a 'grassy' odour to kiwifruit. Therefore, the experimental pulp was characterised by a 'grassy/green' odour and flavour. The high concentration of E-2-hexenal and low concentration of ethyl butanoate in pulp from both years of the study suggest that the fruit may not have been fully ripe at the time of pulping.

An increased ripening period has been linked to a rapid increase in the concentrations of odour compounds, in particular esters (Young and Paterson, 1985). During

ripening, the odour compound composition of kiwifruit changes dramatically (Paterson *et al.*, 1991). Aldehydes, in particular E-2-hexenal decrease and a radical increase in esters is observed (Young and Paterson, 1985). In response to the increased ripening period after receiving ethylene treatment, the Year Two fruit harvested in 1999 has a greater presence of esters, particularly ethyl butanoate, and less aldehydes than the fruit harvested in 1998, which was allowed a shorter period of ripening.

During ripening, concentrations of organic acids decrease (Matsumoto *et al.,* 1983). As a consequence of the increased ripening period, the Year Two fruit had significantly less malic acid than fruit on both days of consumer testing in Year One. A comparison of the major organic acids from the current study with those found by MacRae *et al.,* (1989b) in eating ripe fruit harvested at 5.0° Brix, stored for six weeks at 0° C and ethylene ripened, showed that there were slightly higher levels of the major organic acids present in the pulp. MacRae *et al.,* (1989b) found 1.3 mg/g malic acid, 7.5 mg/g quinic acid and 8.4 mg/g citric acid in experimental fruit, compared to an average of 2.0 and 1.1 mg/g malic acid, 9.9 and 8.8 mg/g quinic acid and 10.3 and 10.9 mg/g citric acid in Years One and Two respectively. The organic acids in the current fruit at 'eating ripeness' are considerably higher than the ripe fruit harvested at the same maturity analysed by MacRae *et al.,* (1989b). This is further evidence that the fruit from both years of the current study may not have been fully ripe at the time of pulping.

The composition of sugar in the pulp was similar to the sugar composition of the fruit analysed by MacRae *et al.,* (1989b). Fruit from the two studies had similar glucose and fructose levels at 'eating ripeness'. However, sucrose levels were considerably higher in the fruit from the current study, 16.2 and 15.9 mg/g in Years One and Two respectively compared to 1.8 mg/g in fruit from the MacRae *et al.,* (1989b) study. Okuse and Ryugo, (1981) found that the fruit sugars, fructose and glucose increased during ripening and the concentration of sucrose actually decreased as the fruit reached maturity. The presence of high levels of sucrose further suggest that the fruit in the current study were not fully ripe at the time of pulping. Fruit were considered

to be 'eating ripe' when a firmness of 0.7 kgf was reached. At this firmness, fruit were soft enough to pulp. Lallu *et al.,* (1989) consider fruit to be 'eating ripe' when they have a firmness within the range 0.4 – 0.8 kgf and a Brix level between 13.0 and 14.5°. The fruit in the current study were considered to be 'eating ripe' when they reached a firmness of 0.7 kgf, however at this firmness the Brix level of the fruit averaged 11° and 13° in Years One and Two respectively. Therefore both Brix level and fruit firmness are important 'eating ripeness' parameters and should be used together to determine the point at which a kiwifruit is 'eating ripe'.

Consumer's 'overall liking' ratings of the pulp increased with rising Brix. The results of this study indicate that sweeter kiwifruit are likely to be more acceptable. Within the range of Brix included in the study, there was no indication of this trend having reached a plateau. Jordan *et al.,* (1996) (unpublished) using whole, fresh fruit have also demonstrated that consumer acceptance of kiwifruit was positively correlated with Brix level. Fruit studied by Jordan *et al.,* (1996) (unpublished) were harvested at the recommended harvest maturity of 6.2° Brix and would presumably have ester levels typical of this harvest maturity. A true confirmation of the results found by Jordan *et al.,* (1996) (unpublished) would be if the same correlation between Brix and consumer acceptability were found in a pulp model system with ester levels typical of fruit harvested at the recommended harvest maturity of 6.2° Brix.

Sweetness liking was found to increase linearly as the Brix level increased. Using aqueous sugar solutions, Moskowitz, (1971) reported that pleasantness of sweetness does not increase ceaselessly with increasing sugar concentration but actually decreases at a certain concentration and eventually becomes unpleasant. Within the range of Brix included in this study, there was no indication that 'sweetness liking' might begin to decline at a certain Brix level. It is worth considering that GC analysis of sugars, acids and odour compounds in the pulp have suggested that the fruit may not have been 'eating ripe' at the time of pulping. Therefore, it would follow then that 'sweetness liking' in the pulp would increase with increasing Brix for some time as the increased sugar would be acting to mask the harshness of the 'grassy/green' flavour notes imparted in the pulp as a result of the high aldehyde content. Barnes *et*

al., (1991) have demonstrated the importance of sweetness using fruit flavoured yoghurts, they found that the sweeter the yoghurt the higher the overall liking. The importance of sweetness to fruit quality and acceptance is well established in tomatoes (Malundo *et al.,* 1995) and in cherries and plums (Vangdal, 1985).

Perception of 'sweetness intensity' was shown to significantly increase with increasing Brix. This differed from the findings of McMath *et al.,* (1991b) who used whole, fresh fruit to show that Brix level had little effect on perceived sweetness intensity at levels greater than 12° Brix. The fruit in the current study had not been allowed to complete the ripening process and consequently had higher concentrations of organic acids. The more acidic pulp may have suppressed the perception of sweetness at low Brix levels. Suppression of sweetness in the presence of high acid has been described in fruit flavoured beverages (Stampanoni, 1993). Another explanation for the lack of agreement between the findings of McMath *et al.,* (1991b) and the results of the current study may have been an effect of the use of whole, fresh fruit by McMath *et al.,* (1991b). The whole fruit could have introduced confounding factors such as variation between fruit, in particular amongst volatile components and acids (Young *et al.,* 1995). In the current experiment use of a homogenous kiwifruit pulp allowed control of naturally occurring fruit to fruit variations.

A decrease in perceived 'acid intensity' was observed as Brix increased from 11 to 14°. This interaction shows that Brix is able to influence consumer perception of acid intensity in kiwifruit. The 14° Brix treatment was perceived as less acidic than the low Brix treatment (11° Brix) even though the acid level was unchanged. This observation is the suppression of perceived acidity by sweeteners such as sugars. This phenomenon of sourness suppression, has been demonstrated by Schifferstein and Frijters, (1990) who used a trained panel to rate the intensities of sweetness and sourness perception in model solutions of citric acid and sucrose. In a study using whole, fresh fruit with higher acids and lower sugar levels than normal, MacRae *et al.,* (1990) has suggested that the minimal relationship found between perception of acidity and acid concentration is the result of acid suppression by sugars. Research into the perception of sweetness and acidity in lemon drinks by McBride and Johnson,

(1987) has demonstrated that sucrose can readily suppress acid perception, whereas only the highest concentration of acid can suppress sweetness perception. The levels of sugars, which occur naturally in kiwifruit at eating ripeness, are normally high enough to cause a suppression of the sourness caused by organic acids. Therefore the relatively small changes in acid levels as fruit ripen should have little influence on consumer liking.

Consumer liking of the acidity of the pulp has been found to increase with increasing Brix, this is likely to be a result of sourness suppression by sugars. However such a theory assumes that consumers prefer kiwifruit that is less acidic, which was not confirmed by this study.

Results show that there is also an interaction between sugars and acids on the perception of sweetness intensity. Consumer ratings of sweetness intensity in the presence of acid vary according to the sugar content. Perception of sweetness intensity is also influenced by the concentration of acid present in the system. At (11° Brix), the lowest Brix levels of the study, the sweetness intensity of the treatments with high levels of acidity was significantly less than treatments with lower levels of acidity, even though the concentration of sugars remained constant. Acid at high levels has suppressed the perception of sweetness. This confirms the research of McBride and Johnson, (1987) in which a lemon juice drink was used to demonstrate tastant relationships. It was shown that only a high concentration of acid would suppress sweetness. Lemon juice is similar to kiwifruit pulp in that it has a high level of acid and a relatively low sugar content. Suppression of sweetness was not observed at levels above 11° Brix, indicating that if the concentration of sugar was high enough sweetness suppression by acids is resisted.

The TA of kiwifruit at 'eating ripeness' ranges between 10.0 and 16.0 mg citric acid/g (Beever and Hopkirk, 1990). Hence the TA of some kiwifruit may be equivalent to the TA of the 'high' acid level in this study that demonstrated sweetness suppression by acids. The average Brix level of 'eating ripe' kiwifruit is between 13.0 and 14.5° (Lallu *et al.*, 1989). At these Brix level, the sugar concentration is high enough to

resist sweetness suppression by acids. Only poor quality kiwifruit would have a Brix of 11° or less at 'eating ripeness', therefore suppression of sweetness intensity by acids is not likely to impact on the eating experience of most kiwifrut.

Perceived flavour intensity was not influenced by increasing Brix levels nor acid levels. Hence, neither Brix nor acid are sole contributors to perceived flavour intensity. The importance of ester odour compounds on the perception of flavour intensity in kiwifruit has been demonstrated by Esti *et al.,* (1998), who found that flavour intensity was more intense in softer fruit, and that firmer fruit were characterised by a lack of flavour. The concentration of odour compounds increase as fruit soften (Paterson *et al.,* 1991). This is supported by the findings of Gilbert *et al.,* (1996) that odour compounds have a major influence on perceived intensity and acceptability of kiwifruit flavour. The development of 'ripe fruit' odour compounds was retarded in this current study by harvesting fruit early. No additional odour compounds were incorporated into the pulp in this study, therefore it is likely that perceived flavour intensity is reliant on odour compounds, qualitatively and quantitatively.

It must be emphasised that the findings in this study apply to 'eating ripe' kiwifruit with low concentrations of esters. It is not known whether relationships found in this study can be applied to kiwifruit with ester levels typical of fruit harvested at the recommended harvest maturity of 6.2° Brix, as the effect of odour compounds on tastant relationships are not known.

4.5 CONCLUSION

Within the Brix range 11 - 16°, consumer acceptance of kiwifruit increased with increasing Brix. This effect appeared to be linear and did not plateau. Therefore it is not possible to conclude from these results the Brix level at which consumer liking might stop increasing. An interaction between sugars and acids has been demonstrated on the perception of sweetness intensity. At Brix levels of less than 12°, acid was able to suppress sweetness perception in kiwifruit. Brix levels were found to influence the perception of acid intensity in kiwifruit. This study also shows that at the sugar levels found in kiwifruit, Brix has little influence on 'flavour intensity'. A more effective way to increase 'flavour intensity' may be to manipulate the odour volatiles.

CHAPTER FIVE

The effect of high ester levels on the sugar and acid relationship established in kiwifruit with low levels of esters

5.1 INTRODUCTION

Flavour is a complex attribute made up of odour, taste and other chemical sensations such as irritation and chemical heat (Lawless and Heymann, 1998). Thus odour and taste are components of flavour. Tastes are perceived mainly on the tongue. Unlike odour compounds, tastants must be in solution (saliva aids this process) (Shallenberger, 1993). The five recognisable tastes are sweet, sour, salty, bitter and umami. Odour is perceived when volatile odour compounds come in contact with nerve endings in the olfactory area of the nose (Lawless and Heymann, 1998). Much of the flavour perceived in the mouth is due to the interaction of these odour compounds (Lawless and Heymann, 1998).

The major classes of odour compounds found in kiwifruit have been identified as alcohols, esters and aldehydes (Young and Paterson, 1985). It is well established that aldehydes impart a 'grassy/vegetative' aroma to the fruit and that esters are responsible for the 'fruity' aromas prevalent in kiwifruit (Young and Paterson, 1985). The perception and acceptability of selected kiwifruit odour compounds were investigated by Gilbert *et al.,* (1996) using a model solution system. They demonstrated that increasing levels of the ester, ethyl butanoate, increased 'overall liking', 'liking of aroma' and 'liking of flavour', and also increased perceived intensities of 'kiwifruit aroma' and 'kiwifruit flavour'. In contrast, increasing levels of the aldehyde, E-2-hexenal decreased the degree of liking of aroma and flavour and decreased 'overall liking', but increased the perceived intensity of 'kiwifruit aroma' and 'kiwifruit flavour', similar to ethyl butanoate.

There are many physiochemical factors that can influence the odour compound profile of a kiwifruit and thus alter the flavour profile such as harvest maturity and storage

conditions (Stec *et al.*, 1989). Fruit harvested before the minimum harvest maturity standard of 6.2° Brix, have lower levels of ester compounds at 'eating ripeness' than fruit harvested at the recommended harvest maturity (Young and Paterson, 1985).

The flavour profile of kiwifruit changes dramatically during ripening. With progressive ripeness, there is a decrease in aldehydes resulting in a loss of the 'green' flavour character associated with aldehydes, and a concomitant rise in esters providing the 'fruity' notes to the fruit (Young and Paterson, 1985). Fruit firmness at the time of consumption also influences the flavour profile of kiwifruit, due to the increase in esters with progressive softening of the fruit (Paterson *et al.*, 1991). The flavour of kiwifruit has been found to be more intense in softer fruit (Esti *et al.*, 1998).

Relationships found between acceptability attributes of kiwifruit, and sugar and acid levels were explored in Chapter 4. In the previous study, fruit were harvested early, to minimise the concentration of esters in the fruit at 'eating ripeness' (Young and Paterson, 1985). Therefore those findings apply only to fruit with low levels of ester compounds at 'eating ripeness'. The current study examines whether the relationships found in fruit with low ester compounds can be applied to fruit with typical levels of esters at eating ripeness. In addition to sugars and acid being added to kiwifruit pulp, odour compounds were also added to simulate kiwifruit with typical ester concentrations. The odour compounds selected to incorporate into the pulp were ethyl butanoate and E-2-hexenal, as these two odour compounds were identified by Gilbert *et al.*, (1996) as having the greatest influence on consumer perception.

5.2 METHODS AND MATERIALS

5.2.1 Fruit

The harvesting, and postharvest storage and ripening conditions of the fruit is detailed in section 4.2.1. Only fruit harvested in 1999 were included in this study.

5.2.2 Sample Preparation

Pulp was made according to the method developed in section 3.2.2.

Pulp was adulterated using sugar and acid stock solutions following the methods described in section 4.2.2.

All pulp treatments were separated in half and odour compounds were added to one half only. Thus the sugar and acid relationship could be examined with low and high levels of ester odour compounds.

A stock solution of ethyl butanoate and E-2-hexenal was prepared by dissolving 103.8 mg of ethyl butanoate and 46.4 mg of E-2-hexenal in 7.5 mL of ethanol and 7.5 mL of 'Microlene™' filtered water according to Gilbert *et al.*, (1996). The solution was shaken vigorously and stored at -15° C overnight in an airtight jar.

Based on the study by Gilbert *et al.*, (1996), 167 μL/100 g of the odour compound stock solution was incorporated into the kiwifruit pulp treatments. The pulp was immediately sealed in an air-tight jar and shaken vigorously. The pulp was stored at 4° C until required, the maximum length of time that each batch was stored for was eight hrs. Pulp treatments containing odour compounds were sealed in airtight jars.

5.2.3 Storage trial of odour compound enhanced pulp

A benchtop triangle test was carried out to ensure that there was no difference between fresh odour compound-enhanced pulp and stored odour compound-enhanced pulp as a result of the loss of volatiles from the pulp system or chemical changes over the storage period. The triangle test was used to determine whether a difference

existed between fresh odour compound enhanced pulp and odour compound enhanced pulp that had been stored for nine hrs at 4° C. Three 30 mL samples of pulp were presented to a total of 15 panellists simultaneously. Two of the samples were taken from one of the pulp treatments, and were therefore identical, the other sample was taken from the other of the pulp treatments. The samples were coded with a 3-digit random number and were presented in lidded, plastic crème cups. Panellists were asked to identify the odd sample by circling the corresponding code on a paper ballot. The total number of correct responses were collated and compared to 'The table of minimum number of correct assessments to establish significant differentiation' (Poste *et al.*, 1991) to establish if a significant difference existed between the samples.

5.2.4 Instrumental analysis using GC of sugars, acids and odour compounds in unadulterated pulp

On Day One of consumer testing, a sample of pulp was taken for odour compound analysis by GC-FID according to the methods outlined in 2.5.1. An additional sample was taken and frozen in liquid nitrogen and stored at -15° C for subsequent analysis of sugars and acids following the methods outlined in section 2.5.2. Unfortunately the sample for instrumental analysis on Day Two of consumer testing was inadvertently lost and resampling was not possible.

5.2.5 Consumer Demographics

The consumer panel participating in this study is the Year Two panel described in section 4.3.5. The demographics of the consumer panel are presented in Table 4.7.

5.2.6 Consumer evaluation of kiwifruit pulp adulterated with sugars, acid and odour compounds

Consumer evaluation of the kiwifruit pulp samples was carried out as outlined in section 4.2.4. Sample presentation differed so that the relationship between pulp with low ester levels and pulp with ester levels typical of fruit at 'eating ripeness' could be explored. There was a total of 12 treatments in the first part of the consumer evaluation session consisting of: kiwifruit pulp ranging from 13 - 16° Brix at three levels of acidity. The second part of the consumer evaluation session tested a total of

12 different treatments also. These samples consisted of: kiwifruit pulp ranging from 13 - 16° Brix at three levels of acidity with added odour compounds.

In the first part of the consumer testing session each of the 120 consumers was presented monadically with four samples from the group of treatments without added odour compounds. The panel then returned to the discussion room for a compulsory 10 min break to prevent sensory fatigue. During the break, consumers read magazines and relaxed. On returning to the tasting booths for the second part of the session, consumers were presented monadically with a further four samples from the group of treatments with added odour compounds. Consumers were asked to assess each sample for 'overall liking', 'sweetness liking' and 'acidity liking' and also their perceived intensity of flavour, sweetness and acidity. A total of eight samples was assessed by each consumer.

Samples were allocated to panellists according to a split plot design that was completely balanced to minimise order and carry-over effects (Ball, 1997).

5.2.7 Data Analysis

The data were analysed as described in section 4.2.5.

5.3 RESULTS

5.3.1 Storage trial of odour compound enhanced pulp

The benchtop triangle test was conducted to determine if there was a significant difference between fresh odour compound-enhanced pulp and stored odour compound-enhanced pulp. Results of the benchtop triangle test showed that eight out of the 15 panellists correctly identified the odd sample. A comparison of these results to table values show that no significant difference ($p > 0.05$) exists between fresh odour compound-enhanced pulp and stored odour compound-enhanced pulp. As there was no perceived differences between the two samples, it was assumed that the pulp did not change during the storage period on consumer testing days.

5.3.2 Instrumental analysis using GC of sugars, acids and odour compounds in unadulterated pulp

The sample for instrumental analysis on Day Two of consumer testing was inadvertently lost, as resampling was not possible, comparisons of sugars, acids and odour compounds in the pulp could not be made between the two days of consumer testing. Quantitatively the major sugars present in the unadulterated pulp were fructose and glucose (Table 4.2). Citric and quinic acids were the major organic acids present in the unadulterated pulp (Table 4.3). The compound dominating the odour compound profile was E-2-hexenal (Table 4.5).

5.3.3 Titratable acidity of kiwifruit pulp adulterated with sugars, acid and odour compounds.

The pulp was adulterated with malic acid to achieve three distinct levels of acidity. The TA of the Low, Medium and High acid levels was 11.8, 12.8 and 14.4 mg citric acid equivalent/g respectively (Table 4.6).

5.3.4 Consumer response to pulp adulterated with sugars, acids and odour compounds

Consumer response to increasing Brix levels in pulp with added odour compounds followed a similar trend to increasing Brix levels in pulp without added odour

compounds (Figures 5.1 – 5.5). 'Overall liking' (Figure 5.1), 'sweetness liking' (Figure 5.2), 'acid liking' (Figure 5.3) and perception of 'sweetness intensity' (Figure 5.4) increased with increasing Brix levels.

Figure 5.1. 'Overall liking' of kiwifruit pulp with and without added odour compounds between 13 and 16° Brix.

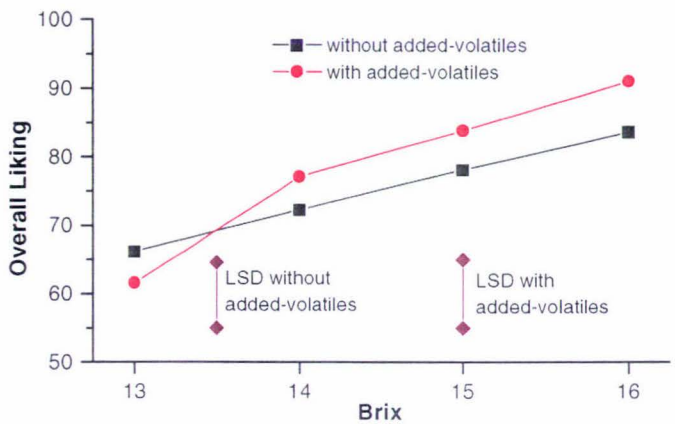


Figure 5.2. 'Sweetness liking' of kiwifruit pulp with and without added odour compounds between 13 and 16° Brix.

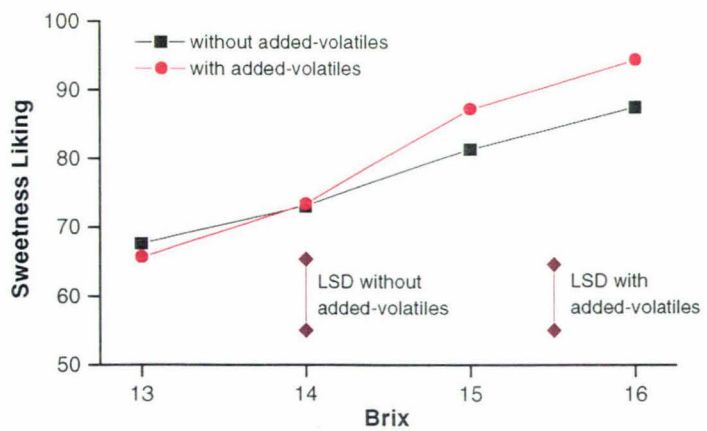


Figure 5.3. 'Acid liking' of kiwifruit pulp with and without added odour compounds between 13 and 16° Brix.

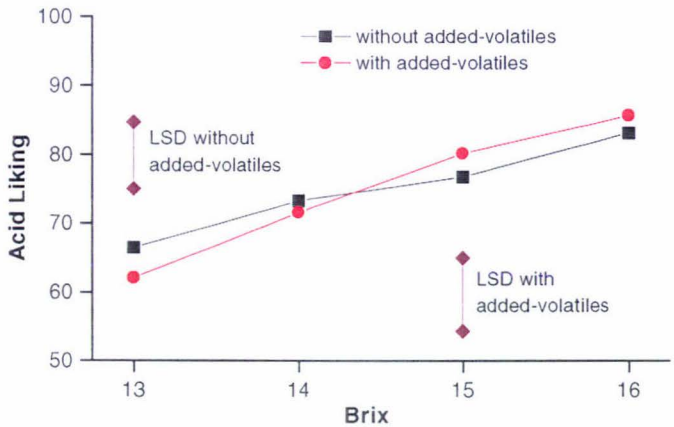
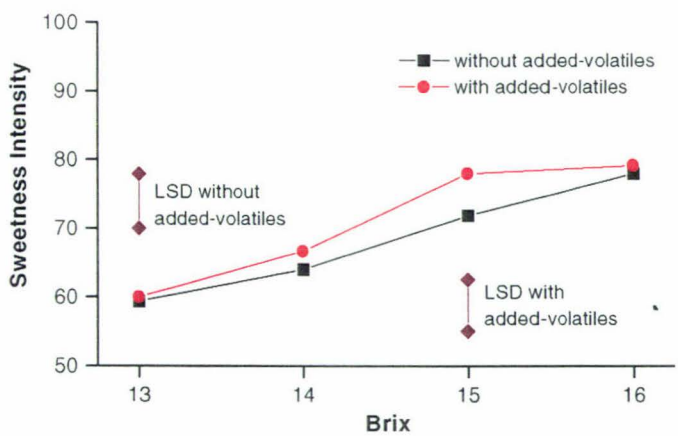
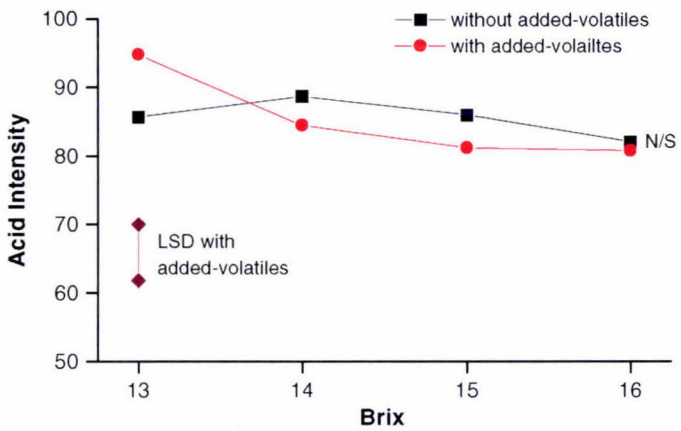


Figure 5.4. Perceived sweetness intensity of kiwifruit pulp with and without added odour compounds between 13 and 16° Brix.



There were no significant differences in the perception of 'acid intensity' with increasing Brix in pulp without added odour compounds (Figure 5.5).

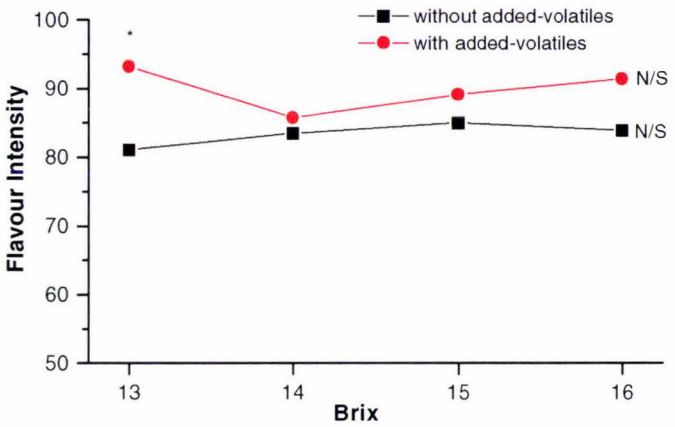
Figure 5.5. Perceived acid intensity of kiwifruit pulp with and without added odour compounds between 13 and 16° Brix.



However, in pulp with added odour compounds there was a significant decrease ($F_{(3,117)} = 8.36$, p value = <0.001) in the perception of acid intensity as Brix increased from 13 to 14°, further increases had no effect on perceived 'acid intensity' (Figure 5.5).

Perception of 'flavour intensity' was unaffected by increasing Brix levels (Figure 5.6).

Figure 5.6. Perceived flavour intensity of kiwifruit pulp with and without added odour compounds between 13 and 16° Brix (N/S = not significant).

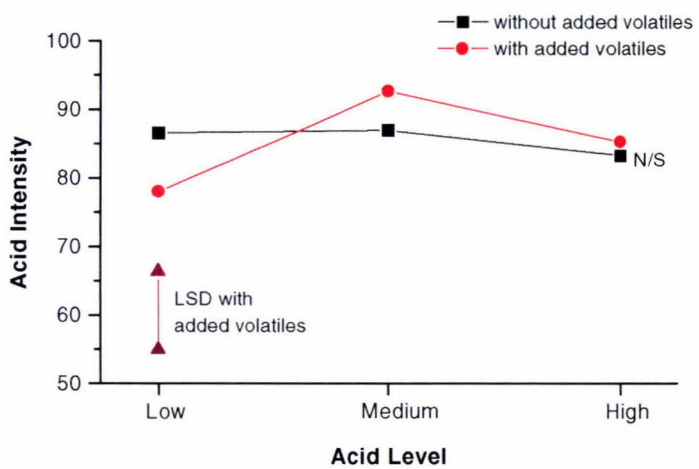


* = significant difference in perceived flavour intensity of pulp without added volatiles and pulp with added volatiles at the same Brix level.

At each Brix level, the only significant difference in consumer response between odour compound enhanced pulp and pulp without added odour compounds is in the perception of 'flavour intensity'. Flavour intensity was perceived to be significantly more ($F_{(3,817)}$ = 2.270, p value = 0.079) intense in odour compound enhanced pulp at 13° Brix (Figure 5.6). However there was no significant difference in perceived flavour intensity at greater Brix levels. At each acid level, there were no significant differences in consumer response between pulp with added volatiles and pulp without added volatiles for any of the attributes tested (Appendix 5, Figure A5.1a - f).

In pulp both with, and without added odour compounds there were no significant differences in consumer response to increasing acid levels on 'overall liking', 'sweetness liking', 'acid liking' and the perception of sweetness and flavour intensity (Appendix 5, Figure A5.1a – d, and A5.1f). Acid level had an effect on perceived acid intensity in pulp with added odour compounds (Figure 5.7).

Figure 5.7. Perceived acid intensity of kiwifruit pulp with and without added odour compounds at three different levels of acidity



Odour compound enhanced pulp with medium acid levels was perceived to be significantly more ($F_{(2,117)}$ = 4.640, p value = 0.011) acidic than the same pulp with low levels of acidity, this effect was not observed in pulp without added odour compounds.

5.4 DISCUSSION

The odour compound composition of kiwifruit is very sensitive to harvest maturity. Fruit harvested at low Brix levels ripen with low levels of esters, the predominant odour compounds in ripe kiwifruit (Young and Paterson, 1985). In order to manipulate pulp odour compound composition, kiwifruit were harvested prior to the minimum harvest maturity standard of 6.2° Brix. It was desirable that the kiwifruit pulp based model system had minimal concentrations of esters so that through the addition of odour compounds to a portion of the base pulp, a high ester treatment and a low ester treatment could be created. This allowed relationships found between sugars and acids in fruit with typical ester levels to be compared and contrasted with fruit with low ester levels.

Due to the early harvest of the fruit from the current study, the ester levels are considerably lower than in fruit harvested at the recommended harvest maturity. A comparison of the major esters in the base pulp with those found by McMath *et al.*, (1991b) in fruit harvested at the recommended harvest maturity of 6.2° Brix, shows that the major kiwifruit esters, ethyl butanoate and methyl butanoate were lower in the pulp than in the fresh, whole fruit studied by McMath *et al.*, (1991b) (352 and 946 ng/g versus 4249 and 1773 ng/g respectively). Thus the early harvest of fruit was successful in maintaining low concentrations of esters in the pulp after ripening. Therefore the unadulterated kiwifruit pulp system simulated ripe kiwifruit, but with low levels of esters. A high ester treatment was produced through the incorporation into the pulp of an odour compound stock solution comprising of ethyl butanoate and E-2-hexenal. The treatments with added odour compounds simulated kiwifruit with ester levels typical of 'eating ripe' fruit harvested at the recommended maturity of 6.2° Brix.

Consumer response to the pulp with added odour compounds was similar to pulp without added odour compounds. Generally, increasing Brix in pulp with added odour compounds followed the same trend as increasing Brix in pulp without added odour compounds. The previous study on fruit with low esters, described in Chapter

four, determined that consumer acceptance of kiwifruit increased with increasing Brix. As consumer response to pulp with added odour compounds mirrored pulp without added odour compounds, the interactions between sugars and acids and their effect on consumer acceptability found in Chapter Four can be applied to kiwifruit with levels of esters found in ripened fruit when harvested at the recommended harvest maturity of 6.2° Brix. Jordan *et al.*, (1996) (unpublished) demonstrated that Brix level was positively correlated with consumer acceptance. This has also now been confirmed in a model kiwifruit system representing fruit with ester levels typical of fruit harvested at the recommended harvest maturity of 6.2° Brix.

Not only did consumer response to pulp with added odour compounds follow the same trends as consumer response to pulp without added odour compounds, but at each Brix and acid level, consumers gave the same attribute ratings for both sets of pulp treatments. At each Brix and acid level there was no significant difference between pulp with added odour compounds and pulp without added odour compounds, except for perceived flavour intensity. Flavour intensity was perceived to be more intense in 13° Brix pulp with added odour compounds than in pulp without added odour compounds. This confirms the conclusions of Gilbert *et al.*, (1996) where flavour intensity was found to increase with increasing concentrations of ethyl butanoate. Although the flavour intensity was perceived to be higher in pulp with added odour compounds than without, at Brix levels of 14, 15 and 16°, the perceived flavour intensity was not significantly different. Volatile odour compounds sensed by the olfactory receptors, make the largest contribution to the perception of flavour (Lawless and Heymann, 1998). The results of this study using a kiwifruit pulp model system imply that sweetness, not odour makes the largest contribution to the perception of flavour in kiwifruit. It is possible that the odour compounds incorporated into the pulp were not at high enough levels and therefore they did not contribute to flavour intensity. If the presence of esters in the pulp were at higher concentrations it would seem likely that odour would contribute more to the perception of flavour intensity.

The perceived sweetness intensity of pulp with higher ester levels was the same as pulp with low ester levels, therefore addition of esters to the pulp had no effect on the perception of sweetness intensity. Volatile esters have been strongly correlated with perceived sweetness intensity in kiwifruit (Paterson *et al.,* 1991). Esters have been reported to positively affect sweetness perception in other fruits also, e.g. in apples (Young *et al.*, 1996) peaches (Narain and Thomas, 1990) and guavas (Binder and Flath, 1989). In this study, increasing ester concentration did not affect sweetness perception of kiwifruit. This may be attributed to the high concentrations of aldehydes present in the pulp. McMath *et al.*, (1991b) found that E-2-hexenal had a negative linear relationship with sweetness perception. The aldehydes in the pulp may be interacting with the esters, causing their perception to be suppressed.

Increasing Brix level increased 'overall liking' of kiwifruit. Increasing acid, and odour compounds in the pulp had no effect on consumer 'overall liking' ratings. Therefore acceptance of kiwifruit appears to be driven solely by sweetness. This disagrees with Esti *et al.,* (1998) who found that the overall acceptability of kiwifruit depends more on the odour of the fruit than on the taste. The sensory panel used in the study by Esti *et al.,* (1998) were Italian, and there may be cross cultural differences in terms of sensory perception and preferences of kiwifruit between New Zealand and Italian consumers. Cross-cultural differences in flavour perception of various food products has been reported by Prescott *et al.,* (1997). Also, there may be differences between the flavour profile of kiwifruit cultivars.

The lack of differentiation in consumer's overall liking, sweetness liking, acidity liking and perceived intensities of sweetness, flavour and acid, between pulp without added odour compounds and pulp with added odour compounds was unexpected. Although every effort was made to ensure that the pulp samples were held in air-tight vessels at all times, it is possible that the odour compounds incorporated into the kiwifruit pulp system escaped, or it may be that they were unsuccessfully incorporated into the system in the first instance.

5.5 CONCLUSION

Variations in Brix and acid level elicited the same consumer response to pulp with added odour compounds as to pulp without added odour compounds. Therefore the interactions observed between sugars and acids and their effect on consumer response when Brix was increased from 11 - 16° in fruit with low levels of esters (as detailed in Chapter four), can be applied to kiwifruit with levels of esters found in ripened fruit when harvested at the recommended harvest maturity of 6.2° Brix.

CHAPTER SIX

Concluding Discussion and Recommendations

A model system using kiwifruit pulp has been developed so that sugar and acid relationships could be explored in a homogenous product where natural variation between fruit and within fruit is eliminated. Previously, research into sugar and acid relationships have been investigated using whole, fresh kiwifruit (Stec *et al.*, 1989; McMath *et al.*, 1991b; Paterson *et al.*, 1991). There is tremendous variation between fruit (Young *et al.*, 1995) and within fruit (Hopkirk *et al.*, 1986). Hence any relationship found between chemical composition and sensory attributes using fresh, whole fruit may not be real. To overcome the confounding influences introduced by the use of whole, fresh fruit, Gilbert *et al.,* (1996) experimented with a model solution system. Use of model solutions enabled Gilbert *et al.,* (1996) to explore perception and acceptability of selected kiwifruit odour compounds in a controlled system. While this study has provided a valuable insight into kiwifruit flavour, in reality, it provided information on key odour compounds in solution and not in a kiwifruit. It is not surprising then that there were many discrepancies of results between the current research using a kiwifruit pulp model system and the results observed by Stec *et al.*, (1989); McMath *et al.*, (1991b); Paterson *et al.*, (1991) and Gilbert *et al.,* (1996).

A kiwifruit pulp model system was desired that would best mimic the experience of eating a whole, fresh kiwifruit. Therefore a processing time was necessary that caused minimal cell rupture. The pulp from various processing times was viewed under a light microscope to assess cell damage. A processing time of 30 secs was selected as this length of processing produced a pulp that allowed a maximal number of intact cells to be introduced into the oral cavity during mastication and ensured a sufficiently uniform product with respect to taste and texture. The mastication process gradually ruptures vacuolar membranes, and consequently organic acids are released into the mouth. The major difference between the experience of consuming the kiwifruit pulp model system and the experience of consuming a fresh, whole kiwifruit, is that additional acids have been incorporated into the pulp system, whereas the only acid in the fresh, whole kiwifruit are contained in vacuoles.

Therefore the consumer of the kiwifruit pulp model system experiences instantly the intensity of the additional acids plus the acids released into the system as a result of cell rupture during processing. As the rupture of vacuoles during mastication is a gradual process, the consumer of a fresh, whole kiwifruit will experience a prolonged, lower intensity perception of acid. It is recommended that future research focus on a time-intensity study comparing acid perception in an adulterated kiwifruit pulp model system, to acid perception in a fresh, whole kiwifruit.

Use of a kiwifruit pulp model system enabled the sugar and acid level in 'eating ripe' kiwifruit to be manipulated in a controlled fashion through the incorporation of sugar and acid stock solutions. The sugar stock solution was developed based on the sugar composition naturally occurring in 'eating ripe' kiwifruit and was comprised of: glucose 509.1 g/L, fructose 455.7 g/L and sucrose 35.6 g/L dissolved in 'Microlene™' filtered water at the ratio of 14.3:12.8:1.0 of glucose, fructose and sucrose. The sugar stock solution was incorporated into the pulp at appropriate volumes to achieve final levels of 11, 12, 13 and 14° Brix in Year One of the study and 13, 14, 15 and 16° Brix in Year Two. The pulp was further adulterated using a 7.5 molar malic acid stock solution, prepared by dissolving 100 g of D-L-malic acid in 100 mL of 'Microlene™' filtered water. Within each of the Brix levels, the malic acid stock solution was incorporated into the pulp to create three distinct levels of acidity; Low, Medium and High. The ratio of major organic acids occurring naturally in 'eating ripe' kiwifruit harvested prior to the recommended harvest maturity, is 0.2:1.0:0.9 of malic, citric and quinic acids respectively (MacRae *et al.*, 1989b). For further insight into acid perception and acceptability of naturally occurring acids in kiwifruit, it is recommended that the perceived acidity of the kiwifruit pulp model system be manipulated using an acid stock solution based on ratios occurring naturally in 'eating ripe' kiwifruit.

Young and Paterson, (1985) have shown that fruit harvested at low Brix levels ripen with low levels of esters, the predominant odour compounds in ripe kiwifruit. The fruit used in the model system were harvested early (5.0° Brix) in relation to the minimum harvest maturity standard of 6.2° Brix used commercially. It was desired that the fruit have low ester levels at 'eating ripeness' so that the influence of esters on

the consumer evaluations of the pulp would be minimal. GC analysis was carried out on the pulp to investigate the concentrations of major odour compounds present. A comparison of the major esters in the base pulp with those found by McMath *et al.*, (1991b) shows that the major kiwifruit esters, ethyl butanoate and methyl butanoate were considerably lower in the pulp (352 and 946 ng/g versus 4249 and 1773 ng/g respectively). The aldehyde, E-2-hexenal was higher in the current fruit (9657 ng/g and 3870 ng/g versus 187 ng/g). Using Gas Chromatography Olfaction (GCO) techniques, Young *et al.*, (1983) found that esters such as ethyl butanoate impart a 'fruity' odour and aldehydes impart a 'grassy' odour to kiwifruit. Therefore, the kiwifruit pulp model system was characterised by a 'grassy/green' odour and flavour.

Consumer's 'overall liking' ratings of the pulp increased with rising Brix. The results of this study indicate that the sweeter the kiwifruit is, the more acceptable it is likely to be. Within the range of Brix included in the study, there was no indication of this trend having reached a plateau. Increasing Brix level was also shown to increase 'sweetness liking', 'acidity liking', and perception of sweetness intensity. There were no significant changes in perceived flavour intensity with increasing Brix or at differing acid levels, hence neither Brix level nor acid level are sole contributors to perceived flavour intensity.

Perceived acid intensity decreased significantly as Brix increased above 11°, demonstrating that a change in Brix was able to influence consumer perception of acid intensity in kiwifruit. Kiwifruit at 'eating ripeness' are characterised by a Brix level between 13.0 and 14.5° (Lallu *et al.*, 1989). At these Brix levels, the sugar concentration in a kiwifruit would be high enough to cause a suppression of the sourness caused by organic acids. Therefore the relatively small changes in acid levels as fruit ripen should have little influence on consumer liking.

Consumer ratings of sweetness intensity in the presence of acid varied according to the sugar content. At the lowest Brix levels of the study, the sweetness intensity of the treatments with high levels of acidity was significantly less than other acid levels. This indicates that at low (11°) Brix, if titratable acidity is high enough, sweetness suppression will occur in kiwifruit. Suppression of sweetness was not observed at

levels above 11° Brix, indicating that if the concentration of sugar is high enough sweetness suppression by acids is resisted. The average Brix level of 'eating ripe' kiwifruit is between 13.0 and 14.5° (Lallu *et al.*, 1989). Within this Brix range, the sugar concentration is high enough to resist sweetness suppression by acids. Only poor quality kiwifruit would have a Brix of 11° or less at 'eating ripeness', therefore suppression of sweetness intensity by acids is not likely to impact on the eating experience of most kiwifruit.

To establish whether the relationships found in fruit with low ester compounds could be applied to fruit with typical levels of esters at 'eating ripeness', the pulp was further adulterated with odour compounds. Thus simulating kiwifruit with ester levels typical of fruit harvested at the recommended harvest maturity of 6.2° Brix.

Variations in Brix and acid level elicited the same consumer response to pulp with added odour compounds as to pulp without added odour compounds. Therefore the interactions observed between sugars and acids and their effect on consumer response in fruit with low levels of esters, can be applied to fruit with typical levels of esters, harvested at the recommended harvest maturity. It is recommended that further research using a kiwifruit pulp model system to explore perception and acceptability of selected odour compounds be undertaken. Individual odour compounds could be incorporated into the model system as opposed to the incorporation of an odour compound stock solution comprised of more than one odour compound as in the current research. This would enable perception and acceptability of individual odour compounds to be explored without the influence of other odour compounds introduced into the system at the same time.

As much as 85% of kiwifruit produced in New Zealand is exported (Belrose Inc., 1999). As the largest importer of New Zealand kiwifruit is Japan, future research should investigate the acceptability of sugars, acids and selected odour compounds using a Japanese consumer panel. Identification of preferred combinations will provide valuable information to guide kiwifruit breeding programs towards producing a kiwifruit that Japanese consumers consider to be 'ideal'. With the advent of genetic engineering it is becoming possible to develop fruit with 'ideal' combinations of any

flavour component. Transgenic fruit in which genes responsible for specific tastants or odours can be turned off or on, or even replaced. Thus information from the use of an idealised model such as the kiwifruit pulp model system used in the current study is more valuable than ever.

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APPENDICES

Appendix 1.

Preliminary trial of the incorporation of sugar stock solution into the pulp to alter Brix level

Preliminary laboratory work was carried out to determine the levels of sugar stock solution to be added to pulp to achieve the desired Brix levels

Methods

From a 1 kg batch of kiwifruit pulp, 100 g was weighed into each of four beakers. Treatment 1 had no added sugar and treatments 2, 3 and 4 had 0.1 mL, 1.0 mL and 10.0 mL of sugar stock solution added respectively (Table A1.1). Constant viscosities were maintained across all treatments with the addition of water to ensure each treatment received equivalent volumes of fluid.

Table A1.1. The volume of sugar stock solution added to kiwifruit pulp to alter pulp Brix levels

	Treatment			
	T1	**T2**	**T3**	**T4**
Pulp (g)	100	100	100	100
Sugar Soln[1] (mL)	0.0	0.1	1.0	10.0
H_2O (mL)	10.0	9.9	9.0	0.0

[1]Sugar stock solution: 14.3:12.8:1.0 of glucose, fructose and sucrose

The treatments were mixed thoroughly on a magnetic stirrer for 2 min and the Brix level was determined using a digital refractometer 'Atago™' following the methods

outlined in section 2.2. Results were analysed by ANOVA ('Minitab 12.1©', 1998) to determine if a significant difference existed in Brix level between the treatments.

The preliminary laboratory trial was repeated and the levels of sugar stock solution were expanded to focus on the addition into the pulp of between 0 mL and 10 mL of stock solution in increments of 1 mL. From a fresh, 1 kg batch of pulp, 100 g was weighed into eleven separate beakers. The sugar stock solution was added to each treatment as shown in Table A1.2.

Table A1.2. The volume of sugar stock solution added to kiwifruit pulp to alter pulp Brix levels

	Treatment										
	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**	**T11**
Pulp (g)	100	100	100	100	100	100	100	100	100	100	100
Sugar Soln[1] (mL)	0.0	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0
H_2O (mL)	10.0	9.0	8.0	7.0	6.0	5.0	4.0	3.0	2.0	1.0	0.0

[1]Sugar stock solution: 14.3:12.8:1.0 of glucose, fructose and sucrose

Results

Addition of either 0.1 mL or 1.0 mL of the sugar stock solution (treatments 2 and 3, Table A1.3) did not significantly alter the Brix level of the kiwifruit pulp.

Table A1.3. The effect on Brix level in kiwifruit pulp after addition of sugar stock solution.

	Treatments			
	T1	**T2**	**T3**	**T4**
Pulp (g)	100	100	100	100
Sugar Soln[1] (mL)	0.0	0.1	1.0	10.0
H_2O (mL)	10.0	9.9	9.0	0.0
° Brix	9.9 +/- 0.4[2]	9.7 +/- 0.3	9.8 +/- 0.4	14.4 +/-0.2

[1]Sugar stock solution: 14.3:12.8:1.0 of glucose, fructose and sucrose
[2]n = 3

When 10 mL of sugar stock solution (treatment 4) was incorporated into the pulp, the Brix level increased by nearly 5°. Thus in order to augment control on the increases in Brix level of the pulp, the effects of adding sugar stock solution in 1 mL increments within the range of 1.0 - 10.0 mL was examined.

With the addition of the sugar stock solution in volumes between 1.0 – 10.0 mL, the Brix level of the pulp increased significantly ($F_{(3,8)} = 161.2$, p value = <0.001) (Table A1.4).

Table A1.4. The effect on Brix level of kiwifruit pulp after addition of sugar stock solution

	Treatments										
	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**	**T11**
Pulp (g)	100	100	100	100	100	100	100	100	100	100	100
Sugar Soln[1] (mL)	0.0	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0
H_2O (mL)	10.0	9.0	8.0	7.0	6.0	5.0	4.0	3.0	2.0	1.0	0.0
° Brix	8.1 +/-0.4[2]	8.9 +/-0.1	9.2 +/-0.0	9.8 +/-0.1	10.9 +/-0.5	11.1 +/-0.4	11.2 +/-0.1	11.6 +/-0.4	12.2 +/ 0.0	12.7 +/-0.3	13.0 +/-0.5

[1]Sugar stock solution: 14.3:12.8:1.0 of glucose, fructose and sucrose
[2]n = 3

In general, with the addition of 2 mL of the sugar stock solution to 100 g of pulp, there was an approximate imcrease in the Brix level by 1° (Table A1.4). This occurred regardless of the amount of sugar stock solution already present in the pulp. For example, with addition of 2 mL to treatment 1 the Brix level changed from 8.1° to 9.2°, similarly when a further 2 mL of sugar solution were added to treatment 7, the Brix level changed from 11.2° to 12.2°.

Appendix 2.

Preliminary trial of malic and citric acid stock solutions into the pulp to achieve three distinct levels of acidity

To establish a method for altering acid levels in the kiwifruit pulp model system, preliminary laboratory work trialed the incorporation of 7.5 molar solutions of citric and malic acid into the pulp to achieve three levels of acidity.

Methods

From a 1.0 kg batch of kiwifruit pulp, 100 g was weighed into each of seven beakers. Malic acid solution was added into each treatment and mixed for 2 min on a magnetic stirrer (Table A2.1).

Table A2.1. The volume of acid stock solution added to kiwifruit pulp to alter acidity

	Treatment							
	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**
Pulp (g)	100	100	100	100	100	100	100	100
Acid Soln[1, 2] (mL)	0.0	1.0	2.0	3.0	4.0	5.0	6.0	7.0
H_20 (mL)	7.0	6.0	5.0	4.0	3.0	2.0	1.0	0.0

[1] Trial 1: Acid stock solution: 1.0 kg/L malic acid
[2] Trial 2: Acid stock solution: 1.4 kg/L citric acid

The acid intensity of each treatment, was rated by five HortResearch Sensory Science Staff on a 150 mm linescale, with anchors labelled, absent and extreme at 0 and 150 mm, respectively. In addition to rating the acid intensity, descriptors were sought for each treatment. The trial was repeated using citric acid added to the kiwifruit pulp. Constant viscosities were maintained across all treatments with the addition of water to ensure each treatment received equivalent volumes of fluid.

Results

When the citric acid stock solution was added to the pulp, the viscosity of the model system was dilute and watery. This was due to the fact that a substantial amount of stock solution was required to get a change in perceived acidity (Table A2.2).

Table A2.2. Effects of acid addition to pulp on perceived acidity

	Treatment							
	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**
Pulp (g)	100	100	100	100	100	100	100	100
Acid Soln (mL)	0.0	1.0	2.0	3.0	4.0	5.0	6.0	7.0
H_2O (mL)	7.0	6.0	5.0	4.0	3.0	2.0	1.0	0.0
Mean Sensory Score* - Citric acid[1]	50	50	50	50	60	60	90	90
Mean Sensory Score* - Malic Acid[2]	50	50	80	90	120	130	150	150

[1] Acid stock solution: 1.4 kg/L citric acid
[2] Acid stock solution: 1.0 kg/L malic acid
* scored on a 150 mm linescale

Results in Table A2.2 show that at least 4.0 mL of citric acid stock solution was required to achieve a 10 mm difference in sensory perception of acidity (from 50 mm at 0.0 mL stock solution addition to 60 mm at 4.0 mL stock solution addition). Another disadvantage of using citric acid to alter perceived acidity was saltiness, as noted anecdotally during preliminary testing by the HortResearch Sensory Science Staff. When a malic acid stock solution was incorporated into the pulp, the kiwifruit pulp model system did not require as much solution to affect acidity perception.

Appendix 3.

Questionnaire presented to consumers for evaluations of kiwifruit pulp

CONSUMER EVALUATION OF KIWIFRUIT PULP

Name: _____ Date: _____

Please tick the appropriate boxes:

Gender: ☐ Male

☐ Female

Age group: ☐ 18 - 30 years old

☐ 31 - 45 years old

☐ 46 - 60 years old

☐ 60+ years old

How often do you eat kiwifruit when it is in season?

- At least once per day ☐
- At least once per week ☐
- At least once per month ☐
- At least once per year ☐

When you eat fresh kiwifruit, do you prefer the fruit to be:

- Hard ☐
- Firm ☐
- Soft ☐
- Very soft ☐

Please,

- Work through the questionnaire one page at a time - Do NOT go back and change any of your responses on previous pages.
- Answer the questions by placing a small mark **through** the line.
- You will be presented with a total of four samples, **ONE AT A TIME**.

Please check you have the following sample _____

OVERALL

How much do you LIKE or DISLIKE the sample OVERALL?

Dislike very much

Like very much

CHECK

☐ Have you marked your sample on the linescale above?

OVERALL FLAVOUR INTENSITY

How intense do you think the OVERALL FLAVOUR of your sample is?

Very weak Very strong

CHECK

☐ Have you marked your sample on the linescale above?

SWEETNESS LIKING

a) How much do you LIKE or DISLIKE the sweetness of your sample?

Dislike very much

Like very much

b) How STRONG do you think the SWEETNESS of your sample is?

Very weak

Very strong

CHECK

☐ Have you marked your sample on the linescale above?

ACIDITY LIKING

a) How much do you LIKE or DISLIKE the acidity of your sample?

Dislike very much

Like very much

b) How STRONG do you think the ACIDITY of your sample is?

Very weak

Very strong

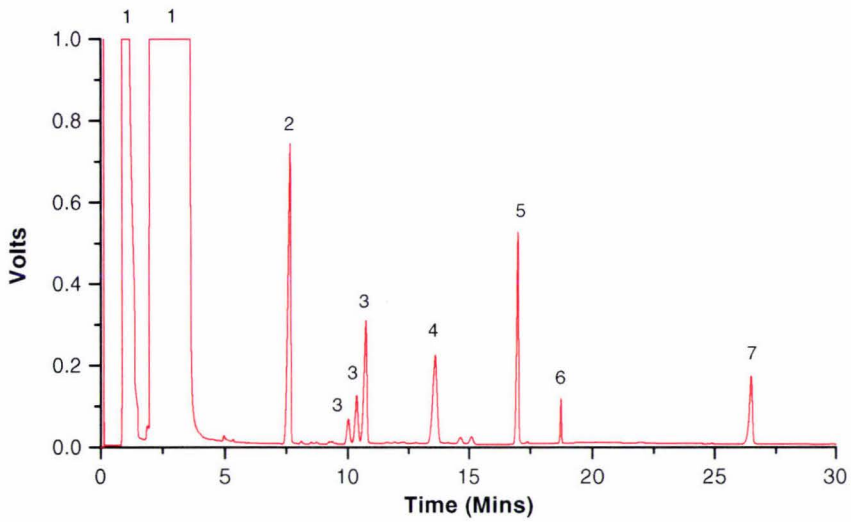
CHECK

☐ Have you marked your sample on the linescale above?

When you have finished please pass this form on your tray back through the hatch.

Appendix 4

Figure A4.1. Typical Gas Chromatogram of sugars isolated from unadulterated kiwifruit pulp



1 = solvent

2 = arabitol

3 = fructose

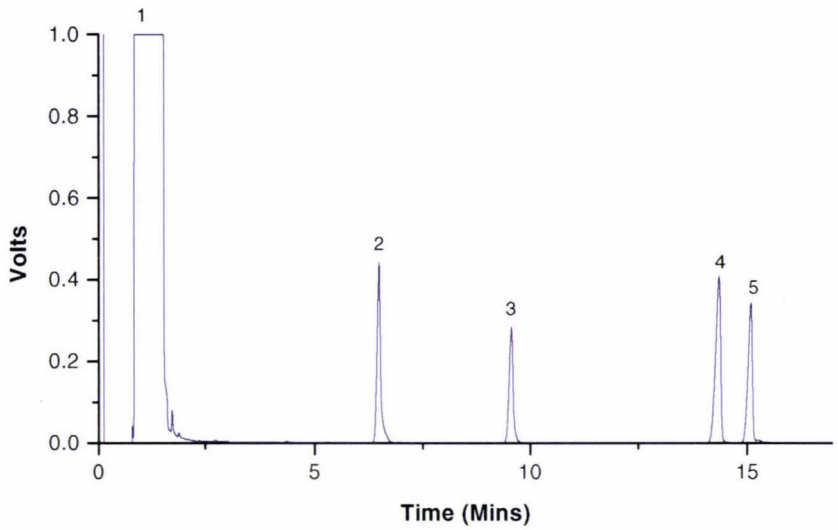
4 = α - glucose

5 = β - glucose

6 = inositol

7 = sucrose

Figure A4.2. Typical Gas Chromatogram of acids isolated from unadulterated kiwifruit pulp



1 = solvent

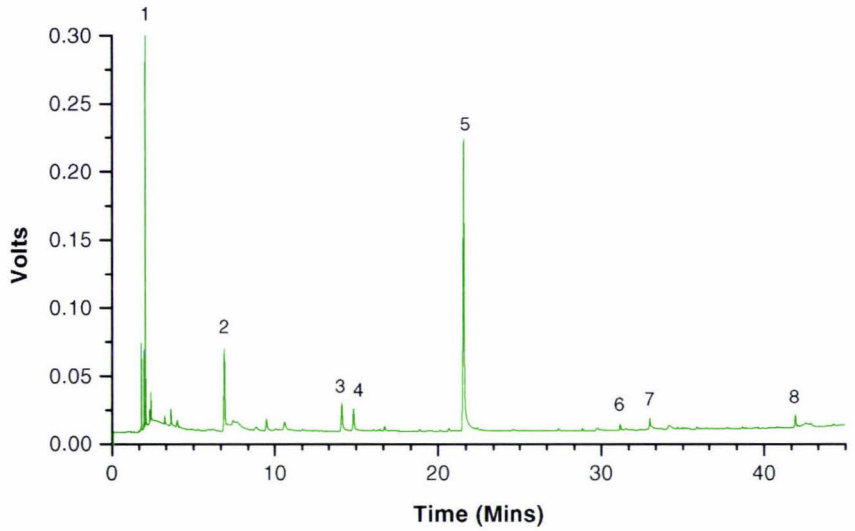
2 = malic acid

3 = tartaric acid

4 = quinic acid

5 = citric acid

Figure A4.3. Typical Gas Chromatogram of odour compounds isolated from unadulterated kiwifruit pulp



1 = contaminant

2 = ethanol

3 = ethyl butanoate

4 = hexanal

5 = E-2-hexenal

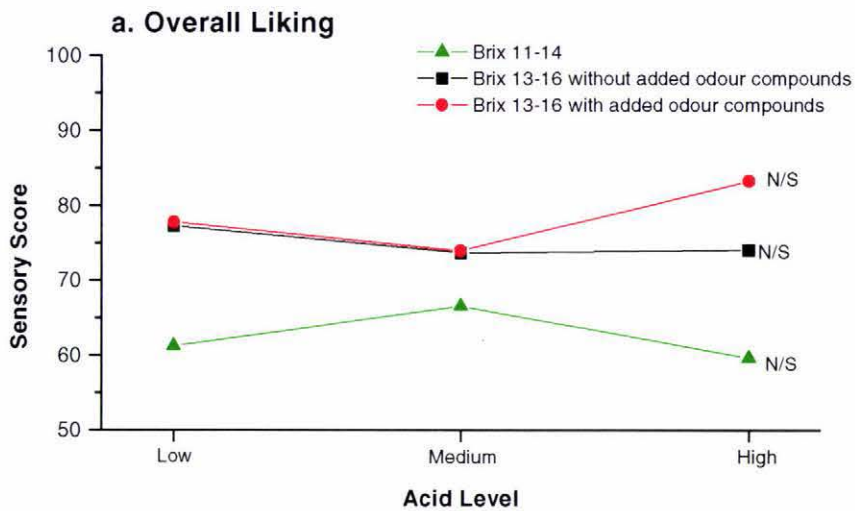
6 = hexanol

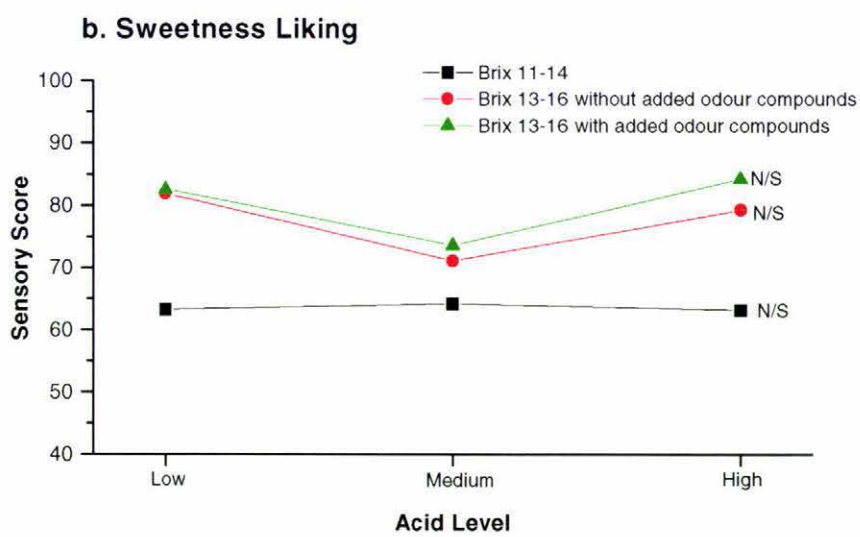
7 = E-2-hexanol

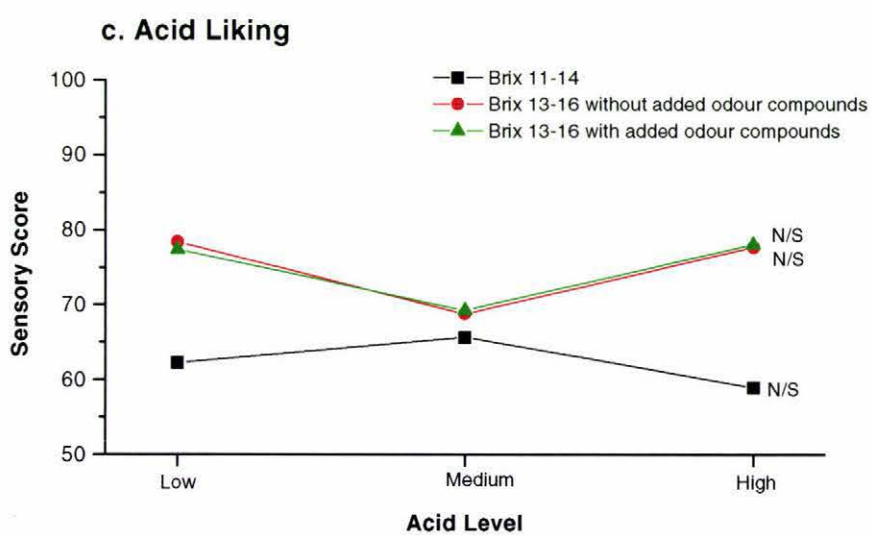
8 = methyl benzoate

Appendix 5

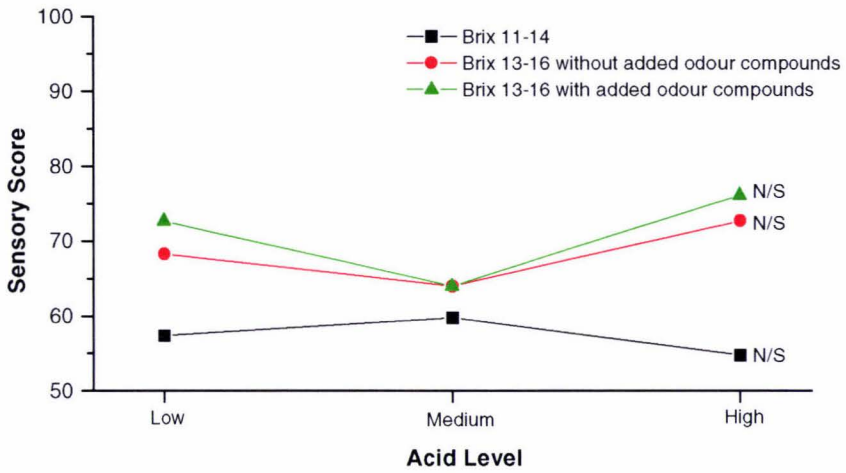
Figure A5.1. Consumer response to kiwifruit pulp adulterated with an acid stock solution to achieve 3 distinct levels of acidity; low, medium and high. N/S = not significant



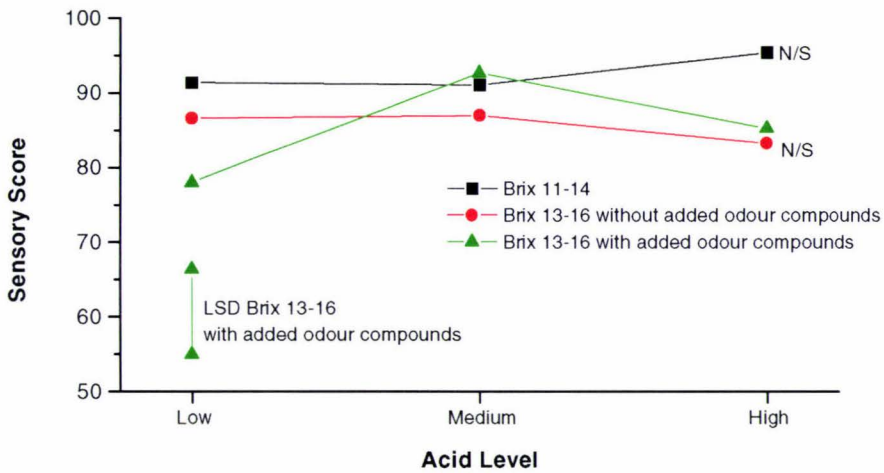




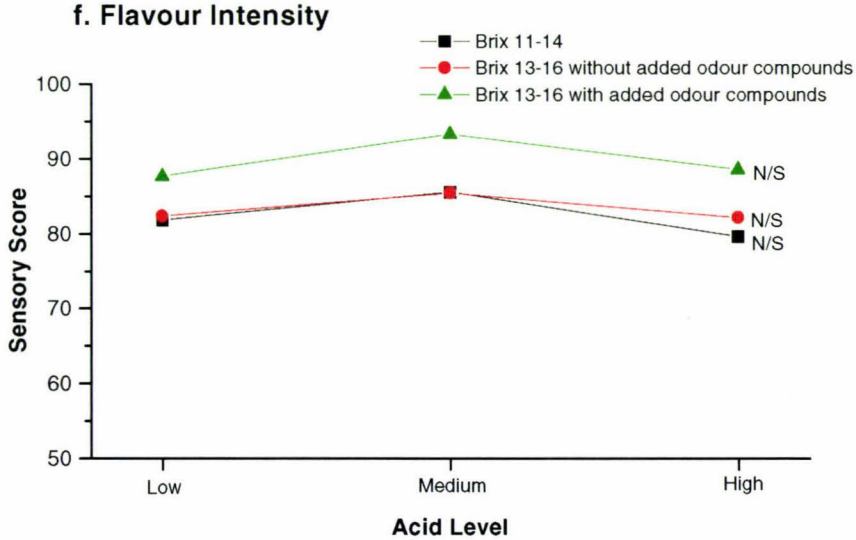
d. Sweetness Intensity



e. Acid Intensity

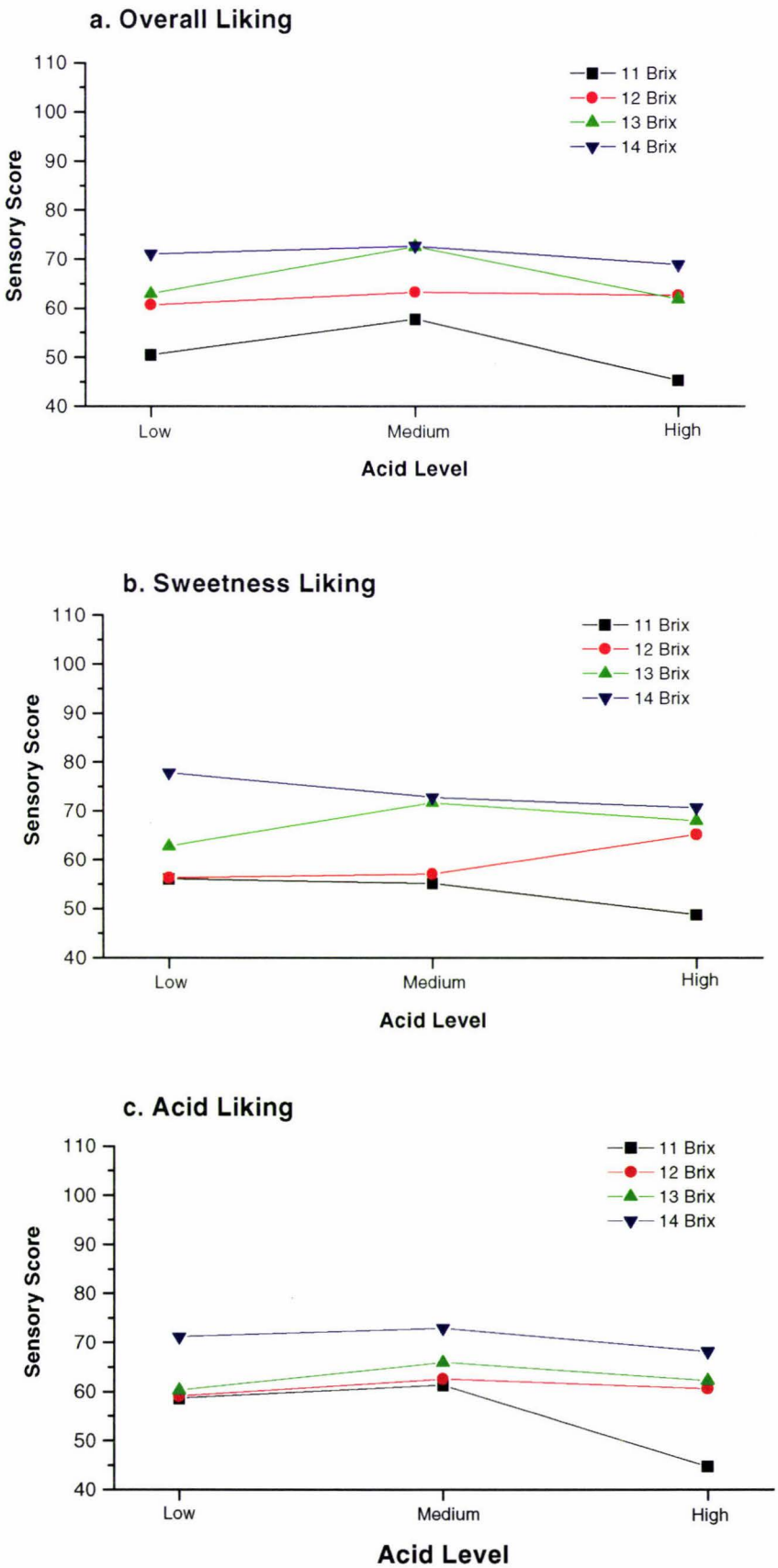


f. Flavour Intensity

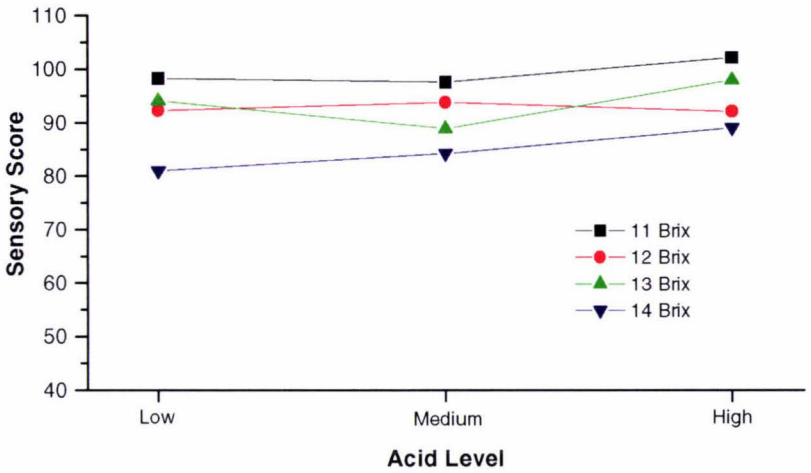


Appendix 6

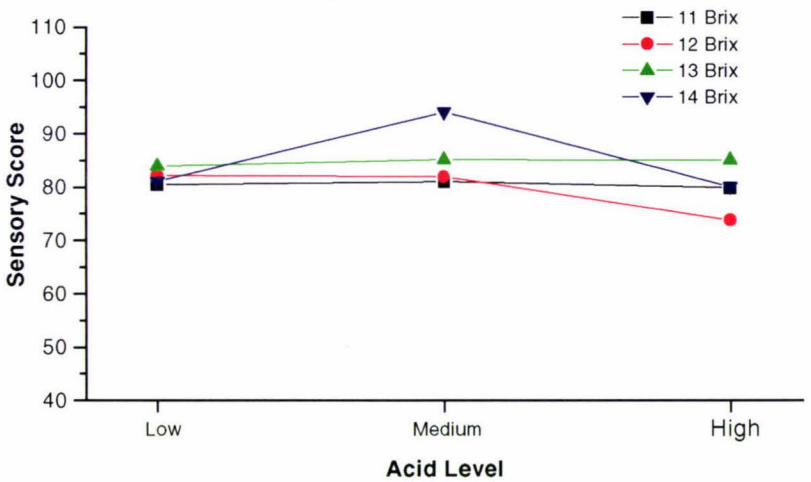
Figure A6.1. Interaction between Brix level and acid level on sensory attributes of kiwifruit pulp adulterated with sugars and acids in Year 1 (1998). Not significant.



d. Acid Intensity

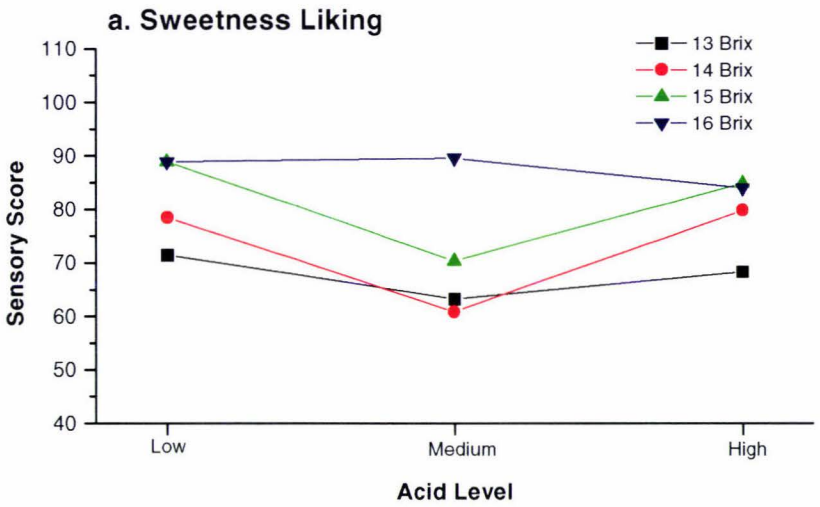


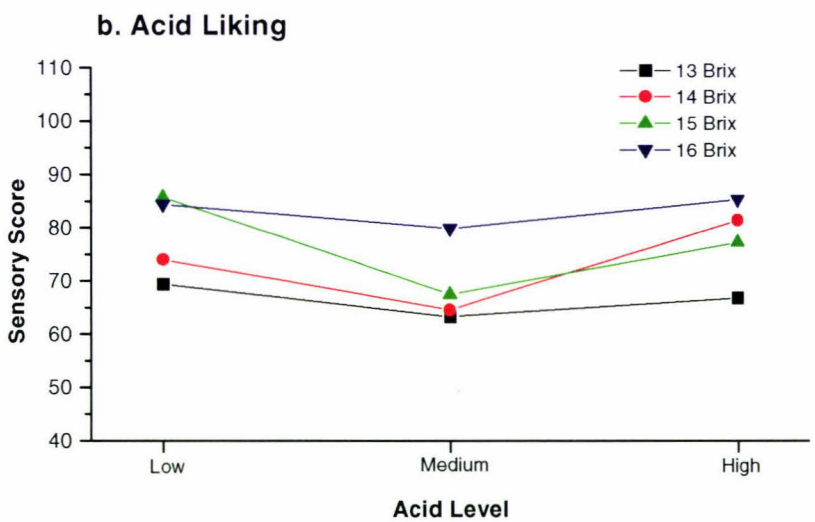
e. Flavour Intensity

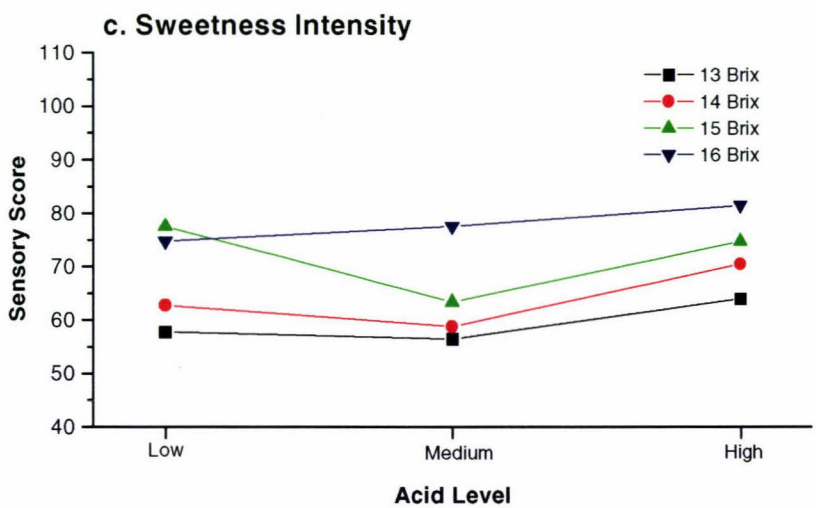


Appendix 7

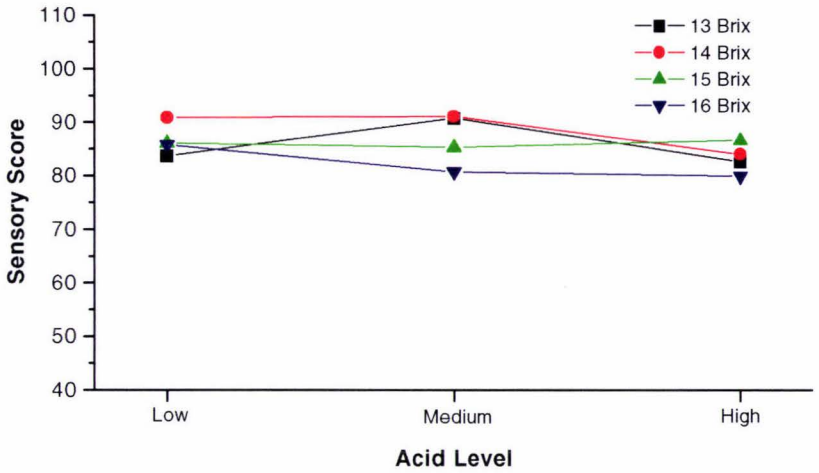
Figure A7.1. Interaction between Brix level and acid level on sensory attributes of kiwifruit pulp adulterated with sugars and acids in Year 2 (1999). Not significant.







d. Acid Intensity



e. Flavour Intensity