Production and Characterisation of ZESPRI™ Gold Kiwifruit Vinegar

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Abstract

Gold kiwifruit (Hort 16A) is a relatively new entrant into the international fresh fruit market and is a controlled variety only marketed by Zespri™. Zespri™ gold and the traditional green 'Hayward' kiwifruit are mainly marketed as fresh whole fruit; however there is interest in extending the range of processed products for gold fruit to provide further opportunities to utilize the increasing volumes now becoming available. Vinegar was selected for investigation as it plays an important role in food processing as a condiment, acidulant and preservative, and has also been identified to have various health benefits.

The aims of the project were:

(1) To evaluate the effect of juice extraction techniques and conditions on juice yield and quality.

(2) To evaluate the effects of pre-fermentation treatment and fermentation conditions on the fermentation behaviour and quality of Zespri™ gold kiwifruit mashes.

(3) To identify suitable conditions for acetifcation of Zespri™ gold kiwifruit wines and investigate the effect of the vinegar elaboration technique on the quality of the resultant kiwifruit vinegars.

Ripe peeled or unpeeled gold kiwifruit was processed in a hammer mill and the juice was extracted using a laboratory scale hydraulic press. Yield was measured for four pressurization cycles, to a maximum pressure of 250MPa. Press aid, and pre-(cellulase) and post-pressing (pectinase) enzymes were used to improve juice yield and quality. Juice yield increased through the first three pressing cycles, but there was little gain in the fourth cycle. A juice of suitable clarity and consistency, and yield of 3.8 L.(5 kg pulp)$^{-1}$ was obtained with the recommended process conditions of: 2 or 3%(w/w) press aid, 0.15mL.kg^{-1} pre-press enzyme held at 50°C for 2h, 0.035mL.kg^{-1} post-press enzyme.

Repeated pressing was found to increase total phenolics but reduced colour intensity in juice. The free-run juice was superior in colour and TP; other physico-chemical parameters were not affected by repeated pressing. Hand peeling and holding pomace at 30-50°C for 2-6h slightly reduced total acidity and significantly ($P<0.05$) reduced vitamin C. Skin contact and temperature (30-50°C, 2-6h) significantly ($P<0.05$) increased total phenolics. The character impacting aromatic compounds, ethyl butanoate, hexanal and trans-2-hexanal, were identified in the juice at 10.8, 4.2 and 9.8mg.L^{-1}, respectively. Proteolytic activity attributed to actinidin was about 45% of that observed in 'Hayward' green kiwifruit juice.

Alcoholic fermentation behaviour was evaluated at 20, 30 and 37°C for natural juice and juice supplemented with sucrose to 18°Brix using a wine yeast strain of *Saccharomyces cerevisiae*. Juices obtained from peeled and unpeeled fruit, filtered and unfiltered, were fermented. With sucrose enrichment, wines with 8.1%w/v or 8.0%w/v were obtained at efficiencies of 88% and 87% and productivities of 1.3 and 1.6g.L^{-1}h^{-1} at 20 and 30°C, respectively. Natural juice at 20°C gave a similar yield but efficiency and productivity

varied from 84-96% and 1.1-0.8g.$L^{-1}h^{-1}$, respectively. Both sucrose enrichment and high fermentation temperature reduced total vitamin C and total acidity in wine. Many esters which impact positively were identified by GC-MS in the gold kiwifruit wines. These included isoamyl acetate, ethyl acetate, ethyl butanoate, 1-hexy hexanoate, ethyl decanoate and ethyl octanoate.

Gold kiwifruit wines with up to 7.5%w/v ethanol were subjected to acetic acid fermentation using a commercial cider vinegar as the inoculum. A start up protocol for a simple semi-continuous fermentation system was developed. The best fermentation conditions identified were 29±2°C with flow rate of 0.8L.min^{-1} of oxygen enriched (40%) air. A yield of up to 5.8% w/v acetic acid was obtained at an efficiency of 85% and productivity of 1.2g.$L^{-1}h^{-1}$. A sensory panel described the gold kiwifruit vinegar as having stronger wine character than commercial cider vinegar, and equal to cider vinegar in terms of fruity aroma, ethyl acetate aroma and overall impression. The vinegar was found to have a meat tenderizing effect comparable to commercial papain enzyme and left the meat in good eating condition. Gold kiwifruit vinegar could find a niche market as marinating vinegar.

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1. 1. History of kiwifruit and development of Zespri™ gold kiwifruit

Kiwifruit is produced on a large, deciduous vine, taxonomically referred to as *Actinidia chinensis* Planch. Individual plants are dioecious, either staminate (male) or pistillate (female) (Ferguson, 1985), so to obtain berries it is necessary to have both male and female plants (Ferguson, 2004). Kiwifruit vines can be propagated by both grafting seedlings or by root cuttings. Seedlings are normally grown in nurseries and they are ready for grafting after almost one year (Ferguson, 1985). Cropping usually begins 3 years after planting with full bearing 3-5 years later (Miller, 1975). Harvest maturity takes 5-6 months from full bloom, when total soluble solids are 8-10 %(w/w). This ensures longest storage life (Sale and Lyford, 1985). The minimum average soluble solid requirement for harvest of kiwifruit is 6.2% (w/w) (Sale and Lyford, 1985). In New Zealand fruit are harvested at only 6% and kept in cold storage for up to 9 months awaiting shipment (Sale and Lyford, 1985). The harvesting period in New Zealand is usually April-June (Sale and Lyford, 1985).

The origin of kiwifruit is said to be the Yangtze valley in Southern China. Seeds were brought from China to New Zealand in 1904 (Ferguson and Bollard, 1990) and grown for the first time by enthusiasts in the Bay of Plenty near the city of Tauranga. These seeds gave rise to all important cultivars grown in New Zealand. Commercial production began in 1930, with the fruit sold only on the domestic market until 1952 when the first consignment sailed to the United Kingdom. Most crops were consumed domestically until 1976, when as result of superior marketing strategies of New Zealand, the market grew in Europe, USA and Japan, and consequently domestic consumption dwindled as exports boomed. This success encouraged growers to plant more kiwifruit (Seager, 1999).

From 1965, the area planted in kiwifruit grew rapidly and the value of exports continued to rise until 1992 when returns to growers were reduced due to the oversupply of fruit in the international markets. The reason for the reduced export returns was largely attributed to the increasing level of world production and from competition from Chile, Italy and California. Besides, there has been a change in consumer attitude to kiwifruit in many countries. Kiwifruit, which was once considered as a rare exotic seasonal crop hence meriting a high

price, had become a year-round mainstream fruit. The reduction in export returns consequently resulted in a remarkable decrease in acreage from about 14,000ha in 1985 to about 10,000 ha in 1995 (Bollard, 1996). Since then, the acreage and number of growers has remained fairly stable. Table 1.1 shows the situation of kiwifruit production in New Zealand for a period of 10 years (1993-2003).

Table 1.1 New Zealand kiwifruit sector profile for the period of (1993- 2003) (Compiled from Kiwifruit NZ and Zespri™ Group annual reports)

Season (ends 31st March	Crop volumes (millions)						
	1993	1998	1999	2000	2001	2002	2003
Trays submitted	55.3	60.6	63.1	54.2	65.5	70.0	64.1
Trays sold	49.2	56.2	59.4	51.8	61.5	64.7	61.3
General statistics							
Yield (trays.ha^{-1})	4,516	5,919	6,305	5,295	6,445	6,933	6.175
Area planted (ha)	12,256	10,243	10,015	10,234	10159	10,100	10,376
Growers or suppliers (no.)	2,748	2,723	2,681	2,541	2,506	2,504	2,719
Packhouses (no.)	252	126	118	113	102	106	101
Cool stores (no.)	146	111	106	103	98	101	99

However, kiwifruit has remained a significant export fruit in New Zealand. In the period 2002/03 kiwifruit valued at $NZ539 million was exported to 44 countries. The crop comprised 47.6 million trays Zespri™ green (82%), 8.1million trays Zespri™ gold (14%) and 2.4 million trays Zespri™ Green Organic ™ (4%) (Zespri™ Group Annual report 2003). Currently, Zespri™ International Ltd is the single marketer of kiwifruit. It comprises 2,506 shareholders and is a NZ$ 800 million business (Martin 2003, in Huang 2003).

Following the introduction of kiwifruit from China in 1930, and few exports in 1940s and 50s, many varieties were selected and propagated mainly by individual nurserymen. However, after a few years of exporting, a strong preference for the 'Hayward' variety became apparent because of its bigger size and good keeping quality (pers. comm.. Pat Sale, 2004). From 1970 the 'Hayward' variety became very much the predominate variety and the industry has been a single variety for green fruit for a number of years (Pat Sale, 2004). It was thought that with

the single variety being grown in many countries in the world, this was not a sustainable business, and therefore scientists begun working towards developing other varieties from the genus *Actinidia*. Several types of gold kiwifruit also grow wild in China. These tend to be small and not commercially viable. Horticulturalists from New Zealand's Bay of Plenty imported *Actinidia chinensis* seeds from China in 1987 and began the eleven-year process of developing Zespri™ gold kiwifruit (http://www.zesprikiwi). Through natural cross-pollination with select gold kiwifruit vines and grafting with vigorous green kiwifruit vines, New Zealand horticulturalists were able to develop the complex flavour of Zespri ™ gold kiwifruit while increasing shelf life with each subsequent generation.

In 1991 one seedling was identified as having particularly good fruit, with a pointed shape quite different in appearance to 'Hayward' and bright yellow flesh when harvested at the right maturity. This was registered under the Plant Variety Right (PVR) name of Hort 16A and is now marketed as Zespri™ gold kiwifruit (http://www.zesprikiwi). Orchard production of Zespri™ gold began in 1996 (Warrington and Weston, 1990). A total of 105,000 trays of Zespri™ gold was first tested marketed in Japan, UK and in New Zealand in 1997 and commercially launched in 2000 (http://www.zespri.com)

1.2 Justification for the research

Kiwifruit is mainly marketed as fresh whole fruit, both in export and domestic markets. The quality standard for the fresh fruit market is high and as a result a significant proportion of fruit is rejected. The gold fruit that cannot meet fresh fruit market standards are currently processed into individual quick frozen (IQF) slices, puree or juice (pers.com. Grainger, 2004). Other kiwifruit products include canned slices, leather, nectar, unclarified juice and wines (Burns, 1981). As other countries are adopting or increasing production of kiwifruit, the competition in the export market and even the domestic market become much stiffer. For example, by 2006 China is expected to emerge as the biggest kiwifruit producer in the world (Huang and Ferguson, 2003), consequently there may be a need for processing kiwifruit into a variety of consumer products, not only from culled fruit but also from high quality fruit to combat the expected rise in competition. Overall, the trend of kiwifruit production in New Zealand is towards a slight increase. The predictions of total production for the 2004 and 2005 figures by both MAF and Zespri™ are 300,000 tonnes and 305,000 tonnes, respectively. This

is challenge to the processing sector to come up with innovative processed products for both 'Hayward' and, increasingly, Gold (Hort 16A)

Gold kiwifruit has a distinctive taste, finer flesh, and some researchers have already reported it to have even higher vitamin C content than green-fleshed kiwifruit (McGhie *et al.*, 2002). Gold kiwifruit is also sweeter than green and therefore its products may be more likely to be accepted by consumers.

The overall objective of this project was to develop gold kiwifruit vinegar suitable for commercial production so that it could serve to utilise the culled kiwifruit. A suitable vinegar could serve either as a bulk product or a gourmet or specialised vinegar.

This project also aimed to contribute to methods of extracting quality juice, and fundamental knowledge about alcoholic and acetous fermentation of Zespri^{TM} Gold kiwifruit mashes. Vinegar was selected as, in addition to its key role in sauces dressings and other foods, vinegar has also been identified to have various health benefits. These include: enhancing digestion of vegetable based foods (Wandu *et al.*, 2003 in Costabeber and Ibañez 1999), reduction of blood pressure (Kondo *et al* 2001), hypoglycemic effects (Brighenti *et al* 1995; Liljeberg and Bjorck 1998; Ogawa *et al.*, 2000), calcium absorption stimulating effect (Kishi et al 1999) and enhancement of glycogen depletion in liver and skeletal muscle (Fushimi *et al* 2001).

Vinegar plays an indispensable role in food processing as a condiment, as an acidulant and as a preservative (Entani *et al* 1998; Vijayakumar and Wolf-Hall 2002). Therefore, with the increase in knowledge in the benefit that vinegar has to offer, and being a product of low cost (Adams 1998), it is an excellent option for utilising the culled fruit. In addition, a specialty product such as a gourmet vinegar which will capture some of the unique features of the kiwifruit (e.g. high level of vitamin C and antioxidants, golden yellow colour, high levels of arginine and potassium, or proteolytic activity) could potentially find a market among the tourist centres in New Zealand and overseas, and in the food service and restaurant sector.
In summary, the aim is to develop vinegars from Zespri^{TM} gold kiwifruit for domestic and export market.

The specific objectives were identified as:

(1) To evaluate the effect of juice extraction techniques and conditions on juice yield and quality.

(2) To evaluate the effects of pre-fermentation treatment and fermentation conditions on the alcoholic fermentation behaviour and quality of Zespri™ gold kiwifruit mashes.

(3) To establish optimal conditions for acetification of Zespri™ gold kiwifruit wines.

(4) To investigate how vinegar elaboration techniques affect the quality of the resultant kiwifruit vinegars.

2. Literature review

2.1 Introduction

Kiwifruit is one of New Zealand's major horticultural exports (Martin, 2003 in Huang 2003): New Zealand (NZ) produces 26.3% of all world production and exports 91-93% of its total production. Table 2.1 gives an overview of NZ kiwifruit production, export and import from 2001 to 2003.

Table 2.1: An overview of NZ kiwifruit production, export and consumption over the past 3 years (calculated from USDA, 2003 data base)

Year	Planted (acreage)	Production (tonnes)	Export (tonnes)	%	Import (tonnes)	%	Consumption (tonnes)	%
2001	10,750	229068	213,033	93	150	0.065	16,185	7
2002	10,750	271,000	246,610	91	150	0.065	24,150	9
2003	11,470	247, 320	227,534	92	150	0.065	24,1950	8

The fruit is exported, mainly unprocessed, to fresh markets. At present most processed kiwifruit are prepared as slices, juices or purees and in small volumes, wine. There is still a need to broaden the options available to the processor so that much a wider range of products may be sent to the marketplace and thus absorb the excess kiwifruit available due to increased production. This will also protect the fresh fruit market by reducing the temptation to export fruits which do not meet the fresh market standard.

This review considers the techniques of producing quality juice and wine from Zespri^{TM} gold kiwifruit, and techniques of developing vinegars from the wine.

2.2 Kiwifruit and its diversity

Originally kiwifruit was a wild plant, which grew in the southern part of China. Two major varieties have been developed and are now produced in various countries. The green-fleshed *Actinidia deliciosa*, ('Hayward') cultivar was the first to be developed and is well established in many countries; important producers are Italy, NZ, Chile, China, France and the USA..

Besides the green kiwifruit, the yellow-fleshed Zespri™ gold (Hort16A) kiwifruit has recently been developed in New Zealand (Ferguson, 1999). The Arguta (or kiwi berry) variety has also been commercialised (Ferguson, 1999). This indicates that there is still a potential of developing more edible fruits from this plant. Fig 2.1 below shows the diversity of kiwifruit.

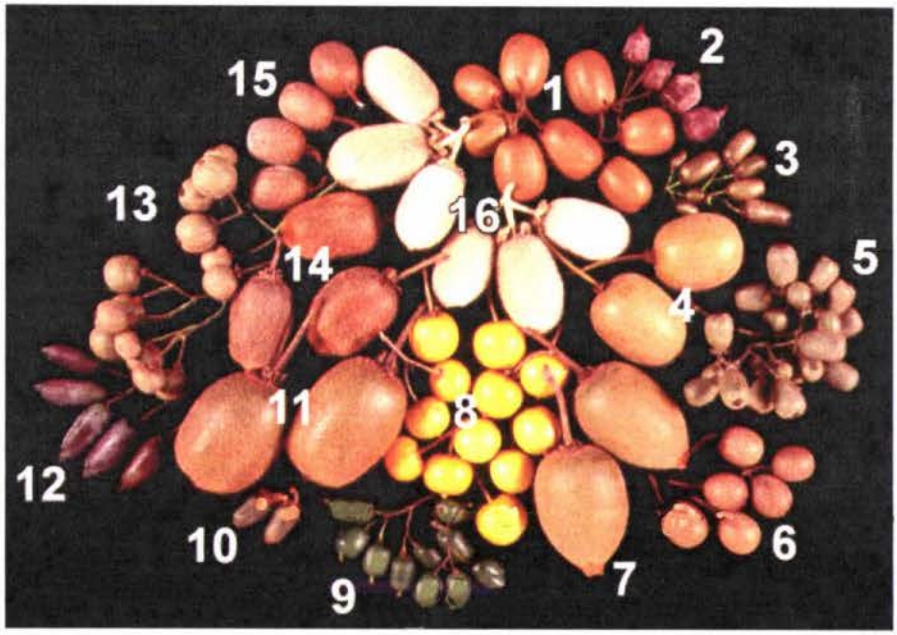


Fig 2.1: Kiwifruit diversity (from Ferguson, 1999)

Notes: 1 *A. rufa*, 2 *A. melanandra*, 3 *A. glaucophylla*, 4 *A. chinesnsis*, 5 *A.latifolia*, 6 *A.indochinensis*, 7 *A.chinesnsis*, 8 *A. macrosperma*, 9 *A. arguta*, 10 *A. fulvicoma*, 11 *A. deliciosa* 'Hayward', 12 *A. arguta* var. *purpurea*, 13 *A. guilinensis*, 14 *A. setosa*, 15 *A. chrysantha*, 16 *A. eriantha*

2.3 Kiwi fruit properties and composition

2.3.1 Gross characteristics

Actinidia deliciosa fruit is oval in shape with a hairy, dull-brown exterior (Fig 2.2). Inside, the flesh is bright green with rows of black, edible seeds aligned mainly at the central core. Fruit texture is similar to strawberry and the flavour is sweet or tart to acid, which resembles a blend of strawberry and pineapple (Ferguson, 1999).

Until 1984 *A. chinensis* and *A. deliciosa* were classified together in the one species. After that these fruits were classified into different species because, from a horticultural point of view, the two fruits are distinctly different. In wild plants of *A. chinensis*, the fruit are generally much smaller, more rounded, and less cylindrical than those of cultivated kiwifruit and initially it was feared that fruit size would be too small for commercial development. However, because of the considerable variation of the fruit size and shape, breeders, through carefully selection, have been able to develop *A. chinensis* to a size and shape comparable to, or even exceeding the average size of *A. deliciosa*, 'Hayward' fruit. This variety, which is now marketed as Zespri™ gold kiwifruit is almost hairless at maturity, and whatever hair remains is usually much shorter and finer than on the green fleshed fruit. Flesh colour can vary greatly from bright green, shading through lime green to a clear, intense yellow. The flavour of good selections of the *A. chinensis* is considered by many to be much better than that of 'Hayward'. The fruit is sweeter and more aromatic, with a flavour reminiscent of some subtropical fruit (Ferguson, 1999). Fig 2.2 shows the clear physical outwardly differences between the 'Hayward' and Zespri ™ gold kiwifruit (Hort 16A).

Fig 2.2 'Hayward' (left) and Hort 16A (right), from Ferguson, (1999)

2.3.2 General Composition.

As there is not yet much information on Zespri™gold kiwifruit (Hort 16A) (which is can only be provided by growers licensed by Zespri™) available in the literature, the data provided here is mainly that for 'Hayward' unless stated otherwise. The main chemical parameters that characterises kiwifruit are quinic acid (mean value 0.538g.100g^{-1}), L-ascorbic acid (mean value 106.7mg100g^{-1}, potassium (mean value 300mg100g^{-1}) and arginine mean (value 107 mgkg^{-1}) (Gherardi *et al.*, 1992). Titrable acidity ranges from 1.25 and 1.79g.100g^{-1} and is due mostly to citric acid, quinic acid and malic acid (Gherardi *et al.*, 1992). The major sugars in kiwifruit are fructose (average 4.7g.100g^{-1}) and glucose (average 4.3g.100g^{-1}); sucrose occurs only in small amounts (Gherardi *et al.*, 1992). The low level of sucrose may be explained by the presence in the fruit of the invertase enzyme, which might hydrolyse sucrose during fruit preparation. The composition of various components reported in literature show a high variability, for example Table 2.2 shows the variation of vitamin C as reported by different researchers.

Table 2.2 Variation of vitamin C in kiwifruit ('Hayward')

Amount (mg100g^{-1})	Author
160-200	Guenther, 1970
70	Westin, 1974
70-100	Heatherbell, 1975
38-54	Selman, 1983
80-300	Ferguson, 1984
120	Venning *et al.*, 1989

This wide range could be attributed to variation due to different harvest season, geographical location, difference in the methods of juice extraction and differences in analytical techniques. Storage history of the fruits could also affect the level of vitamin C observed. Wilson and Burns, (1983) reported that various kiwifruit juices had 60-65 mg.100g^{-1} of ascorbic acid after 20 weeks of storage at 15°C. However, even at this lower level they are still an excellent source of vitamin C and better than any other commercial fruit (Ferguson, 2003). More interesting, the newly developed kiwifruit (Hort16A) has been claimed to contain more vitamin C than the amount reported in 'Hayward' (MacGhie *et al.*, 2002; Ferguson, 2003).

Experiments carried out on the chemical composition and the enzyme activity of 'Hayward' kiwifruit) by Guenther *et al.* (1970) and Gherardi *et al.* (1992) give average data for the peeled fruit (Table 2.3).

Table 2.3: Composition of peeled 'Hayward' (green) kiwifruit from (modified from Guenther *et al.*, 1970 and Gherardi, *et al.*, 1992)

Component	Amount
Water	83.9%b
Dry matter	16.1%b
Total sugar	8.8%b
Invert sugar	7.9%b
Sucrose	0.9%b
Fat	0.9%b
Protein (N x 6.25)	1.2%b
Fibre	1.7%b
Total acid (as citric acid)	1.6%b
Minerals	1.5%b
Potassium	428mg/100g[a]
Sodium	4mg/100g[a]
Magnesium	12.3mg/100g[a]
Potassium	15.3mg/100g[a]
Calcium	21.4mg/100g[a]
Ash	73.1mg/100g[a]
pH	3.6[b]
Formol values	2.3[b]
Caloric value	50kcal[b]
Weight	59.2-79.6g[b]

Notes: a = from Gherardi *et al.*, 1992, b = from Guenther *et al.*, 1970.

In optimal acid condition (pH between 5.6 and 5.8), enzyme activity (phosphatase) of the fruit juice was tenfold the activity of apples (Guenther *et al.*, 1970). Chlorophyll contents reported in literature are 4.17mg.100g^{-1} (chlorophyll A) and 2.28mg.100g^{-1} (chlorophyll B) (Guildas, 2003). Total pectin content in Turkish kiwifruit was reported to be 61.0g kg^{-1} (Guildas, 2003), whilst, Gherardi *et al.* (1992) reported a mean value of 6.99g.kg^{-1} in Italian kiwifruit.

Among the microelements, Cu, Fe, Mn and Zn and Fe are present in high concentration whereas among the macro-elements, Ca, Mg, K, and Na, are present in higher concentration. Elemental analysis of different parts of kiwifruit revealed that there is a higher concentration of Fe and Ca in the shell than other elements, in the central core there is more Zn and Mg than other elements, while K is almost equally distributed throughout the fruit (Samadi-maybodi and Rzia Shariat 2003). Processing of kiwifruit, which involves removal of the skin, is likely to give products lower in Fe and Ca content.

Although kiwifruit has been reported (Ferguson, 2003) to contain large amount of vitamin E (α-tocopherol), it is unlikely to be bioavailable as it is confined in the seeds. The same applies for vitamin K; although kiwifruit could provide up to 50% of its RDI, most of it could be bound in the seeds (Ferguson, 2003).

2.3.3 Proteolytic activity

Actinidin is the key proteolytic enzyme found in 'Hayward' kiwifruit (Arcus, 1959). This contains free sulfhydryl as a functional group (Arcus, 1959) and is grouped with plant thiol proteases such as papain, ficin and steam bromelain (Glazer and Smith, 1971). It affects taste, allergenic properties and processing characteristics (Nishiyama and Oota, 2002). For example kiwifruit slices cannot be set in gelatine-based desserts since the enzyme in the fruit degrades the protein (Burns, 1981). It has a broad range of pH for optimum activity, which varies depending on substrate. Arcus (1959) reported a range of pH 4.0 – 4.3 with 4% gelatine whereas McDowall (1970) reported a range of pH 5 – 7 with benzoly – L – arginine ethyl ether. The molecular weight is 26 kDa (Boland & Hardman, 1972).

The distribution of actinidin in the skin, under the skin and in the flesh is shown in Table 2.5 (Lewis and Luh 1987). The flesh is the best source for the extraction of the actinidin from kiwifruit (Table 2.4). The enzyme may find application in meat tenderisation (Wada *et al.*, 2001). The specific activity of actinidin is similar to that of papain and actinidin hydrolysis of Z-Lys-pNP, which follow first order kinetics (Boland & Hardman, 1972).

Table 2.5: Distribution of actinidin in different portions of green kiwifruit 'Hayward' (modified from Lewis and Luh, 1987)

Portion	Protein mg g fruit^{-1}	Activity (U g^{-1})	Specific activity U.g^{-1}	% Total Protein
Skins	0.65	0.72	1.11	17.8
5 –10mm under skins	1.52	6.93	4.56	41.5
Bulk	4.48	6.23	4.21	40.7

Since there is no published data yet available there is a need for evaluating the level of proteolytic activity in gold kiwifruit.

2.3.4 Volatile compounds in kiwifruit

Volatile compounds identified in 'Hayward' kiwifruit are mainly esters, aldehydes and alcohols (Peterson & Young, 1990). Following an investigation by Gilbert *et al.* (1996) on consumer perception and acceptability, it was evident that the major compounds imparting on sweet aroma and flavour of kiwifruit are ethyl butanoate, trans-2-hexenal and hexanal. They reported that ethyl butanoate and trans-2-hexenal greatly influence perception and acceptability of kiwifruit flavour. Ethyl butanoate alone positively affected overall liking, liking of aroma and liking of flavour. In the absence of ethylbutanoate increasing levels of trans-hex-2-enal and hexanal increase perceived intensity of kiwifruit aroma. Trans-hex-2-enal separately and negatively affected overall acceptability but increased the perceived intensity of kiwifruit aroma and acid flavour (Gilbert *et al.*, 1996). However, in a different study by Young *et al.* (1995) it was found that increasing levels of trans-hex-2-enal increased intensity of sweet flavour.

2.3.5 Summary of kiwifruit chemical characteristics

Overall 'Hayward' kiwifruit is an excellent source of vitamin C and arginine, with reasonably good levels of other antioxidants, sugars, vitamins and minerals. High level of vitamin C attracts special attention given the current interest in vitamin C as an antioxidant and its immune simulation properties. Additionally, a good balance of the chemical composition and desirable flavour make kiwifruit an attractive fruit to process. The high level of quinic acid,

however, does contribute to astringency in juices. The high level of actinidin is of concern with some people who might be allergic, and the presence of aroma compounds such as hexanal and trans-2-hexanal which may contribute to off flavour poses another challenge in kiwifruit processing. The importance of these properties now needs to be investigated for gold kiwifruit, as being a new variety this might have different characteristics.

2.4 Kiwifruit products

To date the most common processed kiwifruit products are canned slices in syrup, frozen pulp, frozen or dried slices, juice and wines, and kiwifruit powder which can be used in bakery products such as bread and cookies (Venning *et al.*, 1989; pers. comm. J. Grainger, 2004); Others products on commercial scale in New Zealand include chocolate, jellies, jams and marmalade, marinade, cream, soaps and perfumes.

Juice and wine produced from the green 'Hayward' kiwifruit has been extensively reported in the literature (Heatherbell *et al.*, 1980; Withy and Lodge, 1982 and Wilson and Burns, 1983); data from Heatherbell *et al.* (1980) is given in Table 2.6.

Table 2.6: Chemical composition of typical green kiwifruit juice and wines (modified from Heatherbell *et al.*, 1980)

Determination	Units	Press juice	Wine
Soluble solids	% Brix	13.9	-
pH		3.24	3.23
Titratable acidity	$g.100mL^{-1}$	1.44	0.75
Glucose	$g.100mL^{-1}$	4.85	0.51
Fructose	$g.100mL^{-1}$	4.21	1.21
Sucrose	$g.100mL^{-1}$	0.83	0.0
Total sugar	$g.100mL^{-1}$	8.98	1.72
Malic acid	$g.100mL^{-1}$	0.27	0.15
Citric acid	$g.100mL^{-1}$	1.10	0.54
Quinic acid	$g.100mL^{-1}$	0.90	0.51
Total acidity	$g.100mL^{-1}$	2.27	1.20
Ascorbic acid	$mg.L^{-1}$	850	360
Dehydroascorbic acidity	$mg.L^{-1}$	124	20
Total phenol	$mg.\ L^{-1}$(GAE)	690	289
Flavonoid	$Mg.\ L^{-1}$ (GAE	50	Trace
Alcohol	% v/v	NAD	10.1
Methanol	$mg.\ L^{-1}$	NAD	181
Acetaldehyde	$mg.\ L^{-1}$	NAD	15
Volatile acidity	$g.100mL^{-1}$ (as acetic acid)	NAD	0.01
Pectin	$mg.\ L^{-1}$, (galacturonic acid)	706	Trace
Free SO_2	$mg.\ L^{-1}$	5	25
Bound SO_2	$mg.\ L^{-1}$	10	96
Sulphate	$mg.\ L^{-1}$	NAD	189

Notes: NAD mean not detectable

Free and bound SO_2, as well as sulphates arise from additions during processing.

From the composition data given in Table 2.6, it can be concluded that the composition of green kiwifruit juice is characterised by its high acidity; its relatively low soluble solids and its high concentration of ascorbic acid and quinic acids.

2.5. Processing of kiwifruit to juice and related products

2.5.1. Flow chart

Fig 2.3 represents a representative flow chart for processing juice and wine from kiwifruit

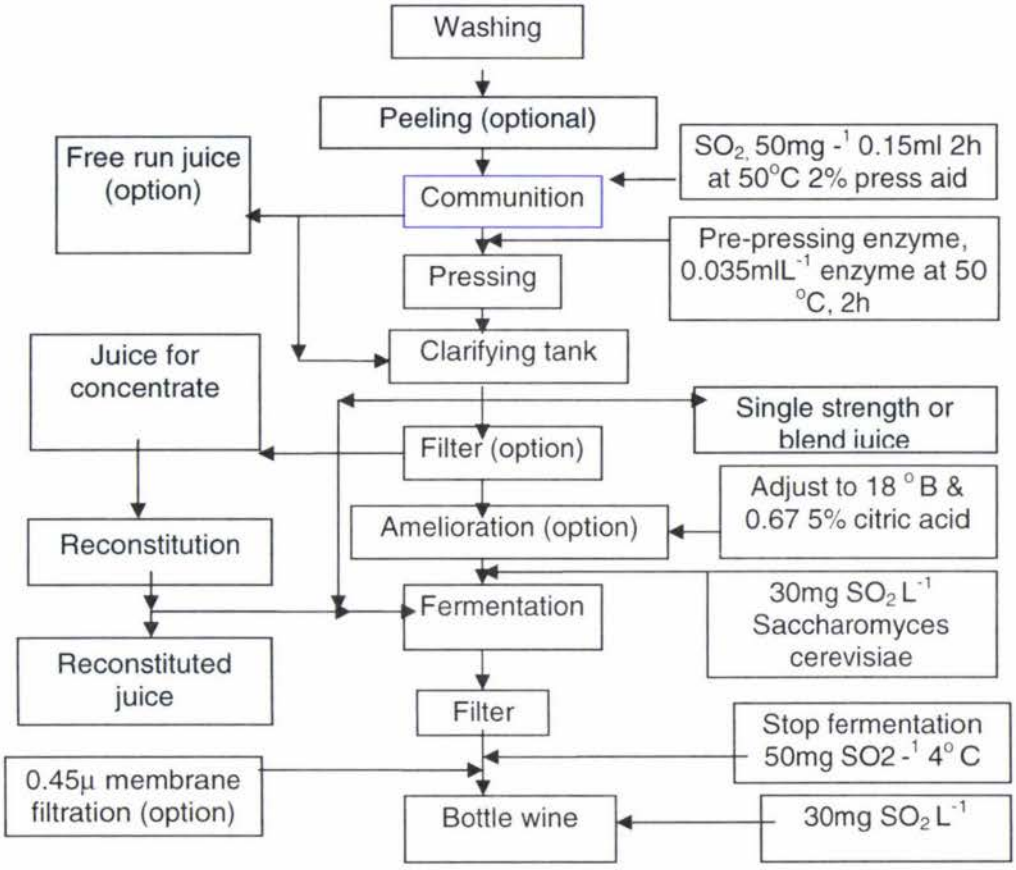


Fig 2.3: Flow chart for processing kiwifruit juice and wine (modified from Withy and Lodge, 1982)

2.5.2. Peeling

'Hayward' kiwifruit is normally harvested when mature but not ripe (6.5 -8 % soluble solids). They are typically stored at 0 °C under 85-90% relative humidity. Prior to processing, the fruit should be stored at room temperature and, may be treated with ethylene to ripen and acquire a soluble content of 13 – 16% (Wilson and Burns, 1983).

'Hayward' has a hairy skin that must be peeled before processing (Beutel., 1976; Luh and Zhang Wang, 1984; Gauthier-Jaques and Bortlik, 1999; Lidster et al., 2003; Guldas, 2003). In

contrast the yellow-fleshed Zespri™ gold kiwifruit has a smoother skin and peeling may not always be necessary (although this is required for IFQ frozen slices).

Researchers have investigated several peeling techniques. These including the gas flame technique, hand peeling and lye peeling (Beutel *et al.*, 1976). Of these, lye peeling using a boiling 15% lye solution for 90s, followed with cold water washing, was the most successful. The lye peeled fruit retained a higher concentration of vitamin C (1040mg.kg$^{-1)}$ compared to hand peeled fruits (813mg.kg $^{-1}$). A Higher concentration of vitamin C is located near the skin than in the inner parts of the fruit (Guldas, 2003). Hand peeling with a knife removes more of the tissue alone with the skin, whereas the lye treatment removes only the very thin skin membrane as indicated by the smaller weight loss of the lye peeled fruit (9.0%) compared to the hand peeled fruit (13.7% Beutel *et al.*, 1976).

Boiling the fruit for 45 minutes reduces weight loss by hand peeling (Simmons, (1978) and gives comparable results with lye peeling in 10% sodium hydroxide (Dawes, 1972). As far as lye peeling is concerned, overmature fruit peels too deep resulting in loss of flesh (and spoiling the appearance of the flesh-based product) (Dawes, 1972).

Sometimes, with certain fruits, skin contact is deliberately allowed during fruit processing to enhance extraction of desirable aroma compounds from the skin. For example Cabaroglu *et al.*, 2001 reported a considerable increase of aroma compounds in white wine when grapes (cv. Muscat of Alexandria) were allowed skin contact of 7 hour at 15°C. This skin contact time was reported to cause significant increase of alcohols, C-6 compounds, terpene, esters, fatty acids, phenolics and carbonyl compounds. However, skin contact may also increase concentration of total phenolics TP which may be detrimental to wine quality (Arnold and Noble 1979; Singleton *et al.*, 1980; Test *et al.*, 1986); besides, skin contact may result to higher colour intensity and sensitivity to oxidation in wines (Macheix *et al.*, 1991).

2.5.3 Crushing or communition and pressing

The goal in juice manufacture is to remove as much of the desirable components from the fruit as possible whilst minimising extraction of the undesirables. Crushing of the fruit enhances juice extraction and thorough communition maximises the yield, but by so doing extracts substances from everything, i.e. seed, skin, core, etc. Thus the compromise between juice yield and quality dictates the juicing and subsequent processing steps selected.

Fruit with unpalatable skin (e.g. green kiwifruit) and seeds must be treated more cautiously than one that can be completely pulverized. It is possible to minimize extraction of skin and seed components by a suitable crushing regime. However some fruit must be carefully peeled and deseeded or cored prior to juicing. Hand labour is the usual method with many minor fruits, although there is an economic incentive to mechanize if possible. Various types of crushers exist in the market; some are tailored for particular type of fruits, e.g. the Bellmer Apple crusher (Schiller, 1997), grapes crushers and crusher for oranges (Quiroz, 1970) whilst others are designed to accommodate different types of fruits (Gelb, 1981).

The range of pressing equipment includes hydraulic pressers, mechanical screw type presses, rack and cloth, pneumatic and continuous worm pressers, and membrane pressers (Cumming, 1985; Rieger, 1993; Delpeuch, 1994; Pinnow, 1996; Zamach, 1996; Colesan, 1999; Brinkman 2002; Brovelli *et al.*, 2004). All these different type of press will affect juice yield and quality differently, depending on the nature of the fruit being pressed. Therefore for each given fruit, the press or pressing system that give the best results needs to be determined

2.5.4. Use of press aid (PA) and enzymes in juice processing

Press aid is a cellulase material which are used to extraction and filtration of juice
The use of press aid (PA) in combination with pre-pressing (macerating) enzymes has been reported to increase juice yield by up 24-29% (Heatherbell *et al.*, 1979) in Hayward green kiwifruit. Similarly, increase of apple juice yield with the use pre-pressing enzyme preparations has been widely reported in the literature (Pilnk et al., 1970; Meischak; 1971; Bevers, 1995; Szymczuk *et al.*, 1997; Pagan *et al.*, 1997) and pectinase enzymes up to 0-0.1% have significantly increased juice yield in soursop and plums (Tung-sun chang et al., 1994).

Traditionally, two types of enzyme are used in juice processing. Pre-pressing (macerating) enzyme are used to break down polymeric carbohydrates, while post-pressing (clarifying) enzymes are used to hydrolyse protein and clear the juice of sediments and debris. (Note that the words 'pre-pressing' or 'macerating' and 'post pressing' or 'clarifying' are used interchangeably in the literature).

The use of pre-and post-pressing enzymes has a range of important effects, which can include:

(1) Reducing juice viscosity and improving clarity and filtrability (Brown and Ough, 1981; Tung-sun chang *et al.*, 1994; Chopda and Berrett, 2001; Beveridge *et al.*, 2002; Fundira *et al.*, 2002).

(2) Modifying colour (Tung-sun chang *et al.*, 1994; Helbig, 2001; Essa, 2002; Mihalev *et al.*, 2004).

(3) Alteration of the sugar and acid composition of the juice, and the resulting brix: acid ratio, all of which can impact on the flavour perception of the juice (Pilnik *et al.*, 1975; Fellers *et al.*, 1988; Fellers, 1991; Guyer *et al.*, 1993; Tung-sun chang *et al.*, 1994; Salmah and Nurzarina, 1994).

(4) Altering the phenolic composition of the juice.

Phenolic compounds are secondary metabolites that contribute significantly to flavour, colour, and aging characteristics of fruit products ((Lea and Timberlake, 1978; Macheix *et al.*, 1990; Spanos *et al.*, 1992; Spanos and Wrolstad, 1992; Shahid and Naczk, 1995) and health benefits of fruits and fruit juices have been ascribed, in part, to polyphenolics antioxidants (Gokmen *et al.*, 2003). Phenolic compounds are subject to oxidation by both enzymes inherent in fruits and those in commercial enzyme preparations used as processing aids. For example, oxidation of phenolic compounds in pressed apple juice by the endogenous enzyme polyphenoxidase prior to pasteurization resulted in loss of hydroxycinnamic acids (Spanos *et al.*, 1990) and hydrolysis of cinnamic acid derivatives into free acids after use of clarifying enzyme have been attributed to the presence of esterase activity in the commercial enzyme preparations, (which is also naturally present in the fruit) (Spanos *et al.*, 1990).

While phenolics have important functional and health benefits in food, they have also been associated with juice instability as they are involved in the formation of undesirable sediments (Heatherbell, 1994) and yellow and brown pigments in juice (Montgomery, 1993).

It can be concluded that since the commercial enzyme preparation differ widely in composition, and since enzyme activity differ with temperature, pH and ionic strength it will be necessary to evaluate the effect of enzyme type and enzyme concentration for gold kiwifruit.

2.5.5. Juice filtration and clarification

Juice clarification is done to reduce viscosity and remove suspended particles including colloidal haze, microorganisms, and unwanted proteins, acids and tannins, leaving a crystal clear juice. Many techniques have been used in juice clarification; these include the use of fining agents, clarifying enzymes, and dead end on cross flow membrane filtration. Just as for any other processing techniques, these techniques can all affect the quality of the resultant juice and derived products.

Yokotsuka *et al.* (2003), reported clarification of kiwifruit juice depended strongly on the use of pectinase and proteinase in post-pressing enzymes preparation. They reacted pressed juice with the post pressing enzymes for 15h at 30°C and reported that the juice, which could be filtered easily through filter paper, was used as high quality must in wine production.

The potential of pectinase and chitosan to clarify kiwifruit juice was investigated by Liang *et al.* (1998). A transparency greater that 98% was achieved at the following conditions of concentration, temperature and pH, 110mg.kg^{-1}, 45°C, pH 3-4, and 0.6mg.kg^{-1}45°C, pH 3.5, for pectase and chitosan, respectively (*Liang et al.*, 1998). They reported no precipitation occurred during storage for 3 and 6 month for pectase and chitosan treated juices, respectively.

By using an enzyme concentration of 500mg.kg^{-1} and incubating at 60°C for 20 and 60min, removed 73 and 82% of the proteins in 'Hayward' kiwifruit juice and concentrates prepared from this juice had reduced browning and haze formation compared to a control (Dawes *et al.*, 1994). The authors reported that the molecular size and concentration of the protein are important factors in haze formation.

Gauthier-Jaques and Bortlik (1999) reported that peeling kiwifruit at 100-400MPa (steam) for 10 seconds, holding the peeled fruit more than 24h at refrigeration temperature, pureeing and

treating the puree with clarifying enzyme produced juice that maintained the colour of the fruit.

The conventional methods which use fining agents such as bentonite and gelatine (Withy and Lodge, 1982) and enzymes to clarify fruit juice were not satisfactory in clarifying kiwifruit juice as sediments form during storage.

Heat treatment and ultrafiltration techniques have also been employed to process kiwifruit juice (Wilson and Burns, 1983). The partially clarified juice was heated at 90° C for 5 minutes and then cooled to 30-40 ° C in a heat exchanger. After the heat denatured protein settled, the top fraction and cloudy fraction were mixed with 0.5 and 1% Celite, respectively, and then filtered through washed Carlson –Ford filters (grade 2A) layered with 2-3 mm Celite. The resulting clear juice was filled hot at 85 ° C into cans.

The ultrafiltration technique proved to have the advantages of producing a clear sterile juice retaining its kiwifruit flavour without use of high temperature, removal of most of the protein from the juice thereby rendering unlikely any subsequent protein precipitation caused by heat or any other factor and besides, concentrating actinidin as a by-product of possible commercial importance (Wilson and Burns, 1983). However during storage fine sediments formed in the juices processed by both the conventional techniques and by ultrafiltration. This could be due to protein (actinidin) interaction with pectin or polyphenolics.

Wilson and Burns,(1983) attempted to blend apple and kiwifruit juices in equal volumes but this failed as haze formed immediately. Apple procyanidin – kiwifruit protein interaction was assumed to be responsible. The authors proposed that equal amount of the two fruits could be milled together to pre-empt the problem of haze formation.

Heatherbell *et al.* (1980) and Lodge (1980) reported on the procedures for vinification of green kiwifruit. The effect of clarification of kiwifruit juice on the quality of wine was investigated by Heatherbell *et al.* (1980), who reported that wines prepared from juice clarified by pectolytic enzymes possessed an intense fruity and "Riesling Sylvaner-type" aroma. Wines prepared from unclarified juice or clarified by settling or centrifugation possessed "grassy green stalky" aroma, and astringent and harsh taste that was not acceptable (Heatherbell *et al.*, 1980). The wines made from pectolytic enzyme treated juice had 112mg.L^{-1} of total phenol

less than the ones made from unclarified or centrifuged juice. The astringency and harshness detected in the wines made from unclarified juice could be due to high levels of phenolics. It has been reported that an increase of 85-100mgL^{-1} in TP produced a threshold difference in astringency (Heatherbell, 1975).

Overall, Hayward kiwifruit is characterised by high levels of vitamin C, quinic acid, potassium and arginine and high levels of protein (especially actinidin). The use of press aid and pectolytic enzymes in juice processing improves both juice yield and quality although results vary from one fruit to another even among varieties of the same fruit. Hence, the effects of these factors need to be determined for each new fruit or variety.

2.6 Alcoholic fermentation

2.6.1 Biochemical pathway

Alcoholic fermentation is the conversion of fermentable sugars by wine yeast into carbon dioxide gas (CO_2) and ethanol. Numerous other products, which are known as secondary products because they are present only in small quantities, are by-products of this major reaction sequence. Fig 2.4 outlines the enzymic breakdown of glucose to pyruvate and the decarboxylation of pyruvate to acetaldehyde and its reduction to ethanol and carbon dioxide.

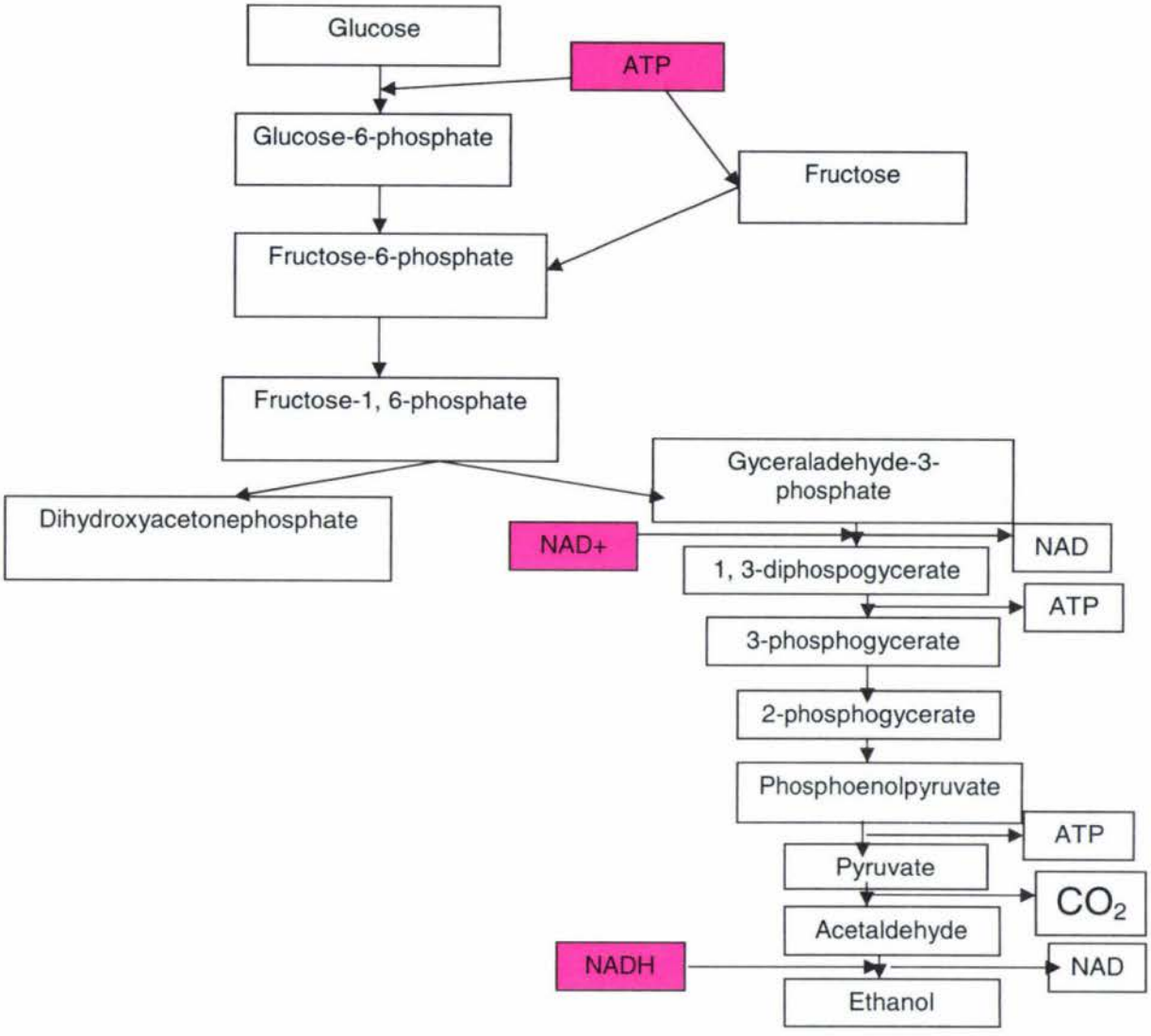


Fig 2.4 Glycolytic pathway and ethanol (modified from Linda, 2001)

The typical distribution of the end products is as follows: 95% ethanol and carbon dioxide, 1% new cell growth and 4% other products which include pyruvate, acetaldehyde, glycerol and lactate. The overall stoichiometric equation is given below. From this equation 1g of fermentable sugars produces 0.51g of ethanol (Adams, 1980).

$$\underset{\text{Glucose/ Fructose}}{C_6H_{12}O_6} \xrightarrow[\text{anaerobic condition}]{\text{yeast}} \underset{\text{Ethanol}}{2\,C_2H_5OH} + \underset{\text{Carbon dioxide}}{2\,CO_2} \qquad (2.1)$$

MW 180 46 44

2.6.2. Factors affecting fermentation of wine must

The most important factors affecting fermentation of wine must are temperature, level and type of sugars in the must, pH, nutrient supply, and type of yeast. Where applicable other factors such as length of skin contact or filtration or clarification of the wine musts may also be important, as can be the type of fermenter and condition and preparation of the juice (Ribeareau, 1999)

The type of fruit from which the juice/must is derived is an important factor in alcoholic fermentation since fruits differ significantly in physicochemical composition e.g. the amount and type of acids, sugars, minerals, polyphenolics, vitamins and minerals. These will determine both the level of nutrient supply as well as the level of inhibitory substances.

2.6.3 Effect of temperature, must composition and pH on the rate of fermentation and wine quality

During alcoholic fermentation, yeast growth determines not only the rate of fermentation but also the quality of the resultant wine. They are involved in the formation of esters which are important for flavour (Suomalainen, 1980). In fact the body of flavour, in part, is formed during fermentation by yeast (Nyakanen, 1985). In most alcoholic fermentation involving natural matrices such as fruit juice and wine musts, besides the wine yeast *Saccharomyces cerevisiae*, indigenous yeast in the genera *Kloeckera, Hanseniaspora, Candida and Pichia*, grow during the early stages (Fleet, 1997; Fleet and Heard, 1993). For fruit juices that are inoculated with pure culture the main effect is likely to be the yeast added and the process treatments employed e.g. application clarifying enzymes.

The effect of temperature, sugar concentration and pH on yeast growth and cell biomass were investigated by Charoenchai *et al.* (1998) among others, who reported that, for all strains growth increased with temperature with most strains giving fastest growth at 25°C and that for most strains, cell biomass increased substantially between 10 and 15°C. Two strains of wine yeast, *S. cerevisiae* HH350 and V1118 gave similar growth at 15, 20 and 25°C and that the optimal range of temperature for *S. cerevisiae* was 15-25°C. The rate of growth for some yeast strains decreased with increase of sugar concentration from 20-30% w/v, whilst pH in the range of 3.0-4.0 had no significant effect on yeast growth.

High initial sugar concentration in grape juice can affect yeast growth by increasing the lag phase, decreasing the growth rate, decreasing maximum cell population and decreasing the ethanol tolerance in later stages of fermentation (Monk and Cowley, 1984).

The esters concentration and type in an alcoholic beverage can be altered by changing temperature, pH or alcohol concentration or the amount and type of yeast (Suomalainen, 1980) hence affecting the aroma profile of the alcoholic beverage. Apart from esters, other groups of compounds which affect aroma profile are carbonyl compounds, alcohols and fatty acids. So many compounds affect the flavour of alcoholic beverages including wine that it is rare that one single compound responsible for a nuance of a specific flavour can be identified (Nykanen, 1985). Acetaldehyde and other short chain alphatic aldehydes along with β- keto acids are the major compounds formed when yeasts form fusel alcohols from sugars and amino acids (Nykanen, 1985). Some aldehydes impart positive flavours while others give negative flavour notes to wine e.g. hexanal is associated with off-flavour in kiwifruit products (Young *et al.*, 1991).

While the presence of some carbonyl compounds in a certain wine may be due to the presence of their precursors in the wine must (musts composition), the formation and alteration of most of these compounds is associated with alcoholic fermentation by yeast. The highest level of aldehyde is typically reached during fermentation when the action of yeast is in the most vigorous phase (Nykanen, 1985). The total aldehyde content depends not only on the type of yeast used but also the nutrient composition (Nykanen, 1985). With a deficiency of amino acids, the path from carbon source to the fusel alcohol is clearly diverted to β- keto acids whereas in the abundance of amino acids, the amino acids are deaminated and decarboxylated respectively, to form fusel alcohols (Nykanen, 1985) Fermentation rates increase with available nitrogen, if the selected amino acids are corrected for the total sugar content (Llaurado *et al.*, 2002).

Low temperature fermentation is feasible in musts with greater 20% w/v sugar but a reduction in fermentation rate should be expected. A low temperature fermentations yields wine with low levels of acetic acid, acetaldehyde and ethyl acetate and this is considered positive for wine quality (Llaurado *et al.*, 2002). Prolonged fermentation may encourage yeast to metabolise organic acid such as malic, lactic and succinic acids which will result in formation

of high levels of acetate (Herrero *et al.*, 1999). Therefore control of the duration of fermentation is necessary to avoid deleterious changes in organic acid composition.

The deleterious effect of temperature can be overcome by choosing an appropriate strain of yeast; supplementing poor musts with appropriate nutrients, and increasing cell density (D'Amore *et al.*, 1989)

2.6.4 Malolactic fermentation

This is a secondary fermentation in which lactic acid bacteria metabolise malate to produce lactate. This has the effect of replacing two acidic groups in L-malate by only one in L-lactate and hence decreasing the acidity of the wine. This is normally a spontaneous process in alcoholic fermentation although in some circumstance it is deliberately induced through addition of selected strains *Leuconostoc, Pediococcus* or *Lactobacillus*. This fermentation may be beneficial in enhancing the sensory attributes of the wine, through lowering the acidity (Henschke, 1993) and production of organoleptically active compounds (especially diacetyl and acetoin) and it may also increase microbial stability (Varsari *et al.*, 1999) of wine through removal of growth stimulating nutrients (Henschke, 1993).

2.6.5 Kiwifruit wine

Wine has been successfully produced from Hayward kiwifruit. Use of PA did not affect wine quality, but the enzyme treatment improved the appearance, flavour and aroma of wine. Firmness of the fruit affected the sensorial profile of wine with fruit of intermediate firmness scoring high (Yokotsuka *et al.*, 2003). Formerly, kiwifruit wine was described by a sensory panel as being too acid and of an unacceptable astringency and harshness, but later through amelioration with sucrose syrup and use of gelatin, a wine of an acceptable sensorial quality was produced (Heatherbell *et al.*, 1980). Lodge *et al.*, (1986) employed deionization technique to reduce acidity in kiwifruit wine and a wine was described as being very fruity in flavour and aroma, by a sensory panel.

2.6.6 Summary for alcoholic fermentation

Two of the most the critical factors in alcoholic fermentation are must composition and fermentation temperature. These affect both the rate of fermentation and the quality of the resultant wine, mainly through their effect on the performance of the wine yeast. Therefore for good results an oenologist need to take into account the type and quality of the fruit and juicing techniques, as these will affect the composition of the juice. The choice of a strain of wine yeast should take into consideration the fermentation temperature and the initial sugar concentration in the fermentation must as some strains perform better than others at a given temperature. Most strains have been reported to perform well in temperature range of 15-25°C. Poor musts may need nutrient supplementation for optimal performance by the wine yeast.

2.7 Vinegar Production

2.7.1 Introduction

The etymology of the word vinegar is derived from two French word *vin* and *aigre* which has the meaning of "sour wine", but now the term is applied to the products of acetous fermentation of ethanol from a variety of sources. Vinegar is defined as "a liquid fit for human consumption, produced from a suitable raw material of agricultural origin, containing starch, sugar, or starch and sugars by the process of double fermentation, alcoholic and acetous, and containing a specific amount of acetic acid" (Joint FAO/WHO Food Standards Programme, 1987). Traditionally vinegar elaboration required maturation in wood. This takes a long time and is relatively expensive. Modern methods involve the use of selected bacterial cultures and continuous aeration systems and result in products of similar quality but which are less expensive and more suited to large-scale commercial production (Troncoso *et al.*, 2002).

The art of wine making and thus, vinegar production is older than 10,000years. It has been claimed that Babylonians prepared vinegar from the sap of date palm, date and from raisin wine and from beer (Conner and Allgier, 1976).

Vinegar has long been used as condiment acidifying and preserving agent, and sometimes diluted and taken as a drink. Tannahill (1973) cited several references to the use of vinegar in food from the time of the Greeks to the 17thcentury and the earliest cookbook in modern

Europe, "*Le Viandier de Taillevent*" compiled in 1375 by Guuillaume Tirel contains a recipe for the preparation of cameline and listed vinegar as one of the ingredients. Recently vinegar has acquired an important role in salad dressing, ketchup and other sauces (Troncoso *et al.*, 2002).

2.7.2. Biochemistry and substrate

Until the beginning of the 1800s it was believed that vinegar was derived from spontaneous acidification of wine, however, in 1864 Pasteur discovered that microorganisms were responsible for producing vinegars. Vinegar is produced by two-stage fermentation process:

(1) Conversion of fermentable sugars to ethanol by yeast (e.g. *Saccharomyces spp*) (equation 2.1, as above).

(2) Oxidation of ethanol by bacteria (*Acetobactor spp)* (Adams, 1998). In the acetic acid fermentation ethanol is oxidised to acetic acid by acetic acid bacteria using atmospheric oxygen. The basic equation is represented as in equation 2.2:

$$C_2H_5OH + O_2 \xrightarrow[oxygen\ (air)]{acetic\ acid\ bacteria} CH_3COOH + H_2O \qquad (2.2)$$

The most common vinegar is alcohol vinegar, which is produced from diluted purified ethyl alcohol, also commonly referred as white vinegar or spirit vinegar. Mashes obtained by alcoholic fermentation of natural sugar-containing liquids also serve as raw materials for vinegar production and the designation of the vinegar depends on the particular raw material, wine vinegar from acetous fermentation of grapes wine, cider vinegar from fermented apple juice, malt vinegar from infusion beer of barley or malt of other cereals, and rice vinegar from saccharified rice starch, following by alcoholic and acetous fermentation (Tesfaye *et al.*, 2002).

Many natural materials do not need addition of extra nutrients but some, which are low in nitrogenous compounds such as apple cider and grape wines, require the addition of ammonium phosphate (Troncoso *et al.*, 2002). Generally, when producing vinegar with up to 15% acetic acid, the acetic acid bacteria will certainly require glucose, potassium, sodium, and ammonium as ammonium phosphate, sulphate and chloride. In addition trace minerals such as

iron, manganese, cobalt, copper, molybdenum, vanadium, and zinc are also needed for optimal acetic acid fermentation. Supplements of yeast extracts may also be required to quickly restart fermentations in event of disturbances such as power failure. However, nutrients should be added sparingly to exert selection pressure directing to a low requirement for nutrients (Tesfaye *et al.*, 2002).

A typical concentration of acetic acid in vinegar is 4% (w/v) although wine vinegar in European countries may contain up to 6%(w/v). In theory one gram of glucose produces 0.67g of acetic acid, but practically 8% (w/v) fermentable sugar is required to produce a minimum strength (4%w/v acetic acid) vinegar (Adams, 1980).

Commercial vinegar is normally produced at higher strength up to 10%(w/v) and sometimes even up to 20% (w/v) by using a strong sugar solution . The vinegar can then be diluted down to the required acidity with water (Adams,1980). The alcohol concentration in the mash (i.e. the alcohol containing solution) is normally expressed as percentage volume per volume (% v/v) whereas the acetic acid in it is expressed in grams of acetic acid per 100mL (% w / v). The sum of ethanol (% v/v) and acetic acid (g $100mL^{-1}$) is called total concentration because this indicates the maximum concentration of acetic acid that can be obtained by complete fermentation. The ratio of the total vinegar concentration over the total mash sugar concentration designates the concentration yield (Troncoso *et al.*, 2002).

2.7.3. Microorganisms

The microorganisms oxidizing ethanol to acetic acid are commonly called acetic acid bacteria (Tesfaye *et al.*, 2002). Two genera of acetic acid bacteria are known in the production of vinegar: *Acetobacter* and *Gluconobacter*. *Acetobacter* oxidize ethanol more strongly than glucose, whereas Gluconobacter oxidize glucose more strongly than ethanol. The oxidation of ethanol by acetic acid bacteria is catalyzed by a two-step reaction: the oxidation of ethanol to acetaldehyde followed by the oxidation of acetaldehyde to acetate. *Acetobacter aceti* was the bacteria frequently used in vinegar industries, however, it has been demonstrated that *Gluconobacter oxydans* subsp. *sphaerricus* is advantageous for the production of vinegar containing a large amount of gluconate (Saeki, 1993). The use of strict and costly sterility controls is generally not necessary and most acetifications are carried out by mixed and often undefined cultures.

2.7.4. Factors affecting vinegar production

Tesfaye *et al.* (2003) classified the factors that affect vinegar elaboration into two main groups: main factors and secondary factors.

Main factors are those involved in the metabolism of acetic acid bacteria and enzymatic activity (Nieto *et al.*, 1993 Takemura *et al.*, 1993; Tesfaye *et al.*, 2003). These are temperature, oxygen supply and substrate supply, and unless they are closely controlled will halt fermentation in a very short interval (Arnold *et al.*, 2002; Tesfaye *et al.*, 2003).

Secondary factors are those, which increase the metabolic efficiency of the acetic acid bacteria and determine the acetification rate and production cost of the finished product (Tesfaye *et al.*, 2003). These include fermenter design, working volume, loading proportion, and loading rate and starter culture. These factors however, are interdependent and will govern the overall aceticification process (Nieto *et al.*, 1993). Each is describe in more detail below.

Substrate

This will play a vital role and its composition and supply rate determines the growth and multiplication of acetic acid bacteria and the quality of the finished vinegar (Tesfaye *et al.*, 2002). The end point of each production cycle is marked by a minimum of ethanol content (0.1-0.3% v/v) (Adams 1998) and which point new substrate is added.

The composition and quality of the base wine (Tesfaye et al., 2003), depends on the raw material from which it is derived (e.g. type and quality of fruit), juice processing conditions, and fermentation conditions. The yeast species used can also affect the acetic fermentation. For example it has been claimed that strains of *Saccharomyces cerevisiae* are not always the best for vinegars base wine (Ciani, 1998). To obtain best results it is necessary to ensure completion of the alcoholic fermentation prior the acetous fermentation.

Generally, wine vinegars are elaborated from uncontaminated wine with an alcohol content of between 10 and 12 % (v/v). The level of total sulphur dioxide in the base wine will severely impact on the acetic acid bacteria (Tesfaye *et al.*, 2000). Base wine for acetifcation to vinegar should not contain more than $10mgL^{-1}$ and $15^{-1}mgL^{-1}$ free and total SO_2 respectively.

Therefore, little SO_2 should be added prior alcoholic fermentation to wines that are specifically for vinegars production, and ideally no SO_2 should be added at the end of alcoholic fermentation (Bartowsky *et al.*, 2003).

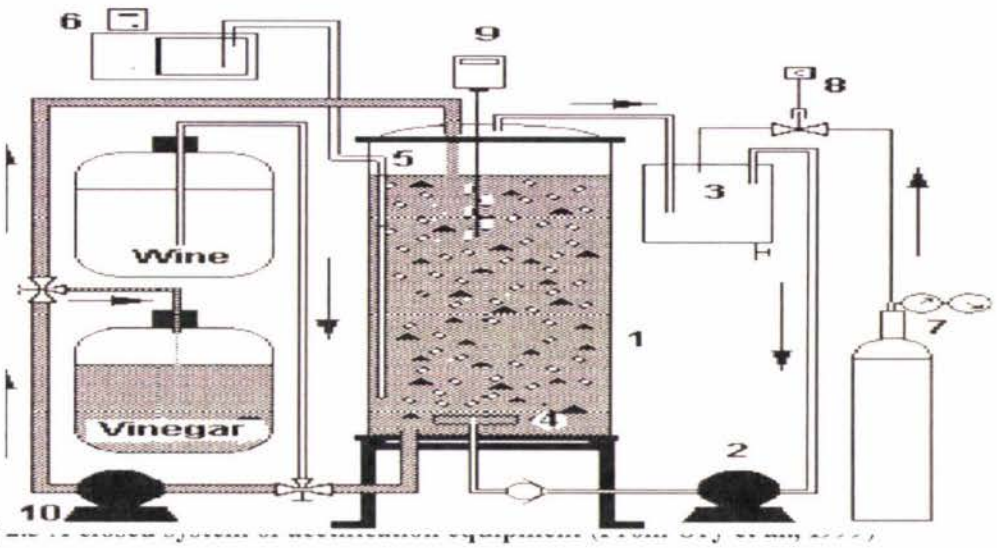
Oxygen supply

Acetification is a strictly aerobic process where fermentation of 1L of ethanol requires about 430L of oxygen at 30°C (Tesfaye *et al.*, 2003). An interruption of oxygen supply for even one minute may stop the fermentation process (De Ley *at al.*, 1984) the effect being more severe when other factors such as temperature and substrate supply are not at their optimal values (Adams 1998; Nieto *et al.*, 1993).

The oxygen transfer rate and purity of the introduced air all affect the efficiency of acetification. The extent of oxygen consumption in the fermenter and hence oxygen concentration in the broth depends on the stage of the process (Nieto *et al.*, 1993). Romero *et al.*, 1994) found that the maximum growth rate of acetic acid bacteria is obtained at oxygen values between 1 and 3ppm and the rate drops to 10% for the maximum value for oxygen concentrations around saturation (around 8ppm). Recent research has focused on improving diffusion rates and developing better mixing systems in reactors (Fregapane *et al.*, 1999). The submerged technique for vinegar elaboration is the subject of many patents. (Ory *et al.*, 1999) covering such devices as the Frings acetifier, which allows large volume fermentation with a low air up take. It has an excellent oxygen transfer rate and thus high fermentation efficiency, which ensures increased yield (Troncoso *et al.*, 2002). Other popular acetification systems include the tower or bubble column fermenters, the cavitator, and the Jet fermenter (Ory *et al.*, 1999).

When systems are open to the atmosphere high evaporation losses of volatile compounds, such as ethanol, acetic acid or ethyl acetate occur with the subsequent reduction of yield (10-30%) and increases in operational costs (Romero and Cantero, 1998). To solve this problem, gas re-circulation systems have recently been developed (Gomez *et al.*, 1994). These systems are fitted with a closed gas recycling system, in which the volatile compounds in the gas stream is impelled by air pump to the bottom of the fermenter after passing through an expansion tank; they are admitted again into the broth through two sinterized stainless steel diffusers. The reactor is therefore fully closed to the atmosphere, oxygen being supplied from industrial

oxygen cylinder (and there is no gas that leaves the system (Ory *et al.*, 1999). The system is illustrated in Fig 2.5.



Fig

Notes: 1 Reactor
2 Gas recycling pump
3 Expansion tank
4 Air diffusers
5 Heat exchange
6 Thermostatic bath
7 Oxygen cylinders N-50
8 Electrovalve ON/OFF
9 Dissolved oxygen sensor
10 Feed inlet and effluent outlet

Temperature

Acetification is an exothermic reaction where 8.4MJ of heat is produced for every litre of ethanol oxidised (Adams 1998). It has been demonstrated that the optimal temperature for acetification is 30-31°C (Ory *et al.*, 1998). Excess temperature will kill the acetic acid bacteria or limit enzyme activity, and besides, losses of ethanol and volatile compounds will increase at high temperature, lowering both yield and quality (Tesfaye *et al.*, 2003).

Working volume

In both surface and submerged culture acetification systems the working volume affects the performance of the acetic acid bacteria. The working volume is always less than total capacity of the fermenter to allow adequate aeration, i.e. to accommodate gas hold up and foaming. In surface culture, typically 1/3 of the full capacity of the fermenter is recommended (Tesfaye *et al.*, 2003) whilst in submerged culture 2/3 of total volume has demonstrated to be optimal (Tesfaye *et al.*, 2000).

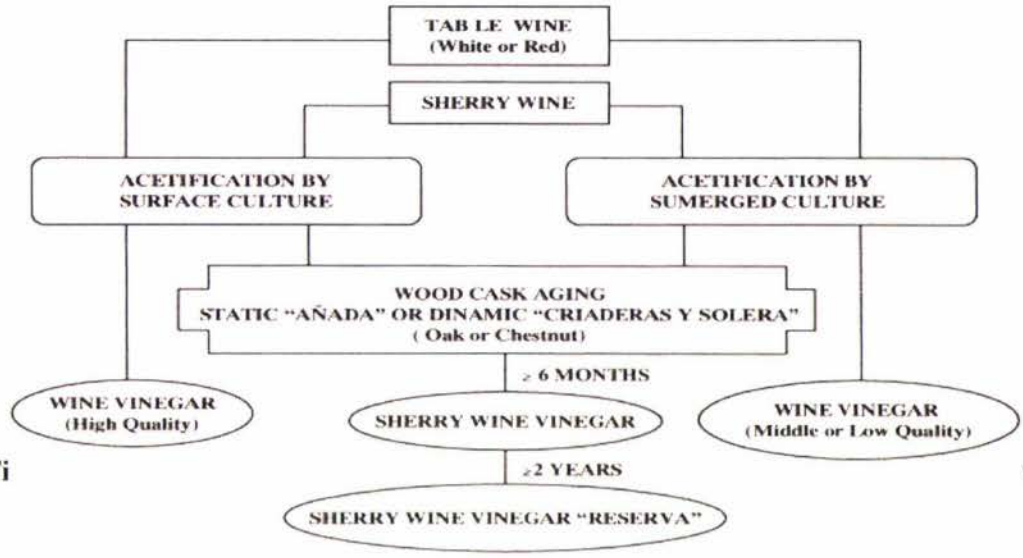
The unloading proportion

In semi continuous batch production, better rates of acetification were observed when fermenters were unloaded by half of their finished product (Nieto *et al.*, 1993; Tesfaye *et al.*, 2000), i.e. at the end of every cycle typically half of the reactor's total volume is unloaded and the remaining volume is normally used as an inoculation for the subsequent cycle. The loading proportion of substrate is directly related to the concentration of alcohol, acetic acid and the sum of both (Tesfaye *et al.*, 2002). Thus, the concentration of ethanol in the medium as well as the final metabolic product (acetic acid) must be controlled and maintained within certain limits since an elevated concentration of these will inhibit bacterial growth (Romero *et al.*, 1994). The absence of ethanol leads to the death of part of the culture and acetate peroxidation may occur when the bacteria use acetic acid as a carbon source for its own metabolism leading to formation of carbon dioxide and water (Saeki *et al.*, 1997).

It is recommended that loading of substrate must be done when ethanol concentration is 0.1-03% (w/v. It has been reported that in a laboratory fermenter, both the fermentation rate and the fermentation yield of the process increased when the loading proportion of the substrate was 1:1) (Adams, 1998; Tesfaye *et al.*, 2000 and Tesfaye, *et al.*, 2000).

2.7.5 Industrial Processes

Early production systems used trickling filters, but submerged systems are more commonplace in modern processing (Troncoso *et al.*, 2002) point of view there are two, commonly used, methods for vinegar production, the traditional slow surface process and the rapid modern submerged process. Troncoso *et al.* (2002) summarised the two methods for vinegar production as shown in Fig 2.6.



Fi **'ation, surface**

Semi-continuous submerged vinegar fermentation

The submerged culture system implies that the acetic acid bacteria are suspended in the acetifying liquid in which a strong aeration is applied to satisfy the metabolic oxygen demand. It is used for the elaboration of most commercial vinegars for consumption (Troncoso *et al.*, 1998). The system consists of stainless steel fermentation tanks typically with a capacity of 10,000 to 40,000 L, air supply and cooling systems foam controlling systems and loading and unloading valves (Nieto *et al.*, 1993). Fig 2.6 below illustrates three typical submerged systems used for elaboration of vinegars.

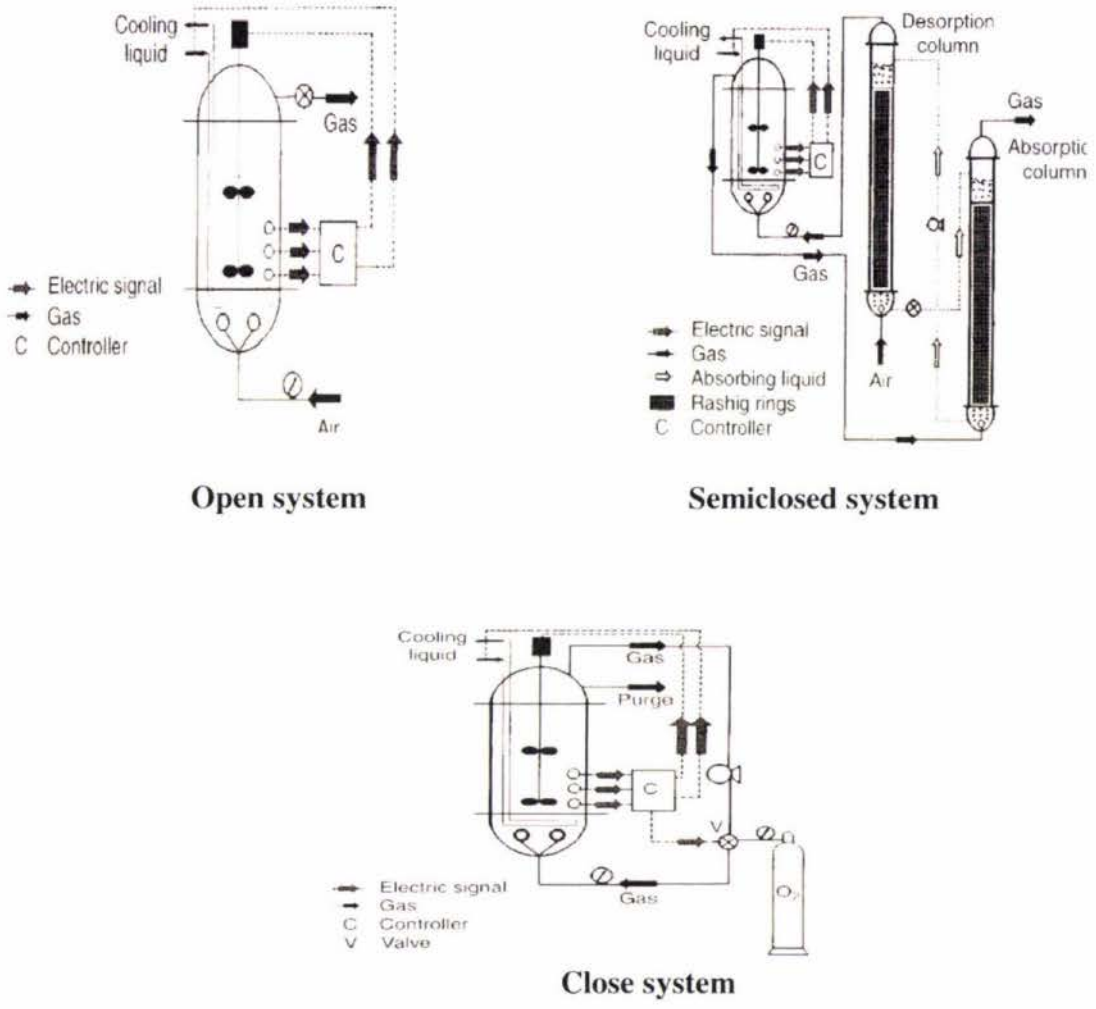


Fig 2.7 Typical submerged systems for vinegar elaboration (from Romero and Cantero, 1998)

The open system consists of an automatic, thermostatically controlled fermenter equipped with both mechanical agitation and aeration completely open to the atmosphere. The semiclosed system is similar to the open system but adapted by adding two absorption columns. The gas out flow from the fermenter is passed through the absorption column which contains water, this water is then recirculated through the desorption column through which the clear air is introduced into the fermenter and recirculated by means of peristaltic pump. An electronic gauge controls the level of water in the pump. The closed system consists of a fermenter that operates in a closed gas circuit, thus preventing the leakage of volatiles into the gas outflow.

Discrete quantities of O_2 gas are injected into the recirculation gas to compensate for the consumption in the fermenter

Table 2.7 give data for the systems discussed above for at laboratory, pilot plant and industrial scale

Table 2.7 Typical data for acetic fermentation parameters for laboratory, pilot plant and industrial scale (modified from Romero and Cantero, 1998)

Scale	System	Temperature (°C)	Air (vvm)	Initial ethanol %(w/v)	Final ethanol (w/v)	Initial acetic acid %(w/v)	Final acetic acid %(w/v)
Laboratory							
5L	Open 1	26	0.2	8.0	2.5	0.04	3.3
	Semiclosed	26	0.2	8.0	2.5	0.04	3.3
	Open 2	26	0.5	8.0	0.0	0.15	5.8
	Semiclosed	26	0.5	8.0	0.0	0.15	5.8
Pilot plant							
(1,000L	Open 1	30	0.2	4.8	1.5	4.0	7.4
	Closed	28	0.2	4.8	2.2	4.0	7.0
Industrial	Open 1	20	0.0032	5.0	2.0	4.0	8.0
	Open 2	25	0.0032	5.0	2.0	4.0	8.0

From these data it is likely the closed system even at unfavorable conditions, is the most effective in avoiding loss of ethanol and high efficiency and productivity. The semiclosed system seems to be suitable at laboratory scale at low temperature and aeration rate (Romero and Cantero, 1998).

In recent years it has been possible to produce vinegar of up to 19% acetic acid in a modified single stage process (Tesfaye *et al.*, 2002) concentration as mentioned above alcohol is slowly added at a constant alcohol level of 2 to 3% in the fermentation liquid creating a corresponding increase of the total concentration. Addition of alcohol is stopped when the

desired total concentration has been reached. When the concentration of ethanol approaches zero, part of the fermentation liquid is discharged and replaced with mash with lower total concentration to bring the acetic acid concentration and total concentration back to the starting conditions, thereby enabling the bacteria to multiply faster. Later total concentration is increased further by addition of alcohol rich must (Tesfaye *et al.*, 2002).

Continuous fermentation submerged vinegar fermentation

Continuous fermentation is only possible up to a maximum of 9 to 10% acetic acid because the specific growth rate of the bacteria decreases with the decreasing ethanol concentration, and to obtain high yield the fermentation must be carried out at a low alcohol concentration. In this system the biotransformed product is removed continuously and feed is added at the same rate, which provides a constant composition with time. The success of the process depends on the maintenance of the bacterial culture in the exponential phase of growth. To do this the medium must provide the nutrients and oxygen necessary for the survival of the bacteria (Troncoso *et al.*, 1998).

Vinegars with a higher percentage of acetic acid (more than 15%), can be obtained using a two-stage process (Troncoso *et al.*, 2002). In the canning industry vinegar of high acidity is required in order to save storage and transport costs. During submerged fermentation with a total concentration below 15% in the first fermenter, alcohol is added slowly to increase the total concentration up to about 18.5%. After the acetic acid has reached 15%, about 30% of the fermenting liquid is transferred into a second fermenter. The first fermenter is re-supplied with a new mash of lower total concentration. In the second fermenter, the fermentation continues until the alcohol is almost depleted. The whole quantity of the finished vinegar is discharged. The fermentation liquid in the first fermenter is supplied with alcohol at the appropriate time and later divided again (Michael and Stephen, 1999).

Since the end of 1993, vinegar of more than 20% acetic acid has been produced using this process. The means of automatically controlling the process was developed by Enenkel (1988). In 1981, a similar two stage process for the production of vinegar was described (Kunimatsu *et al.*, 1981) in which the second fermentation stage was carried out at a reduced temperature of only 18 to 24° C, whereas in the first fermentation stage, the more common temperature range of 27 to 32° C was used (Tesfaye *et al.*, 2002).

Surface fermentation

In the surface culture fermentation process the acetic acid bacteria are grown at the air liquid interface in a direct contact with the atmospheric air. The presence of the bacteria is limited to the surface of the acidifying liquid and it is thus considered as a static method. This method, which is currently employed for the production of traditional and specialty vinegars, requires very long time to obtain a high degree of acetification and as a result production costs are high (Troncoso *et al.*, 1998).

However, a new approach to surface fermentation has been tried (Toda *et al.*, 1990) in which a slow, horizontal flow of the medium was introduced under a bacterial film with a surface of a few hundred cm^2 was used. Liquid depth was limited to 10mm or less and the acetic acid concentration in the effluent was 5.76%. The oxygen absorption rate through the microbial film was found to be very high. Another surface fermentation process was described (Kaisa, 1973). The liquid depth under the layer of acetic acid bacteria was about 50mm. The inflow liquid had 2% acidity and contained 3.5% alcohol. The acidity of the effluent stabilised at 5% acetic acid. The residence time was about 21h. Continuous surface fermentation in a single vessel with a working volume of 16 L was subsequently reported (Kaisa, 1984). Under optimal conditions with a liquid depth of 100mm, 2 to 3% acidity and 20 to 30 g L^{-1} alcohol concentration of the inflow liquid, vinegar with 4.5% acetic acid was produced during 80 days. The flavour of this vinegar was claimed to be better than that of vinegar produced by other processes (Tesfaye *et al.*, 2002). As a consequence of the effort to produced rice vinegar at low acidity with better quality, surface fermentation is being developed in Japan into an automated process at low cost (Tesfaye *et al.*, 2002).

2.7.6. Factors that affect the quality of vinegar

The chemical, physico-chemical composition and sensory properties of vinegar are influenced by three main factors namely: elaboration techniques, the raw material used as substrate and the duration of aging (if any) in wood. As mentioned earlier, vinegar is produced from a wide range of raw materials and thus, there are diverse types of vinegar. Each type of vinegar contains chemical compounds derived from the raw material used as substrate. Therefore, for each type of vinegar it is important to determine which is the factor that most contributes to the final quality, either the elaboration technique or the raw material (Garcia- Parrilla *et al.*, 1997).

Ageing of vinegar in wood

Traditionally, the type and amount of aromatic compounds present in it, rather than its nutritional value, judge the quality of vinegars. The constituents formed during both the alcoholic and acetous fermentation determine the flavour of vinegar. But also the aroma compounds evolved during ageing in wood for specified periods of time decisively impacts on the quality of the final vinegar. During the ageing of sherry wine vinegar in wood, only methyl acetate, ethanol, diacetyl acetate and ethyl acetate changed significantly within six months (Morales *et al.*, 2002). Significant increases of methyl acetate, methanol, diacetyl, acetoin, hydroxyacetone, butyrolactone, and diethyl succinate, methyl acetate, ethyl acetate, methanol, ethanol, diacetyl, 2-methyl-1-propanol, isoamyl alcohols (2-methyl-1-butanol and 3-methyl-1-butanol butyrolactone, and 2-phenylethanol occur after 24 months.

2.7.7 Volatiles compounds in vinegar

Organoleptic properties such as flavour or aroma are important in characterising and differentiate different kinds of vinegars (Signore, 2000).

After extraction and concentration, the volatile compounds are either identified with gas chromatography coupled with mass spectrometry (GC-MS) or gas chromatography – olfactometry (GC-O). Major volatile components samples may be injected directly into the chromatograph whereas the minor components may need to be fractionated for example by using simultaneous distillation extraction techniques (SDE). The high concentration of acetic acid and water are the main interfering substances in the chromatograph, which ought to be eliminated (Gracia *et al.*, 1992).

The major group of volatile compounds identified in vinegars includes alcohols, carbonyl compounds, esters and volatile acids. The concentration of these in vinegars is affected by both composition of the base wine, acetification process and whether the vinegar is aged in wood or not. The major compounds identified in traditional vinegars (Balsamic and sherry vinegars, and conventional vinegars (e.g. wine vinegars) are summarised in Table 2.8.

Table 2.8: Major volatile compounds identified in conventional and traditional vinegars

Major volatiles	Conventional vinegars	Traditional vinegars	Reference
Alcohols			Gracia, (1992)
	Ethanol		Signore,(2000)
	Propan -2-ol		
		Propan-1-ol	Signore,(2000)
		Isobutyl alcohol	Signore,(2000)
		Isoamyl alcohol	Signore,(2000)
		Hexan-1-ol	Gracia, (1992)
		Methyl-1-butanol	Gracia, (1992)
		Pentan-3-ol	Gracia, (1992)
	Methyl-2-propan-1-ol	Methyl-2-propan-1-ol	Gracia, (1992)
	Butan-2,3-diol	Butan-2,3-diol	Natera et al.(2003)
		Octanol	Signore,(2000)
Carbonyl compounds			
	Acetaldehyde	Diacetyl	Gracia, (1992)
		Hydroxy-3-butan-2-one	Gracia, (1992)
		Furan-2-carboxyaldehyde	Gracia, (1992)
		Benzaldehyde	Gracia, (1992)
		Hexanal	Gracia, (1992)
		Heptanal	Gracia, (1992)
Esters			
		Ethyl acetate	Troncoso, (2001)
	Ethyl -2-methylpropanoate	Ethyl -2-methylpropanoate	Gracia, (1992)
	ethylbutanoate	Ethylbutanoate	Gracia, (1992)
	Phenyl acetate	Phenyl acetate	Gracia, (1992)
	Isoamyl acetate	Isoamyl acetate	Natera et al.(2003)
	Diethyl succinate	Diethyl succinate	Natera et al.(2003
	Methyl octanoate	Methyl octanoate	
Volatile acids			Troncoso, (2001)
	Acetic acid	Acetic acid	Troncoso, (2001)
	Methyl-3-butanoic acid	Methyl-3-butanoic acid	Troncoso, (2001)
	Isopentanoic acid		Natera et al.(2003)
	Decanoic acid	Decanoic acid	Natera et al.(2003

2.7.8. Fruit vinegars

In general, most fruit have been used for production of vinegars as a way of using excess or waste fruit; despite of this, there are only few reports in literature about kiwifruit vinegars. Fruit vinegars reported in literature include: cider apple vinegar and kiwifruit vinegars, and vinegars from mango, pineapple, and guava, banana, tamarind, camias and santol (Molina, 1981; Karuwanna *et al.*, 1987; Ethira, 1992; Kim lee, 2000; Bortolini, *et al.*, 2003; Xiuzhu *et al.*, 2003).

For the fruit processing industry, vinegar production is a wonderful way of maximizing profit from economical utilization of the raw material, even from culled fruit or fruit waste. For example vinegars were produced from mango peels and cores, and from pineapple peels and cores (Karuwanna *et al.*, 1987; Ethira, 1992).

Kiwifruit (*Actinidia deliciosa* 'Hayward') vinegar was produced using submerged and vertical generator methods (Bortolini, *et al.*, 2003). The juice was treated in various ways: natural juice, juice supplemented with ammonium or magnesium sulphate and enriched with sucrose up to 18°Brix and 22 °Brix, were fermented to wine and the wine acetified in a vertical generator and submerged culture at 20-25°C.

The submerged culture was carried out in a 2L fermenter at 25°C with oxygen supply at 0.05vvm and agitation at 500rpm. Half of the starting volume was discharged every 12h and the fermenter topped with same volume (Bortolini, *et al.*, 2003). The submerged culture fermentation was completed within 12h producing 1.00 and 1.78% (p/v) acetic acid, at an efficiency of 93-98% and productivity 0.83- 1.73g/L.h. Sensory analysis showed that vinegars obtained by the vertical generator were better than those obtained by submerged culture with acceptability indexes above 70% (panel score) (Bortolini, *et al.*, 2003)

Kiwifruit beverage has been formulated using kiwifruit vinegar in China. Kiwifruit vinegar was prepared by the liquid-state fermentation and used to make an acetic acid beverage. The quality of the kiwifruit beverage was found to be high in terms of aroma and colour. Single factor and orthogonally designed experiments revealed that the optimal composition of 100 ml kiwifruit beverage included 27 ml kiwifruit vinegar, 4.3 mg sweetening agent, 27 mg malic

acid, 15.9 mg citric acid, 12 ml ginger juice, 51 mg calcium lactate, 41.5 mg salt, 31 mg sodium cyclamate and 5 mg apple ethyl maltol (Xiuzhu *et al.*, 2003)

2.8. Overall conclusion

Fruit vinegar processing in fruit processing industries is a feasible means of adding value through from the utilisation of inferior fruits and fruit wastes. Kiwifruit is an exceptional source of vitamin C and arginine, and contain good levels of sugar and minerals. Kiwifruit has been described as having a good balance of nutrients and vinegar is likely to be produced from it without any nutrient supplementation. 'Hayward' kiwifruit has successfully been used to produce wine and there is evidence in literature that it can produce suitable vinegar. Gold kiwifruit volume is expected to increase, so there is a need of other products and vinegar is a good option to explore. Juicing, alcoholic fermentation and acetic acid fermentation, all influence the final quality of vinegar. This work has been undertaken to provide preliminary information, on these factors with a view of promoting the utilisation of the crop for production of bulk vinegars or vinegar that might occupy a especially niche in the fruit vinegar market.

3. Materials and methods

3.1 Materials

3.1.1 Fruit

Zespri™ International Ltd (Mt Maunganui NZ) supplied all the fruit (Gold kiwifruit, *Actinidia chinensis*, Hort16A) used in this study. Some of the fruit were not ripe enough for juice extraction and these were ripened by mixing them with ripe apples in the (number) ratio of 3: 1 and storing them at ambient temperature for 2-3 days prior to processing. The firmness and total soluble solids for the fruit processed were about 14.7±3.7N (measured using a penetrometer; HortPlus, NZ) and 15.4±0.59 Brix (by Abbe refractometer), respectively.

3.1.2 Enzymes

Pre-pressing (macerating) enzyme, Celluzyme® LX, and post-pressing (clarifying) enzyme, Kleerase® 100XL were purchased from Zymus International Ltd, Auckland. Celluzyme® LX is of food grade standardized for pectinase, hemicellulase and cellulase activity. The enzyme is derived from controlled fermentation of selected strains of *Aspergillus niger* and *Trichoderma reesei*. It is permitted for general use as a processing aid under FSANZ standard 1.3.3 E.C 3.2.15, 3.2.1.4. Kleerase® 100XL is a food grade pectic enzyme system obtained from non-genetically modified *Aspergillus niger*. These enzymes hydrolyse both soluble and colloidal pectic substances to reduce viscosity of the juice; it also contains arabinase sufficient to hydrolyse most arabans present in most fruit. This is permitted for general use as a food processing aid under FSANZ standard 1.3.3. E. C. 3.2.15.

3.1.3 Press aid

Press aid ('Celite' diatomaceous earth, 580) was purchased from Mintech New Zealand Ltd, Auckland.

3.1.4 Wine yeast

Active dry wine yeast, *Saccharomyces cerevisiae* var. *bayanus*, (Maurivin® PDM) was purchased from Mauri Yeast Australia Ltd. Yeast sachets were stored in a fridge at 4°C.

3.1.5 Vinegar culture

Mother vinegar culture was purchased from Coral Tree Organic Products Ltd (Waihou Road Levin NZ) and stored in a cold room at 4°C. This is organic apple cider vinegar, which has not been pasteurised, containing 4.9% w/v acetic acid.

3.1.6 Chemicals

All reagents were of analytical grade and obtained from Sigma Company U.S.A and BDH Chemicals Ltd, (England), unless otherwise specified.

3.2 Processing Methods

3.2.1. Juice extraction

Zespri™ Gold kiwifruit of eating ripeness (14.7±3.7 N) were pulped in a hammer mill and treated with the pre-pressing (macerating) enzyme (Celluzyme®) for a given time at a given temperature before samples of 5kg were pressed each time in a hydraulic press (Fig 3.1). Sulphur dioxide in the form of potassium metabisulphate was also added at 30-50mg.L^{-1} to prevent oxidation during pulp holding time and, separately, in the juice. In some treatments press aid ('Celite' diatomaceous earth, 580) was added immediately before pressing.

The pressure employed in the press was approximately 250MPa, for 10 to 12 minutes each cycle. The pressure was built gradually by manual pumping using the handle (Fig 3.1). The inside of the press was covered with cheesecloth to effectively retain the pulp. The juice was collected at the end of each cycle and metered using a measuring cylinder. Juice yield was expressed as liters of juice per kg of fruit mass (pulp).

Fig 3.1 Laboratory hydraulic press

3.2.2 Alcoholic fermentation

Gold kiwifruit juice prepared as above was fermented in 5L laboratory glass fermenters at 20, 30 or 37°C. Maurivin® PDM yeast was added at 2% v/v. Preliminary results showed that fermentation at 20°C gave good results and therefore later experiments were conducted at 20°C. These included fermentation of must which had been ameliorated by adding sugar to increase the soluble solids to 18° or 20°B, free-run versus press juice, skin on versus skin off juice, as well as fermentation of filtered and unfiltered juice.

During fermentation, cell growth was determined by plate count using potato dextrose agar ($39g.L^{-1}$, Biolab Ltd, NZ). The agar was boiled, autoclaved and then cooled to 50°C in a water bath before pouring. Standard peptone water ($5g.L^{-1}$, Biolab Ltd, NZ) was prepared in the same way. Ten mL of must was thoroughly mixed with 90mL of peptone water and the mixture was serially diluted to 10^{-6}. One mL of each dilution was then spread on solidified agar with a sterilised spreader and incubated at 20°C for 72h. Counting of the colony forming units was done with the aid of a magnifying glass and a maker pen.

During fermentation samples were taken every 24h and analysed for ethanol and residual sugars by HPLC (see sections 3.3.5-6), and total soluble solids, pH and TA as per section 3.3.2.

Ethanol yield was calculated according Bortolini *et al.* (2003):

$$\text{Ethanol yield}\,(\%) = \frac{\text{Ethanol produced}\quad (g/L)}{\text{Total sugars consumed}\quad (g/L)} \times 100 \qquad (3.1)$$

Efficiency of alcoholic fermentation was calculated by comparing the experimental yield to the theoretical value calculated from the stoichiometric equation (equation 2).

$$C_6H_{12}O_6 \xrightarrow{yeast} 2\,C_2H_5OH + 2\,CO_2 \qquad (3.2)$$

Glucose/ Fructose	Ethanol	Carbon dioxide
MW 180	46	44

From this equation 1g of fermentable sugars produces 0.511g of ethanol (Adams, 1980).

Efficiency of fermentation is given by equation 3

$$\textit{Efficiency}\,(\%) = \frac{\text{Experimental yield}}{51.1} \times 100 \qquad (3.3)$$

Productivity was calculated as the amount of ethanol produced per total fermentation time ($g\ L^{-1}h^{-1}$) according to Bortolini *et al.* (2003)

3.2.3 Acetic acid fermentation

A start up protocol tailored to Zespri™ gold kiwifruit wines was developed for semi-continuous submerged culture fermentation.

A 2L Duran bottle was used as a fermenter. This was maintained at 29 ± 2°C in a water bath and connected to two Quickfit glass condensers in series (Fig 3.2). Circulation of chilled water (0°C) in the condenser was maintained by a Hi-tech aquarium pump (Guang Dong Risheng Group Co. Ltd, Shenzen, China), which pumped the water from an ice slurry tank through the

condensers (Fig 3.2). Enriched air (40±3% O_2) was achieved using a gas mixer in which commercial oxygen (BOC Gas, PN) and compressed air flow rates were set at a ratio of 1:3 and this was distributed in the reactor using a 30cm long High-tech aquarium air diffuser set at the bottom of the reactor. The flow rate of the gas mixture was set at 0.5 or 0.8Lmin^{-1}.

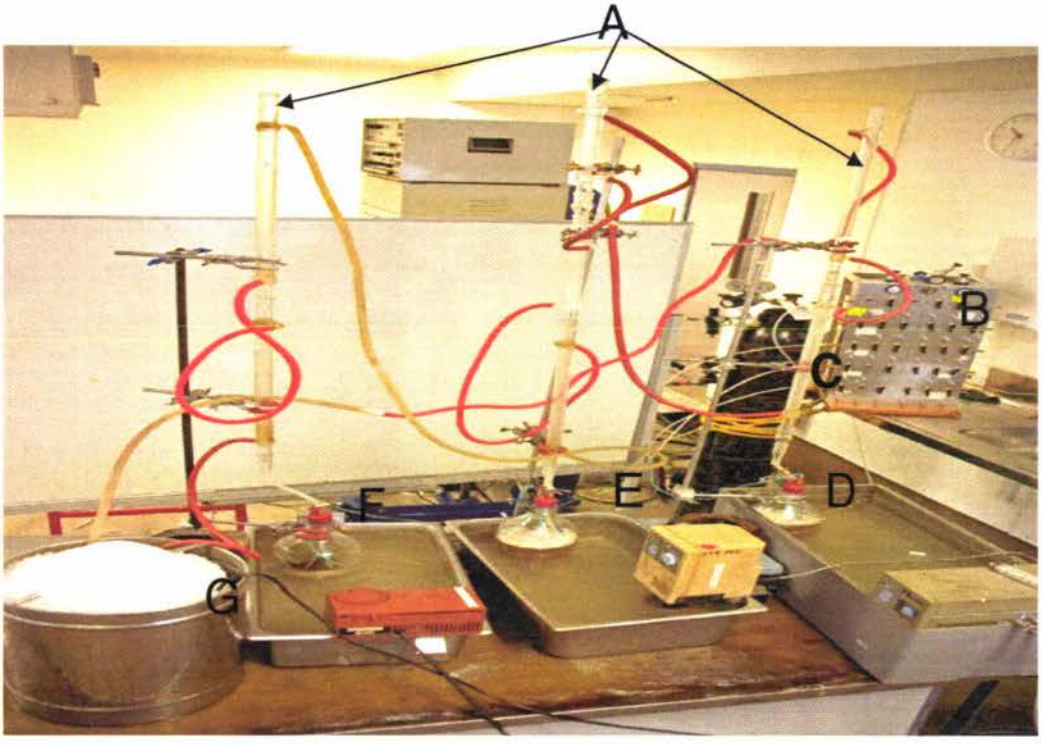


Fig 3.2: Set up for acetic fermentation

Legend: A = Quickfit condensers (3 in series), B= Gas mixer, C= Oxygen tank, D- F = Reactors, G = Ice slurry tank

The substrate for vinegar production was Zespri™ gold kiwifruit wine with 7.5±0.5% w/v ethanol and the mother culture was cider vinegar (Coral Tree Organic Products Ltd, Waihou Road, Levin, New Zealand).

The protocol was developed by maintaining ethanol and acetic acid in the ratio of 1:1.3 at the beginning of fermentation. Fermentation continued until ethanol fell to 0.5%w/v, then 50% or more of the initial volume was decanted and an appropriate volume of the same wine was added to restore the same ratio of ethanol and acetic. This was repeated until a final acetic acid concentration of about 4.5%w/v was obtained (see section 6.2.3)

Estimation of biomass in the acetic acid fermentation was done spectrophotometerically (Pharmacia Ultra Spectrophotometer, USA) by measuring optical density of the fermenting must at 650nm every 24h. The mass concentration of the bacteria was estimated using a calibration curve prepared earlier using the classical gravimetric technique.

To prepare the calibration curve, cider mother vinegar was mixed with Zespri ^{TM} gold kiwifruit wine to obtain ethanol and acetic acid in the ratio of 1:1.3 and fermented under conditions detailed above. Samples were taken at the exponential stage (just before ethanol drops to 0.5%) and 20ml of this culture was serially diluted to 10^{-4} Optical density was measured using the spectrophotometer for the sample and each dilution. Aliquots (10-30 ml) of the sample and each dilution were then centrifuged in 50ml tubes at 5,000 rpm for 10min. The supernatants were discarded and the sedimented bacteria were re-suspended in deionised water, and then re-centrifuged. The precipitates were quantitatively washed into glass weighing bottles, which had previously tared after being dried for at least 2 hours at 105 ±2°C before being cooled in a desiccator, covered and weighed. The precipitates were dried in an oven at 105°C overnight and were then weighed to obtain the cell dry weight. A calibration curve was then prepared by plotting the optical densities versus the dry weight. The calibration is included in Appendix 1.

3.3 Analytical methods

3.3.1 Determination of insoluble cloud matter, alcohol insoluble solids and total pectin in kiwifruit juice

Insoluble cloud matter

Enzyme treated juice, prepared as per section 3.2.1, was stored at –40°C. After thawing at room temperature, 15mL of each sample was centrifuged at 27,000 rpm for 15min. The juice serum was then decanted to obtain the insoluble cloud matter (ICM). The precipitate was re-dispersed in distilled water, stirred and centrifuged three times, and then freeze dried overnight and weighed to obtain dry matter (DM) content of the juice.

Alcohol insoluble solids

To separate the alcohol insoluble solids (AIS) from the serum obtained above, analytical ethanol was added up to 80% (v/v) (by mixing 53ml of 95% ethanol with 10ml of the serum) and the mixture was centrifuged at 15,000 rpm for 15min. The supernatant was then decanted and the precipitate re-dispersed in ethanol (80%) and centrifuged three times. The precipitate was freeze dried overnight and weighed to obtain the alcohol insoluble solids.

Total pectin (TPn)

Determination of total pectin (as galacturonic acid) was done by colorimetric assay using m-hydroxydiphenyl reagent according to Blumenkrantz and Asboe-hansen (1973), with the following modifications.

Galacturonic standard solutions: 10mg of pure D-galacturonic acid (Sigma Company, USA) was dissolved in 100mL of distilled water to make 100-$\mu gm.L^{-1}$. Dilutions were made from this solution to contain 5 to 75μgmL^{-1} of galacturonic acid by transferring 5, 15, 25, 35, 45, 55, 65, and 75mL into a 100mL volumetric flask and making up to volume with distilled water.

Meta-hydroxydiphenyl solution: A solution of m- hydroxydiphenyl (0.15%) was prepared by dissolving 0.15g of (99%) (Sigma Aldrich Chemie, Germany) in 98.85mL of distilled water

Sulphuric acid / sodium tetraborate solution: Sulphuric acid / sodium tetraborate solution (0.00125M) was prepared by dissolving 0.48g of disodium tetraborate crystals (BDH Chemicals ltd, England) in 100ml of 0.1M sulphuric acid

To measure total pectin, 5mg of the AIS obtained above was weighed into a 20mL beaker containing a magnetic stirrer bar. 2mL of chilled concentrated sulphuric acid was added dropwise and stirred for 5min. The beaker was then placed in a water-ice bath sited on a magnetic stirrer. The content of the beaker was stirred gently as 0.5mL of distilled water was added dropwise. The mixture was allowed to stir for 5min before a further 0.5mL of distilled water was added to ensure complete dissolution. The sample was transferred into a 10mL volumetric flask that was filled to volume through repeated water washing of the beaker.

Then, 2mL of the sample or standard was transferred into a test tube and 1.2mL of 0.00125M solution of tetraborate in concentrated sulphuric acid was added. The tubes were refrigerated in crushed ice for 5 min. The mixture was shaken in a vortex mixer at moderate speed with the mixer being stopped several times during mixing to ensure thorough mixing of the content. The tubes were then heated in a water bath at 100°C for 5min. After cooling in a water-ice bath, 0.2mL of the 0.15% *m*-hydroxydiphenyl reagent was added. The tubes were agitated and for 5 min and allowed to settle at room temperature for 15min and absorbance was measured at 520nm in a Beckman DU spectrophotometer (Beckman, USA)

Since carbohydrates produce a pinkish chromogen with sulphuric acid-tetraborate solution at 100°C, a blank sample was run without addition of the reagent, which was replaced by 0.2mL of 0.5% NaOH. The absorbance of the blank sample was subtracted from the total absorbance. A calibration curve was constructed using the absorbance of the standards and total pectin was calculated from the calibration curve (in Appendix 1).

3.3.2. Total soluble solids, pH, and titratable acidity and dissolved oxygen

The soluble solids (Brix) were determined using an Abbe Refractometer in a reflection mode. The pH was determined using a pH meter (model SA 520; Orion Research Incorporated, USA).

Titratable acidity was determined by titration using a method described by Amerine and Ough (1988) with the following modifications. 50mL sample of kiwifruit juice or wine was first vacuum degassed and then, while being continuously, stirred 0.1M sodium hydroxide was added slowly to an end point of pH 8.2. The titratable acidity was calculated as grams per litre of citric acid (equation 4).

$$\text{TA (g/L as citric acid)} = \frac{V \times 0.1 \times 64}{(v)} \qquad (3.4)$$

Where: V = volume of sodium hydroxide solution used for titration (mL)

v = sample volume (mL),

0.1 = normality of sodium hydroxide,

64 = gram equivalent weight of citric acid

Dissolved oxygen (DO) during the acetic acid fermentation was measured using a YSI Model 57 Oxygen Meter (Yellow Spring Instrument Co. In. Yellow Springs, Ohio, USA).

3.3.3 Determination of total phenolics (TP)

The Folin-Cioalteau method for TP relies on elective oxidation (Slinkard and Singleton, 1977). However, easily oxidised substances, when present, could contribute to the apparent total polyphenolic content. These potential competing substances include aromatic amines, sulphur dioxide, ascorbic acid and other endiols, and reductones and ferrous ion (Singleton and Rossi 1965, Slinkard and Singleton, 1977). An attempt to measure TP in kiwifruit juice and wine using the Folin-Cioalteau method appeared to overestimate the amount of TP, probably due to the high levels of ascorbic acid in kiwifruit. Therefore, kiwifruit juice and wine were treated with bromine to remove ascorbic acid before analysis using the Folin-Cioalteau method. Juice samples were diluted 10 times with distilled water; 5mL of the diluted juice was added into a 10mL conical flask and 2-3 drops of bromine was added and shaken until the mixture turned pink. Nitrogen gas was passed into the mixture for 3-5min to remove bromine and obtain a clear juice.

Gallic acid stock solution was prepared by dissolving 0.50g of dry gallic acid in 10mL of ethanol (80%) in a 100mL volumetric flask and then making to volume with distilled water.

Sodium carbonate solution was prepared by dissolving 200g of anhydrous sodium carbonate in 800mL of distilled water. The solution was boiled and after cooling, a few crystals of sodium carbonate were added. The solution was then left at ambient temperature for 24 hours, then filtered using a (Watman no.14) filter paper and make up to 1L using distilled water.

The phenol stock solution was added at 0, 1, 2, 3, 5, 10 mL into 100mL volumetric flasks, and then diluted to volume with distilled water to give phenol concentrations of 0, 50, 150, 150, 250 and 500mg of gallic acid equivalent, respectively.

The Folin-Cioalteau method was adopted from that used by (Alonso *et al.* (2002), which involves consecutive introduction of the following chemicals in the reactor (25mL flask):

25µL of sample or standard, 12.5mL of distilled water, 1.25mL of Folin-Cioalteau reagent and 5mL of a solution of sodium carbonate at 20%, and then distilled water to volume. The solution was agitated to homogenise, then left to stand for 30min for the reactions to take place and stabilise. Absorbance was determined in a 1cm cuvette at 750nm. A standard curve was constructed by plotting the absorbance of the stock phenol solutions against their concentrations and the concentration of phenolics in the juice or wine samples were calculated by comparing their absorbance to the standard curve (in Appendix 1).

3.3.4 Determination of sulphur dioxide in gold kiwifruit wine

Both free and bound sulphur dioxide was determined by the aspiration method. This involves the removal of free sulphur dioxide by passing a stream of air through an acidified sample of the wine. Orthophosphoric acid (H_3PO_4) was used to acidify the wine sample. The sulphur dioxide released by the aspiration was then passed into a neutral hydrogen peroxide solution. The hydrogen peroxide oxide the sulphur dioxide to sulphuric acid:

$$SO_{2(g)} + H_2O_{2(aq)} = H_2SO_{4(aq)} \qquad (3.5)$$

The sulphuric acid formed was then titrated with a 0.1M solution of sodium hydroxide-

$$H_2SO_{4(aq)} + 2NaOH_{(aq)} = Na_2SO_{4(aq)} + H_2O_{(aq)} \qquad (3.6)$$

Both bound and free sulphur dioxide are expressed in $mg.L^{-1}$

3.3.5 HPLC analysis of methanol, ethanol and acetic acid

Liquid chromatography system

A Waters 2690 (Milford Massachusetts) system with refractive index (RI) detector was used to separate the methanol, ethanol and acetic acid in wine and vinegar.

A four point standard was prepared to contain final concentration of 0.5%, 1.0%, 1.5% and 2% methanol 2.5%, 5.0%, 7.5% and 10% ethanol and 1.875%, 3.75% and 7.5% acetic acid, respectively. The standards were chromatographed with 300 × 7.8mm ID Aminex HPX-87H

column (BIO-RAD Laboratories, USA), which is tailored for small molecule organic acids. The chromatographic conditions were 0.0018M H_2SO_4, at a flow rate of 0.6ml/minute, at 60°C.

Sample preparation

Juice, wine and vinegar samples were filtered through a 0.45µm filter (Sartorus AG, Goettingen, Germany) prior to HPLC analysis. A 10µL sample was injected using the fixed volume loop method.

3.3.6 HPLC Analysis of sugars

Standard curves

A four-point external calibration curve was constructed by preparing four standard solutions, each containing sucrose (AJAX Chemicals, Australia), glucose (Sigma Chemical Company, USA) fructose, (Sigma Chemical Company, USA). The final concentration of each of the sugars in the standard solution was 1%, 1% and 0.1%; 0.75%, 0.75% and 0.075%; 0.5%, 0.5% and 0.05%; and 0.25%, 0.25% and 0.025% for the four standards, for fructose, glucose and sucrose, respectively. The standards were passed through a 0.45µm membrane filter (as above) prior to HPLC analysis.

Liquid chromatography system

The same system described in section 3.3.7 was used to separate sugars in kiwifruit juice and wine except that the column was an Aminex HPX-87P 300 x 7.8mm carbohydrate analysis column. The samples were eluted isocratically with Milli-Q water (Milli-Q apparatus, Millipore Corporation, Bedford, MA, USA) at a flow rate of 0.6ml.min^{-1}. Column temperature was set at 60°C. A 10µL sample was injected using the fixed volume loop method.

Sample preparation

Kiwifruit juice that had been kept at -40°C was thawed at room temperature for about 6h and filtered through 0.45µm membrane prior to HPLC analysis.

3.3.7 HPLC Analysis of vitamin C and carboxylic acids

As there is no one method available that can simultaneously resolve ascorbic acid, dehydroascorbic acid, quinic, citric, malic, and fumaric, some method development work was undertaken. Details of the method developed and its validation are given in Appendix 2.

Standard curves

A calibration curve was prepared by using four standard solutions containing 0.3-1.2%w/v citric acid (MERCK, Germany), 0.275-1.1%w/v quinic acid (BDH chemicals Ltd), 0.1-0.4 L-malic acid (Sigma Chemical Company USA), 0.275-1.1%w/v ascorbic acid (Cleveland Ohio, USA) and 0.0275- 0.11%w/v DHAA (prepared from ascorbic acid by reduction, Appendix 8). Fumaric (0.00164-0.00657%w/v) was also present as an impurity from malic acid. All standards were passed through a 0.45μm membrane filter prior to HPLC analysis.

Liquid Chromatography system

The HPLC system described in section 3.3.7 was also used for the analysis of vitamin C and carboxylic acids. The column system consisted of a Brownlee^{TM} Labs (Applied Biosystems, Santa Clara, CA, USA) 30× 4.6mm reverse phase C_{18} guard column and a 220 × 4.6mm Spheri-5ODS reverse phase C_{18} column coupled together in series both were packed with 5μm spherical particles with 300A° pores. The eluting phase was 0.02 M sulphuric acid (BDH Chemical Company). The flow rate was 0.25ml.min^{-1} for 10min before being increased to 0.5ml.min^{-1} for 20min. The column was maintained at 32°C by placing in a stainless water jacket. Detection was carried out at 214nm and 265nm, and samples of 10μml were injected using the fixed volume loop method.

Sample preparation

A 5mL sample of kiwifruit juice, which had been kept at -40°C, was thawed at room temperature and filtered through a 0.45μm membrane filter prior to HPLC analysis.

Peak quantification: This was done by comparing peak heights of the juice samples to peak height of the standard. The standard was chromatographed three times and peak area and peak height were compared. Peak height generally gave a higher linear regression coefficient than peak area and therefore peak height was used for quantification of each carboxylic acids,

ascorbic acid and dehydroascorbic acid. Details of the experiments for peak quantification are given in Appendix 2.

Accuracy and reliability

Accuracy of the method was determined by measuring the recovery of the individual acids. Recoveries of between 92-104% for quinic, citric and ascorbic acid, respectively, and 68-93% for malic acid were obtained indicating acceptable accuracy. DHAA and fumaric were difficulty to quantify, as they were present in small concentrations. Reliability of the method was determined by chromatographing standards and juice samples three times and a standard error of less than 0.15 was obtained for quinic, citric, AA, malic, DHAA and fumaric acids. Further details for the recovery and reproducibility tests are given in Appendix 2.

3.3.8 Colour measurement

The colour for juice, wine and vinegar was measured with a Hunter automatic colour difference meter (Minolta chromameter, model, CR 200), (Biolab Ltd. Auckland), and colour was represented by the L* a* b* colour notation. This is a 3-D colour presentation method in which L* is the lightness of colour and equal to 0 for black and 100 for white, a* is the amount of red (0 to 60) or green (0 to -60) whereas b* is the amount of yellow (0 to 60) or blue (0 to -60) (Mallikarjunan and Mittal, 1994). The equipment was first calibrated with a white pad and black background. Samples were presented on Petri dishes at approximately 0.5 cm depth. Means of three measurements taken at different portions of the sample were recorded. The net colour change (ΔE), chroma (C^*) and hue angle (H^*) were obtained analytically as shown below:

$$\Delta a^* = a^*_o - a^* \tag{3.7}$$

$$\Delta b^* = b^*_o - b^* \tag{3.8}$$

$$C^* = \sqrt{a^{*2} + b^{*2}} \tag{3.9}$$

$$\Delta L^* = L^*_o - L^* \tag{3.10}$$

$$H^* = 180^\circ + \arctan\left(\frac{b^*}{a^*}\right) \tag{3.11}$$

$$\Delta E = \sqrt{(\Delta a^*)^2 + (\Delta b^*)^2 + (\Delta L^*)^2} \qquad (3.12)$$

$$\Delta H^* = \sqrt{(\Delta E^*)^2 - (\Delta L^*)^2 - (\Delta C^*)^2} \qquad (3.13)$$

Where: 'o' indicates initial average colour values for 20 fruits sorted from each batch of fruit processed and Δ means "change of":

Δ a* = change in greenness-redness with respect to the fresh fruit

Δ b* = change in yellowness-blueness with respect to the fresh fruit

Δ C* = change in chroma with respect to the fresh fruit

Δ L* = change in lightness with respect to the fresh fruit

Δ E = net colour change the fresh fruit

Δ H^* = change in hue angle with respect to the fresh fruit

3.3.9 GC and GC-MS analysis of volatile compounds in Zespri ^{TM} gold kiwifruit Juice, wine and vinegar

Sample preparation

Into 5mL of juice, wine or vinegar, 1.0g of sodium chloride was added along with small magnetic stirrer and the vial was capped with a septum. Sodium chloride was added to "salt out" the organic compounds (Whiton and Zoecklenein, 2000), increasing their partition coefficients (Arthur and Pawliszyn, 1990; Buchholz and Pawliszyn, 1994; Steffen and Pawliszyn, 1996). In addition, the vinegar was neutralized with 1M KOH to pH 6.5 to reduce the masking effect of acetic acid, which is dominant in vinegar, thus enhancing detection of other volatile compounds, which are present in smaller concentrations. The vials were placed in a thermostatted water bath (35°C) on a stirrer and a solid phase micro-extraction fibre (SPME), 85µm, coated with polydimethylsiloxane (PDMS), (Supelco, Sigma USA) was inserted into the headspace for 30min with the samples being constantly stirred at 250rpm. After completion of sampling the fiber was removed from the sample vial and inserted into the injection port of the GC for 10min. Prior to use, the fibre was conditioned at 250°C for 30min.

GC conditions

The samples were analysed with GC (Carlo Ebre 600, US). The GC was equipped with a 30m × 025mm (ID) Zebron capillary column (ZB-5) O®Phenomenex®, USA, 0.25µm coating and a flame ionisation detector (FID). The carrier gas was N_2 at a flow rate of 2ml.min^{-1}. The

injector temperature was 250°C, split ratio of 1:60 and the column was initially held at 40°C for 2min, and then programmed at 5°mn^{-1} to 100°C, held for 2min, then ramped to 190°C at 10°C min^{-1} and held for 1min.

GC-MS

Samples for juice wine and vinegar were analysed with GC-MS (GCMS-QP50505A, Shimadzu, US) at AgResearch, Palmerston North, to identify unknown peaks prior GC analysis. The conditions for the GC-MS were the same as for the GC except that the carrier gas was helium. The injection mode was splitless for 5min. The MS was operated in full scan mode using Autotune conditions with 70eV electron impart ionization. The compounds were identified by comparing with retention time against the NST107 and Wiley 139 libraries. This analysis was done with the assistance of Dr. Karl Fraser, AgResearch.

3.3.10 Determination of proteolytic activity

The proteolytic activity in juice and wine was measured and compared to that observed for *Actinidia deliciosa* 'Hayward' variety and commercial papain (Sigma Chemical Company, USA).

The casein digestion method commonly used to determine bromelain activity in pineapple (Sriwatanapongse *et al.*, 2000), was used with the following modifications as explained below.

Preparation of stock solution:

A stock solution of casein was prepared by suspending 1g of Hammerstein-grade casein in 100mL 0.1N phosphate buffer (pH 7.6) in a 150mL beaker. The suspended casein solution was heated for 15 min in a boiling water bath to completely dissolve the casein. The solution was then cooled to room temperature and refrigerated until the time of enzyme activity measurement.

Measurement of enzyme activity:

2mL of the 1% casein stock solution was pipetted into a 15mL test tube and put in a 35°C water bath for 5 min. 1mL of gold kiwifruit juice was added to the warm casein solution and

mixed thoroughly before incubation at 35° C for 20min. At the end of the incubation period, 6mL of 5% (w/v) trichloroacetic acid (TCA) was added and mixed thoroughly, and left at room temperature for 1 hour before being centrifuging at 4400rpm for 20min.

Blank test solution preparation:

The casein stock solution was incubated in 35°C water bath for 25min, then 3mL of TCA was added, mixed thoroughly and the mixture left at room temperature for 1 hour before centrifuging at 4400rpm for 20min.

Measurement of absorbance:

Absorbance was measured for the supernatant solution of the casein using a spectrophotometer against the blank at 280nm. The enzyme unit was calculated as the amount of enzyme that gives an increase of absorbance at 280mn of 0.100 following 20 min incubation as described above (Scocea and Lee, 1969)

3.4 Statistical analysis

Statistical Analysis System Software (SAS) that uses the method of least square to fit a general linear model to an unbalanced data set was used to perform two way (ANOVA) analysis of variance for the effect of difference levels of the pre-pressing enzyme (Celluzyme ®) and the post-pressing enzyme (Kleerase® 100XL) on juice yield, TPn, TP, sugars, carboxylic acids and vitamin C. Three way ANOVA was performed for the effect of skin contact, time and temperature on TP, sugars, carboxylic acids, vitamin C and colour values for juice and wine, whilst one way ANOVA was used for the meat tenderisation and sensory evaluation data (see section 6.4 and 6.5).

4. Effect of extraction techniques on juice yield and quality

4.1. Introduction

The goal in juice manufacture is to obtain maximum yield as well as extracting as much of the desirable health-promoting and flavour and aroma compounds without also extracting undesirable compounds. Thorough comminution and extended pressing maximizes the yield, but by so doing extracts from everything, that is from seeds, skin, core and flesh. This may impact negatively on juice quality, and therefore, the compromise between juice yield and quality determines the juicing and subsequent steps in juice processing

The use of pectolytic enzyme and press aid (PA) has been reported to increase juice yield for the green kiwifruit ('Hayward') variety from 55-60% to 84% (Heatherbell *et al.*, 1979). Also pectinase has been reported to increase juice yield, clarity, soluble solids, titratable acidity (TA), total acidity, total anthocyanin, and total phenolics (TP) but decreased pectin and viscosity in plum and soursop juices (Tung-sun chang *et al.*, 1994), among others.

Since Zespri^{TM} gold kiwifruit is a new variety it was necessary to evaluate the effect of processing conditions on juice yield and quality.

The objectives of this study were to evaluate:

(1) The effect of press aid, and pre-and post-pressing enzymes on Zespri ^{TM} gold kiwifruit juice yield and quality;

(2) The effect of repeated pressing using a laboratory hydraulic press on Zespri ^{TM} gold kiwifruit juice yield and quality;

(3) The effect of pressing Zespri^{TM} gold kiwifruit with the skin on or off following different holding time-temperature regimes on the juice yield and quality

4.2 Effect of different pressing and enzyme treatments on juice yield and quality.

4.2.1 Introduction

The use of press aid (PA) and enzymes has been reported to improve both yield and quality in the production of 'Hayward' kiwifruit and in apple juices (Meischak, 1970; Bevers1971; Heatherbell *et al.*, 1979, Pilnk et al., 1970; Szymczuk *et al.*, 1997; Pagan *et al.*, 1997). Similarly, post-pressing enzymes (pectinases) have been reported to improve juice clarity and quality in banana, grapes, soursop, plums, apple, pears, apricot, banana, carrot and sweet berries (Pilnik *et al.*, 1975; Brown and Ough, 1981; McClellan *et al.*, 1985; Feller 1991; Gorsel *et al.*, 1992; Guyer *et al.*, 1993; Tung –sun chang *et al.*, 1994).

In this study the effect of PA and pre- and post-pressing enzymes were evaluated for their effect on juice yield and quality.

4.2.2 Material and methods

4.2.2.1 Material

Press aid, and pre-and post-pressing enzymes used were as described in section 3.1. 2-3

4.2.2.2 Methods

Use of PA

Zespri^{TM} gold kiwifruit was pulped in a hammer mill and treated with 0.15ml.L^{-1} of pre-press enzyme and held at 50^{o}C for 1 hour before samples of 5kg were pressed each time in the hydraulic press described in section 3.2.1. The press aid was added at 0%, 1%, 2%, and 3% w/w to determine the effect of press aid on juice yield. The pressure employed was approximately 250MPa, for 10 to 12 minutes each cycle. The pressure was built gradually by swirling the handle of the press to and fro. The juice was collected at the end of each cycle and metered using a measuring cylinder. In this experiment two different batches of fruit were pressed in two consecutive days.

Use of pre- and post- pressing enzymes

A second batch of fruit was pressed with the optimal level of PA (2 %(w/w) established earlier to determine the separate effect of the pre-pressing enzyme on juice yield. The pre-pressing enzyme (Celluzyme®) was used at the range of 0.05 to 0.15% (v/v) with the pulp held at 50°C for 1h; the pulp was then pressed and the juice yield was determined as described above. Further batches of fruit pulp were treated with combinations of pre- and post-pressing enzymes to determine their effects on juice quality. The fruit was pulped and divided into three 15kg portions and these were treated with 0.05, 0.1 and 0.15mL.kg^{-1} of the pre-pressing enzyme, respectively, and then held at 50°C for 1h. Each 15kg was further divided into 5kg portions and pressed as described in section 3.2.1. The three portions of 5kg from each level of the pre-pressing enzyme were treated with 0.015, 0,025 and 0.035mL^{-1} of the post-pressing enzyme, respectively, and maintained at 50°C for up to 2h. After cooling to room temperature, 100ml samples were taken from each treatment and kept at -40°C for analysis on a later date.

Effect of repeated pressing

Once the optimal levels of press aid and pre-and post-pressing enzymes were established, fruit pulp was pressed repeatedly for up to 4 cycles to determine the effect of repeated pressing on juice yield and quality. In this technique, the pulp was pressed at low pressure by building the pressure slowly to 250MPa, then the pressure was released, and another cycle was started.

4.2.3 Results and discussion

4.2.3.1 Effect of PA and repeated pressing on juice yield

Table 4.1 gives yield data for free run juice and for each pressing cycle, total juice yield per 5kg of fruit, and percentage yield based on initial fruit mass. From Table 4.1 it is evident that more free-run juice was obtained without PA. Free-run juice yield decreased markedly with increasing PA whereas overall juice yield increased significantly ($P<0.05$) with increasing PA, especially in cycles 1, 2 and 3. The resulting overall yield increased significantly ($P<0.05$) with PA addition.

The data indicate that three pressing cycles are necessary for near maximum yield and that, after the 3rd cycle, little benefit is gained by further pressing. This is illustrated in Fig 4.2. These results are in agreement with the literature: a combination of press aid and pectolytic enzyme has been reported to increase juice yield from 55-60% to 84% for the green kiwifruit ('Hayward') variety (Withy *et al.*1982), and a second pressing of enzyme-treated apple fruit pulp was reported to increased juice yield from 65-70 to >90% (Deelen & Steinbuch., 1983)

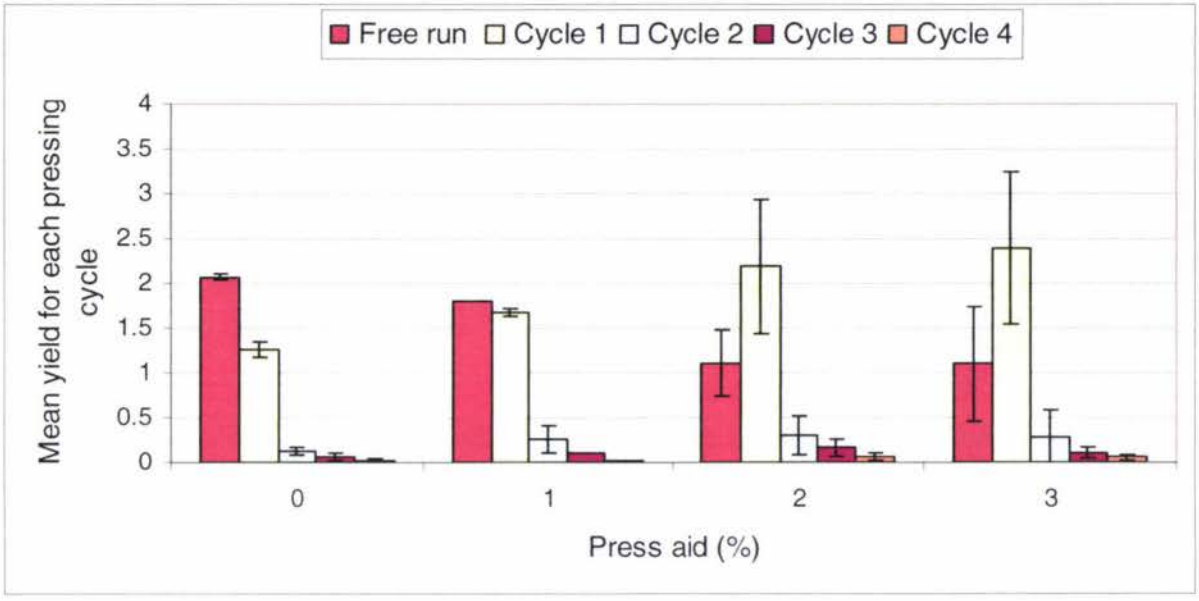


Fig 4.2: Effect of repeated pressing on juice yield at each level of press aid

The juice yields obtained on two different days of processing using different batches of fruit did differ noticeably (Batch 1 vs Batch 2, Table 4.1). The degree of ripening of the fruits was different. The first batch was ripened by mixing the kiwifruit with apple fruits in the ratio of 4:1(w/w) for 3 days before juicing, whereas the second batch was used as it arrived. The level of maturity, ripening and storage history may affect juice yield and further research should be conducted to investigate the possible effects.

4.2.3.2 Effect of repeated pressing on juice quality

Although repeated pressing increased total yield as demonstrated in section 4.2.3.1 it might also affect the levels of substances extracted from the flesh and skin and hence the physico-chemical and sensory properties of the juice and derived products. Therefore, in addition to the effect of repeated pressing on juice yield, its effect on physico-chemical and sensory properties was also investigated.

4.2.3.3 Effect of repeated pressing on juice colour

The colour of the free-run and press juice was measured for Hunter colour values a*, L*, b* and compared with measurements taken in the same way for the fruit flesh (means for 30 fruits sample). The average Hunter values L*, a* and b* for the 30 fruits were 66.69, -6.75 and 30.48, respectively. Table 4.2 presents data for the change in colour during juice processing.

Table 4.2: Effect of repeated pressing on juice colour attributes

	L*	a*	b*	ΔE	Hue angle	C
Fresh fruit	66.69±3.4	6.75±2.7	29.72±0.6	NA	103 ±1.5	30.48 ±2.7
Cycle	ΔL*	Δa*	Δ b*	ΔE	Hue angle	ΔC*
Free run	10.17±2.27	-4.39±1.53	25.66±0.23	27.95±1.27	99.7±1.2	26.03±0.94
cycle 1	12.52±0.44	-5.77±0.18	26.22±0.40	29.62±0.20	102±1.2	26.85±0.43
cycle 2	8.92±0.41	-5.41±0.29	26.50±0.66	28.48±0.54	101.5±0.1.1	27.05±0.72
cycle 3	9.44±0.22	-5.53±0.22	27.10±0.70	29.23±0.76	101.5±1.05	27.66± 0.73

The free-run and first cycle juices were less bright than juice in the subsequent pressing cycles as indicated by the slightly larger ΔL*(Table 4.2) The Hue angle decreased slightly in juice compared to the fresh fruit. The juice was also less saturated (less chroma), but there was no other major difference in colour parameters with repeated pressing.

4.2.3.4 Effect of repeated pressing on total phenolics

Total phenolics were higher in the free run than in the press juices, and increased in the press juice with successive pressings (Fig 4.2). During repeated pressing of apple

mashes phenolic acids were reduced with successive pressing, whereas flavonoids and tannins increased (Dumazert, 1974). In kiwifruit the main phenolics are flavonoids, chlorogenic acid, protocatechuic acid and derivatives of 3, 4-dihydroxybenzoic acid, epicatechin, catechin and procyanidins (Dawes and Keene, 1999), but determining possible changes in the levels of these was outside the scope of this project.

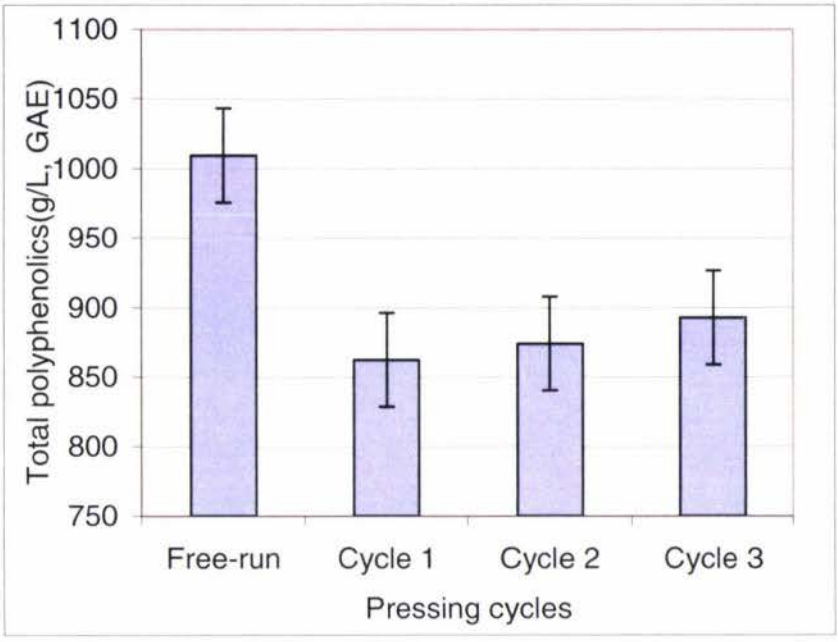


Fig 4.3: Effect of repeated pressing on total phenolics

4.2.3.5 Effect of repeated pressing on physico-chemical properties of Zespri™ gold kiwifruit juice

Total soluble solids (TSS) and titratable acidity (TA) increased slightly but significantly ($P<0.05$). Total acidity, pH, and total reducing sugars (TRS) were not significantly ($P>0.05$) affected by repeated pressing (Table 4.3). Thus, the total acidity /sugar ratio which is an important index in juice quality as it contributes to the development of flavour and provides a thirst quenching effect (Yusof and Ibrahim, 1994), was also not affected by repeated pressing.

Total vitamin C (TVC) decreased with later pressings (Table 4.3). During pressing, tissue disruption occurs and vitamin C can be affected in the same way as other

antioxidants by esterase present in the fruit ((Dietrich, *et al.*, 20002). Losses of ascorbic acid during grinding and centrifugation of apple fruit pulp has been reported to range from 0-8% within 5 to 90 minutes (Bi and Zhang, 1998).

Therefore, in conclusion, repeated pressing significantly increased juice yield up 85%. The free-run juice was superior in vitamin C and TP. Repeated pressing had minor effects on colour intensity, decreased vitamin C and increased TP.

Table 4.3: Effect of repeated pressing on physico-chemical properties

Pressing Cycles	pH	TSS	TA(gL^{-1}) citric acid)	Quinic ($g100mL^{-1}$)	Citric ($g100mL^{-}$)	Malic ($g100mL^{-1}$)	Total acidity ($g100mL^{-1}$)	TVC $C(g100mL^{-1})$	TRS ($g100mL^{-1}$)	Acid/sugar ratio
Free-run	3.52±0.05	14.20±0.57	10.01±0.33	1.12±0.014	0.91±0.036	0.04±0.01	2.07±0.05	0.11±0.001	11.60±0.21	0.18±0.04
Cycle1	3.57±0.05	15.40±0.07	11.7±0.30	1.15±0.027	0.87±0.03	0.04±0.01	2.06±0.05	0.11±0.002	10.40±0.01	0.20±0.07
Cycle 2	3.61±0.06	15.05±0.28	12.4±0.27	1.10±0.003	0.82±0.01	0.04±0.002	1.96±0.01	0.10±6E-04	9.10±0.07	0.22±0.02
Cycle3	3.53±0.10	16.00±1.84	15.6±0.27	1.18±0.028	0.86±0.03	0.11±0.14	2.14±0.19	0.09±0.005	11.50±0.15	0.19±0.04

Notes:

TSS Total soluble solids, TVC Total vitamin C, TRS Total reducing sugar

4.2.3.6 Effect of pre-pressing enzymes on juice yield

To separate the effect of pre-pressing enzymes, varying levels of the pre-pressing enzyme (Celluzyme®) was used with the optimal level of PA and pressed repeatedly as in section 4.3.1. Table 4.4 presents data on the effect of varying levels of pre-pressing enzyme with 2% PA on juice yield.

Table 4.4: Effect of pre-pressing enzyme and press aid on juice yield

Amount of Fruit pulp (kg)	Enzyme concentration ($ml.kg^{-1}$)	Juice yield (L)	Juice yield (% v/w)
13	0.00	5.77±0.35	44.4
13	0.05	10.50±0.40	80.8
13	0.10	10.87±0.46	83.6
13	0.15	11.00±0.55	84.6

The range of 0.05-0.15 is effective for pressing Zespri ™ gold kiwifruit and increased juice yield by about 90% (i.e. from 5.7-11L). Similar results were obtained for soursop where pectinase enzymes up to 0-0.1% was reported to increase juice yield by up to 41% (Yusof and Ibrahim, 1994) and plum juice yield improved significantly ($P<0.05$) with pectinase concentration in the range of 0.05-0.6% (Tung-sun chung *et al.*, 1994). By comparing the results obtained by fixing enzyme concentration at 0.15ml/L with different levels of PA (Table 4.1) and that obtained by fixing the level of PA at 2% w/w (Table 4.4) it was observed that it is the pre-pressing enzyme, which contributed most remarkably to the increase in juice yield in gold kiwifruit.

It can also be seen that there was a noticeable variation in juice yield using the same conditions. For example, from Table 4.1 and 4.4, the equivalent juice yields using 2% PA and $0.15ml.kg^{-1}$ for 3 different batches of fruit were 74%, 80% and 85%, respectively. Perhaps the level of maturity and storage time affected the juice yield and it is therefore recommended that further research be conducted to investigate the effect of maturity and storage time on juice yield.

4.2.3.7 Effect of pre-and-post pressing enzyme on physico-chemical properties of Zespri ™ gold kiwifruit juice

After ascertaining the effective range of the pre-pressing enzyme on juice yield, the effect of the combinations of different levels of the pre-and post-pressing enzyme on physico-chemical properties of Zespri™ gold kiwifruit juice was undertaken. The juices obtained were evaluated for insoluble cloud matter (ICM) dry matter (DM), alcohol insoluble solids (AIS), titratable acidity (TA), pH, total vitamin C(TVC), total soluble solids (TSS), viscosity, carboxylic acids, sugar content, total phenols (TP), colour change and aroma compounds as described in section 3.3.2 -3.5.

Effect of pre-and post-pressing enzymes on juice consistency and clarity

Viscosity, insoluble cloud matter (ICM), alcohol insoluble solids (AIS), dry matter (DM) and total pectin (TPn) data are shown on Table 4.5. The value for viscosity is that after 3h of holding at 50°C; however viscosity was measured after every 30min for up to 3h.

Table 4.5: Effect of enzymes concentration on juice consistency and clarity

Celluzyme ($ml.kg^{-1}$)	Kleerase ($ml.L^{-1}$)	Viscosity (mPa.s after 3 h)	ICM ($g.L^{-1}$)	AIS (mg/L)	DM (mg/L)	TP_n (mg/L, GAE)
0.05	0.015	30	14.06	1580	2913	605
0.05	0.025	20	12.98	1520	473	397
0.05	0.035	20	12.90	1487	540	205
0.1	0.015	30	9.89	1106	393	161
0.1	0.025	20	9.91	1187	420	133
0.1	0.035	20	9.25	1147	400	127
0.15	0.015	20	8.38	533	373	103
0.15	0.025	10	8.26	327	373	58
0.15	0.035	10	8.09	300	353	48

Legend: ICM = insoluble cloud matter, AIS = Alcohol insoluble solids, DM = Dry matter, TP_n = Total pectin, Celluzyme and kleerase = pre and post pressing enzymes respectively.

($P< 0.05$) S.E. for lsmeams

Viscosity decreased significantly ($P<0.05$) with the increase in incubation time and enzyme concentration. Statistical analysis showed that both the pre-and-post pressing enzymes separately contributed significantly ($P<0.05$) in reducing the viscosity. The interaction of the two enzymes in reducing viscosity was not significant ($P>0.05$) (also see Fig 4.3). The pre-pressing enzymes at a concentration of $0.15 ml L^{-1}$ with

0.025 or 0.035 $ml.L^{-1}$ of post-pressing enzyme were effective in reducing viscosity. At this concentration ($0.15mlL^{-1}$), with a long holding time of up to 3 h, the pre-pressing enzyme is capable of reducing viscosity in the absence of the post-pressing enzyme. However, in order to avoid a long holding time, which might damage the sensory qualities of the juice or promote undesirable flavours, the post-pressing enzyme is recommended. The reduction of viscosity caused by the pre-pressing enzyme is due to cellulases present in commercial enzyme preparation, which degrade both soluble and insoluble carbohydrates (Gerhartz, 1990).

The reduction of viscosity in juice processing is important not only to provide juice of acceptable consistency but also allows a high degree of juice concentration should this be required. This confers the further advantages of longer shelf life and lower storage and transport costs. Fig 4.3 illustrates the co-operate effect of the pre-and post-pressing enzymes on viscosity

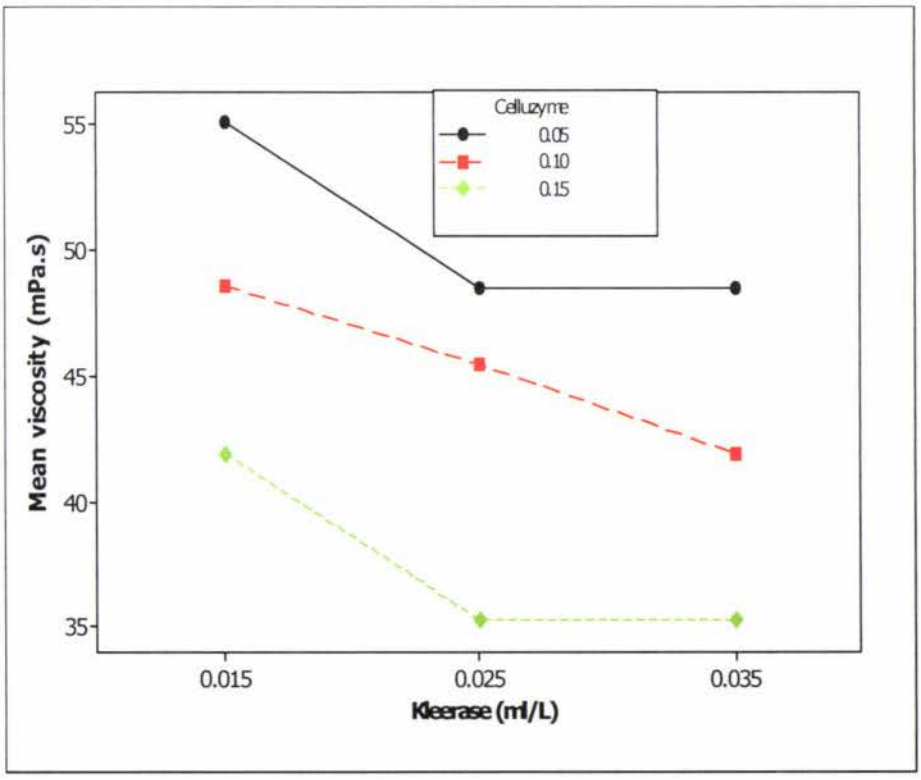


Fig 4.3: Effect of pre and post pressing enzymes on juice viscosity at 50°C for up to 2h

Notes: Celluzyme = pre-pressing enzyme, kleerase = post pressing enzyme

The pre-pressing enzymes degrade soluble and insoluble pectins, which are major constituents of most fruits, and this enhances pressing as well as lowering viscosity. Results in Table 4.3 show AIS, DM and TP_n decreased with an increase of enzyme concentration. Of the concentration tested, $0.15ml.L^{-1}$ of pre-pressing enzyme was most effective with any level of the clarifying enzyme (Kleerase®). Statistical analysis (F-test) revealed that both pre-pressing and post-pressing enzymes contributed significantly ($P<0.05$) towards reduction of TP_n. Using least significant difference (LSD) to separate means it was evident that $0.15mlL^{-1}$ of the pre-pressing enzyme reduced total pectin by about 10-fold (from ca. 650 mgL^{-1} to ca. $70mgL^{-1}$), whereas $0.035mlL^{-1}$ of the post pressing enzyme (Kleerase®) reduced TP by only 5-fold, i.e. from about $650mgL^{-1}$ to ca. 127 (also illustrated in Fig 4.4).

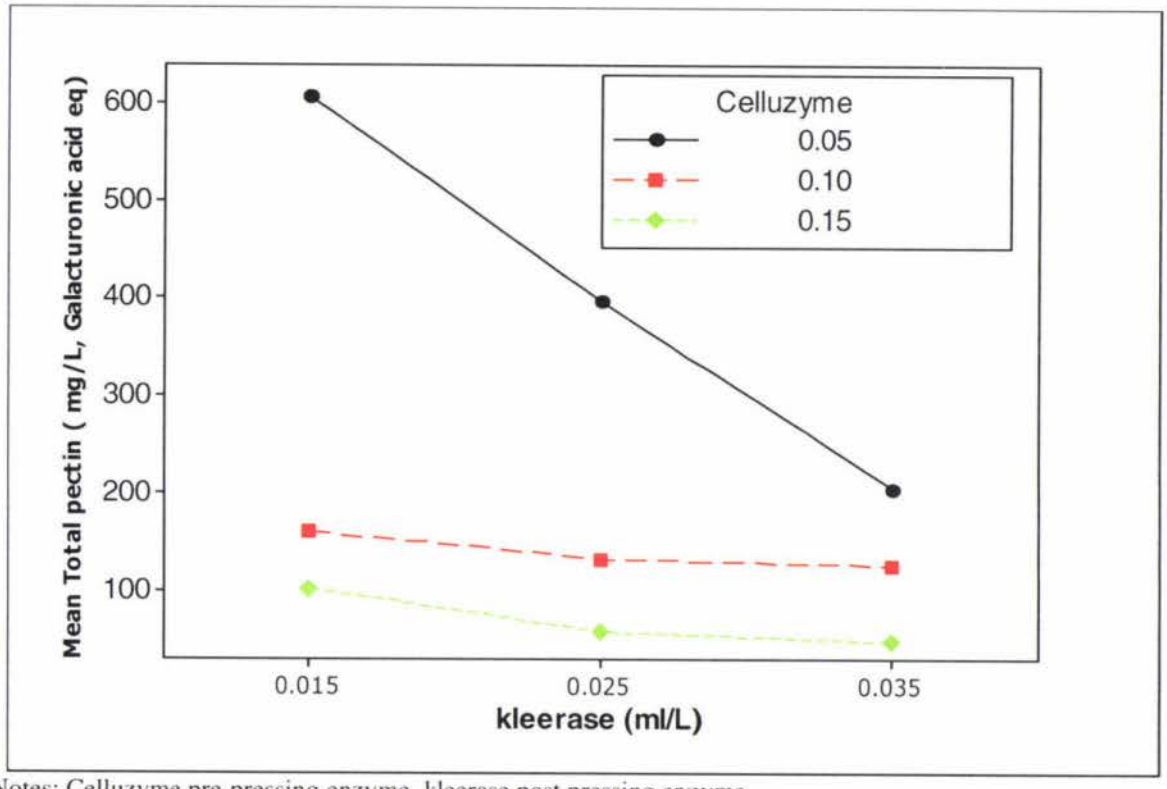
These results demonstrate that the commercial pre-pressing enzyme (Celluzyme®) is effective in breaking down pectin and other carbohydrates hence reducing viscosity of Zespri™ gold kiwifruit, even without the presence of the post-pressing enzyme. However, the use of the post-pressing (clarifying) enzyme is necessary for reducing cloudiness in the juice and will improve shelf life.

The commercial clarifying enzymes contain mixtures of pectinase, polygalacturonase and pectin methylesterase. These particles contribute towards breaking down pectin contained in fragments of plant cells and insoluble particles of skin and seeds that remain in juice (Gerhartz, 1990). The commercial enzymes (pectinase and cellulase) are commonly employed together in juice processing for the purpose of both enhancing juice extraction and clarity (Essa, *et al.*, 2002). Notwithstanding, where a stable cloudy product is desired any enzyme preparation containing pectinase should be avoided, and the pre-pressing (cellulase) is capable of effecting complete degradation of both soluble and insoluble carbohydrates, and ensuring a product of low viscosity.

From Fig 4.4, both the pre-pressing and post-pressing enzyme similarly contributed to the reduction of total pectin. Of the levels of pre-pressing enzyme tested 0.1-$0.15ml.L^{-1}$ were effective in reducing TPn at any level of the post-pressing enzyme with the

0.15ml.L^{-1} being most effective, but 0.05ml.L^{-1} was effective only at the higher levels of the post-pressing enzyme (0.035mlL^{-1}).

Such treatments are recommended as in addition to the benefits of increasing the volume of juice produced, beneficial changes in the flavour and, in the case of winemaking, as from grapes, shorter fermentation times may result. Insoluble plant material is easily removed by filtration, or settling and decantation once the stabilising effect of the pectins on the colloidal haze has been removed (Chaplin, 1990).



Notes: Celluzyme pre-pressing enzyme, kleerase post pressing enzyme

Fig 4.4: Effect of pre-and post-pressing enzymes on total pectin in Zespri^{TM} Gold kiwifruit juice

Dry matter and alcohol insoluble solids followed a similar pattern to total pectin (Table 4.5). This is in agreement with the work of Thomas *et al.* (1987) who found that AIS and TPn decreased with enzyme concentration up to 0.04% for apple puree. It is therefore concluded that in order to improve juice yield and obtain juice of acceptable consistency and clarity, it is important to use both the pre-and post-pressing enzymes in the range of 0.1-0.15ml/L and 0.025-0.035ml/L, respectively.

Effect of pre and post pressing enzymes on TA, total acidity, reducing sugars and vitamin C

Besides their effect on yield, consistency and clarity on juice, cellulase and pectinase enzymes can also affect the yield of substances contained in fruit (Gerhartz 1990). Their effect however depends on substrate characteristics, e.g. pH (Dennella *et al.*, 2000), and processing conditions such as temperature, holding time and technique of juice expression. In this work the effect of pre-and post-pressing enzymes were evaluated for pH, titratable acidity, reducing sugars, carboxylic acids, and vitamin C for Zespri™ gold kiwifruit.

Table 4.6 presents data for the measured parameters. There was no significant ($P>0.05$) change in pH, TA, TSS or reducing sugars. Total acidity increased from about 1.9 to 2.3g 100mL $^{-1}$ for combination of low and high enzyme concentration, respectively. Essa (2000) reported similar results for pH and titratable and total acidity when guava and plum juices were treated with pectinase. It has been reported that in the course of the enzymatic depectinization of apple juice, galacturonic acid is the main hydrolysis product of the cell wall pectin. It is released by the combined action of different pectinases, such as pectinmethylesterases and polygalacturonases, and cellulase (Will *et al.*, 2002). This acid then contributes to the increase in acidity. When the enzyme treated juice was compared to the natural cloudy apple juice it was found that the latter was dominated by usual fruit acids such as malic and quinic. Therefore, besides hydrolysis of polymeric carbohydrates, which cause an increase in TA, the pectinase enzyme also enhances release of the fruit acids into the juice Will *et al.* (2002).

Ascorbic acid and total vitamin C decreased slightly with increasing in enzyme concentrations (Table 4.6) from 0.089g.100mL^{-1} and 0.125g.100mL^{-1} to 0.074g.100mL^{-1} (and 0.118g.100mL^{-1} (1180mg.L^{-1}), respectively. Vitamin C content was reported to increase with pectinase treatment in guava but decreased in plum and banana juices (Essa, 2000). In the contrary, Ching Chun Pong *et al.* (1996) reported a decrease of vitamin C when pectinase was used to extract guava juice and Salmah and Nurzarina, (1994) reported that there was no significant change in ascorbic acid when pectinase up to 0.1% was used in extraction of soursop juice. Therefore the effect of enzymes on vitamin C is variable and may depend on substrate and

processing conditions. This work has demonstrated a minimal loss in vitamin C for the given pre-treatment of gold kiwifruit juice.

The composition of Zespri™ gold kiwifruit juice was observed to be similar to 'Hayward' kiwifruit in titratable acidity, total acidity, total phenolics, but noticeably higher in total vitamin C and total reducing sugars than 'Hayward' kiwifruit (Compare Table 2.6 and 4.6).

Table 4.6: Effect of pre and post pressing enzymes on physico-chemical properties of Zespri [TM] gold kiwifruit juice

PPE	POPE	pH	TSS (Brix)	TA gL^{-1}, (Citric acid)	Quinic ($g.100mL^{-1}$)	Citric ($g.100mL^{-1}$)	Malic ($g.100mL^{-1}$)	ToA ($g100mL^{-1}$)	TVC ($g.100mL^{-1}$)	TRS ($g.L^{-1}$)	ASR
0.05	0.015	3.42	16.5	13.9	1.11	0.89	0.05	2.05	0.13	16.2	0.13
0.05	0.025	3.43	16.6	14.9	1.13	0.75	0.04	1.92	0.13	16.6	0.12
0.05	0.035	3.4	16.5	14.4	1.12	0.74	0.04	1.90	0.12	15.9	0.12
0.1	0.015	3.44	16.7	14.4	1.10	0.80	0.04	1.94	0.12	15.3	0.13
0.1	0.025	3.43	16.7	14.4	1.15	0.93	0.05	2.13	0.13	14.9	0.14
0.1	0.035	3.44	16.7	14.3	1.17	0.96	0.04	2.17	0.12	15.8	0.14
0.15	0.015	3.42	16.5	14.0	1.10	1.10	0.04	2.23	0.10	15.3	0.15
0.15	0.025	3.45	16.4	14.1	1.20	1.13	0.05	2.37	0.12	16.0	0.15
0.15	0.035	3.42	16.1	14.1	1.20	1.04	0.05	2.28	0.12	15.3	0.15

Data on table are means for 3 replications LSM = 0.05

Notes: PPE and POPE = pre-and post-pressing enzyme respectively, TA Titratable acidity, ToA Total acidity, TVC Total vitamin C, TRS Total reducing sugars, ASR Acid sugar ratio

4.2.3.8 Effect of pre-and post-pressing on total phenolics

Total phenolics (TP) decreased significantly ($P<0.05$) with the increase in concentration of both the pre-and post-pressing enzyme. The interaction of the two enzymes was also very strong (Fig 4.5). This differ from reports in some literature that pectinase and cellulase lead to increase in phenolics and antioxidants during juice processing (Dietrich *et al.*, 2002; Will *et al.*, 2000; Mehrlaender *et al.*, 2004 and Essa. *et al.*, (2002). It is possible that the effect of pectinase and or cellulase depends on the phenolics composition of the fruit being processed. For example, while the cited literature showed an increase of TP in apples and blackberry, Canal-Llauberes (2003) reported a decrease of TP with pectinase treatment in Muscadelles, semillion and sauvignon grapes. Also raw juice obtained from 'Jonagold' apples had an antioxidant activity after pulp enzyme treatment that was only 3% of the activity of the fresh apples (Sluis *et al.*, 2002). The levels of flavonoids and chlorogenic acid in the juice had been reduced to between 50% (chlorogenic acid) and 3% (catechins). It was also claimed that most of the antioxidants were retained in the pomace rather than being transferred into the juice (Sluis *et al.*, 2002).

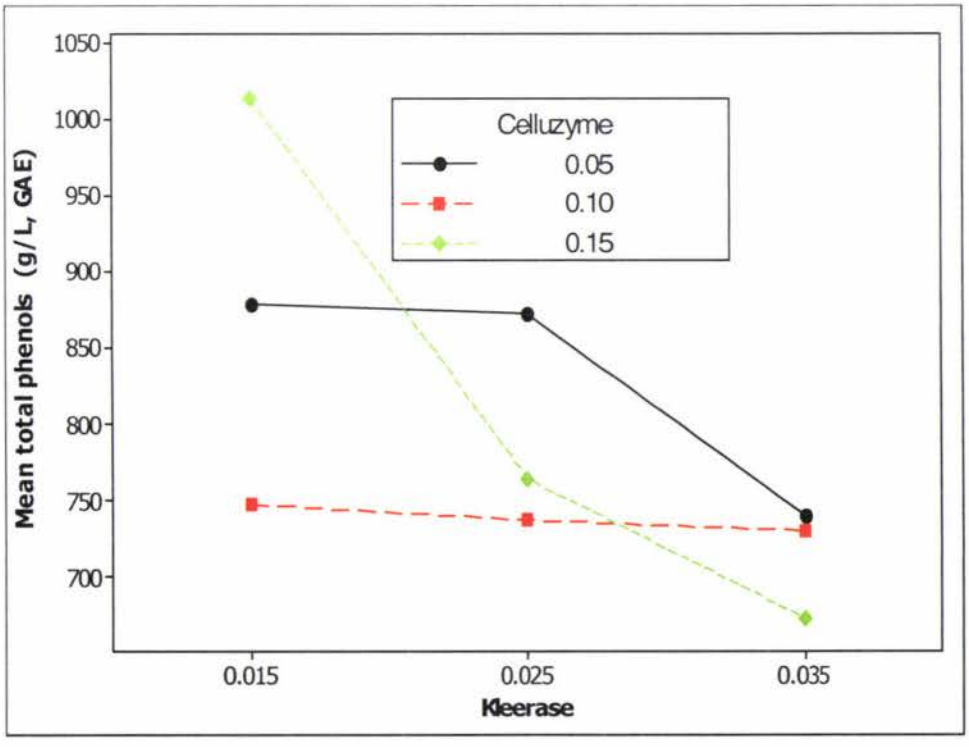


Fig 4.5: Effect of pre and post pressing enzymes on TP in Zespri™ gold kiwifruit juice

Notes: Celluzyme = pre-pressing enzyme, kleerase = post pressing enzyme

Kiwifruit contains a considerable amount of chlorogenic acid and catechin (Dawes and Knees, 1999). Hydrolysis of cinnamic acid derivatives to the free acid after use of clarifying enzymes has been attributed to the presence of esterase activity in commercial enzyme preparation (Spanos *et al.*, 1990). Therefore there could be a significant loss of phenolics, especially during enzyme treatment, when holding at high temperature and continuous stirring.

Phenolics in fruits contribute considerably to the colour, flavour and aging characteristic of fruits. They also contribute to antioxidant activity, which confers potential health benefits to consumers of kiwifruit products (Dawes and Knees, 1999). Since the level of phenolics in kiwifruit is already low compared to other fruits (Dawes and Knees, 1999), it is recommended that future work to be done on the effects of processing methods of kiwifruit on both the levels of active compounds and biological activity of different fractions occurring during processing. This will provide a means for developing processing techniques that will improve kiwifruit products with respect to their potential health promoting properties.

4.2.3.9. Effect of pre-pressing and clarifying enzymes on juice colour attributes

Table 4.7 presents data for the change of lightness ΔL*, chroma and hue angle.

Table 4.7: Effect of enzyme treatment (50°C for 2h) on juice colour

		L	E	C*	Hue angle
Fresh fruit		66.7	72.1	27.4	103
PPE	POE	Δ L*	Δ E	Δ C	Hue angle
0.05	0.015	9.3±1.2	26.5±0.6	23.4±10	114.4±0.3
0.05	0.025	11.0±1.2	26.3±0.7	22.4±10	113.8±1.7
0.05	0.035	11.4±1.6	27.3±0.8	23.3±10	112.8±1.3
0.1	0.015	10.5±01.0	28.0±0.5	24.5±10	118.5±0.5
0.1	0.025	12.1±0.7	27.9±0.5	23.6±10	114.9±0.8
0.1	0.035	14.0±0.5	29.1±0.3	24.0±10	117.4±0.9
0.15	0.015	13.1 ±2.1	28.9±1.2	24.3±10	116.8±4.1
0.15	0.025	13.5±1.4	28.6±0.8	23.7±10	114.8±1.2
0.15	0.035	11.4±2.7	28.5±1.3	24.6±10	117.5±5.1

Notes: PPE and POE = pre and post pressing enzymes respectively.

Overall, there was a marked decrease of the Hunter colour parameters in the enzyme treated juice compared to the fresh fruit (Table 4.7). The Hunter colour parameters decreased noticeably with enzyme concentration, specifically decreasing with the post-pressing enzyme (Kleerase®) concentration. Overall the data visually confirmed this and these observation are supported by the reports of Essa. (2002) and Singh *et al.*, 2000) who reported decrease of colour values in plum, guava and mango juice followed enzyme treatment.

4.3. Effect of skin contact on juice yield and quality

The effects of pressing Zespri™ Gold kiwifruit with skin on or off and different time-temperature holding regimes on juice yield and quality were investigated.

4.3.1. Material and methods

4.3.2. Material

Fruit, PA and enzymes employed were as described in section 3.1

4.3.3. Methods

After pulping of peeled or unpeeled fruit, 0.15ml.L^{-1} of pre-pressing enzyme (Celluzyme®) was added and the pulp held at 30°C or 50°C for 2 or 6h before pressing then post-pressing enzyme (Kleerase®) was added to the juice and held at 50°C for 2 hours. Another batch of fruit was treated in the same way but held at 30 or 50 or 70°C for 6 or 12h, respectively. For each treatment 100mL samples were taken at end of holding time and kept at -40°C for analysis on late date.

Juice quality parameters examined were viscosity, insoluble cloud matter (ICM), alcohol insoluble solids (AIS), total pectin (TPn), total soluble solids (TSS), pH, titratable acidity (TA), total phenols (TP), vitamin C, carboxylic acids, sugars, colour and aroma compounds as described in section 3.3.0-3.5.10.

4.3.4. Results and discussion

4.3.4.1. Effect of skin contact, temperature time regime on juice yield

Fig 4.6 illustrates the effect of these skin contact and time –temperature regime on juice yield for the 30 and 50°C runs.

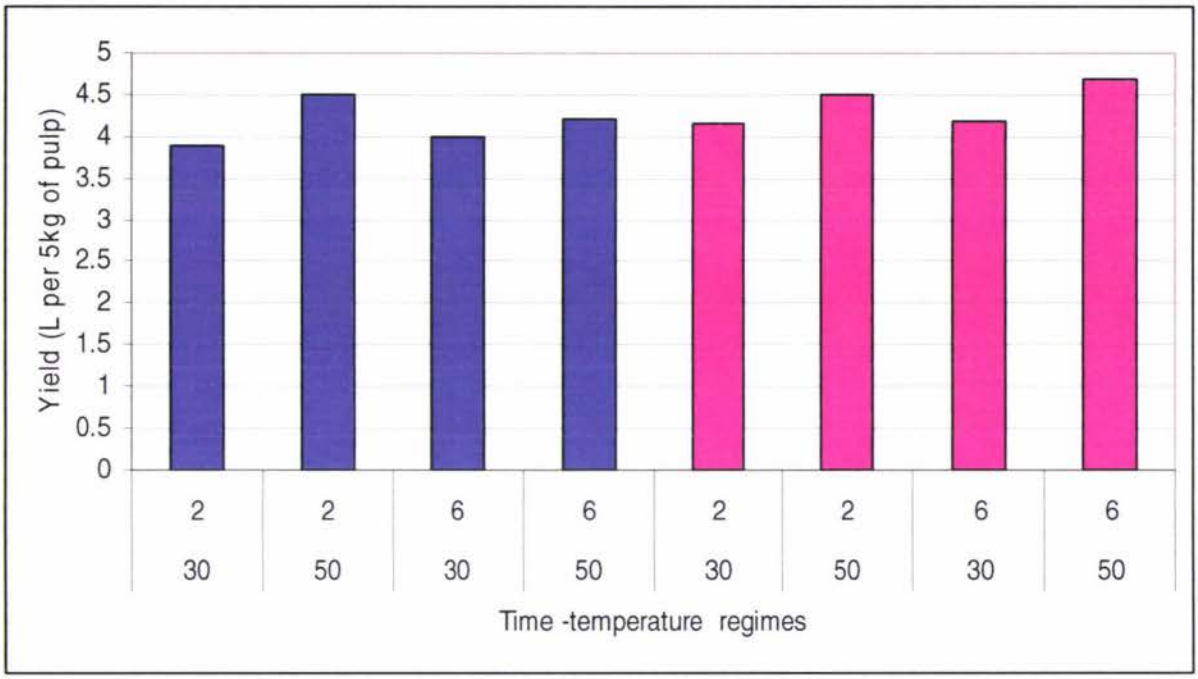


Fig 4.6 Effect of skin contact time at 30 and 50°C on juice yield (red bars for skin on and blue bars for skin off)

The pulp pressed with skin off produced a significantly higher yield ($p<0.05$), and yield also increased significantly ($P<0.05$) at higher holding temperature (Fig 4.6), but variability between batches could not be discounted. Generally, pulp pressed with skin off produced more juice per fruit mass than pulp pressed with skin on at both 30°C and 50°C (Fig 4.6). Juice yield of up to 4.68L (93.6%) from initial 5kg of fruit pulp was obtained with the skin off pulp held at 50°C (Fig 4 6). The fine comminution and optimal temperature (50°C) for the cellulase enzyme (Heatherbell *et al.*, 1979) ensured efficient extraction of juice from the pulp. Table 4.8 and 4.9 provide data for pH, TA, total soluble solids, titratable acidity, carboxylic acids, total acidity and

vitamin C, total reducing sugars and acid to sugar ratio and other physico-chemical parameters.

4.3.4.2. Effect of skin contact time –temperature regime on total soluble solids, reducing sugars, titrable acidity and total acidity

Titratable acidity, total acidity and soluble solids content were slightly higher in the skin on juice than in the skin off juice (Table 4.8). Reducing sugars (glucose and fructose) increased slightly with skin contact time and temperature in the low temperature-time treatment (30 –50°C for up to 6h), but decreased noticeably with high temperature - long time treatment (50 and 70 °C for up to 12h; Tables 4.8 and 4.9). The decrease of total reducing sugars with long holding at high temperature could be due to caramelisation. This leads to low acid to sugar ratio, which affects sweetness and liking of fruit juice (Krueger *et al.*, 2003).

The pH was higher by 0.1 in all skin off juice samples (Table 4.8). It was reported that skin contact of grapes even at very low temperature (2°C) for 24h before pressing increased levels of carboxylic acids compared to immediate pressing (King *et al.*, 1988). The levels of all carboxylic acids (quinic, citric, and malic) were higher in skin on juice than in skin off juice (Table 4. 8) except at low temperature and short holding time (30°C for 2h). This suggests that a reasonable amount of these acids are located in the skin and that effective extraction require a greater extent of enzyme action. Temperature (50°) and holding (6h) time also noticeably increased titratable acidity, total acidity and total soluble solids. When temperature and holding time was increased up to 70°C and 12h, respectively, the results were similar except that slightly higher levels of titratable acidity were observed in skin off samples held at 50 and 70°C for 12h.

Total vitamin C (ascorbic acid and dehydroascorbic acid) levels were generally higher in skin on juice samples (Table 4. 8 and 4.9). Literature reports that lye peeled 'Hayward' kiwifruit fruit retained a higher concentration of vitamin C (0.104g.100g^{-1}) compared to hand peeled fruits (0.0813g.100g $^{-1}$; Simmons, 1978). This implies that a considerable amount of vitamin C is located near the skin and therefore scooping

with a spoon, which was the technique employed in this study, leaves a reasonable amount of vitamin C with the skin. From literature lye treatment removes only a very thin skin membrane as indicated by small weight loss of the lye peeled fruit (9.0%) compared to the hand peeled fruit (13.7%; Simmons, 1978). Temperature and holding time up to 70°C for 12h reduced TVC noticeably (Table 4. 9), but there were noticeable effect on TVC at 30-50°C for up to 6h.

It should be noted here that these were two separate experiments using different batches of fruit. The results for the two batches, although showing a similar pattern with regard to the treatments employed, do differ noticeably. For example, batch 1 (Table 4.8) has higher levels of TA, TVC, TRS and ToA than the second batch (Table 4.9) whereas the second batch has slightly higher levels of TSS. This implies that the results of any set of treatments could be affected by differences in season, orchard location, maturity and storage history so care is required in extrapolating or generalising the effects noted.

Table 4.8 Skin contact (at 30 or 50°C for 2 or 6h) effect on physico-chemical properties

Skin contact	time (h)	Temperature	pH	TSS	TA	Quinic	Citric	Malic	ToA	TVC	TRS	ASR
son	2	30	3.5	15.95	12.22	0.86	0.92	0.03	1.82	0.13	14.72	0.12
son	2	50	3.5	15.59	12.17	0.86	0.89	0.04	1.79	0.13	10.64	0.17
son	6	30	3.5	15.15	12.08	1.05	1.21	0.03	2.28	0.14	14.07	0.16
son	6	50	3.5	15.15	11.97	0.78	0.88	0.02	1.69	0.12	13.24	0.13
soff	2	30	3.6	15.06	11.83	0.73	0.82	0.03	1.58	0.09	12.97	0.12
soff	2	50	3.6	15.16	10.95	0.84	0.9	0.03	1.77	0.1	11.41	0.16
soff	6	30	3.6	15.3	11.62	0.88	0.8	0.03	1.71	0.08	12.89	0.13
soff	6	50	3.6	14.83	11.43	0.85	0.89	0.02	1.76	0.08	11.15	0.16

Notes: (1) TA; Titratable acidity (gL^{-1}, as citric acid), ToA; total acidity $g100mL^{-1}$, TVC, Total vitamin C, $mg100mL^{-1}$, TSR, Total reducing sugar, gL^{-1}, ASR, Acid-sugar ratio.

(2) Units for the individual acids are in mg100mL

Table 4.9 Skin contact (at 30, 50 or 70°C for 6 or 12h) effect on physico-chemical properties

Skin contact	Time (h)	Temperature	pH	TSS	TA	Quinic	Citric	Malic	ToA	TVC	TRS	ASR
Son	6	30	3.40	16.30	11.65	1.00	0.895	0.03	1.93	0.12	10.56	0.18
Son	6	50	3.38	16.40	11.78	0.73	0.574	0.02	1.32	0.11	12.37	0.01
Son	6	70	3.50	15.10	11.30	0.91	0.561	0.03	1.49	0.09	9.41	0.01
Son	12	30	3.40	16.40	11.01	0.88	0.767	0.02	1.68	0.11	12.85	0.01
Son	12	50	3.45	16.60	11.01	1.03	0.920	0.03	2.25	0.12	10.83	0.01
Son	12	70	3.42	16.40	11.30	0.85	0.506	0.02	1.37	0.08	9.45	0.02
Soff	6	30	3.52	16.40	10.24	0.88	0.826	0.02	1.73	0.07	10.62	0.01
Soff	6	50	3.44	16.30	11.40	0.65	0.594	0.01	1.26	0.07	12.39	0.01
Soff	6	70	3.45	16.60	11.30	1.11	0.785	0.03	1.92	0.06	9.64	0.01
Soff	12	30	3.40	15.30	11.01	0.97	1.015	0.03	2.01	0.07	12.3	0.02
Soff	12	50	3.40	15.00	12.16	0.72	0.640	0.02	1.38	0.08	12.51	0.01
Soff	12	70	3.52	16.00	12.16	0.77	0.496	0.02	1.29	0.07	11.50	0.01

Notes:

(1) TA; Titratable acidity (gL^{-1}, as citric acid), ToA; total acidity $g100mL^{-1}$, TVC, Total vitamin C, $mg100mL^{-1}$, TSR, Total reducing sugar, gL^{-1}, ASR, Acid-sugar ratio.

(2)Units for the individual acids are in $mg.100mL^{-1}$

4.3.4.3 Effect of skin contact and time temperature regime on total phenols in Zespri™ gold kiwifruit juice

TP in Zespri™ Gold kiwifruit pulp increased significantly ($P<0.05$) due to skin contact and with holding temperature (Fig 4.7), but decreased noticeably with holding time. It have been reported that skin contact increases total phenolics in grape juice and wine made from it (Marais and Rapp, 1988; Marais, 1998; Cabaroglu, 997; Dubourdieu *et al.*, 1986). It is probable that peeling removes some phenolic compounds with the skin and that temperature enhances extraction of phenolic compounds from the skin. The decrease of TP with holding time could be accounted for by the action of the enzymes used to macerate the pulp. In this study it has been demonstrated that TP decrease significantly ($P<0.05$) with both pre-and post-pressing enzymes concentration (section 4.3.4). For example, at the low temperature where the enzymes are less active the effect of skin contact time in increasing phenolics was evident. For example at 30°C for 6h skin on TP was 933mg.L^{-1}, GAE whereas the skin off TP was 680mg.L^{-1} GAE. At higher temperature (50 or 70°C) there was no remarkable difference in TP between skin on and skin off juice. In this experiment these enzymes were added at 0.15ml/L, the effective levels established in previous experiments in this study (see section 4.3.6).

Fig 4.7 illustrates the effect of skin contact time- temperature regime on TP in Zespri™ Gold kiwifruit juice. When temperature was increased up to 70°C and holding time up to 12 hours, a similar trend of increasing reduction in TP was observed.

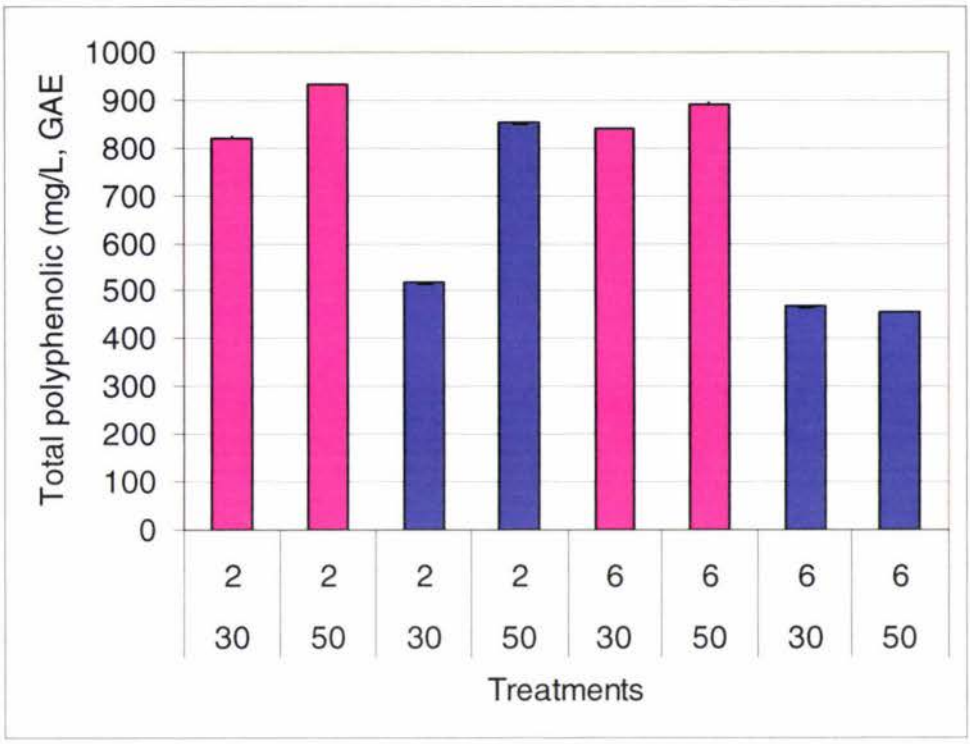


Fig 4.7 Effect skin contact time- temperature regime on total phenols in Zespri™ gold kiwifruit juice (red bars for skin on and blue bars for skin off)

4.3.4.4 Effect of skin contact and time temperature regime on colour attributes in Zespri™ gold kiwifruit juice

The main challenge in processing the 'Hayward' variety has been to keep the green colour in the processed products as the chlorophyll pigments are degraded by the processing conditions (Lodge, 1990). In this study the effect on colour of pressing Zespri™ gold kiwifruit pulp with and without skin after holding at 30, 50 or 70°C for 2, 6 or 12 hours was investigated. The pulp was also treated with 0.15mL.kg^{-1} of the pre-pressing enzyme and juice with 0.035ml.L of the post-pressing enzymes, respectively. Table 4.10 presents data for the change of Hunter colour values, as compared to average values for 30 fresh fruits.

Table 4.10: Effect of skin contact time –temperature regime on Hunter colour values

			L	C	E	Hue angle
Fresh fruit		20	66.69	30.48	73.32	103
Skin contact	Time	Temperature	ΔL	ΔC	Δ E	Hue angle
Son	2	30	26.7	28.8	39.3	109
Son	2	50	24.8	29.8	39.0	110
Son	6	30	25.3	29.8	39.2	112
Son	6	50	26.8	28.3	39.0	109
Soff	2	30	26.2	29.2	39.2	117
Soff	2	50	27.8	28.7	40.0	111
Soff	6	30	25.5	29.8	39.5	127
Soff	6	50	26.1	29.8	39.7	127

LSM = 0.05, Son = skin on juice, Soff = skin off juice

Generally the colour values LCH decreased remarkably compared to the fresh fruit colour as observed earlier (Tables 4.7, 4.10). The hue angle appears to increase with both temperature and holding time, especially for the skin off samples. The data showed huge decrease of the b* value with very small decrease of a* with increase of temperature and time. Therefore, long holding at high temperatures leads to decrease of the golden yellow colour of Zespri™ gold kiwifruit.

The other Hunter colour parameters (lightness and chroma) did not show any significant trend as a result of temperature and holding time. The change in chroma was greater than observed earlier but the differences were not significant due to the variability of the data.

4.3.4.5 Volatile compounds

Volatile compounds were analysed with GC and GC-MS as detailed in chapter 3. Table 4.11 present these compounds that were identified in juice. Only hexanal, trans-2-hexanal, ethyl butanoate, ethyl acetate, methanol were quantified; the rest were only identified with GC-MS and are given in their relative amounts. The GC-MS chromatograms are included in Appendix 5.

Table 4.11 Volatile compounds in gold kiwifruit juice

	Compounds	Amount (mgL^{-1})	Relative amounts (%)
1	5-hydroxymethyl-2-furancarboxyaldehyde		5.75
2	4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6methyl		0.57
3	Cyclohexene, 1,3,5-tris (methylene)		17
4	Pentanedioic acid		14.1
5	Bicylo [3.1.1] heptane, 6,6-dimethyl-2-methylene		19.7
6	Eucalyptol		3.98
7	D-limonene (cyclohexene, 1-methyl-4-(1-methylenethenyl)		1.11
8	Ethyl butanoate	10.8	7.96
9	Ethanol		10.8
10	Methanol	4.3	10.2
11	Hexanal	4.2	1.11
12	Trans-2-hexanal	9.1	1.7
13	Triethyl orthoacetate		3.76
14	Ethyl acetate	19.1	2.21
15	3-methylbutanol		5.75
16	Acetic acid	0.10	0.57

The most prominent compounds identified in juice were mainly carbonyl compounds (aldehydes) and esters. Other compounds detected were organic acids and alcohols. Among the carbonyl compounds only hexanal and trans-2-hexanal were quantified. The amounts reported in Table 4.11 are less than those reported in literature for 'Hayward' kiwifruit (Young *et al.*, 1983). For example by using vacuum distillation, head space sampling and GC-MS, Young *et al* (1983), could identify up to 76 compounds and reported relative amounts of 5.08%, 37.92% and 19.74% for hexanal, trans-2-hexanal and ethyl acetate respectively, those compounds identified. The reason for this difference could be that, volatiles compounds were lost during juice extraction, when the pulp was held at 50°C for 2h, prior to pressing. On the other hand 5-hydroxymethyl-2-furaldehyde appeared in relatively high amount in the juice in this study, which indicates some partial caramelisation of sugars during heat of pulp prior juice extraction and juice clarification.

Esters were in relatively high amount in the juice and the most abundant esters identified were ethyl butanoate and ethyl acetate (Table 4.11). Ethylbutanoate is attributed to fruity aroma, and in kiwifruit more of ethylbutanoate relative to trans-2-hexanal leads to a more appealing fruity aroma (Young *et al.*, 1983) as the former has low odour threshold. In a sensory study of kiwifruit aroma Gilbert *et al.* (1996) reported that ethylbutanoate and trans-2-hexanal synergistically influence kiwifruit flavour, and that ethylbutanoate alone positively affected overall liking, liking of aroma and liking of flavour. In contrast trans-2-hexanal separately

negatively affected all acceptability attributes. In this study, Zespri™ gold kiwifruit juice was found to have higher levels of ethyl butanoate relative to trans-2-hexanal, an indication that its flavour is likely to be acceptable. The sensory characteristics of the juice were not investigated in this study and further research is recommended to investigate how the composition of Zespri™ gold juice affects its sensory profile.

4.3.4.6 Amount of proteases activity in Zespri™ gold kiwifruit juice

Actinidin is an important enzyme found in kiwifruit and affects taste, allergenic properties and processing characteristics (Nishiyama and Oota 2002). Some researches have reported that there is varietal difference in the amount of actinidin present in kiwifruit (Nishiyama and Oota, 2002). The aim of this experiment was to measure the activity of actinidin in Zespri™ gold kiwifruit juice and compare to its activity in 'Hayward' and commercial papain.

The protease activity was evaluated using the casein digestion method as described in section 3.3.10.

Table 4.12 compares the levels of proteases activity in Zespri™ Gold kiwifruit, 'Hayward' kiwifruit and commercial papain.

Table 4.12 Protease activity (Actinidin) Gold ™ kiwifruit, green 'Hayward' and commercial papain

Samples	Zespri ™ Gold Kiwifruit (Unitsg^{-1})	Kiwifruit (Green) Hayward (Unitsg^{-1})	Commercial Papain (units g^{-1})
1	8.58 ± 0.5	19.02 ± 0.5	21.15 ± 0.05
2	9.2 3± 0.5	19.43 ± 0.5	20.85 ± 0.05
3	7.95 ± 0.5	20.31 ± 0.5	21.536 ± 0.05
4	9.11 ± 0.5	19.3 1± 0.5	21.23 ± 0.05
5	8.62 ± 0.5	19.21 ± 0.5	20.86 ± 0.05
Mean	8.7±0.51	19.5±0.5	21.1±0.28

This result shows that the amount of protease activity in Zespri™ gold kiwifruit to be significantly ($P<0.05$) less than that found in 'Hayward' variety. On average the activity was only 45% of that for green. This means that it is likely there will be less concern regarding allergy from consumption of Zespri™ gold kiwifruit or its products. Also it means that it

should be easier to process Zespri™ gold kiwifruit into juice and other beverages than the green 'Hayward' variety as there will be less problem of haze formation if no other protein is increased to compensate.

4.4. Overall Discussion and Conclusion

The combination of press aid (2%) and celluzyme enzyme (0.15mlL^{-1}) increased juice yield by 93%. However, there was a wide variation on juice yield (74%, 85%, and 93%) with different batches of fruit for similar conditions. It could be that the extent of maturity and storage history contributed to these variations. Further research is therefore recommended to quantify these effects.

DM, AIS and TPn decreased significantly with enzymes concentration. The optimal concentration of these values tested appeared to be 0.1-0.15ml.kg^{-1} Celluzyme®, and 0.025-0.035ml.L^{-1} Kleerase®. Both pre-and post-pressing enzyme contributed to the reduction of viscosity, the effective range being 0.1-0.15mlL^{-1} Celluzyme® and 0.025-0.035mlL^{-1} Kleerase®. Enzyme treatment had no noticeable effect on pH, TSS and reducing sugars, but slightly increased levels of TA and total acidity, perhaps due to hydrolysis of polymeric carbohydrates. Vitamin C and TP decreased slightly with enzyme concentration. The combined effect of polyphenoxidase inherent in the fruit and esterase present in the commercial enzyme preparation could have caused reduction of TP. Enzyme concentration did not seem to have major effects on Hunter colour attributes.

Pressing with skin gave a lower yield than pressing with skin off. Skin contact time and temperature increased pH and total acidity. Removal of could be beneficial where single strength juice from kiwifruit is desired. However, removal of skin by scooping flesh significantly ($P<0.05$) decreased vitamin C. Thus, if peeling is to be done a suitable peeling technique which removes very little of the underskin flesh needs to be sought. Skin contact and temperature increased TP but the effect seem to be concealed by the effect of enzymes, which appeared to reduced TP. Low temperature up to 50°C for up to 6h of skin contact improved colour intensities and juice quality in general. Therefore if skin contact is to be allowed it should be at low (≤ 50°C) temperatures.

Repeated pressing significantly ($P<0.05$) increased juice yield but the initial free-run juice was superior in colour and vitamin C. Results might also vary with different types of press and different extraction techniques. Therefore, in order to maximise yield without compromising quality, the effect of both press type and the extraction techniques to be used will need to be investigated in the commercial equipment to be used.

Zespri™ gold kiwifruit juice contains a good balance of aroma compounds and is likely to be at least as acceptable in terms of flavour as the traditional 'Hayward' kiwifruit juice.

Zespri™ gold kiwifruit is likely to be easier to process and may be more acceptable to consumers as the level of proteolytic activity is less than half that observed in 'Hayward' variety.

5. Alcoholic fermentation behaviour and wine quality

5.1. Introduction

Alcoholic fermentation may be affected by a number of factors including temperature (Moreno *et al.*, 1986; Julien, 2001; Torija *et al.*, 2003; Ananta and Shukla, 2004); level of fermentable sugars (Ananta and Shukla, 2004), nutrient supply, pre-fermentation treatments of musts such as filtration or clarification (Ribereau-Gayon, 1999; Delteil, 2004), juice expression techniques, and reactor sizes and volume fraction (Hildalgo, 1981). The relative importance and effects of these factors depend on the substrate and the strain of yeast employed; for example some strains of *Saccharomyces* perform better at low temperature while other perform better at high temperatures (Torija *et al.*, 2003). Therefore for each new substrate, the effects of these key factors affecting alcoholic fermentation need to be evaluated.

The current study was to explore alcoholic fermentation behaviour of gold kiwifruit juice so as to establish appropriate conditions for fermentation and to assess the effects of various juice extraction treatments on wine yield and quality.

The specific objectives were:

(1) To study the effects of temperature and sucrose enrichment on fermentation behaviour of musts and wine quality.

(2) To assess the effects of pressing with skin on or skin off and holding on pomace at different time- temperature regimes on the fermentation behaviour and wine quality.

(3) To evaluate the effect of repeated pressing on the fermentation behaviour and quality of the finished wine

(4) To evaluate the effect of filtration of juice and reactor size or volume fraction of must on fermentation behaviour and wine quality.

5.2 Effect of fermentation temperature and sucrose addition

5.2.1 Introduction

Fermentation temperature and substrate concentration affects the rate of fermentation and composition of the finished wine (Moreno *et al.*, 1986; Mauricio *et al.*, 1989; Gil-Munoz *et al.*, 1997; Charoenchai *et al.*, 1998; Julien, 2001; Torija *et al.*, 2003; Ananta and Shukla, 2004). The effects of these, however, are in turn influenced by the intrinsic characteristics of the musts and strains of yeasts employed in the fermentation (Benda, 1982; Heard and Fleet, 1988; Lafon-Lafourcade, 1983). Whereas high temperature may be favoured for high productivity, low temperature might be desirable for evolution of flavour compounds. Therefore, a compromise should be obtained between sensorial quality and productivity.

5.2.2 Material and methods

Zespri^{TM} gold kiwifruit juice was obtained as detailed in section 3.2.1 and fermented in a 5L glass fermenter at 20, 30 and 37°C with and without sucrose addition, to determine the effect of substrate concentration and fermentation temperature on the fermentation behaviour and quality of the finished wine. The press juice was 16±0.2° Brix. In the sucrose-enriched samples, this was 20°Brix. The dry wine yeast, *Saccharomyces cerevisiae* (see section 3.1.4), was first cultured in 1% sucrose solution at 20°C and pitched at 2% w/v; this achieved an initial cell concentration of ca. 10^4 cfu.mL^{-1}. The fermenters were filled to 0.75 volume fraction (VF). Each experiment was done in triplicate to determine experimental variability.

During fermentation, 20mL samples were removed every 24h and analysed for pH, TA, TSS, residual sugars and ethanol. At the end of fermentation the wine was also analysed for alcohols, carboxylic acids, vitamin C, and colour and aroma compounds as detailed in section 3.3. The total fermentable sugars (sum of sucrose, glucose and fructose) theoretical ethanol yield, actual ethanol yield, efficiency and productivity were calculated as described in section 3.2.2

5.2.3 Results and discussion

5.2.3.1 Effect of temperature and sucrose enrichment on fermentation behaviour

Table 5.1 presents data of the effects of temperature of 20, 30 and 37°C, with or without sucrose enrichment, on ethanol production, yield, efficiency and productivity of Zespri™, gold kiwifruit musts.

Table 5.1: Effect of temperature and sucrose enrichment on ethanol production, yield, efficiency and productivity of Zespri™ Gold kiwifruit must

Treatments	Temperature (°C)	Fermentable sugars (%)	Actual Ethanol (%)	Yield (%)	Efficiency (%)	Productivity ($gL^{-1}h^{-1}$)
JS	20	18.0	8.1	44.9	87.9	1.3
NSJ	20	14.6	6.3	43.5	84.3	1.2
JS	30	18.1	8.0	44.1	87.1	1.6
NSJ	30	14.5	6.4	43.8	84.5	1.2
JS	37	17.9	7.9	43.8	85.5	1.2
NSJ	37	14.6	6.7	45.7	88.6	1.1

Notes: **(1) JS Sucrose enriched juice, NSJ Non sucrose enriched juice,**

(2)Values on the Table are means of 3 replications, LSM = 0.05

(3)Productivity was calculated at 72h

Alcoholic fermentation yield varied from 43-45%w/v efficiency from 84-89% w/v and productivity between 1.1-1.6gL^{-1} h^{-1}. These results are very similar to those reported for alcoholic fermentation of 'Hayward' green kiwifruit in which the musts were enriched with sucrose to 18°Brix and 22°Brix, and ammonium and magnesium sulphate, and fermented at 28°C. For those the yield ranged from 38-47%, efficiency from 75-92% and productivity from 0.74-2.0$g.L^{-1}h^{-1}$) (Bortolini *et al.*, 2001).

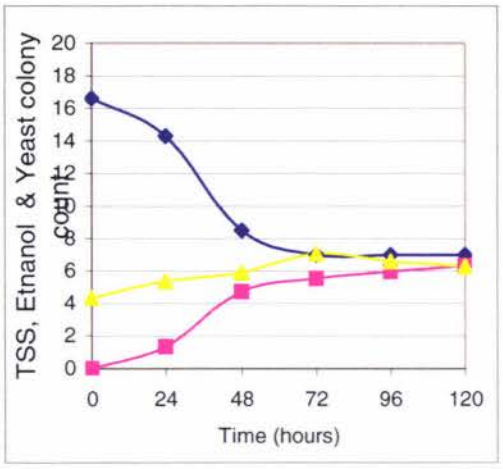
The addition of sucrose led to higher efficiency and productivity except at 37°C where the efficiency was lower (Table 5.1). At 37°C high efficiency was obtained for the unsupplemented juice but at a lower productivity. It could be that, high substrate concentration increases the sensitivity of the yeast cells to the increase in fermentation temperature and thus, slowed metabolism. It has been reported that increase of temperature from 10-30°C increases the rate of fermentation but temperatures above 35°C can lead to sluggish fermentation (Dharmadhikari, 1999), and ethanol production decreased significantly during fermentation of Semillon wines as

temperature was increased from 15-35°C (Reynolds *et al.*, 2001). It seems that the optimal temperature is in the range from 20-30°C, and also that the effect of temperature on the yeast metabolism may be affected by substrate concentration. Addition of sucrose should take into account the carbon-nitrogen ratio (C: N ratio) balance as it is important for the yeast cell's metabolism (Cramer *et al.*, 2002).

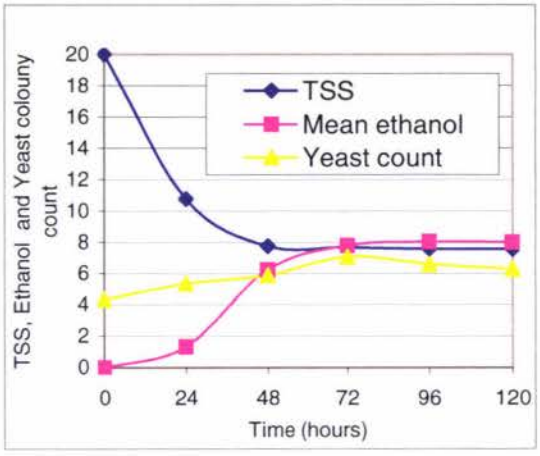
From the fermentation profiles (Fig 5.1) it was evident that most of the ethanol was formed within 48h with slight variation among the treatments and that the fermentation were essentially complete after 72h.

Alcoholic fermentation is closely linked to the growth and metabolic activity of wine yeast. In batch fermentations such as winemaking, four main steps normally characterize the yeast growth pattern. Following inoculation, yeast cells experience an adjustment period. The growth is temporarily halted and this phase is referred to as the lag phase. However, this stage was not observed in this study because the dry yeast cells were grown first in 1% of sucrose syrup and pitched at their exponential stage. Generally, in musts inoculated with a pure culture of active dry yeast, the lag phase is short.

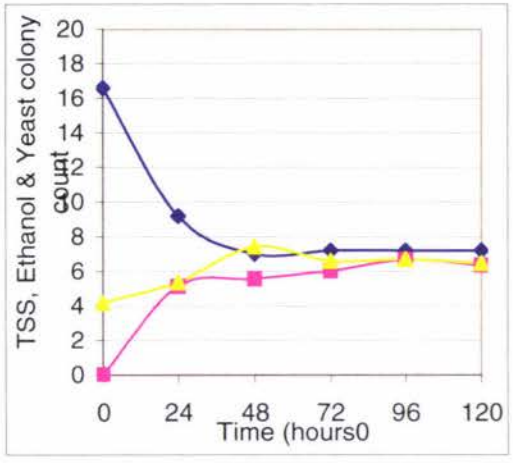
In the second phase called the exponential or log phase, the cells proliferate exponentially, and the population density can reach as much as $2x10^8$cells.mL^{-1} (Dharmadhikari, 1999), when growth levels off in the stationary phase. In this study the highest population (7.4×10^6 cells.mL^{-1}) was observed at 30°C in the must with no sucrose and lowest population number was at 20°C in the must with sucrose. During the stationary phase nutrient level continue to decrease and toxic metabolites accumulate. The yeast's fermentation activity continues (Dharmadhikari, 1999); although there is no cell growth, ethanol may continue to increase until ethanol and toxic metabolites builds up to lethal levels or all substrates are depleted. The yeast population then declines progressively; this is termed as the decline phase.



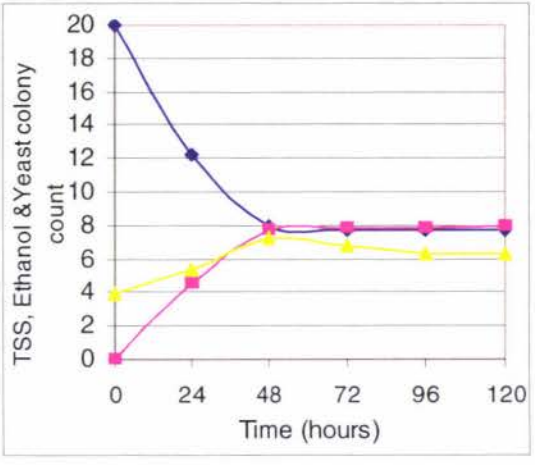
Non-sucrose enriched mash at 20°C



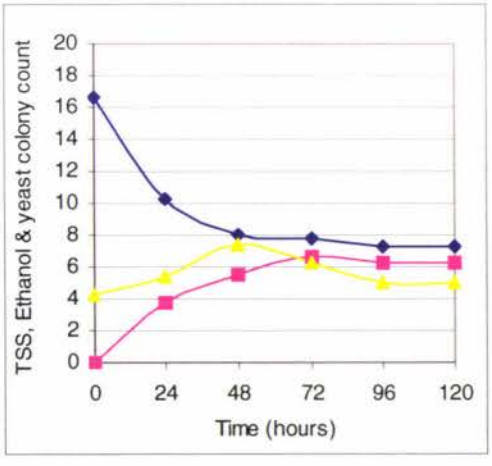
Sucrose enriched mash at 20°C



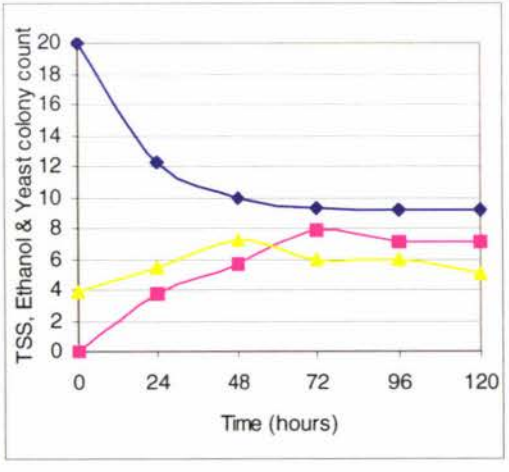
Non-sucrose enriched mash at 30°C



Sucrose enriched mash at 30°C



Non-sucrose enriched mash at 37°C



Sucrose enriched mash at 37°C

Fig 5.2 Fermentation profiles for Zespri™ gold kiwifruit mashes

Notes: TSS as Brix, Ethanol in %w/v and colony count in log CFU.ml^{-1}

5.2.3.2. Effect of temperature and sucrose enrichment on the physico-chemical properties of Zespri™ gold kiwifruit finished wine

Physico-chemical properties were evaluated every 24 hours throughout the fermentation period, up to 144h. At the end of fermentation the finished wine was analysed for total polyphenolics (TP), carboxylic acids, vitamin C, colour attributes and volatile compounds. Their mean values at the end of fermentation are presented in Table 5.2. Data obtained through the fermentation is placed in Appendix 2.

Titratable acidity and total acidity were significantly ($P<0.05$) higher in non sucrose enriched must than in sucrose-enriched must (Table 5.2). Addition of sucrose syrup concurrently elevates the level of total soluble solids and the dilution effect lowers the acidity of the juice (Withy and Lodge, 1982) and this has been reflected in the finished wines. This may be particularly important for kiwifruit wine as it will reduce the astringency and harshness caused by high level of acids in the fruit.

The values for volatile acidity were similar at 20 and 30°C but much lower at 37°C. Kourkoutas *et al.* (2001) and Mauricio *et al.*, (1989) indicated that volatile acidity decreased with temperature.

There was no significant change of TA caused by temperature, however, the values for TA obtained in this study were lower for wine than for juice as reported in literature (Saulleros *et al.*, 2001) (see the initial data for TA in Appendix 2). However these results differ from those reported for grape wines, where TA increase with fermentation temperature (15 to 35°C) (e.g. Reynolds *et al.*, 2001). The batch of fruit used for this experiment had relatively high acidity compared to most other batches (see Tables 4.3, 4.8 and 4.9 except that used for the enzyme experiment in Table 4.6) which was uniquely higher.

The finished wines had high levels of TP (450-740mg.L^{-1}, GAE) and vitamin C (615-920mg/L), with higher levels of polyphenolics in the 37°C wines and higher levels of vitamin C in the 20°C (Table 5.2). In a separate experiment, in which musts were prepared in the same way and fermented at 20°C and wines stored in sealed plastic containers, it was observed that wines stored up to 4 month had low levels of vitamin

C (compare Table 5.7 and 5.8). It seems that a considerable amount of vitamin C is lost during aging and storage of wines. Although some polyphenolics compounds such as syringic acid, *p*-coumaric acid, sinapic and 3,4-dihydroxybenzoic acids have been reported to increase with fermentation temperature (14 to 19°C) for Malvasia Fina wine, the same compounds were reported to decrease in Códega and Gouveio wines (Ramos *et al.*, 1999). Therefore, the effect of fermentation temperature on TP varies depending on substrate and yeast used.

Table 5.2 Effect of temperature and addition of glucose on the physico-chemical properties of Zespri™ gold kiwifruit finished wine

Tmt	Tmp (°C)	pH	TA ($g.L^{-1}$)	TSS	RS (%)	VA (%)	ToA	TP (g/L.GAE)	BI (b*/a*)	TVC (mg/L
JS	20	3.40	11.90	7.70	0.0	0.5	2.0	453.2	-0.61	700.0
NSJ	20	3.40	13.20	7.20	0.0	0.3	2.3	591.8	-0.50	920.0
JS	30	3.41	12.16	7.70	0.0	0.6	2.1	456.5	-0.80	550.9
NSJ	30	3.50	13.02	7.20	0.0	0.4	2.1	740.6	-0.48	725.0
JS	37	3.50	11.80	9.20	0.2	0.01	2.1	645.9	-0.65	615.0
NSJ	37	3.48	13.15	7.73	0.2	0.01	2.5	629.0	-0.63	695.0

Data on Table are means of 3 replications, (P= 0.05)

Notes: Tmt treatments, Tmp temperature, JS Sucrose enriched juice, NSJ Non sucrose enriched juice, TA titratable acidity, TSS total soluble solids, ToA total acidity, TP total phenolics, BI browning index, TVC total vitamin C

During fermentation the main reaction that affects the level of polyphenolics is hydrolysis of glycosides and aglycons (Romero *et al.*, 2004). Vigorous reaction in the high sugar concentration might have enhanced the hydrolysis reaction in the sucrose-enriched mashes. It has been reported that red wines obtained at low temperatures (16-18°) had significantly low levels of polyphenolics than wines obtained at high temperatures (30-32°) (Lorinz *et al.*, 1998) which is consistent with the general trend here.

Overall the composition Zespri™ gold kiwifruit wines was observed to have higher levels of TP, TVC and TSS than 'Hayward' kiwifruit wines reported in literature (compare Tables 2.6 and 5.2)

Fermentation temperature is one of the factors that affect colour and sensory characteristics of wine (Gomez-Miguez and Heredia, 2004). Fig 5.3 shows there was effect of fermentation temperature and sucrose addition on the wine colour.

Wines fermented at 20°C with sugar (left) and with no sugar enrichment (right)

Wines fermented at 30°C with sugar (left) and with no sugar enrichment (right)

Wines fermented at 37°C with sugar (left) and with no sugar enrichment (right)

Fig 5.3: Photos of wines fermented at 20^{0}C, 30°C and 37°C with or without sucrose enrichment

There was no significant difference in the change of the Hunter colour parameters; L*, a* and b*, caused by the fermentation temperature but the browning index was slightly increased in the sucrose enriched replicates.

5.3. Effect of juice pressing technique

5.3.1 Introduction

The method of juice expression from fruit affects the juice composition, and hence may influence the fermentation behaviour of the juice and quality of finished wines. For example hard pressed apple juice had higher levels of TP and organic acids than free-run juice (Kin *et al.*, 1988), and during pressing of bunch grapes, it was observed that after the free run volume was obtained, pressure and number of rotations dramatically increased TP concentration (Dunford and Sneyd, 1990). In chapter 4 it has been observed that repeated pressing significantly ($P<0.05$) increased the level of TP and carboxylic acids (section 4.4.3-4) of Zespri^{TM} gold kiwifruit juice. Therefore, in juice processing, the free run juice could be collected prior to pressing for use in premium products. The objective of this experiment was to evaluate the effect of pressing of Zespri^{TM} gold kiwifruit on the fermentation behaviour of the resultant total juice as compared to the free run juice.

5.3.2 .Material and methods

Free run and total press juice obtained as per section 4.4 were fermented as described in section 5.1 at 20°C to evaluate the effect of repeated pressing on the fermentation behaviour and quality of the finished wine. Sucrose syrup was added to both juices to increase TSS to about 18.0±0.1°Brix. During fermentation sampling was done every 24h and analysed as described in section 5.1.1. All fermentations were conducted in triplicate.

5.3.3. Results and discussion

Table 5.3 present data for ethanol produced, yield, efficiency and productivity. There was no significant difference in fermentation rate but the free run juice attained a significantly ($P<0.05$) higher efficiency than the press juice.

For the press juice it was observed later in the analysis that the finished wine had slightly higher levels of phenolics than the free-run wine (Table 5.4). Grape phenolics have been blamed for stuck and sluggish fermentation (Bisson, 1999), and this could be the reason for the lower efficiency observed in the press juice in this study.

Table 5.3 Ethanol production yield, efficiency and productivity for free run and press juice

Tmt	TFS (%)	AE (%w/v)	Yield (%)	Efficiency (%)	Productivity ($gL^{-1}h^{-1}$)
Free run	17.8	8.0	44.7	87.7	1.1
Press	19.9	8.3	51.0	81.3	1.2

Notes: (1) Data in the Table are means of 3 replications, LSM = 0.05

(2)Tmt treatments, TFS total fermentable sugars, AE Actual ethanol

(3) Productivity was calculated at 72h

In addition, separation of the free-run juice from the press juice could have changed the composition of both in a manner that did not favour fast cell growth and as a result fermentation took longer time (72h) (Fig 5.4) compared to the earlier fermentations (completed after 48h) (section 5.2.1).

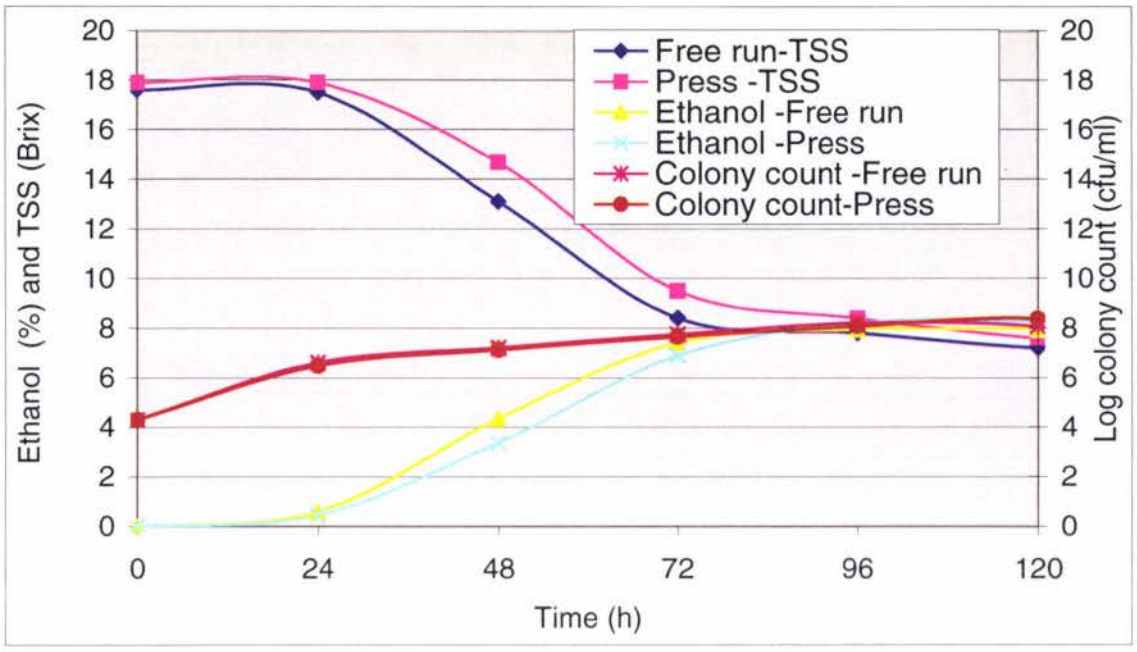


Fig 5.4: Fermentation profile for free-run and press juice

Table 5.4 Physico-chemical properties of free run versus total juice wine of Zespri™ gold kiwifruit

Tmt	pH	TA	TSS	ToA	VA (acetic acid)	Vitamin C (mg/L)	DHAA (mg/L)	TP ($g.L^{-1}$,GAE)	BI (b*/a*)
Free-run	3.45	15.04	7.17	1.70	0.44	500.00	15.84	372.00	-0.47
Press	3.49	13.82	7.63	1.80	0.43	510.00	20.00	384.00	-0.64

Notes: (1) Data in the Table are means of 3 replications, LSD = 0.05

(2) Tmt treatment, TA Titratable acidity, Volatile acidity, TP Total phenolics, BI Browning index

In contrast to the juice (Table 4.3), TA and VA were slightly higher in the free-run wine than in the press wine. It is not clear if this is due to differences in fermentation behaviour or an indication that the free run juice was contaminated with microbes (bacteria or wild yeast), which metabolized the nutrients in the musts and produced acids, most likely acetic acid or lactic acid. During white wine vinification, a significant ($P<0.05$) decrease in phenolic compounds present in free run juice was observed, mainly caused by hydroxycinnamic acids (Betes-Saura, 1996).This effect was not evident for gold kiwifruit and vitamin C levels were also similar. The BI (browning index) was however, higher in the free-run wine (Table 5.4).

5.4 Skin contact effect on fermentation behaviour and quality of the finished wine

5.4.1 Introduction

Fruit skin contact time at certain temperatures may affect the composition of the juice and hence fermentation and quality of finished wine (Noble 1979; Singleton *et al.*, 1980; Test *et al.*, 1986 and Cabaroglu *et al.*, 2001). The aim of this experiment was to investigate the effect of different temperature-time holding regimes on the fermentation behaviour of Zespri^{TM} gold kiwifruit mashes and the quality of the finished wine

5.4.2 Material and methods

Zespri^{TM} gold kiwifruit was crushed with or without skin, held at different temperature –time regimes and juice expressed as described in section 3.2.2 and 4.5.1. Fermentation was conducted at 20°C using the same fermenter as in section 5.1. Two separate experiments were conducted using different batches of fruit. Results for the two experiments are presented in Tables 5.5 and 5.7, and 5.6 and 5.8, respectively.

5.4.3 Results and discussion

5.4.3.1 Ethanol yield, efficiency and productivity

Tables 5.5 and 5.6 presents data for ethanol yield, efficiency and productivity for juice obtained with and without skin and pre-treated as detailed in section 5.1. The final ethanol concentration varied from 5.7 to 6.2%, and yield varied from 36 to 49% at an efficiency of 71 to 97%, productivity was low at 0.77 to 0.86 ($gL^{-1}\ h^{-1}$) (Table 5.5).

Table 5 .5 Effect of skin contact, holding time and temperature on fermentation of Zespri^{TM} gold kiwifruit must

Tmt	Time (hours)	Temperature (°C)	TFS (%)	AE (%)	Yield (%)	Efficiency (%)	Productivity (gL^{-1} h^{-1})at 72h
Son	2	30	13.00	6.2	47.6	93.5	0.86
Son	2	50	11.20	5.5	49.2	96.5	0.77
Son	6	30	14.10	6.0	42.7	83.7	0.84
Son	6	50	15.20	5.9	38.5	75.5	0.81
Soff	2	30	15.83	5.7	36.1	70.9	0.79
Soff	2	50	13.20	5.6	42.4	83.2	0.79
Soff	6	30	12.70	5.5	43.4	85.1	0.77
Soff	6	50	12.70	5.7	45.1	88.5	0.80

Date on Table are means for 3 replications LSM = 0.05
Notes: son = Juice pressed with skin on, soff = Juice pressed with skin off, Tmt treatment, TFS Total fermentable sugars, Actual ethanol.

Temperatures holding time of 30 or 50°C for up to 6 h respectively did not have a significant effect on fermentation efficiency (Table 5.5). But when holding time was increased up to 12h and temperature up to 70°C, fermentation efficiency decreased noticeably with both time and temperature, being generally higher in the skin off wines (Table5.6). Long skin contact time, caused more extraction of tannins from the skin, which can inhibit the wine yeasts (Ribeareau, 1999), leading to low efficiency and productivity.

Table5. 6 Effect of skin contact, longer holding time and higher temperature on fermentation of Zespri^{TM} gold kiwifruit must

Tmt	Time (h)	Temperature (°C)	TFS (%)	AE (%)	Efficiency (%)	Yield (%)	Productivity (g/L/h)
Son	6	30	21.6	8.6	78.0	39.8	0.98
Son	6	50	22.4	8.8	76.8	39.1	1.00
Son	6	70	22.7	9.0	77.9	39.7	0.88
Son	12	30	22.6	8.5	73.9	37.7	0.85
Son	12	50	21.3	7.5	68.8	35.1	0.87
Son	12	70	21.7	8.4	75.5	38.5	0.84
Soff	6	30	20.9	8.4	78.7	40.2	0.92
Soff	6	50	20.9	9.5	88.8	45.3	1.00
Soff	6	70	21.8	9.2	82.6	42.1	1.00
Soff	12	30	20.5	8.5	81.4	41.5	0.96
Soff	12	50	20.2	7.7	75.0	38.2	0.66
Soff	12	70	23.5	7.9	76.8	33.5	0.84

Data on Table are means for 3 replications, LSM = 0.05

Notes: son = Juice pressed with skin on, soff = Juice pressed with skin off, Tmt treatment, AE Actual ethanol, Total fermentable sugars

Fig 5.5 and 5.6 illustrates the fermentation profiles for pulp held at 30 and 50°C for 6h prior pressing. All samples started off well with a lag phase of about 24h, a log phase of about 48h and stationary phase of about 24h. Long skin contact caused slow fermentation and finished wines had high levels of residual TSS (Fig 5.6).

Skin off mashes held at 50 and 70°C for 6 and 12h attained noticeably high yield and efficiency compared to their skin on counterparts (Table 5.6). For mashes held at 30°C there was no noticeable difference in yield and efficiency.

The skin on mashes attained a noticeably high biomass and productivity than their skin off counterparts at the same temperatures (Fig 5.5 and 5.6, and Table 5.6). However, in the later stages of fermentation due to relatively high levels of TA, total acidity and TP (Table 5.7), build up of fermentation metabolites and increase of temperature might have prevented sustained cell growth and consequently, short stationary phase and hence low yield and efficiency.

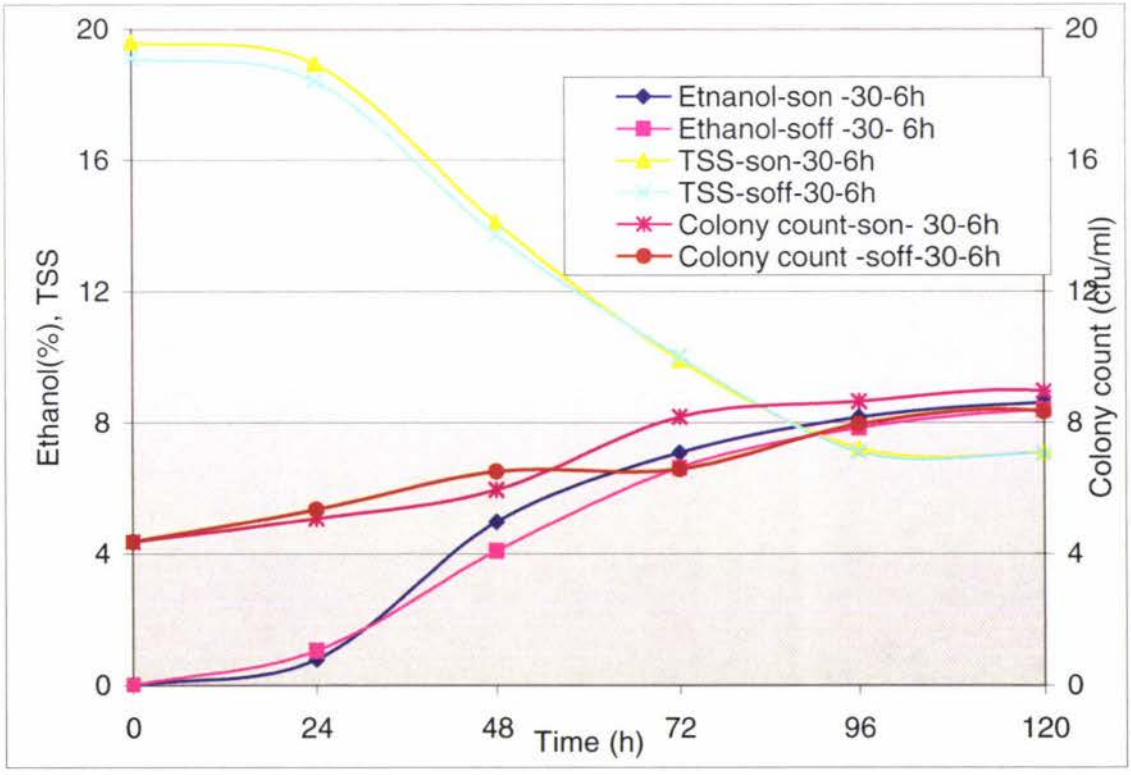


Fig 5.5 Fermentation profile for samples held at 30° for up to 6h before pressing

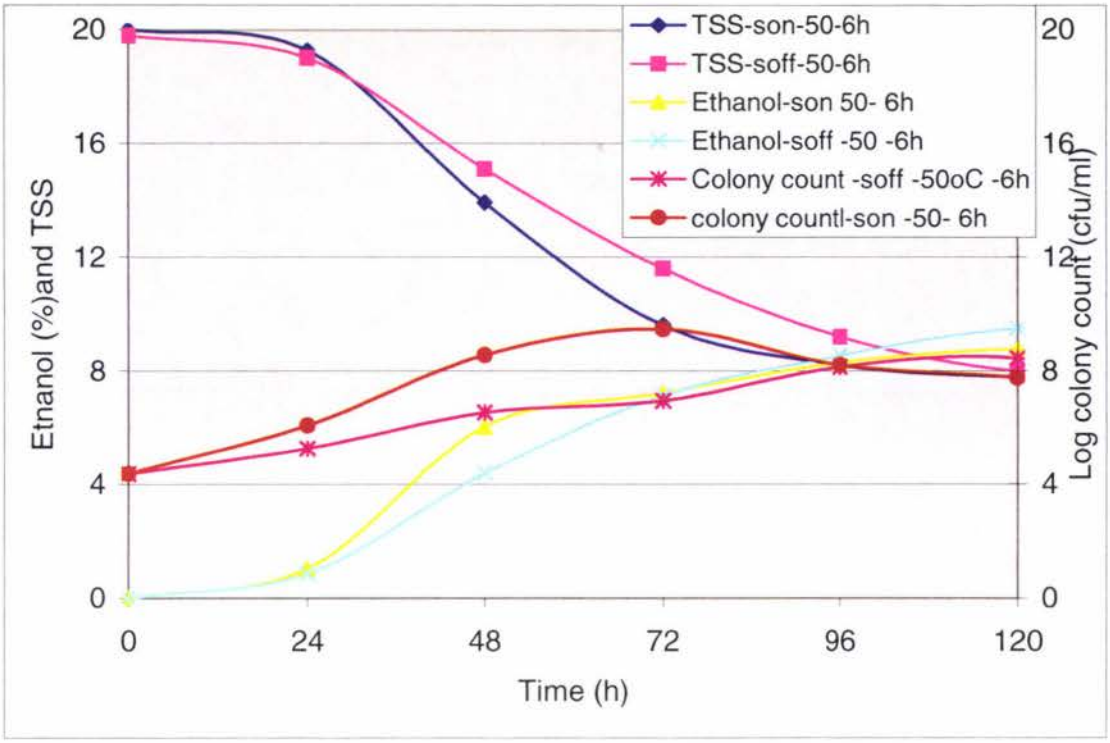


Fig 5.6 Fermentation profile for samples held at 50° for up to 6h before pressing

5.4.3.2 Effect of skin contact time and temperature on physico-chemical properties of Zespri™ gold kiwifruit wine

The pH of the wine was not affected by the pre-fermentation treatments. Meanwhile, TA was low in this batch of fruit compared to other batches of fruit processed later (compare Table 5.7 and 5.8).

Wines from juices whose pulp was held at 30°C finished with slightly higher TSS than those from juices whose pulp was held at 50°C (Table 5.7) whilst total acidity was significantly ($P<0.05$) higher in the wines of which pulp was allowed skin contact prior to pressing than those of which skin was removed prior to pressing. Likewise, total acidity increased noticeably with holding time and temperature. Even when temperature was increased to 70°C and holding time up to 12h (in a different experiment with a different batch of fruit) a similar pattern was observed (Table 5.8) This show that when pulp was allowed skin contact prior pressing more of the fruit acids were extracted from the skin into the juice.

These results are similar to Bates *et al.* (1980), who reported that, acidity of muscadine grape wine increased steadily with holding time and heating of grape pulp up to 60°C increased dramatically the total acidity. Temperature above 50°C might have caused tissue breakdown and enhanced release of the fruit acids into the juice.

Table 5.7: Analytical data for Zespri™ gold kiwifruit wine from juice extracted with skin on or off and held at 30 or 50 °C for 2 or 6 hours

Tmt	Time (h)	Temperature (°C)	pH	TA ($g.L^{-1}$)	TSS (Brix)	ToA ($g.100mL^{-1}$)	VA ($g.100mL^{-1}$)	TVC ($g.100mL^{-1}$)	TP ($g.L^{-1}$),GAE	BI (b*/a*)
Son	2	50	3.45	8.70	5.60	2.73	0.05	0.07	583.35	-0.53
son	2	30	3.37	11.05	6.20	2.7	0.06	0.05	679.73	-0.37
son	6	50	3.48	9.42	6.00	2.5	0.05	0.05	630.02	-0.42
son	6	30	3.44	9.29	6.10	2.74	0.06	0.02	715.24	-0.49
soff	2	50	3.45	9.11	5.80	2.59	0.06	0.02	467.70	-0.50
soff	2	30	3.37	9.81	5.85	2.4	0.06	0.03	536.69	-0.51
soff	6	50	3.43	9.24	5.90	2.24	0.06	0.04	532.63	-0.52
soff	6	30	3.43	9.02	6.00	2.10	0.07	0.02	503.51	-0.674

Data on Table are means for 3 replications LSM = 0.05

Notes: son = Juice pressed with skin on, soff = Juice pressed with skin off, Tmt treatment, A Titrable acidity, TP, Total phenolics, BI Browning index, VA, Volatile acidity as acetic acid

Wines from juice with skin contact (2, 6, 12h) prior to pressing had significantly ($P<0.05$) higher levels of TP than those with the skin was removed (Table 5.7 and 5.8), Skin contact, holding time and temperature contributed significantly ($P<0.05$) towards increase in TP in the finished wine with strong interaction between time and temperature.

It has been reported in literature that, long skin contact time prior fermentation (8-18 h at 18-22°C and 5-48h at 16°C) with Semillon, Sauvignon and Muscadelle grapes caused considerable increase of TP (Dubourdieu, *et al.*, 1986). Without skin contact, a grape fermented with temperature of 20°C gave good wine quality. With skin contact, a lower fermentation temperature was desirable, (Marais, 1998). Therefore, long skin contact at low temperature lead to good results in terms of maintaining colour, desired levels of polyphenolics and liberation of aroma compounds.

Skin contact prior to fermentation significantly ($P<0.05$) increased vitamin C in the finished wines i.e. vitamin C was remarkably higher in wines whose pulp was held for either for 2, 6, or 12 h at 30, 50 or 70°C, than wines whose pulp was held at the same conditions without the skin. A temperature up to 50°C for up to 2h increased total vitamin C, but longer holding times (6 and 12h) at 50°C and above caused decrease in total vitamin C (Table 5.7 and 5.8). The same trend was observed in juice (Table 4.8 and 4.9)

This result suggests that relatively high temperature for short time might be advantageous in releasing nutrient bound in complex fruit structures. As noted above higher amount of vitamin C were observed in wines for pulps pressed with the skin on, compared to wines for pulp pressed with the skin off, suggesting that considerable amount of the vitamin C exists near the skin. Blanching might soften and loosen the skin and allow its removal with minimum amount of under skin flesh. In addition to possible benefits in nutrient extraction this may worth further investigation.

Skin contact did not noticeably affect colour values (Hunter L*, a*, b*) however, holding time and temperature caused a considerable decrease in the yellow (b* values) aspect of colour of the finished wine. Holding at 50°C for up to 6h best preserved the yellow colour of the finished wine (Table 5.7 and 5.8) as indicated by higher b* values for colour.

Table 5.8: Analytical data for Zespri™ gold kiwifruit wine from juice extracted with skin on or off and held at 30, 50,0r 70°C for 6 or 12 hours

Tmt	Time (h)	Tmp (°C)	pH	TA ($g.L^{-1}$)	TSS Brix	ToA ($g.100mL^{-1}$	VA $g.100mL^{-1}$	TV C $g.100mL^{-1}$	TP $g.L^{-1}$,GE	BI b*/a*
Son	6	30	3.48	14.5	7.9	2.00	Trace	0.087	439.63	-0.59
Son	6	50	3.45	14.1	7.8	1.97	Trace	0.053	456.54	-0.50
Son	6	70	3.50	11.7	8.2	1.94	Trace	0.048	466.69	-0.81
Soff	6	30	3.41	11.1	7.1	1.89	Trace	0.065	368.61	-0.77
Soff	6	50	3.43	12.0	8.0	1.87	Trace	0.05	443.01	-0.13
Soff	6	70	3.50	11.5	7.6	2.12	Trace	0.082	439.63	-0.62
Son	12	30	3.45	14.1	8.2	1.76	Trace	0.076	426.10	-0.56
Son	12	50	3.45	11.4	9.1	1.95	0.004	0.086	294.21	-0.29
Son	12	70	3.50	12.16	8.13	1.97	Trace	0.087	507.26	-0.66
Soff	12	30	3.44	14.21	7.76	1.96	Trace	0.085	294.21	-0.56
Soff	12	50	3.45	12.16	8.00	1.92	Trace	0.071	324.64	-0.69
Soff	12	70	3.40	12.16	7.63	2.06	0.003	0.064	338.17	-0.27

Notes: (1) Data on Table are means for 3 replications LSM = 0.05

(2) son = Juice pressed with skin on, soff = Juice pressed with skin off , TA Titratable acidity, ToA Total acidity, VA Volatile acidity, TVC Total vitamin C, TP Total phenolics, BI Browning index.

5.5 Juice filtration and reactor fraction volume effects on the fermentation behaviour and wine quality

5.5.1 Introduction

Filtration or clarification of fruit mashes may affect yeast population and hence fermentation rate (Delteil, 2004; Ribeareau, 1999). Also adequate aeration at the beginning of alcoholic fermentation is advantageous (Colas *et al.*, 1983), this will be affected by the working volume relative to the reactor size. The aim of this experiment was to investigate the effect of these factors on the fermentation behaviour and quality of the finished wine for Zespri™gold kiwifruit mashes.

5.5.2. Material and methods

Filtered versus unfiltered juice was fermented at two fermenter volume fraction, 0.5% and 0.8%. Sampling and analysis of musts during the fermentation and analysed as per section 5.1.1

5.5.3. Results and discussion

5.5.3.1 Effect of filtration and fraction volume on the fermentation behaviour

Table 5.9 gives data on ethanol production, yield, efficiency and productivity. Higher yield, efficiency and productivity were obtained with 80% volume (0.8Vf) filled fermenters than with the 50% (0.5Vf) volume fermenters. Also best results in terms of yield and efficiency were obtained with the filtered juice (Table 5. 9).

Table 5.9 Filtration and reactor fraction volume effect on yield, efficiency and productivity

Tmt	VF	TFS (%)	AE (%)	Yield (%)	Efficiency (%)	Productivity ($gL^{-1}\ h^{-1}$)
Filtered	0.5	14.95	6.	46.3	90.8	0.96
Filtered	0.8	15	7.43	49.5	97.1	1.03
Unfiltered	0.5	16.3	7.0	43.2	84.7	0.98
Unfiltered	0.8	16.09	7.43	46.12	90.5	1.03

Data on Table are means of 3 replications, LSM = 0.05

Notes: Tmt, Treatment, VF Fraction volume, Actual ethanol,

High fraction volume (0.8 or 80% of the size of the reactor) attained slightly higher efficiency and productivity than the lower fraction volume (0.5 or 50% of the size of the reactor) (Table 5.9. Filtration of juice significantly ($P<0.05$) improved efficiency and yield but did not affect the rate of fermentation. However yield was higher than many other runs, indicating variation in batches of fruit, but differences due to fraction volume could still be observed. Table 5.9 present physicochemical data for these wines.

Table 5.9 Filtration and reactor fraction volume effect on physico-chemical properties during fermentation of Zespri™ gold kiwifruit

Tmt	VF	pH	TA ($g.L^{-1}$)	TSS	ToA $g.100ml^{-1}$	VA $g.100ml^{-1}$	TP mgL^{-1},GAE	TVC $g.100ml^{-1}$	BI b*/a*
Filtered	0.5	3.42	13.44	7.4	2.08	0.01	621.9	0.09	-1.28
Filtered	0.8	3.45	13.12	9.1	2.16	0.01	641.2	0.09	-0.44
Unfiltered	0.5	3.45	13.18	7.5	3.12	0.00	721.3	0.14	-0.46
Unfiltered	0.8	3.39	13.28	7.6	2.54	0.01	714.6	0.10	-0.13

Notes: (1) Data on Table are means of 3 replications, LSM = 0.05
(2) Tmt treatment, VF Fraction volume, TA Titratable acidity, ToA Total acidity, VA volatile a cidity, TP Total phenolics, TVC Total vitamin C.

TA and pH were not affected by filtration or fraction volume. The unfiltered juice had higher levels of total acidity, total vitamin C and TP at the end of fermentation compared to those observed for the filtered juice (Table 5.10). Filtration might remove some fruit tissues, which contain phenolics and vitamin C and organic acids compounds that are released during fermentation. This may also explain why the filtered juice attained higher efficiency than the unfiltered juice (Table 5.9).

Most browning occurred in the low volume fraction probably due to excessive aeration (Table 5.10). Therefore, for efficient fermentation and quality wine, 80% working volume with filtered or clarified juice is suitable.

5.6. Volatile compounds identified and / quantified in Zespri™ gold wine

Volatile compounds in wine were analysed with GC/ GCMS as detailed in section 3.3.9. Table 5.11 presents the quantitative amounts quantified with GC as well relative amount of compounds which were only identified with GCMS.

Table 5.11 Volatile compounds identified and or quantified in Zespri™ gold kiwifruit wine using GC and GC-MS

Compound	Relative amount (%)	Quantitive amount (mg/L)
Cyclo-and aromatic hydrocarbons		
Cyclohexene -1-ol, 4-methyl-1-(methylethyl)	0.47	
Bicyclo[3.1.1]heptan-3-one, 2,6,6-trimethyl	0.93	
Bicyclo[2.2.1]heptan-2-one, 2,7,7--trimethyl	0.23	
Bicyclo 3.1.1heptan-3-ol-6,6-dimethyl-2-methylene	0.23	
Bicyclo[2.2.1]hepatn-2-ol, 1,3,3trimethyl	0.02	
Hydroxyethylbenzene	0.70	
Dodecane	0.70	
Esters		
Methyl octanoate	0.14	
Ethyl propionate	0.14	6.20
Ethyl acetate	5.35	29.70
1-hexyl acetate	0.23	
Ethyl hexanoate	10.00	
Isoamyl acetate	16.50	
Ethylbutanoate	1.63	3.50
Isoamyl methanoate	0.02	
Butyl acetate	0.47	
Ethyl decanoate	2	
Ethyl octanoate	11.90	
Alcohols		
Ethanol ($g.L^{-1}$)	21.9	8.00
1-hexanol	0.23	
3-methyl butanol	12.3	
2-ethylhexanol	0.70	
Eucalyptol	10.00	
Methanol	0.03	1.30
2-propanol, 1-(1-ethylnylcyclohexyl)oxy	0.02	
2-methyl propanol	0.02	
Carbonyl compounds		
Nonanal	0.02	
Ethylidene diethyl ether	2.56	
3-methylbutanal	0.23	
Hexanal	0.07	2.90
Trans-2-hexanal	0.15	6.60
Volatile acids		
Butyric acid	0.05	
Acetic acid	0.06	2.40

The main volatile compounds of interest in wine identified in Zespri^{TM} gold kiwifruit wines can be classified into aldehydes, esters alcohols and organic acids. In the GC-MS analysis more compounds were identified in wine than in juice or vinegar. This implies that some compounds which evolve during alcoholic fermentation are lost during acetic acid fermentation. This is discussed in Chapter 7.

Aldehydes

Aldehydes identified in wine included hexanal, trans-2-hexanal, 3-methylbutanal and nonanal among these only the first two were quantified (Table 5.11). These compounds (hexanal and trans-2-hexanal) are character imparting in kiwifruit (Gilbert *et al.*, 1996) increasing the perception of kiwifruit aroma intensity. They have also been identified in 'Hayward' kiwifruit juice by Young *et al.* (1991), but surprisingly they were not identified in Greek kiwifruit wine (mixture of culls of 'Hayward' and 'Bruno', 'Abbot' and 'Monty' cultivars were used for those wines) when Soufleros *et al.* (2001) carried out instrumental analysis of volatile compounds for samples they fermented in 1993 and 1996. It could be that these compounds disappeared during long time storage of those wines.

Esters

Large numbers of esters were identified in the wine including, ethyl butanoate, ethyl acetate, ethyl propionate, isoamyl acetate, isoamyl methanoate, ethyl hexanoate, 1-hexyl acetate, butyl acetate, ethyl octanoate, methyl octanoate and ethyl decanoate. Among these only the first 3 were quantified with GC. The concentrations for ethyl acetate observed in this study were slightly lower (29.7mgL^{-1}) than that reported for Greek kiwifruit wines (38-85.2 mgL^{-1}), but ethyl butanoate (6.2mgL^{-1}) was much higher that reported for Greek kiwifruit wines (0.10-0.27mgL^{-1}). Bartley and Schwed (1989) noted the existence of ethylbutanoate in the significant percentage (14.6%) of volatile compounds in ripe 'Hayward' kiwifruit and at too high level (64.4%) in very ripe kiwifruit. Craig (1988) found that the quantity of ethyl acetate in green kiwifruit wine is nearly double that of white wines produced from the Muller-Thurgau variety. Ethyl butanoate is an essential component of fruits, being responsible for the fruity

aroma, and in kiwifruit it works synergistically with trans-2-hexanal and hexanal to enhance the intensity of kiwifruit aroma and kiwifruit flavour (Gilbert *et al.*, 1996). Ethyl propionate which was observed in significant amount in Zespri^{TM} gold kiwifruit wine has not been reported for 'Hayward' kiwifruit juice or wines.

Acetic acid esters and higher esters

The higher esters with C6, C9 and C10, though were not quantified, some appeared on the GC-MS chromatogram in significant amounts, isoamyl acetate 16.5%, ethyl octanoate 11.9% ethyl hexanoate 10%, and ethyl decanoate 2%, 1-hexyl-acetate 0.23% (Table 5.11). None of these were identified in juice except acetic acid in very small amount (0.1%w/v).

The acetic acid esters, ethyl acetate, isoamyl acetate, and 1-hexyl acetate have been identified in significant amounts as shown above, with the isoamyl acetate being dominant. Isoamyl acetate has been quantified in Greek kiwifruit wines in the range of 0.17-0.79mgL^{-1} and hexyl acetate in the range of 0.02-0.11mgL^{-1} (Soufleros *et al.*, 2001). According to Craig (1988), isoamyl acetate was found in quantities 6-7 times lower in kiwifruit wines than in wines from the white variety Muller-Thurgau, whereas the amounts for 1-hexyl acetate were similar for kiwifruit and white wines. The acetic acid esters, even in small amounts in wines, their characteristic of flower and fruit aroma make they presence favourable (Soufleros, 2001).

The other higher esters were identified in small proportion of volatiles (Table 5.11). But in spite of their low concentration aroma compounds determine, to a high degree, the aromatic characteristic of wines and spirits (Soufleros, 2001).

Alcohols and volatile acids

Besides, ethanol, other alcohols identified were methanol, phenyl alcohol, 3-methyl butanol, hexanol, among these 3-methylbtanol was present in significant proportion in the volatile component of the wine. Craig (1988) reported that higher alcohols occur in much higher concentration in wines from the variety Muller-Thurgau than in

kiwifruit wines. 2-methyl propanol was observed in very small proportion in the volatile components, though it has been reported to occur in higher concentration in kiwifruit wines than in grape wines (Soufleros *et al.*, 2001).

Apart from acetic acid only butyric acid, was observed and in very small proportion in the volatile component in the GCMS analysis (Table 5.11). Butyric isobuytric and isovaleric acids have been reported to enhance acetic acid flavour in wines and spirits (Soufleros *et al.*, 2001).

5.7 Overall discussion and conclusion

Temperature in the range of 20-30°C is suitable for the fermentation of Zespri™ gold kiwifruit mashes with the wine yeast *Saccharomyces cerevisiae*. It can achieve high efficiency (up to 97%) and productivity ($1.6gL^{-1}h^{-1}$), with a final ethanol concentration up to $8.1g100ml^{-1}$ ($81gL^{-1}$). In commercial terms, this appears economically sound, as should not involve significant cooling costs. However, the fermentation performance varied widely with different batches of fruit and this needs further investigation. These conditions ensure retention of vitamin C, good amount of phenolics (antioxidants) and the fresh golden yellow colour of the fruit in the finished wines. Hence a product that is visually attractive and with good levels of antioxidants which confer health benefits to consumers.

Addition of sucrose to raise the level of fermentable sugars, which is a common industrial practice, at this particular range of temperature, does not only increase productivity and efficiency, but also lowers the acidity, hence should reduce the bitterness and harshness noted in kiwifruit wines. By using the Pearson square technique, sucrose syrup can be added in such a manner that, it concurrently raises the level of brix as well as lowering the acidity to half the amount present in press juice.
There was no remarkable difference in fermentation behaviour between the free run juice and the total pressed juice, but there were noticeably higher levels of TP, total vitamin C, and total acidity at the end of fermentation for the press juice. It was also observed that the free run juice produced wine with slightly higher in TA, volatile acidity and yellow colour (Hunter b* values). The level of acidity and TP in the separated press juice might affect the taste of the wine, especially, in kiwifruit with

high levels of quinic acid (ca. 1.2g/100ml), which may lead to an unacceptable stringency and harshness. Therefore, for Zespri^{TM} gold kiwifruit, if the free-run juice has to be separated for the purpose of producing special products, then nutritional quality of those products needs to be considered. Furthermore, the effect of the increased acidity and TP and reduced yellowness need to be taken into account in the use of the press juice.

According to this study, long skin contact and or high temperature could possibly lower fermentation efficiency and productivity, and increase in TP and total acidity. Moreover it leads to increased loss of vitamin C and colour degradation. This may contribute to a wine of poor quality with unacceptable astringency and harshness. On the other hand removal of the skin results into wine with low levels of TP and better colour but a considerable amount of vitamin C is removed along with the peel. Overall however, a skin contact time of up to 6h at low temperature (up to 50°C) does not significantly affect the fermentation behaviour of the fruit mashes. When skin removal is necessary, a peeling method which remove minimal amount of the under skin is required. Blanching might soften and loosen the skin to ensure minimal removal close to the skin tissue and hence minimal loss of vitamin C.

6. Acetic fermentation of Zespri™ gold kiwifruit

6. 1 Introduction

The production of vinegar by acetic acid bacteria requires conditions to be optimised to take into account the activating and inhibiting effect of both substrate (ethanol) and product (acetic acid) on the growth of *Acetobacter* bacteria, as well as conditions of temperature and oxygen supply. Commercially, this often involves an intricate set of equipment such as a gas circulation system, a cooling system, oxygen sensors, heat exchanger, and thermostats, and refilling and discharging devices. For small-scale production a compromise must be found between simplicity, with likely lower productivity and efficiency, and the high capital requirements of more sophisticated systems. The laboratory apparatus used in this study aimed to identify suitable conditions for a semi-continuous acetification system towards the simpler end of the operating spectrum.

The rationale for this was that at least initially, any commercial production of kiwifruit vinegar is likely to be on a small scale making use of existing equipment (e.g. wine fermenters) where possible.

The most common technology for vinegar elaboration is submerged culture, which is normally equipped with aeration, stirring and heating devices (Ory, *et al.*, 2002), so as to maximize production. Variation in the type of equipment set up and nature of a particular substrate can lead to different results. Therefore although starting up protocol for vinegar elaboration with particular acetifiers has been recently reported in literature (Ory *et al.*, 1999), since this is the first attempt to produce vinegar from Zespri™ gold kiwifruit and on a simpler system, it was necessary to first develop a starting up protocol for this kind of vinegar.

Specific objectives were:

(1) To develop a protocol for producing Zespri^{TM} gold vinegars

(2) To establish optimal conditions for acetic fermentation of gold kiwifruit vinegars

(3) To investigate the effect of juice extraction techniques effect on the acetic fermentation and the quality of the finished vinegars.

6.2. Material and methods

6.2.1 Material

The starting material was Zespri gold ^{TM} kiwifruit wine obtained by fermenting juice with *Saccharomyces cerevisiae* at 20°C following methods outlined in previous chapters. This wine contained 7.5±0.5% ethanol (w/v). The starting culture was organic cider vinegar (Coral Tree Organic Products Ltd, Waihou road Levin, NZ) with 4.5% (w/v) acetic acid. Fermentation samples were taken every 24h and analyzed for total acidity, pH, ethanol and acetic acid. A mass balance was performed to check the content of the reactor at any given time.

6. 2.2 Equipment set up.

A 2L Duran bottle, (Schott, West German) was used as a fermenter. This was connected to a condenser to minimise the loss of volatiles. Oxygen enriched air (40%) was supplied by a Hi- tech aquarium air pump model 4500 and air diffuser (Guang Dong Risheng Group co., Ltd Shenzen, China). The air diffuser, which is a porous rubber tube 30cm long, was coiled to form a round loop and placed at the bottom of the bottle. The bottle was then placed in a 29±2 °C water bath. The airflow rate was set at 0.5 or 0.8L.min^{-1}. The experimental set up is detailed in section 3.2.31

6.2.3 Start up protocol

Semi-continuous operation is the operational mode used most often for vinegar elaboration in industry. This operational mode involves developing successive cycles of acetification, each one with the conversion of substrate (ethanol) to product (acetic acid). At the end of each cycle, 50% of the total volume is discharged and the reactor is charged with the same proportion of fresh wine (Ory *et al.*, 2002).

An attempt to adopt this mode to produce vinegar from gold kiwifruit using a 2L laboratory fermenter did not initially produce satisfactory results. Therefore a starting up protocol was designed in which defined proportions of substrate (gold kiwifruit wine) and culture (cider mother vinegar) are introduced into the reactor, followed by two successive additions of fresh wine when the level of ethanol concentration decreased below 0.3-0.5%w/v to bring the relative concentration of substrate and product back to the about the initial proportions. In the third stage, when ethanol is below 0.3-0.5%w/v, half of the reactor's volume is harvested and the reactor is topped up with fresh wine to restore the initial proportions of substrate and product. Samples were taken every day and analysed for dissolved oxygen, TA, pH, ethanol and acetic acid as described in sections 3.2.3.3, 3.3.2 and 3.5.

Initially, 200ml of gold kiwifruit wine (7.32%w/v) ethanol was introduced along with 300ml of cider mother vinegar (4.5% w/v) acetic acid, to give a ratio of 1:1.1 ethanol and acetic acid respectively.

6.2.3 Protocol performance evaluation

The starting up protocol was evaluated at three temperatures (22, 25 and 29°C) and two flow rates (0.5 and 0.8Lmin^{-1}).

6.2.4 Production of various vinegars

After the protocol was developed various vinegars were produced for evaluation. This included vinegars made from skin-on, skin-off wines, free-run and press wines

described in section 5.1. These wines had been stored at -1°C up to six month before being submitted to acetic fermentation.

6.2.5 Meat tenderisation

Some of the Zespri™ gold kiwifruit vinegars produced were tested for their potency to tenderise meat and compared with commercial cider vinegar and commercial papain enzyme.

Meat cube roll (3.5kg) was purchased from a bulk meat supply store in Palmerston North, New Zealand. The meat was cut with a slicing machine to give 20mm thick slices, each piece weighing about 100g. These were injected with 10mL of either Zespri™ kiwifruit vinegar containing 5.1% acetic acid or the same vinegar diluted 50:50 by adding 10% juice from which the vinegar was made (this juice had been stored frozen at –40°C), cider vinegar, papain solution ($2.2gL^{-1}$) or nothing. For each treatment 3 replicates were done. The treated pieces were immediately heat-sealed in polythene bags and stored at 4°C for 2 h before being cooked at 75°C in a waterbath until the internal temperature was 70°C. The meat pieces were then stored at 4°C overnight. Eight rectangular samples of 1 cm^2 cross-section were cut out from each cooked sample, parallel to the muscle fibre direction and analysed with a MIRINZ tenderometer. The force that the tenderometer required to cut through the meat was taken as the degree of tenderness of the meat, the bigger the force the tougher the meat.

6.2.6 Sensory evaluation

Sensory evaluation for the various vinegars was carried out including a comparison with commercial cider vinegar. The first test was a triangle test utilisng different combinations of the kiwifruit and commercial cider vinegars. A panel of 30 assessors was used for this, drawn form Massy staff and postgraduates. Samples were assessed on one day in the roder they were presented, using booths in a sensory laboratory.

The second evaluation was descriptive analysisin which the gold vinegars were characterised and again compared to commercial cider vinegar. Ten assessors were selected from the previous triangle test and compriede Massey staff and postgraduates who were experienced in wine or vinegar testing. One day of training preceded the evaluation during which the descriptors ot be used were discussed and agreed upon.

6.3 Results and discussion

6.3.1 Protocol for acetic fermentation

Data for ethanol and acetic acid for the 3 consecutive stages of fermentation are presented in Table 6.1. The initial volume refers to the volume of ethanol and acetic acid present in the fermenter at the beginning of the day whereas the actual volume is volume remaining in the fermenter after sampling. These volumes were calculated to aid in the calculation of the actual amount of ethanol and acetic acid remained in the fermenter after sampling.

From Table 6, 1 during the first phase there was considerable loss of ethanol and slight loss of acetic acid indicating that there were very few live cells in cider mother vinegar and that for the first 48h they consumed the ethanol to grow and increase in number. Increase in acetic acid began after 72h. In the second phase after topping up with another 200ml wine at 96h, the cells were at their exponential phase and there was rapid conversion of ethanol to acetic acid. In the 3rd phase (from 168-240h), especially after 24h the ethanol: acetic acid ratio was far greater than the optimal range (1:1-1:1.3) (Tesfaye *et al.*, 2002) and the rate of acetification began to fall.

Table 6.1: Mass balance for start up protocol

Top up phases	Time (h)	WA ml	Culture Or VR ml	Initial volume	Ethanol ($g100ml^{-1}$)	Acetic acid $g100ml^{-1}$	Sampling ml	Actual volume	Ethanol ($g\ 100ml^{-1}$)	Acetic acid ($g100ml^{-1}$)	TEA	DO mgL^{-1}
1	0	200	500	500	2.93	2.70	15	485	2.81	2.62	5.5	2.3
	24			485	2.26	2.60	16	469	2.14	2.46	4.6	2.3
	48			469	1.83	2.57	20	449	1.75	2.46	4.2	2.4
	72			449	1.290	2.53	18	438	1.26	2.47	3.7	2.4
	96			438	0.31	3.43	16	422	0.30	3.30	3.6	2.5
2	0	200	422	622	2.39	2.43	15	607	2.33	2.37	4.7	3.2
	24			607	0.63	3.91	15	592	0.61	3.8	4.4	3.3
	72			592	0.32	4.23	15	587	0.32	4.20	4.1	3.2
3	0	305	587	892	2.76	2.77	16	876	2.71	2.72	5.4	3.4
	24			876	1.56	3.62	15	861	1.54	3.55	5.1	3.4
	48			861	0.88	4.28	15	846	0.86	4.21	5.1	3.3
	72			846	0.57	4.61	15	831	0.55	4.53	5.1	3.3

Notes: TEA= Total equivalent acetic acid which is the sum of the ethanol (w/v) and acetic acid (w/v).

After satisfactory results were obtained using the system described above, the effect of temperature and air flow rate on the acetification of Zespri ™ gold kiwifruit wine was investigated. Acetic acid yield, efficiency and productivity were calculated as detailed in section 3.2.2. The data given in Table 6.2 are means for three consecutive top ups which were considered as replications of the acetification process. Theoretical yield, actual yield, efficiency and productivity was calculated as described in section 3.2.3.3

Table 6.2 Acetification performance of Zespri™ gold kiwifruit

Batch	Temperature (°C)	Air flow rate (Lmn^{-1})	Theoretical yield (gL^{-1})	Yield acetic acid (gL^{-1})	Efficiency (%)	Productivity ($gL^{-1}h^{-1}$)
1	29±2	0.5±0.2	52.5±7.03	43.4±8.10	82.4±0.90	0.90±0.2
1	25±2	0.8±02	46.1±5.35	34.14±4.50	74.1±3.68	0.71±0.1
1	22±2	0.8±0.2	56.8±6.24	43.3410.81	76.1±5.91	1.02±0.6
2	29±2	0.8±0.2	69.7±2.00	58.5±3.24	84.7±4.02	1.22±0.1
2	25±2	0.5±0.2	59.4±10.45	48.7±12.93	54.1±40.07	0.62±0.1
2	22±2	0.5±0.2	61.7±5.34	52.5±0.21	75.2±2.22	0.73±0.0

Notes: Data are means S.E for 3 replications

The rate and efficiency of formation of acetic acid were noticeably increased with the increase of temperature and airflow rate (Table 6.2). The highest temperature (29°C) in combination with either 0.5 or $0.8Lmin^{-1}$ of airflow rate was the best of the conditions tried for acetification of Zespri™gold kiwifruit musts.

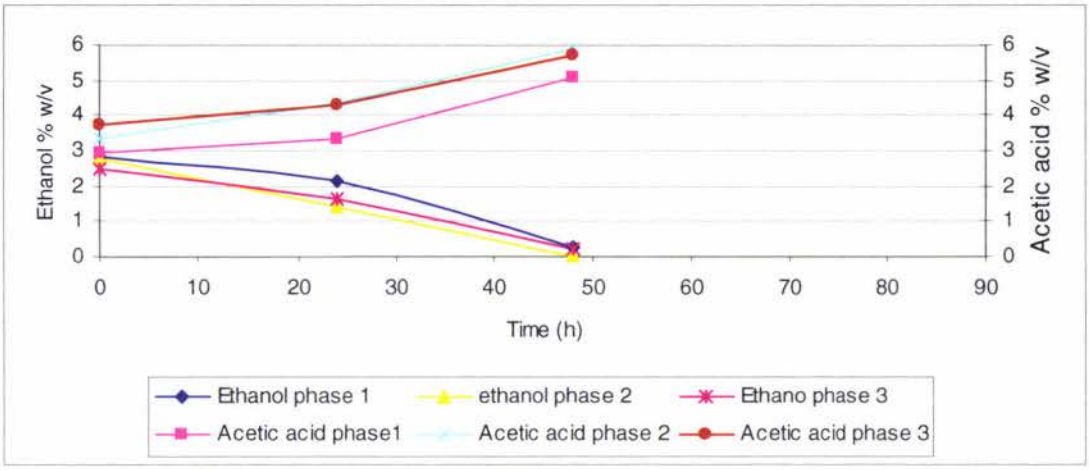
The results attained for yield, efficiency and productivity in this study are very similar to those reported for vinegar production using the green kiwifruit, ('Hayward') variety (Bortolini *et al.*, 2003) who used a vertical generator to acetify ammonium enriched musts at 20 and 25°C, using pure oxygen at 0.05vvm. The productivity is slightly lower ($0.73\text{-}1.22gL^{-1}h^{-1}$ versus $0.83\text{-}1.73gL^{-1}h^{-1}$). The efficient was in similar range to the one attained for gold kiwifruit in this study.

Therefore suitable conditions for acetification of Zespri™ gold kiwifruit musts are 29±2°C with 40% oxygen enriched air at $0.5\text{-}0\text{-}8L.min^{-1}$. From the mass balance done during the fermentation it was observed that best results were obtained when the initial ratio of ethanol and acetic acid in the reactor was 1:1.3 (ethanol% w/v to acetic acid %w/v) and this was restored at every top up, and with maximum a working volume of 75% (1.5L in the 2L

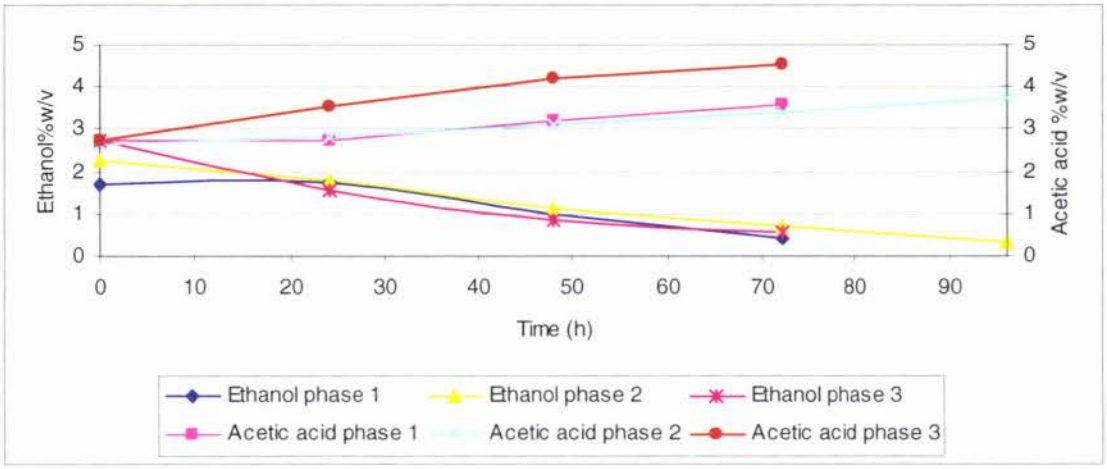
reactor) of the total reactor volume. Higher levels of acetic acid were obtained when the base wine was at least 7.0% w/v ethanol (see mass balance sheet in appendix 4).

Fig 6.1 shows the fermentation profile for three experiments. The differences seen in the fermentation rate at 22 and 25°C (second and third graph on Fig 6.1) among the three top ups explain the effect of differences in working volume. In these fermentations the amount of acetic acid remaining in the reactor after harvesting the previous stages was different and hence in the mass balance calculation the working volume exceeded the optimal volume which is ¾ of the reactor volume (see mass balance sheet in appendix 4). As a result the fermentation period was doubled and this reduced severely the productivity. Although ethanol supply is the most important factor as an imbalance might intoxicate the bacteria and stop fermentation, volumetric imbalance may slow down the rate of fermentation by thedilution effect. To avoid this problem one needs to use wine with a high initial concentration ethanol. In literature it has been reported that at least 8%w/v ethanol is necessary to produce minimum strength vinegar (Adams 19980). The maximum ethanol concentration for the wines used in this study was 7.5%w/v.

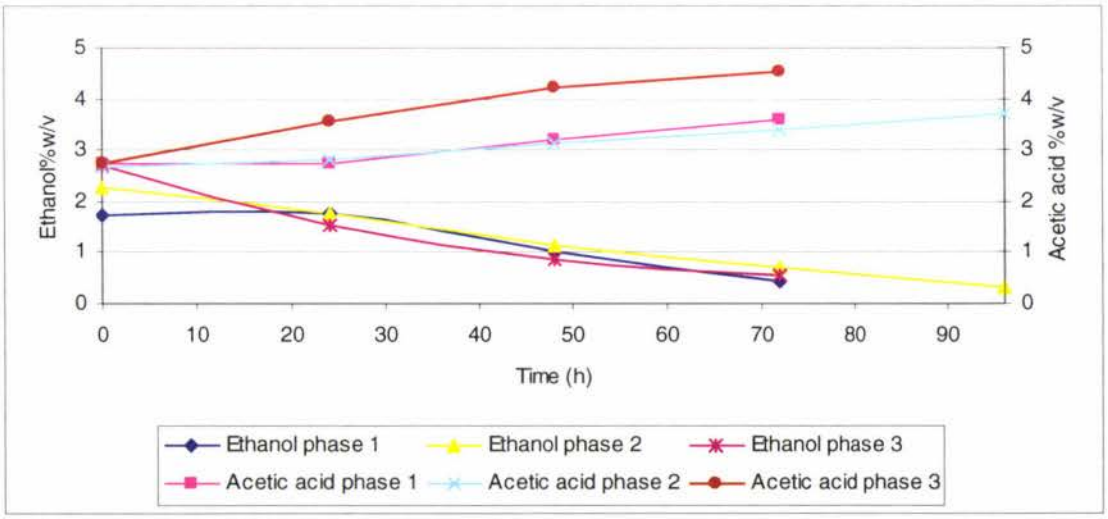
Fig 6.1 illustrates typical acetic fermentation behaviour of Zespri™ gold kiwifruit wine and the effect of fermentation temperature and oxygen supply. The fermentation profiles are only given for the first batch, complete data for the fermentation at the conditions employed is included in appendix4.



Fermentation 29°C and air flow rate of 0.5Lmn^{-1}



Fermentation at 25°C and airflow rate of 0.8Lmn^{-1}



Fermentation at 22°C and airflow rate of 0.8Lmn^{-1}

Fig 6.1 Acetic acid fermentation profile for Zespri^{TM} gold kiwifruit wine

6.3.2. Zespri™ gold kiwifruit vinegars effect of juice extraction techniques

Several vinegars were produced using the wines from free-run juice, press juice, skin-on and skin-off juice produced by fermentation at 20°C using juice without supplementation. In all cases Strong vinegars were obtained with acetic acid concentration ranging from 4.7 to 5.2g.100mL^{-1} (Table 6.2).

Table 6.3 Composition of Zespri™ gold kiwifruit vinegars made from different juice treatments

Pre-treatment	TA (g.L^{-1})	pH	Final ethanol (g.100ml^{-1})	Final acetic acid (g.100ml^{-1})	TEA (g.100ml^{-1})
Free-run juice	5.87±0.3	3.02±0.20	0.46±0.04	4.67±0.30	5.13±0.30
Press juice	6.40±0.4	3.02±0.10	0.12±0.07	5.20±0.43	5.32±0.43
Skin on juice	5.30±0.9	3.04±0.01	0.25±0.03	4.92±0.60	5.17±0.50
Skin off juice	6.08±0.7	3.03±0.01	0.64±0.03	4.88±0.20	5.52±0.30

Notes: TA = Titratable acidity, TEA= Total equivalent acetic acid

The TEA is an important index as it is used to predict from the beginning the amount of acetic acid that will be produced, and also provides a quick means of estimating the amount of losses that may have occurred during fermentation. In an ideal system the TEA value should remain constant through the fermentation period. Table 6.1 shows this was not always the case, indicating some potential for further improvement of the process

There was no significant ($P>0.05$) effect with regard to juice extraction techniques on the acetification process described.

6.3.3. Carboxylic acids and vitamin C, effect of juice extraction techniques on resultant vinegars

The possible effects of juice preparation on the physicochemical and sensory properties of the resultant vinegars were investigated. In Table 6.3, data are presented for carboxylic acids and total vitamin C for the resultant vinegars.

Table 6.4 Carboxylic acids and vitamin C

Pre-treatments	Quinic[a] (g/100ml)	Citric[a] (g/100ml)	Malic [a] (g/100ml)	Tartaric[b]	Succinic[b]	TVC[a] (g/100ml)
Free-run juice[c]	0.881±0.001	0.664±0.002	0.287±0.034	0.329±0.011	0.058±0.001	0.017±3E-04
Press juice[c]	1.028±0.0001	0.768±0.004	0.28±0.006	0.421±0.004	0.11±0.002	0.025±5E-04
Skin on juice[c]	1.21±0.004	0.986±0.006	0.373±4E-04	0.226±0.012	0.084±0.007	0.025±2E-05
Skin off juice[c]	0.856±0.002	0.655±0.003	0.279±0.02	0.324±0.007	0.069±0.001	0.02±5E-05

Notes: Superscript a = analysed with reverse phase columns, b = analysed with HPLC using carboxylic acid column, c = juice treated as detailed in 4.4.2 and 4.5,

As indicated earlier in sections 4.4.3, 4, 4.5 and 5.2.3 and 5.2.6, juice extraction techniques do affect the levels of carboxylic acids in juice and wine. This effect has been carried over to the resultant vinegars. The free-run wine vinegar has slightly low levels of quinic, citric and tartaric acid and total vitamin C than the press wine vinegars (Table 6.3). As described in section 5.2.5 hard pressing extracts more acids from the skin and this will be reflected in the final products. The wine vinegar for juice pressed with skins on had noticeably higher levels of quinic, citric, malic and succinic acids, and total vitamin C.

Removal of skin prior juicing may be advantageous as it reduces acidity and hence harshness in the juice and derived products, but it also reduces vitamin C. Therefore, considering also extra labour costs involved in peeling, one needs to consider how to balance the pros and cons for peeling or not peeling. Although the free-run wine vinegar seems to have high levels of acidity, it was described by a descriptive sensory testing panel (see following sections) as being fruitier than the rest of the wines. However, the skin off wine vinegar with low levels of fruit acids and vitamin C scored highest for overall impression (see section 6.5.2). Therefore, the techniques for juice extraction need to take into account the type of products that are to be processed from the juice.

The vinegars were also analysed for total phenolics (TP) to assess the pre-juice extraction treatments effect of the TP of the final vinegar. Fig 6.2 illustrates the effect of juice extraction techniques on the polyphenols of the resultant vinegars.

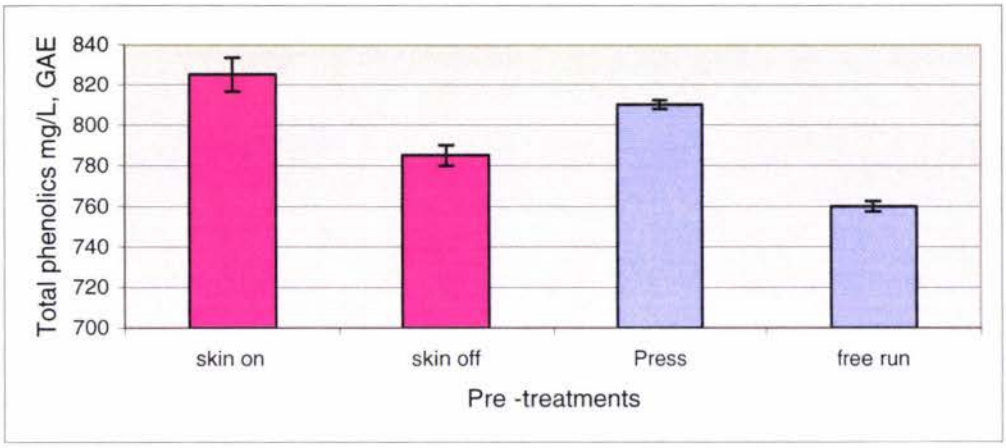


Fig 6.2: Effect of pre-treatments on juice extraction on the total phenols of the resultant vinegar

Skin-on vinegar had a noticeably higher level of TP than the skin off vinegar and the free-run vinegar had more TP than the press wine vinegar. To this end removal of skin and separation of free-run juice leads to lower levels of total vitamin C and TP in the resultant vinegar. Phenolics and vitamin C are both antioxidants which researchers have recently attached them to a number of health benefits (Veloz-Garcia *et al.*, 2004; Helo *et al.*, 2004; Olsson *et al.*, 2004; Anon, 2004). Thus, with vinegar being an ingredient of many food recipes it is may be worth to explore processing techniques that keep these antioxidants in the vinegar.

The colour for the vinegars was measured with Minolta colorimeter as detailed in section3.3.8. The aim for this analysis was determined how much of the fresh fruit colour remained in the final vinegar. Table 4.6 gives data for change of the colour parameter as compared to colour of the fresh fruit. The hue for the juice from which the vinegars were derived is also given on the last column to emphasise the change of colour from fresh fruit to vinegar through juice.

Table 6.4 Effect of juice extraction techniques on the colour of the resultant vinegar

Pre-treatment	ΔL*	Δ C*	Δ E	Hue angle	Hue angle for juice
Total vinegar	26.18±0.32	19.13±0.09	32.64±0.20	91.11±1.2	102.30±1.5
Skin on vinegar	23.22±0.46	22.39±0.16	32.31±0.44	92.68± 2.1	102.20±1.4
Skin off vinegar	22.45±0.20	21.94±0.15	31.44±0.25	96.73±1.3	101.34±1.6
Free-run vinegar	22.14±0.06	21.84±0.09	31.13±0.11	97.37±0.16	109.00±0.94
Press vinegar	22.33±0.11	22. 5±0.05	31.74±0.03	96.73± 2.0	104.00±1.2

Notes : Total vinegar = vinegar from a batch of fruits processed without removal of skin, fermented at 20°C and the wine stored at 1°C for 8month before acetification.
Values in bracket for hue angle are for the juice from which the vinegar was made.

Wines stored for up to 8 month before acetification produced vinegars with less of the fresh fruit colour remaining than the fresh wines as shown by large changes for ΔL*, ΔC*, ΔE*, and lower hue angle (Table 6. 4). However, there was no noticeable difference in lightness, chroma or total colour change (ΔL*, ΔC*, ΔE*) attributable to the pre-juice extraction treatments, although there was a noticeable deviation from the juice colour. The hue angle was slightly higher for free-run than the press vinegars. Vinegar production is a long processes which at same stage involving oxidation of the raw material to ethanol then to acetic acid and although most wide anaerobic conditions, other oxidation and browning reactions can be expected especially in the acid environment. Such vinegars are not much expected reflect the colour of the raw material, rather, their darker colour reflects similar changes that occur during grape wine fermentation and aging.

6.3.3 Volatile aroma compounds identified in Zespri™ gold kiwifruit vinegar

Volatile aroma compounds in Zespri™ vinegar were analysed with GC and GCMS as detailed in section 3.3.9. Four main classes of volatile compounds were identified; esters, alcohols, carbonyl compounds and organic acids.

Esters

Esters identified included ethyl acetate, ethyl butanoate, isoamyl acetate, ethyl pentanoate, ethylcyclohexanepropionate, β-phenyl acetate, and ethyl lactate and ethyl succinate. The last four of these were not quantified (i.e. they were only identified with GC/MS).

Ethyl acetate was the most abundant among the esters. This is an important compound as it presents a wine note in vinegar. This correlates well with sensory descriptive analysis, where the kiwifruit vinegar was described as having moderate wine character, whereas the commercial cider vinegar was described as having weak wine character. High levels of ethyl acetate have been similarly reported in 'Hayward' kiwifruit wines compared to grape wines (Graig 1988).

Ethyl acetate, ethyl lactate and diethyl succinate are esters that are mainly produced from bacterial conversion of various wine components such as ethanol, sugars and tartaric acids (Souflerous *et al.*, 2001).

Acetic acid esters (isoamyl acetate) were the second most abundant. These together with phenyl acetate (not quantified), have been reported in literature to be favourable when present in wine as they are characterised by the aroma of 'flowery and fruity'. The amounts of these esters observed in Zespri^{TM} gold vinegar is higher than those reported for Hayward kiwifruit wines (Souflerous *et al.*, 2001).

Ethyl butanoate was the third most abundant esters (Table 6.5). This is an important character-imparting compound in kiwifruit (Gilbert *et al.*, 1996), as it represent fruity aroma. In a descriptive sensory testing, the kiwifruit vinegar was ranked as having moderate to strong fruity aroma.

Alcohols

Among alcohols, ethanol was dominant, followed by 2-methyl –n-butanol. Methyl butanol and neopentyl glycol identified in the Zespri^{TM} gold kiwifruit vinegars are classified as higher alcohol and their present in small quantities in wines generally has a positive effect on quality (Soufleros *et al.*, 2001).

Volatile acids

The only volatile acid identified apart from acetic acid in vinegar was 2-methyl butanoic acid. The presence of short chain fatty acids, though in minor quantities enhances the acetic acid aroma

Table 6.5 Volatile compounds in Zespri™ gold kiwifruit vinegar by GC-MS

Esters	**mg/L**	Relative amount (%)
Beta-phenyl acetate		0.498318
Ethyl cyclohexanepropionate		0.498318
Ethyl acetate	381.8	20.6802
Ethyl butanoate	34.5	1.868693
Ethyl pentanoate		0.311449
Isoamyl acetate	69	3.737386
butyl ethanoate		1.868693
Ethyl lactate		0.398655
Ethyl succinate		0.498318
Phenyl acetate		1.868693
Alcohol		
Neopentyl glycol		0.498318
2-methyl n-butanol	46	2.491591
Ethanol	174.8	9.468045
methanol	4.6	0.249159
Volatile acids		
2-methylbutanoic acid		2.491591
Acetic acid (g/L)	46	24.91591
Carbonyl compounds		
Hexanal	67.94	3.737386
Trans-2-hexanal	412.53	22.17516
3-methoxyl -Propanal		3.114489

Carbonyl compounds

Among the carbonyl compounds, trans-2-hexanal was the most dominant, followed by hexanal (Table 6.5). These compounds have been identified in 'Hayward' kiwifruit and linked with increase in kiwifruit aroma intensity (Gilbert *et al.*, 1996). The presence of all three character impacting compounds; hexanal and trans-2-hexanal and ethyl butanoate is required for ideal intensity of kiwifruit aroma (Gilbert *et al.*, 1996). Trans -3-hexanal which have also been associated with 'cooked gooseberry' aroma or 'hay' note in 'Hayward' kiwifruit (Young *et al.*, 1991), was not detected with GC-MS although a descriptive sensory testing of the Zespri™ gold kiwifruit vinegar using a trained panel scored a threshold level of hay note in these vinegars (see section 6.5.2).

In conclusion, Zespri™ gold kiwifruit vinegar is rich in aroma compounds, of which the esters are likely to impact favorably on the flavour of the vinegar. It is possible that the vinegar would occupy a special niche in the fruit vinegar market. As far as analysis of volatile compounds is concerned, it should be noted that it was not exhaustive for this vinegar. Vinegar is a complex matrix, and identification and quantification of all aroma compounds require diverse analytical techniques, which were beyond the scope of this project. Therefore, to fully characterise this vinegar, more comprehensive aroma analysis is recommended.

6.4 Tenderising effect of Zespri™ gold kiwifruit vinegars compared with cider vinegar and commercial papain

Proteolytic activity in Zespri™ gold kiwifruit vinegar was investigated as detailed in section 6.2.5. An experiment was conducted to compare the tenderising effect with a standard commercial tenderising enzyme (papain). Ordinary fruit vinegar of a comparable acidic strength (cider vinegar) was also included in the experiment so to assess the effect that could be due to acetic acid. It was found that a reasonable amount of the proteolytic activity observed in juice (section 4.3.4.6) was carried over to the vinegar. Fig 6.3 illustrates the effect of commercial papain, Zespri™ gold kiwifruit vinegar 5.1% (acetic acid) without and with 10% juice added, cider vinegar compared to a control to which nothing was added.

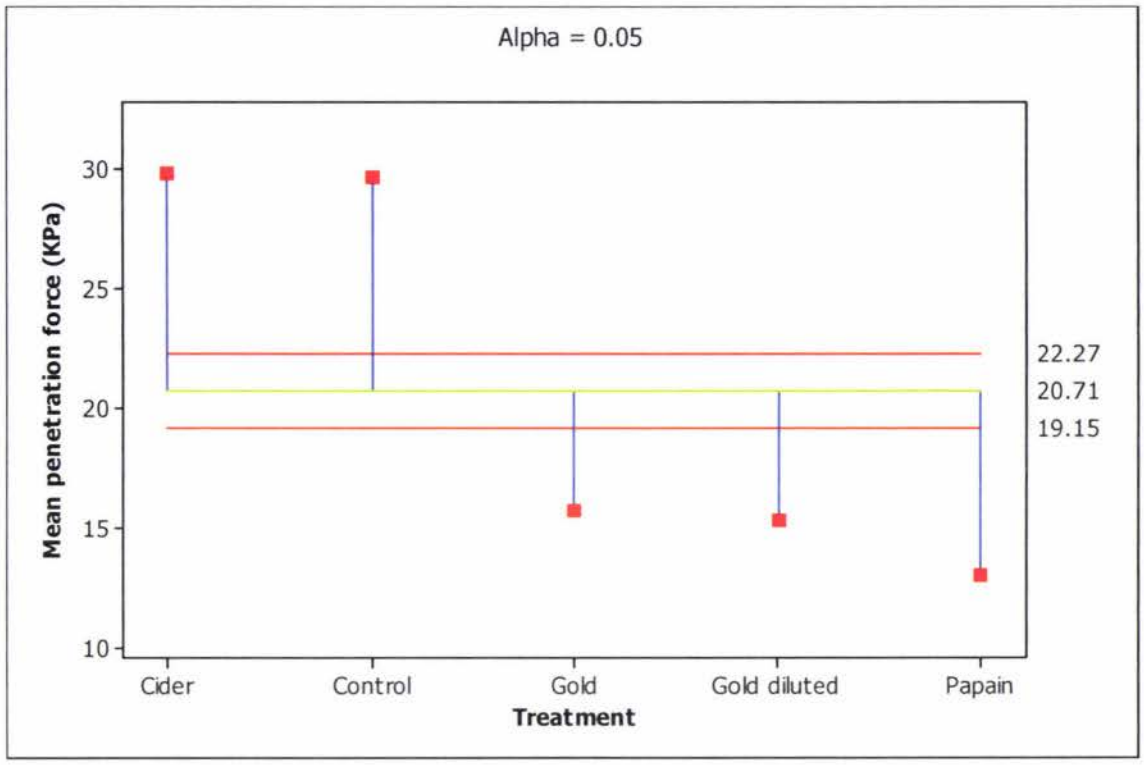


Fig 6.3 Tenderising effect of Zespri^{TM} Gold kiwifruit vinegar compared to cider vinegar and commercial papain

Notes: Cider = Cider mother vinegar, Control = untreated meat, Gold = Zespri^{TM} Gold kiwifruit vinegar, Gold diluted = Zespri^{TM} gold vinegar diluted with 10% juice, Papain = commercial papain enzyme.

The results showed that the meat treated with Zespri^{TM} gold kiwifruit vinegar with or without dilution with juice and papain were significantly ($P<0.05$) more tender than those treated with cider vinegar and the control. The figure to the right of the graph, show mean for all data, clearly indicating the difference of the replicate samples for the mean. There was no significant difference between the meat treated with cider vinegar and the control. This indicates that the tenderness of the meat was caused by enzyme activity rather than acetic acid present in the vinegar. These results demonstrate that kiwifruit vinegar offer a special niche opportunity as a marinating vinegar in the vinegar market. Papain was slightly stronger than the kiwifruit vinegar, but there was no remarkable difference between the meat treated with undiluted vinegar and that treated with the diluted vinegar.

6.5. Sensory evaluation

Sensory evaluation was done for chosen vinegars on taste and aroma as using a triangle test and descriptive analysis as detailed in section 6.2.6.

6.5.1 Triangle test

Table 6.6 present results for a triangle test to evaluate possible difference between fermentation with the skin on versus skin off and free run or press wine vinegars. The skin on / off wines that were submitted to acetic fermentation were those whose juice was held with skin on or off at 50°C for 6h prior pressing (see section 4.5)

Table 6.6 Results for triangle test for skin on versus skin off and free run versus press wine vinegars

	Skin on versus skin off wine vinegars			Free-run versus press wine vinegars		
Attribute	# Correct answers	# Incorrect answers	Total	# Correct answers	#Incorrect answers	Total
Taste	13	17	30	20	10	30
Aroma	26	4	30	25	5	30

Skin-on and skin-off wine vinegars were not significantly ($P>0.05$) different in taste, but they were significantly ($P<0.05$) different in aroma with the skin-off vinegars aroma being described by assessors as being less harsh and having strong 'yeasty' aroma. The harshness detected in the skin on wine vinegars is likely due to the presence of more fruit acids as was observed in the HPLC analysis of juice and wine (section 4.5.2 and section 5.2.6.2). It has also being reported in literature that skin contact increase acidity in juice and wine (Bates et al., 1980; King *et al.*, 1998).

The free-run wine vinegars were significantly ($P<0.05$) different from the press wine vinegars in both taste and aroma. Assessors described the free run vinegar as having strong aroma and strong 'yeasty' and 'fruity' wine note than the press wine vinegars.

Therefore the pre-treatments for juice extraction or alcoholic fermentation can affect sensory quality of the resultant vinegar. Since vinegar is mostly judged on by its sensory quality, in the production of vinegar it is important to consider how juice extraction or alcoholic fermentation pre-treatments will affect the resultant vinegars.

6.5.2. Descriptive analysis

In order to obtain more information on the Zespri™ gold kiwifruit vinegars, descriptive analysis was conducted on attributes which were selected previous by the panel. The panel consisted of 10 judges, 8 of whom were Massey staff experienced in wine and vinegar sensory testing and 2 postgraduate students experience in general sensory testing but not in wine and vinegar. The panel was selected based on their performance in the previous triangle test and individual interview, regrading their experience in wine and vinegar sensory testing. Because the panel appeared to be generally experienced, long training sessions were not done. Instead the panel met one day and evaluated solutions of ethyl butanoate 10ppm (which represent 'fruit' aroma), ethyl acetate for ethyl acetate aroma (10ppm) hexanal 0.1ppm for hay note, and wine for wine note (1000ppm). All solutions were prepared in 4% acetic acid so that they could be perceived in the same way as in vinegar.

The panel ranked the solutions individually from 'not present' to 'very strong' and then they come together for discussion and agreement on what each attribute meant and the way it is normally perceived. Initially, in the discussion, the judges differed in the way 'pungent' sensation is perceived, same saying that it is a strong sensation perceived at the back of the palate and same saying it is an aroma character perceived in the olfactory organ. After discussion and smelling of acetic acid it was agreed that pungent sensation is an aroma character and it is perceived in the olfactory organ. All members of the panel could easily detect the rest of the attributes and there was no discrepancy in the way they were perceived. The panel also agreed that important attributes in characterising fruit vinegar are fruity aroma, wine note, ethyl acetate aroma, woody aroma, acetic acid sensation or pungent sensation, but 'woody' aroma was dropped, as these vinegars were not aged in wood.

The following day, the panel met and characterised four samples of Zespri™ gold kiwifruit vinegar, differentiated only by the way the juice was extracted, to evaluate the effect of juice extraction technique on the quality of the resultant vinegar. Alongside these, commercial cider vinegar purchased from Coral Tree Organic Ltd was also evaluated to compare the new vinegar with cider vinegar. Table 6.7 present means for the panel's scores for each attribute on a scale ranged from 0 for 'not present' to 5 for 'very strong'.

Table 6.7 Descriptive sensory analysis results for Zespri™ gold kiwifruit vinegars compared to commercial cider vinegar

Vinegar samples	Attributes analysed					
	Wine character	Ethyl acetate aroma	Fruity aroma	Pungent sensation	Hay note	Overall impression
Cider	2.2[a]	2.4[a]	2.4[b]	2.7[a]	0.4[a]	2.5[a]
Free-run	2.8[b]	1.9[b]	2.5[b]	3.2[b]	1.0[b]	2.1[b]
Press	2.7[b]	2.1[b]	2.4[b]	3.3[b]	1.0[b]	1.7[b]
Skin off	2.5[b]	1.7[b]	2.0[b]	3.3[b]	1.0[b]	2.5[a]
Skin on	2.8[b]	1.9[b]	2.2[b]	3.6[b]	1.0[b]	1.9[b]

Means marked with the same letter superscript are not significantly different (P=0.05)

Statistical analysis of results revealed that gold kiwifruit vinegars were ranked as having moderate wine character whereas the cider vinegar was ranked as having weak wine character (Table 6.7). But there was no significant (P>0.05) difference in wine character among the gold kiwifruit vinegars attributed to the juice extraction techniques.

There was also no significance difference (P>0.05) noted in ethyl acetate between the gold kiwifruit vinegars, but the cider was marked as having more of ethyl acetate aroma than the gold kiwifruit vinegars. It has been reported that ageing vinegars in wood increases their ethyl acetate aroma (Tesfaye *et al.*, 2000). The cider vinegar was aged in wood whereas the gold kiwifruit vinegar was not. There was no significance (P>0.05) different in fruity aroma between the cider and the kiwifruit vinegars, but the kiwifruit vinegars had moderate to strong 'pungent' sensation whereas the cider vinegar was marked as having weak to moderate 'pungent' sensation. Although it has been reported in literature (Tesfaye *et al.*, 2000) that ageing vinegars in wood increases their pungent sensation, high level of fruit acids in kiwifruit vinegar increase its pungency. It could also indicate an inability of some of the judges to distinguish between astringency a taste detected at the back of the tongue and 'pungency' a strong sensation detected by the olfactory cells as had been observed in the preliminary session.

Despite all the vinegars being diluted to the same level of acetic acid (1%), the panel commented that the skin-on and the press gold vinegars had slightly high acidity than the skin off and free run vinegars, respectively. This could reflect the higher level of the fruit acids in these vinegars as observed in section 6.3.2.

Unsurprisingly, 'hay' note or 'old grass' aroma was not detected in cider vinegar, but was detected at a threshold level in all kiwifruit vinegars. Young *et al.* (1991) studied the aroma compounds in kiwifruit by monitoring the eluent during gas chromatography of ether extract at a sniffing port and associated the 'hay' note with trans- 3-hexenal, an aromatic compound which has been detected in kiwifruit. The purpose of testing for hay note was to see whether the hay note caused by trans-3-hexenal is detectable in the vinegar. However in instrumental analysis of the vinegars (CG-MS) trasns-3-hexanal was not detected. Young *et al.* (1991) had reported 0.74% and 0.16% as relative amount for 'Hayward' kiwifruit harvested in 1988 and 1989, respectively. Such a small amount in juice indicates that there could be very small amount of it remaining the in vinegar. However, since this has been detected only at a threshold level it may not be a concern for commercial production of kiwifruit vinegars. Since vinegar is only used as an ingredient in most food recipes more sensory studies with actual food recipes in the form in which vinegars are normally used should be conducted to see whether this note would still be detected.

The overall impression of the skin off kiwifruit vinegar was similar for the commercial cider vinegar, which was rated with medium to high overall impression. The rest of the kiwifruit vinegars were ranked as medium overall impression. The free-run vinegars were commended by assessors as having a fruitier aroma than the rest of the kiwifruit vinegars.

6.6 Overall discussion and conclusion

The protocol developed successfully produce vinegar and it is suitable to small scale with low costs of production. For successful acetic fermentation require maintenance of optimal levels of temperature and oxygen supply.

Removal of skin tends to lower the level of total phenolics and total vitamin C in the final vinegar

Juice extraction techniques slightly affect sensory quality of the resultant vinegar. Kiwifruit vinegar is of acceptable sensory quality, and if allowed to age in wood could result in a product of excellent sensory quality. Furthermore, this vinegar should find a special application as marinating vinegar.

7. Overall discussion and conclusion

7. 1 Juice extraction and quality

The use of pre-and post-pressing enzymes containing cellulases and pectinases improves juice yield and quality, but this use does need to be optimised as excessive action of these enzymes may lead to increased acidity, probably due to hydrolysis of carbohydrate polymers. The enzymes used in this study (Celluzyme® and Kleerase® from Zymus International) were found to be effective in the range 0.1-0.15 and 0.025-0.035ml.L^{-1}, respectively. In this range these enzymes were effective in breaking down pectin and clarifying the juice and lowering viscosity. Enzymes from other suppliers may show different activity and the best conditions for their use will need to be confirmed before introducing these to a commercial process. Viscosity reduction is important as it facilitates a greater degree of subsequent juice concentration if this was required, which reduce costs in terms of juice handling and shipping, as well as extending shelf life.

The decrease of total phenolics observed with an increase in enzyme concentration could be due to hydrolysis of cinnamic acid derivatives to free acid by esterase present in the commercial enzymes preparations. Phenolics contribute significantly to antioxidant activity and hence to health benefits which researchers have recently associated with antioxidants. It is therefore recommended that more work to be done on the effect of processing conditions and enzyme addition on antioxidant activity and biologically active compounds in kiwifruit. This will help in developing processing techniques which maximise the health benefit of the fruit or derived products, and the processing of the juice.

Peeling of Zespri™ gold kiwifruit prior processing reduces acidity and help in retaining the golden yellow colour of the fruit, which may be beneficial especially were single strength juice is desired. However a considerable amount of vitamin C may be lost if hand peeling is used depending on the skill of the operator. Considering the extra cost incurred in terms of labour, it is recommended that mechanised peeling technique which causes minimal loss of the flesh adjacent to the skin be investigated. The development of an effective automated peeling procedure would also be invaluable in the development of further added-value gold kiwifruit products. On the

other hand skin contact at low temperature (up to 50°C) for short time (up to 6h) may be beneficial in maximising the level of antioxidants in kiwifruit juice. Possibly, if an effective peeling process were developed, some juice could be separately contacted with the skin then added back to the juice to optimise the desired characteristics.

7.2 Alcoholic fermentation

The wine yeast, a strain of *Saccharomyces cerevisiae*, performed efficiently within the temperature range of 20-30°C for Zespri™ gold kiwifruit mashes. The enrichment of kiwifruit juice with sucrose syrup can be done in such a manner that both increase fermentable sugars and decreases of acidity. This appears to reduce the astringency and harshness caused by high natural levels acidity in kiwifruit.

Fermentation temperature affects the evolution of aroma compounds in wine. This also depends on the strain of wine yeast used. It is therefore recommended that further work to conduct to explore the effect of temperature in relation to yeast strain to provide products suited to different consumers.

Delayed pressing of crushed fruits increases the level of acidity, total phenol and appeared to lower fermentation efficient and productivity.

The use of gold kiwifruit juice concentrates to produce wine and vinegar could reduce time and cost and would be an option with further investigation. No major difficulties are identified with the approach although there will be a loss of aroma compounds during juice extraction

7.3 Acetic acid fermentation

The production of vinegar as a subsidiary product in fruit processing factories appears worthwhile; however, a compromise between simplicity and extra costs incurred for intricate vinegar elaboration systems will need to be established. A simple system, like the one employed in this study, could be economically feasible in small scale vinegar production, though, it may not

attain the level of efficiency and productivity observed with large scale sophisticated systems incorporating gas recycle and intense agitation, which can attain up to 100% of the theoretical yield (Ory *et al.*, 2004). In this work at 29°C and with an airflow rate of 0.5-0.8Lmn^{-1} (40% O_2 enriched air) an efficiency of up to 85% and productivity of up 1.2gL^{-1}h^{-1} acetic acid was attained. Maintaining a ratio of about 1:1 for ethanol and acetic acid in the reactor at all times was found to encourage favorable acetification. This is important for optimal performance of the *Acetobacter* strains, which are inhibited by high levels of both ethanol and acetic acid.

The most difficult step in acetification is to build enough biomass to start the acetification process. Once enough biomass has been established, the process should not be stopped i.e. the air supply can not be cut or the temperature allowed falling below 15°C or rising above 31°C. By doing so most of cells will die and it takes time to start up the culture again.

The Zespri™ gold kiwifruit vinegar produced in this study was of good sensory quality, described as being fruitier and higher in wine notes than commercial cider vinegar. The gold kiwifruit vinegar has high amounts of isoamyl acetate and phenyl acetate which characterise the aroma of flower and 'fruity'. It is also rich in ethyl butanoate which represents fruit aroma.

Vinegar is a complex matrix containing a wide category of compounds which can not all be identified by one set of analytical equipment and conditions. To full characterise gold kiwifruit vinegar further work is recommended, e.g. to. identify all the key aroma compounds in it.

Aging of vinegar in wood barrel, a traditional method for sherry wine vinegars, results in increase of aroma components such as vanillin, volatile phenols, and whisky-lactone, and hence improves the sensorial complexity of the vinegar. The aging of vinegar in wood barrels involve changes to colour, phenolic profile, and aroma which are usually highly appreciated by consumers. This technique, however, takes a long time and tends to result in a relatively expensive product. The ageing time could be significantly reduced and yet produce vinegars richer in aroma component that ageing in barrel through the use of toasted oak chips (Morales *et al.*, 2004). It is therefore recommended that further work to be done to investigate the effect of aging Zespri™ gold kiwifruit vinegar in toasted wood chips on aroma profile.

One of the special benefits of gold kiwifruit vinegar observed was its meat tenderising effect as the vinegar contained about a 25% of protease activity observed in a commercial papain. The vinegar offers the advantage of not only tenderising the meat and producing an acceptable texture, but also of seasoning. Thus, this vinegar may have a unique role in marinades and the value of this characteristic in the market should be exploited.

Overall, ZespriTM gold kiwifruit appears to be amenable to process to food beverages and its products are likely to be more acceptable than the traditional 'Hayward' variety because of its lower level of protein and protease activity.

REFERENCES

Adams, M.R. (1980). Small-scale vinegar production from bananas. *Tropical Sciences,* 20, 11-19

Adams, M. (1998). Vinegar. In B. Wood (Ed.), *Microbiology of Fermented Foods* (2nd ed., Vol. 1, pp. 1-44). London: Blackie Academic and Professional.

Akimoto, K., Kuroda, S., & Sakurai, H. (1984). Peeling kiwi fruit (Actinidia chinensis Planch.) utilizing high pressure steam. *Research Bulletin of the Faculty of Agriculture, Gifu University [Gifu Daigaku Nogakubu Hokoku]*

Alonso, A. M., Guillen, D.A., barrosso, C. G., Puetras, B & Garcia, A. (2002) Determination of antoxidant activity of wine byproducts and its correlation with polyphenolic content. *Journal of Agricultural & Food Chemistry. 5 0*(21) 5832-5836), 1237 - 1241

Ananta, D., & Shukla, S. S. (2004). Effects of treatments on reducing sugar and titratable acidity of low-alcoholic beverage prepared from guava (Psidium guajava). *Journal of Food Science & Technology Mysore, 41*(2), 210-213.

Anon. (1997). Fruit research in New Zealand 1996. *New Zealand Fruitgrowers Federation,* 48.

Anon. (2004). Plant polyphenols for functional foods. *Innovations in Food Technology.*

Arcus, A. C. (1959). Proteolytic enzyme of *Actinidina chinensis. Biochemical Biophysica Acta. 33*, 242-244.

Arnold, R. A., & Noble, A. C. (1979). Effect of pomace contact on the flavor of Chardonnay wine. *American Journal of Enology & Viticulture, 30*(3), 179-181.

Arnold, S., Becker, T., Delgado, A., Emde, F., & Enenkel, A. (2002). Optimizing high strength acetic acid bioprocess by cognitive methods in an unsteady state cultivation. *Journal of Biotechnology, 97*(2), 133-145.

Arthur and Pawliszyn. (1990). Inorganic chemical pollution of freshwater. *Water Pollution Control Research Series.* No. DPV, 180, 10 US Environmental Protection Agency Washington, DC

Bartowsky, E. J., Xia, D., Gibson, R. L., Fleet, G. H., & Henschke, P. A. (2003). Spoilage of bottled red wine by acetic acid bacteria. *Letters in Applied Microbiology, 36*(5), 307-314.

Bartley, J.P. P & Schwed, A.M. (1989). Production of volatile compounds in ripening kiwifruit (*Actinidia chinensis*). *Journal of Agricultural & Food Chemistry.37*1023-1025.

Bates, R. P., Mills, D., Mortensen, J. A., & Cornell, J. A. (1980). Prefermentation treatments affecting the quality of muscadine grape wines. *American Journal of Enology & Viticulture, 31*(2), 136-143.

Benda, I. (1982). Wine and brandy. In *Prescott & Dunn's Industrial Microbiology*, 4th edition. G. Reed (Ed.). pp 293 -402. AVI Publishing Co. Westport, CT (1982).

Betes-Saura, C., Andres-Lacueva, C., & Lamuela-Raventos, R. M. (1996). Phenolics in white free run juices and wines from Penedes by high-performance liquid chromatography: changes during vinification. *Journal of Agricultural & Food Chemistry, 44*(10), 3040-3046.

Beutel, J. A., Winter, F. H., Manners, S. C., & Miller, M. W. (1976). A new crop for California: kiwifruit. *California Agriculture, 30*(10), 5-7.

Beveridge, T., Harrison, J. E., & Drover, J. (2002). Processing effects on the composition of sea buckthorn juice from Hippophae rhamnoides L. cv. Indian Summer. *Journal of Agricultural & Food Chemistry, 50*(1), 113-116.

Bevers, D. J. (1995) Fruit development and fruit physiology. In kiwifruit: Science and Management, eds I. J. Warrington & G.C. Weston. Ray Richards, New Zealand Society for Horticultural Science, Auckland, pp. 97 – 126.

Bi, D. C., & Zhang, G. Q. (1998). Losses of ascorbic acid in production of concentrated apple juice. *Shipin Kexue, 19*(6), 25-27.

Bisson, L. F. (1999). Stuck and sluggish fermentations. *American Journal of Enology & Viticulture, 50*(1), 107-119.

Blumenkrantz, N. & Asboe-hansen. (1973). New methods for quantitative determination of uronic acid. *Analytical Biochemistry*, 54, 584-489.

Boland, M. J., and Harman, M. J. (1972). Kinetic studies of the thiol protease from *Actinidia chinensis. FEBS Letters, 27*, 282.

Bollard, E.G. (1996) Further prospects for horticulture: The continuing importance of research. New Zealand Fruit-growers Charitable Trust. pp. 94.

Bortolini, F., Sant'Anna, E. S., & Torres, R. C. (2003). Behaviour of alcoholic and acetic fermentations of kiwi mashes (Actinidia deliciosa); composition of mashes and production methods. *Ciencia e Tecnologia de Alimentos, 21*(2), 236-243.

Brighenti, F., Castellani, G., Benini, L., Casiraghi, M. C., Leopardi, E., Crovetti, R., & Testolin, G. (1995). Effect of neutralized and native vinegar on blood glucose and acetate responses to a mixed meal in healthy subjects. *European Journal of Clinical Nutrition, 49*, 242-247.

Brinkmann, G. (2002). Citrus press. *German Patent Utility Model, DE, 298*(24).

Brovelli, E. A., Boucher, A. Y., Arnason, J. T., Menon, G. R., & Johnson, R. M. (2004). Effects of expression technology on physical parameters and phytochemical profile of Echinacea purpurea juice. *Hortscience, 39*(1), 146-148.

Brown, M. R., & Ough, C. S. (1981). A comparison of activity and effects of two commercial pectic enzyme preparations on white grape musts and wines. *American Journal of Enology & Viticulture, 32*(4), 272-276.

Buchholz, K.D and Pawliszyn, (1994). Solid-phase micro-extraction for determination of phenols. *Analytical Chemistry*, 66, 160

Buren, J. v., Vos, L. d., & Pilnik, W. (1973). Phenolic materials on 'Golden Delicious' apple juice. *Abstracts of Papers American Chemical Society, 16675.*

Burns, D. J. W. (1981). *Juices, nectars, wines and enzymes from kiwifruit.* Palmerston North, N. Z: Dept. of Food Technology, Massey University.

Cabaroglu, T., & Canbas, A. (2001). The effect of skin contact on the aromatic composition of the white wine of Vitis vivifera L. cv. Muscat of Alexandria grown in southern Anatolia. *Acta Alimentaria, 31*(1), 45-55.

Cabaroglu, T., Canbas, A., Baumes, R., Bayonove, C., Lepoutre, J. P., & Gunata, Z. (1997). Aroma composition of a white wine of Vitis vinifera L. cv. Emir as affected by skin contact. *Journal of Food Science, 62*(4), 680-683.

Canal-Llauberes, R. M (2003). Prefermentation processes in white winemaking: results of trials on enzyme treatment during pressing. *Revue des Oenologues et des Techniques Vitivinicoles et Oenologiques*, 109, 16-18.

Chaplin, M. F. B. (1990). *Enzyme Technology.* London: Cambridge University Press.

Charoenchai, C., Fleet. G & Henschke. P. (1998) Effect of temperature, pH, and sugar composition on the growth rates and cell biomass of wine yeasts. *American Journal of Enology & Viticulture, 49* (3), 283-288.

Ching Chun, P., Yann Hi, L., & Chung-May, W. (1996). Effects of pectinase treatment on guava juice quality and volatile constituents. *Food Science, 23*(1), 77-87.

Chopda, C. A., & Barrett, D. M. (2001). Optimization of guava juice and powder production. *Journal of Food Processing & Preservation, 25*(6), 411-430.

Ciani, M. (1998). Wine vinegar production using base wines made with different yeast species. *Journal of the Science of Food & Agriculture, 78*(2), 290-294.

Colas, S., Cuinier, M., & Daumas, M. F. (1983). Optimization of alcoholic fermentation: application. *Vignes et Vins*, 322, 24-28.

Colesan, F., & Joehrer, P. (1999). Flottweg belt presses, decanter and separators for fruit and vegetable processing. *Fruit Processing, 9*(6), 200-204.

Conner, H. A., & Allgeier, R. J. (1976). Vinegar: its history and development. *Advances in Applied Microbiology.*

Craig, J.T. (1988). A comparison of the headspace volatiles of kiwifruit wine with those of wine of Vitis vinifera variety Muller-Thurgau. *American Journal of Enology & Viticulture.* 39 (4) 321-324.

Cramer, A. C., Vlassides, S., & Block, D. E. (2002). Kinetic model for nitrogen-limited wine fermentations. *Biotechnology & Bioengineering, 77*(1), 49-60.

Cumming, D. B. (1985). Latest developments in mechanical presses. *Fluessiges Obst, 52*(12), 630-634.

D'Amore, T., Celotto, G., Russel, I and Stewart G. (1989) Selection and optimization of yeast suitable for ethanol production at 40°C, *Enzyme & Microbial technology*, 11: 411-415.

Dawes, N. (1972). Processing potential and composition of New Zealand subtropical fruits. *Food Technology New Zealand.* 7 (4) 22-27

Dawes, H. M., & Keene, J. B. (1999). Phenolic composition of kiwifruit juice. *Journal of Agricultural & Food Chemistry, 47*(6), 2398-2403.

Dawes, H., Struebi, P., & Keene, J. (1994). Kiwifruit juice clarification using a fungal proteolytic enzyme. *Journal of Food Science, 59*(4), 858-861.

Deelen., Van, W., & Steinbuch, E. (1983). Application of the ENSINK-belt-press for juice production from stored apples. *Fluessiges Obst*, 50, (9):394-397; 423-424

Delteil, D. (2004). Fermentation properties of rose wines. The main factors in alcoholic fermentation of red wines. *Revue Francaise D'Oenologie*, 204, 26-29.

Delpeuch, F. (1994). Batch press, especially applicable to pressing of grapes for Cognac manufacture. *French Patent Application, FR, 2*(701).

De Ley, J., Gillis., M and Swings, J (1984) Acetobacteraceae. In: N.R. Krieg and J.G. Holt, (Ed) *Bergey's Manual of Systematic Bacteriology*, Williams and Wilkins Co, Baltimore (1984), pp. 275–284.

Dharmadhikari, M. (1999). Small Scale Wine Production. *Vineyard and Vintage View, 43*(3), 1-3.

Dietrich, H., Rechner, A., Patz, C. D., Bitsch, R., Boehm, V., & Netzel, M. (2002). Polyphenols and antioxidant capacity of blackcurrant juice during processing. *Obst , Gemuese und Kartoffelverarbeitung, 87*(5), 16-23.

Dinnella, C., Sio, G.,Monteleone, E.,Palmieri, L., & Impembo, M. (2000) Effects of enzyme treatment on characteristics of carrot juices. *Industrie Delle Bevande,* 29, (169):490-493

Dubourdieu, D., Ollivier, C., & Boidron, J. N. (1986). Effect of prefermentation treatments on chemical composition and sensory properties of dry white wine. *Connaissance de la Vigne et du Vin, 20*(1), 53-76.

Dumazert, G. (1974). Effects of pre- and post- fermentation operations on polyphenol contents of Mauzac white wines and musts. *Industries Alimentaires et Agricoles, 91*(1), 3-9.

Dunford, M. D. and Sneyd, S1990) Polyphenols in Golden Delicious apple juice in relation to method of preparation. *Journal of Agriculture and Food Chemistry,* 50, (25), 448-451

Ebner, H. (1967). An acetator with a defoamer for elaboration of vinegars *US Patent*, 252, 262.

Ebner, H. (1999) Acetic acid fermentation. *United States Patent, 3, (445): 245,*

Ebner, H., Pohl, H., & Enenkel, A. (1978). Self priming Aerator and mechanical defoamer for microbiological processes. *Biotechnology & Bioengineering,* 9, 357 – 364

Enenkel, A. (1988).Two stage process for the production of vinegar of more than 20 percent. *U.S. Patent*, 4, 315, 773.

Entani, E., Asai, M., Tsujihata, S., Tsukamoto, Y., & Ohta, M. (1998). Antibacterial action of vinegar against food-borne pathogenic bacteria including Escherichia coli O157:H7. *Journal of Food Protection, 61*(8), 953-959.

Essa, H. A., & Salama, M. F. (2002). Effect of macerate enzymes on the yield, quality, volatile compounds and rheological property of prickly pear juice. *Nahrung, 46*(4), 245-250.

Ethira, S. S., E. R. (1992). Studies on the utilization of mango processing wastes for production of vinegar. *Journal of Food Science & Technology-Mysore, 29*(1), 48-50.

Fellers, P. J. (1988). A review of limonin in grapefruit (Citrus paradisi) juice, its relationship to flavour and efforts to reduce it. *Journal of the Science of Food & Agriculture, 49*(4), 389-404.

Fellers, P. J. (1991). The relationship between the ratio of degrees Brix to percent acid and sensory flavor in grapefruit juice. *Food Technology, 45*(7), 72-75.

Ferguson, A. R. (1999). New temperate fruits: *Actinidia chinensis* and *Actinidia deliciosa*. In J. Janick (Ed.), *Perspectives on New Crops and New Uses.* (pp. 342-347). Alexandria,VA: ASHS Press.

Ferguson, A. R. & Bollard, E.G. (1990). Domestication of the kiwifruit. pp. 165–246, In: Warrington I. J. and Weston G.C. (eds.), *Kiwifruit: Science and Management.* Ray Richards, Publisher in association with the New Zealand Society of Horticulture Science, Auckland.

Ferguson, A. R., & Eiseman, J. A. (1984). Estimated annual removal of macronutrients in fruit and prunings from a kiwifruit orchard. *New Zealand Journal of Agricultural Research, 26*(1), 115-117.

Ferguson, A. R. (2004) Kiwifruit history and development: www.zesprikiwi.com

Ferguson, A.R. (1985). Kiwifruit (*Actinidia*). *Acta Horticulturae, 290*, 601–653. Acta

Ferguson, A. R. (2003). The year 1904, kiwifruit came to New Zealand. *New Zealand Journal of Crop and Horticulture Science*, 32, 3-27.

Fleet, H. (1997). Yeasts-what reactions and interactions really occur in natural habitats. *Food Technology & Biotechnology, 36, (4): 285-289.*

Fleet, G. H. & Heard, G. M. (*1993).* The effects of temperature and pH on the growth of yeast species during the fermentation of grape juice. *Journal of Applied Bacteriology, 65, (1): 23-28.*

Fregapane, G., Rubio-Fernandez, H., Nieto, J., & Salvador, M. D. (1999). Wine vinegar production using a noncommercial 100-litre bubble column reactor equipped with a novel type of dynamic sparger. *Biotechnology & Bioengineering, 63*(2), 141-146.

Fundira, M., Blom, M., Pretorius, I. S., & Rensburg, P. v. (2002). Comparison of commercial enzymes for the processing of marula pulp, wine, and spirits. *Journal of Food Science, 67*(6), 2346-2351.

Fushimi, T., Tayama, K., Kukaya, M., Kitakoshi, K., Nakai, N., Tsukamoto, Y., & Sato, Y. (2001). Acetic acid feeding enhances glycogen repletion in liver and skeletal muscle of rats. *Journal of Nutrition*, 131 (7) 1973 -1977.

Garcia-Parrilla, M. C., Gonzalez, G. A., Heredia, F. G., and Troncoso, A. M. (1997). Differentiation of Wine Vinegars Based on Phenolic Composition. *Journal of Agriculture & Food Chemistry, 45*, 3487-3492.

Gauthier-Jaques, A., & Bortlik, K. (1999). Process for production of kiwifruit juice. *European Patent Application, EP, 0* (953).

Gelb, G. (1981). Apparatus having a rotor for extracting the juice from fruits. *French Patent Application, 2* (473).

Gerhartz, W. (1990). *Enzyme in Industry: Production and Application.* New York: VCH Publication.

Gherardi, S., Castaldo, D., Lo Voi, A and Trifiro, A. (1992). Composition of Italian Kiwi (*Actinidia chinensis*) Puree. *Journal of Agriculture & Food Chemistry, 40*, 594-598.

Gilbert, J.M., Young, H., Roderick, D. B., & Shona , H. M. (1996) Volatile flavour compounds affecting consumer acceptability of kiwifruit.*Journal of Sensory Studies*, 11, 247-259

Gil-Munoz, R., Gomez-Plaza. E., Martinez, A., & Lopez-Roca, M. (1997). Evaluation of phenolic compounds during wine fermentation and post-fermentation: Influence of grape temperature. *Journal of Food Composition and Analysis*, 12, 259 – 272.

Gil, J. V., Mateo, J. J, Jimenez, M., Pastor, A., & Huerta, T..(1996). Aroma compounds in wine as influenced by speculate yeasts. *Journal of Food Science.* 61, 1247 -1250

Glazer, A. M., and Smith, E.L. (1971). *Papain and Other Sulfhydryl Proteolytic* in *The Enzymes* (3rd. ed), ed. P. D. Boyer. Academic Press, New York, vol 3, pp.501-546.

Gokmen, V., Acar, J., & Kahraman, N. (2003). Influence of conventional clarification and ultrafiltration on the phenolic composition of Golden Delicious apple juice. *Journal of Food Quality, 26*(3), 257-266.

Gomez, J. M., Romero, L. E; & Cantero, D. (1994). Application of a gas recirculation system to industrial acetic fermentation proceses. *Biotechnology, 8*, 711-716.

Gomez-Miguez, M., & Heredia, F. J. (2004). Effect of the maceration technique on the relationships between anthocyanin composition and objective color of Syrah wines. *Journal of Agricultural & Food Chemistry, 52*(16), 5117-5123.

Gorsel, H. v., Li, C., Kerbel, E. L., Smits, M., & Kader, A. A. (1992). Compositional characterization of prune juice. *Journal of Agricultural & Food Chemistry, 40*(5), 784-789.

Gracia, P. B., Tabra, J., Sanz, J., Herraiz, M. & Regelero, G. (1992). Volatile composition of vinegars. Simultaneous distillation – extraction and gas chromatograph –mass spectrometric analysis. *Journal of Agricultural & Food Chemistry,* 40(6), 1046-1049

Graig, G. (2004), http//: www.zesprikiwi. Kiwifruit

Guenther, F., Burckhart, O., Ostinga. I. (1970). Contribution to the knowledge of the kiwi fruit. *ndustrielle Obst- und Gemueseverwertung, 55*(4).

Guldas, M. (2003). Peeling and the physical and chemical properties of kiwi fruit. *Journal of Food Processing & Preservation, 27*(4), 271-284.

Guyer, D. E., Sinha, N.K., Chang, T.S. & Cash, J.N. (1993). Physicochemical and sensory characteristics of selected Michigan sweet cherry (*Prunus avium* L.) cultivars. *Journal of Food Quality, 16*, 635-370.

Heard, G.H. & Fleet, G.H. (1988). The effects of temperature and pH on the growth of yeast species during the fermentation of grape juice. *Journal of Applied Bacteriology* 65, 23–28.

Heatherbell, D. A. (1975). Identification and quantitative analysis of sugars and non- volatile organic acids in Chinese gooseberry fruit (Actinidia chinensis Panch). *Journal of the Science of Food & Agriculture., 26*, 815.

Heatherbell, D. A., Struebi, P., Eschenbruch, R., & Withy, L. M. (1979). A new fruit wine from kiwifruit: a wine of unusual composition and Riesling Sylvaner character. *American Journal of Enology & Viticulture, 31*(2), 114-121.

Heatherbell, D. A., Struebi, P., Eschenbruch, R., Eschenbruch, R., & Withy, L. M. (1980). A new fruit wine from kiwifruit: a wine of unusual composition and Riesling Sylvaner character. *American Journal of Enology and Viticulture*, 31 (2), 114 – 121.

Heatherbell, D., Ngaba, p., Fombin, F., Watson, Jr., Garcia, Z., Flores, J., & Hsu, J. (1984). Recent developments in the application of ultrafiltration and protease enzymes to grape juice and wine processing. *Proceedings International symposium on cool climate viticulture and Oenology.* D.A. Heatherbell, P.B. Lombard, F.W. Bodyfelt and S.F. Price (Ed), p. 418-445, Oregon state University. Corvallis.

Helbig, J. (2001). Production of colour-intensive and colour-stable coloured juices. *Fruit Processing, 12*(9), 342-347.

Henschke, P. A. (1993). An overview of malolactic fermentation research. *Australian & New Zealand Wine Industry Journal, 8*(1), 69-79.

Helo, H. J., Kim, D. O., Choi, S. J., Shin, D. H., & Lee, C. Y. (2004). Apple phenolics protect in vitro oxidative stress-induced neuronal cell death. *Journal of Food Science, 69*(9), S357-S360.

Herrero, M., Garcia, L. A., & Diaz, M (1999). Organic acids in cider with simultaneous inoculation of yeast and malolactic bacteria: effect of fermentation temperature. *Journal of the Institute of Brewing,* 105, (4):229-232.

Hildalgo Togores, J. (1981). The heat problem in alcoholic fermentation. *La Semana Vitivinicola, 36*(1846), 5239-5241.

Hongwen, H. (2003). Proceedings of the Fifth International Symposium on Kiwifruit, *Acta Horticulturae* (Vol. 610). ISHS: Wageningen.

Huang. H & Ferguson, A.R, (2003) Kiwifruit production trends. In Hongwen, H. (2003). In the Proceedings of the fifth international symposium on kiwifruit, *Acta Horticulturae* (Vol. 610).

Myung-Hwa- Kim. B.-Y. K. A. M.-H. K. (2003). Utility of post-mature kiwi fruit powder in bakery. *Journal of Korean Society of Food Science and Nutrition. 32*(4), 581-585.

Jain, S. K & Khurdiya. (2002). Studies on juice extraction of Aonla (*Emblica officinalis* Gaertn.) cv. 'Chakaiya'. *Journal of Food Science and Technology*, 39 (5) 515 – 516.

John, W., & Michael, C. (1995). Oxidation of dilute ethanol solution by *Acetobacter. Biotechnology: A Dictionary of Terms.* 2nd Ed. pp.285. American Chemical Society, Washington.

Joint FAO/WHO Food Standards Programme (1987). Codex standards for sugars, cocoa products and chocolate and miscellaneous. Codex standard for vinegar. In Codex alimentarius. Regional European standard, Codex Stan 162. Ginebra.

Julien, A., Triolli, G., & Dulau, L. (2001). Control of alcoholic fermentation: yeast growth factors and prevention of stuck fermentations. II. Management of alcoholic fermentation and prevention of stuck fermentation. *Revue des Oenologues et des Techniques Vitivinicoles et Oenologiques.*

Kaisa, K.D. (1984). Continuous fermentation of vinegar in a single vessel. *Food Research International.* 28 (6) 243-251

Kaisa, K.D. (1973).Surface acetification of vinegar. *Applied Biotechnology Food Science and Policy* 1(3) 116-125.

Karuwanna, P., Boonyaratanakornkit, M., Wongkhalaung, C.,& & Faungfupong, P. (1987). Wine vinegar from skins and cores of pineapple. *Food, 17*(3), 137-145.

King, R. C., Sims, C. A., Moore, L. F., & Bates, R. P. (1998). Effects of maturity, skin contact and carbonation on the quality of sterile-filtered white muscadine grape juice. *Journal of Food Science, 53*(5), 1474-1476.

Kishi, M., Fukaya, M., Tsukamoto, Y., Nagasawa, T., Takehana, K., & Nishizawa, N. (1999) Enhancing effect of dietary vinegar on the intestinal absorption of calcium in overiectomized rats. *Bioscience Biotechnology Biochemistry*, 63, 905-910.

Kondo S, T. K., Tsukamoto Y, Ikeda K, & Yamori Y. (2001). Anti-hypersensitive effects of acetic acid and vinegar on spotenesiouly hypersensitive rats. *Bioscience Biotechnological Biochemistry, 65*(12), 1690-2694.

Kourkoutas, Y., Komaitis, M., Koutinas, A. A. & Kanellaki. (2001) Wine production using yeast immobilization on apple pieces at low and room temperature. *Journal of Agricultural & Food Chemistry,* 49(3), 1417-1425.

Krueger, D.A. & Krueger, H.W. (2003). "Isotopic composition of carbon in vinegars". *Journal of AOAC,* 68, 449 – 452.

Kunitz, M. (1974) Crystaline soyabean inhibitor: General properties (Laboratories of the Rockefeller Institute for Medical Research, Princetion, 300-301

Kunimatsu, H. O., Masai, H. Yamada, K., & Yamada, M. (1981). Two stage process for the production of vinegar. *U.S. Patent*, 4, 257, 282

Lafon-Lafourcade, S. (1983).Wine and brandy. In: Rehm, H.J., Reed, G. (Eds.), *Food and Feed Production with Microorganisms. Biotechnology*, vol. 5. Verlag Chemie, Weinheim, pp. 81– 163.

Lea, A. G. H., & Timberlake, C. F. (1978). The phenolics of ciders: effect of processing conditions. *Journal of the Science of Food & Agriculture, 29*(5), 484-492.

Lee, D.-H. & K. J.-S. (2000). Vinegar production from subtropical fruits. *Journal of the Korean Society of Food Science and Nutrition, 29*(1), 68-75.
Molina, P. I. (1981). Title

Lema, C., Garcia-Jares, C. Orriols, I., & Angulo, L. (1996). Contribution of Saccharomyces and non-Saccharomyces populations to the production of some components of Albarino wine aroma. *American Journal of Enology and Viticulture,* 47, 206 – 216.

Lewis, D. A., & Luh, B. S. (1987). Development and distribution of actinidin in kiwifruit (Actinidia chinensis) and its partial characterisation. *Journal of Food Biochemistry, 12*, 109-116.

Liang, L., Wang, H. F., Shi, J. L., & Yi, J. H. (1998). Effects of pectase and chitosan on the clarification of kiwifruit juice. *Food Science and Technology*.

Lidster, P., O'Donovan, M., Cheong Kit, L., & Powrie, W. D. (2003). Process for the preservation of fresh quality attributes of peeled, whole and peeled, cut kiwifruit. *United States Patent Application Publication, US, 0091705*.

Linda, B (2001). The Alcoholic Fermentation: University of California at Davis, University Extension

Liljeberg, H & Bjorck, I. (1998) Delayed gastric emptying rate may explain improved glycaemia in health subjects to a starchy meal with added vinegar. European *Journal of Clinical Nutrition*, 52, 368-371

Llaurado, J., Constanti, M., Rozes, N., Mas, A., Velazquez, A., & Bobet, R. (2000). Fermentation at low temperatures (13[degrees]C). Effects of yeast strains and addition of nutrients. *Alimentacion Equipos y Tecnologia, 19*(2), 87-92.

Lodge, N. (1980). Kiwifruit: two novel processed products. *Food Technology in New Zealand, 16*(7), 37-38.

Lodge, N., Nguyen, T., Hogg-Stec, M., & Stanton, D. (1986). Production of kiwifruit wine using a deionization technique. *New Zealand Journal of Technology, 2*(4), 225-230.

Lo¨rincz, G. K. I., M.; & Pa´sti, G. (1998). Effect of carbonic maceration on phenolic composition of red wines. *Acta Alimentaria, 27*(4), 341-355.

Luh, B. S., & Zhang, W. (1984). Kiwifruit. *Advances in Food Research*. 29, 279-309

Macheix, J. J. F., A., Billot, J. (1991). *Fruit Phenolics*. Boca Raton, FL: CRC Press Inc.

Mallikarjunan, P. & Milttal, G.S. (1994). Colour kinetics during beef chilling. *Trans. ASAE*, 37, 203 - 209

Mallouchos, A., Komaitis, M., Koutinas A. & Kanellaki, M. (1996) Wine fermentations by immobilized and free cells at different temperatures. Effect of immobilization and temperature on volatile by-products. *Food Chemistry, 80*(1), 109-113

Marais, J., & Rapp, A. (1988). Effect of skin-contact time and temperature on juice and wine composition and wine quality. *South African Journal for Enology & Viticulture, 9*(1), 22-30.

Marais, J. (1998). Effect of grape temperature, oxidation and skin contact on Sauvignon Blanc juice and wine composition and wine quality. *South African Journal for Enology & Viticulture, 19*(1), 10-16.

Martin (2003). Kiwifruit marketing. In Hongwen, H. (2003). In the Proceedings of the fifth international symposium on kiwifruit, *Acta Horticulturae* (Vol. 610). Wageningen.

Mauricio, C. J.; Moreno, J.; Zea, L.; Ortega, M. J.; & Medina, M. (1989). The effects of grape must fermentation conditions on volatile alcohols and esters formed by *Saccharomyces cerevisiae. Journal of the Science of Food and Agriculture. 75*, 155-160.

McDowall, M. A. (1970). Anionic protease from actinidin chinensis preparation and properties of the crystalline enzyme. *European Journal of Biological. Chemistry. 14*, 214-221.
McGhie, T. K., Montefiori, M., Costa, G., &Ferguson, R. ((2002. Pigments in the fruit of re-fleshed kiwifruit (*Actinidia chinensis and Actinidia deliciosa*), *Journal of Agriculture and Food Chemistry*, 53 (24), 9526-9530

McLellan, M. R., Kime, R. W., & Lind, L. R. (1985). Apple juice clarification with the use of honey and pectinase. *Journal of Food Science, 50*(1), 206-208.

Mehrlaender, K., Will, F., Dierich, H., Sembries, S., & Dongowski, G (2004). Analytical characterisation of grape juices and polyphenol extracts produced with a 2-step enzymatic process. *Deutsche Lebensmittel-Rundschau*, 100, (3):77-83.

Meischak, G. (1970). Commercial apple production presents the processing industry with problems. *Gartenbau, 23*(12), 369-371.

Michael, F.C. & Stephen W. D. (1999). Vinegar: acetic acid production. In the Encyclopedia of Bioprocess technology, fermentation, biocatalysis and bio-separation vol. 5. 2637-2645

Michelet, E., Celotti, E., & Branca, G. (2004). Fermentative capacity of some yeast strains at low temperatures. *Australian Grape Grower & Winemaker.*

Mihalev, K., Schieber, A., Mollov, P., & Carle, R. (2004). Effect of mash maceration on the polyphenolic content and visual quality attributes of cloudy apple juice. *Journal of Agricultural & Food Chemistry, 52*(24), 7306-7310.

Miller, T. (1975). Export potential of kiwifruit. *New Zealand. Journal of Agriculture* 131 (2), 20-21, 35, 37

Molina, P. I. (1981). Local fruits as media in producing vinegar and their effects on food as seasoning. *TCA Research Journal, 3*(1), 40-52.

Monk, P. R & Cowley, P. J. (*1984*) Effect of nicotinic acid and sugar concentration of grape juice and temperature on accumulation of acetic acid during yeast fermentation. *Journal of Fermentation Technology; 62, (6): 515-521.*

Montgomery, M. W. (1993). Cysteine as an inhibitor of browning in pear juice concentrate. *Journal of Food Science, 48*(3), 951-952.

Morales, M. L., Tesfaye, W., Garcia-Parrilla, M. C., Casas, J. A., & Troncoso, A. M. (2002). Evolution of the aroma profile of sherry wine vinegars during an experimental aging in wood. *Journal of Agricultural & Food Chemistry, 50*(11), 3173-3178.

Morales, L. G., Benita B.B., Ana, M., & Troncoso, G., (2004) Accelerated Aging of Wine Vinegars With Oak Chips: Evaluation of Wood Flavour Compounds. *Food Chemistry*, 88 (2), 305-315

Moreno, J., Mauricio, J. C., Ortega, J. M., & Medina, B. (1986). Choice of fermentation temperature best suited to production of white wines from 'Pedro Ximenez' var. grapes in southern Spain. *Connaissance de la Vigne et du Vin, 20*(2), 97-106.

Nieto, M., Gonza'lez-Vinas, M.A., Barba, P., Martin-Alvarez., Aldave, L., Garcia-Romero, E & Cabenzudo, M.D. (1993). Recent progress in wine vinegar and some indicators for the future. In Charalambous (Ed), *Food Flavors, Ingredients and Composition* pp. 469-500) Elsevier Science Publishers B.V.

Nishiyama, I., & Oota, T. (2002). Varietal difference in actinidin concentration and protease activity in kiwifruit juice. *Journal Japanese Society for Food Science and Technology, 49*(6), 401-408.

Noble, A. C. (1979). Pectic enzyme effects on red grapes. *American Journal of Enology and Viticulture.* 26, 195 - 2000

Nykanen, L. (1985). Formation and occurrence of flavor compounds in wine and distilled alcoholic beverages. *American Journal of Enology & Viticulture, 37*(1), 84-96.

Ogawa, N., Satsu, H., Watanabe, H., Fukaya, M., Tsukamoto, Y., Miyamoto, Y., & Shimizu, M. (2000). Acetic acid suppress the increase in disaccharidase activity that occurs during culture of Caco-2cells. *Journal of Nutrition*, 130, 1507 - 1513

Olsson, M. E., Gustavsson, K. E., Andersson, S., Nilsson, A., & Rui-Dong, D. (2004). Inhibition of cancer cell proliferation in vitro by fruit and berry extracts and correlations with antioxidant levels. *Journal of Agricultural & Food Chemistry, 52*(24), 7264-7271.

Ory, I.., Romero, L. E., & Cantero, D. (1998). Modelling the kinetics of growth of Acetobacter aceti in discontinuous culture: influence of the temperature of operation. *Applied Microbiology & Biotechnology, 49*(2), 189-193.

Ory, I., Romero, L. E., and Cantero, D. (1999). Maximum yield acetic acid fermenter. Comparative fed-batch and continuous operation studies at pilot plant scales. *Bioprocess Engineering*, 21, 187–190.

Ory, I., Romero, L. E., and Cantero, D. (2002). Optimum starting-up protocol of a pilot plant scale acetifier for vinegar production. *Journal of Food Engineering*, 52, 31–37.

Ory, I., Romero, L. E., & Cantero, D. (2004). Operation in semi-continuous with a closed pilot plant scale acetifier for vinegar production. *Journal of Food Engineering, 63*(1), 39-45.

Pagan, J., Casane R, A., Pique, M. T., & Ibarz, A. (1997). Extraction of apple juice using liquefaction enzymes. *Alimentaria.*

Pinnow, D. (1996). Self-optimizing fruit presses from Bucher. *Fruit Processing, 6*(9), 350-352.

Quiroz, F. A. (1970). Method and device for crushing fruit. *West German Patent Application, 1*(517).

Radford, G. C. (1997). *Determination of individual sugars and organic acids of New Zealand varietal apple juice and their use in evaluating authenticity.* Unpublished Masters of Philosophy in Food Technology, Massey University, Palmerston North.

R adler, T. (1973). Formation of carbonyl compounds in alcoholic fermentation. *Weinberg. U Keller*, 20, 339 – 350.

Reynolds, A., Cliff, M., Girard, B., & Kopp, T. G. (2001). Influence of fermentation temperature on composition and sensory properties of Semillon and Shiraz wines. *American Journal of Enology & Viticulture, 52*(3), 235-240.

Ribereau-Gayon, P. (1999). Observations related to the causes and consequences of stuck fermentation in vinification. *Journal International des Sciences de la Vigne et du Vin, 33*(1), 39-48.

Rieger, H. (1993). Container and process for treatment of mash. *German Federal Republic Patent Application, DE, 42*(00).

Romero, L. E., Gomez, J. M., Caro, I., & Cantero, D. (1994). A kinetic model for growth of Acetobacter aceti in submerged culture. *Chemical Engineering Journal, 54*(1), B15-B24.

Romero, L. E & Cantero, D (1998). Evaluation of ethanol evaporation losses in acetic acid fermentation. *Bioprocess Engineering* 18: pp. 289–291

Romero, E., Cantero. D., & Ignacio.O. (2004). Operation in semi-continuous with a closed pilot plant scale acetifier for vinegar production. *Journal of Food Engineering* 63, 39–45

Saeki, A., Taniguchi, M., Matsushita, K., Toyama, H., Theeragool, G.,& Lotong, N. (1993). Microbiological aspects of acetate oxidation by acetic acid bacteria, unfavorable phenomena in vinegar fermentation. *Bioscience Biotechnology & Biochemistry, 61*(2), 317-323.

Sales, P (2004). Development of Gold Kiwifruit. Personal communication

Sale, P. R. & Lyford. A. (1985*). Kiwifruit Culture*, 2nd revised edition. Government Printer, Wellington.

Salmah, Y. & Nurzarina, I. (1994). Quality of soursop juice after pectinase enzyme treatment. *Food Chemistry* 51: 8-88.

Samadi-Maybodi, A., & Reza Shariat, M. (2003). Characterization of elemental composition in kiwifruit grown in northern Iran. *Journal of Agricultural & Food Chemistry, 51*(10), 3108-3110.

Soufleros, E. H., Irini Pissa, P., D. Lygerakis, M. Mermelas, K. Boukouvalas G. & Tsimitakis E. (2001) Instrumental analysis of volatile and other compounds of Greek kiwi wine; sensory evaluation and optimization of its composition. *Food Chemistry*, 75 (4), 487-500

Schiller, H. G. (1997). Influence of comminution systems on the economics of the overall dejuicing line. *Flussiges Obst, 64*(6), 296-297.

Schobinger, U., & Duerr, P. (1977). Quantitative analysis of important components and aroma compounds of apple juices produced by a combined pressing-extraction process in pilot-scale experiments. *Fluessiges Obst, 44*(7), 275-278.

Scocea, J., & Lee,Y. C. (1969). The composition and structure of the carbohydrateof pineapple stem bromelain. *Journal of Biological Chemistry, 244*(18), 4852-4863.

Seager, N. (2003) Kiwifruit: a New Zealand case study: *Acta Horticulturae 495,* 449 – 455.

Selman, J. D. (1983). The vitamin C content of some kiwifruits (*Actinidia chinensis* Planch., variety 'Hayward'). *J ournal of Food Chemistry, 11*(1), 63-75.

Shahid, F. N.,& Naczk, M. (1995). *Food Phenolics: Sources, Chemistry, Effects, Applications.* Lancaster PA: Technomic Publishing Co.

Signore, A. D. (2000). Chemometric analysis and volatile compounds of traditoional balsamic vinegars from Modena. *Journal of food Engineering, 50*(2), 77 - 90.

Simmons, I. L. (1978). Drying and candying of Chinese gooseberries. *Food Technology in Australia*. 30: 236

Singleton, V.L., John, Z., & Eugene T. J.A. (1980). White Table wine quality and polyphenol composition as affected by must SO_2 content and pomaca contact time. . *American Journal of Enology and viticulture*, 31 (1), 14-20.

Singleton, V. L. & Rossi, J. A. (1965). Colorimetry of total phenolics with Phoshotungstic acid reagents. *American Journal of Enology and Viticulture*, 37, 144–158.

Singh, N. I., Dhuique-Mayer, C., & Lozano, Y. (2000). Physico-chemical changes during enzymatic liquefaction of mango pulp (cv. Keitt). Journal of food processing and preservation 24, 73 – 85.

Slinkard, K. & Singleton, V. L. (1977) Total phenol analysis: Automation and comparison with manual methods. *American Journal of Enology and viticulture*, 28 (1), 49-55.

Sluis, A. A. v. d., Dekker, M., Skrede, G., & Jongen, W. M. F. (2002). Activity and concentration of polyphenolic antioxidants in apple juice. 1. Effect of existing production methods. *Journal of Agricultural & Food Chemistry, 50*(25), 7211-7219.

Spanos, G. A., & Wrolstad, R. E. (1992). Phenolics of apple, pear, and white grape juices and their changes with processing and storage - a review. *Journal of Agricultural & Food Chemistry, 40*(9), 1478-1487.

Spanos, G. A., Wrolstad, R. E., & Heatherbell, D. A. (1992). Influence of processing and storage on the phenolic composition of apple juice. *Journal of Agricultural & Food Chemistry, 38*(7), 1572-1579.

Spanos, G. A., Wrolstad, R. E., & Heatherbell, D. A. (1990). Influence of processing and storage on the phenolic composition of apple juice. *Journal of Agricultural & Food Chemistry, 38*(7), 1572-1579.

Sriwatanapongse, A., Balaban, M., & Teixeira, A. (2000). Thermal inactivation kinetics of bromelain in pineapple juice. *Transactions of the ASAE, 43*(6), 1703-1708.

Steeffen, A. & Pawliszyn, J. (1996). Analysis of Flavor Volatiles Using Headspace Solid-Phase Microextraction. *Journal of Agricultural and Food Chemistry, 44 (8), 2187 -2193*

Suomalainen, H. (1980). Yeast esterases and aroma esters in alcoholic beverages. *Journal of the Institute of Brewing, 87*(5), 296-300.

Szymczuk, J. A., Plocharski, W., & Markowski, J. (1997). Effect of enzymic treatment on the yield of juices and the quantity and quality of pectic substances in apple cake. Przemysl Fermentacyjny i Owocowo-Warzywny, 41, (11):28-31.

Takashi Fushimi, K. T., Fukaya, M. Kitakoshi, K., Naoya N., Yoshinori T. & Yuzo S.. (2001). Acetic Acid Feeding Enhances Glycogen Repletion in Liver and Skeletal Muscle of Rats. *Journal of Nutrition, 131*, 1973-1977.

Tannahill, R. (1973). *Food in History* Stein and Day, New York.

Takemura, H., Kondo, K., Horinouchi, S., & Beppu, T. (1993). Induction by ethanol of alcohol dehydrogenase activity in Acetobacter pasteurianus. *Journal of Bacteriology, 175*(21), 6857-6866.

Tesfaye, W., Garcia-Parrilla, M. C., & Troncoso, A. M. (2002). Sensory evaluation of sherry wine vinegar. *Journal of Sensory Studies, 17*(2), 133-144.

Tesfaye, W., Lourdes-Morales, M., Garcia-Parilla, M. d. C., & Troncoso, A. M. (2003). Optimising wine vinegar production: fermentation and ageing. *Applied Biotechnology, Food Science and Policy, 1*(2), 109-114.

Tesfaye, W., Morales, M. L., Garcia-Parrilla, M. C., & Troncoso, A. M. (2002). Evolution of phenolic compounds during an experimental aging in wood of sherry vinegar. *Journal of Agricultural & Food Chemistry, 50*(24), 7053-7061.

Tesfaye, W., Garcı´ a-Parrilla, M. C., & Troncoso, A. M. (2000). Set up and optimization of laboratory scale fermenter for the elaboration of wine vinegar. *Journal of the Institute of Brewing*, 106, 215–219.

Test, S. L., Noble, A. C., & Schmidt, J. O. (1986). Effect of pomace contact on Chardonnay musts and wines. *American Journal of Enology & Viticulture, 37*(2), 133-136.

Thomas, R.L., Gaddis, J.N., Westfall, P.H., Titus, T.C. & Dells, N. D. (1987). Optimization of apple juice production by single pass metallic membrane ultrafiltration. *Journal of Food Science*, 52 (5) 1263-1266.

Toda, K., Ohtaka, H & Parker, Y.S. (1990).A kinetic study of acetic acid production. *Applied Microbiology-Biotechnology*, 33, 259-263.
Torija, M. J., Rozes, N., Poblet, M., Guillamon, J. M., & Mas, A. (2001). Effects of fermentation temperature on the strain population of Saccharomyces cerevisiae. *International Journal of Food Microbiology, 80*(1), 47-53.

Troncoso, A.M., Tesfaye, W., Morales, M.L., & Garcı´ a-Parrilla M.C. (2002). Wine vinegar: technology, authenticity and quality evaluation. *Trends in Food Science & Technology* 13: 12–21

Troncoso, A. M., Garcia-Parrilla, M. C., & Heredia, F. J. (1998). The influence of the acetification process on the phenolic composition of wine vinegars. *Sciences des Aliments*, 18, (2):211-221

Tung-Sun, C., Siddiq, M., Sinha, N. K., & Cash, J. N. (1994). Plum juice quality affected by enzyme treatment and fining. *Agricultural & Food Chemistry, 59*(5), 1065-1069.

Varsari, A., Parpinello, G. P., & Cattaneo, M. (1999). *Leuconostoc oenos* and malolactic fermentation in wine: a review. *Journal of Industrial Microbiology, 23*(6), 447-455.

Veloz-Garcia, R. A., Marin-Martinez, R., Veloz-Rodriguez, R., Munoz-Sanchez, C. I., Guevara-Olvera, L., & Miranda-Lopez, R (2004). Antimutagenic and antioxidant activities of cascalote (Caesalpinia cacalaco) phenolics. *Journal of the Science of Food & Agriculture, 84*(13), 1632-1638.

Venning, J. A., Burns, J.W., Hoskin, K.M., Nguyen, T. & Stec M.G.H. (1989). Factors influencing the stability of frozen kiwifruit pulp. *Agricultural & Food Chemistry. 42*(2), 396-400.

Vijayakumar, C., & Wolf-Hall, C. E. (2002). Minimum bacteriostatic and bactericidal concentrations of household sanitizers for *Escherichia coli* strains in tryptic soy broth. *Food Microbiology, 19*(4), 383-388.

Wada, M., Suzuki, T., Yaguti, Y., Hasegawa, T. (2001). The effeect of pressure treatments with kiwifrut protease on adult cattle semitendinosus muscle. *Food Chemistry, 78*(2), 167-171.

Warrington, I. J., &. Weston. G. C. (1990). *Kiwifruit: Science and Management.* Wellington: New Zealand Society of Horticultural Science.

Wendu, T., Morales, L., Garcia- Parrilla, M. C., & Troncoso A. M. (2003). Optimizing vinegar production: fermentation and ageing. *Applied Biotechnology, Food Science and Policy.* 1 (2) 109 -114.

Weston, I. J, & Wang. A. G. C. (1990). *Kiwifruit: Science and Management*

Westin, S. I. (1974). Chinese gooseberry or kiwi. *Var-Foda, 25*(6), 99-101.

Will, F., Bauckhage, K., & Dietrich, H. (2002). Apple pomace liquefaction with pectinases and cellulases: analytical data of the corresponding juices. *European Food Research & Technology, 211*(4), 291-297.

Wilson, E. L. & Burns, D. J. (1983). Kiwi juice processing using heat treatment techniques and ultrafiltration. *Journal of Food Science, 48*, 1101-1105.

Whiton & Zoecklenein (2002). Growth and compositional changes in kiwifruit berries from three Californian locations. *Annals of Botany,* 66 285- 298

Withy, L. M., & Lodge, N. (1982). Kiwifruit wine: production and evaluation. *American Journal of Enology & Viticulture, 33*(4), 191-193.

Xiuzhu, Y. L. Z., & Du Shuangkui. (2003). Study on the preparation of the kiwifruit vinegar in liquid-state fermentation and its acetic acid drink. *Shipin Yu Jixie, 5*(519-21).

Yokotsuka, K., Nakanishi, K., Hori, I., & Fukui, M. (2003). Studies on the production of kiwifruit wine. I. Effects of press design and enzymatic treatment of crushed kiwifruit on juice yield and clarity. *Journal of the Brewing Society of Japan, 98*(2), 139-147.

Yokotsuka, K., Nakanishi, K., Seki, T., & Fukui, M. (2003). Studies on the production of kiwifruit wine. III. Quality of wines produced from fruits with various maturities. *Nippon Jozo Kyokaishi, 98*(11), 798-809.

Young, H., Peterson, V., & Burns, D.J.W. (1983). Volatile aroma constituents of kiwifruit. *Journal of the Science of Food & Agriculture*, 34, 81-85.

Young, H., Perera, C. O., & Paterson, V. J. (1991). Identification of E-hex-3-enal as an important contributor to the off-flavour aroma in kiwifruit juice. *Journal of the Science of Food & Agriculture, 58*(4), 519-522.

Young, H., Gilbert, J.M., Murray, S.H., & Ball, R.D. (1995). Factors contributing to kiwifruit flavour. HortResearch Client Report 95/112

Yusof, S., & Ibrahim, N. (1994). Quality of soursop juice after pectinase enzyme treatment. *Food Chemistry, 51*(1), 83-88.

Zumbach, G. (1996). Experiences with the Bellmer WPX angle press, a high-performance belt filter press. *Flussiges Obst, 63*(2), 63-66.