FACTORS AFFECTING COLOUR AND CLOUD STABILITY IN A WILDBERRY HERBAL DRINK

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ABSTRACT

An investigation was undertaken into the stability of the natural colour, from anthocyanins, and cloud in a Wildberry Herbal fruit drink. The fruit drinks consisted of cloudy apple and berry fruit juice with natural herb extracts and flavours. The objectives of the research were to identify the cause of cloud instability and sediment formation in the drink; determine the effect of ascorbic acid, berryfruit juice volume, storage temperature and light on anthocyanin stability; investigate the use of stabilisers to prevent sediment formation and determine consumer acceptability of a modified drink. The cause of sediment formation was determined by analysing the contribution of the major ingredients to the total amount of sediment formed. To minimise the sediment, a range of commercially available polysaccharide stabilisers were added to the drink and the amount of sediment formed determined. A consumer sensory evaluation was undertaken to determine consumer acceptability of drinks in which stabilisers had been added to improve the cloud stability. The factors affecting the anthocyanin's in the drink were analysed using a fractional factorial experimental design. The effect of the commercial pasteurisation process on the colour was also investigated. The formation of sediment was identified as being the result of complexing between the unstable cloud of the cloudy apple juice and polyphenolics, including anthocyanins, in the berryfruit juice. No sediment formed during eight weeks storage when clarified apple juice was substituted for cloudy apple juice. The sediment was reduced by approximately 45% using stabiliser systems consisting of either xanthan or a xanthan/propylene glycol alginate mixture. Consumer sensory evaluation of the modified drinks found no significant difference in liking from the standard drink. The anthocyanin loss in the drink was found to be significantly affected by increased storage temperature. Elderberry juice was found to have better colour stability over blackcurrant juice. Pasteurisation did not initially affect the colour stability of the drink. It was recommended that the composition of the Wildberry Herbal drink remain unchanged. The product should be stored at as low a temperature as possible. The drinks should be cooled to ambient temperature as quickly as possible after the pasteurisation process.

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CHAPTER 1

INTRODUCTION

The non-alcoholic beverage market in New Zealand is worth $760 million a year (Vercoe, 1998). The fruit juice sector is worth approximately $80 million while the fruit drink sector is worth approximately $250,000 (Vercoe, 1998). The majority of fruit juices (91%) sold in NZ are shelf stable with the remainder having a limited shelf life and chilling requirement (Vercoe, 1998).

By value, orange juice is the leading fruit juice with approximately 46% of sales, followed by apple juice blends with approximately 36% of sales (Vercoe, 1998). Blended beverages have widespread consumer appeal as the blending process allows beverages to be developed which have unique flavours and colours (Hicks, 1990).

At present, the key driver of the beverage market is increasingly health conscious consumers who desire beverages which provide a myriad of health benefits and are perceived to help cope with consumers' busy lifestyles (Corbett, 2000). These drinks have been variously described as "new age" or "functional" beverages. Manufacturers are meeting the demand of consumers by providing an increasing range of products with "functional" ingredients such as herbs, vitamins and minerals.

These "functional" ingredients have been incorporated into many fruit juice based products in the New Zealand market; "Thextons" (Rio Beverages Ltd., NZ) fruit juice drinks include vitamins A, C and E and the herb Echinacea; Arano Fruit Juice Ltd. produces a chilled smoothie with Acerola and Echinacea; Frucor Beverages Ltd. produce "Muse", a combination of fruit juice, mineral water and herbs.

Phoenix Natural Foods Ltd. produces a range of blended fruit drinks each with a combination of herbs which have been traditionally used as therapeutic aids. The range includes an Apple and Guava, an Orange and Mango, and a Wildberry Herbal fruit drink.

The research for this project focuses on one of the drinks from this functional beverage range, the Wildberry Herbal fruit drink. This drink consists of a cloudy apple and berryfruit juice blend with herbal extracts and natural flavours. The Wildberry Herbal fruit drink is unique in the New Zealand market as it is the only fruit drink which has a cloudy apple and berryfruit juice blend. All other blended drinks on the market are made from a clarified apple juice base.

The Wildberry Herbal fruit drink is visually appealing as it is red in colour with a cloudy appearance. Cloud is important in a drink as it has been found that consumers desire cloud in certain drinks, such as fruit juices, and associate cloudiness with "naturalness" (Hicks,1990). The desirable colour of the drink is obtained solely from the berryfruit juice in the blend. Berryfruit juices contain anthocyanins, naturally produced compounds, which have an intense colour, most often red. As well as providing a very desirable colour, anthocyanins are also known to be powerful antioxidants. Much research is being undertaken into the ability of anthocyanins to be beneficial for certain health ailments (Smellie, 2000). Hence, the Wildberry Herbal drink not only has a blend of herbs which have been used as therapeutic aids but is also a source of anthocyanins, which are also thought to be beneficial to health.

This research focuses on three phenomena associated with the drink. The first phenomenon is that a sediment forms in the drink soon after pasteurisation. The second phenomenon is the loss of the bright red colour of the drink, obtained from the berryfruit juice, to a dark brown colour during storage. The third phenomenon to be investigated is the texture of the drink. It is desired that the drink's mouthfeel be improved to increase the "natural" juice perception of the drink.

Haze and sediment formation in fruit juices can be due to a number of chemical reactions, which produce insoluble complexes (Heatherbell, 1984). Proteins and phenolics present in blends of fruit juices can form insoluble complexes leading to an unsightly sediment. The loss of colour due to the degradation of red pigments (anthocyanins) in fruit products can be due to a number of factors including pH, temperature, oxygen concentration, ascorbic acid concentration and the presence of metals (Jackman & Smith, 1996). Addition of natural antioxidant compounds has

potential for providing more colour stable juices (Clegg & Morton, 1968; Shrikhande & Francis, 1974).

To minimise quality loss in the Wildberry Herbal drink the factors (composition, processing conditions) responsible for the observed changes must be first determined. Once these factors have been determined solutions to the problems can be proposed and tested. The aim was to be able to make recommendations on product formulation and processing conditions in order to (i) eliminate or reduce the sediment, (ii) stabilise the colour, (iii) to increase the body (texture) of the drink.

The primary objectives of this project were to:

(i) Identify the cause of the sediment formation in the Wildberry Herbal drink and potential ingredients for reformulation of the drink to reduce/eliminate the sediment formation.

(ii) Determine the effect of processing on colour stability.

(iii) Determine the effect of added antioxidants on colour stability.

(iv) Determine the acceptability of the texture of the drink to consumers and if necessary reformulate the drink to give a more acceptable texture.

CHAPTER 2

LITERATURE REVIEW

2.1 Anthocyanins

Anthocyanins are part of the very large and widespread group of plant constituents known as flavonoids (Mazza & Miniati, 1993). Anthocyanins belong to the flavonoid sub group as they have the characteristic $C_6C_3C_6$ carbon skeleton which is the basic chemical structure of the flavonoid group. The base structure of the anthocyanin is the 2-phenylbenzopyrylium of flavylium salt (Figure 2.01). Anthocyanins exist as glycosides of polyhydroxy and/or polymethoxy derivatives of the salt. Anthocyanins differ in the number of hydroxyl and/or methoxy groups present, the types, numbers, and sites of attachment of sugars to the molecule, and the types and numbers of aliphatic or aromatic acids that are attached to the sugars in the molecule (Mazza & Miniati, 1993).

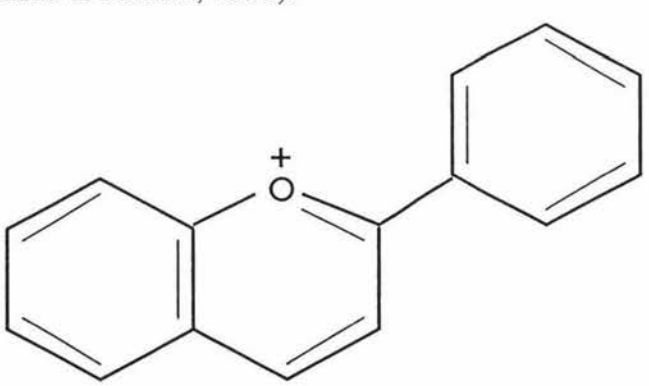


Figure 2.01: The base structure of an anthocyanin, 2-phenylbenzopyrilium.

When the sugar moiety of an anthocyanin is hydrolysed, the compound formed is referred to as an anthocyanidin. The colour of anthocyanins and anthocyanidins results from excitation of a molecule by visible light. The ease with which a molecule is excited depends on the relative electron mobility in the structure. Double bonds, which are abundant in anthocyanins and anthocyanidins, are excited very easily, and their presence is essential for colour. Anthocyanins occurring in nature contain several anthocyanidins, but only six occur commonly in foods namely

pelargonidin, cyanidin, delphinidin, peonidin, petunidin and malvidin (Mazza & Miniati, 1993).

Anthocyanins are very widespread in the plant kingdom and are the source of the orange, red, and blue colours found in many fruits, vegetables, leaves and roots (Francis, 1989). Since the presence of aanthocyanins is associated with attractive colourful and flavourful fruits, such as grapes, strawberries, blackcurrants, elderberries etc., they are often used for the natural red colouring of food products including many beverages (Jackman & Smith, 1996).

2.1.1 Anthocyanins of blackcurrants (*Ribes nigrum*)

Blackcurrant berries are very strongly coloured due to anthocyanins and consequently blackcurrant juice has a very high anthocyanin content (Mazza & Miniati, 1993). Cyanidin-3-rutinoside and delphinidin-3-rutinoside are the major anthocyanins in blackcurrants and the relative proportions of four anthocyanins which characterise the blackcurrant are shown in Table 2.01.

Table 2.01: Anthocyanin composition of ripe blackcurrants (Macheix *et al.*, 1990).

Anthocyanin	Concentration (% of total)
Cyanidin-3-rutinoside	36
Cyanidin-3-glucoside	15
Delphinidin-3-rutinoside	34
Delphinidin-3-glucoside	15

Analysis of blackcurrant syrup by high pressure liquid chromatography (HPLC) found cyanidin-3-rutinoside and delphinidin-3-rutinoside to be the major anthocyanins in nearly equal quantities, and smaller amounts of cyanidin-3-glucoside and delphinidin-3-glucoside (Skrede *et al.*, 1992).

2.1.2 Anthocyanins of elderberries (*Sambucus nigra)*

Elderberries are black soft skinned berries which are a rich source of anthocyanins with four different anthocyanins and few other phenolic compounds (Mazza & Miniati, 1993). Elderberries are used to manufacture a variety of food products including juices, jams, preserves and food colours.

The anthocyanin composition of elderberries is shown in Table 2.02.

Table 2.02: Anthocyanin composition of elderberries (Dradk & Daucik, 1990).

Anthocyanin	Concentration (% of total)
Cyanidin-3-glucoside	33
Cyanidin-3-sambubioside	55
Cyanidin-3-sambubioside-5-glucoside	10
Cyanidin-3,5-diglucoside	2

Bronnum-Hansen & Hansen (1983) isolated the anthocyanin pigments from elderberries using HPLC and liquid chromatography. Their results found twice the concentration of cyanidin-3-glucoside of that found by Dradk and Daucik (1990) and the minor pigments consisted of less than 2% of the total concentration. It has been suggested that these observed differences were due to different varieties of elderberry being used in the studies (Mazza & Miniati, 1993).

2.2 Factors influencing anthocyanin colour and stability

Anthocyanins are inherently unstable being more stable under acidic conditions, and may degrade by any number of possible mechanisms to soluble colourless and/or brown coloured and insoluble products (von Elbe & Schwartz, 1996). The major factors influencing anthocyanin colour stability are pH, temperature, the presence of oxygen and light, interactions with other food components (such as ascorbic acid, sugars, metal ions) and enzymic degradation (Jackman & Smith, 1996).

Being electron deficient, the flavylium nuclei of anthocyanins are highly reactive, and these compounds therefore readily undergo undesirable structural and colour changes in food environments such as fruit juices (Francis, 1989). Several kinetic investigations have been made to determine the factors that affect the rate of pigment breakdown in strawberry (Decareau *et al.*, 1956; Lukton *et al.*, 1956; Markakis *et al.*, 1957), raspberry (Daravingas and Cain, 1968) and cranberry products (Starr and Francis, 1968) and in model systems (Tinsley and Bockian, 1960).

Nebesky *et al.* (1949) undertook research into the stability of a number of fruit juices including currant juice. Studies were undertaken to determine the effect of storage length and temperature on colour stability. They also investigated the relationship between oxygen, light, sugar, pH or ascorbic acid on deteriorative changes in the colour of currant juice. Similar studies were made on solutions of purified anthocyanin pigments which were isolated from currants. This early study found that in currant juice storage temperature and oxygen content were directly responsible for deterioration of colour during storage of both juices and isolated pigments.

2.2.1 Anthocyanin structure

Due to the vast array of anthocyanin structures, anthocyanin degradation rates vary greatly between different anthocyanins. Increased hydroxylation decreases stability while increased methylation increases stability (Francis, 1989). The colour of foods containing anthocyanins that are rich in pelargonidin, cyanidin, or delphinidin

aglycones are less stable than those that contain anthocyanins which are rich in petunidin or malvidin aglycones (von Elbe & Schwartz, 1996).
Taylor (1989) found that during storage of blackcurrant juice there was significant variation in the rate of loss between the major and minor pigments of blackcurrant juice. Delphinidin-3-rutinoside was found to be more stable than cyanidin-3-rutinoside and both of these major anthocyanins were more stable than the minor pigments, delphinidin-3-glucoside and cyanidin-3-glucoside.

Daravingas and Cain (1968) found that black raspberry anthocyanins had higher stability in a model system than in a natural juice. It was suggested that some cellular constituents were influential in this pigment destruction. Cyanidin was found to be much more unstable than cyanidin-3-diglucoside in both model systems and black raspberry juice. This demonstrated that blocking of the hydroxyl group of the 3-position with a sugar retarded destruction of the desirable red pigment.

2.2.2 pH

Anthocyanins have a characteristic amphoteric nature due to the presence of the oxonium ion adjacent to the C-2 position (Mazza & Miniati, 1993). Anthocyanins exhibit intense red colour only in a very limited, strongly acidic pH range, between 1 and 3 (Francis, 1989). They readily undergo reversible structural transformations with loss of red colour and/or formation of undesirable colour characteristics (Hrazdina, 1981).

The major anthocyanin pigment of black raspberries is cyanidin-3-diglucoside. In model systems of cyanidin 3-diglucoside, under an oxygen or nitrogen atmosphere at 50°C, a decrease in pH from 4.25 to 0.95 enhanced anthocyanin retention (Daravingas & Cain, 1968). However, the degradation reaction proceeded faster at pH 0.95 than at pH 2.15. At lower pH's the more stable cation form of the anthocyanin is present. At pH values less than 1.8, the overall stability is decreased because of the hydrolysis of the anthocyanin to sugar and the highly unstable anthocyanidin moiety. Additional experiments by Daravingas and Cain (1968) found

that freshly extracted juice at various pH levels, showed that lowering the pH from 4.15 to 2.15 increased the stability of the anthocyanin pigment in the juice.

In anaerobic environments anthocyanin degradation is virtually pH independent (Francis, 1989). However, in the presence of oxygen increased methoxyl, glycosyl and/or acyl substitution generally leads to an increase in the pH at which the maximum thermal stability occurs.

2.2.3 Temperature

The colour stability and rate of degradation of anthocyanins is greatly influenced by temperature. Early research by Kertesz and Sondheimer (1948) studied time temperature relationships with anthocyanin colour degradation. This research determined 18°C to be a critical temperature for the storage of strawberry preserve, above which accelerated loss of red colour and subsequent formation of brown color occurred.

In general anthocyanin degradation follows first order kinetics (Jackman & Smith, 1996). The mechanism of anthocyanin degradation appears to be temperature dependent. At storage temperatures, <40°C, activation energy (E_a) and z values of around 70 kJ mol^{-1} and 25°C, respectively, have been reported; while at processing temperatures, >70°C, E_a values of 95 to 115 kJ mol^{-1} and z values of around 28°C have been reported (Markakis, 1974).

These values indicate that anthocyanins have relatively greater stability at higher temperatures, despite that the higher temperatures favour the formation of the colourless anthocyanin species, particularly the chalcones (Jackman & Smith, 1996). It has been recommended that high-temperature short-time processing be used to achieve maximum pigment retention in anthocyanin-containing food products (Markakis *et al.*, 1957).

Simard *et al.* (1981) found that anthocyanin monomers stability decreased with increasing storage temperature such that juices stored at 15°C and 20°C were more

stable than juices at 37°C, stored for 12 weeks. At these incubation temperatures the anthocyanin monomers were slightly influenced by pH.

Palamidis and Markakis (1975) have investigated the stability of grape anthocyanins in a carbonated beverage. They found that increasing the storage temperature greatly accelerated the pigment destruction. After 135 days in darkness, at 38°C, only 23% of the original pigment was retained, while under the same conditions of storage, at 3.5°C, 92% of the pigment was retained.

Kearsley and Rodriguez (1981) investigated the effect of temperature on the stability of anthocyanin powder prepared in distilled water at a concentration of 100mg/100ml. The anthocyanin solutions were subjected to heat treatments over the range 20°C – 100° C for a period of 240 minutes and their absorptions measured over this period. At 20°C and 50°C there was very little change in absorbance with time. At 75°C and 100°C there was marked decrease in the absorption indicating a loss in anthocyanins. The highest proportion of loss occurred in the first 60 minutes.

Studies undertaken by Adams and Ongley (1973) have shown that canning red fruit juices at 100°C for less than 12 min resulted in negligible anthocyanin loss in comparison to losses occurring during slow cooling and subsequent ambient temperature storage. They stated that colour deterioration rendered canned or bottled red fruit juices unsaleable after one to two months storage at 0-5°C.

2.2.4 Ascorbic acid

The interaction of ascorbic acid with anthocyanin pigments, which results in degradation of both, and a decrease in the colour and nutritional quality of products, has been investigated by many researchers (Sondheimer and Kertesz, 1953; Pratt *et al.*, 1954; Markakis *et al.*, 1957; Starr and Francis, 1968; Harper *et al.*, 1969; Shrikhande and Francis, 1974; Poei-Langston and Wrolstad, 1981).

Sondheimer and Kertesz (1953) investigated the participation of ascorbic acid in the destruction of the main anthocyanin of strawberries, pelargonidin-3-monoglucoside,

in model systems and strawberry juice. They presented evidence for an indirect ascorbic acid induced destruction of the anthocyanin pelargonidin-3-monoglucoside. They found that the factors that decrease the rate of ascorbic acid oxidation, lack of oxygen and the addition of thiourea (a metal complexing agent), also decreased the rate of anthocyanin destruction. Hydrogen peroxide, or related substances, was believed to play a significant role in this process possibly by cleaving the pryrilium ring to form colourless esters and coumarins (Jackman & Smith, 1996).

Pratt *et al.* (1954) investigated the interaction of ascorbic acid, riboflavin and anthocyanin pigments from strawberry juice. Spectrophotometric determinations found that as the length of storage increased there was a corresponding increase in the amount of brown colour present. They also found that the greatest loss of ascorbic acid was in samples containing ascorbic acid, riboflavin, and anthocyanin pigments. They concluded that ascorbic acid and anthocyanin pigments react, causing destruction of the pigment.

Starr and Francis (1968) investigated the effect of headspace oxygen and added ascorbic acid on individual pigments and on total pigment content of cranberry juice. In this study the total pigment content decreased rapidly during the first several weeks of storage, with approximately 40% of the loss occurring in the first four weeks. This loss was found to be faster and greater with increasing levels of oxygen or ascorbic acid. The greatest pigment loss was found to occur when oxygen and ascorbic acid were present simultaneously, while the least loss occurred in their absence. This loss was much faster than was expected and it was suggested that part of this loss may be due to dissolved oxygen rather than headspace oxygen since an appreciable amount may have been present when the product was bottled at 88°C.

In Concord grape juice addition of ascorbic acid at a level of 50mg/100ml produced a lighter red and less purple juice than in a juice with no added ascorbic acid. After ascorbic acid addition the juice was browner but the total anthocyanin content was not affected after 18 months storage (Sistrunk and Gascoigne, 1983).

Ascorbic acid (~ 80 mg/100ml) had a negative effect on strawberry syrup pigment stability with increased degradation rate of anthocyanins, deterioration in Hunter *a*

and hue values, and a significant increase in the browning index (Skrede *et al.*, 1992). The fortification of strawberry syrup with anthocyanins and ascorbic acid increased stability to a level similar to that of blackcurrant syrup.

Poei-Langston and Wrolstad (1981) investigated the interactions between ascorbic acid, pelargonidin-3-glucoside, and catechin in pH 3.4 citrate-phosphate buffer at 20°C, under oxygenated and anaerobic conditions. Poei-Langston and Wrolstad (1981) found that anthocyanin stability in samples containing ascorbic acid was greater in oxygen treated samples than in the corresponding nitrogen-treated samples. The reverse would be expected if anthocyanin degradation was due to the presence of hydrogen peroxide from ascorbic acid oxidation. It was thus proposed by Poei-Langston and Wrolstad (1981) that the direct condensation mechanism predominates in anthocyanin destruction. These findings agreed with Jurd (1972) who had earlier proposed a mechanism whereby ascorbic acid condenses directly with anthocyanins.

Iversen (1999) investigated the degradation of anthocyanins and ascorbic acid in blackcurrant nectar during storage at 20°C. They found that the degradation rates of the four main anthocyanins (cyanidin-3-rutinoside, cyanidin-3-glucoside, delphinidin-3-glucoside and delphinidin-3-rutinoside) were not significantly different. The degradation of total anthocyanin and ascorbic acid in light and dark storage was compared. Anthocyanin destruction was much greater than ascorbic acid destruction, both under natural lighting and in the dark. Rate constants were significantly different for anthocyanin degradation in light and dark storage but not for ascorbic acid degradation. The degradation of anthocyanin and ascorbic acid was described as a first order reaction, although the correlation coefficient for ascorbic acid was lower than for the anthocyanins. The degradation rate of total anthocyanin appeared to be four times faster than ascorbic acid degradation during storage in light, and about three times faster during storage in the dark.

2.2.5 Oxygen

Due to the unsaturated nature of the anthocyanidin structure it is very susceptible to molecular oxygen (von Elbe & Schwartz, 1996). Early work with Concord grape juice found that pasteurised juice packed in bottles with little headspace underwent little colour deterioration. Juices in partially filled bottles deteriorated quickly with increased browning and sediment formation (Nebesky *et al.*, 1949).

Oxygen may cause degradation of anthocyanins by a direct oxidation mechanism and/or by indirect oxidation whereby oxidised constituents of the medium react with anthocyanins. Daravingas and Cain (1968) found that molecular oxygen was a very important factor in the destruction of the anthocyanin pigments of black raspberry when subjected to thermal degradation.

A study by Skrede (1985) on the colour stability of blackcurrant syrups during storage, found that packaging materials had the greatest influence on both free anthocyanin content and colour stability. Hence, it was concluded that colour stability was affected by oxygen availability. Both colour parameters were best maintained in glass bottles where anthocyanin half life was about 35% longer than in PET bottles and about 80% longer than in PVC bottles.

Dissolved oxygen has been found not to have a significant effect on anthocyanin stability in blackcurrant nectar and syrup (Skrede, 1985; Iversen, 1999).

2.2.6 Light

There are conflicting reports on the role of light on the degradation of anthocyanins. Adams (1973) states that the consensus of opinion is that light has little effect on the rate of anthocyanin degradation, however, it is generally agreed now that light accelerates the degradation of anthocyanins (Skrede, 1985; Francis, 1989; von Elbe & Schwartz, 1996; Iversen, 1999). Different anthocyanins have different stabilities to light, with anthocyanins substituted at the C-5 hydroxyl groups being more susceptible to photodegradation than those unsubstituted at this position (von Elbe &

Schwartz, 1996). Skrede (1985) found that in blackcurrant syrups, those stored under dark conditions were more colour stable than those stored in light. Iversen (1999) found that the half life of the total anthocyanin concentration, in blackcurrant nectar, was thirty times greater when stored in dark conditions compared with light storage conditions.

Exposure to light was found by Palamidis and Markakis (1975) to accelerate the degradation of grape pigment in a carbonated beverage. After 135 days storage in the dark, at 20°C, approximately 30% of the pigment was lost. For the same time and temperature with exposure to light during daylight hours, the pigment loss was 50%. Under continuous fluorescent light for 135 days, at 22°C, the pigment loss was found to be 70%.

2.2.7 Sugars

Sugars can have a protective effect on anthocyanins if present in sufficient quantity to affect the water activity (a_w), as is found in preserves (Francis, 1989). If sugars are present in much smaller quantities, such that they have little effect on a_w, then they or their degradation products can sometimes accelerate anthocyanin degradation (von Elbe & Schwartz, 1996).

At low concentrations, fructose, arabinose, lactose and sorbose have a greater degradative effect on anthocyanins than do glucose, sucrose, and maltose (Francis, 1989). The rate of degradation of the anthocyanins followed the rate of degradation of sugars to furfural (Meschter, 1953). Furfural and hydroxymethyl furfural are products of the Maillard reaction and ascorbic acid degradation. These compounds readily condense with anthocyanins forming brown compounds. The mechanism of this reaction is not known however it is hastened by the presence of oxygen and is very noticeable in fruit juices (von Elbe & Schwartz, 1996).

Debicki-Pospisil *et al.* (1983) found that the presence of 0.012 M furfural or 5-hydroxymethylfurfural, in blackberry juice and a model system, accelerated degradation of cyanidin-3-glucoside (the primary pigment of blackberries). This

degradation was found to be directly temperature dependent, more pronounced in the blackberry juice and considerably decreased in nitrogen conditions.

2.2.8 Total anthocyanin content

Skrede *et al.* (1992) found that the rate of free anthocyanin degradation was about twice as high in strawberry syrup than in blackcurrant syrup. Skrede *et al.* (1992) investigated colour stability after fortifying strawberry syrup with anthocyanins to levels similar to the blackcurrant syrup. They found that the stability of pelargonidin-3-glucoside (the main pigment of strawberries) was similar to the delphinidin-3-glucosides and cyanidin-3-glucosides of blackcurrant when anthocyanin levels were similar. This indicates that the total anthocyanin concentration is an important factor in stability.

2.2.9 Flavonols

Several researchers (Timberlake, 1960; Clegg and Morton, 1968; Shrikhande and Francis, 1974) have shown that flavonols such as quercetin and kampferol will retard the oxidation of ascorbic acid in blackcurrant juice and other anthocyanin containing systems.

Clegg and Morton (1968) investigated the stability of ascorbic acid in blackcurrant juice, lemon juice and comparable model systems. They found that with and without additional Cu^{2+}, the blackcurrant juice contained protective agents for ascorbic acid, whereas, in the natural lemon juice the ascorbic acid was oxidised faster than in comparable model systems. It was concluded from the investigation that phenolic rich extracts of blackcurrant juice, or pure flavonols, may be added advantageously to less stable natural sources of ascorbic acid such as lemon juice.

Shrikhande and Francis (1974) investigated the action of flavonols in retarding the oxidation of ascorbic acid and also anthocyanin breakdown. They undertook their studies using citrate phosphate buffer model systems with quercetin and quercitrin,

as the flavonols and anthocyanins isolated from cranberries. They found that both quercetin and quercitrin inhibited the oxidation of ascorbic acid in the presence of anthocyanins. Quercetin at the concentration of 3 and 6 mg/100ml and quercitrin at the concentration of 9mg/100ml had almost similar activities and retarded the oxidation by an average of 20%, over a 72 hour period. Shrikhande and Francis (1974) also found that the flavonols that reduced the oxidation of ascorbic acid also reduced the loss of anthocyanin pigment.

Shrikhande and Francis (1974) attempted to increase the stability of anthocyanins in cherry juice by adding quercetin and quercitrin. This did not improve anthocyanin stability in the juice because the added flavonols could not be maintained in solution. After pasteurisation of the cherry juice, the flavonols crystallised out immediately. Shrikhande and Francis (1974) suggested that the ability to exploit the protective effect of flavonols in juices would require judicious blending with a juice in which the flavonols are normally in high concentrations and are maintained in solution possibly by association with other compounds naturally present. They also suggested that flavonols with a higher solubility, in a given juice, be used.

The addition of 0.4, 0.8 and 1.2 g/l of polyphenol to blackcurrant juices has been found to increase anthocyanin stability (Simard *et al.*, 1981). Also, at identical polyphenol concentrations, pH exerted an important influence on stability, with juices at pH 2 more stable than those at pH 3.5.

2.2.10 Metals

Anthocyanins are very reactive towards metals, forming complexes with tin, copper and iron (Markakis, 1982). Simard *et al.* (1981) found that the addition of 2, 6 and 10 mg/l of cupric ions to blackcurrant juices in which pH varied from 2 to 3.5, and were stored in darkness at 20°C, showed no significant difference in the stability of the anthocyanin monomers.

2.2.11 Blending of fruit juices

Spayd *et al.* (1984) investigated the factors contributing to haze and colour instability in apple and pear juices, blended with anthocyanin pigmented juices (cherry, grape, raspberry) at concentrations of 5%, 10% and 20% v/v. In this study total anthocyanin concentration decreased more rapidly as the % of anthocyanin pigmented juice increased. After 24 weeks of storage, anthocyanin concentration in 20% blends continued to decrease. For the 5% and 10% blends anthocyanin concentration stopped declining and had levelled off after 24 weeks of storage, except for the blend containing blackberry juice which continued to decline up till the 24th week. As the anthocyanins degraded, during storage of the juices, they condensed and polymerised to produce increased polymeric colour and sediment. This was shown by a high correlation between turbidity and polymeric colour within a given base juice.

Spayd *et al.* (1984) concluded that a combination of many factors probably contributed to the anthocyanin loss and browning in the blends used. They recommended the removal of reactive phenolic compounds, sugar degradation products and ascorbic acid degradation products from the base juices prior to blending to help reduce the rate of colour degradation and turbidity development. They also recommend the use of an acidulant such as citric acid to lower the pH of the blends. Their final conclusion was that further research was required to determine what treatments or combination of treatments would be most effective in stabilising colour and preventing turbidity development in blended juices during commercial storage and marketing.

2.3 Haze and sediment formation in apple juice

2.3.1 Composition of cloudy apple juice

The chemical composition of apple juice varies according to the cultivar, growing region, climate and maturity of the apples used (Lee and Mattick, 1989). Processing conditions can also contribute to variations in apple juice composition. On milling and disruption of cellular components, enzymes and substrates come in contact with each other and rapid changes can occur with certain juice components. The most susceptible to change are pectin, polyphenols and volatile flavour components (Lea, 1990). The proximate composition of apple juice is shown in Table 2.03.

Table 2.03: Composition of a clarified apple juice (Burlingame, 1994).

Composition	Weight per 100g
Water	89g
Protein	1.1g
Total fat	Trace
Dietary fibre	0.1g
Total average sugar (monosaccharide)	9.7g
Starch	Trace
Vitamin C	11mg

2.3.1.1 Protein

Lea (1990) found the protein level to be between 10 and 250ppm in a range of cloudy apple juices. This small concentration of protein is sufficient to play a major role in the formation of post bottling haze and sediment when complexed with

polyphenols. These hazes have a composition of up to 50% each of protein and polyphenol on a dry weight basis.

2.3.1.2 Starch

In freshly pressed apple juice starch is present in the form of insoluble granules about 1-16μm in diameter which derive from storage vacuoles in the fruit and can constitute as much as 2% of the fruit on a fresh weight basis (Lea, 1990). The starch in apple juice consists of α 1,4 glucoside and α 1,6 glucoside, about 30 units in length. The granules tend only to be present in early season or under ripe fruit, and their small size means that they often escape filtration procedures (Lea, 1990).

2.3.1.3 Pectin

Pectin is released from the middle lamella of apple cell walls by the mechanical action of milling and pressing and is thus present in all freshly pressed apple juice. Pectin content varies in apples depending on when they are picked and subsequently processed. For example, in early season fruit the amounts of soluble pectin may be low in the order of 0.1% by weight of juice, while in later season fruit or in stored fruit from cold store, pectin levels may rise as high as 1-2.5% in the juice (Lea, 1990).

The structure of native apple juice pectin contains predominantly polymerised galacturonic acid chains which are methoxylated up to a level of 95%, forming the smooth regions of the pectin molecule. At intervals along the chain, the so called hairy regions are incorporated, containing a variety of polymers based on neutral sugars such as arabinose, rhamnose and galactose (Hicks, 1990).

2.3.1.4 Polyphenols

Polyphenols are always present in apple tissue, but their concentration decreases as the apple ripens, with a further decrease during storage. Common apple cultivars contain 0.05 – 0.1% polyphenol at maturity (Lea, 1990). Apple polyphenols are composed of epicatechin's combined in higher-molecular-weight oligomers. These oligomers are not insoluble but during storage polymerise to larger in-solouble particles resulting in haze and sediment formation (Lea, 1990).

2.3.2 Cloud stability in cloudy apple juice

Two mechanisms help stabilise particles in juices – the net charge on the particles and the presence of protective colloids. In juices all particles tend to have the same net electrical charge and thus, as like charges repel, aggregation is prevented (Beveridge, 1999).

In cloudy apple juice the action of endogenous pectinmethylesterase in the apple juice is sufficient to demethylate the pectin, giving up some free galacturonic acid groups which are negatively charged (Beveridge, 1999). These complex with native apple protein to form a stable hydrated floc which remains suspended in the viscous juice. This is responsible for the stable cloud in cloudy apple juice, which is not due to cellular debris as often believed (Lea, 1990).

Conventional in-bottle pasteurisation of a cloudy juice almost inevitably leads to undesirable characteristics. The long heating times give cooked flavours accompanied by polyphenol oxidation, pectin degradation and subsequent flocculation. The heavily sedimented brown product which results has little consumer appeal (Lea, 1990).

If polygalacturonase (PG) activity is present in the juice system, the long pectin chains will be broken down and the viscosity of the system will diminish markedly. Polygalacturonase also affects the charge distribution of the pectin-protein complexes, causing them to aggregate into much larger particles. Thus any

macroscopic debris or pectin-protein complexes which have already formed will drop through the juice as sediment (Lea, 1990).

2.3.3 Types of apple juice haze

2.3.3.1 Microbial

The presence of microorganisms in a juice, whether dead or alive, will produce a haze. Microbial sediment can be identified microscopically, with the sediment appearing to be composed of small, uniformly sized indistinct particles at a magnification of around 200 times (Van Buren & Kilara, 1989). Rapid daily increases in haze of a microbial nature indicate that live microorganisms are present.

2.3.3.2 Starch and dextrin

Soluble starches and dextrin's in apple juice may undergo retrodegradation which results in these compounds becoming insoluble and leading to haze formation (Heatherbell, 1976). Retrodegradation occurs when individual starch molecules combine to form large complex insoluble compounds.

2.4.3.3 Polyphenol

Hazes composed principally of polyphenols can form in juices low in or lacking proteins and starch (Heatherbell, 1976). Van Buren and Way (1978) hypothesised that apple juice hazes might be primarily polyphenol condensation products. They investigated this hypothesis by looking at haze development in deproteinated apple juice. They found that for deproteinated apple juice, stored at 50°C, haze development increased 15 times faster at pH 2.5 and 5 times faster at pH 3.0 than at pH 3.5. Selective removal of polyphenols with gelatin decreased the rate of haze formation. The hazes and sediments formed in this research were brown in colour.

Addition of pectin and gum arabic (0.1%) also retarded haze development. The haze-forming ability of the juice polyphenols is closely related to their molecular weight or degree of esterfication

Due to the long time it takes for polyphenolic hazes to form in apple juices, it is rarely necessary to take special precautions against polyphenolic hazes except to avoid long storage of apple juice under warm conditions (Van Buren, 1989).

2.3.3.4 Protein-polyphenol

Protein-polyphenol complexes are the most frequent cause of haze in apple juice often appearing within six months of bottling (Van Buren, 1989). The haze or precipitate is usually dark brown in colour. Sediments that appear amorphous almost always contain significant amounts of polyphenols and protein. The proteins and polyphenols have a strong affinity for each other, and eventually form large complexes containing nearly equal percentages of each component (Van Buren, 1989). Other materials such as starch and metals are often minor components of protein-polyphenols complexes as seen in Table 2.04.

Table 2.04: Composition of various apple juice sediments

Sediment dry weight (mg/L juice)	Composition of dry sediment (%)				Reference
	Protein	Polyphenol	Copper	Other	
22	49	10	9	15% ash	Keiser *et al.* 1957
62	11	39	1.4	2% ash	Keiser *et al.* 1957
11	39	16	0.08	0.24% iron	Keiser *et al.* 1957
20	49	35	8.9	0.13% iron	Keiser *et al.* 1957
9-70	7-30	-	0.07-0.3	2-11% starch; 0.08-0.18% iron	Johnson *et al.* 1968
20	3.5	3.2	-	90% starch	Heatherbell 1976
0.3-1.7	11.4-29	45.7-75.8	-	-	Beveridge and Tait 1993

Beveridge and Tait (1993) analysed hazes from commercial apple juice over the 1991 season. They found that they contained from 11.4% to 29.0% (w/w) protein, with trace quantities of both metal cations and polymeric carbohydrates. The hazes gave strong response to tests for polyphenolic compounds indicating a protein-polyphenol haze. Beveridge and Tait (1993) investigated the structure of haze in apple juice using electron microscopy. They found the existence of two internal substructures in protein-polyphenol haze particles. One substructure consists of large, spherical bodies. These bodies, which appear to have a subunit structure of their own, are imbedded within a matrix of another material. Beveridge and Tait (1993) suggested the spherical particles probably represent the protein portion of the protein-polyphenol complex of the haze, since spherical shapes are typical of denatured proteins. The second structure was thought to consist of material

polymerised in such a way as to from chain-like aggregates. This may have consisted of polymerised polyphenols, or polyphenol-protein complexes.

Siebert *et al.* (1996a) investigated the formation of protein-polyphenol haze in beverages. They found that beer was high in haze-active proteins. Apple juice and red wine were high in haze-active polyphenols. Apple juice hazes in which amino acid analysis was carried out, had a significant amount of proline, ranging from 4.6% to 15.9%, was found (Johnson *et al.*, 1968). Model system results show that proline is apparently required for a peptide to demonstrate haze-forming activity (Siebert *et al.*, 1996b).

2.4 Stabilisers

Polysaccharides are a class of biopolymers consisting of simple sugar monomers (Walter, 1998). Polysaccharides are added to foods to increase stability by thickening and/or gelling aqueous solutions and to modify or control the flow properties and textures of liquid food and beverage products (BeMiller and Whistler, 1996).

2.4.1 Carboxymethylcellulose

Carboxymethylcellulose (CMC) is a derivative of cellulose and is manufactured by treating cellulose with aqueous sodium hydroxide followed by reaction with monochloroacetic acid (Zecher & Gerrish, 1999). Depending on the conditions of use, CMC can have different functions in a food product such as a binder, thickener, stabiliser or suspension agent. CMC has been used in fruit drink products in combination with propylene glycol alginate to lessen the separation of solids (DeLeon and Boak, 1984; Lerchenfeld *et al.*, 1998).

2.4.2 Locust bean gum

Locust bean gum (LBG) is obtained from the pods of the locust bean plant. The main polysaccharide component of LBG is a galactomann. LBG molecules can interact with exposed portions of cellulose derivatives to form junctions, which produce an increase in viscosity (Fox, 1999). LBG also interacts with xanthan and carrageenan helices to form rigid gels. LBG is used extensively in dairy and frozen dessert products. It is rarely used alone but used in combination with other gums such as CMC, carrageenan, xanthan and guar gum (Fox, 1999).

2.4.3 Pectin

Pectins are the partial breakdown products of complex structures in plant cell walls and are invariably heterogeneous (May, 1999). Pectins which are produced commercially for food applications show a distribution in molecular weight and degree of esterfication, and these distributions influence their properties. Pectin is modified by reducing the degree of esterfication. Pectins with a degree of esterfication of 60% are capable of gelation only in systems with a high sugar content. Once the degree of esterfication is reduced to below 50% the pectin's are known as low methoxyl and can be gelled under lower soluble solids conditions, provided an appropriate amount of available calcium is present (May, 1999).

Pectins are used in a wide variety of foods including jams, fruit preparations, desserts and confectionery (May, 1999). In fruit juices, high methoxy pectins have been found to act as a thickener, rather than gel, when the solids level is less than 55% (May, 1999).

2.4.4 Propylene glycol alginate

Propylene glycol alginate (PGA) is made by reacting moist alginic acid with propylene oxide to produce a partial ester with 50-85% of the carboxyl groups esterified (Onsoyen, 1999). PGA possesses valuable functionality at low pH. The presence of the lipophilic propylene glycol ester groups provides PGA with emulsifying capability and makes it acid tolerant and less calcium reactive than standard alginates. These PGA properties are utilised in the stabilisation of milk proteins under acidic conditions, in yoghurt and lactic acid drinks and for pulp stabilisation in acidic fruit drinks (Onsoyen, 1999).

Blends of PGA and carboxymethylcellulose have been successfully used for lessening the separation of solids from juice drinks (De Leon and Boak, 1984; Lerchenfeld, *et al.*, 1998). PGA is often used in conjunction with xanthan in low calorie dressings (BeMiller & Whistler, 1996).

2.4.5 Xanthan

Xanthan is a polysaccharide which is produced by a bacterium *Xanthomonas campestris*. Xanthan is widely used as a food gum for stabilisation of aqueous dispersions, suspensions and emulsions. It has many characteristics which make it widely used in the food industry: solubility in hot or cold water; high solution viscosity at low concentrations; no discernable change in solution viscosity within the temperature range of 0°C – 100°C; solubility and stability in acidic systems, pH > 2.5 ; and interaction with other gums (BeMiller & Whistler, 1996). These properties result from the unique rigid, rod-like conformation of xanthan in solution and from its very low molecular weight

Xanthan has very good suspension properties due to its high yield value at low concentrations. Yield value is the minimum shear stress required for a solution to flow (Urlacher & Noble, 1999). This yield value is the result of interactions, between the xanthan marcromolecules which are not permanent, and are totally shear-reversible.

Xanthan gum has been used in many food products at the concentrations shown in Table 2.05.

Table 2.05: Function and concentration ranges of xanthan gum in food products (Walter, 1998).

Food product	Function	Concentration %
Bakery jellies	Gelation, thickening	0.1-0.3
Fruit drinks	Pulp suspension	0.02-0.06
Cream cheese	Gelation	0.1-0.2
Baked goods	Moisture retention	0.1-0.2
Dressings	Stabilisation	0.2-0.3

Xanthan is also used in combination with other stabilisers in food products. Blends of xanthan and locust bean gum and/or guar gum are used as ice cream stabilisers. Xanthan in combination with propylene glycol alginate and guar gum has been successfully used as a beverage thickener system (Bunger *et al.*, 1995).

2.5 Influence of viscosity on consumer perception of drinks

Sensory acceptability factors of foods is extremely important because people obtain great enjoyment from eating and drinking. One of the most important parameters for the sensory properties of beverages is viscosity (Danisco, 2000). Viscosity is defined as the internal friction of a fluid or its tendency to resist flow (Bourne, 1982).

The main difficulty in correlating perceived texture of beverages with objective viscosity is that most fluid foodstuffs are shear thinning. Their apparent viscosity decreases with increasing shear rate, and thus appropriate viscosity measurement depends on the shear regime in the mouth (Cutler *et al.* 1983). Wood (1968) suggested that the effective oral shear rate was about $50s^{-1}$. It was later found by Shama & Sherman (1973) that the effective oral shear rate increases with decreasing fluid viscosity. For extremely non-Newtonian solutions, the viscosity, at low rates of shear, greatly exceeds that of less shear thinning materials and therefore, makes a disproportionate contribution to overall perceived thickness.

Szczesniak & Farkas (1962) found that hydrocolloid stabilisers could be characterised into three groups based on their shear thinning characteristics. They found that stabilisers which exhibited a sharp decrease in viscosity with increasing shear rate were judged by a trained panel to be non-slimy. Gums that showed a small to moderate change in viscosity with increasing shear rate were considered by the trained panel to range in mouthfeel from slimy to extremely slimy.

In model fluid systems, where hydrocolloid solution dispersions have been used, it has been demonstrated that an increase in viscosity of the system decreased the perceived sweetness (Rao, 1999). Different hydrocolloids have been found to affect sweetness to differing extents (Pangborn *et al.*, 1973). Pangborn *et al.* (1973) observed that the influence of different hydrocolloids on the perception of some basic taste intensities (saltiness, bitterness, sourness) appeared to be more dependent on the nature of the hydrocolloid and the taste of the substance than on the viscosity level.

The effect of different kinds and levels of hydrocolloids, including xanthan, on selected aromatic flavour compounds was also investigated (Pangborn *et al.*, 1978). Xanthan was found to be the most effective stabiliser in lowering intensity of odour flavour and sweetness in orange juice.

2.6 Pasteurisation

Pasteurisation is a relatively mild heat treatment, in which food is heated to below 100°C (Fellows, 2000). In acidic foods (pH<4.5) such as fruit juices it is used to extend the shelf life for several months by destruction of spoilage microorganisms and/or enzyme inactivation. There are three main pasteurisation processes used for fruit juices: flash pasteurisation, hot filling and in-pack pasteurisation.

2.6.1 Flash pasteurisation

In flash pasteurisation, raw juice is heated by hot water or steam in a plate or tubular heat exchanger to the desired pasteurisation temperature and held at that temperature for the specified time in a holding tube before being cooled to the filling temperature using chilled water (Lea, 1998).

2.6.2 Hot filling

In hot filling, the juice is heated in a heat exchanger and sent hot to the filler and filled into containers (Lea, 1998). The containers are closed and held at or above the required temperature for a specified time prior to being cooled, usually in a tunnel with water sprays. This system not only heat treats the product but also the container it is filled into.

2.6.3 In-pack pasteurisation

In-pack pasteurisation is the severest and also the most microbially secure form of pasteurisation (Lea, 1998). The juice is filled into a container which is then completely sealed and put into either a batch pasteuriser or a tunnel pasteuriser. In a batch pasteuriser the containers are held in heated water at a set temperature for a set period of time. In a tunnel pasteuriser the containers pass through a tunnel in which they are sprayed with water at various controlled temperatures.

2.6.4 Pasteurisation processing conditions

To calculate the lethal effect of pasteurisation a pasteurisation unit, P, given by

$$P = \exp [2.303(T-RT)/z]$$

Where

T = temperature of product

RT = reference temperature

z = slope of thermal death time curve of microorganism of interest.

Fellows (2000) recommends minimum processing conditions for fruit juice of 77°C for 1 minute. Thus a suitable pasteurisation treatment will have a P value of 1 minute at a reference temperature of 77°C and a z value of 10°C.

2.7 Literature review conclusions

Anthocyanins are distributed widely in the plant kingdom and are the source of orange, red and blue colours found in many fruits and vegetables. The major anthocyanin pigments of blackcurrants are cyanidin-3-rutinoside and delphinidin-3-rutinoside, and for elderberries are cyanidin-3-sambubioside and cyanidin-3-glucoside.

Anthocyanins are inherently unstable due to their electron deficient flavylium nuclei. Their stability is affected by many factors including pH, storage temperature, anthocyanin structure and concentration, the presence of oxygen, light, ascorbic acid, metals and sugars.

The factors which are likely to have the greatest impact on the anthocyanins in a blended juice product are ascorbic acid, anthocyanin concentration, storage temperature and light.

Anthocyanin structure also affects anthocyanin stability, with anthocyanins from different fruit sources having different structures and consequently stabilities. The processing conditions employed during manufacture of anthocyanin containing products can also cause degradation of anthocyanins.

Extensive research has been completed investigating the stability of anthocyanins in berryfruit juices or model systems. The stability of anthocyanins in blended juice systems containing berryfruit juices and other clarified juices has been studied to a limited extent. No literature was available in which the stability of anthocyanins in berryfruit and cloudy fruit juice blends has been investigated.

Haze and sediment formation in fruit juices can be due to many reasons, including microbial growth, starches and dextrins, polyphenol polymerisation and protein-polyphenol complexing.

Haze and sediment in clarified fruit juices is undesirable and the cause of many of these sediments has been identified. Cloudy apple juice is differentiated from clarified apple juice in that is has a stable cloud. The cloudy apple juice cloud is the result of complexing between pectin and native apple protein which forms a stable hydrated floc in the juice. Sediment formation in blended juice products containing berryfruit juices and cloudy apple juice has not been investigated and reported in the literature.

Polysaccharide stabilisers are often added to beverages to increase stability, by increasing viscosity to prevent phase separation and to enhance mouthfeel. Common polysaccharide stabilisers used in beverages include carboxymethylcellulose, locust bean gum, pectin, propylene glycol alginate and xanthan gum. These stabilisers have been successfully used for preventing sedimentation in fruit juice containing beverages.

The addition of polysaccharide stabilisers can affect the sensory properties of the beverage, both texture and flavour. Consumer's perception of texture has been investigated in several drinks and model systems. No literature was found on consumer's perception of texture in blended fruit drinks stabilised with polysaccharides. Investigation has been undertaken into the effect of the polysaccharide stabilisers on flavour however, the observed effect has been found to be dependent on the structure of the flavour compound.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Base ingredients

Cloudy apple juice concentrate

ENZAFOODS NZ Ltd. (Hastings, New Zealand) supplied the cloudy apple juice concentrate used for the manufacture of the drinks. The concentrate had a soluble solids concentration of 55 ± 1°Brix. The cloudy apple juice concentrate used in the experiments was obtained from 200L drums and frozen in smaller batches of 2L before use.

Clarified apple juice concentrate

ENZAFOODS NZ Ltd. (Hastings, New Zealand) supplied the clarified apple juice concentrate used for the manufacture of the drinks. The concentrate had a soluble solids concentration of 70 ± 1°Brix. The clarified apple juice concentrate used in the experiments was obtained from 200L drums and frozen in smaller batches of 2L before use.

Blackcurrant juice concentrate

Horticultural Marketing Ltd. (New Zealand) supplied the blackcurrant juice concentrate used for the manufacture of the drinks. The concentrate had a soluble solids concentration of 65 ± 1°Brix. The blackcurrant juice concentrate used in the experiments was obtained from 18L containers and frozen in batches of 2L before use.

Elderberry juice concentrate

Directus International Ltd. (Auckland, New Zealand) supplied elderberry juice concentrate used for the manufacture of the drinks. The juice concentrate had a soluble solids concentration of 65 ± 1°Brix. The elderberry juice concentrate used in the experiments was obtained from 18L containers and frozen in batches of 2L before use.

Elderberry concentrate I

Christen Hansen (Australia) supplied a water soluble elderberry concentrate. This was produced by extraction and subsequent pasteurisation of the juice from selected elderberries.

Elderberry concentrate II

Salkat New Zealand Ltd. (New Zealand) supplied a Dr Marcus water soluble elderberry concentrate, product number 3266.

Detoxifying herb formulation

Professional Herb Services Ltd. (New Zealand) supplied the herb extract. The herb formulation consisted of five different herbs; Polygonum, Burdock, Dandelion root, Echinacea purpurea and Siberian Ginseng. The herb extract was supplied as a "ready to use" ethanol based extract.

Water

Distilled water was used to manufacture the drinks.

3.1.2 Antioxidants

Ascorbic acid

Food grade ascorbic acid was obtained from Hawkins Watts Ltd (Auckland, New Zealand).

Quercetin

Quercetin was supplied by Nantong Newsmart International Trading Co. Ltd. (China) and distributed through Salkat New Zealand Ltd. (New Zealand). The quercetin was supplied as a yellow homogenous powder with a certificate of analysis indicating it was 98% pure.

Green tea extract - 98% tea polyphenols

A green tea extract containing 98% tea polyphenols was supplied by Nantong Newsmart International Trading Co. Ltd (China), distributed through Salkat New Zealand Ltd. (New Zealand).

Organic green tea extract – 15% tea polyphenols

Organic green tea extract was supplied by Nutri-Zeal Ltd. (New Zealand). The extract contained a minimum of 15% tea polyphenols.

3.1.3 Polysaccharide stabilisers

Carboxymethylcellulose (CMC-LV) – Hercules 12M3IP

Hercules 12M3IP CMC was supplied by Bayer New Zealand Ltd.

Locust bean gum (LBG)

Food grade locust bean gum was supplied by Bronson and Jacobs Pty Ltd. (New Zealand).

Pectin – Grinsted RS 461

A highly esterified (>70%) pectin was supplied by Bronson and Jacobs Pty Ltd. (New Zealand).

Propylene glycol alginate (PGA) – Kelcoloid HVF (PGA HV)

Kelcoloid HVF is a high viscosity PGA which was supplied by Germantown International Ltd. (New Zealand).

Propylene glycol alginate – Protanal Ester CF (PGA LV)

Protanal Ester CF is a low viscosity PGA which was supplied by Bayer New Zealand Ltd.

Xanthan

Food grade xanthan was supplied by Bronson and Jacobs Pty Ltd. (New Zealand).

3.1.4 Flavours

Natural berry flavours were added to all drinks manufactured, unless stated otherwise. The types of flavours and the levels added are confidential to Phoenix Natural Foods Ltd.

3.2 Determination of juice parameters

3.2.1 Total soluble solids

Total soluble solids of the drinks were determined using a Bellingham + Stanley (England) refractometer at 20°C and expressed as °Brix. The readings were uncorrected for acidity. Samples were tested in triplicate. Accuracy ± 0.5°Brix.

3.2.2 Total titratable acidity

Titratable acidity of the drinks was determined using AOAC Official Method (AOAC, 1995). To prepare the sample for titration 10ml of drink was diluted with 25ml of distilled water. The sample was then titrated against standardised 0.1M sodium hydroxide solution (Pauling Industries Ltd., New Zealand). Titratable acidity was expressed as grams of malic acid/100ml of juice using the conversion factor of 0.067. Samples were tested in triplicate.

3.2.3 pH

The pH was determined using a pH meter (Hanna Instruments, U.S.A.) with a refillable double junction combination pH electrode. The pH meter was calibrated with pH 4.0 and 7.0 buffers (Pauling Industries Ltd., N.Z.). Samples were tested at 20°C. Samples were tested in triplicate.

3.3 Experimental procedure for sediment analysis

3.3.1 Procedure

Ten different drink formulations were manufactured as shown in Table 3.01. The drinks were all standardised to 12 ± 0.5°Brix and their pH measured. One hundred milliliters of each formulation was filled into glass bottles. The bottles of drink were then pasteurised in a Grant waterbath (England) for 17 minutes at 70°C ± 2°C. These pasteurisation conditions simulated the pasteurisation conditions carried out by Phoenix Natural Foods Ltd. After 17 minutes, the bottles were cooled in a water bath at 15°C for 5 minutes and then held at ambient room temperature for 1 hour.

After cooling, samples of each treatment were stored in the dark in a refrigerator at 5°C ± 1°C. Two samples from each treatment were analysed for sediment weight and colour at weeks 1, 2, 4, 6 and 8.

Table 3.01: Drink formulations manufactured for sediment formation and analysis

Treatment	Ingredients	Quantity % v/v
Blackcurrant juice	Blackcurrant juice concentrate	16
	Water	84
	Ascorbic acid	400 mg/l
Blackcurrant & herb drink	Blackcurrant juice concentrate	16
	Water	83.6
	Herbal formulation	0.4
	Ascorbic acid	400 mg/l
Clarified apple juice	Clarified apple juice concentrate	15.6
	Water	84.4
	Ascorbic acid	400 mg/l
Clarified apple & herb drink	Clarified apple juice concentrate	15.6
	Water	84
	Herbal formulation	0.4
	Ascorbic acid	400 mg/l
Clarified apple & blackcurrant juice	Clarified apple juice concentrate	13
	Blackcurrant juice concentrate	1.3
	Water	85.7
	Ascorbic acid	400 mg/l
Clarified apple, blackcurrant & herb drink	Clarified apple juice concentrate	13
	Blackcurrant juice concentrate	1.3
	Water	85.3
	Herbal formulation	0.4
	Ascorbic acid	400 mg/l
Cloudy apple juice	Cloudy apple juice concentrate	20.6
	Water	79.4
	Ascorbic acid	400 mg/l
Cloudy apple & herb drink	Cloudy apple juice concentrate	20.6
	Water	79
	Herbal formulation	0.4
	Ascorbic acid	400 mg/l
Cloudy apple & blackcurrant juice	Cloudy apple juice concentrate	20
	Blackcurrant juice concentrate	1.3
	Water	78.7
	Ascorbic acid	400 mg/l
Cloudy apple, blackcurrant & herb drink	Cloudy apple juice concentrate	20
	Blackcurrant juice concentrate	1.3
	Water	78.3
	Herbal formulation	0.4
	Ascorbic acid	400 mg/l

3.3.1 Sediment weight & colour analysis

Preliminary investigation found that drinks made from clarified apple juice did not produce visible sediment within one week of bottling whereas sediment was visible in a week in drinks made from cloudy apple juice. For this reason samples from the different apple juice bases were prepared differently for analysis. For drinks made from cloudy apple juice the samples were prepared for centrifugation by carefully pouring off the juice above the sediment and washing the remaining sediment with distilled water. This sediment solution was then poured onto dry, pre-weighed filter paper circles (Schleicher & Schuell 595) which were placed in the bottom of the centrifuge cups. For drinks made from clarified apple juice concentrate the entire drink sample was poured onto dry, pre-weighed filter paper circles (Schleicher & Schuell 595) which were placed in the bottom of the centrifuge cups.

All the solutions were then centrifuged at 4000rpm for five minutes at 20°C (IEC Centra CL3R centrifuge, rotor system #243).

After centrifugation the supernatant was poured off and the filter paper was carefully removed and placed into preweighed moisture dishes. The filter papers were then dried in an oven (Contherm Digital Series, England) for 1 hour at 50°C ±1°C for subsequent colour measurement of the sediment.

The colour of the sediments, on the filter papers, were measured using a Chroma Meter (Minolta Cr-300, Minolta Co. Ltd., Japan). The sediments were placed directly onto the Minolta measuring system and covered, with a black cover, before measuring. The sediments were measured in duplicate with results expressed using the Hunter *L a b* colour system. After colour measurement the filter papers were placed back into the Contherm drying oven at 105°C ± 1°C for 5 hours until the dishes and filter papers were at a constant weight. The total dry weight of sediment was calculated by determining the change in the total weight of the pre-weighed filter papers and moisture dishes after drying.

3.3.2 Identification of sediment type

The procedure followed was adapted from Van Buren (1989). This provides a test to distinguish polyphenol and protein-polyphenol hazes from other types of hazes. This test uses 75% dimethyl formamide which greatly diminishes protein-polyphenol hazes while not affecting starch, dextrin, microbiological and inorganic hazes. Dimethyl formamide (99% assay, Sigma, U.S.A.) was diluted with distilled water to give a 75% dimethyl formamide solution. The drinks which produced sediment, from experiment 3.3.1, were remade into 250ml batches and stored at 5°C ± 1°C for 2 weeks. After this time the juices were centrifuged at 4000rpm for five minutes. The supernatant was poured off and the remaining sediment was resolubilised in 250ml of distilled water. Fifteen millilitres of 75% dimethyl formamide was added to 5 ml of the sediment solution. A control was prepared by adding 15 ml of distilled water to 5 ml of sediment solution. The absorbance of these two solutions was then measured using an UV-visible double beam spectrophotometer (Shimadzu, Japan) at 700nm and the % difference in absorbance for each drink calculated. This was performed in triplicate.

3.3.3 Total solids of herbal formulation

Five millilitres of the herbal extract was measured into a preweighed moisture dish. The dish was then placed in the Contherm dying oven and dried for five hours at 105°C ±1°C. The dish was then removed and weighed after the sample had reached constant weight. Analysis was performed in triplicate.

3.4 Stabiliser experiments

3.4.1 Preliminary investigation of stabilisers suitable for prevention of sediment formation

A base drink was made as shown in Table 3.02. Five stabilisers were investigated in this study: xanthan (Bronson & Jacobs), propylene glycol alginate high viscosity (PGA HV) (Germantown), propylene glycol alginate low viscosity (PGA LV) (Bayer), carboxymethylcellulose (CMC) (Bayer), locust bean gum (LBG) (Bronson & Jacobs) and pectin (Bronson & Jacobs). These stabilisers were chosen for this initial experiment after contact with food ingredient suppliers and their recommendations of the most suitable products for fruit juice stabilisation. The concentrations of stabiliser used were developed through recommendations from suppliers and also from literature (DeLeon and Boak, 1984; Peignier and Besnard, 1990; Bunger *et al.*, 1995; Lerchenfeld *et al.*, 1998). The stabiliser treatments used in this experiment are shown in Table 3.03.

Table 3.02: Base juice prepared for stabilised drinks.

Ingredient	Quantity % v/v
Cloudy apple juice concentrate	20
Blackcurrant juice concentrate	1.3
Water	78.3
Herbal formulation	0.4
Ascorbic acid	400 mg/l

Table 3.03: Stabiliser treatments for preliminary investigation.

Treatment	Stabiliser
S1	Xanthan 0.06% w/v
S2	Xanthan 0.05% w/v
	PGA HV 0.5% w/v
S3	PGA HV 0.012% w/v
	CMC LV 0.006% w/v
S4	PGA HV 0.3% w/v
S5	PGA LV 0.5% w/v
S6	Pectin 0.3% w/v
S7	Xanthan 0.03% w/v
	LBG 0.03% w/v
S7	Standard drink – no stabiliser

The stabilisers were dissolved in water, using a Braun high shear mixer (Braun, Germany), to form an aqueous slurry. The slurry was then added to the base juice mix and stirred for 1 minute using a spoon. The juice was standardised to 12 ± 0.5°Brix. The juices were filled into 100ml clear glass bottles and pasteurised in a Grant waterbath at 70°C ± 2°C for 17 minutes. The samples were then stored at 15°C ± 2°C in the dark, and visual observation was made of the sediment formation after 2 weeks. This was a preliminary experiment to determine which stabiliser systems had potential for use in the Wildberry Herbal drink, therefore visual observation was used to identify which stabiliser systems were worth investigating further.

3.4.2 Optimisation of stabiliser systems for the Wildberry Herbal drink.

A base juice was prepared as shown in Table 3.02. The stabilisers under investigation in this study were xanthan, PGA HV and pectin. These stabilisers were identified from section 3.4.1 as having the potential to minimise sediment formation. The concentration of stabilisers to be investigated was decided using the results from section 3.4.1. Seven stabiliser systems were prepared as shown in Table 3.04.

Table 3.04: Treatments investigated for the optimisation of stabiliser systems in the Wildberry Herbal drink.

Treatment	Stabiliser System
S8	Xanthan 0.04% w/v
S9	Xanthan 0.06% w/v
S10	Xanthan 0.08% w/v
S11	Xanthan 0.02% w/v
	PGA HV 0.1% w/v
S12	Xanthan 0.03% w/v
	PGA HV 0.1% w/v
S13	Pectin 0.5% w/v
S14	Standard drink – no stabilisers added.

The stabilisers were dissolved in water with a Braun high shear mixer until an homogenous aqueous slurry formed. The slurry was then added to the base juice mix and stirred for 1 minute using a spoon. The juice was standardised to 12 ± 0.5°Brix. The juices were filled into 100ml clear glass bottles and pasteurised in a Grant waterbath at 70°C ± 2°C for 17 minutes. The drinks were stored for 2 weeks in the dark at 15°C ± 2°C and then analysed. The weight of the sediment in the drinks was determined by centrifuging the contents of the bottle at 4000rpm for 5 minutes at 20°C. The sediment was collected on a pre-dried filter paper which was then dried to a constant weight in a Contherm drying oven at 105°C ± 1°C , and then weighed. The

weight of dry sediment collected was then measured. Five bottles per treatment were analysed.

3.4.3 Stability of stabilised drinks during storage

A base drink was prepared as outlined in Table 3.02. The stabilisers under investigation in this study were xanthan and PGA HV. The concentration of stabilisers to be investigated was decided using the results from section 3.4.2. Five stabiliser systems were prepared as shown in Table 3.05.

Table 3.05: Treatments investigated to determine the cloud stability of the Wildberry Herbal drink with added stabilisers.

Treatment	Stabiliser System
S15	Xanthan 0.025% w/v
	PGA HV 0.1% w/v
S16	Xanthan 0.03% w/v
	PGA HV 0.1% w/v
S17	Xanthan 0.03% w/v
	PGA HV 0.08% w/v
S18	Xanthan 0.09% w/v
S19	Standard drink – no stabilisers added.

The stabilisers were dissolved in water with a Braun high shear mixer until an homogenous aqueous slurry was formed. The slurry was then added to the base drink mix and stirred for 1 minute using a spoon. The drinks were standardised to 12 ± 0.5°Brix. The drinks were filled into 100ml clear glass bottles and pasteurised in a Grant waterbath at 70°C ± 2°C, for 17 minutes. The drinks were stored for four weeks at 15° C ± 2°C, in the dark, and then analysed. The weight of sediment in the drinks was determined by centrifuging the contents of the bottle at 4000rpm for 5

minutes. The sediment was collected on a pre-dried filter paper which was then dried to a constant weight in a Contherm drying oven at 105°C ± 1°C, and then weighed. The weight of dry sediment formed was then measured. Five bottles per treatment were analysed.

3.5 Consumer sensory testing

3.5.1 Sample preparation

The three juice drinks to be presented to the panellists were the Phoenix Wildberry Herbal drink (standard drink), a Wildberry Herbal drink with a xanthan (0.085% w/v) stabiliser system and a Wildberry Herbal drink with a xanthan (0.03% w/v)/PGA HV (0.1% w/v) stabiliser system.

The standard drink was obtained from product produced, at Phoenix Natural Foods Ltd., for commercial sale. The two drinks with stabiliser systems were manufactured from the base recipe, shown in Table 3.06, at Massey University, Albany. The colourant used in all of the drinks, for this experiment, was the Directus elderberry juice concentrate. This colourant was used, instead of blackcurrant juice concentrate.

Table 3.06: Base recipe used for the manufacture of stabilised drinks.

Ingredient	Quantity % v/v
Cloudy apple juice concentrate	14.9
Directus elderberry juice concentrate	0.7
Herbal formulation	0.4
Ascorbic acid	400 mg/l
Water	84

The water which was used in this base juice, to standardise it to 12°Brix, was used to dissolve the two stabiliser systems. The stabilisers were dissolved using a high shear Braun mixer with water at 20°C ± 2°C. Once the stabilisers had been dissolved the water/stabiliser solution was added to the base juice and mixed thoroughly with a spoon for one minute. The drinks were then taken to Phoenix Natural Foods Ltd. manufacturing premises where they were filled and bottled in 330ml glass bottles

and pasteurised in the Phoenix Natural Foods Ltd. batch pasteuriser at 67°C for 17 minutes.

3.5.2 Consumer sensory testing procedure

To determine the liking of body, berry flavour and overall impression a 9 point hedonic scale was used. A copy of the questionnaire given to the panellists is shown in Appendix 1.

Samples were assigned a random three digit code. Seventy millilitres of each sample were presented in Lily "Cold Cups" (Polarcup NZ Ltd.) on a white tray with a cup of filtered water (5° ± 2°C), for rinsing between samples, and a score sheet. The samples were served at a temperature of 5° ± 2°C. The order of presentation of the samples was randomised. The testing was conducted in the Study Centre Staff Lounge at Massey University, Albany. Panellists were seated at desks facing towards a blank wall. Testing was undertaken between 10.00am –3.00pm.

3.6 Measurement of the rheological parameters of drinks

The three drinks analysed by the consumer sensory panel were also analysed by a Paar-Physica MC 200 (Paar-Physica, U.S.A.) rheometer. A controlled shear rate analysis was undertaken on the drinks using a double gap measuring system (DG 25). The shear rate range was set as a linear ramp from 0-300s^{-1} with a measuring point duration of 10s and a total of 30 measuring points. The drinks were measured at a temperature of 5°C ± 0.5°C. The analysis was controlled and analysed using Physica Rheosolve Software (US 200 v2.10).

3.7 Analysis of factors affecting colour in the Wildberry Herbal drink

3.7.1 Experimental design

A two level fractional factorial design was developed for analysing the effect of four factors on the drink's colour stability: ascorbic acid concentration, blackcurrant juice concentrate volume, storage temperature and light. This experimental design was used to analyse drinks made from two different apple juice bases, cloudy and clarified apple juice concentrate. The experimental design is shown in Table 3.07.

Table 3.07: Experimental design for the determination of the effect of different factors on colour stability.

Treatment	Apple juice base	Ascorbic acid	Blackcurrant concentrate volume	Storage temperature (°C)	Light/Dark storage
A1	Cloudy	None	Normal	5	Dark
B1	Cloudy	400mg/l	Normal	5	Light
C1	Cloudy	None	Double	5	Light
D1	Cloudy	400mg/l	Double	5	Dark
E1	Cloudy	None	Normal	35	Light
F1	Cloudy	400mg/l	Normal	35	Dark
G1	Cloudy	None	Double	35	Dark
H1	Cloudy	400mg/l	Double	35	Light
J1	Clarified	None	Normal	5	Dark
K1	Clarified	400mg/l	Normal	5	Light
L1	Clarified	None	Double	5	Light
M1	Clarified	400mg/l	Double	5	Dark
N1	Clarified	None	Normal	35	Light
O1	Clarified	400mg/l	Normal	35	Dark
P1	Clarified	None	Double	35	Dark
R1	Clarified	400mg/l	Double	35	Light

The two levels for each factor were as follows:

Ascorbic acid:

Low level: no ascorbic acid added to base drink

High level: ascorbic acid added to base juice at a level of 400 mg/l

Blackcurrant juice concentrate volume:

Low level: normal, 1.3% v/v, blackcurrant juice concentrate

High level: double, 2.6% v/v, blackcurrant juice concentrate

Storage temperature:

Low level: 5°C

High level: 35°C

Light/Dark:

Low level: Storage in the dark, in a sealed cardboard box

High level: Storage under fluorescent lighting – Phillips 17W (60W equivalent) "Softone" fluorescent light

3.7.2 Base drink recipes

The drinks were made according to the recipes in Tables 3.08 – 3.15. The drinks were all standardised to 12 ± 0.5°Brix.

Cloudy apple juice base

Table 3.08: The recipe for treatments A1 & E1, drink with no added ascorbic acid and normal blackcurrant juice concentrate volume.

Ingredient	Amount required % v/v
Cloudy apple juice concentrate	14.8
Blackcurrant juice concentrate	1.3
Herbal formulation	0.4
Water	83.5

Table 3.09: The recipe for treatments B1 & F1, drink with 400mg/l ascorbic acid and normal blackcurrant juice volume.

Ingredient	Amount required % v/v
Cloudy apple juice concentrate	14.8
Blackcurrant juice concentrate	1.3
Herbal formulation	0.4
Ascorbic acid	400 mg/l
Water	83.5

Table 3.10:The recipe for treatments C1 & G1, drink with no added ascorbic acid and double blackcurrant juice concentrate volume.

Ingredient	Amount required % v/v
Cloudy apple juice concentrate	14.3
Blackcurrant juice concentrate	2.6
Herbal formulation	0.4
Water	82.7

Table 3.11:The recipe for treatments D1 & H1, drink with 400mg/l ascorbic acid and double blackcurrant juice concentrate volume.

Ingredient	Amount required % v/v
Cloudy apple juice concentrate	14.3
Blackcurrant juice concentrate	2.6
Herbal formulation	0.4
Ascorbic acid	400 mg/l
Water	82.7

Clarified apple juice base

Table 3.12: The recipe for treatments J1 & N1, drink with no added ascorbic acid and normal blackcurrant juice concentrate volume.

Ingredient	Amount required % v/v
Clarified apple juice concentrate	12.2
Blackcurrant juice concentrate	1.3
Herbal formulation	0.4
Water	86.1

Table 3.13: The recipe for treatments K1 & O1, drink with 400mg/l ascorbic acid and normal blackcurrant juice concentrate volume.

Ingredient	Amount required % v/v
Clarified apple juice concentrate	12.2
Blackcurrant juice concentrate	1.3
Herbal formulation	0.4
Ascorbic acid	400 mg/l
Water	86.1

Table 3.14: The recipe for treatments L1 & P1, juice with no added ascorbic acid and double blackcurrant juice concentrate volume.

Ingredient	Amount required % v/v
Clarified apple juice concentrate	11.0
Blackcurrant juice concentrate	2.6
Herbal formulation	0.4
Water	86

Table 3.15: The recipe for treatments M1 & R1, drink with 400mg/l ascorbic acid and double blackcurrant juice concentrate volume.

Ingredient	Amount required % v/v
Clarified apple juice concentrate	11.0
Blackcurrant juice concentrate	2.6
Herbal formulation	0.4
Ascorbic acid	400 mg/l
Water	86

The drinks were filled into 100ml clear glass bottles and then pasteurised in the batch bottle pasteuriser (67°C, 17 minutes) at Phoenix Natural Foods Ltd.

After pasteurisation the bottles were stored according to the experimental design. Each treatment was repeated in duplicate.

Bottles were removed and their colour was analysed at weeks 0, 2, 4, 6 and 8. The titratable acidity and pH of the drinks were measured at weeks 0 and 8. Selection of bottles from each treatment was randomised.

3.7.3 Determination of the total monomeric anthocyanin concentration

3.7.3.1 Reagents

pH 4.5 buffer

400 ml of 1M sodium acetate (BDH) and 240 ml of 1M hydrochloric acid (BDH) was added to 360 ml distilled water and mixed well. The pH of the solution was measured and adjusted with hydrochloric acid, as required, to obtain a final pH of 4.5

pH 1.0 buffer

250ml of 0.2M potassium chloride (Sigma, U.S.A.) was added to 750ml of 0.2M hydrochloric acid (BDH) and mixed well. The pH of the solution was measured and adjusted as required to obtain a pH of 1.0.

3.7.3.2 Sample preparation

Samples with a cloudy apple juice concentrate base were filtered through Whatman #1 filter paper (Whatman, England) before analysis. Samples with a clarified apple juice concentrate base were not filtered.

3.7.4.1 Reagent

20% Potassium Metabisulphite solution

Five grams of potassium metabisulphite (Chem-Supply, 95% assay) was made up to 25 ml with distilled water in a volumetric flask.

3.7.4.2 Procedure

Sample preparation was the same as outlined in section 3.7.3.2. The procedure outlined by Wrolstad (1976) was followed. Two hundred microlitres of 20% w/v potassium metabisulphite solution was added to a 3.0 ml sample of drink and 200µl of water was added to a second 3.0 ml of drink (control sample). If required the drink sample was diluted with water so the absorbance at 420nm and 510nm was below 1.0. The absorbances of the two samples were then measured at 510nm and 700nm. The spectrophotometer and cuvettes used were the same as described in 3.7.3.3.

3.7.4.3 Calculation of colour density

The colour density was determined by summing the absorbances of the control sample at 420nm and 510nm. Turbidity was corrected for by subtracting the absorbance at 700nm. If the sample had been diluted the sum was multiplied by a dilution factor.

The equation used was:

$$\text{Colour density} = [(A_{510nm}-A_{700nm}) + (A_{420nm} - A_{700nm})] * \text{dilution factor} \quad (4)$$

3.7.4.4 Calculation of polymeric colour

The procedure for calculating polymeric colour was the same as described in 3.7.4.3 except the absorbances of the bisulphite treated samples were used in the calculation, using Equation 4.

L = cuvette pathlength

= 10 mm

MW = molecular weight of major anthocyanin pigment

Blackcurrant: cyanidin-3-rutinoside

MW = 595.2 D (Wrolstad, 1976)

Elderberry: cyanidin-3-glucoside

MW = 445.2 D (Wrolstad, 1976)

3.7.4 Determination of colour density, polymeric colour, % contribution of tannin[1] to colour and colour deterioration index in the drinks using the metabisulphite method

The indicators of colour stability to be used in this experiment are the total monomeric anthocyanin concentration, the percentage of colour due to non-monomeric anthocyanins (tannins) and the colour deterioration index. The concentration of monomeric anthocyanins and percentage contribution of tannins is used as an indicator of colour stability, as the mechanism for anthocyanin degradation and subsequent colour loss includes the polymerisation of monomeric anthocyanins to brown polymeric pigments (von Elbe & Schwartz, 1996). The colour deterioration index gives an indication of the amount of browning that has occurred in the product; as the colour deterioration index decreases the brown pigments in the product increases. The colour deterioration index has been found to correlate well with visual observations of browning in grape juice (Sistrunk & Gascoigne, 1983). Blackcurrant syrups have been found to be unacceptable when their colour deterioration index is below 0.7 (Skrede, 1985).

[1] A tannin is defined as a non-monomeric anthocyanin which is resistant to bisulphite bleaching.

3.7.3.3 Procedure

The determination of monomeric anthocyanin concentration using the pH differential method was based on the method described by Wrolstad (1976). Each sample was diluted with buffer pH 1.0 to a level at which the maximum absorbance was between 0.4 and 0.6. The same dilution factor was used to dilute a second juice sample with pH 4.5 buffer. Dilution factors ranged from 3.5-10. Samples were analysed using a double beam UV – visible spectrophotometer (Shimadzu, Japan). The cuvettes used had a pathlength of 10mm and had a grade of G = glass/visible light. Distilled water was used in the reference cell. Absorbances were measured at 510nm (maximum absorption) and 700nm for the pH 1.0 and the pH 4.5 solutions (Wrolstad, 1976). Samples were analysed in duplicate.

3.7.3.4 Calculation of total monomeric anthocyanin content

The total monomeric anthocyanin content was calculated on the basis of the major anthocyanin pigment present in the analysed sample. The equation used to calculate the total monomeric anthocyanin content:

$$Concentration\ \ major\ anthocyanin\ pigment\ \ (mg\,/\,l) = \frac{A}{\varepsilon L} \times 10^3 \times MW \times dilution\ factor \quad (2)$$

Where

$$A = (A_{510nm\ \ pH1.0} - A_{700nm\,pH1.0}) - (A_{510nm\,pH4.5} - A_{700nm\,pH4.5}) \quad (3)$$

ε = molar absorbtivity of main anthocyanin pigment

Blackcurrant: cyanidin-3-rutinoside

ε = 28,800 (Wrolstad, 1976)

Elderberry: cyanidin-3-glucoside

ε = 29,600 (Wrolstad, 1976)

3.7.4.5 Calculation of % contribution of tannin (non-monomeric anthocyanin colour) to colour

The percent contribution of tannin to total colour was determined from colour density and polymeric colour using the following equation:

$$\% \text{ Contribution of tannin} = \frac{polymeric \quad colour}{colour \quad density} \qquad (5)$$

3.7.4.6 Calculation of colour deterioration index

The colour deterioration index was determined using the ratio of the absorbance at the anthocyanin wavelength maximum (510nm) to the absorbance at 420nm.
The equation used was:

$$\text{Colour deterioration index} = \frac{A_{510nm}}{A_{420nm}} \qquad (6)$$

3.7.5 Measurement of ascorbic acid at Week 0 and Week 8 using high pressure liquid chromatography (HPLC)

3.7.5.1 Reagents

Extraction solution – metaphosphoric acid
A 3% w/v metaphosphoric acid (Sigma, U.S.A.) extraction solution was prepared using distilled water.

Mobile phase
A 0.05M potassium dihydrogen phosphate (KH_2PO_4) solution was made by dissolving 13.69g KH_2PO_4 (Sigma, U.S.A.) in two litres of distilled water in a volumetric flask. The pH of the solution was adjusted to pH 2.5 using phosphoric acid (H_3PO_4) (Sigma, U.S.A.).

Ascorbic acid standard solution

A standard ascorbic acid solution was prepared by dissolving 50.6 mg ascorbic acid (Sigma, U.S.A.) in 50 ml distilled water in a volumetric flask. Five millilitres of this solution was then added to 45 ml of extraction solution to give a 101ppm ascorbic acid standard solution which was used as the ascorbic acid standard. The extraction solution is a strong reducing agent therefore minimising the oxidation of ascorbic acid during the analysis.

3.7.5.2 HPLC column

The column used for the analysis was a Hichrom C18 10μm with dimensions of 250mm x 4.6mm internal diameter.

3.7.5.3 Sample preparation

Drinks that were prepared from a cloudy apple juice concentrate base were filtered using Whatman GF/C 125mm filter paper (Whatman, U.S.A.). Samples that were prepared from a clarified apple juice concentrate base were not filtered.

3.7.5.4 Procedure

Twenty microlitres of sample was injected using an auto-sampler with the flow rate set at 1ml/min. The mobile phase was the dihydrogen phosphate solution. The retention time for ascorbic acid was found to be 4.96 minutes with a UV detector set at 245nm. Ascorbic acid concentration was calculated using an integrator which measured peak height. The ascorbic acid standard solution was used to calibrate the integrator every 8^{th} sample analysed. Samples were analysed in duplicate.

3.8 Analysis of pasteurisation process

3.8.1 Determination of the temperature regime used at Phoenix Natural Foods Ltd. for the pasteurisation of the Wildberry Herbal drink

Pasteurisation is defined as a heat treatment in which food is heated to below 100°C to extend the shelf life for several months by destruction of spoilage micro-organisms and or enzyme inactivation (Fellows, 2000).

Fifteen bottles of Wildberry Herbal drink had thermocouples inserted as shown in Figure 3.01. The bottles were 250ml in volume, and for the purposes of this investigation, were assumed to be a perfect cylinder. Bottles of the Wildberry Herbal drink, including those with thermocouples, were then placed into plastic crates (Recrate 47, Reece Plastics, N.Z.). Five bottles, with thermocouples were placed in the positions shown in Figure 3.02 at levels 1,3 and 4. These crates were then placed onto a wooden pallet. A thermocouple was placed in the centre of the pallet, between the plastic baskets, to record the ambient temperature during pasteurisation. The thermocouples were connected to a Squirrel data logger (Grant, U.S.A.) which was set to record the temperature every minute. The pallet was then loaded into the Phoenix Natural Foods custom made batch pasteuriser and the standard pasteurisation process was started (heating 67°C, 17 minutes). At the end of the pasteurisation process (60 minutes after the start of the process) the pallet was removed from the pasteuriser and left to cool at ambient factory temperature. The datalogger continued recording temperatures for four hours after the end of the pasteurisation process.

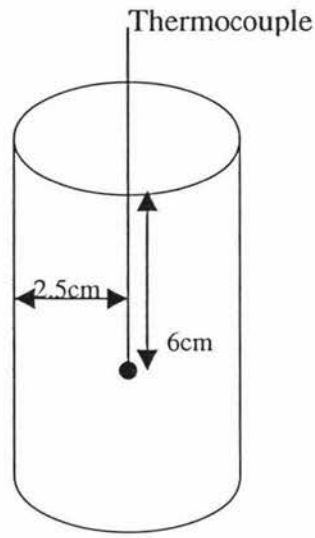


Figure 3.01: Thermocouple positioning in the Wildberry Herbal drink bottle.

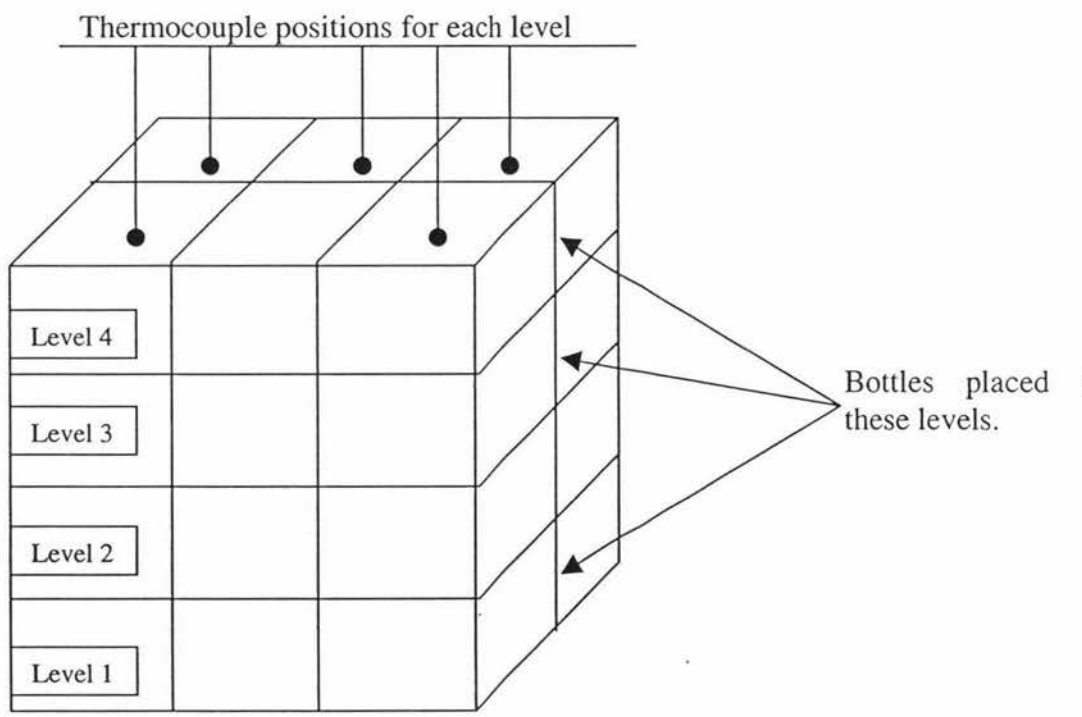


Figure 3.02: Positioning of bottles, with thermocouples, within baskets, and on pallet.

3.8.2 The effect of pasteurisation and cooling process on total monomeric anthocyanin content of the Wildberry Herbal drink

Sample collection

Before pasteurisation, at time 0, two 5ml samples of Wildberry Herbal drink were randomly taken from bottles being filled. These samples were immediately placed into glass containers and cooled in an ice/water slurry. Five millilitre samples were also collected at times 60, 80, 100, 120 and 240 minutes after the initiation of pasteurisation. Sixty minutes corresponds to the time at which the pallets of drinks were removed from the pasteuriser. To obtain these samples two bottles, from level four of the pallet, were chosen at random. The samples were then taken from these bottles at the required time intervals. These samples were put into glass containers and immediately cooled in an ice/water slurry until frozen. Samples were held at –18°C until analysed.

Total monomeric anthocyanin content determination

The samples were analysed using the methodology outlined in section 3.7.3.

3.9 Analysis of colour stability of alternative colourants in the Wildberry Herbal drink

3.9.1 Procedure

Four litres of base juice was prepared using the recipe shown in Table 3.16. This base juice was then separated into four separate batches of 1L. To each of these batches one of four colourants was added. The colourants, and the amount of each added, are shown in Table 3.17. The amount of each colourant to be added was decided using two methods. The blackcurrant concentrate was added at 1.3% v/v, the level at which it was used in the standard Wildberry Herbal drink. The other three colourants were added at the minimum level at which they had a visually comparable

red colour to the standard drink. The amounts were determined visually, as the anthocyanin composition of each was different. Each batch of juice was standardised to 12 ± 0.5°Brix and the pH was measured. The samples were filled into 100ml clear glass bottles and pasteurised in a Grant waterbath for 17 minutes at 70°C ± 2 °C. The bottles were then stored in the dark at 15°C ± 2°C, for 8 weeks. Bottles were removed in duplicate at Weeks 0, 2, 4, 6 and 8 and their colour analysed as described in section 3.7.3.

Table 3.16: Base drink recipe for the addition of colourants.

Ingredient	Quantity % v/v
Cloudy apple juice concentrate	19
Water	79
Herbal formulation	0.4
Ascorbic acid	400 mg/l

Table 3.17: Quantity of colourants added to base drink.

Treatment	Colourant	Concentration % v/v
A2	Blackcurrant juice concentrate	1.30
B2	Directus elderberry juice concentrate	0.70
C2	Christen Hansen elderberry concentrate II	0.35
D2	Dr Marcus elderberry concentrate I	0.38

3.10 Effect of quercetin and tea polyphenols on colour stability

3.10.1 Procedure

A base juice was prepared as shown in Table 3.02. The drink was standardised to 12 ± 0.5°Brix and then divided into 6 x 1L batches. Quercetin and tea polyphenols were added to each batch as shown in Table 3.18. Quercetin was added at a level at which it was soluble and had been found previously to improve anthocyanin stability (Shirkhande & Francis, 1974). Tea polyphenols were added at a level at which they were completely soluble and provided a similar antioxidant potential as the quercetin, using data provided by Rice Evans *et al.* (1997).

Table 3.18: Quercetin and tea polyphenol treatments added to base juice.

Treatment	Quercetin (mg/l)	Tea polyphenol – 98% (mg/l)	Tea polyphenol – 15% (mg/l)
F1	0	0	0
F2	40	0	0
F3	0	150	0
F4	40	150	0
F5	0	0	1005
F6	40	0	1005

Each treatment was filled into 100ml clear glass bottles and pasteurised in a Grant Waterbath at 70°C ± 2°C for 17 minutes. The bottles were then stored in the dark at 15°C ± 2°C for 8 weeks. Bottles were removed at weeks 0, 2, 4, 6 and 8 and their colour analysed in duplicate as described in section 3.7.3.

3.11 Statistical analysis

Statistical analysis of results was undertaken using Minitab® 12.1 (U.S.A) to calculate pooled standard deviations, standard error of means and one way ANOVA's. Statistical analysis of the experimental design, section 3.7, was made using Minitab® 12.1. Minitab was used to determine the significance of the four factors on anthocyanin concentration, colour density, polymeric colour and the anthocyanin degradation.

CHAPTER 4

IDENTIFICATION AND PREVENTION OF SEDIMENT FORMATION AND SENSORY - INSTRUMENTAL EVALUATION OF THE WILDBERRY HERBAL DRINK

The Wildberry Herbal drink manufactured by Phoenix Natural Foods Ltd (N.Z.) forms a heavy sediment soon after manufacture. In the commercial product this sediment is red in colour. However, with time it becomes brown in colour. This sediment can be dispersed with agitation however the sediment reforms again if the drink is left to stand. The base ingredients of this drink are water, cloudy apple juice concentrate, blackcurrant juice concentrate and a herb formulation.

4.1 Identification of ingredients contributing to sediment formation in the Wildberry Herbal drink

Typically sediments form in fruit juices due to the tendency of proteins, polyphenols and pectins to aggregate resulting in particulate formation (Lea, 1990). By identifying the ingredients contributing to the sediment, the mechanism for sediment formation can be proposed. The objective of this experiment was to identify which of the base ingredients contribute to the sediment formation in the Wildberry Herbal drink and the mechanism of formation. The time period over which the sediment formed was also investigated.

The initial pH values of the fruit juice drinks analysed in this experiment are presented in Table 4.01. The fruit juice drinks with the lowest pH were the blackcurrant juice and the blackcurrant juice and herb drink with pH 2.9. The fruit juice drinks with the highest pH were the cloudy apple juice and the cloudy apple and herb drinks with a pH of 3.5. In all cases, the addition of the herbal formulation to the fruit juice drinks did not change the pH whereas the addition of blackcurrant juice concentrate lowered the pH.

The total solid's contributed by the herb formulation, in 100ml of prepared drink, was 278 mg.

Table 4.01: Initial pH measurements of juices analysed in sediment experiment.

Treatment	pH_1
Blackcurrant juice	2.9
Blackcurrant & herb drink	2.9
Clarified apple juice	3.3
Clarified apple & herb drink	3.3
Clarified apple & blackcurrant juice	3.2
Clarified apple, blackcurrant & herb drink	3.2
Cloudy apple juice	3.5
Cloudy apple & herb drink	3.5
Cloudy apple & blackcurrant juice	3.3
Cloudy apple, blackcurrant & herb drink	3.3

1. Mean of triplicate samples (pooled SEM = 0.1; n=30).

The quantity of sediment formed in the drinks during eight weeks storage at 5°C is shown in Figure 4.01. The results show that there are two distinct groups of drinks with regards to the weight of sediment formed.

At week 0, immediately after manufacture, sediment weight was assumed to be zero grams for all the drinks and therefore was not measured. The juices that had a high level of sediment formation all contained cloudy apple juice concentrate. The remaining juices, which did not contain cloudy apple juice concentrate, had sediment levels of approximately 49mg/100ml. The weight of sediment formed in these drinks, made with clarified apple juice concentrate, did not increase during the storage period. Thus the weight obtained was likely to be due to solids in the drinks being absorbed onto the filter papers during centrifugation and remaining on the

filter paper after drying. Visual observation of these drinks during storage found that there was no sediment present in the bottom of the bottles.

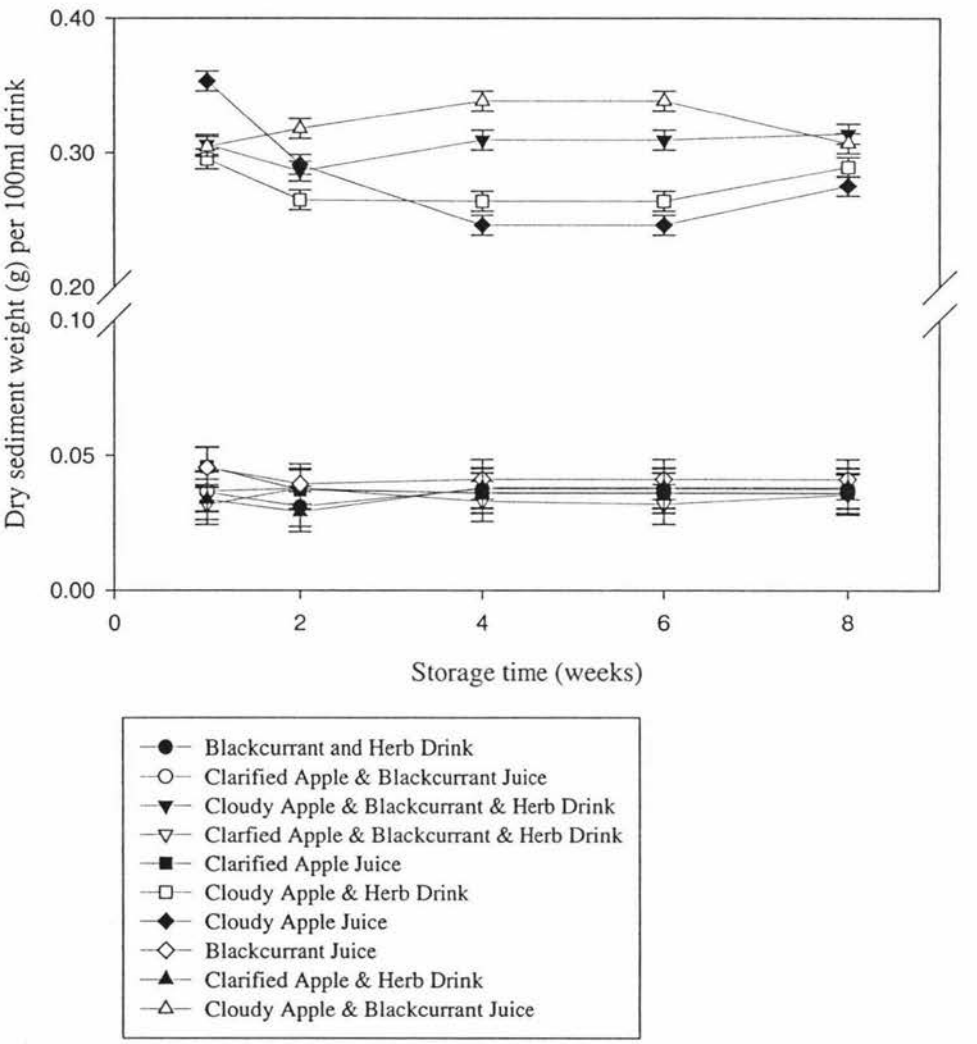


Figure 4.01: The dry weight of sediment formed in fruit drinks during storage in the dark at 5°C for eight weeks.

From Figure 4.01 it can be seen that there was little change in sediment weight with increasing storage time indicating that the majority of sediment forms within the first week of storage. The results in Table 4.02 show the mean weight of dry sediment formed, after eight weeks storage, in the four drinks which contained cloudy apple juice concentrate.

Table 4.02: The mean weight of sediment formed in drinks containing cloudy apple juice concentrate after eight weeks storage at 5°C.

Drink	Mean dry sediment weight (mg/100ml)[1]
Cloudy apple juice	262a
Cloudy apple & herb drink	276a
Cloudy apple & blackcurrant juice	305b
Cloudy apple & blackcurrant & herb drink	321b

1.Numbers followed by the same letter are not significantly different ($p>0.05$, n=40).

The addition of the herbal formulation to the drinks increased the weight of sediment formed; however the increase was not significant ($p>0.05$). The addition of blackcurrant juice concentrate however caused a significant increase in the weight of sediment formed ($p<0.05$).

Figures 4.02 to 4.04 show Hunter *L*, *a* and *b* values of the sediment from the four fruit drinks which formed a high level of sediment. *L* value denotes lightness; *a* value denotes redness or greenness; *b* value denotes yellowness or blueness. During the eight week storage period the *L a b* values of the drinks remained at a relatively constant level. Figures 4.02 – 4.04 clearly show the two distinct fruit drink groups, those with and without blackcurrant juice concentrate. In terms of sediment lightness, *L*, the sediment of the drinks without blackcurrant juice concentrate were significantly lighter ($p<0.05$). There is a significant difference ($p<0.05$) in lightness of the sediments from the cloudy apple juice and the cloudy apple and herb drink for all the weeks tested except week 8. The cloudy apple and herb drink sediment maybe significantly darker due to the dark brown colour of the herb extract which is likely to darken both the drink

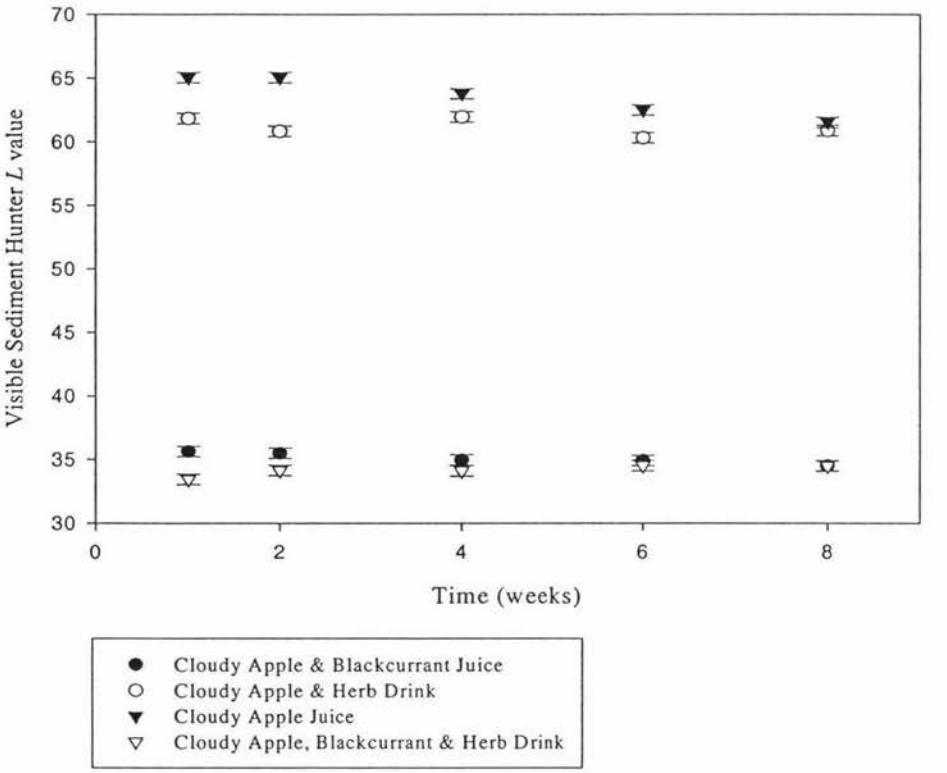


Figure 4.02: The lightness of sediment from drinks containing cloudy apple juice concentrate during eight weeks storage at 5°C.

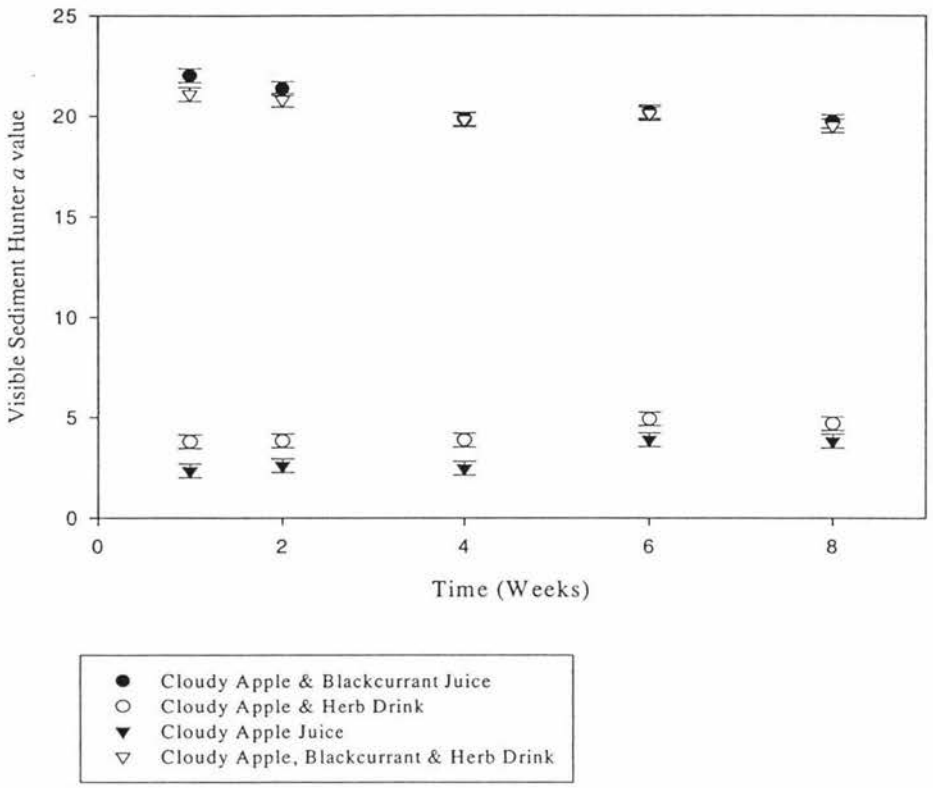


Figure 4.03: The Hunter *a* value of sediment from drinks containing cloudy apple juice concentrate during eight weeks storage at 5°C.

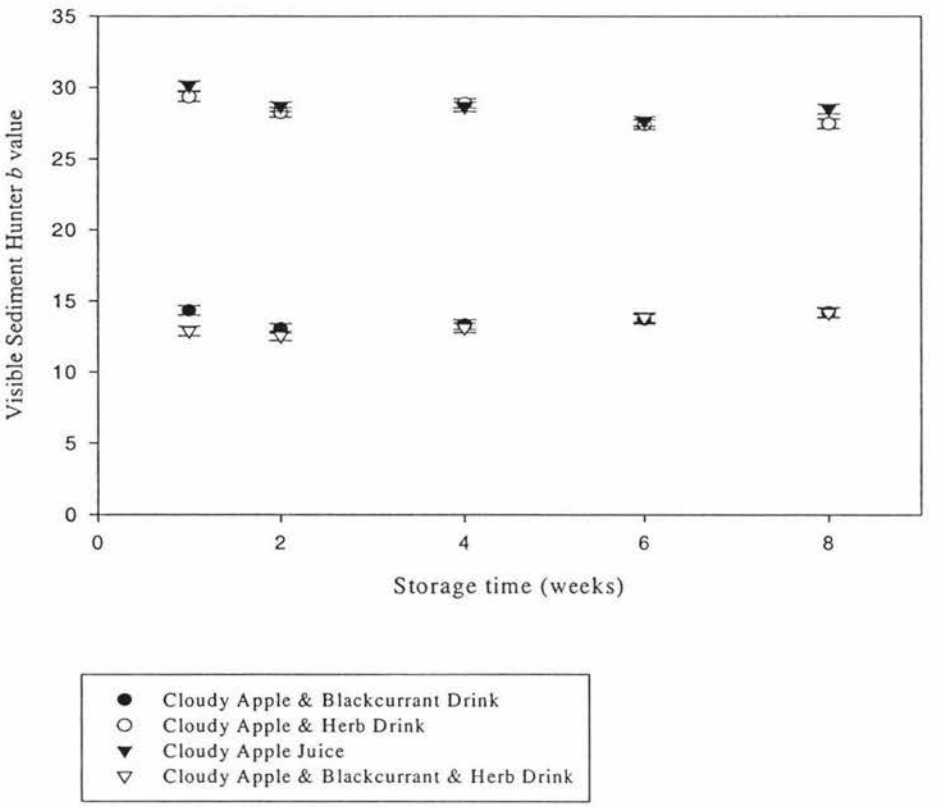


Figure 4.04: The Hunter *b* value of sediment from juices containing cloudy apple juice concentrate during eight weeks storage at 5°C.

and the subsequent sediment. In terms of redness, *a*, and yellowness, *b*, the sediment of the drinks which contained blackcurrant juice concentrate were significantly redder and less yellow ($p<0.05$). From these results it can be concluded that compounds, including anthocyanins, from the blackcurrant juice concentrate were present in the sediment.

The results in Table 4.03 show that the sediment in the four drinks is diminished by suspension in 75% v/v dimethyl formamide (DMF). The greatest loss in absorbance at 700nm was seen in the drinks which contained blackcurrant juice concentrate with losses ≥ 65%. This indicates that the sediment formed in the drinks contained polyphenol and protein-polyphenol associations. Starch, dextrin, microbiological and inorganic hazes are unaffected by DMF (van Buren, 1989).

Table 4.03: Identification of type of sediment in drinks after storage for two weeks.

Treatment	Absorbance of control	Absorbance of DMF_1 sample	% Reduction in $absorbance_2$
Cloudy apple juice	0.45	0.24	52a
Cloudy apple and herb drink	0.17	0.08	57a
Cloudy apple and blackcurrant juice	0.26	0.09	65b
Cloudy apple, blackcurrant & herb drink	0.32	0.11	66b

1. Dimethylformamide (75%v/v) treated sample.
2. Numbers followed by the same letter are not significantly different ($p>0.05$, $n=12$)

From the results in this experiment it was concluded that the cloudy apple juice was unstable when diluted from a concentrate as a sediment formed within one week of manufacture. The cloud in cloudy apple juice has been found to consist of negatively charged, partly demethoxylated pectin wrapped around a core of positively charged protein (Beveridge, 1999). Stability of these particles in cloudy apple juice is maintained because of their small size and electrostatic repulsion (Heatherbell, 1984). If the partly demethoxylated pectin is broken down, either by the action of heat or enzyme activity, agglomeration occurs due to electrostatic attraction of the exposed protein and the pectin, resulting in sediment formation (van Buren & Kilara, 1989; Lea, 1990). Endogenous enzyme activity during the pressing stage of manufacturing of the cloudy apple juice concentrate, used in this experiment, may have resulted in the breakdown of pectin present in the juice.

The sediment formed in cloudy apple and blackcurrant juice in this experiment was observed to be more dense and amorphous than the sediment observed in the cloudy apple juice which looked fluffy and cotton wool like. The mechanism proposed for the formation of this dense sediment in the cloudy apple and blackcurrant juice is a protein-polyphenol interaction. The exposed protein, with a negatively charged oxygen group, in the cloudy apple juice, is strongly attracted to the hydroxyl group of polyphenolic compounds in the blackcurrant juice, especially anthocyanins (Heatherbell, 1984). Large covalently bound complexes are formed which rapidly sediment from the juice serum. It is also likely that other particulates from the apple juice cloud are included in the covalently bound particles (Beveridge, 1999).

Little work has been done on sedimentation in blends of cloudy apple juice and juices with high anthocyanin pigment. Heatherbell (1984) postulated that unstable proteins in apple juice and in other juices that have high polyphenolic content, in particular anthocyanin pigment content, tend to be removed naturally by co-precipitation with the polyphenolic compounds. As was seen in this experiment, clarified apple juice, which has had pectin and protein removed during processing, did not form a sediment during eight weeks storage at 5°C.

Spayd et al. (1984) investigated the stability of clarified apple and pear juices blended with raspberry, cherry and grape juices. They found that, in general,

development of sediment was visually evident during 48 weeks storage at 25°C. They postulated that the sediment produced during storage was due to the degradation of anthocyanins which subsequently condensed and polymerised. No sediment formation was observed in this experiment with the clarified apple and blackcurrant juice blend however the much shorter storage period and storage at 5°C limited the degradation of the anthocyanins thus preventing their condensation and subsequent co-precipitation.

Light microscopy visual examination of the sediment formed in the cloudy apple and blackcurrant juice showed it to consist of an amorphous array of particles. This observation agrees with observations by Beveridge and Tait (1993) who observed protein-polyphenol sediment to have a "ground glass" appearance.

Beveridge and Tait (1993) investigated the structure of a protein-polyphenol haze, from commercial apple juice, using transmission electron microscopy. They proposed a structure for the haze consisting of a envelope filled with innumerable, variously sized micro particles or micro droplets. The surfaces of these envelopes were thought to have sticky surfaces, or sticky patches which cause them to adhere on contact. When this haze particulate was shaken or agitated it broke up into smaller pieces, but when left quiescent, reformed into larger clumps as the smaller pieces came into contact with each other through Brownian motion. This phenomenon was observed with the sediment in the Wildberry Herbal drink where the sediment dispersed into the drink when agitated, however, reformed into a dense sediment when the drink was left to stand.

4.2 Use of stabilisers for preventing sediment formation in the Wildberry Herbal drink

Cloudy apple juice concentrate and blackcurrant juice concentrate were the main contributors to the formation of sediment, due to protein-polyphenolic complexes. Stabiliser systems are typically used in drinks to suspend pulp, and particles, and to avoid phase separation during storage (Danisco, 2000). The stabilisers investigated in this experiment were polysaccharides. Polysaccharide stabilisers are used primarily to modify the flow properties and textures of drinks (BeMiller & Whistler, 1996). The rate of sedimentation of particles in drinks is related to a number of factors such as the size and density of particles, as well as the viscosity of the drink (Hicks, 1990). The objective of this experiment was to investigate a number of stabilisers which may be suitable in decreasing the precipitation of insoluble solids and hence the formation of a sediment in the Wildberry Herbal drink.

4.2.1 Preliminary investigation of stabilisers suitable for prevention of sediment formation

In this experiment eight stabiliser systems were added to a standard drink to determine their effectiveness in reducing the formation of sediment after 2 weeks. Results in section 4.1 found that the majority of sediment formed in the Wildberry Herbal drink within one week of manufacture. The effects of the stabilisers on sediment formation are shown in Table 4.04. From the visual observations the xanthan stabiliser system was the most effective at preventing sediment formation. The two systems containing propylene glycol alginate (PGA) only, also showed less sediment formation than the standard. The drink containing xanthan and PGA HV formed a gel in the bottle and thus was unsuitable as a drink at the concentrations used. The xanthan/locust bean gum (LBG) system was found not suitable for this beverage. The LBG was unstable in the drink and broke down within one week. LBG has an optimum pH range of 4-10 (Walter, 1998). It is likely that the breakdown of the LBG was a result of the pH of the drink (3.4), as it was too low and caused acid hydrolysis of the LBG.

Table 4.04 Visual observation of sediment formed in stabilised Wildberry Herbal drinks after two weeks storage at 15°C.

Stabiliser system	% w/v	Visual observation
Xanthan	0.072	Very light sediment formed.
Xanthan/	0.06	Drink was gel-like.
PGA HV	0.6	
PGA HV/	0.032	Very heavy sediment.
CMC LV	0.032	
PGA HV	0.36	Light/medium sediment.
PGA LV	0.48	Light/medium sediment.
Pectin	0.36	Heavy sediment formed.
Xanthan/	0.03	Drink separated into two phases.
LBG	0.03	
Standard	0	Heavy sediment.

Sediment Definitions:

Light- Small particulates present on bottom of bottle, not completely covering base of bottle. Disperses easily into drink on agitation

Medium- Sediment covers complete base of bottle, but height less than 1mm. Disperses into drink with agitation.

Heavy- Sediment covers complete base of bottle, height greater than 1mm. Sediment breaks into large clumps when agitated.

The drinks containing pectin and PGA/CMC stabiliser systems showed poor sediment prevention at the levels tested.

An informal taste panel, comprising Phoenix Natural Foods staff, tasted the three drinks which had a visually light/medium sediment. All three drinks were found to

have an acceptable texture. The drinks were slightly less viscous than desired by the panellists.

From these results, it was recommended that xanthan be further tested at different concentrations. The xanthan/PGA system should also be investigated at lower concentration levels. Pectin should be further investigated at a higher concentration, as it is the stabiliser that the manufacturers would like to use if it can be found to be effective at preventing sediment formation.

4.2.2 Optimisation of stabiliser systems for the prevention of sediment formation in the Wildberry Herbal drink.

The purpose of this experiment was to further investigate stabiliser systems recommended in section 4.2.1 and determine the weight of sediment formed in the stabilised drinks in comparison with the standard. The results for this experiment are shown in Table 4.05. The sediment of the stabilised drinks ranged from between 65% to 98% of the weight of the standard drink's sediment. The stabiliser system that formed the least sediment was the xanthan 0.03%/PGA HV 0.1% w/v followed closely by xanthan 0.08% w/v. For the drinks stabilised with xanthan the amount of sediment formed decreased with increasing xanthan concentration (Table 4.05). Pectin, at an increased level of 0.5% w/v, was again not effective at reducing the amount of sediment formed. The weight of sediment formed was virtually equal to that formed in the standard. The pectin used in this experiment was a high ester pectin, which in the absence of high soluble solids (>55%) does not form a gel but increases the viscosity and improves mouthfeel (May, 1999). The pectin was not found to be effective at preventing sediment formation in this beverage.

Table 4.05 Quantity of sediment formed and visual observation of sediment formed in stabilised drinks after storage for two weeks at 15°C.

Stabiliser system	% w/v	Dry sediment weight (mg/100ml)	% of sediment relative to standard drink[1]	Visual observations
Xanthan	0.04	205	70	Medium sediment.
Xanthan	0.06	199	68	Medium sediment.
Xanthan	0.08	192	66	Light/medium sediment.
Xanthan/ PGA HV	0.02 0.1	200	68	Medium sediment. Drink colour not as red as other drinks.
Xanthan/ PGA HV	0.03 0.1	189	65	Light/medium sediment. Drink colour not as red as other drinks.
Pectin	0.5	287	98	Very heavy sediment.
Standard	0	293	100	Very heavy sediment.

1. Sediment weight of stabilised drink/sediment weight of standard drink *100

Sediment Definitions:

Light- Small particulates present on bottom of bottle, not completely covering base of bottle. Disperses easily into drink on agitation

Medium- Sediment covers complete base of bottle, but height less than 1mm. Disperses into drink with agitation.

Heavy- Sediment covers complete base of bottle, height greater than 1mm. Sediment breaks into large clumps when agitated.

It was observed that after storage the drinks stabilised with PGA had a much paler red colour than the standard drink. The drinks containing only xanthan stabiliser showed no visual difference in red colour from the standard.

4.2.3 Stability of stabilised Wildberry Herbal drinks during storage

Wildberry Herbal drinks with three combinations of xanthan/PGA and one concentration of xanthan only were investigated to determine their ability to prevent sediment formation. These stabilisers were chosen on the basis of the results of section 4.2.2. Testing was undertaken at 15°C ± 2°C. The most effective stabiliser system was xanthan at a concentration of 0.09% w/v as shown in Table 4.06.

Table 4.06 Quantity of sediment formed and visual observation of sediment formed in stabilised drinks after storage for four weeks at 15°C.

Stabiliser system	% w/v	Dry sediment weight (mg/100ml)	% of control	Visual observations
Xanthan/ PGA HV	0.025 0.1	170	62	Loss of red colour. Light/medium sediment.
Xanthan/ PGA HV	0.03 0.1	187	69	Loss of red colour. Medium sediment.
Xanthan/ PGA HV	0.03 0.08	190	70	Loss of red colour. Medium sediment.
Xanthan	0.09	158	58	Light/medium sediment.
Standard	0	273	100	Heavy sediment.

Sediment Definitions:

Light- Small particulates present on bottom of bottle, not completely covering base of bottle. Disperses easily into drink on agitation

Medium- Sediment covers complete base of bottle, but height less than 1mm. Disperses into drink with agitation.

Heavy- Sediment covers complete base of bottle, height greater than 1mm. Sediment breaks into large clumps when agitated.

The weight of sediment in the drink was 58% of the total weight of sediment formed in the standard. The xanthan 0.025%/PGA HV 0.1% w/v was the next most effective stabiliser in this experiment with sediment formation 62% of the weight of sediment in the standard drink.

It was again found that the drinks, which were made with stabiliser systems containing PGA, were much paler than the standard drink. PGA is much less sensitive to low pH than non-esterified alginates such as sodium alginate because its esterified carboxyl groups cannot ionise (BeMiller & Whistler, 1996). The hydrophobic propylene glycol groups in PGA also give the molecule mild interfacial activity however, and this causes undesirable foaming effects during manufacture. When manufacturing the drink, the use of high shear mixing to dissolve the PGA causes foaming which leads to pumping problems and also increases the amount of air incorporated in the product.

When xanthan is used as a stabiliser, the viscosity changes very little with temperature (BeMiller and Whistler, 1996). Xanthan is also highly pseudoplastic and thus exhibits a decrease in viscosity with increasing shear rate. PGA used in association with xanthan decreases solution viscosity and pseudoplasticity. When xanthan and PGA are used synergistically they give the desired pourability associated with the pseudoplastic xanthan and the body associated with non-pseudoplastic solutions (BeMiller and Whistler, 1996). Szczesniak and Farkas (1962) investigated consumer's perception of hydrocolloids and found that a xanthan solution was perceived as non-slimy, whereas a sodium alginate solution was perceived to be slimy.

A stabiliser system containing xanthan and PGA has been proposed previously, as being a suitable beverage thickener for providing body or mouthfeel at acid pH (pH 3-4) in fruit juices and soft drinks (Bunger *et al.*, 1995). Systems containing PGA/CMC, to prevent the separation of solids in fruit juice containing products, and xanthan/carob gum, to stabilise aqueous suspensions of solid particles have also been proposed (De Leon and Boak, 1984; Peignier and Besnard, 1990; Lerchenfeld *et al.*, 1998). The beverages these proposed stabiliser systems were used in included fruit

juice and high fructose corn syrup bases (De Leon and Boak, 1984; Lerchenfeld *et al.,* 1998) and fruit juice and cane sugar bases (Bunger *et al.*, 1995).

Overall the two most effective stabiliser systems were xanthan 0.025%/PGA HV 0.1% w/v and xanthan 0.09% w/v. However even with these stabilisers sediment still formed in the drink at a level of approximately 60% of the standard drink. The sediment formed was not as dense as the sediment of the standard drink. The stabilisers prevented the protein-polyphenol particles from agglomerating into an amorphous mass. Consequently, when agitated, the sediment of the stabilised drinks dispersed more effectively than the sediment in the standard drink.

4.2.4 Cost of using suitable stabiliser systems

The cost of using selected stabiliser systems is shown in Table 4.07. Overall, the cost of the stabilisers ranged from $1.46 to $5.90 per 100 litres of drink, which is a cost of one to one and a half cents per 250ml bottle. Stabiliser systems containing PGA were the most expensive, however, at the levels used the cost was still below $6 per 100 litres.

Table 4.07 Cost of stabiliser systems.

Stabiliser system	% w/v	Amount of stabiliser required g/L	Stabiliser cost $/kg[1]	Cost $/100L	Total cost $/100L
Xanthan	0.04	0.4	36.5	1.46	1.46
Xanthan	0.06	0.6	36.5	2.19	2.19
Xanthan	0.08	0.8	36.5	2.92	2.92
Xanthan	0.09	0.9	36.5	3.29	3.29
Xanthan	0.02	0.2	36.5	0.73	5.53
PGA HV	0.1	1	48	4.80	
Xanthan	0.03	0.3	36.5	1.10	5.90
PGA HV	0.1	1	48	4.80	
Standard	0	0	0	0.00	0.00

1. Prices based on June 2000 quotes from suppliers.

From the results it was decided that two stabiliser systems, xanthan and xanthan/PGA, would be evaluated by consumer sensory evaluation to determine consumers liking of the modified drinks. While the primary function of the stabilisers is to prevent the formation of sediment in the Wildberry Herbal drink they must also provide a body which is acceptable to consumers. The concentrations of stabilisers to be used in the

consumer evaluation were chosen after consideration of their ability to minimise sediment formation and also provide a suitable mouthfeel.

4.3 Consumer sensory evaluation and instrumental analysis of standard and stabilised Wildberry Herbal drinks.

A consumer's perception of a fruit drink before consumption and when consumed is very important to the consumers overall perception of a drink. Before consumption a consumer's perception is influenced by the colour, aroma and pourability. During consumption a consumer's perception is influenced by the body, aroma and flavour of the drink. For maximum consumer acceptability, the sensory properties of a fruit drink must correspond to the consumer's expectation of the actual fruit and drink type (Hicks, 1990).

The addition of polysaccharide stabilisers to drinks modifies their viscosity and consequently changes the body and flavour release properties of the drink (Pangborn *et al.*, 1973). In section 4.2, stabilisers were added primarily to control the formation of sediment. The objective of this experiment was to determine how much consumers liked the body, flavour and their overall liking of the two stabilised drinks chosen from section 4.2.3 and the standard Phoenix Wildberry Herbal drink. The rheological parameters of the drinks were also measured.

4.3.1 Consumer sensory evaluation

Sensory testing was conducted at Massey University's Albany campus. Ninety nine panellists were recruited for the evaluation. Demographic data was collected from 92 panellists after the sensory evaluation and this is presented in Appendix 2. The panellists were split 46% to 54% female and male, respectively. The majority of panellists, 85%, were in the 17-25 age group. The demographic data shows that the panellists used for the sensory evaluation were frequent fruit juice drinkers with the majority, 77%, consuming at least one to four fruit juice drinks per week. The fruit juice most often consumed was orange juice, with blackcurrant or apple and blackcurrant drinks also being popular. Panellists were asked to indicate which brands of fruit juice they usually consumed, only five panellists indicated that they usually consumed Phoenix (Phoenix Natural Foods Ltd, N.Z.) drinks.

The mean scores obtained from the sensory evaluation are presented in Table 4.08. The results show that there was no significant difference in the liking of the three attributes for the three different drinks ($p>0.05$). The mean scores for the drinks ranged from 5.9 to 6.5, which corresponds on the hedonic scale to a "like slightly" rating. A summary of the comments that panellists made about the drinks is presented in Table 4.09. A full list of comments is shown in Appendix 3.

Table 4.08: Mean nine point hedonic scale scores for body, berry flavour and overall impression of Wildberry Herbal drinks.

Attribute	Mean hedonic scores		
	Standard	Xanthan	Xanthan/PGA
Body[1]	6.3*	6.3*	6.5*
Berry flavour	5.9*	6.2*	6.4*
Overall impression	6.0*	6.3*	6.5*

1. The feel of the drink as it moves over the tongue and around the mouth.

- * not significantly different ($p > 0.05$).
- Scores are the mean for $n = 99$.

In terms of body, the drink with the highest score, 6.5, was the xanthan/PGA stabilised drink (Table 4.08). Comments made by three of the panellists indicated that the body of the drink was very "thick" and one mentioned that it was "like a smoothie". The other two drinks had slightly lower scores for body with little panellist comment made with regard to this attribute.

Table 4.09: Summary of comments made by consumer sensory panellists for standard, xanthan and xanthan/PGA drinks

Drink	Summary of Comments
Standard drink	There were 11 comments that the drink was too sweet however there were also 6 comments that the drink was too sour/acidic. The smell and in particular the taste of the drink was not liked with descriptions such as "dirty" and "nutty" used. Ten panellists gave very favourable comments for this drink, with descriptions such as "very good" and "having a pleasant aftertaste". Neither positive nor negative comments were made about the body of the drink.
Xanthan stabilised drink	The comments about the flavour of this drink were divided quite markedly with 8 making favourable comments about the flavour such as "damn good" and "very pleasant" and 14 who made unfavourable comments with "watery" being the most common descriptor for this dislike. The drink was not found to be as refreshing as the other drinks by five of the panellists who made comments with descriptors such as "flat" and "no kick" being used to describe it.
Xanthan/PGA stabilised drink	Thirteen panellists gave very positive general comments such as "yummy" and "lovely". Six panellists commented that they found the flavour to be too weak and unlike a "normal" berry flavour/aroma. The body of the drink was noted as being thick but was liked and compared by one panellist to that of a smoothie.

- Total number of panellists who made comments on each drink: Standard-58; xanthan/PGA-53; xanthan-50.

The xanthan/PGA stabilised drink also scored highest in terms of berry flavour with a score of 6.4, which was slightly higher than the xanthan stabilised drink with a score of 6.2. From those panellists who made a comment about the berry flavour of the xanthan/PGA drink, 60% made favourable comments about the flavour and 40% made unfavourable comments about the berry flavour such as "too weak" and "unlike normal berry flavour". Of the people who made comments about the flavour for the xanthan, 40% made favourable comments about the flavour and 60% made unfavourable comments such as describing the flavour as "watery".

It is well known that the basic tastes and flavours of drinks are affected by the presence of hydrocolloid stabilisers (Pangborn *et al.*, 1973; Pangborn & Szczesniak, 1974; Pangborn *et al.,* 1978; Hicks, 1990). Xanthan and sodium alginate have been found to significantly decrease sweetness and sourness and are also effective in lowering the intensity of odour and flavour (Pangborn *et al.*, 1973; Pangborn & Szczesniak, 1974; Pangborn *et al.*, 1978). The overall effects of hydrocolloids has been found to be more dependent on the nature of the hydrocolloid and the flavour compound than on the viscosity level imparted by the stabiliser (Pangborn *et al.*, 1978).

To compensate for an anticipated suppression in flavour in the stabilised drinks, the flavours added to the stabilised berry drink's were increased (~30%v/v) to try and achieve a flavour comparable to the standard Phoenix Wildberry Herbal drink. The liking of the standard drink's berry flavour was lower than the stabilised drinks with a score of 5.9 compared to 6.2 and 6.5 for the xanthan and xanthan/PGA drinks, respectively.

Analysis of the comments made by the panellist's gives some indication as to why the standard drink scored lower in flavour than the stabilised drinks. From the comments (Table 4.09), it can be seen that eight panellists commented on the unusual flavour of the drink describing it as "dirty" or "nutty". This "unusual" flavour is attributed to the herb formulation. The panellists scored the flavour of the standard berry drink lower, as the overall flavour profile of the standard drink was not what would be expected in a berry drink such as apple and blackcurrant. When panellists were presented with the drinks, the only description of the drinks given

was that they were berry drinks and when evaluating the flavour the panellists were instructed to evaluate the "berry flavour you taste when drinking". The panellists were not informed that the drinks contained herbal extracts as it was decided that this might create bias in the panellists evaluation. No comment was made about the "dirty/nutty" flavour in the stabilised drinks even though these contained the herbal extract at an equivalent level to the standard drink. Xanthan and PGA are known to have a flavour suppressing effect and this combined with the increased level of berry flavouring is likely to have masked the herbal extract flavour in the two stabilised drinks.

4.3.2 Rheological parameters of standard and stabilised Wildberry Herbal drinks

The rheological parameters of the three drinks, evaluated by the panellists, were measured and the results are presented in Figure 4.05.

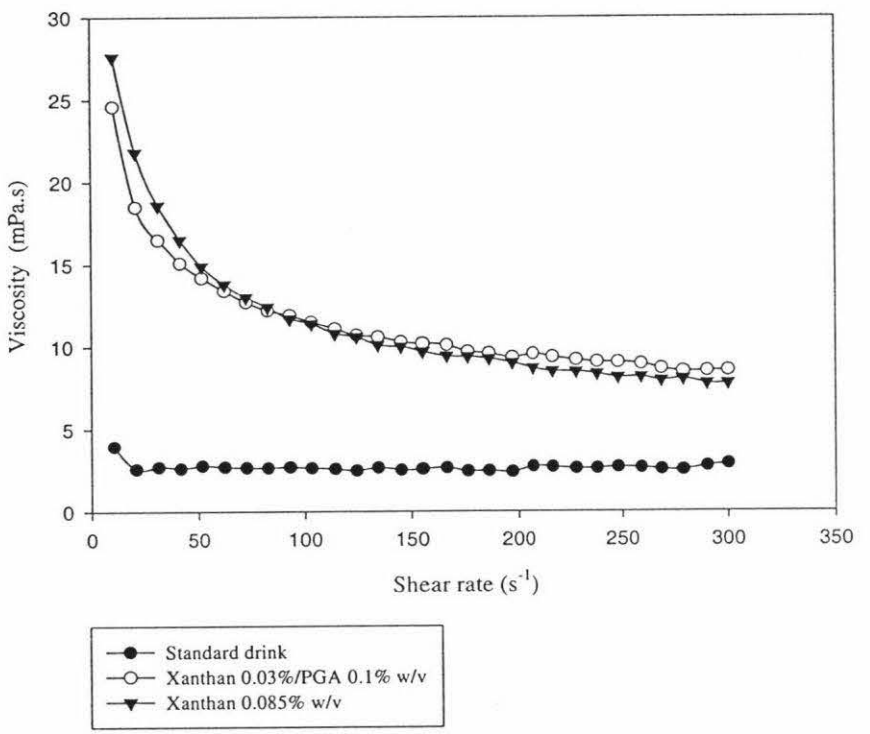


Figure 4.05: Viscosity of the standard and modified Wildberry Herbal drinks at different shear rates.

During sensory evaluation, panellists were only asked to rate their liking of the body of the drinks and thus no quantitative information was obtained with regard to the thickness of the drinks. Panellists did comment however that the two stabilised drinks were much thicker than the standard drink (Table 4.09).

Early work by Wood (1968) suggested that the effective oral shear rate (the shear rate in the mouth during consumption) was about $50s^{-1}$. However it was later suggested by Shama and Sherman (1973) that the effective oral shear rate increases with increasing fluid viscosity due to the fact that fluids that are less viscous are

moved around the mouth more rapidly than more viscous ones. For a sample to reach a given shear rate in the mouth it must first be accelerated from rest through a range of intermediate shear rates. For extremely shear thinning fluids the viscosity at low rates of shear greatly exceeds that of the less shear thinning materials and therefore makes a disproportionate contribution to overall perceived thickness (Shama & Sherman, 1973). Both of the stabilised drinks in Figure 4.05 show pronounced shear thinning behaviour and therefore it can be postulated that this caused the panellists to perceive the drinks to be thicker than the standard drink, when first placed in the mouth.

Szczesniak and Farkas (1962) found that gum dispersions which exhibited a sharp decrease in viscosity, with increasing shear rate, were judged by a trained panel to be non-slimy whereas gums that showed a small to moderate change in viscosity were considered by the panel to range from slimy to extremely slimy. None of the panellists commented that the drinks had any slimy characteristics. The decrease in apparent viscosity with shear rate for the stabilised drinks shown in Figure 4.05, was therefore sufficient for preventing a slimy mouthfeel to the drinks.

The flavour release of a fluid has been found to be affected by both viscosity and binding of flavours within the food matrix (Bourne, 1982). If the viscosity of a drink does not decrease sufficiently with increasing shear rate then the drink will have poor flavour release. It can be seen that for the two stabilised drinks the viscosity at the higher shear rates (200-300s^{-1}) is still much higher than the standard drink (Figure 4.05). Since the viscosity remained higher than the standard drink the flavour release of the stabilised drinks may have been affected. Comments regarding the flavour of the two stabilised drinks can be seen in Table 4.08, where the drinks are described as "watery", "flat" and "not refreshing".

The viscosity of the standard drink remains at a constant low viscosity with increasing shear rate (Figure 4.05). This indicates that the drink has good flavour release however the drink does not impart much body/mouthfeel.

Overall the stabilised drinks were liked slightly more than the standard drink however there was no significant difference between the scores ($p>0.05$). The

panellists liked the thicker bodies of the stabilised drinks however this body did decrease the flavour impact of the drink. If the stabilised formulations were to be used then more work would be required to ensure that the berry flavour was clean and that the drink was refreshing.

CHAPTER 5

FACTORS AFFECTING THE COLOUR OF THE WILDBERRY HERBAL DRINK

5.1 Effect of apple juice concentrate type, ascorbic acid, blackcurrant juice concentrate volume, storage temperature and light on colour stability.

Many factors have been found to influence the stability of anthocyanins in food products (Jackman and Smith, 1996). The major intrinsic and extrinsic factors that affect anthocyanin stability in the Wildberry Herbal drink were identified from the literature. Five factors were investigated to determine their impact on the stability of the anthocyanins in the Wildberry Herbal drink.

The effect of apple juice concentrate on the colour stability parameters was also investigated. The advantage of using a clarified apple juice concentrate base, in terms of sediment prevention, was demonstrated in Chapter 4. Four factors were chosen to be analysed by a factorial experimental design. They were ascorbic acid concentration, blackcurrant juice concentrate volume, storage temperature and light. These factors were to be analysed at low and high levels and their effect on three colour parameters: monomeric anthocyanin concentration, % contribution of tannin and colour deterioration index was investigated. The objective of the experimental design was to determine the effect on colour, during storage, of increasing these factors to their high level in the drink. The experimental design was carried out with drinks made from two different apple juice concentrate bases.

5.1.1 Effect of apple juice concentrate type on colour stability

The effect of apple juice concentrate type (cloudy or clarified) on retention of anthocyanins, % contribution of tannins and colour deterioration in the Wildberry Herbal drink is shown in Figures 5.01-5.12. The drinks were made with two levels of blackcurrant juice concentrate and ascorbic acid, stored at 5 or 35°C for eight weeks.

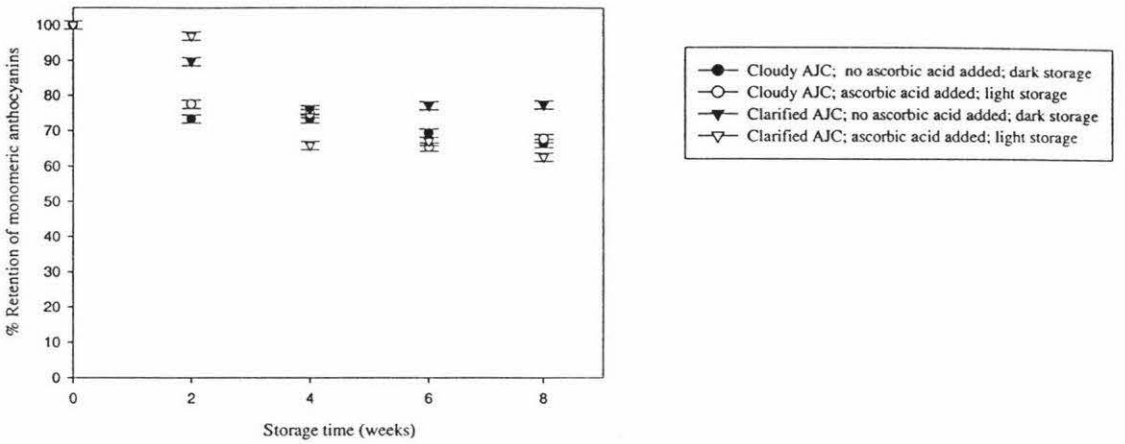


Figure 5.01: % Retention of monomeric anthocyanins in Wildberry Herbal drinks containing 1.3% v/v blackcurrant juice concentrate, stored at 5°C.

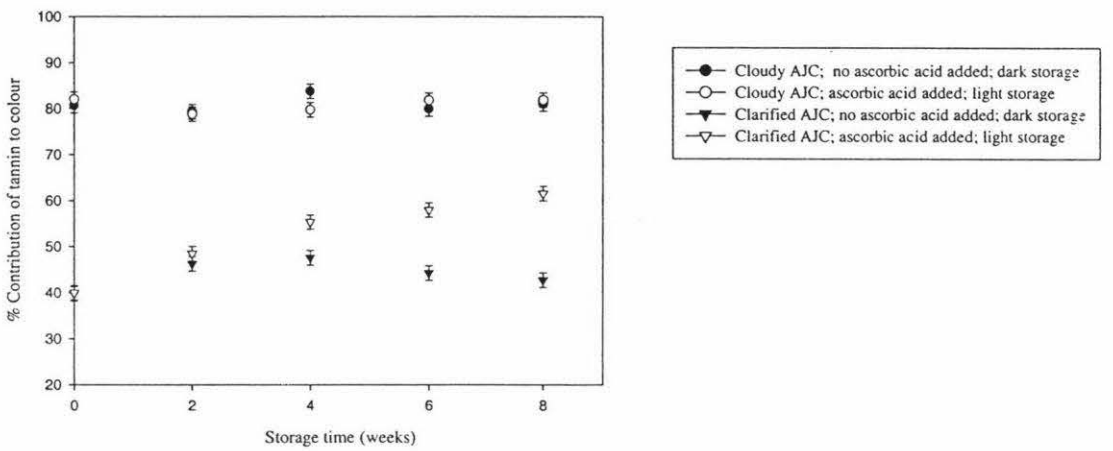


Figure 5.02: % Contribution of tannin to colour in Wildberry Herbal drinks containing 1.3% v/v blackcurrant juice concentrate, stored at 5°C.

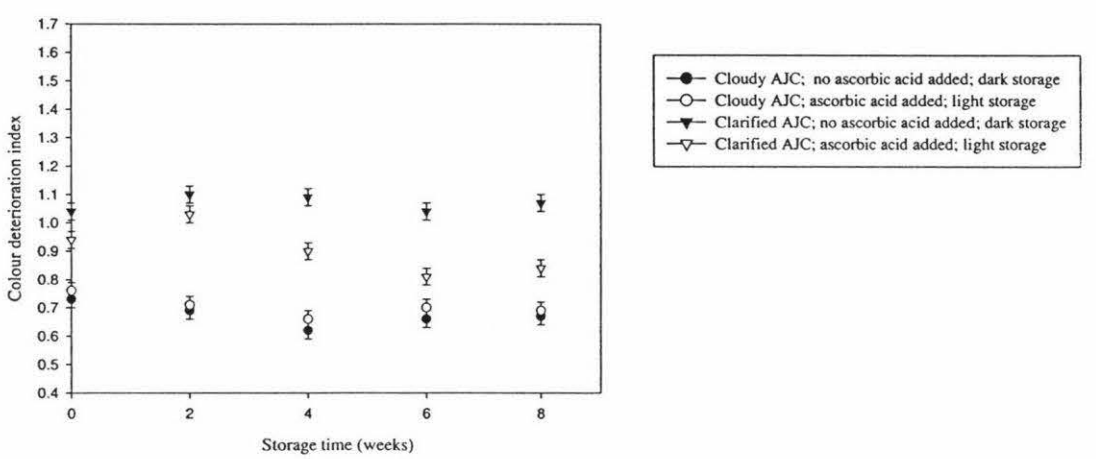


Figure 5.03: Colour deterioration index of Wildberry Herbal drinks containing 1.3% v/v blackcurrant concentrate, stored at 5°C.

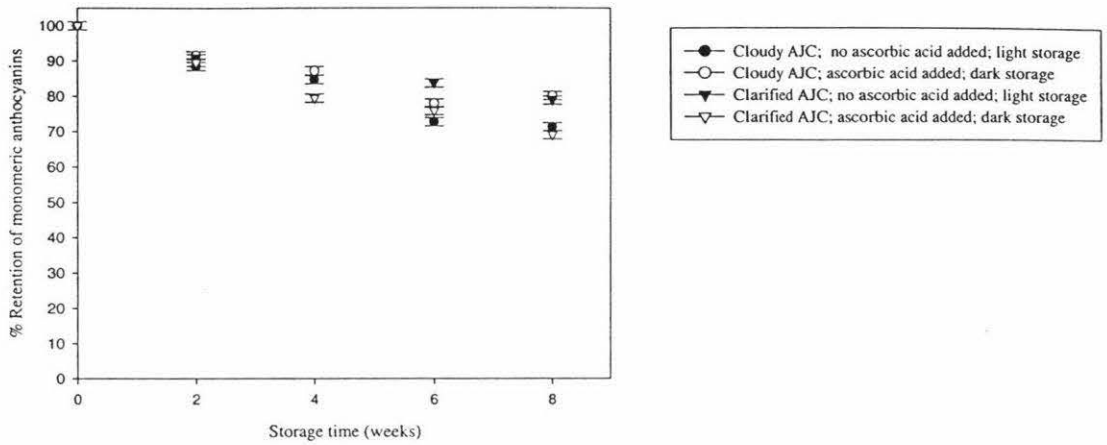


Figure 5.04: % Retention of monomeric anthocyanins in Wildberry Herbal drinks containing 2.6% v/v blackcurrant juice concentrate, stored at 5°C.

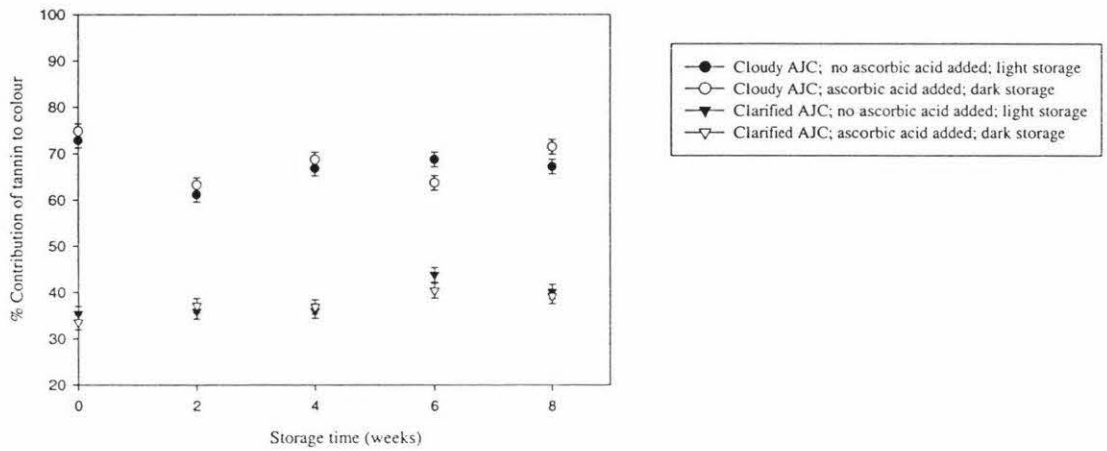


Figure 5.05: % Contribution of tannin to colour in Wildberry Herbal drinks containing 2.6% v/v blackcurrant juice concentrate, stored at 5°C.

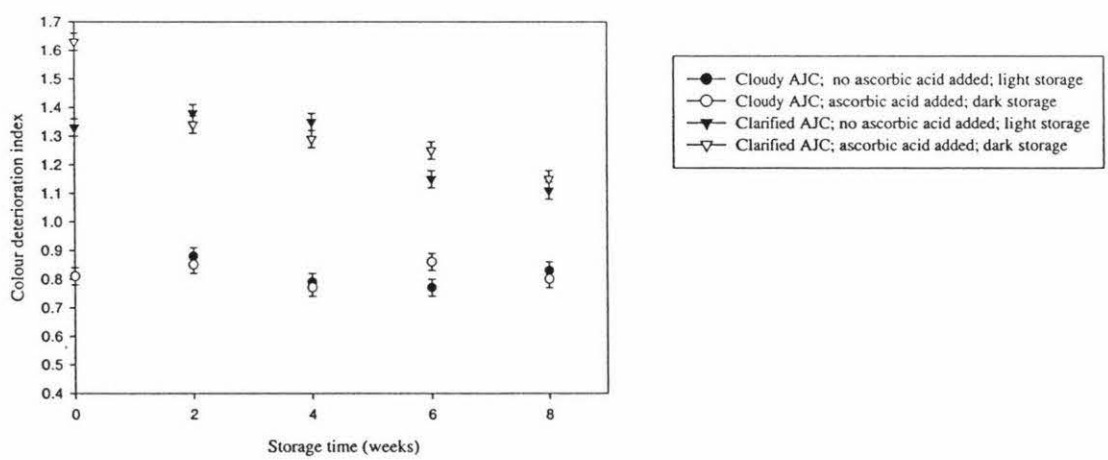


Figure 5.06: Colour deterioration index of Wildberry Herbal drinks containing 2.6% v/v blackcurrant juice concentrate, stored at 5°C.

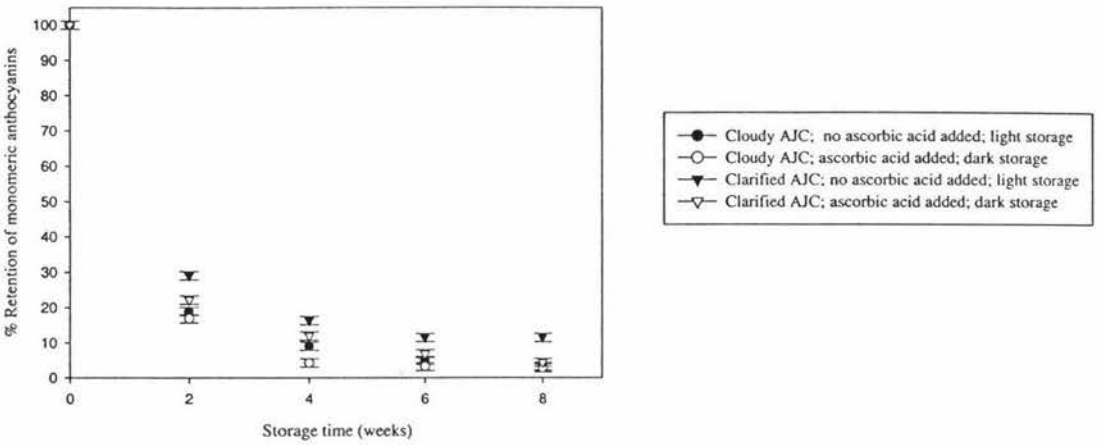


Figure 5.07: % Retention of monomeric anthocyanins in Wildberry Herbal drinks containing 1.3% v/v blackcurrant juice concentrate, stored at 35°C.

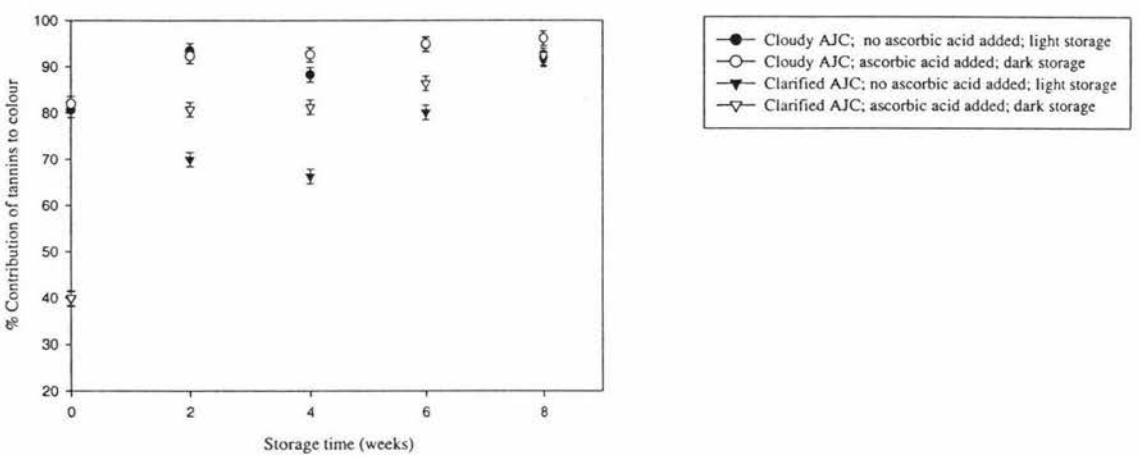


Figure 5.08: % Contribution of tannin to colour in Wildberry Herbal drinks containing 1.3% v/v blackcurrant juice concentrate, stored at 35°C.

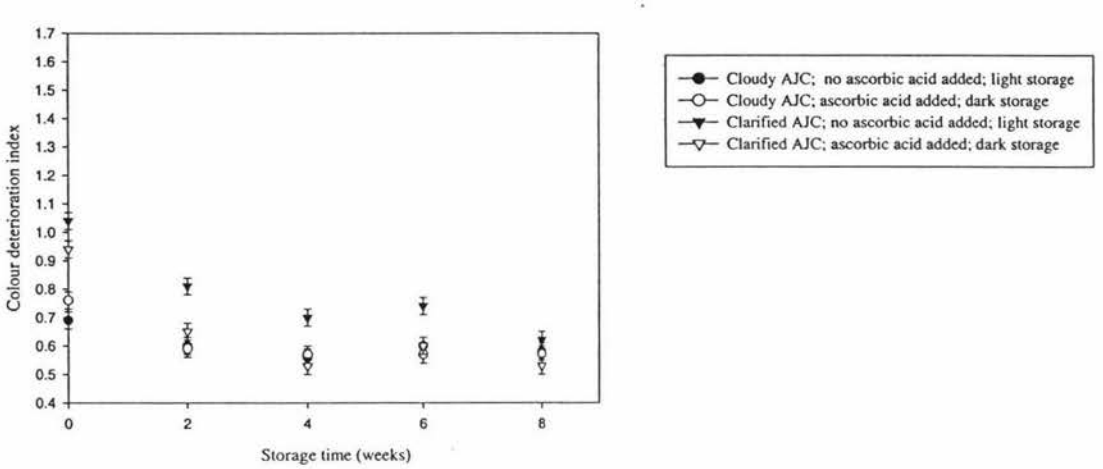


Figure 5.09: Colour deterioratoin index of Wildberry Herbal drinks containing 1.3% v/v blackcurrant juice concentrate, stored at 35°.

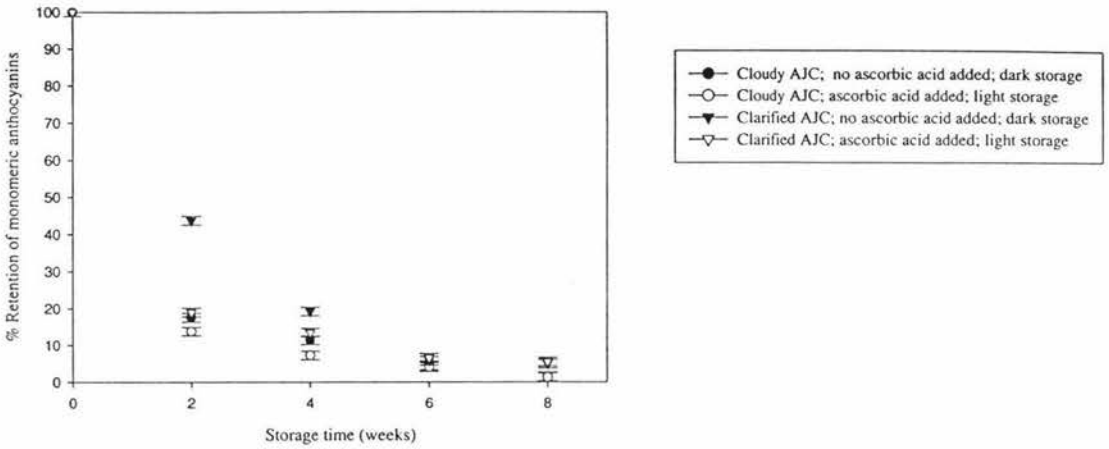


Figure 5.10: % Retention of monomeric anthocyanins in Wildberry Herbal drinks containing 2.6% v/v blackcurrant juice concentrate, stored at 35°C.

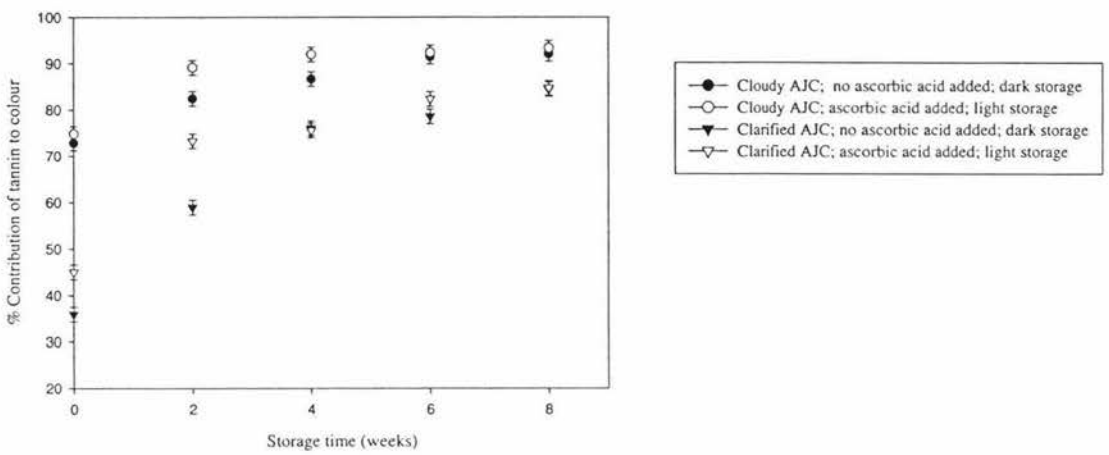


Figure 5.11: % Contribution of tannin to colour in Wildberry Herbal drinks containing 2.6% v/v blackcurrant juice concentrate, stored at 35°C.

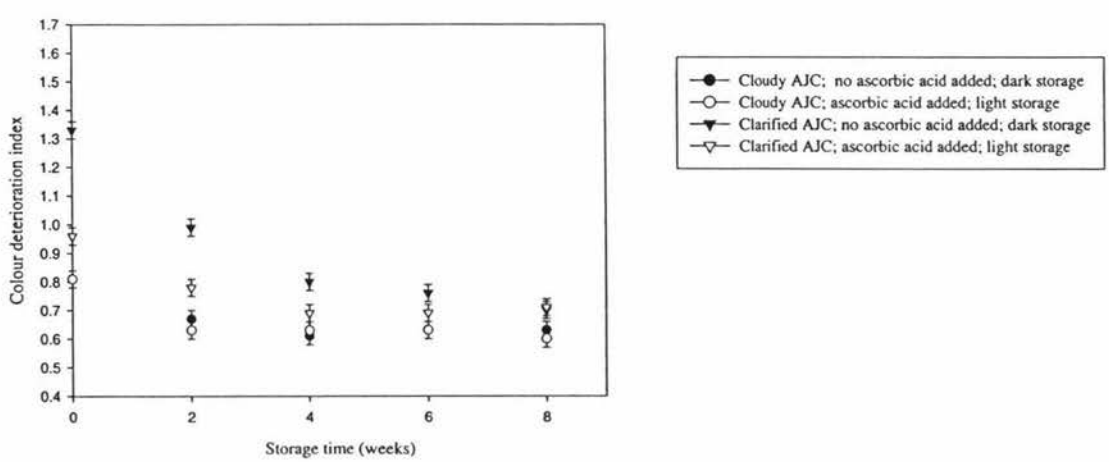


Figure 5.12: Colour deterioration index of Wildberry Herbal drinks containing 2.6% v/v blackcurrant juice concentrate, stored at 35°C.

The pH and titratable acidity of the drinks were measured at week 0 and 8. The pH of the drinks was on average 3.3 ± 0.1 and the average titratable acidity was 0.8 ±0.1g malic /100ml (Appendix 4).

The retention of monomeric anthocyanins decreased with increasing storage time in all the juices. At 5°C, the % retention of monomeric anthocyanins was similar for both cloudy and clarified AJC based drinks at the end of eight weeks (Figures 5.01 & 5.04). For drinks with 1.3% v/v blackcurrant juice concentrate, those made from clarified AJC, without ascorbic acid added had a significantly higher % retention of monomeric anthocyanins than all the other drinks (Figure 5.01) ($p<0.05$). This significant difference was not seen however when the blackcurrant juice concentrate was doubled to 2.6% v/v (Figure 5.04) ($p>0.05$). In the cloudy AJC based drinks, with 1.3% v/v blackcurrant juice concentrate, the % retention is significantly lower at week 2 than in the clarified AJC based drinks (Figure 5.01). The results from Chapter 4 found that anthocyanins were present in the sediment which formed in the drinks made from cloudy AJC. Therefore, this significantly lower retention of monomeric anthocyanins, at week 2, in the cloudy AJC based drinks is likely to be due to the monomeric anthocyanins dropping out of the drink in the sediment. There was no significant difference in % retention of monomeric anthocyanins, at week 2, between cloudy and clarified AJC based drinks with 2.6% v/v blackcurrant juice concentrate (Figure 5.04) ($p>0.05$). The volume of sediment formed in cloudy AJC drinks was relatively constant at 1.3% and 2.6% v/v blackcurrant juice concentrate, therefore at high blackcurrant juice concentrate concentrations the loss of anthocyanins into the sediment was not large enough to be detected by the colourmetric assay used.

The % retention of monomeric anthocyanins was less than 15% for all drinks stored at 35°C. (Figures 5.07 & 5.10). It is difficult to compare differences between the cloudy and clarified AJC bases at this storage temperature because there was approximately 85% reduction in monomeric anthocyanins after two weeks storage for both cloudy and clarified AJC based drinks. At 35°C the clarified AJC based drink, 1.3% v/v blackcurrant juice concentrate, without ascorbic acid added, had a

significantly higher % retention of anthocyanins than the other drinks with this level of blackcurrant juice concentrate (Figure 5.07) ($p<0.05$).

Clarified AJC based drinks had significantly lower % contribution of tannin to colour than cloudy AJC based drinks when stored at 5°C for eight weeks (Figures 5.02 & 5.05) ($p<0.05$). At 35°C, clarified AJC based drinks had an initially lower % contribution of tannin (~40%) which increased during the eight weeks storage to reach levels similar to the drinks made from cloudy AJC (Figures 5.08 & 5.11).

The colour deterioration index of the clarified AJC based drinks was significantly higher than cloudy AJC based drinks at 5°C (Figures 5.03 & 5.06) ($p<0.05$). This indicates that the contribution of browning to colour was much lower in the clarified AJC based drinks. At 35°C the colour deterioration indexes of both cloudy and clarified AJC based drinks decreased, especially during the first two weeks of storage, indicating that rapid browning occurred during these first two weeks of storage.

Significant differences were observed between cloudy and clarified AJC based drinks in terms of % contribution of tannin to colour and colour deterioration index ($p<0.05$). Cloudy AJC based drinks had significantly more contribution of tannins to colour and lower colour deterioration indexes than clarified AJC based drinks ($p<0.05$).

Spayd *et al.* 1984 found that in apple and pear juices, blended with anthocyanin pigmented juices, the pear juice, which had the highest value for % colour due to tannin, had the highest concentration of phenolic compounds.

Another factor that is likely to impact on the colour of the drinks is the Maillard browning reaction. The Maillard reaction is a chemical reaction between reducing sugars and a free amino acid or a free amino acid group of an amino acid that is part of a protein chain (BeMiller & Whistler, 1996). In cloudy apple juice 89% of the total soluble nitrogen has been found to be attributable to free amino acids (Lea, 1990).

Concentration of apple juice increases the concentration of sugars and free amino acids thereby increasing its susceptibility to the Maillard reaction, and the subsequent development of brown polymeric compounds (Lea, 1990). The rate of Maillard browning is increased by increasing storage temperature and also by the addition of ascorbic acid (BeMiller & Whistler, 1996). The addition of ascorbic acid to AJC has been found to enhance the rate of browning because its breakdown products participate in the Maillard reaction (Lea, 1990). Cloudy AJC has high concentrations of ascorbic acid which is added during processing (Lea, 1990).

The cloudy AJC used by Phoenix Natural Foods Ltd. is received in a frozen state and thawed on site. It is important to thaw this concentrate at as low a temperature as possible to minimise browning by Maillard reaction. The concentrate used in this experiment had been thawed twice before use resulting in the formation of polymerised phenolics and browning precursor compounds. Initially, when retention of anthocyanins is high, the intense red colour provided by the anthocyanins masks the browning contributed by the AJC. However, as the monomeric anthocyanins degrade, with a loss of the red colour, the browning of the apple juice base contributes more to the overall colour, and the drink appears brown and unappealing. The polymerisation and degradation of monomeric anthocyanins also contributes to the browning of the drink (von Elbe and Schwartz, 1996).

Using clarified AJC as the base for drinks has the advantage of minimising the levels of browning precursors such as polyphenols, amino acids and ascorbic acid present in the drink. It also has the advantage of preventing the formation of sediment, as shown in section 4.2. Its disadvantage however is it is likely to change the consumer perception of the drink, and in the case of the Wildberry Herbal drink, reduces its uniqueness within the New Zealand beverage market. If cloudy AJC is used, it must be handled carefully before blending in the drinks to reduce formation of brown compounds and drinks must be kept at as low a temperature as possible during storage.

5.1.2 Effect of ascorbic acid concentration on colour stability

The factorial experimental design was analysed using Minitab. The statistical output of the analysis are shown in Appendixes 5 & 6.

Cloudy apple juice concentrate based drinks

The added ascorbic acid had a small significant negative effect on the monomeric anthocyanin concentration and a small significant increase in % contribution of tannin at week 0 (Table 5.01) ($p<0.05$). At week 8, the addition of ascorbic acid had no significant effect on the concentration of anthocyanins, % contribution of tannin or the colour deterioration index (Table 5.01) ($p>0.05$).

The initial concentration of ascorbic acid in the drinks, at 12°Brix, made from cloudy AJC, was approximately 340 mg/l. The ascorbic acid level increased to 610 mg/l - 700 mg/l in drinks which had ascorbic acid added (Table 5.02). At week 8 the % loss of ascorbic acid was highest in drinks which had been stored at 35°C compared with 5°C.

Clarified apple juice concentrate based drinks

At week 0, the addition of ascorbic acid had a significant negative effect on the concentration of monomeric anthocyanins (Table 5.01) ($p<0.05$). The addition of ascorbic also had a significant negative effect on % contribution of tannin however the effect was very small (0.8%). At week 8 the addition of ascorbic acid had a significant negative effect on monomeric anthocyanin concentrations ($p<0.05$). The addition of ascorbic acid also significantly increased the % contribution of tannin and browning in the drinks ($p<0.05$). Drinks made from clarified AJC, which did not have ascorbic acid added, had very low ascorbic acid concentrations of approximately 6 mg/l at week 0. The drinks to which ascorbic acid was added had ascorbic acid concentrations of approximately 250 mg/l at week 0 (Table 5.3). At week 8, the absolute losses of ascorbic acid were greatest for the drinks with added ascorbic acid stored at 35°C. The % loss of ascorbic acid at week 8 was also highest in drinks stored at 35°C.

Table 5.01: Effect of ascorbic acid, blackcurrant concentrate volume, storage temperature, and light on anthocyanin concentration, % contribution of tannin to colour and the colour deterioration index at weeks 0 and 8 for Wildberry Herbal drinks made from cloudy and clarified apple juice concentrate.

	Anthocyanin concentration (mg/l)	% Contribution of tannin to colour	Colour deterioration index
WEEK 0	**Cloudy apple juice concentrate base**		
Ascorbic acid	-1.9*	1.7*	0.01
Blackcurrant volume	47.6*	-7.5*	0.06*
Storage temperature	0	0	0
Dark/Light	0	0	0
WEEK 8			
Ascorbic acid	1.2	2.7	0.01
Blackcurrant volume	19.7*	-6.7*	0.11*
Storage temperature	-45.6*	17.9*	-0.13*
Dark/Light	-1.2	-1.6	0.03
WEEK 0	**Clarified apple juice concentrate base**		
Ascorbic acid	-16.9*	-0.8*	0.10
Blackcurrant volume	75.4*	-5.4*	0.50*
Storage temperature	0	0	0
Dark/Light	0	0	0
WEEK 8			
Ascorbic acid	-12.5*	4.8*	-0.11*
Blackcurrant volume	29.9*	-9.9*	0.19*
Storage temperature	-61.5*	42.3*	-0.44*
Dark/Light	2.4	4.8*	0

* effect is significant at $p < 0.05$.

- Minitab analysis is presented in Appendixes 5 &6.

Table 5.02: Ascorbic acid concentration in drinks made from cloudy apple juice concentrate at week 0 and week 8 of storage.

	Storage temperature	5°C				35°C			
Week	Treatment[1]	A	B	C	D	E	F	G	H
0	Concentration (mg/l)	342	612	343	703	342	612	343	703
8	Concentration (mg/l)	238	493	231	565	48	161	52	159
	% Loss	30	19	33	20	86	74	85	77
	Absolute loss (mg/l)	**104**	**119**	**112**	**138**	**294**	**451**	**291**	**544**

Ascorbic acid pooled standard error = 2; n = 32

1. Treatments as described in Table 3.07.

Table 5.03: Ascorbic acid present in drinks made from clarified apple juice concentrate at week 0 and week 8 of storage.

	Storage temperature	5°C				35°C			
Week	Treatment[1]	J	K	L	M	N	O	P	R
0	Concentration (mg/l)	6	252	6	250	6	252	6	250
8	Concentration (mg/l)	4	191	5	166	1	37	1	53
	% Loss	35	24	11	34	91	85	80	79
	Absolute loss (mg/l)	**2**	**61**	**1**	**84**	**5**	**215**	**5**	**198**

Ascorbic acid pooled standard error = 2; n = 32

1. Treatments as described in Table 3.07.

In Tables 5.02 & 5.03, week 0 starts immediately after pasteurisation. Four hundred milligrams per litre of ascorbic acid was added before pasteurisation and there is a loss of ascorbic acid during the pasteurisation process.

During the manufacture of cloudy AJC large quantities (500ppm) of ascorbic acid are added (Lea, 1990). Subsequently, drinks made from cloudy AJC have a much higher concentration of ascorbic acid than clarified AJC based drinks. This is before the drinks themselves are fortified with ascorbic acid (Table 5.02). In this

experiment, the relative absolute losses of ascorbic acid were much larger for cloudy AJC drinks than clarified AJC drinks. Therefore, in the unfortified cloudy AJC based drinks, sufficient amounts of ascorbic acid were already present to participate in colour loss reactions. The results from the analysis show that, at week 8 significant losses in anthocyanin concentration and increases in browning in the drinks, made from clarified AJC, occurred ($p<0.05$) (Table 5.01).

Many researchers have observed the concurrent disappearance of anthocyanins and ascorbic acid in anthocyanin pigmented products and have suggested a possible interaction between the two compounds (Markakis, 1982; Sistrunk and Gascoigne, 1983; Poei-Langston & Wrolstad, 1981; Skrede *et al.*, 1992; Iversen, 1999).

Several mechanisms have been proposed for the degradation of anthocyanins in the presence of ascorbic acid. In oxidative conditions, in the presence of copper ions, it is postulated that ascorbic-induced degradation of anthocyanins results indirectly from hydrogen peroxide which forms during the oxidation of ascorbic acid (Markakis, 1982). The mechanism for this degradation mechanism is hydrogen peroxide cleavage of the pyrilium ring by a nucleophilic attack at the C-2 position of the anthocyanin. This produces colourless esters and coumarin derivatives which further degrade or polymerise into brown pigmented compounds (von Elbe and Schwartz, 1996). Iacobucci and Sweeney (1983) and Garcia-Viguera and Bridle (1999) have suggested that colour bleaching of anthocyanins by ascorbic acid occurs by ascorbic acid activation of molecular oxygen, producing free radicals which cleave the pyrilium ring.

A different mechanism was proposed by Jurd (1972) and subsequently supported by Poei-Langston and Wrolstad (1981). Jurd (1972) suggested a condensation reaction between anthocyanin and ascorbic acid, similar to that between flavylium salts and dimedone. Poei-Langston and Wrolstad (1981) found that anthocyanin colour decreased more rapidly under oxygen free conditions (nitrogen sparging) than under oxygenated conditions (oxygen sparging), suggesting that a condensation mechanism was the predominant cause of anthocyanin degradation.

In elderberry juice, it has been proposed that ascorbic acid has a protective effect on the oxidative degradation of anthocyanins however Kaack and Austed (1998) did not

propose a mechanism for this observed protection. Work by Shrikhande and Francis (1974) demonstrated the protective anti-oxidative properties of some flavanols (e.g. quercetin) which retarded anthocyanin degradation. Thus ascorbic acid may also have a protective antioxidant effect on anthocyanin degradation.

Overall, it was difficult to ascertain what mechanism was responsible for the loss of monomeric anthocyanins in the clarified AJC based drinks. In cloudy AJC, ascorbic acid addition did not have a significant impact on the colour stability. If clarified AJC was to be used as the base AJC then the use of ascorbic acid will have a beneficial nutritional advantage but a negative impact on the red colour.

5.1.3 Effect of blackcurrant juice concentrate volume on colour stability

Cloudy apple juice concentrate based drinks

Doubling the volume of blackcurrant juice concentrate in the drinks caused a significant increase in anthocyanin concentration at week 0 (Table 5.01) ($p<0.05$). At week 8 high anthocyanin concentration due to added blackcurrant juice concentrate was still significant ($p<0.05$). Doubling the blackcurrant juice concentrate volume significantly reduced the % contribution of tannin by 7.5% and 6.7% at weeks 0 and 8 respectively ($p<0.05$). The doubling of blackcurrant juice concentrate volume significantly reduced the contribution of browning to the overall colour, especially at week 8 where the colour deterioration index effect was 0.11 ($p<0.05$) .

Clarified apple juice concentrate based drinks

The effect of doubling the volume of blackcurrant juice concentrate volume in the drinks made from clarified AJC shows a similar trend to that seen in the cloudy AJC drinks (Table 5.01). The increase in blackcurrant juice concentrate volume caused a significant increase in anthocyanin concentration at both weeks 0 and 8 ($p<0.05$). Doubling the blackcurrant juice concentrate volume significantly decreased the % contribution of tannin. A larger difference was found at week 8 compared with week 0 ($p<0.05$). Doubling the blackcurrant juice volume, at weeks 0 and 8, significantly decreased browning ($p <0.05$).

As well as increasing the anthocyanin concentration significantly doubling the blackcurrant juice concentrate volume resulted in a greater % retention of monomeric anthocyanins in both clarified and cloudy AJC based drinks, at the end of eight weeks storage at 5°C (Figures 5.01 & 5.04).

Skrede *et al.* (1992) found in an investigation of blackcurrant and strawberry syrup that the anthocyanin:ascorbic acid ratio was an important factor in monomeric anthocyanin stability. High anthocyanin:ascorbic acid ratios favoured anthocyanin stability. Taylor (1989) also found that in blackcurrant juice the total concentration of anthocyanins present was more important than the type of anthocyanins, in terms of anthocyanin stability.

Spayd *et al.* (1984) investigated the addition of anthocyanin pigmented juices (grape, cherry and raspberry) to apple and pear base juices at concentrations of 5, 10 and 20% v/v. They found that at 24 weeks storage the 5 and 10 %v/v pigmented juices did not show a further decrease in anthocyanin concentration while the anthocyanin concentration in the 20% v/v drinks continued to decrease till 48 weeks.

The observed decrease in % contribution of tannin and browning when increasing the volume of blackcurrant juice concentrate has been observed previously (Spayd *et al.* 1984; Skrede, 1985; Skrede *et al.*, 1992). Skrede *et al.* (1992) also observed that in syrups with high anthocyanin concentrations, higher browning indexes (absorption of polymeric compounds at 420nm) seemed to develop without affecting the visual colour of the juice. Thus visual browning is not as apparent in juices in with higher anthocyanin concentration.

As well as colour stability, two further issues must be considered when deciding on the level of blackcurrant juice concentrate to be used in the drink. As the volume of concentrate increases, the monomeric anthocyanin content also increases causing a visually darker drink. Increasing the volume of concentrate also increases the unit cost of the drink. Therefore, blackcurrant juice concentrate should be used in the Wildberry Herbal drink at the maximum level which provides an acceptable colour and fits within the cost restraints of the drink.

5.1.4 Effect of storage temperature on colour stability

Cloudy apple juice concentrate based drinks

The results from the statistical analysis in Table 5.01 shows that eight weeks storage at 35°C has a significant negative effect on the concentration of monomeric anthocyanins ($p<0.05$). An increase in the storage temperature had a significant effect on the % contribution of tannin and browning in the drinks ($p<0.05$).

Clarified apple juice concentrate base drinks

In clarified AJC drinks increasing the storage temperature has a significant negative effect on the concentration of monomeric anthocyanins ($p<0.05$). Increasing the storage temperature also had a significant effect on the % contribution of tannin, and significantly increased browning in the drinks ($p<0.05$).

The negative effect of storage temperature on the stability of anthocyanins in model systems and in food products has been reported widely. Simard *et al.* (1981) stored blackcurrant juice at pH 2.0 for eight weeks at 4°C, 22°C and 37°C. All monomeric anthocyanins disappeared at 37°C, 21% remained at 22°C and 60% remained at 4°C. The effect of temperature on the retention of monomeric anthocyanins in the Wildberry Herbal drink can clearly be seen in Figure 5.13. The drink investigated had exactly the same formulation as the Wildberry Herbal Drink sold by Phoenix Natural Foods Ltd. in April 2000. After eight weeks storage the retention of monomeric anthocyanins at 5°C was 66% whereas at 20°C and 35°C the retention was 7% and 3%, respectively.

The large loss of monomeric anthocyanins at 20°C indicates how sensitive the monomeric anthocyanins are to temperature. Hence, storage at these temperatures has a large impact on the colour of the drink. The rate of monomeric anthocyanin degradation at different temperatures is also known to be influenced by the presence or absence of oxygen (von Elbe and Schwartz, 1996).

The exact mechanism of thermal degradation of monomeric anthocyanins is not known and is thought to be dependant on the type of monomeric anthocyanin involved and the degradation temperature. (von Elbe and Schwartz, 1996). In the

presence of molecular oxygen, it is proposed that the flavylium cation form of the anthocyanin is first transformed to a colourless carbinol base, then to a chalcone and finally to brown polymeric degradation products (Jackman and Smith, 1996).

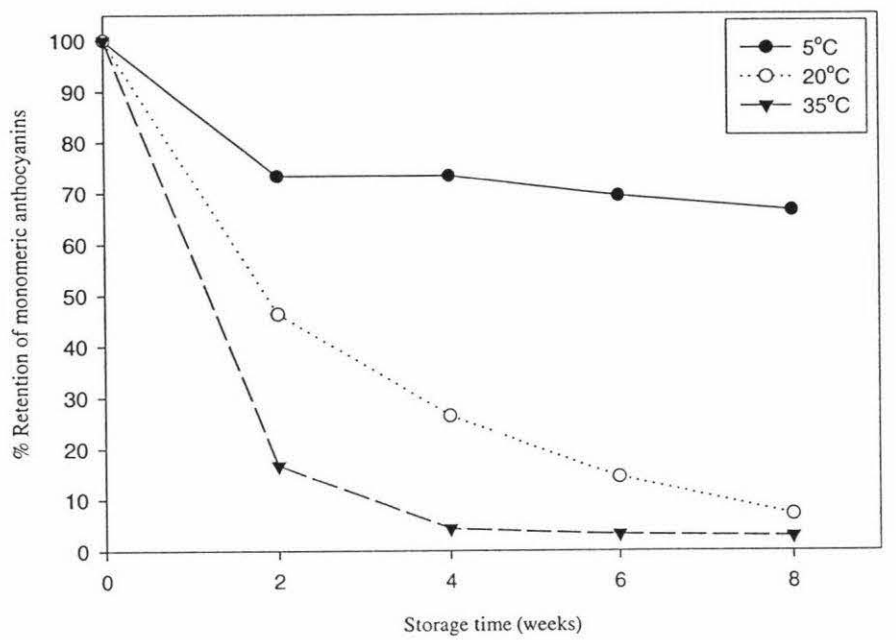


Figure 5.13: The effect of storage temperature (5°C, 20°C and 35°C) on the % retention of monomeric anthocyanins present in a Wildberry Herbal drink.

Storage temperature also had a significantly large effect on the % contribution of tannin and colour deterioration index in the cloudy and clarified AJC drinks (Figures 5.02, 5.03, 5.05, 5.06, 5.08, 5.09, 5.11 & 5.12). With increasing storage temperature non-enzymic browning and polyphenol oxidation from constituents of the cloudy AJC, are likely to be the main contributors to the increase in colour contributed by polyphenolic compounds and browning (Hicks, 1990).

5.1.5 Effect of light on colour stability

Light did not have a significant effect on the monomeric anthocyanin concentration, % contribution of tannin to colour and colour deterioration index in the cloudy AJC drinks after eight weeks storage ($p>0.05$). In clarified AJC drinks, light had a small significant effect on the % contribution of tannin (Table 5.01) ($p<0.05$). It is generally accepted that UV light causes degradation of anthocyanins (von Elbe & Schwartz, 1996) however conflicting results have been found. Palamidis and Markakis (1975) found that continuous fluorescent light (80 ft candles) decreased grape anthocyanin concentration by 70% compared with 50% in direct sunlight, after 135 days storage. Iversen (1999) found sunlight to have no effect on anthocyanins in blackcurrant nectar stored at 20°C, for 200 days, while Skrede (1985) found that daylight storage of blackcurrant syrups lowered half life Hunter *a* values by 10-30% compared with dark storage.

In this experiment the light source was fluorescent (60W), to simulate normal retail storage, and thus only minimal UV would have been emitted. Light emitted by fluorescent light is unlikely to have a significant effect on the colour stability of the Wildberry Herbal drink during its storage life.

5.2 Phoenix Natural Foods Wildberry Herbal drink manufacturing process

The general manufacturing process for the Wildberry Herbal drink is shown in Figure 5.14. It consists of six main steps.

Step 1

Water, which has been carbon filtered, is chilled down to 5°C overnight. Cold storage of the raw ingredients, particularly the juice concentrates, is important to prevent degradative reactions occurring which will affect the colour, taste and haze stability of the final drink. A proportion of this water is then mixed with the juice concentrates and other ingredients and this mixture is pumped into the main storage tank and mixed with the remaining water. From this tank the juice mixture is pumped to the filler.

Step 2

The juice is filled into the bottles using an overhead filler and capped with a screw on capper. Dissolved oxygen can be incorporated into the juice at several points in the process before filling including the pumping from the mix tanks and when being filled into the bottles. No attempt is made to remove oxygen from the process. The dissolved oxygen content of a packaged juice drink is an important parameter in its storage stability (Hicks, 1990).

Steps 3 & 4

After bottling the juice is packed into plastic crates and is stacked on a pallet. This pallet is then placed into the pasteuriser and the pasteuriser is filled with steam heated water and the bottles are held at a minimum of 67°C for 17 minutes. The hot water is then drained out of the pasteuriser and the pallet is given a quick cold water rinse before being removed from the pasteuriser. Pasteurisation is undertaken primarily to inactivate microorganisms present in the product. In the case of fruit juice the most prevalent microorganisms are yeasts (Fellows, 2000). Phoenix Natural

Foods Ltd. does not use any preservatives in its products and thus the only hurdle to microorganism growth in the fruit drink is the low pH (< pH 3.5).

Steps 5 & 6

The bottles are cooled at ambient factory temperature on the pallet. After cooling the bottles are labelled and packed in lots of 12 into corrugated cardboard boxes which are stamped with a batch code. The boxes are then stored at room temperature before distribution.

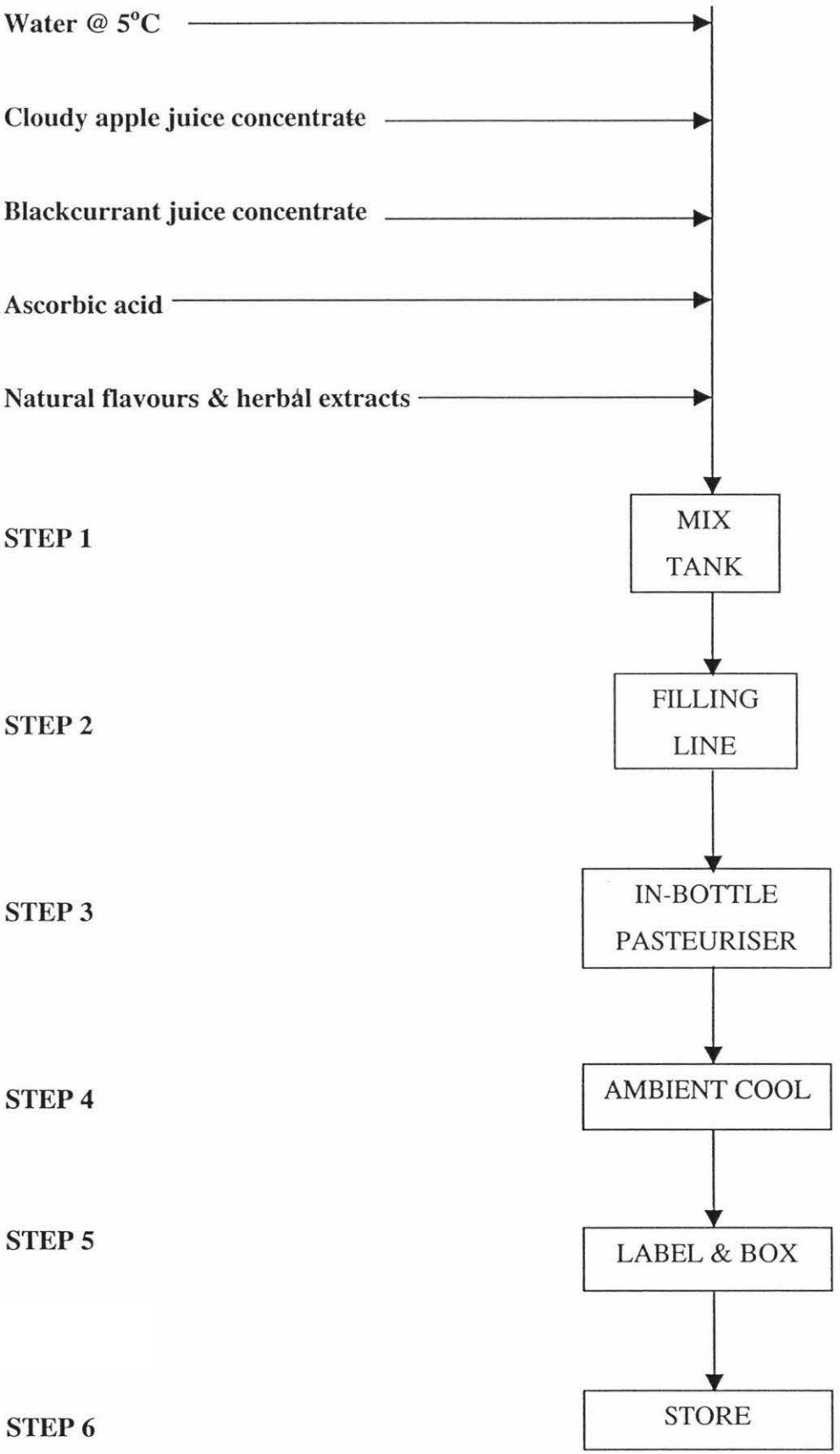


Figure 5.14: Phoenix Wildberry Herbal drink process block diagram

5.3 The effect of the pasteurisation process on anthocyanin stability in the Wildberry Herbal drink

In acidic foods (pH <4.5) pasteurisation is a process used to extend the shelf life for several months by destruction of spoilage micro-organisms (yeasts and moulds) and/or inactivation of enzymes (Fellows, 2000). Typically, in fruit juices the main purpose of pasteurisation is enzyme inactivation (polyphenol oxidase, pectinesterase and polygalacturonase) with the subsidiary purpose of the destruction of spoilage micro-organisms (Fellows, 2000).

The concentrates used by Phoenix Natural Foods for the manufacture of the Wildberry Herbal drink have already undergone thermal treatment and subsequent inactivation of endogenous and exogenous enzymes. Thus the main purpose of the pasteurisation process for the Wildberry Herbal drink is the destruction of yeasts and moulds that may be present in the drink and lead to spoilage. The process employed at Phoenix Natural Foods Ltd. for the Wildberry Herbal drink is an in-bottle pasteurisation process. The objective of this experiment was to determine the thermal regime of the current pasteurisation process and its impact on the monomeric anthocyanins in the Wildberry Herbal drink.

5.3.1 Determination of the thermal regime during the pasteurisation of the Wildberry Herbal drink

The thermal treatment applied to the Wildberry Herbal drink during pasteurisation and cooling was monitored (Figure 5.15). The water used for pasteurisation is pumped into the tank at a temperature of 80°C ± 2°C, which then cools in the tank to a temperature of 65-67°C. The pasteurisation process for the Wildberry is set so that the bottles are in the water bath at 67°C for 17 minutes. At the end of this process the bottles are given a cold water wash (20°C). After this the water is drained from the pasteuriser and then the bottles are taken out. Figure 5.15 shows that the cooling water is introduced 49 minutes after the start of pasteurisation and the bottles are removed after 59 minutes. Figure 5.15 shows that the during the cold water wash the bottle centre temperatures decreased by approximately 15°C to 52°C. At this point

they were then removed from the pasteuriser and left to cool at ambient factory temperature.

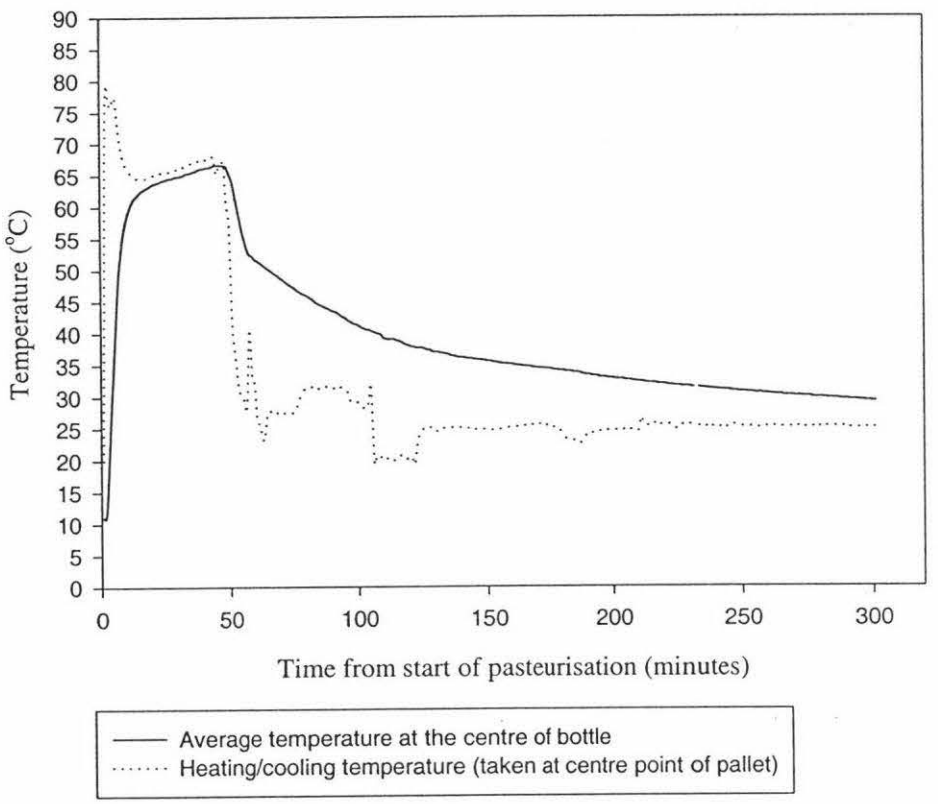


Figure 5.15: Temperature – time profile for the pasteurisation process and cooling for the Wildberry Herbal drink.

After 240 minutes of cooling outside of the pasteuriser the centre temperature of the bottles was 29°C ± 1°C.

The effect of bottle position on centre temperatures during and after pasteurisation was investigated (Figure 5.16). During the pasteurisation process the centre temperatures of the three different bottle positions were very similar. The top level (level 4) was 1-2°C higher than the other two levels. During cooling, the top level had the greatest exposure to the ambient air cooling, hence, cooled the fastest. After 240 minutes the centre temperatures of all the bottles on levels 1,3 and 4 were at 29°C ± 1°C.

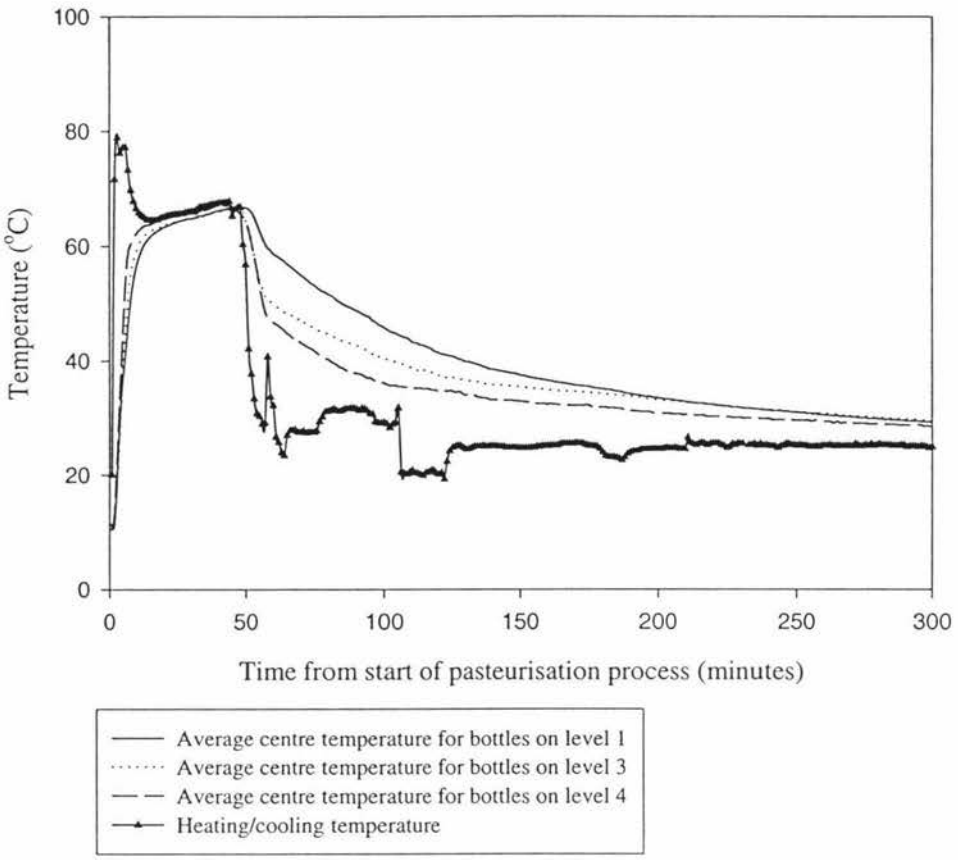


Figure 5.16: Time-temperature profile of average centre temperatures for drinks placed at three different levels during pasteurisation and cooling in the bottle pasteuriser at Phoenix Natural Foods Ltd.

At present the pasteurisation regime employed by Phoenix Natural Foods exposes the bottles of Wildberry Herbal Drink to a minimum of 67°C for 17 minutes in a batch pasteuriser. Fellows (2000) recommend a P value (pasteurisation value) of 1 minute, at a reference temperature of 77°C, for the pasteurisation of fruit juice.

The required P value (1 minute) was reached in the drinks at approximately 32 minutes after the start of the pasteurisation and a final P value of 2.6 minutes was reached for the entire pasteurisation process (Figure 5.17).

The pasteurisation process used at present at Phoenix Natural Foods is therefore more than adequate in providing a heat treatment to destroy yeasts and moulds that may be present in the drink.

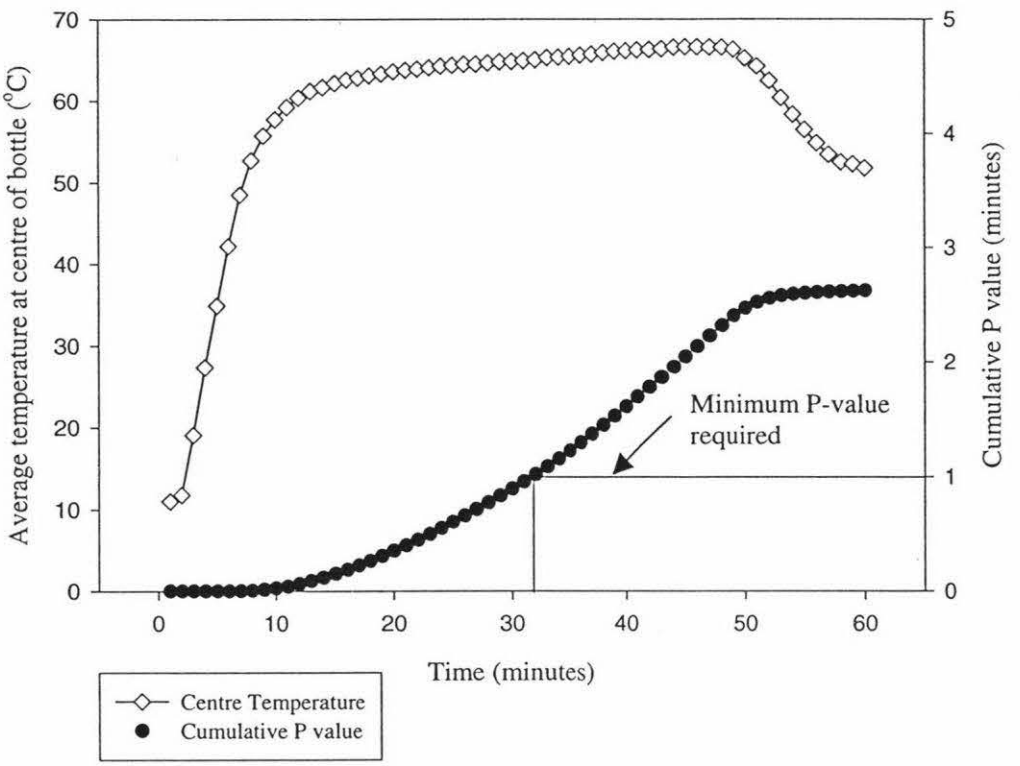


Figure 5.17: Cumulative P-value and corresponding centre temperature in the Wildberry Herbal drink during the pasteurisation process (T_{ref} = 77°C; P_{ref} = 1 minute; z = 10°C).

5.3.2 Effect of pasteurisation on the monomeric anthocyanin content of the Wildberry Herbal drink

The total anthocyanin content of the Wildberry Herbal Drink decreased less than 1% during the pasteurisation process (Table 5.04). Therefore the pasteurisation thermal treatment employed at Phoenix Natural Foods Ltd. did not have an immediate impact on the colour of the Wildberry Herbal drink. During the cooling of the drink there was a decrease in the anthocyanin content. There was 91% total anthocyanin retention three hours after the pallet was removed from the pasteuriser (Table 5.04).

Table 5.04: Total monomeric anthocyanin content in Wildberry Herbal drink pre-pasteurisation and after the pasteurisation process.

Time from start of pasteurisation process	Total anthocyanin concentration (mg/L)	% Retention of anthocyanins	Average centre temperature (°C)
0	86.5	100	5
60[a]	86.4	99.9	52
80	86.3	99.8	46
100	83.5	96.5	41
120	82.2	94.9	38
240	78.5	90.7	29

a. 60 minutes denotes the time at which the pallet was removed from the pasteuriser and began cooling at ambient factory conditions.

Pasteurisation is a relatively mild heat treatment and there are only minor changes to the nutritional and sensory characteristics of most foods (Fellows, 2000). The rate of anthocyanin degradation in natural and model systems has been found to be influenced markedly by processing and storage temperatures (Nebesky *et al.*, 1949; Markakis *et al.*, 1957; Daravingas and Cain, 1966; Adams and Ongley, 1973; Simard *et al.*, 1981; Markakis, 1982; Havlikova and Mikova, 1985; Taylor, 1989; Rommel *et al.*, 1992; Jackman and Smith, 1996).

It has been found that the mechanism of anthocyanin degradation is temperature dependent (Jackman and Smith, 1996). At storage temperatures less than 40°C activation energy (E_a) and z values of approximately 70 kJ mol^{-1} and 25°C, respectively have been reported (Markakis, 1974). At processing temperatures greater than 70°C, E_a values of 95-113 kJ mol^{-1} and z values of around 28°C have been reported (Markakis, 1982; Havlikova and Mikova, 1985). This indicates that anthocyanins have relatively greater stability at higher temperatures.

Adams and Ongley (1972) showed that canning red fruit juices at 100°C for less than 12 minutes resulted in negligible anthocyanin loss in comparison to losses occurring during slow cooling and subsequent ambient temperature storage. Havlikova and Mikova (1985) found that the stability of anthocyanins, in elderberry concentrate and in an anthocyanin preparation, did not significantly differ within the temperature range 50-80°C, whilst at higher temperatures (90-100°C), anthocyanins in elderberry concentrate were more stable. Havlikova and Mikova (1985) recommended that anthocyanins in the form of elderberry concentrate be used for the colouring of foods subject to heat treatment.

It can be concluded that the thermal pasteurisation treatment used at Phoenix Natural Foods Ltd. is more than adequate for spoilage microorganism destruction and does not have a detrimental effect on the anthocyanin content of the Wildberry Herbal drink. It may be beneficial to reduce the time of thermal treatment, applied to the Wildberry Herbal drinks. Prolonged thermal treatment can affect other sensory properties of the drink, apart from colour, such as flavour and cloud stability (Hicks, 1990). Reducing the thermal treatment time would also increase energy efficiency.

There is a loss of anthocyanins during the cooling phase of the pasteurisation process. Ideally the drinks should be cooled rapidly after pasteurisation and maintained at as low as temperature as possible during storage. If the drinks are packed soon after pasteurisation they will maintain a relatively high temperature (>30°C) for a long period. The cardboard box provides some insulation which will reduce the rate of cooling once packed.

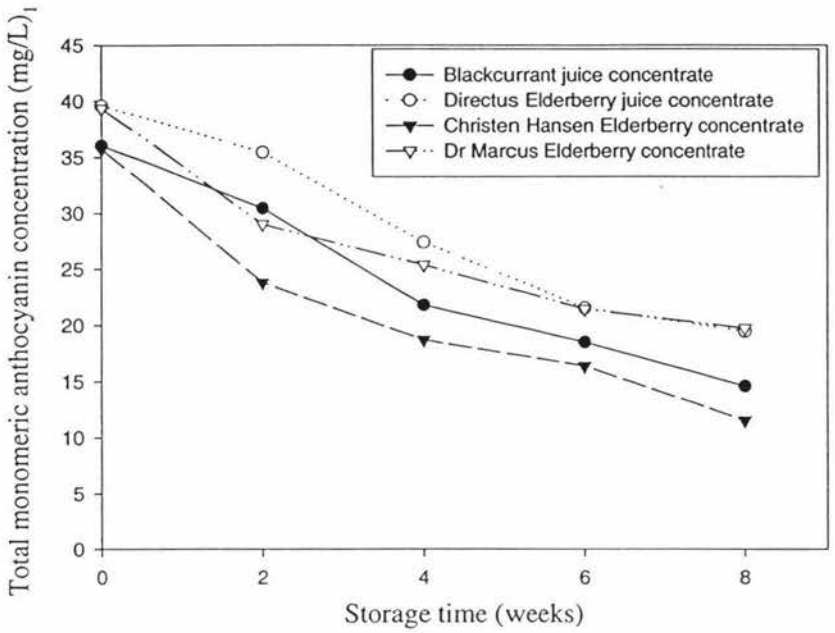
5.4 Investigation of alternative colourants for the Wildberry Herbal drink

At present a blackcurrant juice concentrate provides the red colour of the Wildberry Herbal drink. In sections 5.1 and 5.3 the effect of six factors (apple juice concentrate type, storage temperature, light, ascorbic acid, blackcurrant juice concentrate volume and pasteurisation) on the colour of the drink were investigated. It has been found that the structure of anthocyanins also has an effect on their stability (Markakis, 1982; Taylor, 1989). The objective of this experiment was to investigate the stability of alternative sources of anthocyanin colour compared with the blackcurrant juice concentrate used at present in the Wildberry Herbal drink.

5.4.1 Results and discussion

After storage at 15°C the reduction in monomeric anthocyanin concentration in drinks made with blackcurrant juice concentrate, elderberry juice concentrate and elderberry concentrate I & II was observed after eight weeks, as shown in Figure 5.18. The three new colourants were used at levels, which gave comparable visual red colour

The percentage retention of anthocyanins during eight weeks storage (Figure 5.19) clearly shows the retention of anthocyanins being around 50% or less for the four colourants at the end of eight weeks. The two colourants that had the highest retention of anthocyanins were the Dr Marcus Elderberry concentrate II and Directus Elderberry juice concentrate with 50.1% and 49.1% retention of anthocyanins, respectively. The standard colourant used in the Wildberry Herbal drink at present, blackcurrant concentrate, retained 40.4% of monomeric anthocyanins at the end of eight weeks.



1. Monomeric anthocyanin concentration calculated on the basis of major anthocyanin pigment. Elderberry concentrates - cyanidin-3-glucoside; Blackcurrant concentrates-cyanidin-3-rutinoside.

Figure 5.18: The total monomeric anthocyanin content for four colourants during storage at 15°C for eight weeks.

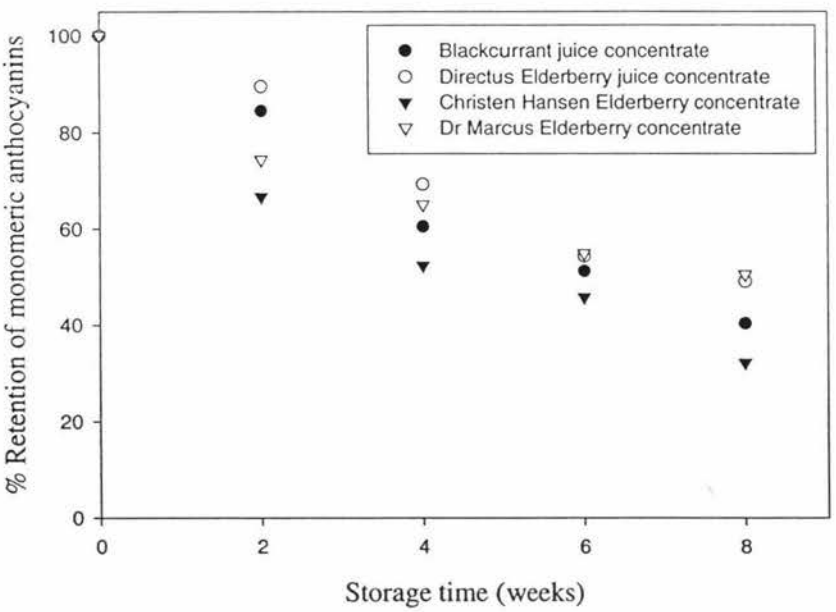


Figure 5.19: The % retention of monomeric anthocyanins in drinks produced with four colourants, during storage at 15°C.

During storage the percentage contribution of tannin compounds to colour showed an increase for all the colourants (Figure 5.20). Blackcurrant showed a very large increase in polymeric colour contribution during the first two weeks of storage and then maintained a fairly constant level for the rest of the storage time.

The colour deterioration index gives an indication of browning in the drinks, with a decrease indication in the colour deterioration index indicating an increase in browning. For all colourants the colour deterioration index decreased with storage time as shown in Figure 5.21. The drink coloured with Christen Hansen elderberry concentrate I showed a continued decrease during the eight week storage period. The other three colourants decreased most during the first two weeks of storage and then maintained a fairly constant degradation level during the rest of the storage period. The continued decrease in the colour deterioration index in the drink made from Christen Hansen elderberry concentrate I corresponded to a continual increase in browning during the storage period.

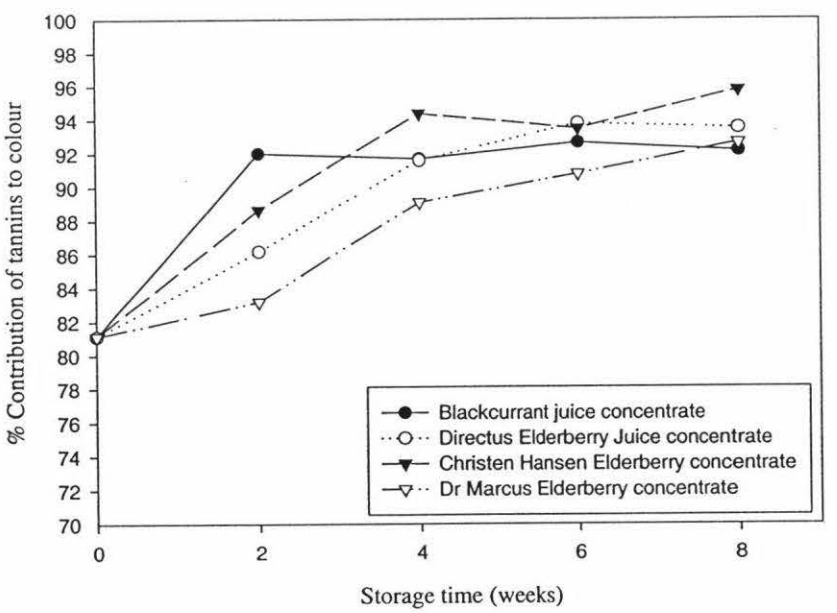


Figure 5.20: % contribution of tannins to the colour of the drinks during storage at 15°C for eight weeks.

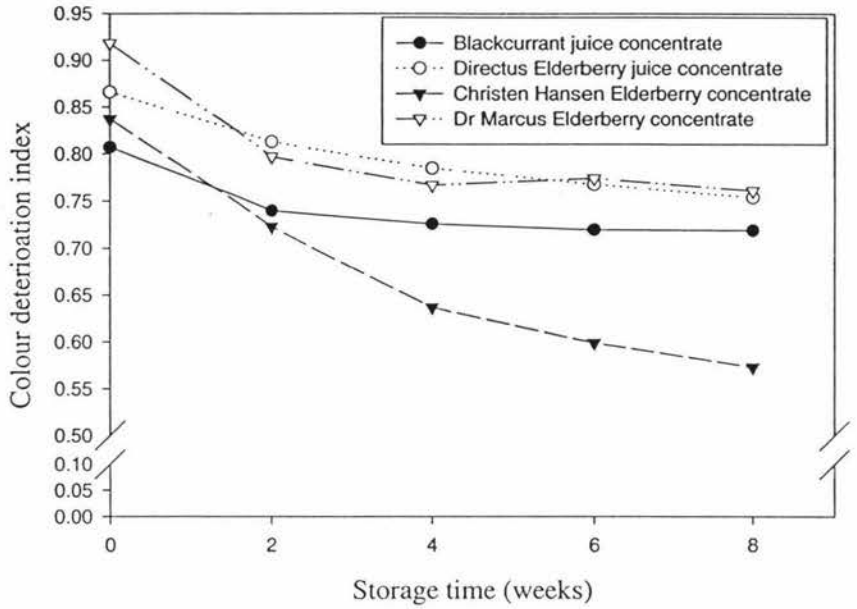


Figure 5.21: Colour deterioration index for the drinks during storage at 15°C for eight weeks.

Blackcurrant juice concentrate has delphinidin-3-rutinoside and cyanidin-3-rutinoside as major anthocyanin pigments in nearly equivalent quantities, and smaller amounts of delphinidin-3-glucoside and cyanidin-3-glucoside (Mazza & Miniati, 1993). In elderberry juice concentrate, the major anthocyanin pigments are cyanidin-3-glucoside and cyanidin-3-sambubioside, with minor anthocyanin pigments cyanidin-3-sambubioside-5-glucoside and cyanidin-3,5-diglucoside (Mazza & Miniati, 1993). The degradation rate of anthocyanins has been found to be dependent on their structure (Markakis, 1982; Taylor 1989). The colour of foods containing anthocyanins that are rich in pelargonidin, cyanidin, or delphinidin aglycones are less stable than that of foods containing anthocyanins that are rich in petunidin or malvidin aglycones (von Elbe and Schwartz, 1996). It has also been shown that the type of sugar moiety influences stability (von Elbe and Schwartz, 1996). Starr and Francis (1967) found that cranberry anthocyanins that contained galactose were more stable during storage than those containing arabinose.

Taylor (1989) concluded that in blackcurrant syrup, delphinidin-3-rutinoside was more stable than cyanidin-3-rutinoside at room temperature. However genotypes with a high delphinidin to cyanidin ratio showed no greater colour stability than genotypes with low ratios. It has been concluded that the differences in stability

between different types of anthocyanins is less important than the total anthocyanin concentration (Skrede, 1987; Taylor, 1989; Skrede *et al.*, 1992).

Of the colourants investigated all but the Christen Hansen elderberry concentrate showed suitable stability during the eight weeks storage. The storage temperature (15°C) meant that the retention of anthocyanins over the eight week period was less than 50%. However both Directus and Dr Marcus elderberry concentrates exhibited better stability than the blackcurrant juice concentrate which is the colourant currently used in the Wildberry Herbal drink.

A cost analysis of using each of the three suitable colourants from this experiment (Table 5.05) shows that at the levels used in this experiment the Directus elderberry juice concentrate has the lowest cost being almost half the price of the blackcurrant concentrate and more than a third of the price of the Dr Marcus elderberry concentrate. Due to its low price it is recommended that the Directus elderberry concentrate be used to replace the current blackcurrant concentrate. It is also recommended that the elderberry concentrate be used at an increased level, from that used in this experiment, as increased pigment concentration has been found to have a positive effect on anthocyanin stability (Taylor, 1989; Spayd *et al.*, 1983)

Table 5.05: Cost of selected colourants (Blackcurrant juice concentrate, Directus elderberry juice concentrate and Dr Marcus elderberry concentrate) when used in quantities suitable for colouring of the Wildberry Herbal drink.

Colourant	Cost/kg[1]	Concentration % v/v	Amount required per litre drink (g)	Cost of colourant per litre drink ($)[1]
Blackcurrant juice concentrate	14	1.3	17.1	0.23
Directus elderberry juice concentrate	15	0.7	8.8	0.13
Dr Marcus elderberry concentrate II	118	0.4	4.0	0.47

1. Prices are based on June 2000 quotes from suppliers.

5.5 Protective effect of flavonoids on anthocyanins in the Wildberry Herbal drink

Flavonoids are effective hydrogen donors, particularly flavonols such as quercetin and flavanols such as catechins and epicatechins. Catechins and epicatechins, found in green tea, and querecetin have antioxidant potential (Miller & Rice-Evans, 1997). These antioxidants can be used to enhance the properties of foods for both nutritional and preservation purposes (Rice-Evans *et al.*, 1997). Flavonols, especially quercetin, have been found to improve anthocyanin stability by retarding the oxidation of ascorbic acid, which has been proposed as detrimental to anthocyanins (Clegg and Morton, 1968; Harper *et al.*, 1969; Shrikhande and Francis, 1974; Simard *et al.*, 1981). The flavanols in teas, especially epigallocatechin gallate and epicatechin gallate, also have similar antioxidant activity as quercetin (Rice-Evans *et al.*, 1997). The objective of this experiment was to determine the effect of quercetin and green tea flavanol extracts on monomeric anthocyanin stability in the Wildberry Herbal drink.

5.5.1 Results and discussion

By week 4 of storage all the flavonoid fortified drinks were unacceptable in colour in comparison with the standard drink. Due to the detrimental effect of the flavonoids on the colour of the drinks, it was decided that only the monomeric anthocyanin content of the drinks would be measured.

After six weeks of storage the % retention of monomeric anthocyanins content of all the Wildberry Herbal drinks had decreased significantly (Figure 5.22). At week 6 the percentage retention of anthocyanins in all the flavonoid fortified drinks was below 30% (Figure 5.22).

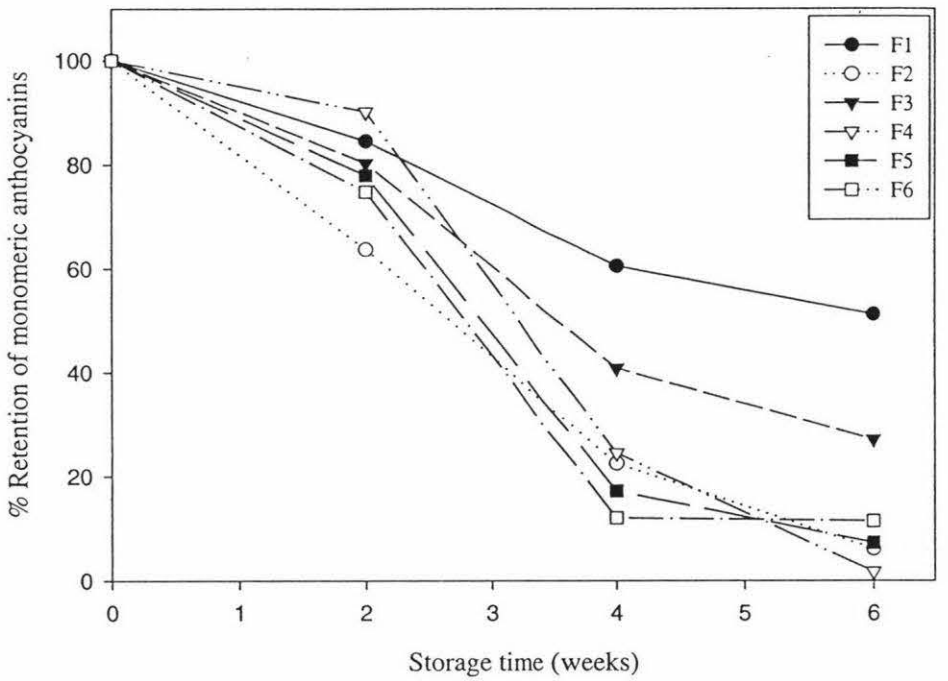


Figure 5.22: % Retention of monomeric anthocyanins in the Wildberry drinks fortified with flavonoids during storage at 15°C for six weeks (F1 standard drink; F2 quercetin 4mg/100ml; F3 98% tea polyphenol 15mg/100ml; F4 quercetin 4mg/100ml and 98% tea polyphenol 15mg/100ml; F5 15% tea polyphenol 105mg/100ml; F6 quercetin 4mg/100ml and 15% tea polyphenol 105mg/100ml).

Beverages which contain green tea solids, which are high in unoxidised flavanols, undergo oxidation during storage which results in precipitation, clouding and further colour changes (Kirksey *et al.*, 1998). It is likely that the oxidation of the flavonoids used in this experiment was responsible for the undesirable colour changes.

Quercetin has been found to have a protective effect on ascorbic acid and subsequently enhanced the stability of anthocyanins (Clegg and Morton, 1968; Harper *et al.*, 1969; Shrikhande and Francis, 1974). This experiment found no evidence of quercetin's ability to enhance the stability of anthocyanins. Quercetin's ability to improve stability of anthocyanins has been attributed to its ability to prevent the oxidation of ascorbic acid and subsequent reaction of degradation

products with anthocyanins (Harper *et al.*, 1969). In this experiment the major contributing mechanism of anthocyanin degradation may have been caused by factors other than ascorbic acid oxidation, negating quercetin's protective ability. The direct condensation mechanism between anthocyanin and ascorbic acid may be responsible for anthocyanin degradation (Jurd, 1972; Poei-Langston & Wrolstad, 1981).

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. The formation of sediment in the Wildberry Herbal drink is due to an unstable cloud in cloudy apple juice concentrate which forms protein-polyphenolic complexes with polyphenolics in the blackcurrant juice concentrate. These complexes drop out of the drink forming a dense sediment. This sediment forms within one week of drink manufacture. The herbal formulation used in the Wildberry Herbal drink does not contribute significantly to the sediment.

2. If clarified apple juice concentrate is substituted for cloudy apple juice concentrate no sediment forms during eight weeks storage.

3. The sediment can be reduced by approximately 45% using stabiliser systems containing either xanthan or a xanthan/proplylene glycol alginate mixture.

4. Consumer sensory evaluation of the stabilised drinks found that the stabilised drinks were not liked significantly more or less than the standard Wildberry Herbal drink in terms of body, berry flavour and overall impression.

5. The red colour of the drink is affected adversely by the storage temperature of the drink. Ascorbic acid addition had a slight negative effect on colour, while increasing total anthocyanin concentration had a positive effect on colour retention.

6. Elderberry juice concentrate II (Directus Ltd, N.Z.) should be used in place of blackcurrant juice concentrate in view of its better colour stability and cost advantage.

7. The batch pasteurisation process does not adversely affect anthocyanin colour however if the drinks are not cooled quickly after pasteurisation anthocyanin colour is negatively affected.

8. Natural flavonoid antioxidants, quercetin and tea polyphenols, are not suitable for the protection of anthocyanins and in this experiment were found to degrade them.

6.2 Recommendations

It is recommended that the composition of the Wildberry Herbal drink remain unchanged. The combination of cloudy apple juice and blackcurrant juice in a drink is unique among products in the New Zealand beverage market place. The addition of stabilisers does help to minimise sediment formation, however it does not eliminate it completely. In this product, the benefits of using stabilisers are most likely to be outweighed by adverse consumer reaction to "additives" being used in the drink. Storage temperatures of below 20°C are recommended and for optimum colour retention the drink should be held at refrigeration temperature (5°C). It is also recommended that the cold wash cycle of the pasteurisation process be extended to cool the drinks sufficiently before packaging.

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APPENDIX

Appendix 1 Consumer sensory evaluation questionnaire

BERRY FRUIT JUICE EVALUATION

Thank you for offering to take part in this study. We would like you to taste three fruit juices and indicate how much you like/dislike their **body**, **flavour** and your **overall liking** of them. For the purposes of this study please use the following definitions when evaluating the drinks-

Body – *the feel of the drink as it moves over your tongue and around your mouth.*

Flavour – *the berry flavour you taste when drinking.*

Please taste the three samples in the **order shown** and put a tick in the box which corresponds to your degree of liking/disliking.

Please do not feel obliged to drink the entire sample provided.

Information from this study will be used as part of a Master's thesis in Food Science

Thank you.

Please start with sample 572

What do you think of the **body** of the drink?

❑	❑	❑	❑	❑	❑	❑	❑	❑
Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither like nor dislike	Like Slightly	Like Moderately	Like Very much	Like Extremely

What do you think of the **berry flavour** of the drink?

❑	❑	❑	❑	❑	❑	❑	❑	❑
Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither like nor dislike	Like Slightly	Like Moderately	Like Very much	Like Extremely

Please tell us your **overall impression** of the drink after having looked at, smelled and tasted it.

❑	❑	❑	❑	❑	❑	❑	❑	❑
Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither like nor dislike	Like Slightly	Like Moderately	Like Very much	Like Extremely

Do you have any comments about this drink that you would like to make?

_____

_____

_____

Now evaluate sample 417

What do you think of the **body** of the drink?

❑	❑	❑	❑	❑	❑	❑	❑	❑
Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither like nor dislike	Like Slightly	Like Moderately	Like Very much	Like Extrer

What do you think of the **berry flavour** of the drink?

❑	❑	❑	❑	❑	❑	❑	❑	❑
Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither like nor dislike	Like Slightly	Like Moderately	Like Very much	Like Extre

Please tell us your **overall impression** of the drink after having looked at, smelled and tasted it.

❑	❑	❑	❑	❑	❑	❑	❑	❑
Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither like nor dislike	Like Slightly	Like Moderately	Like Very much	Like Extre

Do you have any comments about this drink that you would like to make?

_____

_____

_____

Now evaluate sample 381

What do you think of the **body** of the drink?

❑	❑	❑	❑	❑	❑	❑	❑	❑
Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither like nor dislike	Like Slightly	Like Moderately	Like Very much	Like Extremely

What do you think of the **berry flavour** of the drink?

❑	❑	❑	❑	❑	❑	❑	❑	❑
Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither like nor dislike	Like Slightly	Like Moderately	Like Very much	Like Extremely

Please tell us your **overall impression** of the drink after having looked at, smelled and tasted it.

❑	❑	❑	❑	❑	❑	❑	❑	❑
Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither like nor dislike	Like Slightly	Like Moderately	Like Very much	Like Extremely

Do you have any comments about this drink that you would like to make?

_____

_____

_____

OPTIONAL:

Gender Female ❑ Male ❑

Age 17 –25 ❑ 26-40 ❑ 40+ ❑

How often do you consume fruit juice?

More than 5 times a week ❑

1- 4 times a week ❑

1-3 times a month ❑

Occasionally (less than 1- 3 times month) ❑

What type(s) of fruit juice do you usually drink?

Orange ❑ Apple ❑ Apple & Orange ❑ Apple & Mango ❑

Apple & Blackcurrant ❑ Blackcurrant ❑ Apple & Guava ❑

Cranberry ❑ Tropical ❑ Grape ❑ Grapefruit ❑

Fruit & Vegetable Blend ❑ Other ❑ Please specify _____

What brand(s) of fruit juice do you drink?

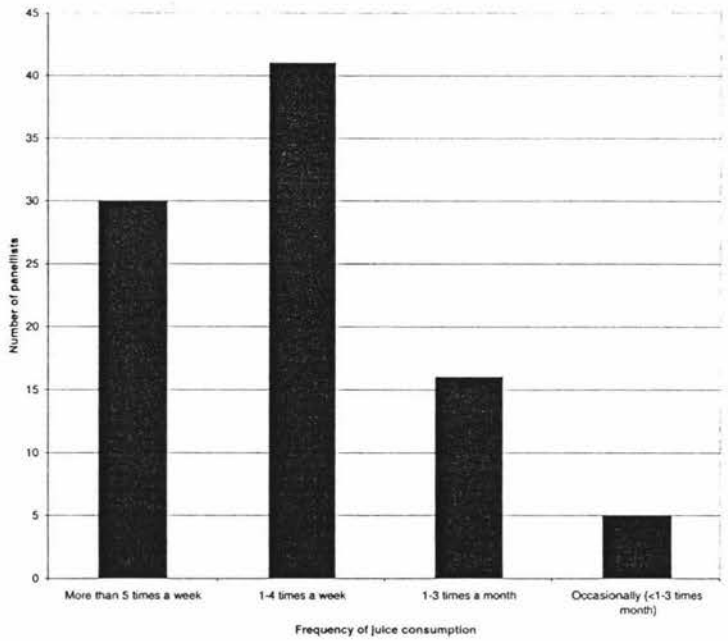
Just Juice ❑ Fresh Up ❑ Rio ❑ Stefans ❑ McCoy ❑

Phoenix ❑ Muse ❑ Arano ❑ Ribena ❑ Keri ❑

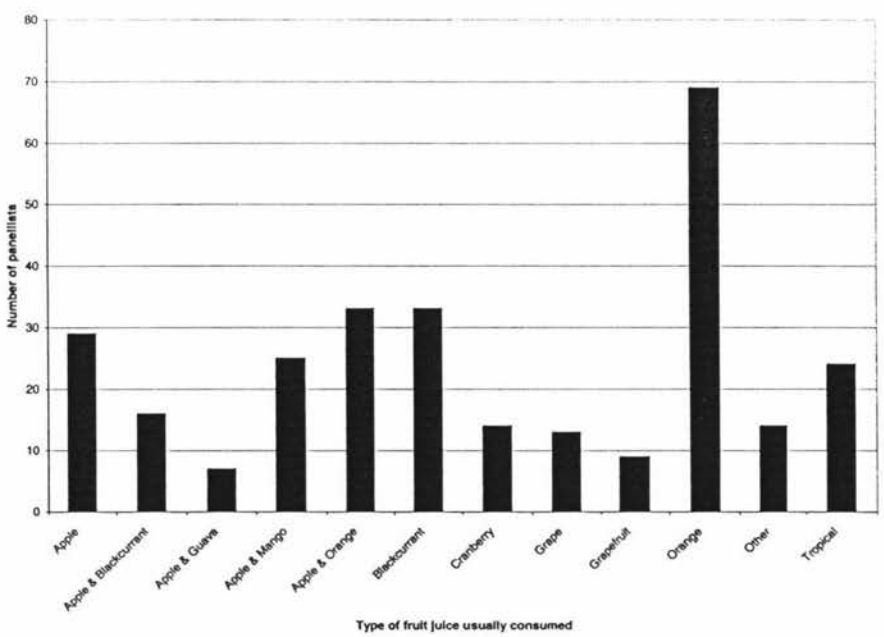
Citrus Tree ❑ Other ❑ Please specify_____

THANK YOU VERY MUCH FOR YOUR COOPERATION

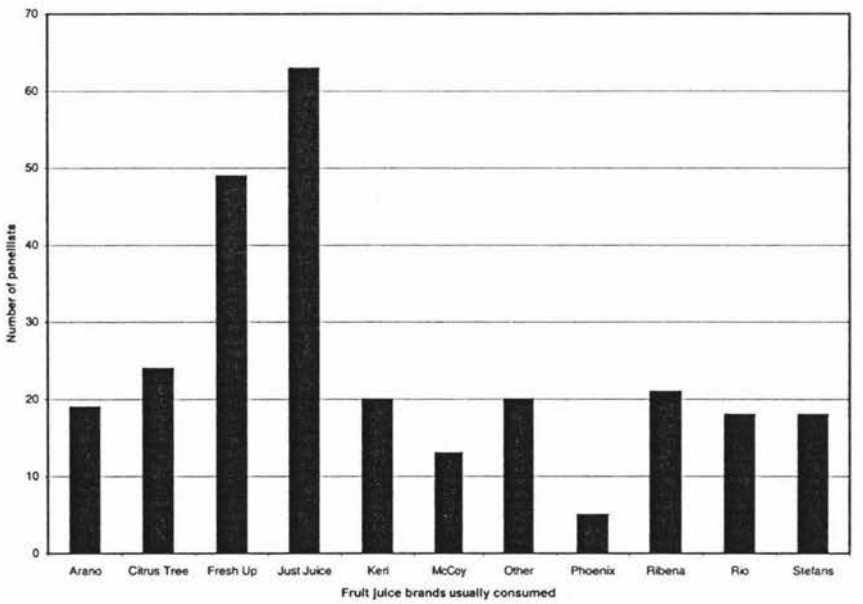
Appendix 2A Panellist fruit juice consumption frequency



Appendix 2B Types of fruit juice consumed by panellists



Appendix 2C Brands of fruit juice consumed by panellists



Appendix 3A Comments made on the Standard Wildberry Herbal drink

1. I like this one
2. A pretty weird taste, different but not dislikable
3. Too sweet.
4. Too sweet
5. A bit too sweet
6.
7. Smells horrible
8. Very good – easy to drink. Not to strong on the berry flavour.
9. Not good.
10. Nice, it's just right
11. The brownish tint makes it look less appealing, but otherwise very enjoyable.
12. The taste is very sweet and sort of sour at the same time-nice though.
13.
14.
15.
16.
17. A strong taste but very nice.
18.
19.
20. It's better. Feels a little drier.
21.
22.
23. Too sweet and syrupy.
24.
25. Very sweet. Weak taste.
26. Too sweet and sugary.
27. Too sweet, not as refreshing as 417.
28. Good.

29. Too sour.
30. Weak in berry flavour, taste a bit sweet.
31. Quite good, not too strong, very similar to the Ribena drink. Very good.
32.
33. Well done.
34. Thin after taste
35. Slightly too tart.
36.
37. This drink is blander and sweeter.
38.
39. Too sweet, slightly tangy.
40. Overall nice, smelled nice and sweet.
41.
42. Has a horrible aftertaste.
43. Tastes funny.
44. It has a strong taste.
45.
46.
47. This one also has a slightly stronger flavour and body to it.
48. Too berry.
49. This juice had a slightly dry aftertaste and was quite acidic to drink.
50. Sweeter than the first (572)
51.
52.
53. I like the tomato flavour at the end.
54.
55.
56.
57. Needs a bit more bite – more punch – more impact.
58. Similar to 417.
59.

60.
61. Nice drink. A bit sour.
62.
63. Not as nice a berry taste – tasted like there was a nutty flavour.
64.
65.
66. Not sweet enough.
67. Better, has a similar taste though.
68. Quite strong, somewhat syrupy – thick.
69.
70.
71. Didn't like the flavour at all.
72. Berry flavour is not ver nice, tastes almost dirty, smell is not that strong either.
73.
74.
75.
76.
77. Too sweet
78.
79. Better than 572, a stronger flavour.
80.
81. Too bitter.
82.
83. Nice sweet flavour but not sickly. Easy to drink and swallow. Pleasant, minor, lingering aftertaste.
84. A bit of a funny smell.
85. Quite strong and maybe a little sweet.
86. I don't like the smell.
87. Quite strong berry flavour.
88.

89.

90.

91. Good taste. I like it. It's cool.

92. Zingy – a little bit too strong.

93. Tingled on the tongue.

94. Didn't like overall taste.

95.

96.

97.

98. A bit funny tasting for a berry drink.

99. Aftertaste is fine but after having drunk a few mouthfuls the taste when drinking starts to worsen.

Appendix 3B Comments made on Xanthan (0.03% w/v)/PGA (0.1% w/v) stabilised Wildberry Herbal drink

1. Too strong a taste.
2. Pretty different taste compared to the other berry drinks I have had previously.
3.
4. Pretty good.
5.
6.
7.
8. All right body – a bit strong.
9. It will be better at getting rid of the thirst.
10. Smelt really nice, however a bit more flavour would be really great.
11. Taste was indistinct- couldn't tell what it was but its still rather nice.
12. Weaker than 381.
13.
14. Not as thick as sample 572, but too thick for my liking.
15.
16. Smells funny- the berry smell.
17. Very refreshing drink.
18.
19.
20. Sweet but not that good.
21.
22.
23. Less sweet, colder
24. Smooth texture and not too sweet.
25. Nice and thick but not too sweet. This is how I think it should taste.
26. Slightly too sweet, need water added.
27. Very refreshing and nice tasting flavour.

28. Just OK.
29.
30. Lovely, just right. I'd drink it anytime. Not too sour not too sweet.
31. Quite weak in the taste, it needs a bit of sugar for a better taste.
32.
33. You have the perfect balance.
34.
35. Could be slightly more concentrated.
36.
37. Appears to have more berry flavour.
38. The berry flavour is a bit weird.
39. A thicker texture.
40. It was thicker than the others – nice.
41.
42. Yummy, I'm going to drink the whole lot.
43. It's nice.
44.
45.
46.
47. This sample had a stronger taste to it than the first (572) and more body to it.
48.
49. This one definitely tasted better and had more flavour than 572.
50. Thicker than the other two.
51.
52.
53.
54.
55.
56.
57. A bit sweet.

58. Quite nice.
59. Very good flavour.
60.
61. Very rich tasting.
62.
63. More syrupy feel in the mouth, felt thicker than 572.
64.
65.
66.
67. Yuck.
68. Sweeter than 381, not as thick in body? More refreshing.
69.
70.
71. Felt quite thick in my mouth.
72. This is good. Berry taste not too overpowering. Smell isn't either. A good balance.
73. It is a bit sweet.
74.
75.
76.
77. Tastes good, better than the first two.
78.
79.
80.
81.
82.
83. Body feels similar to a smoothie. Taste – not distinctive berry flavour, a broader range of flavours.
84.
85. The drink was a bit frothy, didn't really like this feel.
86. It has a weak but pleasant berry taste.

87. Much better that it's a bit creamy and the flavours quite right.
88.
89.
90. Tasted like lollies.
91. Just so. It's too light.
92. Mellow – nice.
93.
94. Drink has more consistency in taste and feels thicker.
95.
96. It tastes like a stronger version of 572.
97.
98.
99. Yum.

Appendix 3C Comments made on Xanthan (0.085% w/v) stabilised Wildberry Herbal drink

1. Little bit too sweet
2. Seems to be a mixture of the last two. Quite a nice taste but not as refreshing as the first drink (417).
3. Middle taste.
4. Interesting body
5. A rather water after taste
6.
7. A bit sour
8. Thick and strong.
9. To be better at quenching the thirst it must be a bit softer.
10. Doesn't have much of a kick to it. Too mild-more berry flavour would be nice.
11. It was Ok only – lovely thick texture, but the taste was rather thin.
12. Feels stronger than the other two.
13.
14. It was very syrupy i.e. too thick.
15.
16. Has that nice tarty taste, but a little too tart. Nice after taste.
17. It's very nice.
18.
19.
20. It tastes weird. Unlike juice.
21.
22.
23.
24.
25. Little watery but generally very pleasant. Love the red berry taste.
26.

27. Very similar to 417 but stronger taste and not as refreshing.
28. Quite natural.
29. Quite sweet.
30. A bit sour.
31. Very, very similar to 381. The taste was very good. I'd like to see what it tastes like warm.
32.
33. The taste is pretty flat, could do with a bit more flavouring.
34.
35.
36.
37. The apple flavour is dominant but the blackberry smell is dominant.
38. Too sour.
39. Thinner texture and slightly bitter in taste towards the end.
40. The liquid looked frothy like it was mixed up a lot or had spit in it. Looked like it was backwashed, taste was not strong.
41.
42.
43.
44. It's too plain.
45.
46.
47.
48. Pleasant
49. Tasted almost watery
50. Thick body not too sweet.
51.
52. Not thick enough.
53. I hope you make this more sour.
54.
55.

56.
57. The best of the three.
58. It's quite strong.
59. Too watery, not enough taste.
60.
61. Very smooth and great tasting.
62.
63. Nicer colour than the others.
64. Slightly sour grape flavour.
65.
66.
67. OK. Not as thick as 417.
68. Tastes thicker than 472 maybe the same as 381. Not as refreshing s 417.
69.
70.
71.
72. Berry smell is quite overpowering.
73.
74.
75.
76.
77.
78.
79.
80.
81.
82.
83. Has a light texture or runnier as opposed to a smoothie. Flavour is sweeter, has a pleasant aftertaste.
84.
85.

86.

87. Tastes a bit watered down.

88.

89. Are 417 and 572 the same?

90.

91. It's good but not sweet enough.

92.

93.

94. It has a very weak flavour and is not very nice to taste.

95.

96. Not too sweet nor sour.

97.

98. Tastes a bit sour, lacks that strong berry taste.

99. Pretty damn good.

Appendix 4A pH values for drinks from section 5.1

Treatment	pH	
	Week 0	Week 8
A1	3.3	3.3
B1	3.3	3.3
C1	3.3	3.3
D1	3.3	3.3
E1	3.3	3.2
F1	3.3	3.2
G1	3.3	3.2
H1	3.3	3.2
J1	3.2	3.2
K1	3.2	3.2
L1	3.2	3.2
M1	3.2	3.2
N1	3.2	3.2
O1	3.2	3.2
P1	3.2	3.2
R1	3.2	3.2

Standard error of mean = 0.1, n=96

Appendix 4B Titratable acidity values for drinks from section 5.1

Treatment	Titratable acidity (g/100ml)	
	Week 0	Week 8
A1	0.7	0.7
B1	0.7	0.7
C1	0.7	0.7
D1	0.8	0.7
E1	0.7	0.7
F1	0.7	0.7
G1	0.7	0.7
H1	0.8	0.8
J1	0.7	0.7
K1	0.7	0.7
L1	0.7	0.7
M1	0.8	0.8
N1	0.7	0.7
O1	0.7	0.7
P1	0.7	0.7
R1	0.8	0.7

Standard error of mean = 0.1, n=96

Appendix 5A Analysis of effect of factors on monomeric anthocyanin concentration for cloudy apple juice based drinks

Estimated Effects and Coefficients for Wk0 (coded units)

Term	Effect	Coef	StDev Coef	T	P
Constant		64.1588	0.2528	253.75	0.000
Asc. Aci	-1.9375	-0.9688	0.2528	-3.83	0.003
Pigment	47.6075	23.8037	0.2528	94.14	0.000
Storage	-0.0000	-0.0000	0.2528	-0.00	1.000
Light/Da	0.0000	0.0000	0.2528	0.00	1.000

Analysis of Variance for Wk0 (coded units)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Main Effects	4	9080.91	9080.91	2270.23	2E+03	0.000
Residual Error	11	11.25	11.25	1.02		
Lack of Fit	3	10.60	10.60	3.53	43.01	0.000
Pure Error	8	0.66	0.66	0.08		
Total	15	9092.16				

Estimated Effects and Coefficients for Wk8 (coded units)

Term	Effect	Coef	StDev Coef	T	P
Constant		24.02	2.997	8.01	0.000
Asc. Aci	1.24	0.62	2.997	0.21	0.840
Pigment	19.72	9.86	2.997	3.29	0.007
Storage	-45.59	-22.79	2.997	-7.61	0.000
Light/Da	-1.19	-0.59	2.997	-0.20	0.846

Analysis of Variance for Wk8 (coded units)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Main Effects	4	9880.8	9880.81	2470.20	17.19	0.000
Residual Error	11	1580.5	1580.46	143.68		
Lack of Fit	3	1572.3	1572.26	524.09	511.49	0.000
Pure Error	8	8.2	8.20	1.02		
Total	15	11461.3				

Appendix 5B Analysis of effect of factors on % contribution of tannin for cloudy apple juice based drinks

Estimated Effects and Coefficients for Wk0 (coded units)

Term	Effect	Coef	StDev Coef	T	P
Constant		77.585	0.09640	804.81	0.000
Asc. Aci	1.700	0.850	0.09640	8.82	0.000
Pigment	-7.480	-3.740	0.09640	-38.80	0.000
Storage	-0.000	-0.000	0.09640	-0.00	1.000
Light/Da	0.000	0.000	0.09640	0.00	1.000

Analysis of Variance for Wk0 (coded units)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Main Effects	4	235.362	235.362	58.8404	395.72	0.000
Residual Error	11	1.636	1.636	0.1487		
Lack of Fit	3	0.360	0.360	0.1200	0.75	0.551
Pure Error	8	1.276	1.276	0.1595		
Total	15	236.997				

Estimated Effects and Coefficients for Wk8 (coded units)

Term	Effect	Coef	StDev Coef	T	P
Constant		84.359	1.131	74.59	0.000
Asc. Aci	2.664	1.332	1.131	1.18	0.264
Pigment	-6.664	-3.332	1.131	-2.95	0.013
Storage	17.949	8.974	1.131	7.94	0.000
Light/Da	-1.589	-0.794	1.131	-0.70	0.497

Analysis of Variance for Wk8 (coded units)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Main Effects	4	1504.7	1504.7	376.18	18.38	0.000
Residual Error	11	225.1	225.1	20.46		
Lack of Fit	3	116.8	116.8	38.94	2.88	0.103
Pure Error	8	108.3	108.3	13.53		
Total	15	1729.8				

Appendix 5C Analysis of effect of factors on colour deterioration index for cloudy apple juice based drinks

Estimated Effects and Coefficients for Wk0 (coded units)

Term	Effect	Coef	StDev Coef	T	P
Constant		0.778750	0.01146	67.94	0.000
Asc. Aci	0.012500	0.006250	0.01146	0.55	0.596
Pigment	0.062500	0.031250	0.01146	2.73	0.020
Storage	-0.000000	-0.000000	0.01146	-0.00	1.000
Light/Da	0.000000	0.000000	0.01146	0.00	1.000

Analysis of Variance for Wk0 (coded units)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Main Effects	4	0.0162500	0.0162500	0.0040625	1.93	0.175
Residual Error	11	0.0231250	0.0231250	0.0021023		
Lack of Fit	3	0.0006250	0.0006250	0.0002083	0.07	0.972
Pure Error	8	0.0225000	0.0225000	0.0028125		
Total	15	0.0393750				

Estimated Effects and Coefficients for Wk8 (coded units)

Term	Effect	Coef	StDev Coef	T	P
Constant		0.68375	0.005449	125.49	0.000
Asc. Aci	0.00750	0.00375	0.005449	0.69	0.506
Pigment	0.10750	0.05375	0.005449	9.86	0.000
Storage	-0.13000	-0.06500	0.005449	-11.93	0.000
Light/Da	0.02500	0.01250	0.005449	0.89	0.642

Analysis of Variance for Wk8 (coded units)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Main Effects	4	0.116550	0.116550	0.0291375	61.34	0.000
Residual Error	11	0.005225	0.005225	0.0004750		
Lack of Fit	3	0.004025	0.004025	0.0013417	8.94	0.006
Pure Error	8	0.001200	0.001200	0.0001500		
Total	15	0.121775				

Appendix 6A Analysis of effect of factors on monomeric anthocyanin concentration for clarified apple juice based drinks

Estimated Effects and Coefficients for Wk0 (coded units)

Term	Effect	Coef	StDev Coef	T	P
Constant		91.476	0.1536	595.72	0.000
Asc. Aci	-16.897	-8.449	0.1536	-55.02	0.000
Pigment	75.437	37.719	0.1536	245.64	0.000
Storage	-0.000	-0.000	0.1536	-0.00	1.000
Light/Da	-0.000	-0.000	0.1536	-0.00	1.000

Analysis of Variance for Wk0 (coded units)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Main Effects	4	23905.4	23905.4	5976.34	2E+04	0.000
Residual Error	11	4.1	4.1	0.38		
Lack of Fit	3	1.1	1.1	0.36	0.93	0.470
Pure Error	8	3.1	3.1	0.38		
Total	15	23909.5				

Estimated Effects and Coefficients for Wk8 (coded units)

Term	Effect	Coef	StDev Coef	T	P
Constant		36.37	4.449	8.18	0.000
Asc. Aci	-12.54	-6.27	4.449	-2.41	0.032
Pigment	29.94	14.97	4.449	3.36	0.006
Storage	-61.47	-30.73	4.449	-6.91	0.000
Light/Da	2.44	1.22	4.449	0.27	0.789

Analysis of Variance for Wk8 (coded units)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Main Effects	4	19350.6	19350.6	4837.64	15.28	0.000
Residual Error	11	3483.0	3483.0	316.63		
Lack of Fit	3	3481.9	3481.9	1160.62	8E+03	0.000
Pure Error	8	1.1	1.1	0.14		
Total	15	22833.5				

Appendix 6B Analysis of effect of factors on % contribution of tannin for clarified apple juice based drinks

Estimated Effects and Coefficients for Wk0 (coded units)

Term	Effect	Coef	StDev Coef	T	P
Constant		37.181	0.1748	212.66	0.000
Asc. Aci	-0.792	-0.396	0.1748	-2.27	0.045
Pigment	-5.403	-2.701	0.1748	-15.45	0.000
Storage	-0.000	-0.000	0.1748	-0.00	1.000
Light/Da	-0.000	-0.000	0.1748	-0.00	1.000

Analysis of Variance for Wk0 (coded units)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Main Effects	4	119.260	119.260	29.8151	60.96	0.000
Residual Error	11	5.380	5.380	0.4891		
Lack of Fit	3	4.558	4.558	1.5194	14.79	0.001
Pure Error	8	0.822	0.822	0.1028		
Total	15	124.641				

Estimated Effects and Coefficients for Wk8 (coded units)

Term	Effect	Coef	StDev Coef	T	P
Constant		67.086	1.079	62.15	0.000
Asc. Aci	4.776	2.388	1.079	2.21	0.049
Pigment	-9.931	-4.966	1.079	-4.60	0.001
Storage	42.344	21.172	1.079	19.61	0.000
Light/Da	4.806	2.403	1.079	2.23	0.048

Analysis of Variance for Wk8 (coded units)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Main Effects	4	7750.14	7750.14	1937.54	103.93	0.000
Residual Error	11	205.07	205.07	18.64		
Lack of Fit	3	200.89	200.89	66.96	128.04	0.000
Pure Error	8	4.18	4.18	0.52		
Total	15	7955.21				

Appendix 6C Analysis of effect of factors on colour deterioration index for clarified apple juice based drinks

Estimated Effects and Coefficients for Wk0 (coded units)

Term	Effect	Coef	StDev Coef	T	P
Constant		1.23375	0.02995	41.19	0.000
Asc. Aci	0.09750	0.04875	0.02995	1.63	0.132
Pigment	0.49750	0.24875	0.02995	8.30	0.000
Storage	0.00000	0.00000	0.02995	0.00	1.000
Light/Da	0.00000	0.00000	0.02995	0.00	1.000

Analysis of Variance for Wk0 (coded units)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Main Effects	4	1.02805	1.02805	0.257012	17.90	0.000
Residual Error	11	0.15793	0.15793	0.014357		
Lack of Fit	3	0.15602	0.15603	0.052008	218.98	0.000
Pure Error	8	0.00190	0.00190	0.000238		
Total	15	1.18597				

Estimated Effects and Coefficients for Wk8 (coded units)

Term	Effect	Coef	StDev Coef	T	P
Constant		0.8613	0.01833	46.99	0.000
Asc. Aci	-0.1100	-0.0550	0.01833	-3.00	0.012
Pigment	0.1900	0.0950	0.01833	5.18	0.000
Storage	-0.4425	-0.2212	0.01833	-12.07	0.000
Light/Da	-0.0025	-0.0012	0.01833	-0.07	0.947

Analysis of Variance for Wk8 (coded units)

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Main Effects	4	0.97605	0.97605	0.244012	45.40	0.000
Residual Error	11	0.05913	0.05913	0.005375		
Lack of Fit	3	0.04503	0.04503	0.015008	8.52	0.007
Pure Error	8	0.01410	0.01410	0.001762		
Total	15	1.03517				