COPIGMENTATION REACTIONS OF BOYSENBERRY JUICE

A THESIS PRESENTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER TECHNOLOGY IN FOOD TECHNOLOGY AT MASSEY UNIVERSITY, ALBANY, NEW ZEALAND

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ABSTRACT

Colour is one of the main sensory characteristics of berry juice and fruit products and this parameter also powerfully impacts on consumer behaviour. However, the colour of berry juices is unstable and degradation occurs during storage. The main objectives of the project were: to determine the mechanism by which boysenberry juice enhances the colour of other berry juices and to determine if its addition to berry juices will also stabilise the anthocyanin pigments and enhance copigmentation. In this study, total anthocyanin, total phenolic acids, hyperchromic and bathochromic shift and the rate of colour degradation was measured by spectrophotometric techniques. Individual anthocyanin and phenolic acid content were measured in each juice by high performance liquid chromatography (HPLC) were evaluated during storage at 5, 20 and 35°C.

Boysenberry juice improved the colour of blackcurrant, cranberry and pomegranate juices immediately after addition; however, only blackcurrant juice colour was stable during storage at 5°C. There was no influence on the stability of total anthocyanins in pomegranate or cranberry juices when boysenberry juice was added. Of the three juices, pomegranate had the highest rate of degradation. The total anthocyanin of blackcurrant enhanced with boysenberry juice was more stable than for cranberry and pomegranate juices. The impact of phenolic acids found in boysenberry juice (kaemferol, quercetin and ellagic acid) on blackcurrant juice colour stability was also investigated.

The colour stability of blackcurrant juice was improved by the addition of ellagic acid at 5°C; however, the colour intensity of blackcurrant enhanced with kaemferol and quercetin decreased with storage. The copigmentations between anthocyanins themselves were not found to be a significant effect on colour stability of blackcurrant juice. Ellagic acid had the strongest colour improvement in blackcurrant juice compared to boysenberry juice. In conclusion, ellagic acid as found in boysenberry juice formed intermolecular copigmentation with blackcurrant juice anthocyanins, so this resulted in stabilised juice colour during storage; however, the effect was found when the juice was stored at 5°C only.

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CHAPTER 1

INTRODUCTION

Anthocyanins are natural pigments widely distributed in nature. Anthocyanin colour molecules are a subclass of flavonoids. They are responsible for the reds, purples and blues in many fruits and berries. Nevertheless, anthocyanin molecules are highly unstable and easily susceptible to degradation. The stability of anthocyanins is influenced by pH, temperature, presence of enzymes, light, oxygen, structure and concentration, and the presence of complexing compounds such as other flavonoids, phenolic acids, proteins, metals and minerals (Markakis, 1982).

The stability of anthocyanin colour can be improved by copigmentation which can occur through intramolecular interactions, in which an organic acid or a flavonoid is covalently linked to an anthocyanin chromophore (Brouillard, 1981; Dangles et al., 1993), or through intermolecular interaction, in which colourless flavonoids or other phenolic acompounds react with weak hydrophobic forces with anthocyanins (Mazza & Brouillard, 1990). Anthocyanin copigmentation gives brighter, stronger and more stable colour than those presented by anthocyanins alone. In food science, copigmentation is considered an important interaction, as colour is one of the quality factors strongly affecting consumer acceptance of food. Copigmentation is detected as both as a hyperchromic effect, where the λ_{max} of the absorption spectrum increases, and as a bathochromic shift, where a shift toward higher wavelength (nm) at λ_{max} of the absorption spectrum occurs (Mazza & Brouillard, 1990; Baranac et al., 1996).

The objectives of this research were to firstly, study the effects of boysenberry juice on the colour stability other berry juices, secondly to investigate the impact of three phenolic acids acting as copigments on colour stability of blackcurrant juice. Finally, the interaction between boysenberry juice and ellagic acid in blackcurrant juice was also examined to try and elucidate if boysenberry juice or ellagic was more effective for maintaining the colour stability of blackcurrant juice.

CHAPTER 2

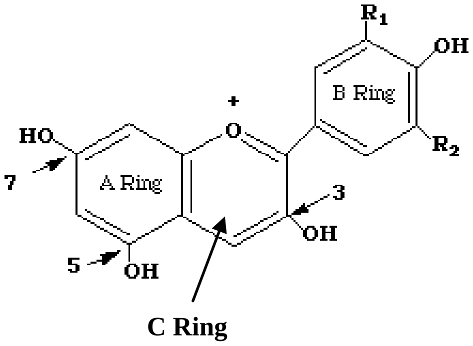
LITERATURE REVIEW

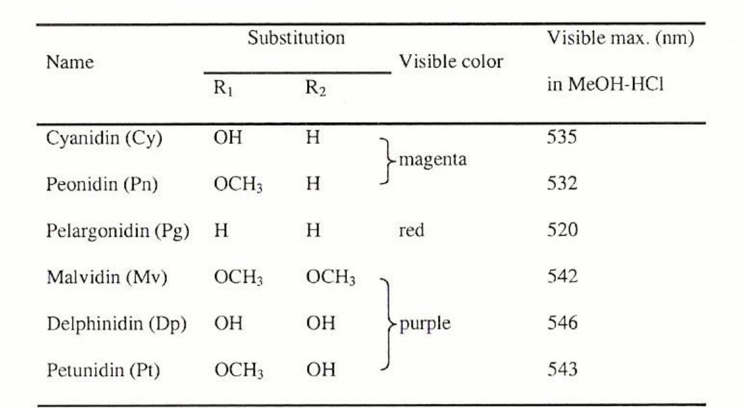
2.1 Anthocyanins

2.1.1 Structure of anthocyanins

Anthocyanins are the largest group of water-soluble pigments in the plant kingdom and are responsible for the blue, red, and purple colours of many fruits, vegetables, grains flowers, and leaves (Mazza et al., 2004). The anthocyanins are part of the group of compounds collectively known as flavonoids. The pigments are hydroxylated and methoxylated derivatives of phenyl-2-benzipyrylium or flavylium salts consisting of an aglycone (anthocyanidin), sugars and in many cases acyl groups. The basic structure of the aglycone shown in Figure 2.1 is a C-6 (A-ring)-C-3 (C-ring)-C-6 (B-ring) carbon skeleton (Harborne, 1998). While 22 monomeric anthocyanidins have been properly identified, nearly all reported anthocyanins (more than 200) are based on only six anthocyanidins (Francis, 1989; Bridle & Timberlake, 1997). The most common anthocyanidins are pelargonidin, cyanidin, peonidin, delphinidin, petunidin and malvidin, as shown in Figure 2.1, where they differ in the number of hydroxyl groups in the B-ring of the flavylium cation (Harbone, 1998; Kong et al., 2003).

The colour of anthocyanins results from excitation of a molecule by visible light. The degree of excitation depends on the relative electron mobility in the structure. Double bonds are excited very easily and cause the presence of colour. Anthocyanins are intensely coloured under acidic conditions because of the long chromophore of eight conjugated double bonds carrying a positive charge. The maximum absorption in the visible region is usually between 465 and 550 nm (Harborne, 1998; Eder, 2000). Approximately 400 individual anthocyanins have been identified (Mazza & Miniati, 1993). The six anthocyanidins commonly found in plants are classified according to the number and position of hydroxyl and methyl groups on the flavan nucleus, and they are named cyanidin, peonidin, pelargonidin, malvidin, delphinidin and petunidin (Mazza & Miniati, 1993; Harborne, 1998) (Figure 2.1). If there are more methoxyl groups, then redness is increased. If there are more hydroxyl groups, then the colour goes toward a more blueish shade (Harborne, 1998; Heredia et al., 1998).





Name	Substitution		Visible color	Visible max. (nm) in MeOH-HCl
	R_1	R_2		
Cyanidin (Cy)	OH	H	magenta	535
Peonidin (Pn)	OCH_3	H		532
Pelargonidin (Pg)	H	H	red	520
Malvidin (Mv)	OCH_3	OCH_3	purple	542
Delphinidin (Dp)	OH	OH		546
Petunidin (Pt)	OCH_3	OH		543

Figure 2.1: Basic structures of anthocyanidins (Harborne, 1998)

2.1.2 Glycosylation

The anthocyanidins are not often found in nature as they are highly unstable (Harborne, 1967; Clifford, 2000). The glucosides of anthocyanidins, called anthocyanins, are more soluble and stable in water than the former (Timberlake & Bridle, 1977). Glycosylation makes anthocyanins are much more soluble and stable in water than anthocyanidins (Robinson & Leon, 1931). The stability is improved by one or more sugar molecules bonded at different hydroxyl positions. Anthocyanins are always glycosylated at C-3 on C ring (Figure 2.2). Beside the C-3 position, other sugars can also be attached at any one of hydroxyls at C-5, C-7, C-3’, C-4’ and C-5’ (Brouillard, 1988; Eder, 2000). The most common sugars of anthocyanin are monoglycerides, especially glucose, but rhamnose, galactose, xylose and fructose are also found. Moreover, rutinose, sambubiose and sophorose are the most common disaccharides present in anthocynins (Clifford, 2000; Kahkonen et al., 2003).

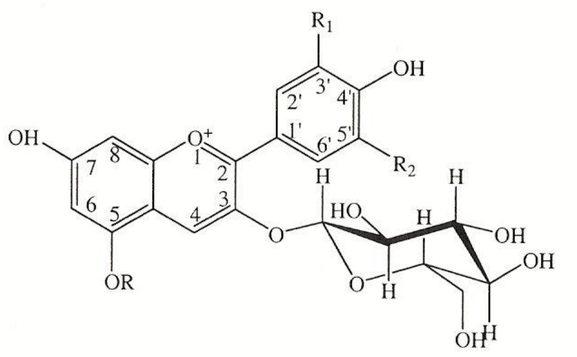


Figure 2.2: Structure of anthocyanidin 3- glucoside (Wu & Prior, 2005)

2.1.3 Acylation

The sugar residues on anthocyanins can be acylated further with organic acids via an ester bond (Eder, 2000). Common acylating units include aromatic phenolic acids such as cinnamic acid, coumaric acid, sinapic acid and caffeic acid (Figure 2.3) as well as a range of aliphatic acids such as acetic, malic, malonic, oxalic and succinic acid (Figure 2.4) (Francis, 1989). Aromatic and aliphatic acylation may occur in the same molecule, and from zero to at least three acylating units may be present (Harborne, 1998; Clifford, 2000). The acylated anthocyanins in berry fruits affect the stability of their colour and significantly diminishes the shelf life of food products derived from berries (Rein, 2005).

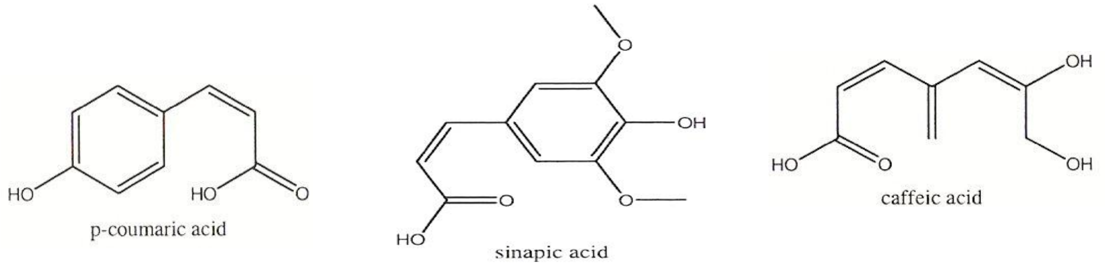


Figure 2.3: Common phenolic acids acylated with sugar units on anthocyanins (Rein, 2005)

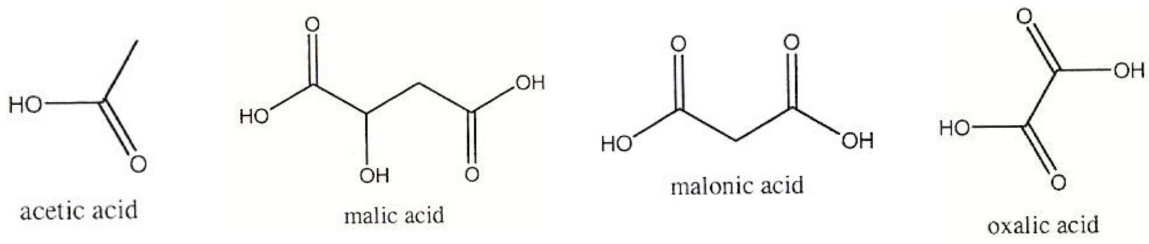


Figure 2.4: Common aliphatic acids acylated with sugar units on anthocyanins (Rein, 2005)

2.2 Anthocyanins in berry fruits

The anthocyanin content in berry fruits contributes to the darkness of the berry colour. The darker blue a berry, the higher its anthocyanin content (Rein, 2005). Table 2.1 is a summary of anthocyanin contents of some berries based on the information from several studies. Among the different types of berry fruits, the total anthocyanin content varies considerably, affected by genetics, light and temperature (Shahidi & Naczk, 2004). Also, the methods used to identify and quantify anthocyanins have an effect on a wide range of anthocyanin concentrations (Clifford, 2000).

Table 2.1: Anthocyanin concentrations of different types of berry fruits

Berry	**Anthocyanin contents (mg/kg of fresh weight)**	**Reference**
Black currant	1300-4000	Timberlake, 1988; Eder, 2000
Boysenberry	1609-2710	Torre & Barritt, 1977; Clifford, 2000
Blueberry	825-5030	Timberlake, 1988; Eder, 2000; Clifford, 2000; Prior, 2004
Blackberry	820-1800	Eder, 2000; Clifford, 2000
Cranberry	460-2000	Timberlake, 1988; Eder, 2000; Prior, 2004
Raspberry (Black)	763-4277	Timberlake, 1988; Clifford, 2000; Prior, 2004
Raspberry (Red)	100-600	Timberlake, 1988; Eder, 2000; Clifford, 2000
Strawberry	127-360	Timberlake, 1988; Eder, 2000; Prior, 2004
Pomegranate	162-387 *	Gil et al., 2000
Blood orange	65.6-488* 2000	Rapisarda et al., 2000 Clifford, 2000

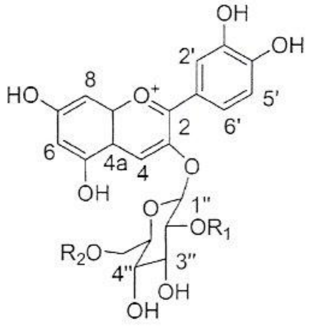
* Anthocyanin contents (mg/L of juices)

Moreover, different berry fruits have different contents of various individual anthocyanins. Four anthocyanins were identified (cyanidin-3-rutinoside, cyanidin-3-glucoside, delphinidin-3-rutinoside and delphinidin-3-glucoside) in blackcurrants; however, the two major anthocyanins are cyanidin-3-rutinoside (36%) and delphinidin-3-rutinoside (34%) (Machiex et al., 1990; Kahkonen et al., 2003). Red raspberry contains six anthocyanins (cyanidin-3-sophoroside, cyanidin-3-glucorutinoside, cyanidin-3-glucoside, cyanidin-3-rutinoside, pelargonidin-3-sophoroside and pelargonidin-3-glucorutinoside) identified by high performance liquid chromatography. The main anthocyanins of raspberry are cyanidin-3-sophoroside and cyanidin-3-glucorutinoside (Withy et al., 1993; Beekwider et al., 2005).

Twenty-five different anthocyanin pigments have been identified in strawberry, most of them containing pelargonidin (Pg) as aglycone; some cyanidin (Cy) derivatives were also found in the fruit. The major anthocyanin of strawberry is pelargonidin-3-sophoroside (77-90%), followed by pelargonidin-3-rutinoside (6-11%) and cyanidin-3-glucoside (3-10%) (Goiffon et al., 1991; Lopes da Silva et al., 2007).

Boysenberry is considered to be derived from a cross between *Rubus loganbaccus* and *Rubus baileyanus Britt* (Cooney et al., 2004). The anthocyanin composition of boysenberry was previously found to contain four main anthocyanins determined by thin-layer chromatography: cyanidin-3-glucoside, cyanidin-3-[2-(glucosyl)glucoside], cyanidin-3-[2(glucosyl)-6(rhamnosyl) glucoside], and cyanidin-3-[6 (rhamnosyl) glucoside] (Figure 5)(Torre & Barritt, 1977). More recently, Wada & Ou (2002) identified boysenberry anthocyanins by using liquid chromatography-mass spectrometry (LC-MS). They found four anthocyanin peaks and confirmed the identity of three of the peaks as cyanidin-3-[2-(glucosyl)glucoside], cyanidin-3-glucoside, cyanidin-3-[6 (rhamnosyl) glucoside], similar to the previous identifications. However, another peak was confirmed by using a reversed-phase high performance liquid chromatography (HPLC) and the structure was assigned as cyanidin-3-[6’-(p-coumaryl)glucoside]. From further analysis of boysenberry anthocyanins by Cooney et al. (2004) using LC-MS, reported that the fruit contains four different types of anthocyanins. The two major components are cyanidin-3-sophoroside and cyanidin-3-glucoside, respectively.

The two less abundant are cyanidin-3-[2-(glucosyl)rutinoside] and cyanidin-3-rutinoside. Similar results were found for boysenberry extracts detected by HPLC (McGhie et al., 2003). More recently, Wu & Prior (2005) used LC-MS to study the anthocyanin composition of red raspberry, which has a similar anthocyanin composition to boysenberry. They suggested that four anthocyanins detected in boysenberry were cyanidin-3-[2-(glucosyl)glucoside], cyanidin-3-glucoside, cyanidin-3-[6 (rhamnosyl) glucoside], and cyanidin-3-[2-(glucosyl)glucoside]-5-rhamnoside (Figure 2.5). McGhie et al. (2006) identified that the two major anthocyanin components of boysenberry by using HPLC are cyanidin-3-[2-(glucosyl)glucoside] and cyanidin-3-[2(glucosyl)-6-(rhamnosyl)glucoside].



	R_1	R_2
1	β-D-glucopyranosyl	H
2	β-D-glucopyranosyl	α-L-rhamnosyl
3	H	H
4	H	α-L-rhamnosyl

Figure 2.5: Chemical structures of the anthocyanins found in boysenberry (Wu & Prior, 2005)

(1) Cyanidin-3-[2-(glucosyl)glucoside]

(2) Cyanidin-3-[2(glucosyl)-6-(rhamnosyl)glucoside]

(3) Cyanidin-3-glucoside

(4) Cyanidin-3-[6-(rhamnosyl)glucoside]

Pomegranate (*Punica granatum*) is one of the main sources of anthocyanins (Wikipedia, 2009). Six anthocyanins were found as being responsible for the pomegranate juice colour: delphinidin-3-glucoside and 3, 5-diglucoside, cyanidin-3-

glucoside and 3, 5-diglucoside, and pelargonidin-3-glucoside and 3, 5-diglucoside (Du et al., 1975; Gil et al., 1995a). However, delphinidin-3-glucoside was identified to be the major anthocyanin present in pomegranate (Miguel et al., 2004). Pomegranate juice also contains 1 g/L citric acid and only 7 mg/L ascorbic acid (El-Nemr et al., 1990).

2.3 Boysenberry Characteristics

2.3.1 Chemical characteristics

Boysenberry is found to have a range of pHs between 3 and 3.5 that is similar to other types of berries such as raspberry and blackcurrant, between 3 and 4 (Byers et al., 1995) (Table 2.2). About 8.5-14g of total sugars are present in 100 g of boysenberry fruits; however, the major sugars identified in the fruit are fructose and glucose, 3.70 and 2.48 g per 100g, respectively, of the berries (Oregon-berries, 2007).

Table 2.2: Chemical characteristics of boysenberries (*Rubus ursinus x idaeus*) (Oregon-berries, 2007).

Properties	Content
pH	3-3.5
Titratable Acid (as % citric acid)	0.9-1.8
Soluble Solids	8.82-11.2 °Brix
Total Sugar	8.5-14 g/100g
Glucose	2.48 g/100g
Fructose	3.70 g/100g
Water	113 g/100g
Ash	0.7 mg/100g

2.3.2 Nutrition information and health benefits

As with other fruits, boysenberry contain a range of micronutrients with are essential for health. In particular, boysenberry contains a high level of vitamin C that has antioxidant activity, which can act as a cofactor in hydroxylation reactions which are required for collagen synthesis (Byers et al., 1995; Jacob et al., 2001) (Table 2.3).

This vitamin also has a role in hormone synthesis, the immune system, iron absorption, platelet aggregation and can have a role in preventing heart disease and a range of cancers (Byers et al., 1995). Moreover, boysenberry can be a significant dietary source of folic acid, a water soluble B vitamin which as well as being essential to prevent neural tube defects in new-borne babies and can also play a role in reducing risk of heart disease and cancers (Key et al., 2002; Verhaar et al., 2002).

2.4 Factors affecting anthocyanin colour stability

2.4.1 Anthocyanin structure

Anthocyanin degradation rates vary greatly between different anthocyanins. The increased hydroxylation of the anthocyanidin stabilises the pigment. Cyanidin is less stable than delphinidin in acidic methanol (Taylor, 1989; Dao et al., 1998). The colour of anthocyanins becomes darker (pink to blue) as a result of an increase in the number of hydroxyl groups (Rein, 2005). Also, cyanidin-3-diglucoside was found to be more stable than cyanidin in black raspberry juice because the blocking of the hydroxyl group of the 3-position with a sugar interrupted damage of the red pigment (Daravingas & Cain, 1968). Moreover, an increase in methylation of the hydroxyl groups weakens the stability of the anthocynins. In a buffer solution, petunidin-3-glucoside was less stable than cyanidin-3-glucoside because the former has an additional 5’-methoxyl (Cabrita et al., 2000).

The sugar substitution of anthocyanidin structure also affects the colour of the anthocyanin pigment. Anthocyanins with a sugar at 3-position were more coloured than glycosylation at other positions (Rein, 2005). An increase in the number of a specific type of sugar (glucose) also influences the colour stability. Giusti et al (1999) reported that increasing the number of glucose molecules on the anthocyanidin caused the colour change to a more yellow pigment. Bassa and Francis (1987) found that acylation of anthocyanin with acids can increase the stability of the pigment. Anthocyanins with more than one acyl group are more stable than monoacylated anthocyanins and they also have higher stability of colour over a wide range of pH (Asen,1976).

Table 2.3: Nutritional information for (frozen, unsweetened,and unthawed) Boysenberries (132g per serving) (Nutrition Data, 2007)

Properties	Content	Properties	Content	Properties	Content
Calories	66.0 Cal (276 kJ)	Lycopene	0.0 mg	**Minerals**	
Calories from Carbohydrate	58.2 Cal (244 kJ)	Lutein+Zeaxanthin	156 mg	Calcium	35.6 mg
Calories from Fat	2.9 Cal (12.1 kJ)	Vitamin C	4.1 mg	Iron	1.1 mg
Calories from Protein	4.9 Cal (20.5 kJ)	Vitamin E (Alpha Tocopherol)	1.1 mg	Magnesium	21.1 mg
Calories from Alcohol	0.0 Cal (0.0 kJ)	Vitamin K	10.3 mg	Phosphorus	35.6 mg
Total Carbohydrate	16.1 g	Thiamin	0.1 mg	Potassium	183 mg
Dietary Fiber	7.0 g	Riboflavin	0.0 mg	Sodium	1.3 mg
Starch	0.0 g	Niacin	1.0 mg	Zinc	0.3 mg
Sugars	9.1 g	Vitamin B6	0.1 mg	Copper	0.1 mg
Protein	1.5 g	Folate	83.1 mg	Selenium	0.3 mg
Total fat	0.3 g	Folic Acid	0.0 mg	Caffeine	0.0 mg
Vitamins		Dietary Folate Equivalents	83.1 mg		
Vitamin A	88.4 IU				

2.4.2 pH

According to Table 2.4, there were four forms of anthocyanin with different colours and stability; flavium cation, carbinol pseudobase, neutral quinonoidol bases and ionized quinonoidol bases. Anthocyanins show red colour only in a very limited pH range, pH less than 2. As the pH is raised, kinetic and thermodynamic competition occurs between the hydration reaction on position 2 of flavylium cation and the proton transfer reactions related to its acidic hydroxyl groups. Also, increasing the pH causes a decrease of both the colour intensity and the concentration of the flavylium cation, as it is hydrated by nucleophilic attack of water, to the colourless carbinol form (Brouillard, 1982). While the first reaction gives colourless cabinol pseudobases that can undergo ring-opening to yellow chalcones, when the pH increases further, the latter reactions give rise to more violet quinonoidal bases (Brouillard, 1982; Francis, 1989). Therefore, it can be noted that anthocyanins are more stable in acidic condition than in alkaline solutions.

Table 2.4: Conformations of anthocyanins in aqueous conditions under varying pH (Brouillard, 1988)

pH	Colour	Forms of anthocyanin
<2	Red to orange	Flavylium cation
3-6	Colourless	Carbinol pseudobase Chalcone pseudobase
Neutral	Purple to violet	Neutral quinonoidol bases
8-10	Blue	Ionized quinonoidol bases

2.4.3 Temperature

The colour stability and rate of degradation of anthocyanins are affected by temperature. 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 ^{o}C, activation energy (E_a) and z values of around 70 kJ/mol and 25 ^{o}C, respectively, have been reported; while at processing temperatures, >70 ^{o}C, E_a values of 95 to 115 kJ/mol and z values of about 28 ^{o}C have been reported (Markakis, 1974).

Processing and storage under low temperature can improve the stability of anthocyanins (Delgado-Vargas & Peredes- Lopes, 2003). When temperature is increased, the unstable formation of the chalcone form is favored, and the chalcone is further degraded to brown products (Jackman & Smith, 1996). Similarly, Simard et al. (1981) found that for anthocyanin monomers stability decreased with increasing storage temperature such that juices stored at 15^{o}C and 20^{o}C were more stable than juices at 37 ^{o}C, stored for 3 months. Also, Palamidis & Markakis (1975) stated that increasing the storage temperature significantly quicken the destruction of grape anthocyanins in a carbonated beverage.

2.4.4 Anthocyanins concentration

Higher colour stability can be improved by an increase in the concentration of anthocyanins (Giusti & Wrolstad, 2003). Skrede et al (1992) reported that the addition of anthocyanins in strawberry syrup can increase the stability of colour. It was found that the stability of the major pigment of strawberry, pelargonoidin-3-glucoside, was similar to the delphinidin-3-glucosides and cyanidin-3-glucosides of blackcurrant once they are at a similar anthocyanin level (Skrede et al., 1992).

2.4.5 Light

Light strongly affects colour stability of anthocyanins. It favors their biosynthesis, but it accelerates their degradation (Markakis, 1982; Skrede, 1985; Francis, 1989). 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). The adverse effect of light on the colour of grape juice in bottles was reported by Tressler & Pederson (1936). Skrede (1985) found that blackcurrant syrups stored under dark conditions were more coloured than those stored in light. Similarly, the half life of the total anthocyanin concentration in blackcurrant nectar was thirty times greater once stored in dark conditions compared with light storage conditions (Iversen, 1999). Palamidis & Markakis (1975) also noted that the half life of grape juice colour was higher in dark, 416 days, than in daylight at 20^{o}C, 197 days.

2.4.6 Oxygen

Anthocyanin is very susceptible to oxygen due to the unsaturated nature of the anthocyanidin structure (Von Elbe & Schwartz, 1996). Therefore, oxygen can be a cause of degradation of anthocyanins via a direct oxidation reaction or by indirect oxidation whereby oxidised constituents of the medium react with anthocyanins. This was supported by Daravingas & Cain (1968) who reported that oxygen affected the destruction of the anthocyanin pigments of black raspberry once subjected to thermal degradation. Moreover, Skrede (1985) studied the effect of different types of packaging on the colour stability of blackcurrant syrups. It was found that the blackcurrant syrups contained in glass packaging material had a half life of colour stability about 35% longer than polyethylene terephthalate (PET) and approximately 80% longer than polyvinyl chloride (PVC). This was due to the permeability of the packaging material to oxygen gas.

2.4.7 Enzymes

Enzymes such as polyphenol oxidase, rich in fruit and vegetables, oxidises phenolic substrates to produce quinones which are polymerized further into browning pigments (Skrede & Wrolstad, 2002). Also, these quinones may react with anthocyanins chemically to form degraded complexes that caused a change in colour of products (Kader et al., 1999; Lee et al., 2002). In addition to polyphenol oxidase, glucosidases are very important in anthocyanin stability because of their degradation impact producing very unstable anthocyanidins and glycosides (Skrede & Wrolstad, 2002; Lee et al., 2002; Delgado-Vargas & Peredes- Lopes, 2003). Juice processing enzymes, such as pectinase and cellulases, can also affect the stability of anthocyanins as a result of glucosidase side activity of some enzyme preparations (Wightman & Wrolstad, 1996). Native peroxidase enzymes may be a cause of damage of anthocyanin in some berry products (Skrede & Wrolstad, 2002).

2.4.8 Sugars

If sugar is present in sufficient quantity to affect the water activity (a_w), it can give a protective effect (Francis, 1989). On the other hand, if sugars are present in much smaller quantities that they have a little effect on a_w, so they can sometimes accelerate anthocyanin degradation (Delgado-Vargas & Peredes- Lopes, 2003). Increased sugar level may affect the rate of anthocynin destruction. Wrolstad et al (1990) reported that the addition of sucrose can protect and improve the colour of frozen strawberries. This may be caused by an association with and hence inhibition of enzyme polyphenol peroxidase and peroxidase which oxidise phenolic substrates to produce quinones which are polymerized into brown pigments (Delgado-Vargas & Peredes- Lopes, 2003).

2.4.9 Metals

Some metal ions, such as tin, copper and iron can react with anthocyanins and form stable complexes and shift the colour of the pigment toward the blue end of the spectrum (Markakis, 1982). For example, cyanidin 3-glucoside forms a stable coloured complex in the presence of aluminium ions at pH 5.5 (Francis, 1989). Similarly, Asen et al. (1969) showed the remarkable effect that pH exerts on the colour and the formation of a complex between cyanidin 3-glucoside and aluminium. Also, calcium, iron, aluminium and tin were found to protect the anthocyanin stability of cranberry juice (Francis, 1989).

2.5 Phenolic acids

Phenolic acid compounds are universally distributed in plants (Haslam, 1982). They have been the subject of a great number of chemical, biological, agricultural, and medical studies. Phenolic acids form a diverse group that includes the widely distributed hydroxybenzoic and hydroxycinnamic acids**.**

2.5.1 Hydroxybenzoic acids

Hydroxybenzoic acids have a general structure of C6-C1 derived directly from benzoic acid (Figure 2.6). Variations in the structures of individual hydroxybenzoic acids are based on the hydroxylations and methylations of aromatic ring (Macheix et al., 1990). Four acids occur commonly: p-hydroxybenzoic, vanillic, syringic, and protocatechuic acid. They may be present in soluble form conjugated with sugar or organic acids, as well as bound to a cell wall part, such as lignin (Schuster &Herrmann, 1985). A common hydroxybenzoic acid is also salicylic acid (2-hydroxybenzoate). Gallic acid is a trihydroxyl derivative and is found free and as part of tannins (Haslam, 1982). Also, ellagic acid is in the form of hydrolyzable tannins called ellagitannins. Ellagitannins are esters of glucose with hexahydroxydiphenic acid; when hydrolyzed, they yield ellagic acid, the dilactone of hexahydroxydiphenic acid (Hollman & Venema, 1993).

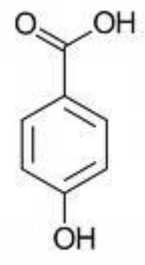


Figure 2.6: Chemical structure of p-hydroxybenzoic acid (Macheix et al., 1990):

2.5.2 Hydrocinnamic acids

The four most widely distributed hydroxycinnamic acids in fruit are p-coumaric, caffeic, ferulic and sinapic acids (Figure 2.7) (Macheix et al., 1990). Hydroxycinnamic acids usually occur in various conjugated forms and the forms are esters of hydroxyacids such as quinic and tartaric acid, as well as their sugar derivatives (Schuster &Herrmann, 1985; Macheix et al., 1990).

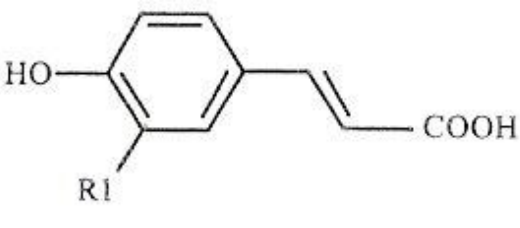


Figure 2.7: Chemical structure of three common hydroxycinnamic acids (Macheix et al., 1990):

p-coumaric acid, R1=H
caffeic acid, R1=OH
ferulic acid, R1=OCH_3

2.6. Phenolic acids in berries

The contents of phenolic acids in berries vary widely according to the literature (Table 2.5). Variations in the content of phenolic compounds within one species are mainly due to differences in the variety of berry, the degree of maturity at harvest, genetic differences, postharvest storage condition or processing (Schuster &Herrmann, 1985; Macheix et al., 1990; Amiot et al., 1995; Prior et al., 1998). Moreover, Zadernowski et al. (2005) stated that about twenty phenolic acids are found in berries. Of these, hydroxycaffeic, m- and p- coumaric, and 3, 4-dimethoxycinnamic acids are the main phenolic acids in blackberries and blueberries. M-coumaric acid is the major phenolic acid in black currant and blue berried honeysuckle.

Only a few studies are available on the content of phenolic acids in berries. For many berries such as bilberry, cranberry and gooseberry, no data on the contents of phenolic acids are available. Daniel et al. (1989) and Hollman & Venema (1993) reported that the content of ellagic acid in red raspberry and strawberry is found to be high, but not higher than that of anthocyanins.

Table 2.5: Phenolic acids content of different types of berries

Berry	**Phenolic acids (mg/kg fresh weight)**					
	p-coumaric	**Caffeic**	**Ferulic**	**Gallic**	**p-hydroxybenzoic**	**Ellagic**
Blueberry[a]	6-20	1860-2090	13-14	3-7	5-6	-
Red raspberry[a, b]	6-25	4-10	3-17	15-59	-	19-38
Strawberry[a, c, d, e]	7-27	0.5-7	2	1-44	10-36	90-402
Black currant[a]	20-44	68-84	18-24	4-13	-	4-11
Red currant[a]	5-15	3-8	1-3	9-13	3-80	-

[a] = Schuster &Herrmann, 1985; [b] = Mosel & Herrmann, 1974

[c] = Stohr & Herrmann, 1975; [d] = Daniel et al., 1989

[e] = Hollman & Venema, 1993

Also, caffeic acid, p-coumaric acid and ferulic acid concentrations are generally high in young fruits of red raspberries, black currants and strawberries, falling first rapidly and then more slowly during maturation and postharvest storage (Stohr & Herrmann, 1975). In strawberry, p-hydroxybenzoic acid appears at a relatively late stage of the fruit development (Stohr & Herrmann, 1975). The ellagic acid content of strawberries is higher in green than in red fruit pulp (Maas et al., 1991).

As mentioned above, a boysenberry is a cross between a blackberry, red raspberry and loganberry (blackberry x raspberry), so the major phenolic compounds in boysenberry may be similar to that in blackberry and red raspberry. According to the Table 2.6, twenty-two phenolic acids are identified in blackberry; however, the main phenolic acids of blackberry are hydroxycaffeic, 3, 4-dimethoxycinnamic, p-coumaric acid, m-coumaric acid, caffeic acid, gallic acid, ellagic and salicylic acid (Schuster &Herrmann, 1985; Macheix et al., 1990; Zadernowski et al., 2005). Red raspberry contains twelve phenolic acids. The major phenolic acids of the raspberry are p-hydroxybenzoic acid, gallic acid, ellagic and p-coumaric acid (Herrmann, 1973; Schuster &Herrmann, 1985; Macheix et al., 1990).

Table 2.6: The major phenolic acids found in blackberry and red raspberry

Phenolic acids	**Blackberry** **(*Rubus fructicosus)***	**Red raspberry** **(*Rubus idaeus*)**
Hydrobenzoic acid derivatives	p-hydroxybenzoic acid Protocatechuic acid Gallic acid Salicylic veratric Gentisic	Gallic acid p-hydroxybenzoic acid Protocatechuic acid
Hydrocinnamic acid derivatives	Caffeic acid 3'-p- coumaroylquinic 3'-p- coumaroylquinic 5'-p-coumaroylquinic 3'-caffeoylquinic 4'- caffeoylquinic Chlorogenic acid 3'-feruloylquinic 5'-feruloylquinic p-coumaric acid m-coumaric acid Ferulic acid hydroxycaffeic 3,4-dimethoxycinnamic	5'-galloylquinic 5'-p-coumaroylquinic 3'-caffeoylquinic 5'-caffeoylquinic 5'-feruloylquinic p-coumaric acid Caffeic acid Ferulic acid
Other phenolic acids	p-hydroxyphenyl-lactic Ellagic	Ellagic
References	Schuster &Herrmann, 1985; Macheix et al., 1990; Zadernowski et al., 2005	Herrmann, 1973; Schuster &Herrmann, 1985; Macheix et al., 1990

2.7 Copigmentation

Anthocyanin interacts with other phenolic compounds in solution to create the effect known as copigmentation. This is a transient interaction; no chemical bonds are formed. It is a result of the chemical phenomenon called charge transfer complex formation, or π-π complex (Waterhouse, 2002). This occurs when there are two aromatic ring substances in solution that have very different electron densities. The phenomenon of copigmentation is because of molecular associations between pigments and other organic molecules in solution, usually non-coloured, such as flavonoids and polyphenols, alkaloids, amino acids and organic acids (Rein, 2005). However, flavonoids (flavonones, flavanones and flavonols) and phenolic acids (hydroxycinnamic acids and hydroxybenzoic acids) are the most studied group of copigments (Waterhouse, 2002). These associations cause the pigments to exhibit far greater colour than would be expected from their concentration (Boulton, 2001; Rein, 2005).

The occurrence of copigmentation is based on at least two effects (Waterhouse, 2002). Firstly, the formation of the π-π complex causes changes in the spectral properties of the molecules in the flavylium form, resulting in hyperchromic shift (an increase in colour intensity) and bathochromic shift (a shift in the wavelength of maximum absorbance toward higher wavelength) (Giusti et al., 1999; Eiro & Heinonen, 2002). Secondly, the stabilisation of the flavylium form by the π complex shifts the equilibrium to better favor the flavylium, so increasing the proportion of anthocyanin molecules in the red-coloured form (Waterhouse, 2002) (Figure 2.8).

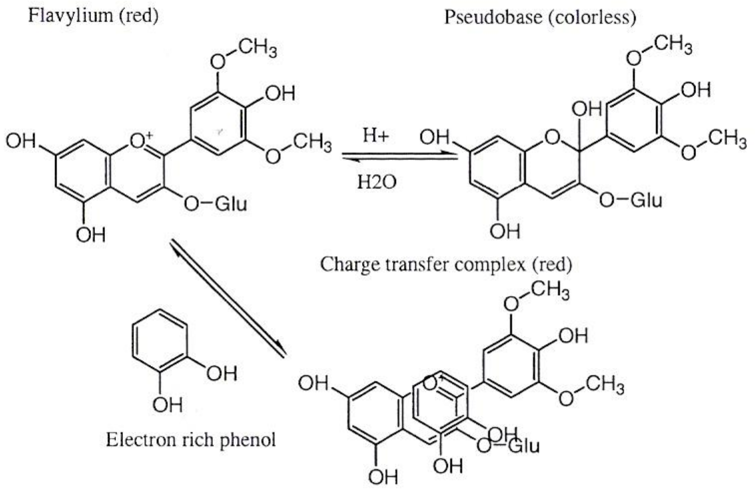


Figure 2.8: Copigmentation of wine anthocyanins (Waterhouse, 2002)

A summary of anthocyanins, copigments, and conditions used in major copigmentation studies is shown in Table 2.7. Also, many studies suggested that stability of anthocyanins can be improved through intramolecular, intermolecular copigmentation, self- association and metal complexion.

2.7.1 Intermolecular copigmentation

Intermolecular copigmentation is defined as the interactions between coloured anthocyanin and a colourless copigment, which is not bound covalently to the anthocyanin molecule. Asen et al. (1972) and Brouillard et al. (1989) reported that the main mechanistic driving forces for intermolecular copigmentation are hydrogen bonding and hydrophobic interactions. Moreover, electrostatic interactions can be a possible means for the intermolecular copigmentation (Chen & Hrazdina, 1981; Clifford, 2000). The structure of anthocyanins also can impact on the degree of copigmentation. For example, for 3-monoglucosides, maximum effect of colour improvement was observed to occur at pH 3.5-4.2; however, for 3, 5-diglucosides and 3-acylglucoside-5-glucosides this occurred at pH 3.1 (Williams & Hrazdina, 1979). In addition, the number of hydroxyl groups in the flavonoid copigment affects the magnitude of copigmentation. More hydroxyl groups present on the copigment, the stronger the copigmentation and complex formation.

Table 2.7: Summary of anthocyanins, copigments and conditions of major copigmentation studies

Anthocyanin	Copigments	Anthocynin concentration	Copigment/ anthocyanin ratio	Buffer system	Colour improvement	References
Cyanidin 3,5-diglucoside	30 cofactors (amino, benzoic and cinnamic acids, flavan-3-ols, flavonols, flavones)	6 mM	Ratio of 3	Citrate-phosphate pH = 3.32	Enhancement of 60-80% caffeic acid, actechin, 220-240% quercetin Wavelength shift (0 to 33 nm)	Asen et al., 1972
Cyanidin 3,5-diglucoside	Quercitin	5 mM, 7.5 mM, and 10 mM	Ratios of 0.1, 0.5, 1.0, 2.0, 3.0, 4.0, and 6.0	Citrate-phosphate pH = 4.38	Enhancement proportional to copigment to pigment and increase more than expected with pigment concentration.	Asen et al., 1972
Malvidin 3,5-diglucosides	Chlorogenic acid	773 µM	Ratio of 0, 1, 5, 10, and 20	Phosphate-acetate pH = 3.65	Effect of temperature 10-60^{o}C. No shift observed	Brouillard et al., 1989
Malvidin 3,5-diglucoside	Epicatechin, catechin, catechin-3-o-gallate, catechol, and phloroglucinol	1, 2 mM	Ratios of 8, 11, 14, 21, 32, and 33	None pH = 3.32	Enhancement of 68% phloroglucinol, 151% Epicatechin, and 221% catechin-3-o-gallate. Shifts of 6 nm to 19 nm	Liao et al., 1992
Malvidin 3-rhamonoside, 5-glucoside	Quercitrin and myricitrin	2.5 mM	Ratio of 3.2 of both copigments	Citrate-phosphate pH = 3.6 and 5.8	Enhancement of 600%. Shift of 50 nm.	Yoshitama et al., 1992
Pelargonidin 3-glucoside (Strawberry) and cyaniding 3-glucosides (chokeberry juice)	Chlorogenic acid	100 µM	Ratios of 0, 1, 10, 25, and 50 (w/w)	Britton buffer pH = 2.6, 3.2, 3.4, 3.6, 4.1, 4.5, and 6.0	Enhancement of 68% chokeberry juice, 49% Strawberry juice at pH =3.4 Shifts of 6 and 8 nm.	Wilska-Jeszka & Korzuchowska, 1996

Two complex formations can occur via intermolecular interactions; interlocked and parallel complex (Figure 2.9). As the flavylium cation and the quinonoidal base are almost planar, with efficiently delocalised π-electrons, this makes the interactions between copigments and the anthocyanin much easier leading to overlapping arrangement of the two molecules, protecting the coloured flavylium cation from the nucleophilic attack of the water molecule (Williams & Hrazdina, 1979; Delgado-Vargas & Peredes-Lopes, 2003). On the other hand, the parallel complex can occur between the keto group of the quinoidal base form and a flavonal copigment through hydrogen interactions (Williams & Hrazdina, 1979).

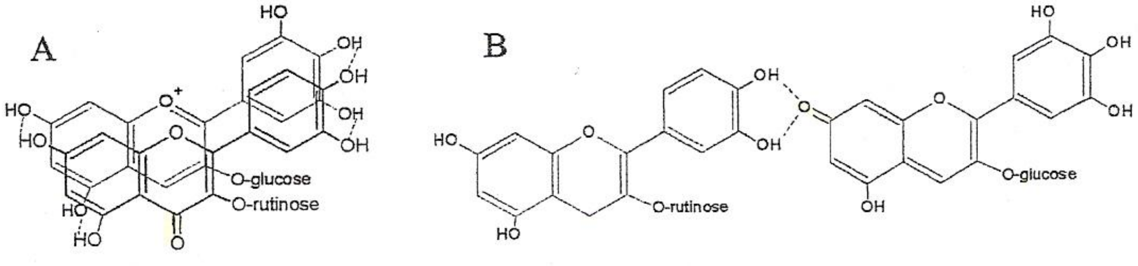


Figure 2.9: Two complex formations via intermolecular interaction: A) Interlocked complex and B) Parallel complex (Williams & Hrazdina, 1979)

In an anthocyanin stability study, Madhavi et al. (1996) attributed the better in- *vitro* stability of the pigment to the production of co-pigmenting agents, such as flavonols, phenolic acids, and tannins. Reaction between anthocyanins and tannins produces a “stabilised” anthocyanin or pigmented tannin, which persists much longer in wine than the initial form, and it is this stabilised colour that persists in most red wines more than a few years old (Waterhouse, 2002). Sweeney et al. (1981) reported that polyhydroxylated flavone, isoflavone, and aurone sulphonates increased the photo stability of anthocyanins. They attributed this effect to extensive H-bonding and ionic bonding between the negatively charged sulphonate and the electron deficient flavylium structure (Sweeney et al., 1981).

2.7.2 Intramolecular copigmentation

Francis (1989) defined intramolecular copigmentation as acylation of the anthocyanin molecule through covalent bonds and this acylation is attached to a sugar molecule of an anthocyanin. Also, the number of acyl groups, their structure, the position of attachment to glycosyl parts, the number and structure of saccharides impact on the intramolecular copigmentation (Dangles et al, 1993). Compared to intermolecular copigmentation, the intramolecular one is considered to be stronger and more effective because of strength of covalent bonds (Brouillard, 1982; Delgado-Vargas & Peredes-Lopes, 2003).

The exceptional colour stability of flower colours near neutral pH values was associated with intramolecular copigmentation, since anthocyanins of flowers are often acylated. This applies also to blackcarrot extracts and other edible plant materials that contain acylated anthocyanins in high amounts (Giusti & Wrolstad, 2003). The aromatic residue of acyl groups of an anthocyanin interact with the positively charged flavylium cation so that the reactivity of the carbon C-2 and C-4 with nucleophilic reactants is hindered (Brouillard, 1981). Brouillard (1981) also stated that the protective stacking is taking place on both sides of the anthocyanin aglycone and it is hydrophobic interactions between the flavylium aglycone and the aromatic residues of the acyl groups that are the driving forces for this stacking conformation.

2.7.3 Self-association

A subset of the interactions involving anthocyanins is the case of self-association of molecules in relatively concentrated (>1 mM) solutions (Asen et al., 1972; Hoshino et al., 1980; Hoshino et al., 1981). Asen et al. (1972) reported that by self-association cyanidin 3, 5-diglucoside solutions at 5 mM displayed twice the expected colour at pH values of 3.16, 4.12 and 5.10. Self-association is characterised by a hyperchromic shift in the wavelength of the absorbance maximum, that is, toward shorter values (Hoshino et al., 1981; Haslam, 1998). The relationship between anthocyanin concentration and the colour of solution in the case of self-association differs from that of copigmentation. It was expected that the equilibrium and colour response in self-association would be second order in nature; whereas for copigmentation it would be the first order with respect to the anthocyanin concentration (Boulton, 2001).

2.7.4 Pigment-metal complex

Robinson & Robinson (1931) have defined the pigment-metal complex as the formation of coloured complexes between some anthocyanins and certain metal cations such as Fe, Al, Sn and Cu at levels of 10 mg/L. The ability to form these complexes is correlated to an arrangement of ortho-dihydroxyl on the B ring, so that while the glycosides of cyanidin and petunidin can form complexes, but malvidin and pelargonidin cannot (Harborne, 1958).

Osawa (1982) stated that the blue colour of blueberries (*vaccinium* sp.) is associated with aluminium complexes of otherwise red anthocyanins. Also, the blueness and colour intensity of juice and wine from some grapes have been attributed to metal complexes because monoglucosides are more ionized (more colourful) than cyanidin 3, 5-diglucoside (Ingalsbe et al., 1963).

2.8. Analytical methods

2.8.1 Anthocyanins

There are a number of methods that can be used to determine anthocyanins, such as spectrophotometric methods and high performance liquid chromotography (HPLC), paper chromatography, thin layer and column chromatography. However, HPLC has been the most widely used method for separation of the individual anthocyanins (Hostettman & Hostettman, 1982; Hong & Wrolstad, 1990). Also, HPLC has been used for determining the anthocyanin composition of berry juices and for determining the authenticity of fruit juice concentrates and colour extracts (Hong & Wrolstad, 1990; Wightman & Wrolstad, 1996). The main benefits of HPLC over other chromatographic methods for phenolic separations is that it is rapid, sensitive automated, simple to clean and to prepare, the column is reusable and provides excellent qualitative and accurate quantitative analysis in the same operation (Strack & Wray, 1989; Hay, 2000).

2.8.1.1 High Performance Liquid Chromatography (HPLC)

The principle of HPLC is the sample is forced through a column of the stationary phase by pumping a mobile phase at high pressure. Then, the sample is introduced in a small volume to the stream of mobile phase and is retarded by specific chemical or physical interactions with the stationary phase as it traverses the length of the column. The amount of retardation depends on the nature of the analyte, stationary phase and mobile phase composition. The time at which a specific analyte comes out of the end of the column is called the retention time and is considered to be a reasonably unique identifying characteristic of a given analyte (Table 2.8) (Eder, 2000; Eder, 2004).

Table 2.8: Retention time and specific data of anthocyanins from blueberries by using reversed-phase liquid chromatography with photodiode array detection (Mazza et al., 2004)

Compound	**Retention time (min)**	**Wavelength maximum (λ max) (nm)**
Cyanidin-3,5-diglucoside	18.0	514
Pelargonidin-3,5-diglucoside	21.9	500
Peonidin-3,5-diglycoside	25.2	515
Cyanidin-3-glucoside	26.6	517
Malvidin-3,5-diglycoside	26.7	524
Cyanidin-3-arabinoside	29.2	517
Pelargonidin-3-glucoside	32.3	502
Peonidin-3-glucoside	36.0	518
Malvidin-3-glucoside	38.5	528
Cyanidin	43.0	526
Pelargonidin	48.5	517
Peonidin	50.6	528

Identification of anthocyanins can be done qualitatively and quantitatively. Qualitative analysis is when the retention time of known standards are matched with retention times of unknown components (Synder et al., 1983). However, quantification of compounds by HPLC is the process of determining the unknown concentration of a compound in a known solution. It involves injecting a series of known concentrations of the standard compound solution onto the HPLC for detection. The chromatograph of these known concentrations will give a series of peaks that correlate to the concentration of the compound injected (Synder et al., 1983; Hay, 2000).

8.1.2 Spectrophotometric methods

The determination of anthocyanin content by spectrophotometric methods is based on the fact that anthocyanins absorb energy when they are exposed to light. The concentration of the absorbing component in a sample solution is directly proportional to the absorbance of that components. Absorption spectra of berry juices are recorded using UV-visible spectrophotometer, scanning the visible range from 450-600 nm. A hyperchromic effect is detected as an increase in the absorbance value at λ_{max} due to the change in colour intensity and a bathochromic shift as a shift in the wavelength (nm) of maximum absorbance toward higher wavelengths (Eiro & Heinonen, 2002). Anthocyanins have absorption maximum in the 510-550 nm wavelength regions (Mazza et al., 2004). Francis (1982) suggested that readings at 2 pH levels can improve the problem of interfering substances that could absorb in a similar absorbance range.

2.8.2 Phenolic acids

The analysis of phenolic acids in foods begins with extraction. The extraction proceduce depends on the types of food to be analysed and the method to be used (Lee & Widmer, 1996). As many phenolic compounds occur as glycosides or esters, the sample preparation may include acid, alkaline or enzyme hydrolysis to free the bound phenolics (Rommel & Wrolstad, 1993).

2.8.2.1 Extraction and hydrolysis techniques

The most common solvents used for the extraction of phenolic acids from plants are ethyl acetate, diethyl ether and, methanol (Torres et al., 1987; Fernandez de Simon et al., 1990). Enzymatic hydrolysis with β-glucosidase has been applied for the analysis of phenolic acids (Kanes et al., 1993). Nevertheless, acid and alkaline hydrolysis is more commonly used for determination of phenolic acids in plants (Lee & Widmer, 1996). Acid hydrolysis has been done by heating the sample with HCl for 2 hours or more (Kuninori & Nishiyama, 1986). Alkali hydrolysis can be performed with NaOH at room temperature for 4-24 hours or 90 minutes at 60°C under N_2 (Torres et al., 1987; Rommel & Wrolstad, 1993). It has been found that the effectiveness of acid and base hydrolysis is similar to each other (Rommel & Wrolstad, 1993).

2.8.2.2 Chromatographic techniques

Thin-layer chromatography (TLC) has been used for quantitative analysis of phenolic acids by using normal phase chromatography on cellulose or silica layers and separating the compounds with a mixture of hydrocarbon carriers and polar organic solvents (Azar et al., 1987). Gas chromatography (GC) was applied for the analysis of phenolic acids in fruits and vegetables (Schuster & Herrmann, 1985). Reverse phase (RP-HPLC) is now commonly used for the separation of complex mixtures of phenolic compounds in plants. However, usually only a group of phenolics has been analyzed (Table 2.9) (Wulf & Nagel, 1978; Hakkinen et al, 1998).

Phenolic compounds absorb in the UV region and the most commonly used detector for HPLC is a variable-wavelength UV (Lee & Widmer, 1996). As phenolics show absorbance maximum at different wavelengths, single wavelengths cannot be found for monitoring all phenolics (Delage et al., 1991). Hydroxycinnamic acids absorb in two UV regions, one maximum being in the range of 225-235 nm and the other in the range of 290-330 nm (Ribereau-Gavon, 1972). However, detection at 280 nm is the best choice for the determination of phenolic compounds (Pussayanawin & Wetzel, 1987).

Table 2.9: High-performance liquid chromatography of phenolic acids in fruits

Compounds	Fruits	Column	Solvent system	Detection	Reference
Hydroxybenzoic acid, hydroxycinnamic acid	Bilberry juice	250 x 4mm LiChrosorb C18	A: 2 % acetic acid in water B: 2 % acetic acid/ water/acetonitrile (2:68:309) 7-100% B in 50 min	UV 280 nm	Azar et al., 1987
Hydroxybenzoic acid, hydroxycinnamic acid, Ellagic acid	Red raspberry	250 x 4.6 mm Spherisob ODS-1	A: aqueous 1 % acetic acid B: acetonitrile (2:68:309) 5-100% B in 77 min	PDA* 260,280, 320,360 nm	Rommel & Wrolstad, 1993
Hydroxycinnamic acid, flavonoids and anthocyanins	Wine	150 x 3.9 mm Novapak C18	A: 50 mM dihydrogen ammonium phosphate (pH 2.6) B: A/acetonitrile (20:80) C: 0.2 M orthophosphoric acid (pH 1.5)	PDA* 280,520 nm	Lamuela-Raventos & Waterhouse , 1994
Hydroxybenzoic acid, hydroxycinnamic acid	Blackberry, blueberry, red raspberry, strawberry	150 x 3.6 mm Novapak C18	A: 50 mM dihydrogen ammonium phosphate (pH 2.6) B: A/acetonitrile (20:80) C: 0.2 M orthophosphoric acid (pH 1.5) (100:0:0) to (0:80:20) in 60 min	PDA* 280,316 nm	Heinonen et al., 1998
Flavonoids, hydroxybenzoic acid, hydroxycinnamic acid	Strawberry and blackcurrant	100 x 4 mm ODS-Hypersil	A: 50 mM dihydrogen ammonium phosphate B: 0.2 M orthophosphoric acid C: A/acetonitrile	PDA* 260,280, 320,360 nm (20:80)	Hakkinen et al., 1998

2.9. Literature review conclusions

Anthocyanins are the largest group of water-soluble pigments in the plant kingdom and are responsible for the blue, red, and purple colours of many fruits, vegetables, grains, flowers, and leaves. In berry fruits, the anthocyanin content contributes to the darkness of the berry colour. The major anthocyanins of blackcurrants are cyanidin-3-rutinoside and delphinidin-3-rutinoside. For raspberries they are cyanidin-3-sophoroside and cyanidin-3-glucorutinoside. Twenty-five different anthocyanin pigments have been identified in strawberry, most of them containing pelargonidin-3-sophoroside. The anthocyanin composition of boysenberry was previously found to contain two main anthocyanins cyanidin-3-[2-(glucosyl)glucoside] and cyanidin-3-[2(glucosyl)-6-(rhamnosyl)glucoside]. Also, delphinidin-3-glucoside was identified to be the major anthocyanin present in pomegranate juice.

Anthocyanin molecules are unstable and highly susceptible to degradation. The colour stability of anthocyanins is influenced by structure, pH, temperature, light, anthocyanin concentration, oxygen, enzymes, sugars and the presence of complexing compounds such as other flavonoids, phenolic acids and metals.

Stability of anthocyanins can be enhanced by intramolecular, intermolecular, self association or pigment-metal complex copigmetation, by protecting the coloured flavylium cation from the nucleophilic attack of the water molecule. Acylated anthocyanins containing two or more aromatic acyl groups may affect the colour through a mechanism called intramolecular copigmentation. Anthocyanins also interact with other flavonoids and phenolic acids to produce an increase in colour intensity (hyperchromic effect) and a shift in the wavelength of maximum absorbance toward higher wavelengths (bathochromic effect). Flavonoids (flavonones, flavanones and flavonols) and phenolic acids (hydroxycinnamic acids and hydroxybenzoic acids) have been of interest for copigmentation researchers, and have shown a prominent effect on the enhancement and stabilization of anthocyanin.

Only a few studies are available on the content of phenolic acids in berries. About twenty phenolic acids are found in berries. Of these, p- and m-coumaric acid, caffeic acid, ferulic acid, gallic acid, p-hydroxybenzoic acid and ellagic acid are the main phenolic acids in most berries. Red raspberry contains twelve phenolic acids. The major phenolic acids of the raspberry are p-hydroxybenzoic acid, gallic acid, ellagic acid and p-coumaric acid. M-coumaric acid is found to be the major phenolic acid in black currant. The content of ellagic acid in red raspberry and strawberry is found to be high, but not higher than that of anthocyanins. The main phenolic compounds present in pomegranate juice are gallagyl-type tannins (punicalagin B and D), ellagic acid and hydrolysable tannins (galloyl glucose).

The determination of anthocyanin content and phenolic aicds by spectrophotometric methods is based on the fact that anthocyanins and phenolics absorb energy when they are exposed to light. The concentration of the absorbing components in a sample solution is directly proportional to the absorbance of those components. HPLC has been the most widely used method for separation of the individual anthocyanins and phenolic acids. Also, HPLC has been used for determining the anthocyanin composition of berry juices and for determining the authenticity of fruit juice concentrates and colour extracts.

CHAPTER 3

MATERIALS AND METHODS

3.1 Analysis of copigmentation reactions of boysenberry juice

3.1.1 Experimental design of the work

Table 3.1 summarises the experimental plan and conditions for each separate study.

Study 1 looked at the anthocyanin and phenolic acid composition of blackcurrant, cranberry, pomegranate and boysenberry juices.

Study 2 studied the enhancement of three juices (blackcurrant, cranberry and pomegranate) with boysenberry juice.

Study 3 investigated the copigmentation effects of phenolic acids: kaemferol, quercetin and ellagic acid (found in boysenberry) on blackcurrant juice.

Study 4 evaluated if there were any anthocyanin copigmentation effects occurring between boysenberry and blackcurrant when ellagic acid was also added to blackcurrant juice.

Table 3.1 Summary of experimental design

Study	Juices	Enhancers	Storage temperature (°C)	Methods
1	*Blackcurrant* *Cranberry* *Pomegranate* *Boysenberry* *(10°Brix)*	-	-	*Spectrophotometry* Total anthocyanins content Colour density and polymeric colour Total phenolics content *HPLC* Anthocyanin and phenolic acid contents
2	*Blackcurrant* *Cranberry* *Pomegranate* *(10°Brix)*	*Boysenberry juice (0,1,2 and 3% (w/v))*	*5, 20 and 35*	*Spectrophotometry* Total anthocyanins content Colour density and polymeric colour Hyperchromic effect Bathochromic effect Total phenolics content *HPLC* Change in anthocyanin, and phenolic content
3	*Blackcurrant* *(10°Brix)*	*Pure phenolic acids (kaemferol, quercetin and ellagic acid) at 0, 0.2, 0.6 and 1mg/L concentrations*	*5 and 20*	*Spectrophotometry* Total anthocyanins content Colour density and polymeric colour Hyperchromic effect Bathochromic effect Total phenolics content *HPLC* Change in anthocyanin, and phenolic content
4	*Blackcurrant* *(10°Brix)*	*Ellagic acid (0, 0.2, 0.6 and 1mg/L concentrations) and boysenberry juice (0,1,2 and 3% (w/v))*	*5*	*Spectrophotometry* Total anthocyanins content Hyperchromic effect *HPLC* Change in anthocyanin, and phenolic content

3.2 Materials

3.2.1 Juices and colour enhancer

3.2.1.1 Samples

Boysenberry juice concentrate was supplied by Berryfruit Export NZ Ltd. (Nelson, New Zealand), at a soluble solids concentration of 65 ^{o}Brix. All juices were enhanced with boysenberry juice (10^{o}Brix) in the concentrations of 0, 1, 2 and 3 % (w/v).

Blackcurrant concentrate

The New Zealand Blackcurrant Co-operative Ltd. (Nelson, New Zealand) supplied the blackcurrant concentrate, at a soluble solids concentration of 65 ^{o}Brix.

Cranberry and pomegranate concentrates were supplied by Northwest Naturals (USA), at a soluble solids concentration of 50 and 65 ^{o}Brix, respectively.
After arrival, all concentrates were stored frozen at -20^{o}C. Before analysis, they were thawed and adjusted to single strength juice (10^{o}Brix) with distilled water.

3.2.1.2 Water

Distilled water was used to produce the drinks.

3.2.2 Pure anthocyanin standards

Cyanidin-3-rutinoside (Extrasyntheses, Genay, France) (Purity>96%)
Cyanidin-3-arabinoside (Polyphenols, Sweden) (Purity>97%)
Cyanidin-3-galactoside (Polyphenols, Sweden) (Purity>97%)
Cyanidin-3-glucoside (Extrasyntheses, Genay, France) (Purity>96%)
Cyanidin-3, 5-diglucoside (Apin Chemicals LTD., UK) (HPLC grade)
Delphinidin-3-glucoside (Extrasyntheses, Genay, France) (Purity>95%)
Delphinidin-3, 5-diglucoside (Apin Chemicals LTD., UK) (HPLC grade)
Delphinidin-3-rutinoside (Apin Chemicals LTD., UK) (HPLC grade)
Peonidin-3-galactoside (Polyphenols, Sweden) (Purity>97%)
Pelargonidin-3-glucoside (Extrasyntheses, Genay, France) (Purity>94%)

3.2.3 Pure phenolic acid standards

Gallic acid (Sigma, Germany) (Purity>97%)
Catechin (Sigma, Germany) (Purity>98%)
Benzoic acid (Aldrich, Germany) (Purity>99%)
Epicatechin (Sigma, Germany) (Purity>90%)
Caffeic acid (Sigma, Germany) (Purity>98%)
Ellagic acid (Fluka, USA) (Purity>96%)
P-coumaric acid (Sigma, Germany) (Purity>98%)
Cinnamic acid (Aldrich, Germany) (Purity>97%)
Ferulic acid (Aldrich, Germany) (Purity>99%)
Myricetin (Sigma, Germany) (Purity>96%)
Quercetin (Sigma, Germany) (Purity>98%)
Kaempferol (Fluka, Germany) (Purity>96%)

3.2.4 Sample preparation

Juice samples (raspberry, strawberry, blackcurrant and pomegranate) were filtered through Whatman #1 filter paper before analysis. Boysenberry juice concentrate was diluted to 10±0.5 ^{o}Brix before filtering. After addition of the copigments as shown in Table 3.1, the juices were kept for storage in sealed brown glass bottles and were then allowed to equilibrate for 30 minutes at room temperature before the first measurement. pH of the juices was measured in the beginning of storage.

3.3 Analytical methods

3.3.1 Determination of the total monomeric anthocyanin concentration

3.3.1.1 Reagents

Buffers for determination of anthocyanin content

pH 4.5 buffer

Four hundred millilitres (400 mL) of 1M sodium acetate (BDH) (136 g/L) and 240 mL of 1M hydrochloric acid (83 mL conc. HCl/L) (BDH) was added to 360 mL distilled water and mixed well. The pH of the solution was measured and adjusted with hydrochloric acid to obtain a final pH of 4.5.

pH 1.0 buffer

Two hundred and fifty millitres (250 mL) of 0.2 M potassium chloride (14.9 g/L) was added to 750 mL of 0.2 M hydrocholoric acid (BDH) (200 mL of 1M HCl/L) and mixed well. The pH of the solution was measured and adjusted with hydrochloric acid to obtain a final pH of 1.0.

3.3.1.2 Procedure

The determination of monomeric anthocyanin concentration using the pH differential method was based on the method described by Wrolstad, et al. (2005a). Each sample was diluted with buffer pH 1.0 to a level at which the maximum absorbance was between 0.4-0.6. The same dilution factor was used to dilute a second juice sample with pH 4.5 buffer. Samples are analysed using a double beam UV-visible spectrophotometer (Shimadzu Scientific Instruments, Japan). The cuvettes used had a pathlength of 10 mm and had a grade of G (glass/visible light). Distilled water was used in the reference cell. Absorbances were measured at 510 nm and 700 nm for the pH 1.0 and the pH 4.5 solutions (Wrolstad et al., 2005a). Samples were analysed in duplicate.

3.3.1.3 Calculation of total monomeric anthocyanin content

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

$$\text{Concentration main anthocyanin (mg/L)} = \frac{A}{\varepsilon L} \times 10^3 \times MW \times \text{dilution factor}$$

Where;

A = $(A_{510nm\ pH\ 1.0} - A_{700nm\ pH\ 1.0}) - (A_{510nm\ pH\ 4.5} - A_{700nm\ pH\ 4.5})$

ε = molar absorbance of major anthocyanin pigment

L = cuvette pathlength

MW = molecular weight of main anthocyanin pigment

3.3.2 Determination of colour density and polymeric colour the juice samples using the metabisulphite method

3.3.2.1 Reagent

20% w/v Potassium metabisulphite solution

Two grams of potassium metabisulphite (BDH, Analar, England) is made up to 10 mL with distilled water.

3.3.2.2 Procedure

The method was based on the method described by Wrolstad et al. (2005a). Two hundred microlitres (0.2 mL) of 20% w/v potassium metabisulphite solution was added to a 3.0 mL juice sample and 200 microliters of H_20 was added to a 3.0 mL control sample. Samples were analysed using a double beam UV-visible spectrophotometer. The cuvettes used had a pathlength of 10 mm and had a grade of G (glass/visible light). Distilled water was used in the reference cell. The absorbances of the two samples were then measured at 420 nm and 700 nm.

3.3.2.3 Calculation of colour density

The colour density was determined by summing the absorbance of the control sample at 420 nm and 520 nm. Turbidity was corrected for by subtracting any absorbance at 700 nm. The equation used to calculate the colour density was:

Colour density $= [(A_{520nm} - A_{700nm}) + [(A_{420nm} - A_{700nm})] \text{ x dilution factor}$

3.3.2.4 Calculation of polymeric colour

The method for calculating polymeric colour was calculated by applying the same procedure as used in determining colour density to the bisulphate treated sample. The equation used to calculate the polymeric colour was:

Polymeric colour $= [(A_{520nm} - A_{700nm}) + [(A_{420nm} - A_{700nm})] \text{ x dilution factor}$

3.3.3 Determination of total phenolic acids using the modified Folin-Ciocalteu procedure

Total phenolic content was determined using the modified Folin-Ciocalteu procedure described by Singleton & Rossi (1965) and expressed as milligrams of gallic acid equivalent per gram (GAE). Extraction of juice samples with acetone precipitates the proteins and ascorbate oxidase treatment is helped to specifically oxidize the ascorbic acid to avoid interferences in the assay (Singleton & Rossi, 1965; Zocca & Ryugo, 1975).

3.3.3.1 Reagents

Folin-Ciocalteu stock reagent (Scharlau, Spain)
The reagent was diluted with distilled water to 1:10.

Sodium carbonate stock solution Anhydrous Na_2CO_3 (Sigma, Germany) was dissolved in distilled water to make up to 75 gL^{-1}.

Gallic acid standard stock solution (Scharlau, Spain)

Dissolve 200mg in 1 litre distilled water. From this, dilute:

5 mL to 100 mL with distilled water, final concentration = 10 µg mL^{-1} gallic acid.

10 mL to 100 mL with distilled water, final concentration = 20 µg mL^{-1} gallic acid.

15 mL to 100 mL with distilled water, final concentration = 30 µg mL^{-1} gallic acid.

20 mL to 100 mL with distilled water, final concentration = 40 µg mL^{-1} gallic acid.

25 mL to 100 mL with distilled water, final concentration = 50 µg mL^{-1} gallic acid.

AR acetone

1 M potassium hydroxide (Scharlau, Spain)

3.3.3.2 Procedure

Extraction

Five millitres (5 mL) of juice samples were mixed with 50 mL AR acetone in 100 mL conical flask and the mixtures were held at room temperature for 30 minutes. A white precipitate of protein formed immediately and were filtered through No. 542 Whatman paper into a 250 mL round bottom flask and the conical flask was rinsed with a further about 25 mL. The organic solvent was removed in 35°C incubator and the residue was dissolved in distilled water (3 x 30 mL). After that, the mixture was filtered through No. 4 Whatman paper into a 100 mL volumetric flask and is made up to volume. This achieves an overall 1:20 dilution of the acetone soluble components of the juice.

Ascorbate oxidase treatment

The pH of the 1:20 dilutions was adjusted to 5.0 with 1 M KOH. Then, a 5 ml of sample was treated with an ascorbate oxidase spatula for 15 minutes and was stirred vigorously for 5 seconds (5 times during the 15 minutes were required).

Assay

A 1 mL of sample (after extraction and ascorbic oxidase treatment) was mixed 5 ml of diluted Folin-Ciocalteu reagent. Within 0.5-8 minutes, 4 mL of aqueous Na_2CO_3 was added to the mixture and mixed. After 2 hours of incubation at 35^oC, the mixture was cooled to room temperature and the absorbance was measured at 765 nm versus H_2O at 20^oC. Results were expressed as milligrams of gallic acid equivalent per gram (GAE).

3.3.4 Determination of hyperchromic and bathochromic shift

The hyperchromic effect means an increase in colour intensity while the bathochromic shift consists of a shift of the wavelength of maximum absorbance (Brouillard, 1982). A wide range of different molecules has been found to act as copigments. The method for hyperchromic and bathochromic shift was the same procedure as colour density. Samples were analysed using a double beam UV-visible spectrophotometer. The cuvettes used had a pathlength of 10 mm and had a grade of G (glass/visible light). Distilled water was used in the reference cell. The juice samples were diluted with distilled water to give an absorbance reading. The absorbance spectra were scanned from 450 to 610 nm for a maximum wave length (λ_{max}) (Wrolstad et al. (2005a). Each spectroscopic measurement was repeated two times. For bathochromic shift measurement, the juices were also scanned the absorbance from 450 to 610 nm to see which wavelength give the maximum absorbance (Chen and Hrazdina, 1981; Mazza and Miniati, 1993).

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3.3.5 Determination of individual anthocyanin pigments using high performance liquid chromatography (HPLC)

3.3.5.1 Reagents and sample preparation

Solvent

Acetonitrile (Merck, Germany)
10% acetic acid (BDH, Analar, England)/ 5% acetonitrile/ 1% phosphoric acid in water (Scharlau, Spain)

3.3.5.2 HPLC column and procedure

The column used for the analysis was a Prodigy ODS-C18 with dimensions of 250 mm x 4.6 mm internal diameter (Phenomenex, New Zealand). Fifty microlitres (50 µL) of sample was injected into an auto-sampler with the flow rate set at 0.8 mL/min. and the detection UV wavelength was at 520 nm. The mobile phrases were acetonitrile (A) and 10% acetic acid/ 5% acetonitrile/ 1% phosphoric acid in water (B). Each sample was filtered through 0.45 um syringe filters before injection to the HPLC (Shimadzu Instruments). The concentration of individual anthocyanins was calculated using an integrator that measured peak area. The anthocyanin standards (delphinidin-3-rutinoside, cyanidin-3-rutinoside, delphinidin-3-glucoside, cyanidin-3-glucoside, peonidin-3-galactoside, cyanidin-3-arabinoside, cyanidin-3-galactoside, delphinidin-3, 5-diglucoside and pelargonidin-3-glucoside) were run as samples. All measurements were done in duplicate.

Table 3.2: HPLC Gradient for anthocyanin separation on Prodigy ODS column (Wrolstad et al., 2005b)

Time (min)	Percent A	Percent B
0	0	100
5	0	100
20	20	80
25	40	60
30	0	100

A: acetonitrile

B: 10% acetic acid/ 5% acetonitrile/ 1% phosphoric acid in water

3.3.6 Determination of individual phenolic acids using high performance liquid chromatography (HPLC)

3.3.6.1 Reagents

Extraction and hydrolysis

50% methanol (Scharlau, Spain)
1.2 M hydrochloric acid (Labserve, New Zealand)

Mobile phase

50 mM dihydrogen ammonium phosphate (BDH, Analar, England)
Acetonitrile
0.2 M Orthophosphoric acid (Merck, Germany)

Ascorbic acid standard solution

Pure phenolic acids

Gallic acid, catechin, benzoic acid, epicatechin, caffeic acid, ellagic acid, p-coumaric, cinnamic acid, ferulic acid, myricetin, quercetin and kaemferol.

3.3.6.2 HPLC column and procedure

The column used for the analysis was a Synergi Fusion RP80 C18 (100x4mm, 3.5um) (Phenomenex, New Zealand). Twenty microlitres (20 µL) of sample was injected an auto-sampler with the flow rate set at 0.5 mL/min. and the detection wavelengths were at 260, 280 and 320 nm, set on the PDA detector (Shimatzu instruments Ltd). Ellagic and benzoic acids were detected at 260 nm, gallic acid, catechin and epicatechin at 280 nm, p-coumaric acid, cinnamic acid, ferulic acid, myricetin, quercetin and kaempferol at 320 nm. The mobile phrases were 50 mM dihydrogen ammonium phosphate, adjusted to pH 2.6 with orthophosphoric acid (A), 0.2 M orthophosphoric acid, adjusted with ammonia to pH 1.5 (B) and 20% A with 80% acetonitrile (C). Each sample was filtered through 0.45 um syringe filters before injection to the HPLC. The concentration of individual phenolic acids was calculated using an integrator that measured peak area. All measurements were done in duplicate.

Table 3.3: HPLC Gradient for phenolic acids separation on Synergi Fusion RP80 C18 column (Hakkinen et al., 1998)

Time (min)	Percent A*	Percent B*	Percent C*
0	100	0	0
5	0	8	92
15	0	20	80
20	0	30	70
25	0	40	60
35	0	80	20
40	0	80	20
50	100	0	0

* Solvent A = 50 mM dihydrogen ammonium phosphate, adjusted to pH 2.6 with orthophosphoric acid; Solvent B = 20% A with 80% acetonitrile; Solvent C = 0.2 M orthophosphoric acid, adjusted with ammonia to pH 1.5.

3.3.6.3 Sample and standard preparation

Extraction and hydrolysis

The samples are extracted and hydrolysed with 50% methanol and 1.2 M HCl for 16 hrs at room temperature (35^oC) under nitrogen.

Sample preparation

Eighty grams (80 g) of ascorbic acid was dissolved in 15 mL of distilled water in a 100 mL round bottomed bottle. The sample is weighed and rinsed with 25 mL of methanol to the bottle. To this extract, 10 mL of 1.2 M HCl is added and then sonicated for 2 minutes. The remaining air in the bottle is replaced by nitrogen gas. Fifty milliliters (50 ml) of total volume of the extract was shaken in a 35^oC water bath in the dark. After 16 hours, the extract is allowed to cool and is then filtered. A 15 ml of the filtrate was evaporated to dryness by using a rotary evaporator and 35^oC water bath. The residue was dissolved in 1.5 ml of methanol and filtered through a 0.45 um filter prior to injection to the HPLC apparatus (Hakkinen et al., 1998).

3.3.7 Calculations of rate constants of degradation (k)

The order of degradation for anthocyanin and phenolics acid was determined to be first order. The equations for calculating shelf life are:

$$\ln C_A \quad = \ln C_0 - kt$$

Where:

C_A = Concentration of component A

C_0 = Concentration at time

T = Time

K = Rate of change of degradation

3.3.8 Statistical analysis

Study 2 & 3

One way ANOVA was conducted using SPSS, version 15.Tukey’s procedure was used to see if there are any significant differences between means of all measurements (duplicate)

Study 4

StepWise Multiple Regression was used to investigate which factor was a higher correlation with colour stability of blackcurrant juice, by comparing R Square or each factor.

CHAPTER 4

BOYSENBERRY JUICE ENHANCEMENT

4.1 Anthocyanin and phenolic acid composition (study 1)

The first stage of this project was to determine the colour and composition of boysenberry, blackcurrant, cranberry and pomegranate juices. The juice concentrates as supplied were all diluted to 10.0 ^{o}Brix before analyses. The juices were analysed by spectrophotometric methods to determine total monomeric anthocyanin content (Section 3.2.2), colour density (Section 3.2.3), polymeric colour (Section 3.2.3) and total phenolic content (Section 3.2.4). The total monomeric anthocyanin content is a measure of total monomeric anthocyanins based on a pH differential method that measures the absorbance at two different pH values (1.0 and 4.5), and relies on the structural transformations of the anthocyanin chromophore as a function of pH. As anthocyanin pigments can undergo reversible structural tranformations with a change in pH resulting in strikingly different absorbance spectra. The coloured flavylium cation form predominates at pH 1.0 and the colourless carbinopseudo-base form at pH 4.5 (Table 2.4). Colour density is a measure of the intensity of the colour and defined as the sum of absorbances at the λ_{max} and at 420 nm. Polymeric colour is the same measurement for the bisulphite treated samples and is a measure of the degree of polymerization of the anthocyanins. Monomeric anthocyanin pigments will combine with bisulphite to form colourless compounds, while polymeric anthocyanin –tannin and melanoidin pigments are resistant to bleaching by bisulphite and will remain coloured at 420 nm (Wrolstad et al., 2005a). Total phenolic content is measured by oxidising phenolic substrates in juice samples with Folin-Ciocalteau reagent and measuring absorbance at 765 nm (Singleton & Rossi, 1965; Zocca & Ryugo, 1975). The juices were also analysed by HLPC techniques to quantify individual anthocyanins and phenolic acids.

The total anthocyanin content, colour density, polymeric colour, total phenolic content and pH of boysenberry, blackcurrant, cranberry and pomegranate juices at 10.0 ^{o}Brix are shown in Table 4.1. The colour density of the juices ranged between 9.7 and 85.1. Blackcurrant juice had the highest colour density (85.1), while for boysenberry juice it was a little lower at 66.4. Cranberry and pomegranate juice had a much lower colour densities. This means that at the same soluble solids concentration, there were less anthocyanins present in pomegranate and cranberry compared to boysenberry and blackcurrant juice. A similar result was found in total anthocyanin content measurements. Blackcurrant juice had the highest concentration of total anthocyanins, followed by boysenberry juice, cranberry juice and pomegranate juice, respectively. The total anthocyanin content calculated from HPLC of boysenberry, blackcurrant, cranberry and pomegranate juices are shown in Table 4.2. The total anthocynins determined from HPLC analysis for boysenberry, blackcurrant and cranberry juices were less than the total anthocyanin value by the spectrophotometric method by 4, 16 and 5%, while pomegranate total anthocyanins by HPLC was higher than the spectrophotometric method by 10%. The reason for the slightly lower values in the boysenberry, blackcurrant and cranberry juices could be because minor anthocyanins were not detected by HPLC or the peaks were too small to quantify.

The polymeric colour indicating the degree of polymerization was greatest in boysenberry juice and least in pomegranate juice (Table 4.1). The pH of the four juices at 10.0 ^{o}Brix were similar to each other and ranged between 2.5 and 3.2. Among these juices, boysenberry juice had the highest pH, 3.2 and cranberry juice had the lowest with pH 2.5.

Table 4.1 Anthocyanin and phenolic contents of four juices (mean ± SD, n=2)

Juices	Total anthocyanin content (mg/L)*	Total phenolic acids content (mg/L)*	Colour density*	Polymeric colour*	pH
Boysenberry	1194.6 ± 4.8	550.6 ± 17.5	66.4 ± 2.7	23.0 ± 0.3	3.2
Blackcurrant	1308.2 ± 63.3	264.3 ± 22.6	85.1 ± 2.0	13.7 ± 0.4	2.9
Cranberry	304.1 ± 5.4	145.4 ± 1.5	27.2 ± 0.7	7.4 ± 0.2	2.5
Pomegranate	90.4 ± 0.0	16.6 ± 0.3	9.7 ± 0.0	1.9 ± 0.0	2.7

* The data was calculated from juices at single strength of 10^{o}Brix

The major anthocyanin and phenolic acids present in all the juices identified by high performance liquid chromatography (HPLC) are presented in Table 4.2.

Table 4.2 Identification of major individual anthocyanin and phenolic acids found in four berry juices (mean ± SD, n=2).

	°Brix	Concentrations of anthocyanins (mg/L)		Concentrations of phenolic acids (mg/L)
Boysenberry	10		**Boysenberry**	
Cyanidin-3-sophoroside		~500.57[a]	Kaemferol	201.90 ± 10.8
Cyanidin-3-glucoside		403.83 ± 0.9	Quercetin	187.14 ± 2.5
Cyanidin-3-glucosyl-rutinoside		~80.24[a]	Ellagic acid	127.45 ± 6.4
Cyanidin-3-rutinoside		15.33 ± 0.1		
Total anthocyanin content		1,000.0	Total phenolic acids	517.0
Blackcurrant	10		**Blackcurrant**	
Delphinidin-3-rutinoside		562.70 ± 9.1	p-coumaric	113.56 ± 0.7
Cyanidin-3-rutinoside		418.78 ± 18.3	Caffeic acid	74.30 ± 2.5
Delphinidin-3-glucoside		174.62 ± 1.0	Benzoic acid	37.40 ± 0.9
Cyanidin-3-glucoside		104.28 ± 0.5	Quercetin	25.26 ± 0.1
Total anthocyanin content		1,258.0	Total phenolic acids	250.7
Cranberry	10		**Cranberry**	
Peonidin-3-galactoside		119.32 ± 1.0	Benzoic acid	55.77 ± 0.6
Cyanidin-3-galactoside		86.17 ± 0.2	Quercetin	42.93 ± 0.8
Cyanidin-3-arabinoside		43.43 ± 0.2	Myricetin	22.76 ± 1.4
Peonidin-3-arabinoside		34.71 ± 0.2	p-coumaric	14.42 ± 0.1
Total anthocyanin content		282.0	Total phenolic acids	135.8
Pomegranate	10		**Pomegranate**	
Cyanidin-3-glucoside		30.47 ± 0.1	Ellagic acid	9.31 ± 0.1
Delphinidin-3,5-diglucoside		21.48 ± 0.4	Gallic acid	6.37 ± 0.0
Pelargonidin-3-glucoside		14.31 ± 0.4		
Delphinidin-3-glucoside		10.60 ± 2.0		
Cyanidin-3-rutinoside		8.56 ± 0.0		
Cyanidin-3-arabinoside		8.60 ± 0.0		
Cyanidin-3-galactoside		5.49 ± 0.0		
Total anthocyanin content		99.5	Total phenolic acids	15.6

[a] The concentration was estimated based on the cyanidin-3-glucoside standard on anthocyanins chromatogram of boysenberry.

Identification and peak assignment of anthocyanins and phenolic acids in the four berry juices was based on comparison of their retention times with those of pure standards and from published data. Boysenberry has been reported to contain four anthocyanins (Wightman & Wrolstad, 1996). Similarly, in this study we were able to detect the same four anthocyanins in boysenberry juice (Table 4.2; Figure 4.1 (A)). They were cyanidin-3-sophoroside (Peak 1), cyanidin-3-glucoside (Peak 3), cyanidin-3-glucosyl-rutinoside (Peak 2) and cyanidin-3-rutinoside (Peak 4); the two major anthocyanins are cyanidin-3-sophoroside (~50%) and cyanidin-3-glucoside (40%), respectively. Also, only three major phenolic acids were found in boysenberry juice, they were kaemferol (Peak3), quercetin (Peak2) and ellagic acid (Peak1) (Table 4.2). This has not yet been reported in literature the phenolic acid composition of boysenberry.

Blackcurrant was reported in the literature to contain four major anthocyanins (cyanidin-3-rutinoside (Peak 4), cyanidin-3-glucoside (Peak 3), delphinidin-3-rutinoside (Peak 2) and delphinidin-3-glucoside (Peak 1)) (Machiex et al., 1990; Kahkonen et al., 2003). Similar results were found in blackcurrant juice in this study. The two major anthocyanins were delphinidin-3-rutinoside (44.5%) and cyanidin-3-rutinoside (33%) (Table 4.2; Figure 4.1 (B)). Other studies have found cyanidin-3-rutinoside at (36%) and delphinidin-3-rutinoside at (34%) were the main anthocyanins (Machiex et al., 1990; Kahkonen et al., 2003). This difference in anthocyanin composition may be due to differences in blackcurrant variety, growing location and growing season. Schuster & Herrmann (1985) had reported that caffeic, p-coumaric, ferulic, gallic and ellagic acids are the five major phenolic acids found in blackcurrant juice; however, in this study, the following four phenolic acids were found in the blackcurrant juice tested. They were p-coumaric acid (Peak3), caffeic acid (Peak2), benzoic acid (Peak1) and quercetin (Peak4) (Table 4.2).

In this study, four major anthocyanins were found in cranberry juice (Table 4.2; Figure 4.1 (C)). They were peonidin-3-galactoside (Peak 3), cyanidin-3-galactoside (Peak 1), cyanidin-3-arabinoside (Peak 2) and peonidin-3-arabinoside (Peak 4), respectively. This result was similar to Wu & Prior (2005) who found that cranberry fruit contained 13 anthocyanins; they found that the main four anthocyanins were peonidin-3-galactoside, cyanidin-3-galactoside, cyanidin-3-arabinoside and peonidin-3-arabinoside. Four major phenolic acids identified in cranberry juice were benzoic acid (Peak1), quercetin (Peak 3), myricetin (Peak4) and p-coumaric acid (Peak2), respectively (Table 4.2).

Du et al. (1975) and Gil et al. (1995) reported six anthocyanins in pomegranate juice: delphinidin-3-glucoside and delphinidin-3,5-diglucoside, cyanidin-3-glucoside and cyanidin-3,5-diglucoside, pelargonidin-3-glucoside and pelargonidin-3,5-diglucoside. Seven anthocyanins were identified in pomegranate juice in this study (Table 4.2; Figure 4.1 (D)). They were cyanidin-3-glucoside (Peak 4), delphinidin-3,5-diglucoside (Peak 2), pelargonidin-3-glucoside (Peak 7), delphinidin-3-glucoside (Peak 1), cyanidin-3-rutinoside (Peak 5), cyanidin-3-arabinoside (Peak 6) and cyanidin-3-galactoside (Peak 3), respectively. The 3-glucosides and 3,5 diglucosides of cyanidin, delphinidin and pelargonidin have been found to be the major anthocyanins present in pomegranate (Du et al., 1975). Phenolic compounds identified in freshly prepared pomegranate juices were gallic acid, protocatechuic acid, chlorogenic acid, caffeic acid, ferulic acid, o - and p -coumaric acids, catechin, phloridzin and quercetin (Poyrazoglu et al, 2002). However, in this study, only ellagic acid (59.2%) (Peak2) and gallic acid (40.8%) (Peak1) were found to be the major phenolic acids present in pomegranate juice (Table 4.2).

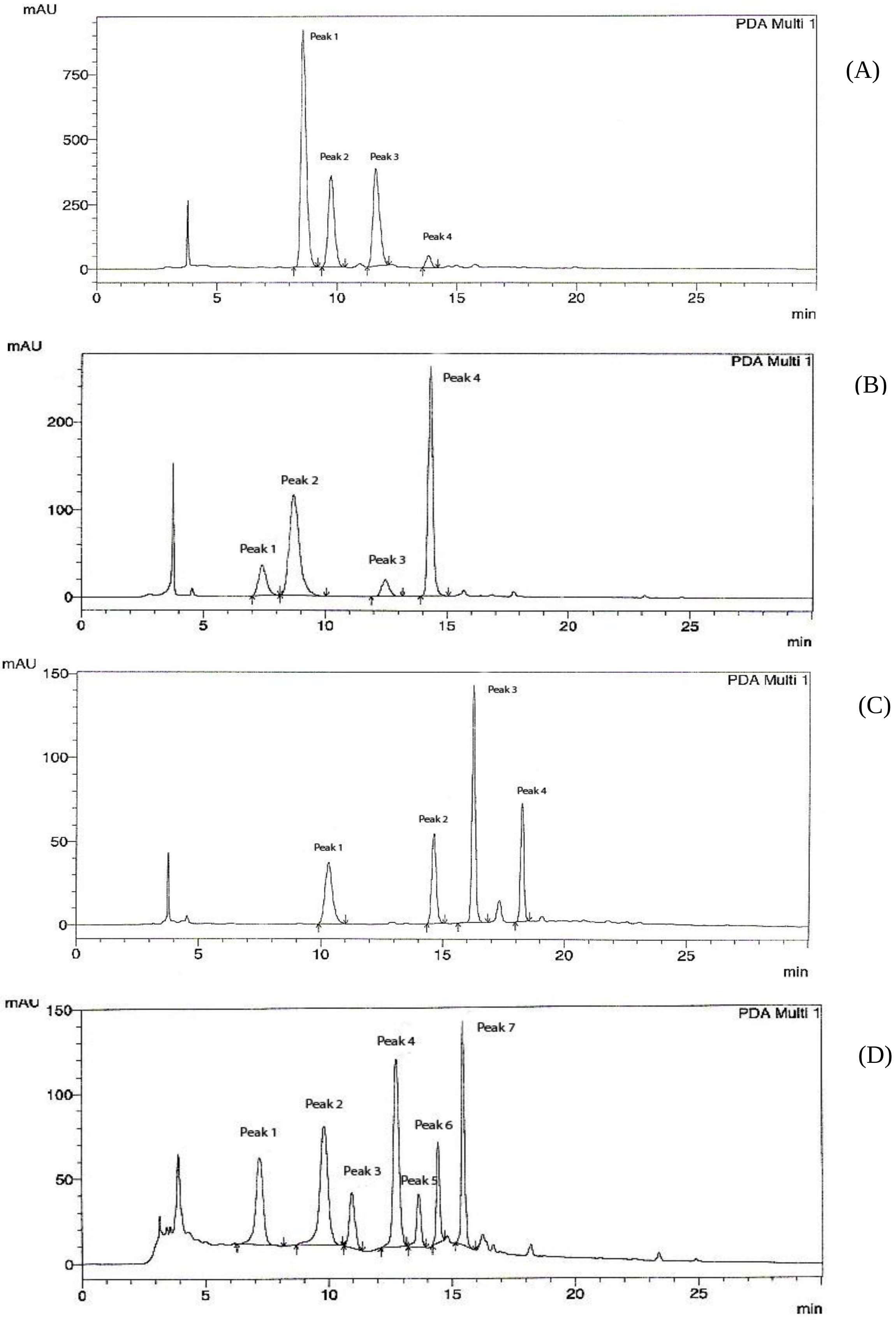


Figure 4.1 HPLC chromatograms of anthocyanin profiles of boysenberry (A), blackcurrant (B), cranberry (C) and pomegranate (D)

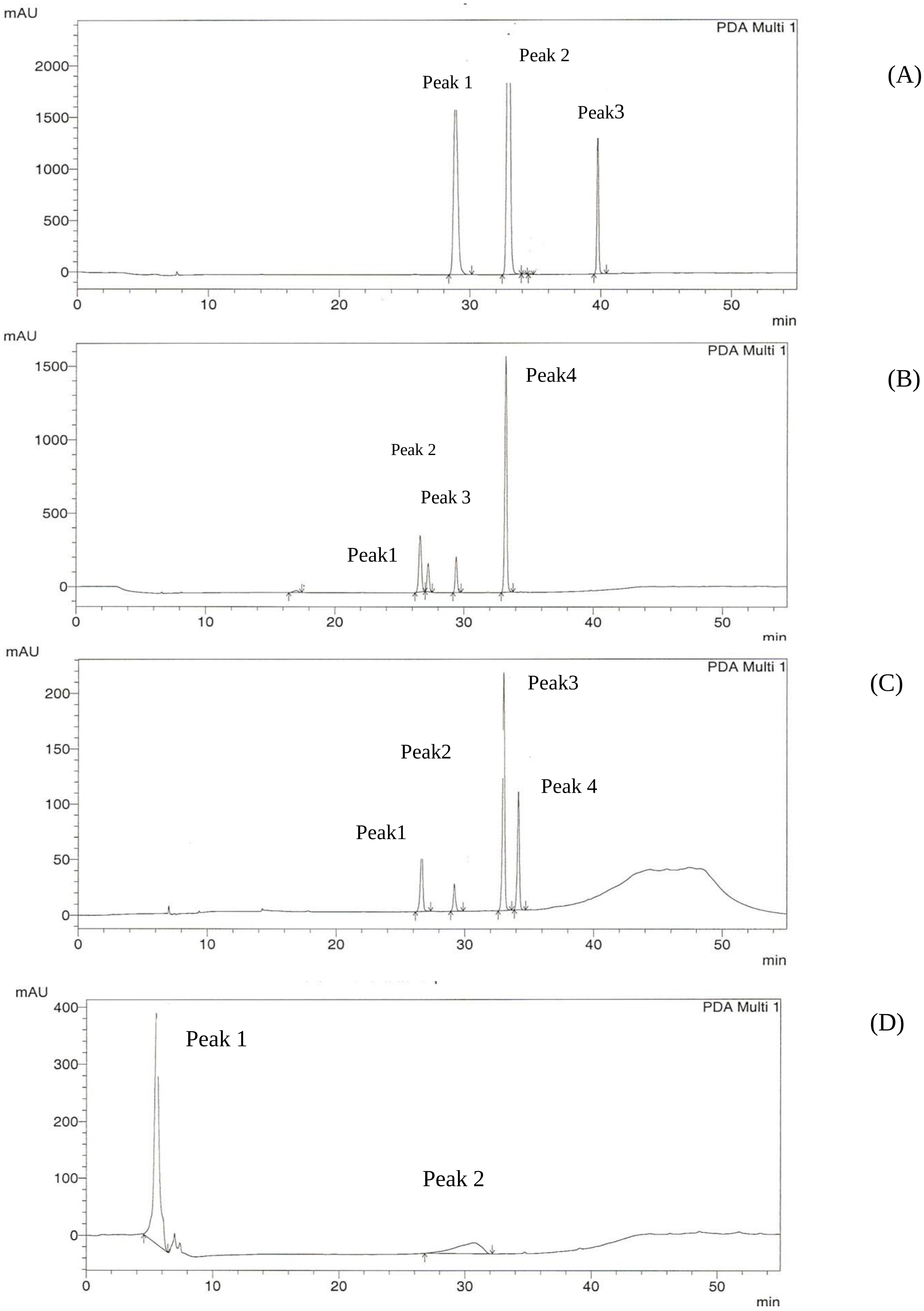


Figure 4.2 HPLC chromatograms of phenolic acid profiles of boysenberry (A), blackcurrant (B), cranberry (C) and pomegranate (D)

4.2 Enhancement of three juices (blackcurrant, cranberry and pomegranate) with boysenberry juice (study 2)

The objective of this step was to determine the effect of boysenberry juice (1, 2 and 3% (w/v)) on the colour stability of blackcurrant, cranberry and pomegranate juices at three storage temperatures (5, 20 and 35 ^{o}C). Colour stabilities of juices were compared using total monomeric anthocyanin content (Section 3.2.2), colour density (Section 3.2.3), polymeric colour (Section 3.2.3), total phenolics (Section 3.2.4), hyperchromic shift (Section 3.2.5), bathochromic shift (Section 3.2.5) and HPLC comparison of anthocyanins (Section 3.2.6) and phenolic acids (Section 3.2.7).

4.2.1 Changes in total anthocyanin content

The anthocyanin contents of the three non-enhanced juices (blackcurrant, cranberry and pomegranate) were not stable during storage at all three storage temperatures.It was found that the addition of boysenberry juice (1, 2 and 3% (w/v)) increased the colour of blackcurrant, cranberry and pomegranate juices immediately after addition; however after 28 days of storage, the anthocyanin content of cranberry and pomegranate enhanced with boysenberry decreased at all temperatures (5, 20 and 35 ^{o}C) (Figure 4.3). There was a significant difference in total anthocyanin content when the enhanced blackcurrant, cranberry and pomegranate juices were stored at all three storage temperatures ($P \leq 0.05$) (Figure 4.4).

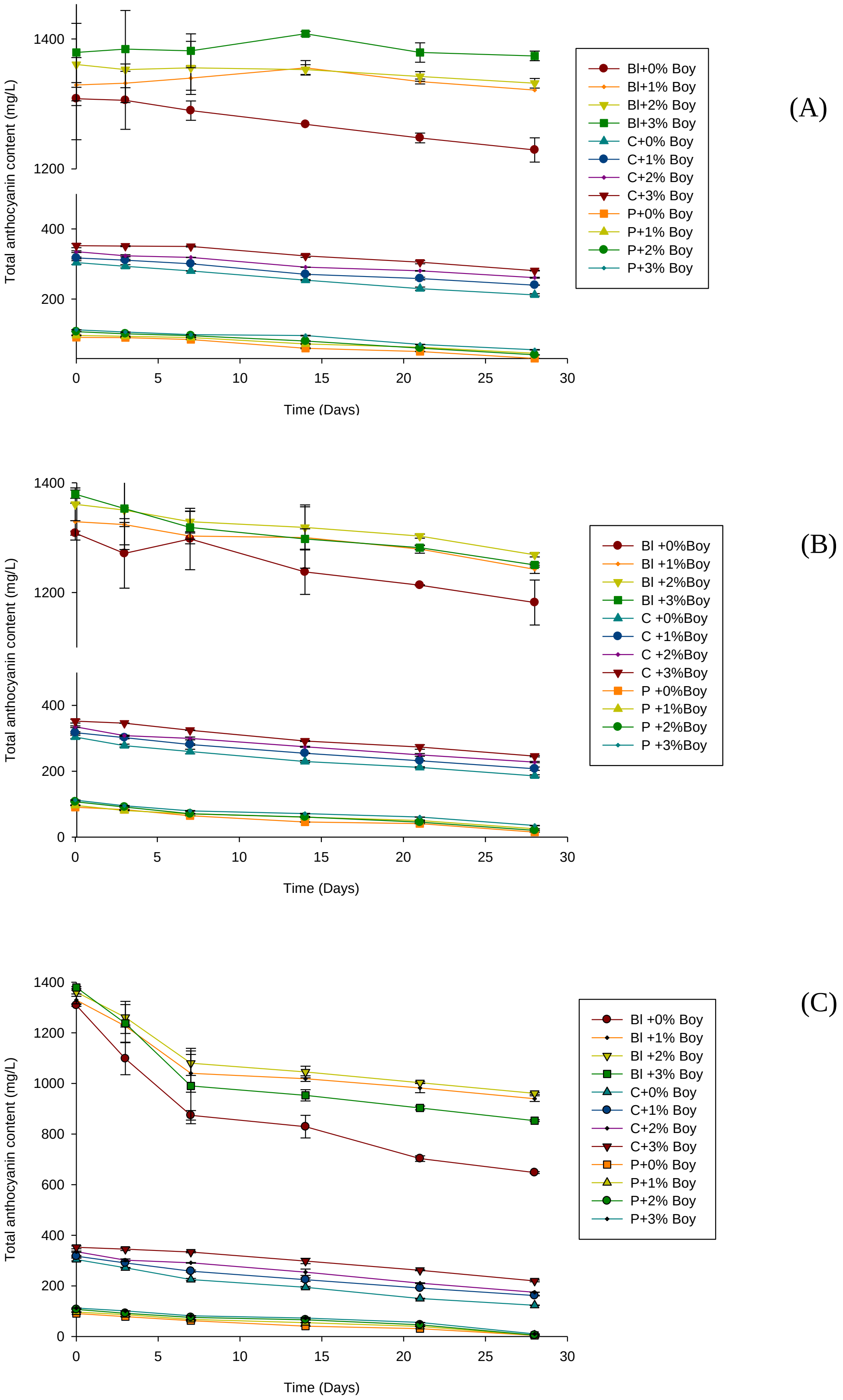


Figure 4.3 Change in total anthocyanin concentration (mg/L) determined by spectrophotometric technique of blackcurrant, cranberry and pomegranate enhanced with 0, 1, 2 and 3% (w/v) concentrations of boysenberry juice stored at (A) 5°C; (B) 20°C and (C) 35°C (Bl = Blackcurrant juice; C = Cranberry juice; P = Pomegranate juice; Boy = Boysenberry) (mean ± SD, n=2)

At the start of storage, the anthocyanin content of non-enhanced blackcurrant juice was 1,308.2 mg/L and of the blackcurrant juice enhanced with 1, 2 and 3% (w/v) concentrations of boysenberry juice the anthocyanin contents initially were 1,329.2, 1,360.8 and 1,379.2 mg/L, respectively. As a check calculation of the total anthocyanin content presented by mass balance calculation found that after addition of boysenberry juice, the total anthocyanin content of enhanced blackcurrant juice calculated was slightly lower than by 5% than actually measured. Similar result was also found in enhanced cranberry juice, but not in pomegranate juice with a little higher in calculation (Appendices 39 &40). After 28 days of storage at 5^{o}C, the pigment content decreased to 1,055 (19.3 % loss) mg/L for non-enhanced blackcurrant juice and to 1,297 (2.4 % loss), 1,305 (4.1 % loss) and 1,321 (4.2 % loss) mg/L for blackcurrant juice enhanced with 1, 2 and 3% (w/v) boysenberry juice (Figure 4.4). At 20°C, there was a small decrease in the pigment content of blackcurrant juices. The concentration of pigment in blackcurrant juice enriched with 0, 1, 2 and 3% (w/v) concentrations of boysenberry juice diminished by 9.7, 6.5, 6.8 and 9.4% of the initial concentrations (Figure 4.4). Therefore, the addition of boysenberry at 1, 2 and 3% (w/v) concentrations in blackcurrant juice was found to keep the anthocyanin pigments stable if the juice was stored at 5°C, but this was not found in blackcurrant juices at 20 and 35 °C temperatures (Figure 4.3). Simard et al. (1981) found that stability of anthocyanin monomers decreased with increasing storage temperature such that juices stored at 15^{o}C and 20^{o}C were more stable than juices at 37 ^{o}C, stored for 3 months. Also, Palamidis & Markakis (1975) stated that increasing the storage temperature significantly quicken the destruction of grape anthocyanins in a carbonated beverage.

In cranberry juice, at the start of storage, the anthocyanin content of non-enhanced cranberry juice was 304.1 mg/L and of the cranberry juices enhanced with 1, 2 and 3% (w/v) boysenberry juice they were 317.1, 335.1 and 352.2 mg/L, respectively (Figure 4.4). After 28 days of 5^{o}C storage, the pigment content of plain non-enhanced cranberry juice reduced by 30.4%. With 1, 2 and 3% (w/v) concentrations of boysenberry juice, the pigment content in cranberry juice diminished by 24.4, 22.2 and 20.2%, respectively (Figure 4.4). It appeared that the addition of boysenberry did not help to maintain the colour of cranberry juice at 5 ^{o}C (Figure 4.3). It was also found to have a similar effect on the pigment content when cranberry juice was stored at 20 and 35 °C (Figure 4.3).

Likewise, the amount of pigment in pomegranate juice decreased during 28 days of storage at all three temperatures. At the end of storage at 5°C, the pigment content of pomegranate juice enhanced with 0, 1, 2 and 3% (w/v) concentrations of boysenberry juice reduced by 65.97, 52.99, 61.81 and 50.7% of initial content, respectively (Figure 4.4; Table 4.3). Also, the pigment concentration of all enhanced pomegranate juices stored at 35 °C retained only about 5% of original anthocyanins at the end of storage and the colour of the juice became brownish (Figure 4.4).

Consequently, addition of boysenberry juices did not help to maintain the anthocyanin stability of the cranberry and pomegranate juices during 28 days at all storage temperatures, although, addition of boysenberry juice did increase pigment content of cranberry juice in the beginning of the study.

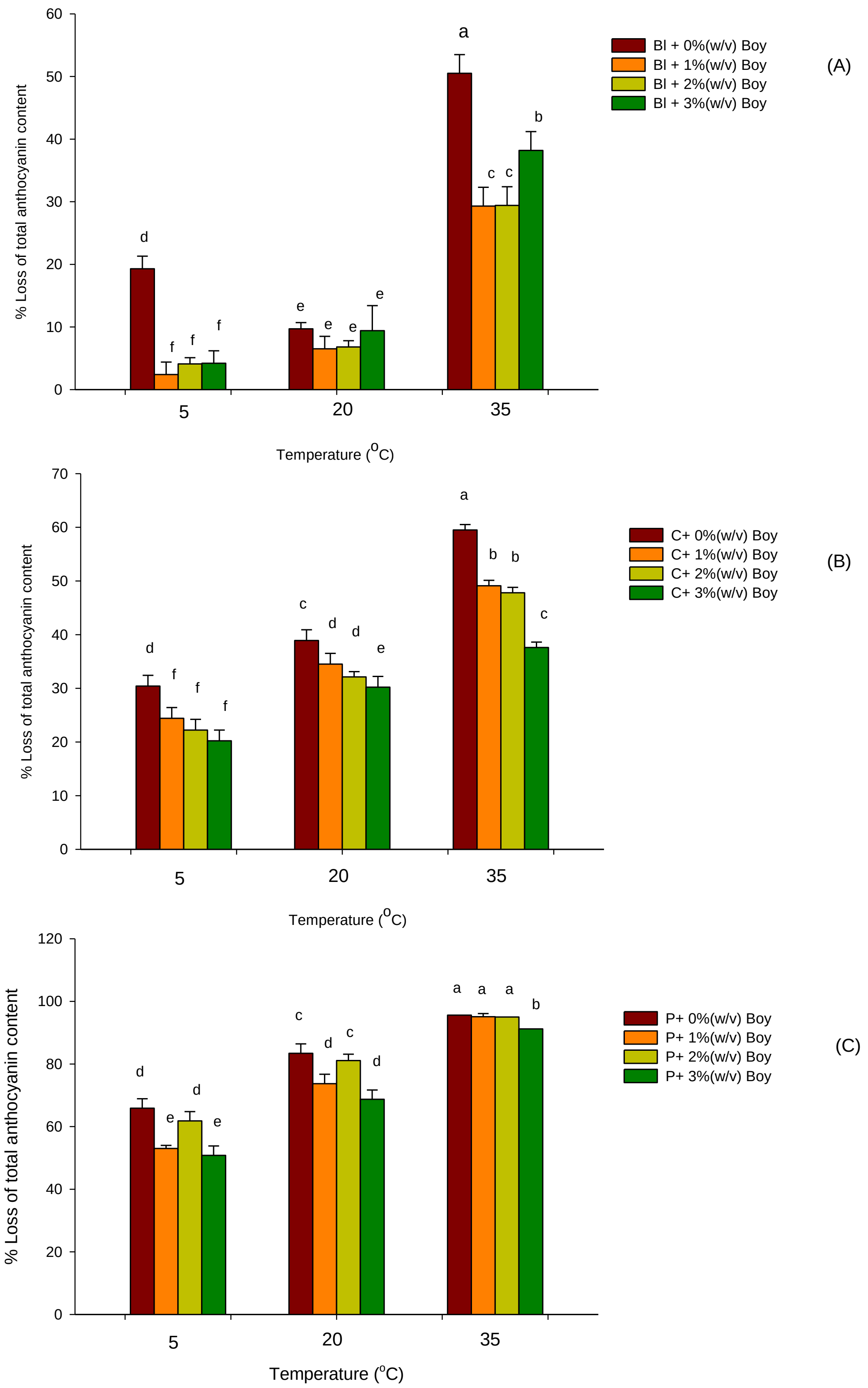


Figure 4.4 % Loss of total anthocyanin concentration determined by spectrophotometric technique of blackcurrant (A), cranberry (B) and pomegranate juices (C) enhanced with 0, 1, 2 and 3% (w/v) concentrations of boysenberry juice stored at 5, 20 and 35^{o}C. (Bl = Blackcurrant juice; C = Cranberry juice; P = Pomegranate juice; Boy = Boysenberry). Values marked by the same letter are not significantly different.

4.2.2 Colour density

Colour density is defined as the sum of absorbances at the λ_{max} (520 nm) and at 420 nm. This means that the greater the colour density the darker the colour of the sample appears. It was found that colour density of blackcurrant, cranberry and pomegranate juices stored at three temperatures were significantly different ($P \leq 0.05$) (Figure 4.6). At the beginning of storage, the colour density of blackcurrant juice enhanced with 1, 2 and 3% (w/v) boysenberry juice was 89.8, 92.4 and 97.9 compared to non-enhanced blackcurrant juice with a colour density of 85.1. After 7 days of storage at 5^{o}C, the colour density of enhanced blackcurrant juice remained stable; however, storage at higher temperatures the colour density of the enhanced juices decreased gradually. After 28 days of 5^{o}C storage, the colour density of non-enhanced blackcurrant juice reduced by 40.2% (Figure 4.5 and 4.6). Storage at 20 and 35°C resulted in a dramatic decrease in the colour density of blackcurrant juices at these two storage temperatures (Figure 4.5 and 4.6).

In cranberry and pomegranate juice, it was found that addition of boysenberry juice did not help to maintain the colour density of juices at any storage temperatures as the colour density decreased gradually during storage. At the end of storage, the colour density of all pomegranate juices enhanced and non-enhanced with boysenberry juice decreased by approximately 50% of the original colour density during storage. The colour density of cranberry juice reduced by 31.2, 18.2, 26.1 and 34.5% at 5^{o}C and by 43.4, 38.6, 35.1 and 52.9% at 35 ^{o}C storage (Figure 4.5 and 4.6)

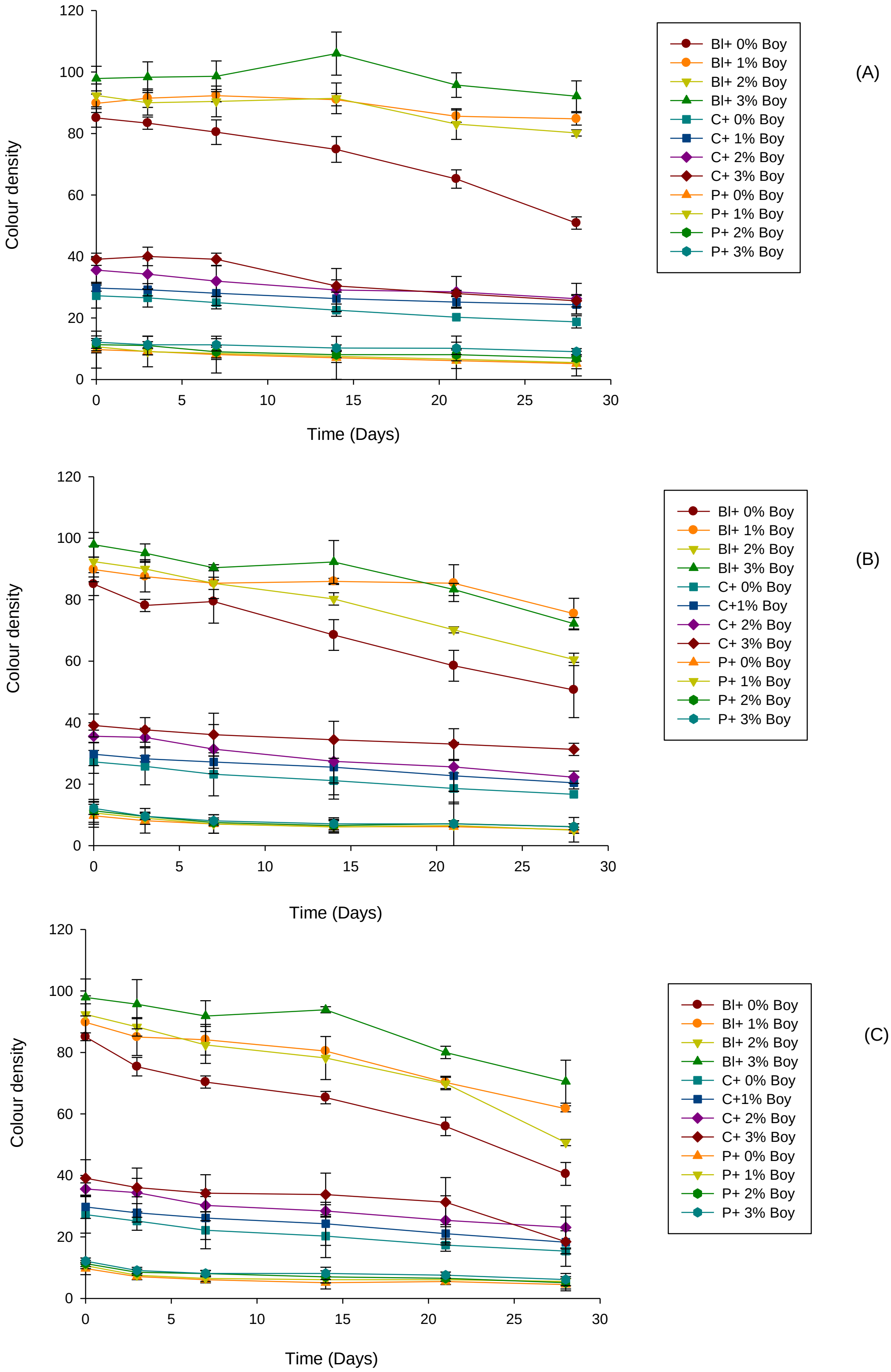


Figure 4.5 Change in colour density determined by spectrophotometric technique of blackcurrant, cranberry and pomegranate enhanced with 0, 1, 2 and 3% (w/v) concentrations of boysenberry juice stored at (A) 5°C; (B) 20°C and (C) 35°C (Bl = Blackcurrant juice; C = Cranberry juice; P = Pomegranate juice; Boy = Boysenberry)

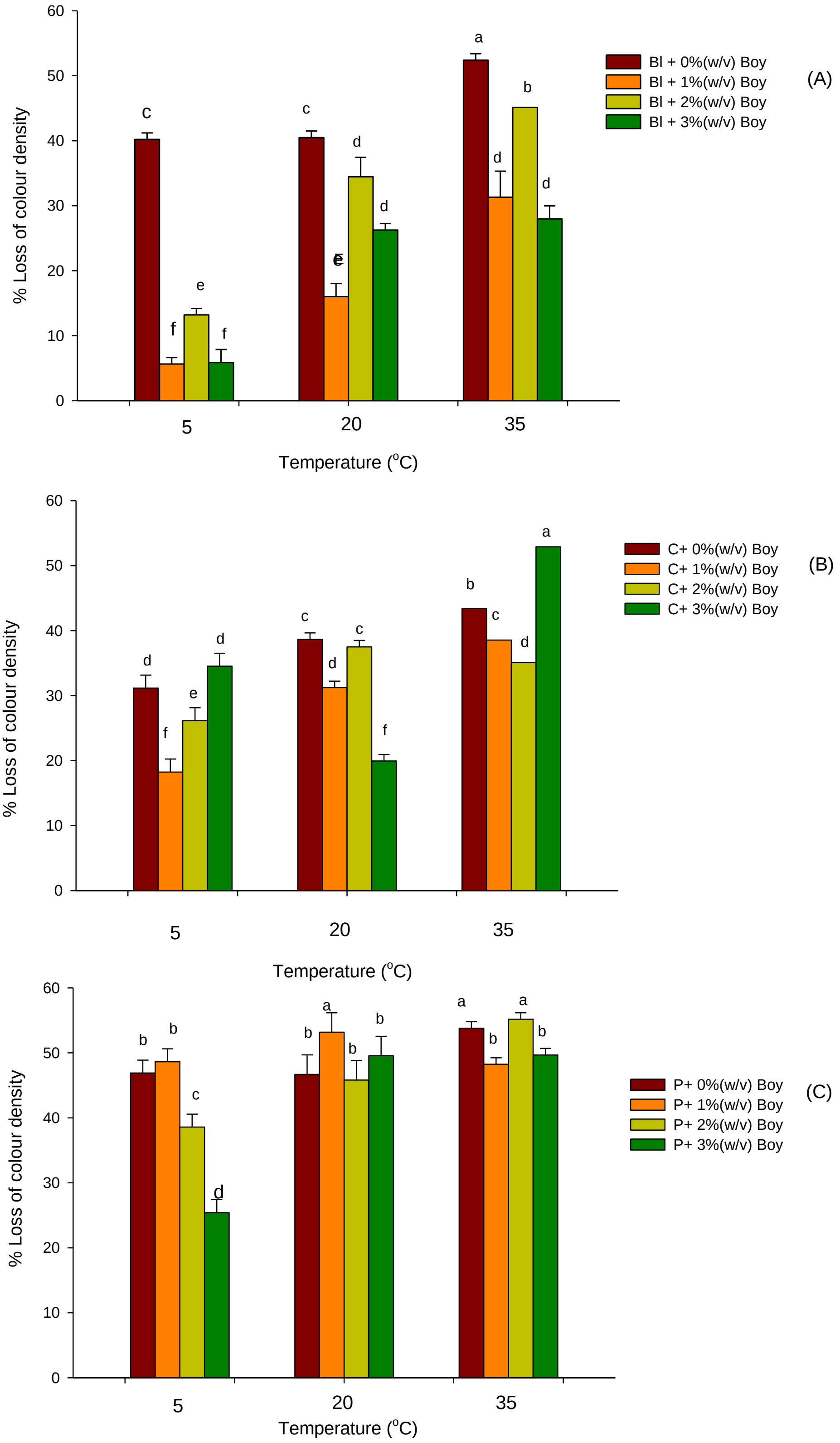


Figure 4.6 % Loss of colour density determined by spectrophotometric technique of blackcurrant (A) , cranberry (B) and pomegranate juices (C) enhanced with 0, 1, 2 and 3% (w/v) concentrations of boysenberry juice stored at 5, 20 and 35°C. (Bl = Blackcurrant juice; C = Cranberry juice; P = Pomegranate juice; Boy = Boysenberry). Values marked by the same letter are not significantly different.

4.2.3 Hyperchromic shift

The hyperchromic shift refers to an increase in absorbance at $\lambda_{max.}$ The bathochromic shift refers to an increase in the wavelength at which the absorbance is greatest, the $\lambda_{max.}$ The shift in λ_{max} and increase in absorbance is called "copigmentation." Copigmentation results in a colour shift toward a longer wavelength and a more intense colour than that seen with the anthocyanin alone (Waterhouse, 2002; Wrolstad et al., 2005a).

According to Figure 4.7, it was found that boysenberry juice increased the absorbance at λ_{max} of blackcurrant, cranberry and pomegranate juice immediately after the addition; however, the hyperchromic shift of enhanced blackcurrant juice stored at 20 and 35 ^{o}C was not more stable during storage than when the juice was stored at 5 ^{o}C ($P \leq 0.05$). The addition of 1, 2 and 3% (w/v) concentrations of boysenberry juice in blackcurrant, cranberry and pomegranate juices did not show any changes in hyperchromic shift at the beginning of study. However, after 28 days of storage at 5 ^{o}C, the absorbance at λ_{max} of blackcurrant juice enhanced with 1, 2 and 3% (w/v) concentrations of boysenberry juice increased by 9.1, 0.0 and 7.7%, respectively, compared to non-enhanced blackcurrant juice which decreased by 45.5% (Figure 4.8). For cranberry and pomegranate juice enhanced with 1, 2 and 3% (w/v) concentrations of boysenberry juice, the absorbance at λ_{max} decreased gradually at three temperatures.

The maximum wavelength (λ_{max}) of all juices ranged between 500 and 503 nm. This maximum wavelength did not shift for each juice during this study indicating that no juices exhibited a bathochomic shift in the maximum wavelength (Appendix 10-12) ($P \geq 0.05$). Boysenberry juice was found to maintain colour stability by maintaining total anthocyanin content and colour density of blackcurrant juice, the maximum wavelengths of enhanced blackcurrant juice were very similar to the values of the maximum wavelength (λ_{max}) with no boysenberry juice addition (Appendix 10).

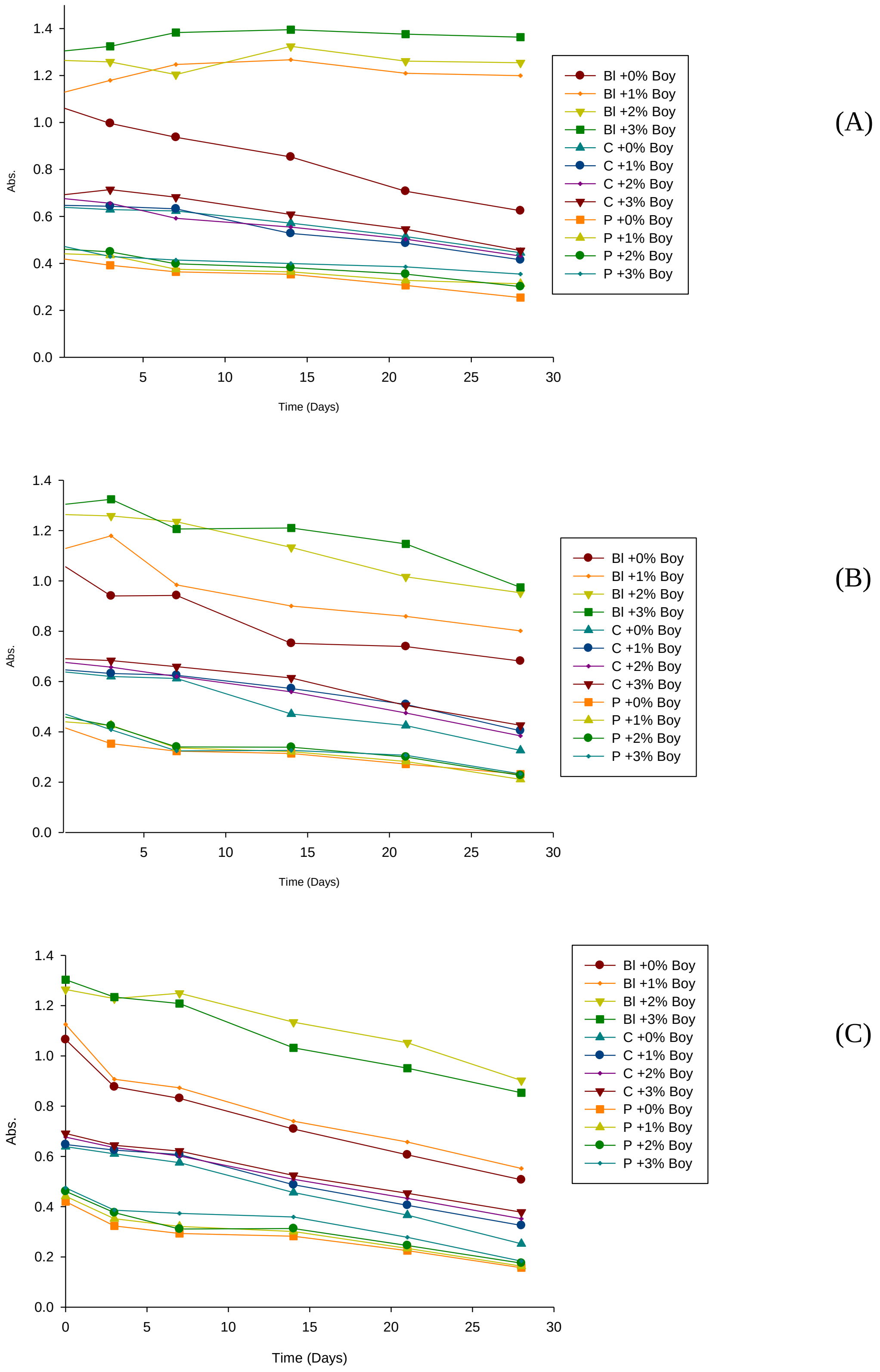


Figure 4.7 Change in hyperchromic shift of blackcurrant, cranberry and pomegranate enhanced with 0, 1, 2 and 3% (w/v) concentrations of boysenberry juice stored at (A) 5°C; (B) 20°C and (C) 35°C (Bl = Blackcurrant juice, $\lambda_{max= 500\ nm}$; C = Cranberry juice, $\lambda_{max= 500\ nm}$; P = Pomegranate juice, $\lambda_{max= 500\ nm}$; Boy = Boysenberry) (mean ± SD, n=2)

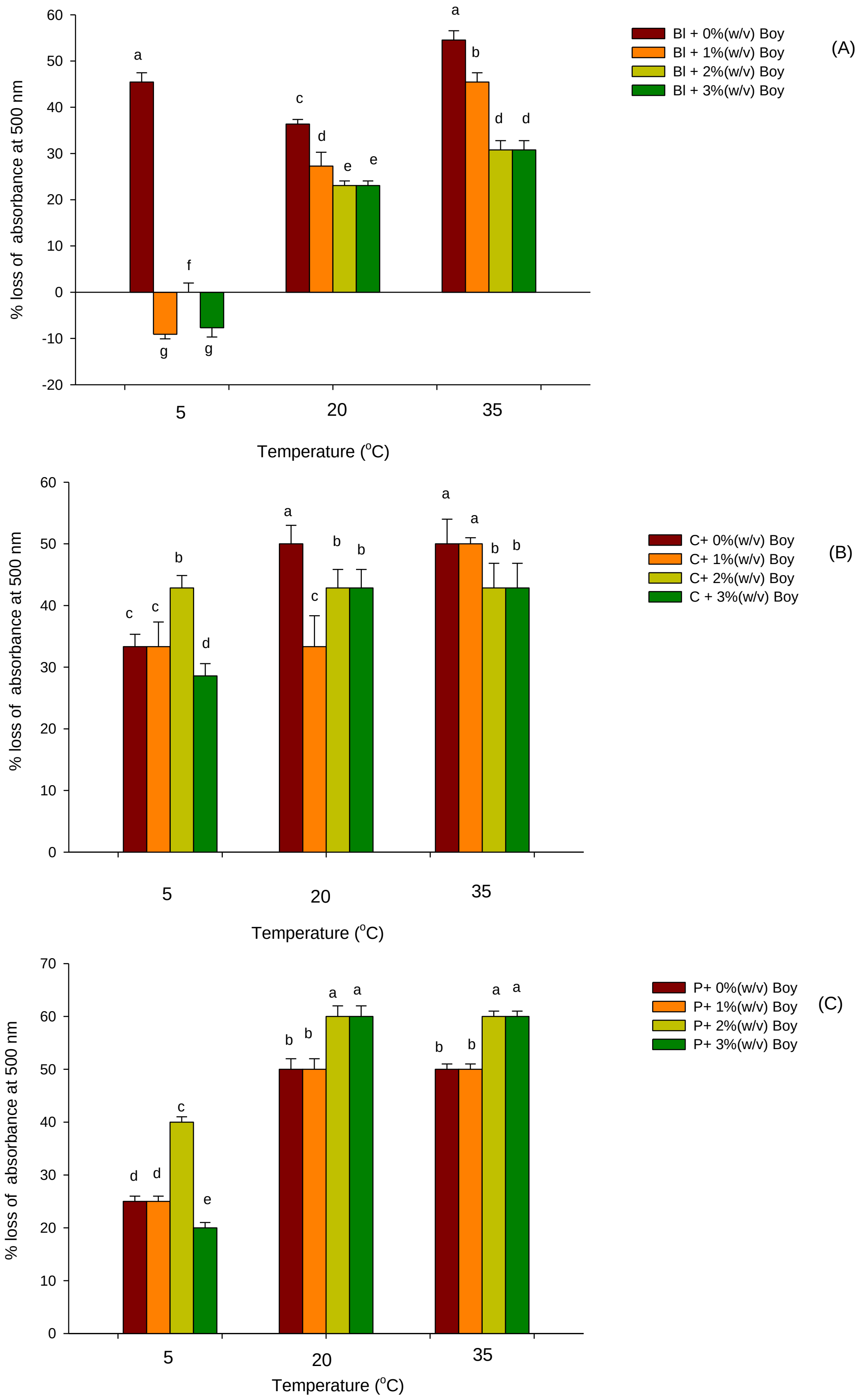


Figure 4.8 % Loss of absorbance at λ_{max} (hyperchromic) of blackcurrant, cranberry and pomegranate enhanced with 0, 1, 2 and 3% (w/v) concentrations of boysenberry juice stored at (A) 5°C; (B) 20°C and (C) 35°C (Bl = Blackcurrant juice, $\lambda_{max= 500\ nm}$; C = Cranberry juice, $\lambda_{max= 500\ nm}$; P = Pomegranate juice, $\lambda_{max= 500\ nm}$; Boy = Boysenberry). Values marked by the same letter are not significantly different.

In summary, the addition of boysenberry juice in blackcurrant gave the highest value of hyperchromic shift at the beginning of the study (followed by pomegranate and cranberry juice), and it could also maintain the hyperchromic shift in blackcurrant juice during storage. The concentrations of boysenberry juice added in blackcurrant also had a positive effect on colour intensity of the juice after storage ($P \leq 0.05$). Higher concentrations of boysenberry juice added, resulted in a stable colour intensity for blackcurrant juice during storage (Figure 4.8).

4.2.4 Polymeric colour

At the start of storage, the polymeric colour increased with the addition of boysenberry juices (Figure 4.8). The polymeric colour of non- enhanced blackcurrant juice was 13.7; however, it increased to 17.8, 20.3 and 23.1 when 1, 2 and 3% (w/v) concentrations of boysenberry juice was added into blackcurrant juice, respectively (Figure 4.9). The rapid increase in polymeric colour in blackcurrant juice after the addition of boysenberry juice may be due to the rapid formation of polymeric anthocyanin –tannins and melanoidin pigments (Wrolstad et al., 2005a). On the other hand, this result was not found in cranberry and pomegranate juices after the addition of boysenberry juice. The polymeric colour of cranberry and pomegranate juice seemed to decrease once boysenberry juice was added.

As shown in Figure 4.10, the polymeric colour of blackcurrant juice enhanced with 1, and 3% (w/v) boysenberry juice with increasing storage temperature ($P \leq 0.05$). Compared to cranberry and pomegranate juice at the same temperature, the polymeric colour of the cranberry and pomegranate juices enriched with boysenberry juice increased rapidly (Figure 4.10) ($P \leq 0.05$). Therefore, at 5^{o}C, blackcurrant juice had the lowest degree of polymerisation of colour compared to other two juices, cranberry and pomegranate juice, respectively. This agreed with the retention of anthocyanins and the maintenance of colour density in blackcurrant juice with the addition of boysenberry.

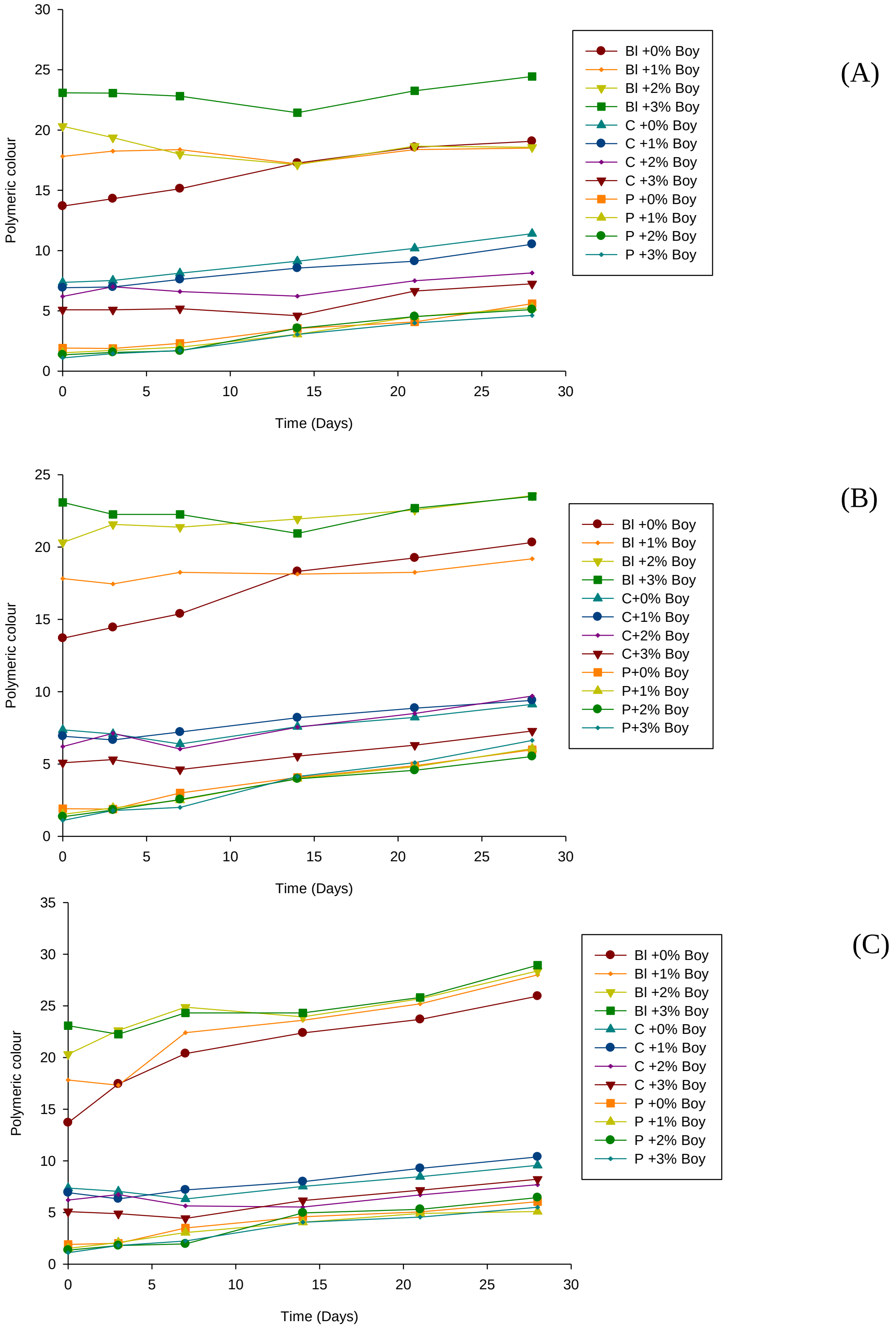


Figure 4.9 Change in polymeric colour of blackcurrant, cranberry and pomegranate enhanced with 0, 1, 2 and 3% (w/v) concentrations of boysenberry juice stored at (A) 5°C; (B) 20°C and (C) 35°C (Bl = Blackcurrant juice; C = Cranberry juice; P = Pomegranate juice; Boy = Boysenberry) (mean ± SD, n=2)

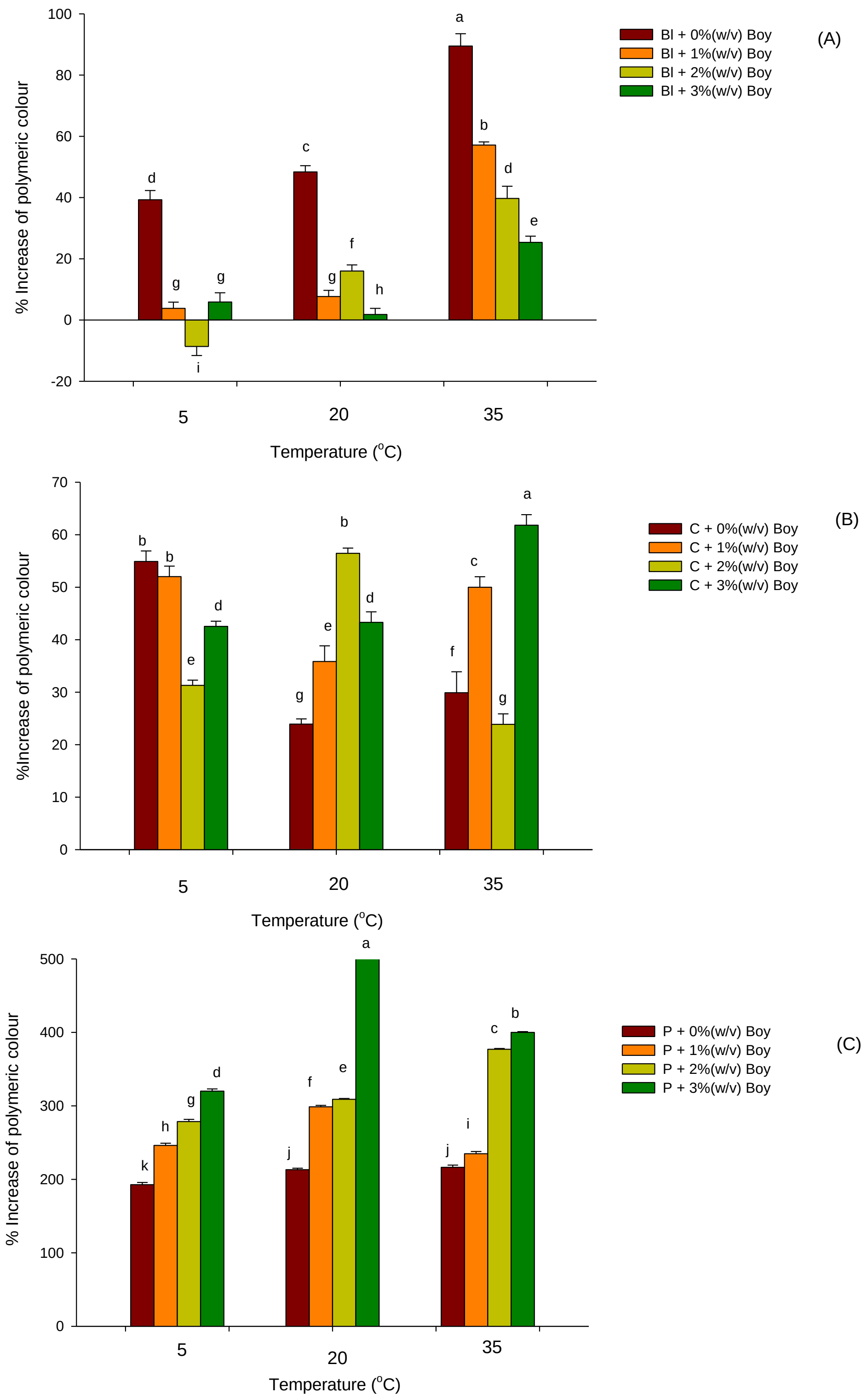


Figure 4.10 % Increase of polymeric colour of blackcurrant, cranberry and pomegranate enhanced with 0, 1, 2 and 3% (w/v) concentrations of boysenberry juice stored at (A) 5°C; (B) 20°C and (C) 35°C (Bl = Blackcurrant juice; C = Cranberry juice; P = Pomegranate juice; Boy = Boysenberry. Values marked by the same letter are not significantly different.

Blackcurrant juices stored at refrigerated temperature (5°C) showed a very low rate of polymeric colour formation, with just detectable levels after 28 days storage. With less formation of polymeric colour, there was less degradation of anthocyanins. (Figures 4.9 & 4.10).

4.2.5 Impact of temperature on anthocyanin stability

The thermal degradation of total anthocyanins (Figure 4.3) in blackcurrant, cranberry and pomegranate juices followed first-order reaction kinetics. The first order rate constants are presented in Table 4.3. Similar results were found in previous studies (Markikis et al., 1957; Daravings & Cain, 1968; Calvi & Francis, 1978; Cemeroglu et al., 1994).

According to Jackman & Smith (1996), the colour stability and rate of degradation of anthocyanins are affected by temperature. It could be seen that the rate constants (k) of total anthocyanin degradation in all juices increased with increasing temperature, indicating that anthocyanin degradation was faster at higher temperature conditions. Non-enhanced pomegranate juice was found to be more sensitive to thermal degradation than the other two juices when the juice was stored at higher temperature, especially at 35 °C which had the highest k value (0.539 day^{-1}), compared to cranberry (0.184 day^{-1}) and blackcurrant juice (0.023 day^{-1}), respectively (Table 4.3).

Table 4.3 Degradation of total anthocyanin content of blackcurrant, cranberry and pomegranate juice enhanced with boysenberry juice at different concentrations, first order reaction rate constants.

Juices	Temperature(^{o}C)	Boysenberry (%w/v)	Rate constant k (Day^{-1})	R^2
Blackcurrant	5	0	0.002	1.00
		1	1x10^{-4}	0.03
		2	0.001	0.88
		3	1x10^{-4}	0.02
	20	0	0.003	0.95
		1	0.002	0.94
		2	0.002	0.97
		3	0.003	0.95
	35	0	0.023	0.91
		1	0.011	0.81
		2	0.012	0.84
		3	0.016	0.82
Cranberry	5	0	0.076	0.97
		1	0.059	0.96
		2	0.051	0.96
		3	0.047	0.88
	20	0	0.097	0.99
		1	0.086	0.98
		2	0.076	0.98
		3	0.075	0.97
	35	0	0.184	0.99
		1	0.136	0.99
		2	0.128	0.95
		3	0.095	0.91
Pomegranate	5	0	0.214	0.88
		1	0.150	0.89
		2	0.033	0.95
		3	0.024	0.93
	20	0	0.328	0.86
		1	0.236	0.88
		2	0.053	0.93
		3	0.036	0.93
	35	0	0.539	0.76
		1	0.503	0.71
		2	0.073	0.77
		3	0.073	0.77

In addition, blackcurrant juice enhanced with boysenberry juice degraded slower than enhanced cranberry and pomegranate juice, respectively, at 5°C (Table 4.3) ($P \leq 0.05$). It was found that the rate of degradation of all three juices was decreased when the juices were enhanced with boysenberry juice at 5°C ($P \leq 0.05$). Without boysenberry juice addition, the rate constants (k) of blackcurrant stored at 5°C was 0.002 day^{-1}; however, when boysenberry juice was added at concentrations of 1, 2 and 3% (w/v) in blackcurrant juice, the k values were only $1x10^{-4}$, 0.001 and $1x10^{-4}$ day^{-1}, respectively (Table 4.3). There was a similar trend in changes of degradation rate of anthocyanins with other the two juices.

Thermal degradation leads to formation of the chalcone (an aromatic ketone that forms the central core) and its subsequent cleavage yields several degradation products which condense to form complex brown polymeric compounds known as melanoidin pigments (Piffaut et al., 1994; Jackman & Smith, 1996) (Figure 4.11). Markakis (1982) stated that the thermal degradation of anthocyanins involved the opening of the heterocyclic molecule and the formation of the chalcone glycoside as first steps, without the need for hydrolysis of the glycosidic moiety and formation of the aglycon (Adams, 1973).

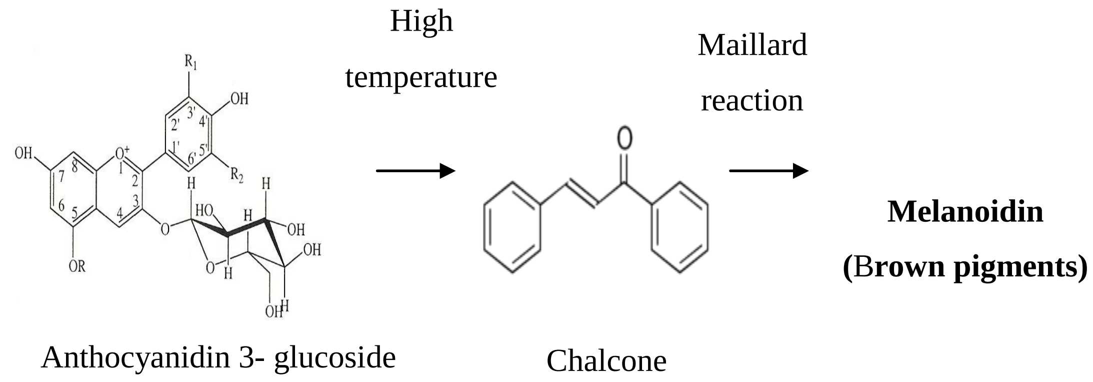


Figure 4.11 An example of anthocyanidin-3-glucoside degradation (Piffaut et al., 1994; Jackman & Smith, 1996)

4.2.6 Changes in individual anthocyanin profile during storage

As discussed in Section 4.1, there were four major anthocyanins found in blackcurrant juice: delphinidin-3-rutinoside, cyanidin-3-rutinoside, delphinidin-3-glucoside and cyanidin-3-glucoside. Boysenberry juices had four anthocyanins: cyanidin-3-sophoroside, cyanidin-3-glucoside, cyanidin-3-glucosyl-rutinoside and cyanidin-3-rutinoside. After addition of boysenberry juice into blackcurrant juice, only cyanidin-3-rutinoside and cyanidin-3-glucoside detected in blackcurrant juice increased immediately (Figure 4.12). The cyanidin-3-rutinoside content of blackcurrant juices enhanced with 1, 2 and 3% (w/v) concentrations of boysenberry juice increased by 7.2, 11.8 and 14.4% of plain blackcurrant juice (Figures 4.12 & 4.13). Also, the cyanidin-3-glucoside content increased after boysenberry juice addition at 1, 2 and 3% (w/v) concentrations by 5.2, 12 and 23%. After addition of boysenberry juice into blackcurrant, cranberry and pomegranate juices, individual anthocyanin content measured by HPLC and mass balance calculations of anthocyanin concentrations of each individual pigment were found to agree (Appendices 39 &40).

The first order degradation rate constant (k) for delphinidin-3-rutinoside in plain blackcurrant juice at 5 ^{o}C was 0.004 (day) $^{-1}$. As temperature increased to 20 and 35 ^{o}C, the rate constant (k) increased to 0.006 and 0.015 (day) $^{-1}$, respectively (Table 4.4). A similar result was also found with the other anthocyanins. Of the four anthocyanins in enhanced blackcurrant juices, delphinidin-3-rutinoside and cyanidin-3-rutinoside were found to be the most stable to thermal degradation at all temperatures than the other two (as seen on the rate constants (k)) ($P \leq 0.05$) (Table 4.4). However, the concentration of delphinidin-3-rutinoside and cyanidin-3-rutinoside still decreased when storage temperature increased. The loss of individual anthocyanins in all the juices is shown in Figure 4.12.

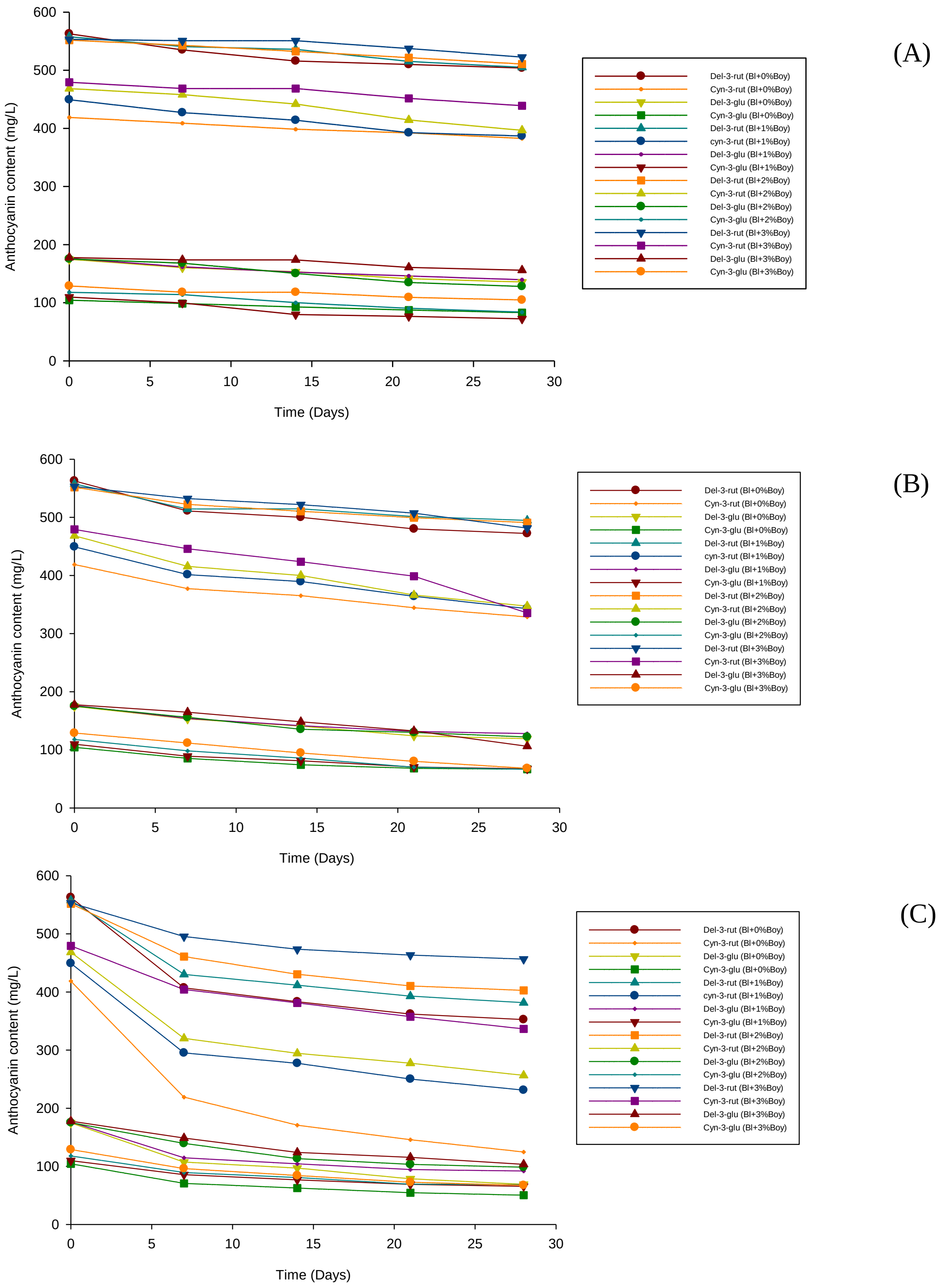


Figure 4.12 Changes in individual anthocyanin concentrations of blackcurrant juice enhanced with 0, 1, 2 and 3% (w/v) concentrations of boysenberry juice stored at (A) 5°C; (B) 20°C and (C) 35°C (Bl = Blackcurrant juice; Boy = Boysenberry) (mean ± SD, n=2)

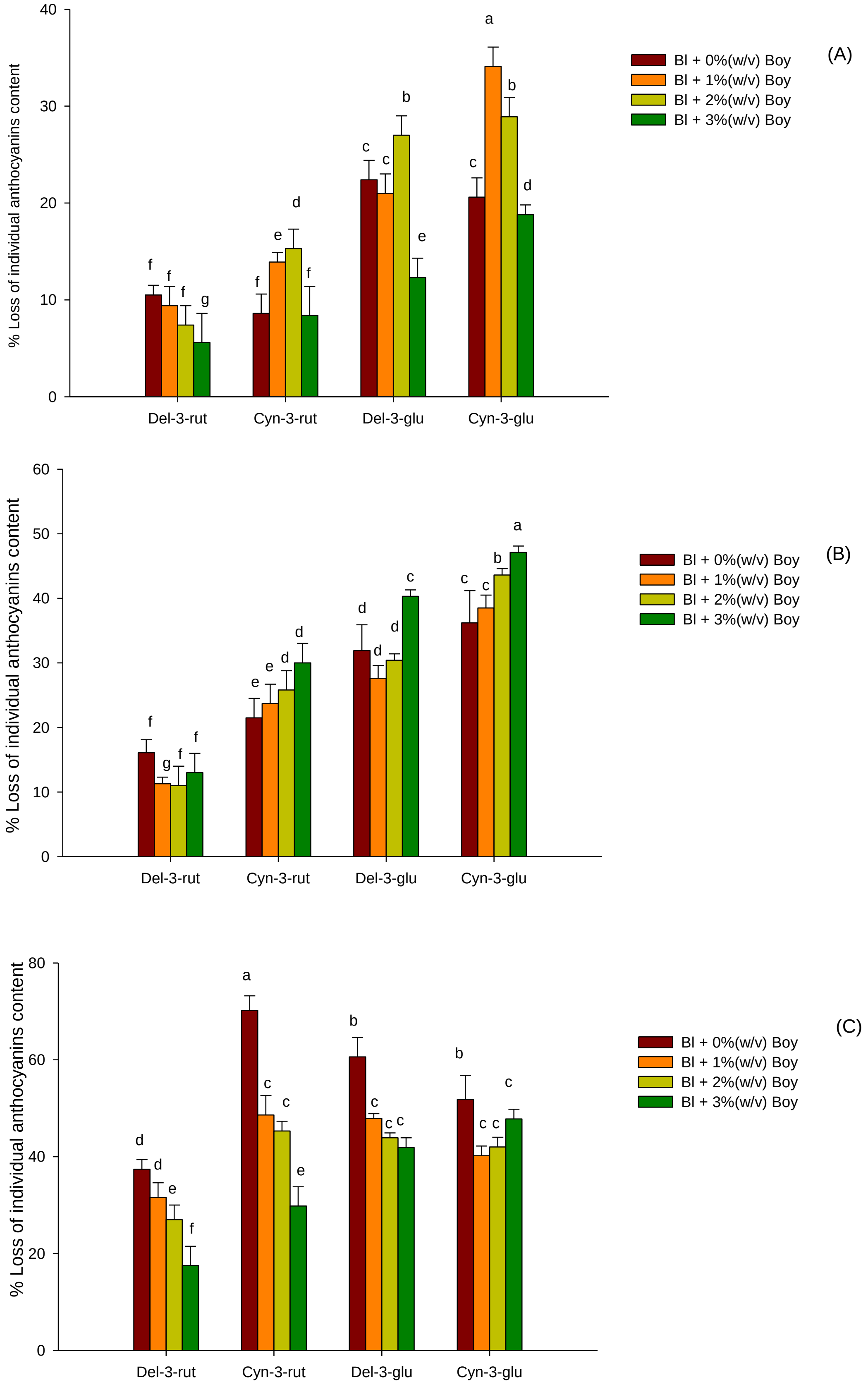


Figure 4.13 % Loss of individual anthocyanins concentration of blackcurrant juice enhanced with 0, 1, 2 and 3% (w/v) concentrations of boysenberry juice stored at (A) 5°C; (B) 20°C and (C) 35°C (Bl = Blackcurrant juice; Boy = boysenberry). Values marked by the same letter are not significantly different.

Table 4.4 Degradation of individual anthocyanin concentrations of blackcurrant juice enhanced with 0, 1, 2 and 3% (w/v) concentrations of boysenberry juice at various temperatures, first order reaction rate constants.

Temp. (^oC)	Boysenberry (%)	Rate constant (k) (Day^{-1})	R^2	Rate constant (k) (Day^{-1})	R^2	Rate constant (k) (Day^{-1})	R^2	Rate constant (k) (Day^{-1})	R^2
		Del-3-rut		Cyn-3-rut		Del-3-glu		Cyn-3-glu	
5	0	0.004	0.902	0.022	0.996	0.063	0.987	0.063	0.987
	1	0.004	0.975	0.006	0.975	0.008	0.978	0.008	0.978
	2	0.019	0.997	0.006	0.973	0.012	0.978	0.012	0.978
	3	0.014	0.825	0.003	0.930	0.005	0.894	0.005	0.894
20	0	0.006	0.898	0.058	0.965	0.098	0.978	0.098	0.978
	1	0.004	0.810	0.009	0.969	0.011	0.946	0.011	0.946
	2	0.028	0.939	0.010	0.974	0.013	0.954	0.013	0.954
	3	0.033	0.974	0.012	0.932	0.018	0.955	0.018	0.955
35	0	0.015	0.775	0.283	0.890	0.217	0.918	0.217	0.918
	1	0.012	0.787	0.021	0.837	0.021	0.804	0.021	0.804
	2	0.075	0.858	0.019	0.824	0.021	0.927	0.021	0.927
	3	0.045	0.848	0.012	0.936	0.019	0.970	0.019	0.970

* Del-3-rut = Delphinidin-3-rutinoside; Cyn-3-rut = Cyanidin-3-rutinoside; Del-3-glu = Delphinidin-3-glucoside; Cyn-3-glu = Cyanidin-3-glucoside

The major anthocyanins found in cranberry juice were peonidin-3-galactoside, cyanidin-3-galactoside, cyanidin-3-arabinoside and peonidin-3-arabinoside and in pomegranate juice those found were cyanidin-3-glucoside, delphinidin-3,5-diglucoside, delphinidin-3, 5-diglucoside, pelargonidin-3-glucoside and delphinidin-3-glucoside. During 28 days of storage, four anthocyanins present in cranberry juice enhanced with boysenberry juice decreased gradually (Appendix 31). Of the four main anthocyanins in cranberry juice, only cyanidin-3-arabinoside and peonidin-3-arabinoside were affected by increasing temperature ($P \leq 0.05$). The other two anthocyanins in enriched cranberry juice did not change at higher temperatures (Table 4.5). The anthocyanins degradation in cranberry juice enhanced with boysenberry juice followed a first–order degradation reaction.

Table 4.5 Degradation of individual anthocyanin concentrations of cranberry juice enhanced with 0, 1, 2 and 3% (w/v) concentrations of boysenberry juice at various temperatures, first order reaction rate constants.

Temp. (°C)	Boysenberry (%)	Rate constant (k) (Day^{-1})	R^2	Rate constant (k) (Day^{-1})	R^2	Rate constant (k) (Day^{-1})	R^2	Rate constant (k) (Day^{-1})	R^2
		Pn-3-gal		Cyn-3-gal		Cyn-3-ara		Pn-3-ara	
5	0	0.024	0.935	0.031	0.784	0.010	0.883	0.008	0.985
	1	0.024	0.859	0.033	0.811	0.011	0.925	0.008	0.982
	2	0.020	0.893	0.032	0.700	0.011	0.892	0.007	0.984
	3	0.014	0.942	0.014	0.992	0.011	0.944	0.007	0.959
20	0	0.021	0.945	0.022	0.990	0.025	0.992	0.013	0.983
	1	0.024	0.993	0.012	0.983	0.013	0.926	0.011	0.983
	2	0.014	0.975	0.013	0.991	0.013	0.834	0.012	0.984
	3	0.013	0.976	0.013	0.999	0.015	0.924	0.013	0.995
35	0	0.027	0.935	0.030	0.994	0.034	0.979	0.016	0.967
	1	0.025	0.950	0.021	0.989	0.031	0.990	0.016	0.992
	2	0.018	0.944	0.019	0.996	0.024	0.970	0.017	0.958
	3	0.017	0.977	0.020	0.996	0.023	0.962	0.018	0.975

* Pn-3-gal = peonidin-3-galactoside; cyn-3-gal = cyanidin-3-galactoside; cyn-3-ara = cyanidin-3-arabinoside and pn-3-ara = peonidin-3-arabinoside

Also, the degradation of anthocyanins in pomegranate juice enhanced with boysenberry juice followed a first–order reaction. It was found that temperature did not affect the anthocyanin profiles (cyanidin-3-glucoside, delphinidin-3,5-diglucoside, pelargonidin-3-glucoside and delphinidin-3-glucoside) in pomegranate juice enhanced with boysenberry juice ($P \geq 0.05$) (Table 4.6), but the remaining anthocyanins decreased with increasing temperature. After addition of boysenberry juice, the anthocyanins in pomegranate juice decreased throughout storage at all temperatures (Appendix 32).

Table 4.6 Degradation of individual anthocyanin concentrations of pomegranate juice enhanced with 0, 1, 2 and 3% (w/v) concentrations of boysenberry juice at various temperatures, first order reaction rate constants.

Temp. (°C)	Boysenberry (%)	Rate constant (k) (Day^{-1})	R^2	Rate constant (k) (Day^{-1})	R^2	Rate constant (k) (Day^{-1})	R^2	Rate constant (k) (Day^{-1})	R^2
		Cyn-3-glu		Dp-3,5-diglu		Pg-3-glu		Dp-3-glu	
5	0	0.006	0.984	0.021	0.980	0.020	0.968	0.030	0.972
	1	0.009	0.984	0.017	0.906	0.015	0.868	0.032	0.949
	2	0.007	0.972	0.011	0.914	0.023	0.851	0.024	0.900
	3	0.006	0.951	0.010	0.900	0.012	0.924	0.025	0.909
20	0	0.008	0.906	0.025	0.920	0.045	0.930	0.033	0.835
	1	0.008	0.976	0.031	0.974	0.033	0.967	0.033	0.902
	2	0.007	0.975	0.028	0.879	0.039	0.962	0.030	0.967
	3	0.034	0.608	0.027	0.889	0.036	0.953	0.027	0.965
35	0	0.008	0.791	0.035	0.953	0.045	0.897	0.041	0.887
	1	0.009	0.945	0.033	0.966	0.035	0.927	0.039	0.955
	2	0.008	0.834	0.031	0.957	0.036	0.939	0.030	0.988
	3	0.009	0.958	0.028	0.990	0.032	0.896	0.029	0.972

*cyn-3-glu = cyanidin-3-glucoside; Dp-3, 5-diglu = delphinidin-3, 5-diglucoside; pg-3-glu = pelargonidin-3-glucoside and dp-3-glu = delphinidin-3-glucoside

4.2.7 Changes in total phenolic content

As determined by the Folin Ciocalteau method for total phenolic content, blackcurrant juice had the highest content of total phenolics (264.3 mg/L), followed by cranberry (145.4 mg/L) and pomegranate juice (16.6 mg/L) at the same single strength of 10.0 °Brix. After the addition of 1, 2 and 3% (w/v) concentrations of boysenberry juice, total phenolics content of blackcurrant juice reduced to 243.9, 234.8 and 234.3 mg/L (Figure 4.15). This may have resulted from the oxidation of phenolic compounds and some chemical reactions occurring between phenolics in blackcurrant juice and some compounds in boysenberry juice (Jackman & Smith, 1996). When boysenberry juice was added into cranberry juice with 1, 2 and 3% (w/v) concentrations, total phenolics contents was found to increase to 147.9, 155.6 and 162.8 mg/L. Similarly, pomegranate juice enhanced with 1, 2 and 3% (w/v) concentrations of boysenberry had a slight increase in total phenolics content with 18.2, 19.1 and 19.6 mg/L (Figure 4.15). As a check calculations of the total phenolic content that should be present by mass balance calculations found that after addition of boysenberry juice the total phenolic content of enhanced blackcurrant juice calculated was a bit higher than actually measured. This may be a result of limitations of spectrophotometric method that cannot measure all phenolic acids content in juices or reactions of phenolics as mentioned above (Appendices 41 &42). Also, for the mass balance calculations of enhanced cranberry and pomegranate juices, their total phenolic acids calculated were higher than the data measured from analysis.

During 28 days of storage, total phenolics content of all three juices enhanced with and without boysenberry juice decreased at all storage temperatures (Figure 4.14) ($P \geq 0.05$). At 5 ^{o}C, the total phenolics content of blackcurrant juice enriched with 0, 1, 2 and 3% (w/v) concentrations of boysenberry juice decreased by 24, 31, 25 and 28% of initial content. At higher temperatures, the total phenolics content of blackcurrant juice was not significantly different to juices stored at 5 ^{o}C. Similar results were also found in enhanced cranberry and pomegranate juice. Cranberry had the greatest loss of total phenolics during storage, followed by pomegranate and blackcurrant juice, regardless of storage temperatures. As previously discussed, boysenberry juice could help to maintain the total anthocyanin content of blackcurrant juice at 5 ^{o}C and results have shown that the total phenolics content of blackcurrant juice at all temperatures decreased throughout storage.

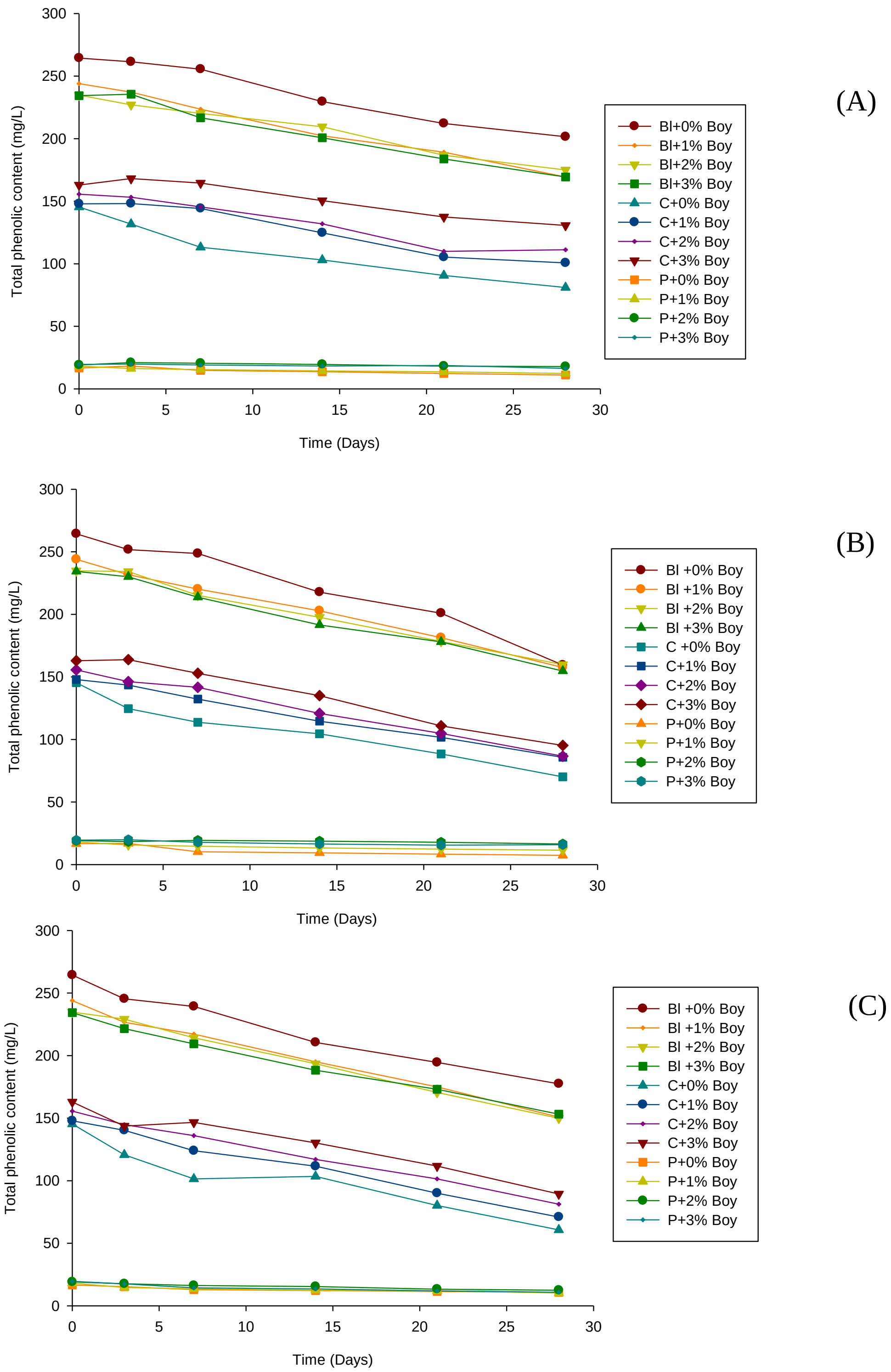


Figure 4.14 Change in total phenolics content of blackcurrant, cranberry and pomegranate enhanced with 0, 1, 2 and 3% (w/v) concentrations of boysenberry juice stored at (A) 5^{o}C; (B) 20^{o}C and (C) 35^{o}C (Bl = Blackcurrant juice; C = Cranberry juice; P = Pomegranate juice; Boy = Boysenberry) (mean ± SD, n=2)

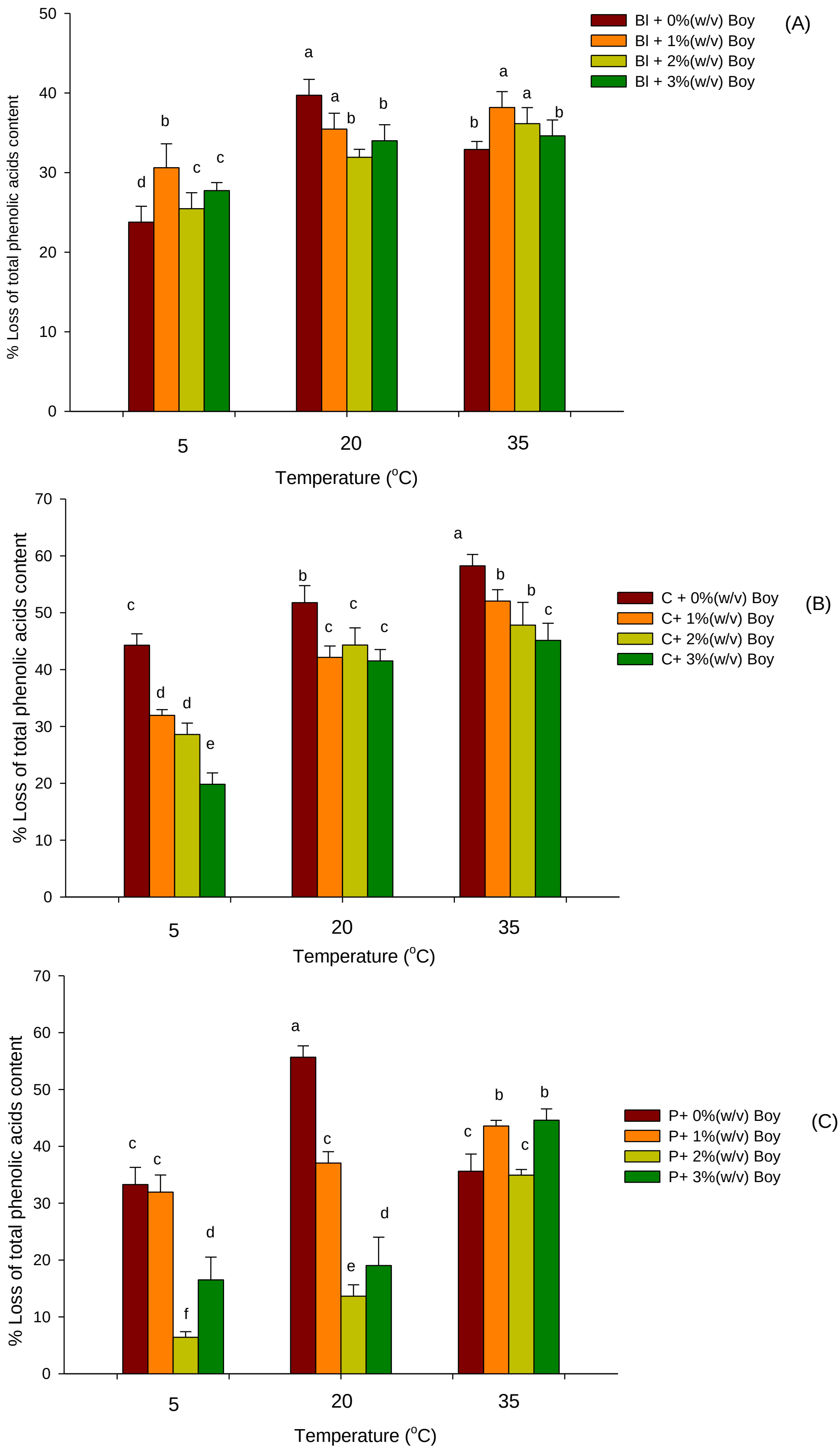


Figure 4.15 % Loss of total phenolic acids concentration of blackcurrant, cranberry and pomegranate enhanced with 0, 1, 2 and 3% (w/v) concentrations of boysenberry juice stored at (A) 5°C; (B) 20°C and (C) 35°C (Bl = Blackcurrant juice; C = Cranberry juice; P = Pomegranate juice; Boy = Boysenberry). Values marked by the same letter are not significantly different.

4.2.8 Changes in individual phenolic acid profile during storage

The results in Section 4.1 showed that the three major phenolic acids found in boysenberry juice were kaemferol, quercetin and ellagic acid and blackcurrant juice contained p-coumaric, caffeic acid, benzoic acid and quercetin as the major phenolics. Benzoic acid, quercetin, myricetin and p-coumaric acidwere identified in cranberry juice and the main phenolic acids present in pomegranate juice were ellagic and gallic acids.

It was found that the concentration of p-coumaric acid and caffeic acid in blackcurrant juice decreased immediately with increasing boysenberry juice concentrations at the first addition ($P \leq 0.05$) (Figure 4.16). The p-coumaric acid content decreased by 12.32, 18.8 and 21.7% when boysenberry juice was added at concentrations of 1, 2 and 3% (w/v) in blackcurrant juice respectively. A similar result was also found with caffeic acid content by 13, 14.8 and 15.6% of the original content. However, the effect was not found in cranberry juice which also contained p-coumaric acid. According to HPLC data (Figure 4.16), after addition of boysenberry juice (1, 2 and 3%) into blackcurrant juice, kaemferol, quercetin and ellagic acid normally present in boysenberry juice were not detected. This may be because of a low concentration of boysenberry juice added into the juice. After addition of boysenberry juice, the individual phenolic content of enhanced blackcurrant, cranberry and pomegranate juices calculated and presented by mass balance was similar to concentrations actually measured. (Appendices 41 &42).

During 28 days of storage, the four main phenolics in blackcurrant juice were not stable at all three temperatures ($P \leq 0.05$) (Figure 4.16). During storage at 5^{o}C, without addition of boysenberry juice, the p-coumaric acid content present in blackcurrant juice decreased significantly by 27.5% from its initial concentration; however, once blackcurrant juice was enhanced with 1, 2 and 3% (w/v) concentrations of boysenberry juice, the phenolic acid loss was reduced to 18.6, 19.0 and 23.4%, respectively (Figure 4.17). Likewise, quercetin present in blackcurrant juice had a higher retention when boysenberry juice was added. The quercetin loss in blackcurrant juice alone was 28.2%; on the other hand, when boysenberry juice at concentrations of 1, 2 and 3% (w/v) was added into the juice, the loss of quercetin was again reduced to 25.7, 21.9 and 23.0% loss, respectively. For benzoic acid, the addition of boysenberry juice did not affect its retention in enhanced blackcurrant juice during storage, as the reduction of the benzoic acid still remained the same as in blackcurrant juices without boysenberry additions. Similar trends were found in blackcurrant juice stored at higher temperature (Figure 4.16).

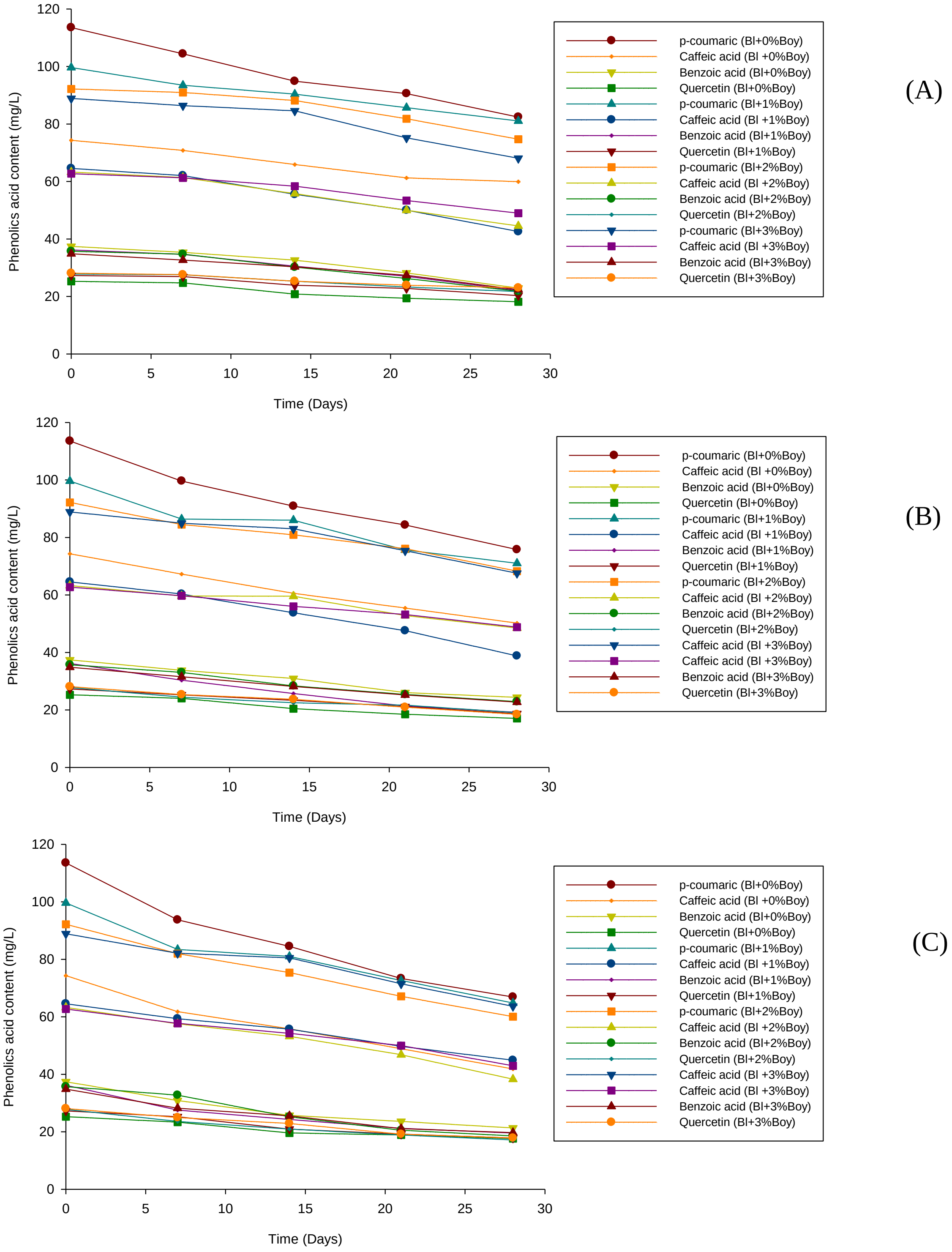


Figure 4.16 Changes in individual phenolic acids in blackcurrant juice enhanced with 0, 1, 2 and 3% (w/v) concentrations of boysenberry juice stored at (A) 5°C; (B) 20°C and (C) 35°C (Bl = Blackcurrant juice; C = Cranberry juice; P = Pomegranate juice; Boy = Boysenberry)

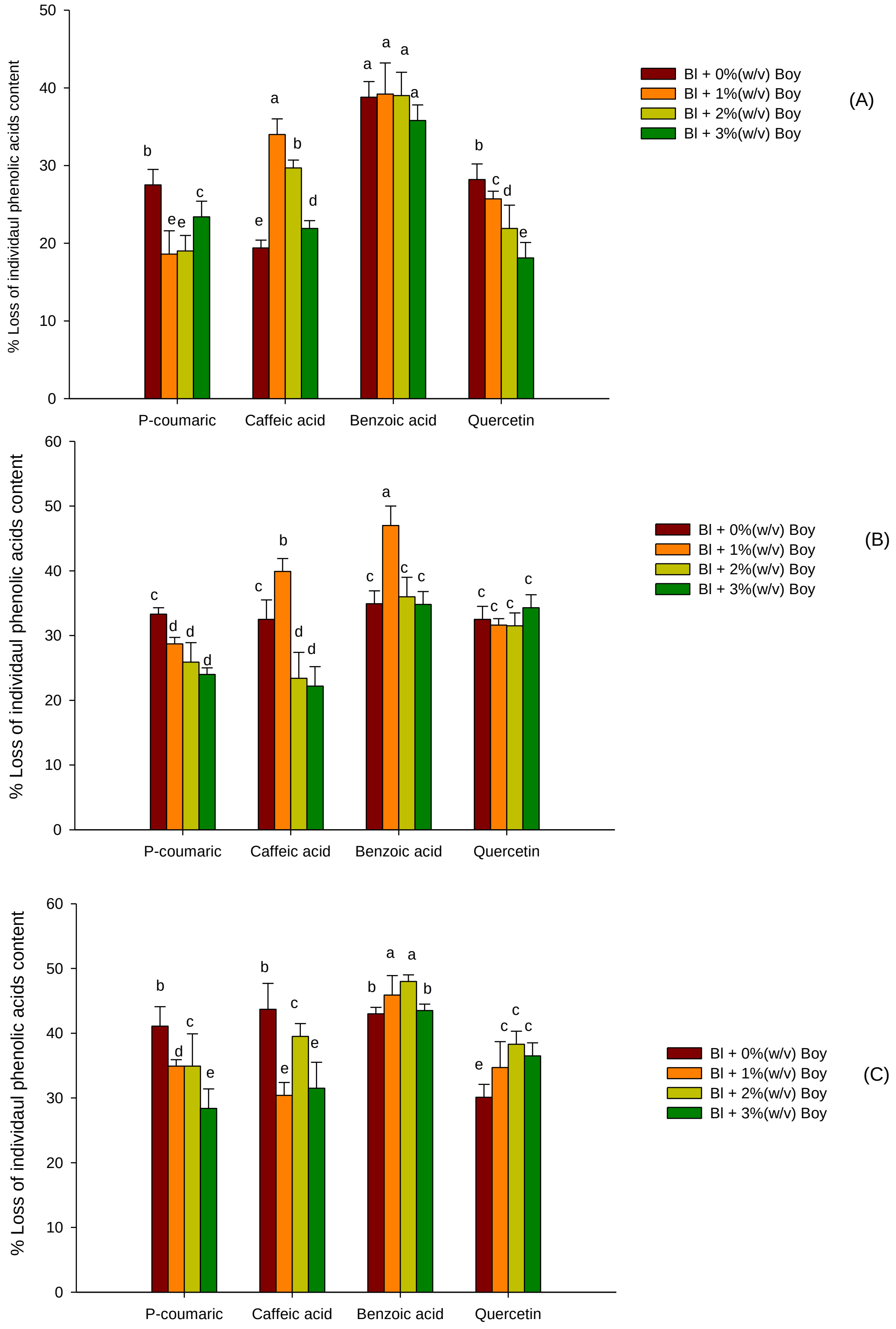


Figure 4.17 % Loss of individual phenolic acids concentration of blackcurrant juice enhanced with 0, 1, 2 and 3% (w/v) concentrations of boysenberry juice stored at (A) 5^{o}C; (B) 20^{o}C and (C) 35^{o}C (Bl = Blackcurrant juice; Boy = Boysenberry). Values marked by the same letter are not significantly different.

At the start of the study, the concentration of quercetin found in cranberry juice increased immediately after boysenberry juice addition with increasing concentrations of boysenberry juice ($P \leq 0.05$). The phenolic acid content in un-enhanced cranberry juice was 42.9 mg/L; however, it rose to 45.5, 50.1 and 54.2 mg/L when boysenberry juice was added at 1, 2 and 3% (w/v) concentrations, respectively, confirmed by mass balance calculations. This was because boysenberry juice contained quercetin as a major phenolic compound, so addition of boysenberry juice in cranberry juice would improve phenolic profile of quercetin in cranberry juice. During storage at all temperatures, the content of myricetin and p-coumaric acid were quite stable, but benzoic acid and quercetin concentrations decreased throughout storage period (Appendix 33) ($P \leq 0.05$).

Boysenberry juice also contained ellagic acid as the most abundant phenolic acid found. Pomegranate juice enhanced with boysenberry juice at 1, 2 and 3% (w/v) concentrations had a higher amount of ellagic acid found in pomegranate juice after the addition. The phenolic acid increased from 9.3 mg/L in non-enhanced pomegranate juice to 10.2, 11.4 and 12 mg/L with 1, 2 and 3% (w/v) concentrations of boysenberry juice, respectively, confirmed by mass balance calculations. At the end of storage, ellagic acid was more sensitive to degradation than gallic acid which was quite stable during storage ($P \leq 0.05$) (Appendix 34).

4.3 Discussion

With the addition of boysenberry juice to the three different berry juices (blackcurrant, cranberry and pomegranate juice), it was found that boysenberry juice improved only the blackcurrant juice colour by enhancing the anthocyanin colour intensity and stabilising the colour during storage. Non-enhanced blackcurrant juice and other juices lost their anthocyanin pigments and redness quickly during storage. The colour density of non-enhanced blackcurrant juice had a slower rate of degradation than cranberry and pomegranate juice, respectively. The study of anthocyanin content of different berry products during storage has been reported before for blackcurrant syrups (Skrede et al., 1992), cranberry juice cocktail (Starr & Francis, 1973), blood orange juice concentrate (Kirca & Cemeroglu, 2003) and grape anthocyanin (Palamidis & Markikis, 1975). Also, the effect of phenolic acids on colour stability of berry juices by phenolic acids has been studied previously (Rein & Heinonen, 2004). However, enhancement and stabilization of the colour of blackcurrant juice by phenolic acids during storage has not been reported yet, therefore, the next part of study will focus this result.

From the previous results, cranberry and pomegranate juices contained less anthocyanins and phenolic acids than blackcurrant juice. This concentration factor might be a reason for copigmentation reactions occurring in blackcurrant juice during storage (Kahkonen et al., 2001; Prior et al., 2001). Also, the different pH values can have an effect on the stabilisation of the blackcurrant juice colour during storage. The result shows that the pH was a little higher in blackcurrant juice (2.9) as compared to cranberry (2.5) and pomegranate juices (2.7). After addition of boysenberry juice, the pH value of all blackcurrant, cranberry and pomegranate juices were 3.4, 2.9 and 3.1, respectively. HPLC data, showed that most anthocyanins found in blackcurrant, cranberry and pomegranate juices were 3-monoglucosides which have a maximum effect of copigmentation at pH 3.5-4.2 (William & Hrazdina, 1979). Therefore, blackcurrant juice enhanced with boysenberry juice resulted in a juice with a more optimum pH value for copigmentation compared to other two juices.

The different anthocyanin profile of the juices is one of the main reasons for the different copigmentation and enhancement reactions (Rein & Heinonen, 2004). From the HPLC data, blackcurrant and pomegranate juice have similar anthocyanin profiles, except delphinidin-3-rutinoside that was found in blackcurrant juice only. Therefore, the copigmentation reactions should be similar within blackcurrant and pomegranate juices compared to cranberry juice which contained different anthocyanin profiles. However, the result showed that only blackcurrant juice could form a copigmentation when boysenberry juice was added. Skrede et al. (1992) reported that colour stability is more dependent on the total anthocyanin content rather than the qualitative anthocyanin composition. This is supported as to why copigmentation with boysenberry juice could occur only in blackcurrant juice but not in pomegranate juice, although both juice contained similar anthocyanin profiles.

CHAPTER 5

PHENOLIC ACID ENHANCEMENT

5.1. Copigmentation effects of phenolic acids on blackcurrant juice (Study 3)

As found previously, the addition of boysenberry juice could improve the colour stability of blackcurrant juice when it was stored at lower temperatures. In this step, the objective was to investigate if the phenolic acids found in boysenberry juice have an effect on colour stability of blackcurrant juice during storage at 5 and 20^{o}C. Phenolic acids identified in boysenberry juice (kaemferol, quercetin and ellagic acid) were each added to blackcurrant juice at 0.2, 0.6 and 1 mg/L based on Rein (2005) who added pure phenolic acids into berry juices such as strawberry, raspberry, lingonberry and cranberry juices. The juices in this study were then stored at 5 and 20^{o}C. Colour stabilities of juices were compared after determining by spectrophotometric methods the total anthocyanin content, colour density, polymeric colour, hyperchromic shift, bathochromic shift and total phenolic content. Individual phenolic acids and anthocyanins were also determined by HPLC.

5.1.1 Changes in total anthocyanin content

The total anthocyanin content of blackcurrant juice enhanced with 0.2, 0.6 and 1 mg/L of kaemferol, quercetin and ellagic acid was not stable during storage at room temperatures (20^{o}C). When the enhanced blackcurrant juices were stored at lower temperature (5^{o}C), only the juices enhanced with ellagic acid (0.2, 0.6 and 1 mg/L) were found to maintain the total anthocyanin content during 28 days of storage ($P \leq 0.05$)(Figure 5.1).

Immediately after addition the anthocyanin content of blackcurrant juice enhanced with 0.2, 0.6 and 1 mg/L ellagic acid increased to 1158.1, 1202.9 and 1255.5 mg/L, respectively, compared to non-enhanced blackcurrant juice with 1137.1 mg/L. When quercetin and kaemferol were added into blackcurrant juice at the same concentrations (0.2, 0.6 and 1 mg/L), the anthocyanin contents of the juices were not found to be change significantly ($P>0.05$). The increase in total anthocyanin may be because of limitations of spectrophotometric method or reactions of anthocyanins with phenolic acids. After 28 days of storage at 5^{o}C, the pigment content of enhanced blackcurrant juice with ellagic acid remained stable; however, storage at higher temperature (20^{o}C) the anthocyanin content of the enhanced juices decreased slowly (Figure 5.1). The anthocyanin content of blackcurrant juice enriched with kaemferol and quercetin decreased during storage at both temperatures ($P\leq0.05$) (Figure 5.2).

At 5°C storage, the anthocyanin content of non-enhanced blackcurrant juice reduced by 14.6 % (Figure 5.2). With 0.2, 0.6 and 1 mg/L concentrations of ellagic acid, the pigment content in blackcurrant juice diminished only by 3.6, 5.0 and 4.4 %, respectively, after 28 days. Compared to storage at 20°C, where there was a remarkable decrease in the anthocyanin content of blackcurrant juices. The anthocyanin content in blackcurrant enhanced with 0.2, 0.6 and 1 mg/L concentrations of ellagic acid stored at 20°C reduced by 20.6, 14.5, 12.7 and 15.1% of the original content (Figure 5.2). Therefore, the addition of 0.2, 0.6 and 1 mg/L concentrations of ellagic acid was found to keep the anthocyanin pigments stable if the blackcurrant juice was stored at 5°C, but not when stored at 20 °C ($P\leq0.05$).

The loss of total anthocyanins in blackcurrant juice enhanced with kaemferol and quercetin, after 28 days of 5^{o}C storage is shown in Figure 5.2. The addition of kaemferol and quercetin did not help to maintain the anthocyanin content of blackcurrant juice at at any storage temperatures as the colour decreased gradually during storage.

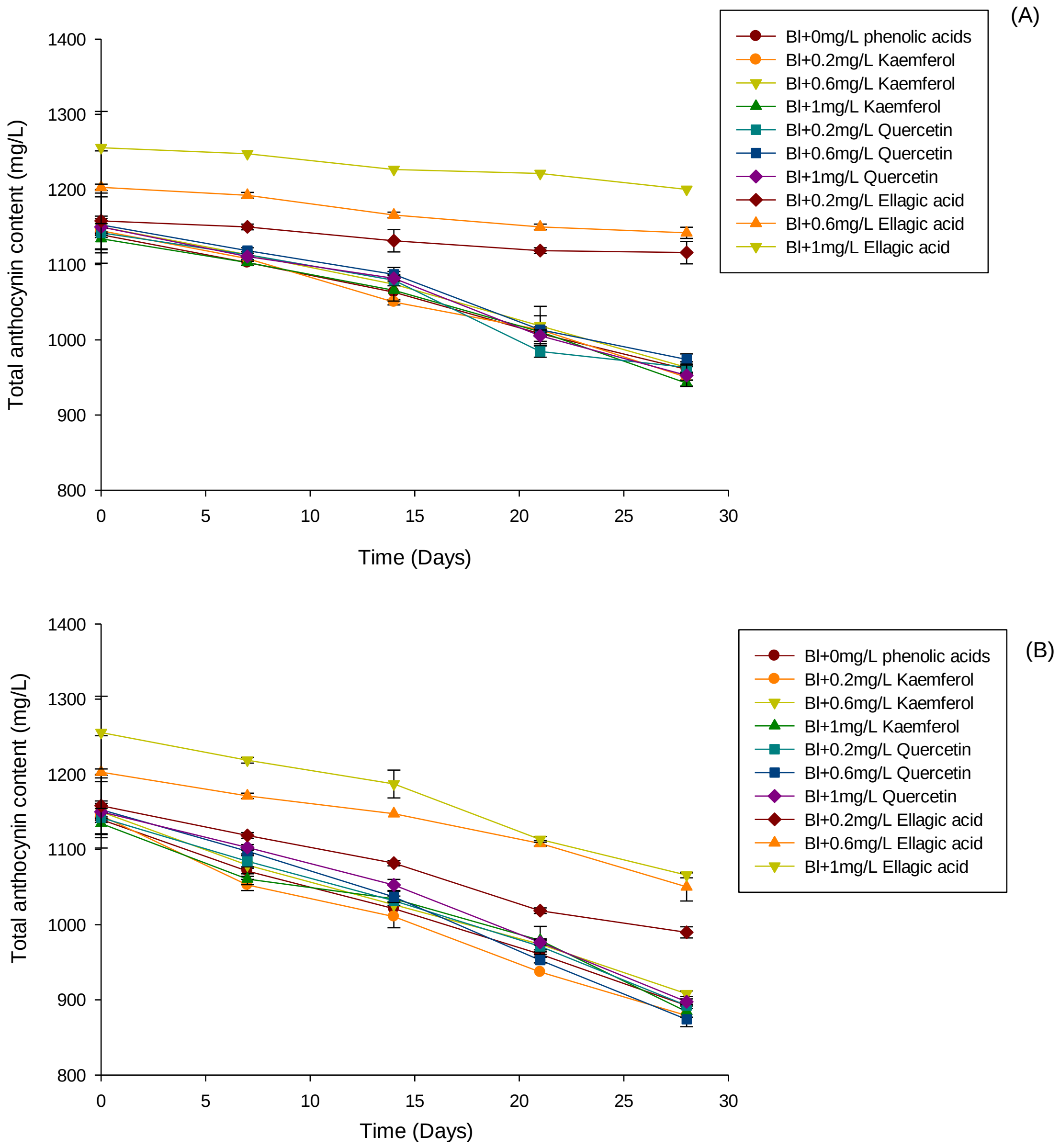


Figure 5.1 Change in total anthocyanin concentration (mg/L) of blackcurrant juice enhanced with 0, 0.2, 0.6 and 1 mg/L of kaemferol, quercetin and ellagic acid stored at (A) 5°C and (B) 20°C (Bl = Blackcurrant juice) (mean ± SD, n=2)

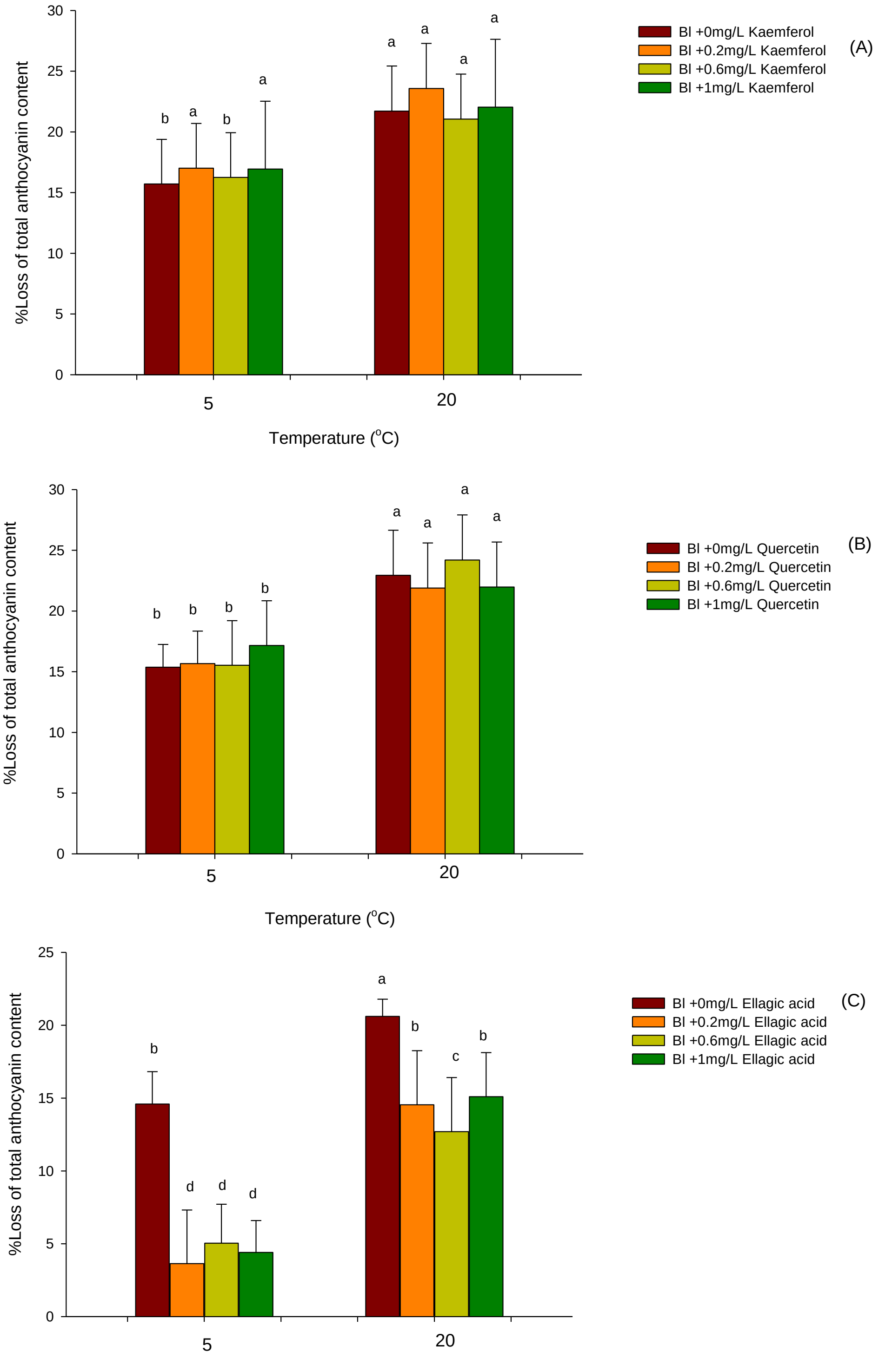


Figure 5.2 % Loss of total anthocyanin concentration determined by spectrophotometric technique for blackcurrant enhanced with 0, 0.2, 0.6 and 1mg/L concentrations of kaemferol (A), quercetin (B) and ellagic acid (C) stored at 5°C and 20°C (Bl = Blackcurrant juice). Values marked by the same letter are not significantly different.

5.1.2 Colour density

The changes in colour density of blackcurrant juice after addition of kaemferol, quercetin and ellagic acid, with storage are shown in Figure 5.3. In agreement with total anthocyanin content, blackcurrant juice enhanced with kaemferol and quercetin did not help to maintain the colour density of blackcurrant juices at any storage temperatures over the 28 days of storage. Only ellagic acid could keep the colour of blackcurrant juice stable when the juices were stored at 5^{o}C ($P \leq 0.05$). It could be seen that blackcurrant juice enhanced with ellagic acid had the smallest loss of colour density after storage of 28 days, followed by quercetin and kaemferol, respectively (Figure 5.4).

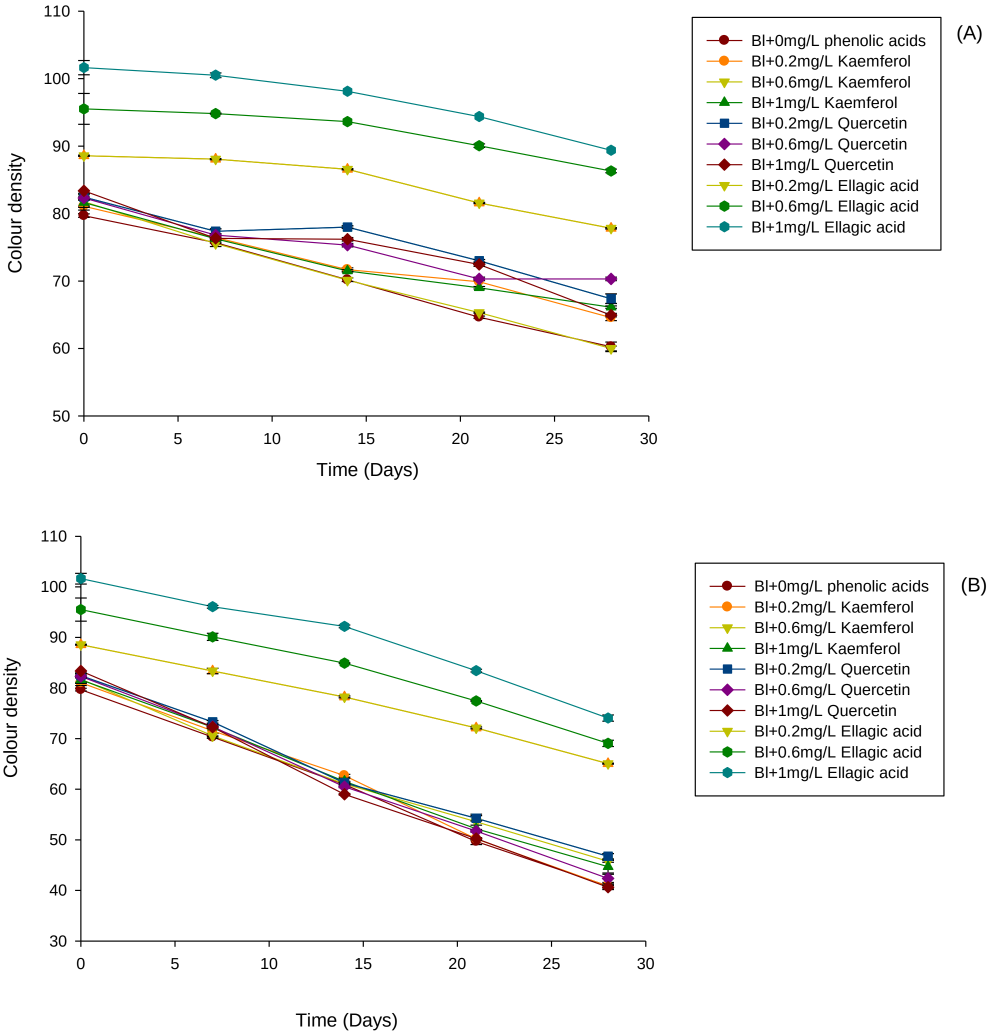


Figure 5.3 Change in colour density of blackcurrant juice enhanced with 0, 0.2, 0.6 and 1 mg/L of kaemferol, quercetin and ellagic acid stored at (A) 5^{o}C and (B) 20^{o}C (Bl = Blackcurrant juice) (mean ± SD, n=2)

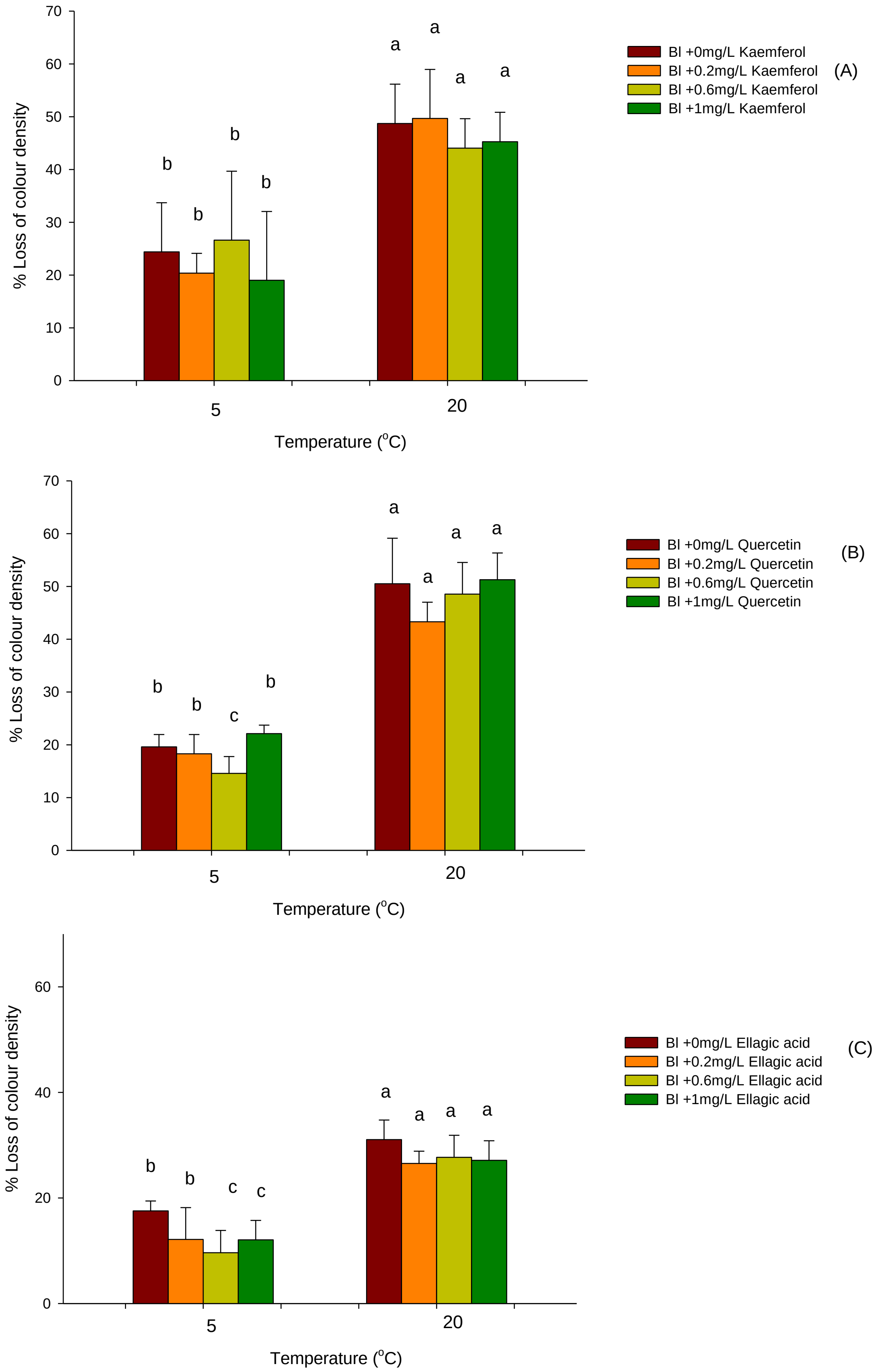


Figure 5.4 % Loss of colour density determined by spectrophotometric technique of blackcurrant enhanced with 0, 0.2, 0.6 and 1mg/L concentrations of kaemferol (A), quercetin (B) and ellagic acid (C) stored at 5°C and 20°C (Bl = Blackcurrant juice). Values marked by the same letter are not significantly different.

5.1.3 Hyperchromic shift

Hyperchromic shift is presented as a change in the absorbance of $\lambda_{max.}$ An increase in the absorbance of λ_{max} can show a rise in the intensity of juice colour. It was found that ellagic acid improved the colour of blackcurrant juice immediately after the addition; nevertheless, the colour intensity of enriched blackcurrant juice stored at 20 ^{o}C was not more stable than during storage at 5 ^{o}C (Figure 5.5), as seen with anthocyanin content.

The addition of ellagic acid in blackcurrant increased the value of colour intensity at the beginning of study, and also maintained the colour stability in blackcurrant juice during storage at 5 ^{o}C ($P \leq 0.05$). During 28 days of storage, it was found that addition of kaemferol and quercetin in blackcurrant juice did not show any improvement the colour stability of blackcurrant juice (Figures 5.5 & 5.6).

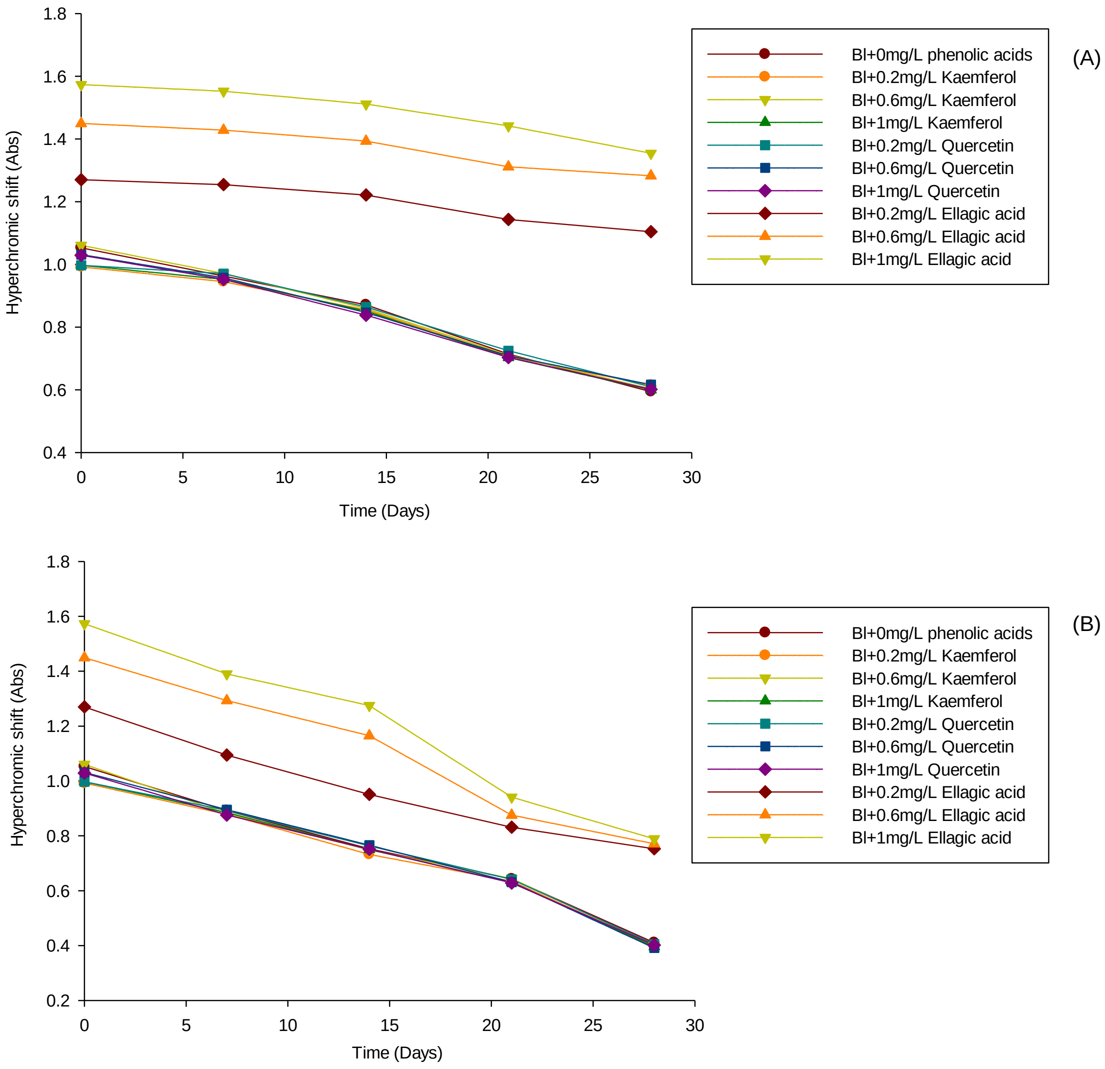


Figure 5.5 Change in hyperchromic shift of blackcurrant juice enhanced with 0, 0.2, 0.6 and 1 mg/L of kameferol, quercetin and ellagic acid stored at (A) 5^{o}C and (B) 20^{o}C (Bl = Blackcurrant juice) (mean ± SD, n=2)

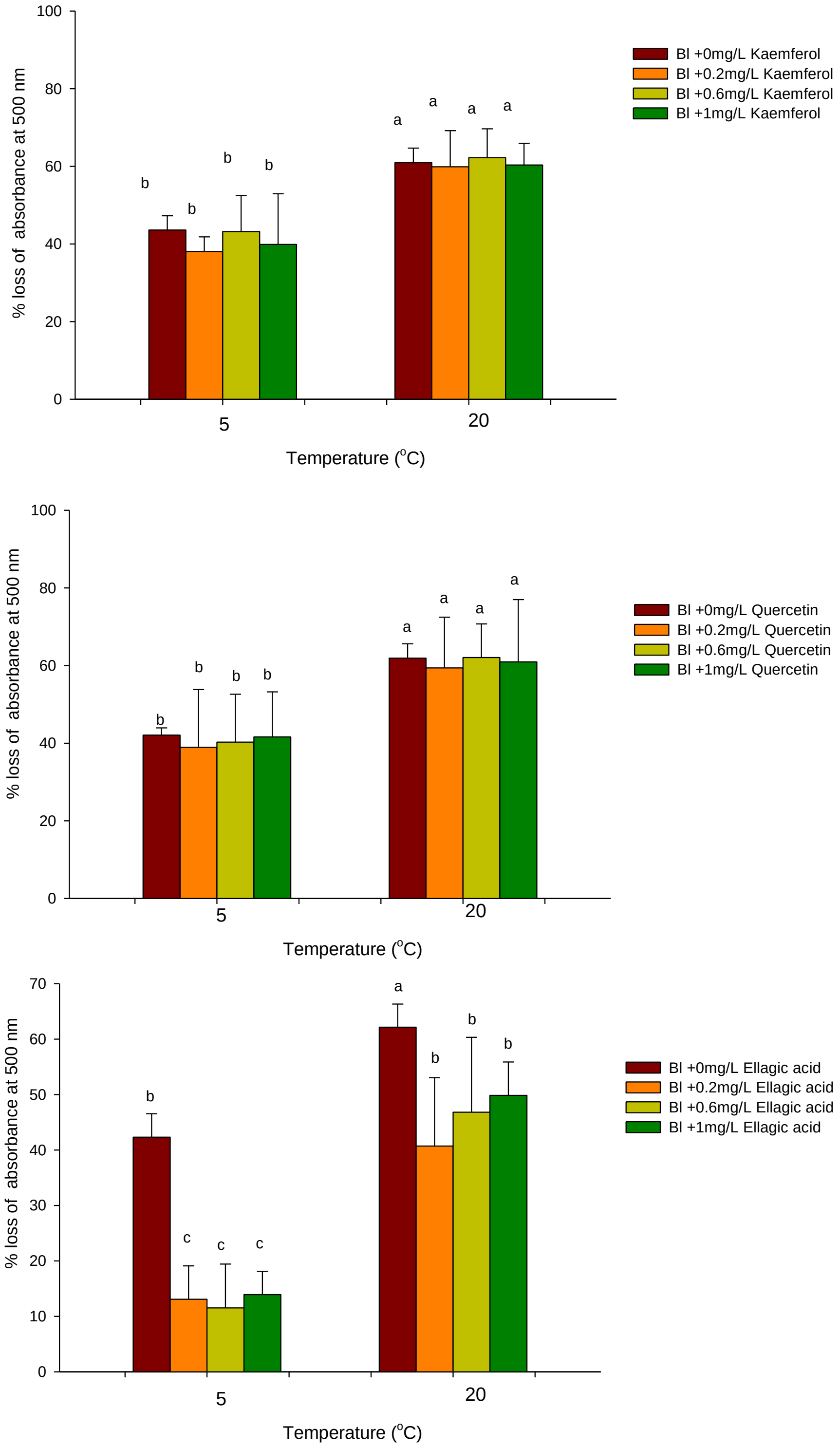


Figure 5.6 % Loss of hyperchromic shift (Abs. At 500 nm) determined by spectrophotometric technique of blackcurrant enhanced with 0, 0.2, 0.6 and 1mg/L concentrations of kaemferol (A), quercetin (B) and ellagic acid (C) stored at 5°C and 20°C (Bl = Blackcurrant juice). Values marked by the same letter are not significantly different.

5.1.4 Polymeric colour

Polymeric colour is a measure of the intensity for the bisulphite treated samples and is a measure of the degree of polymerization of the anthocyanins. Monomeric anthocyanin pigments will combine with bisulphite to form colourless compounds, while polymeric anthocyanin –tannin and melanoidin pigments are resistant to bleaching by bisulphite and will remain coloured at 420 nm (Wrolstad et al., 2005).

Polymeric colour of blackcurrant juice enhanced with and without kaemferol, quercetin and ellagic acid increased gradually during storage at both storage temperatures. The blackcurrant juices enhanced with ellagic acid showed higher polymeric colour than those enhanced with quercetin and kaemferol (Figure 5.7). At the start of storage, blackcurrant juices enriched with ellagic acid showed a greater rise in the polymeric colour compared to juices enhanced with quercetin or kaemferol. At higher temperatures, the polymeric colour of all enhanced and non-enhanced blackcurrant juices showed a higher degree of increase than the juices stored at refrigerated temperature (5^{o}C) (Figure 5.8) ($P \leq 0.05$).

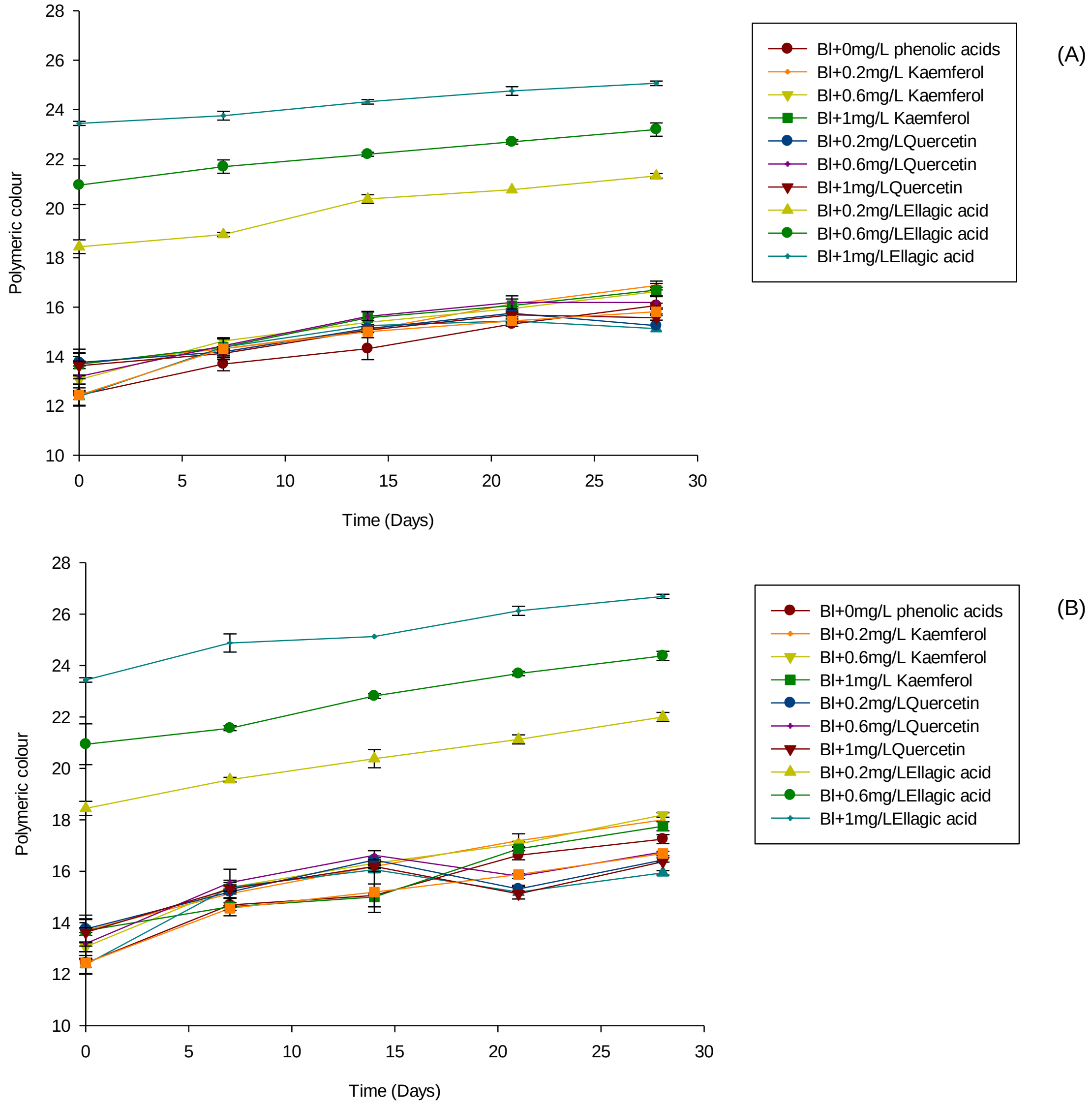


Figure 5.7 Change in polymeric colour of blackcurrant juice enhanced with 0, 0.2, 0.6 and 1 mg/L of kaemferol, quercetin and ellagic acid stored at (A) 5^{o}C and (B) 20^{o}C (Bl = Blackcurrant juice) (mean ± SD, n=2)

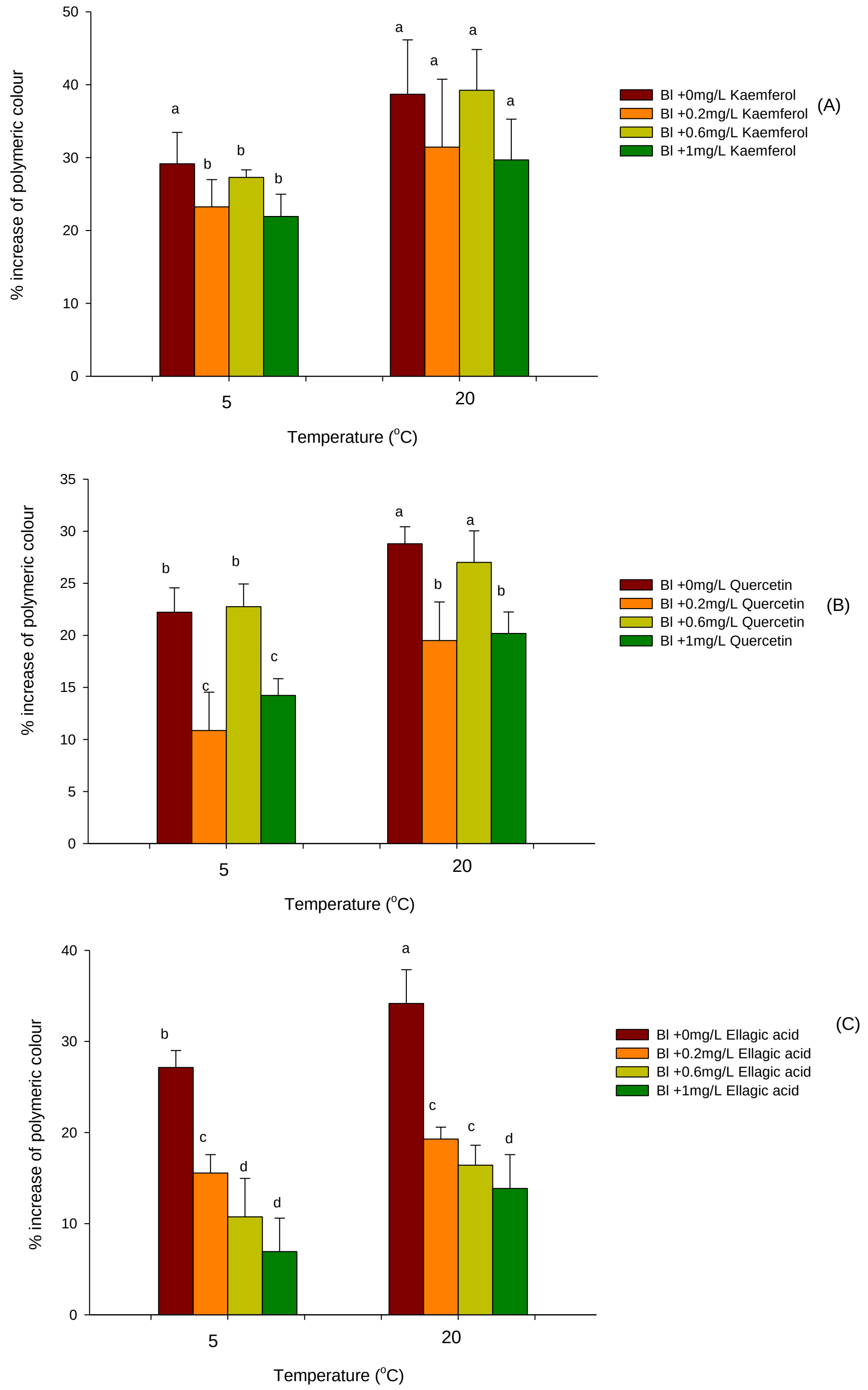


Figure 5.8 % Increase of polymeric colour determined by spectrophotometric technique of blackcurrant enhanced with 0, 0.2, 0.6 and 1mg/L concentrations of kaemferol (A), quercetin (B) and ellagic acid (C) stored at 5^{o}C and 20^{o}C (Bl = Blackcurrant juice). Values marked by the same letter are not significantly different.

The blackcurrant juices enhanced with ellagic acid showed higher polymeric colour than those enhanced with quercetin and kaemferol (Figure 5.7). This may be because of the effect of different chemical structures of each of the phenolic acids. Ellagic acid is a non-flavonoid compound and is formed by the association of two molecules of gallic acid, but quercetin and kaemferol are flavonoids. Therefore, ellagic acid's structure may affect the positions it forms linkages with flavonoids, alcohol or sugar to form tannins in juice The most common covalent linkage occurs between the C4 with C8 of the flavonoid subunit; however, ellagic acid has the linkage at a C6-C3 skeleton which is weaker than C4-C8 position (Hrazdina et al., 1984; Darias-Martin et al., 2002). As a result of the different structures of ellagic acid, this allows ellagic acid to easily to combine with sugar or other compounds via esterification to form a larger polymer of ellagitannin that is a non-soluble compound (Figure 5.9) (Souquet et al., 1996). Therefore, this could be a main reason why there was an increase in polymeric colour when ellagic acid is added into blackcurrant juice compared to the other two phenolic acids.

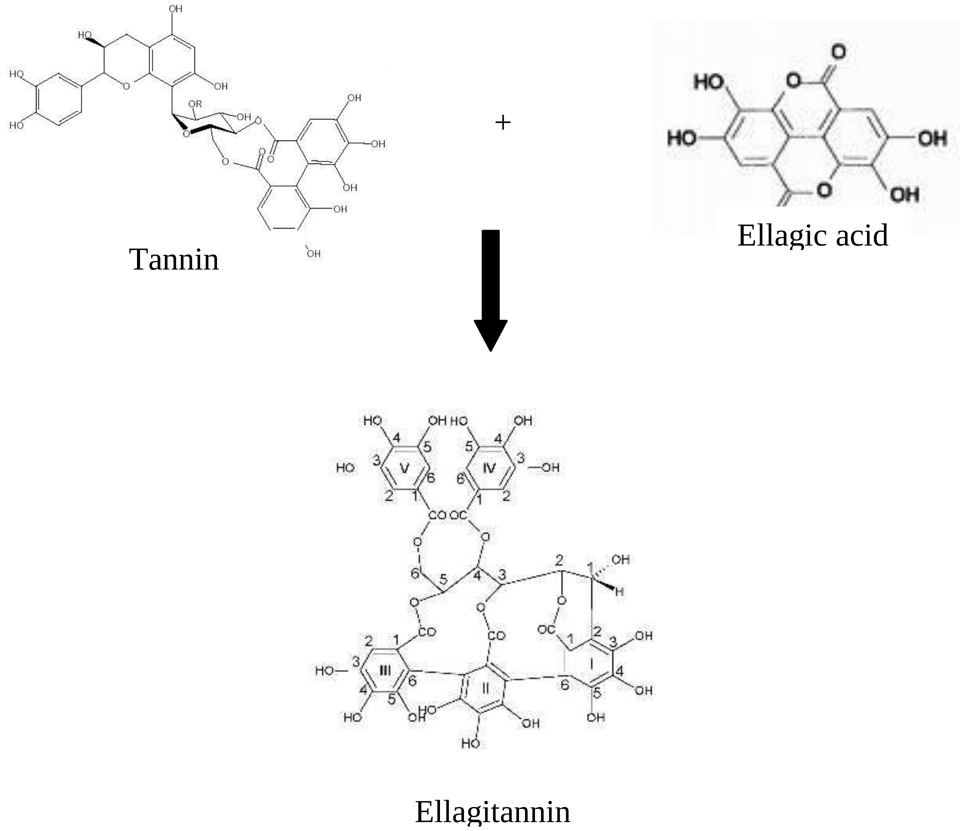


Figure 5.9 The formation of ellagitannins in juice (Barron, 2001)

5.1.5 Impact of temperature on anthocyanin stability

Table 5.5 shows the rate of degradation of the total anthocyanin content of blackcurrant juice enhanced with 0, 0.2, 0.6 and 1 mg/L of kaemferol, quercetin and ellagic acid stored at 5^{o}C and 20^{o}C. The degradation rate of blackcurrant juice enhanced with 0.2, 0.6 and 1 mg/L of kaemferol did not changed at any temperatures. They ranged between 0.006-0.007 day^{-1} at 5^{o}C and 0.008-0.009 day^{-1} at 20^{o}C. Similar results were found with quercetin. In contrast, the rate of degradation of anthocyanin content in blackcurrant juice was slower when ellagic acid was added and stored at 5^{o}C (Table 5.1). Without ellagic acid addition, the rate constants (k) of blackcurrant stored at 5^{o}C was 0.005 day^{-1}; however, when 0.2, 0.6 and 1 mg/L of ellagic acid were added to blackcurrant juice, the k values decreased to 0.002 day^{-1}. The mechanism of thermal degradation was discussed in section 4.2.8.

Table 5.1 Rate of degradation of total anthocyanins in blackcurrant juice enhanced with 0, 0.2, 0.6 and 1 mg/L of kaemferol, quercetin and ellagic acid stored at 5°C and 20°C, first order reaction rate constants.

Phenolic acid standards.	Temperature(°C)	Concentrations of phenolics added (mg/L)	Rate constant (k) (Day^{-1})	R^2
Kaemferol	5	0.0	0.006	0.990
		0.2	0.007	0.988
		0.6	0.006	0.985
		1.0	0.007	0.965
	20	0.0	0.009	0.995
		0.2	0.009	0.992
		0.6	0.008	0.997
		1.0	0.008	0.956
Quercetin	5	0.0	0.006	0.977
		0.2	0.007	0.988
		0.6	0.006	0.973
		1.0	0.007	0.969
	20	0.0	0.008	0.930
		0.2	0.009	0.992
		0.6	0.009	0.945
		1.0	0.008	0.942
Ellagic acid	5	0.0	0.005	0.910
		0.2	0.002	0.955
		0.6	0.002	0.970
		1.0	0.002	0.968
	20	0.0	0.007	0.926
		0.2	0.005	0.943
		0.6	0.004	0.927
		1.0	0.006	0.948

5.1.6 Changes in individual anthocyanin profile during storage

As presented earlier in section 4.1, that delphinidin-3-rutinoside, cyanidin-3-rutinoside, delphinidin-3-glucoside and cyanidin-3-glucoside were the four major anthocyanins found in blackcurrant juice. The addition of any phenolic acids (kaemferol, quercetin and ellagic acid) in blackcurrant juice did not alter the concentration of individual anthocyanins in blackcurrant juice ($P \geq 0.05$) (Figure 5.10). This was supported by comparisons of measured HPLC results and mass balance calculations, found that the concentration of individual anthocyanins of both were similar to each other (Appendices 39 & 40). Although as found previously shown (Section 5.1.3) after the addition of ellagic acid the colour intensity of blackcurrant juice increased immediately after addition.

During storage of 28 days, four anthocyanins (delphinidin-3-rutinoside, cyanidin-3-rutinoside, delphinidin-3-glucoside and cyanidin-3-glucoside) present in blackcurrant juice enhanced with ellagic acid decreased gradually as shown in Figure 5.10 ($P \leq 0.05$). This corresponded to the loss in total anthocyanin content determined in Section 5.1.1. All four anthocyanins were more stable when the juices were stored at 5^oC than storage at higher temperatures (Figure 5.11). After addition of quercetin and kaemferol, the anthocyanin concentrations of delphinidin-3-rutinoside, cyanidin-3-rutinoside, delphinidin-3-glucoside and cyanidin-3-glucoside in blackcurrant juice also decreased throughout storage at all temperatures and faster with increasing temperature ($P \leq 0.05$)(Appendix 20 and 21).

As found previously, the results showed that the degradation rate of anthocyanins increased with increasing temperature and the degradation of anthocyanins in blackcurrant juice enhanced with kaemferol, quercetin and ellagic acid followed a first–order degradation reaction (Table 5.2). The degradation rate constants (k days^{-1}) for each anthocyanin in blackcurrant juice are shown in Table 5.2. Among the four individual anthocyanins found in blackcurrant juice enriched with quercetin and kaemferol, delphinidin-3-rutinoside was also more stable than others, followed by delphinidin-3-glucoside, cyanidin-3-rutinoside and cyanidin-3-glucoside, respectively (Table 5.2). It was found that the addition of ellagic acid only in blackcurrant juice decreased the degradation rate constant (k) of individual anthocyanins in blackcurrant juice (Table 5.2). This result was not found when kaemferol and quercetin were added into blackcurrant juice.

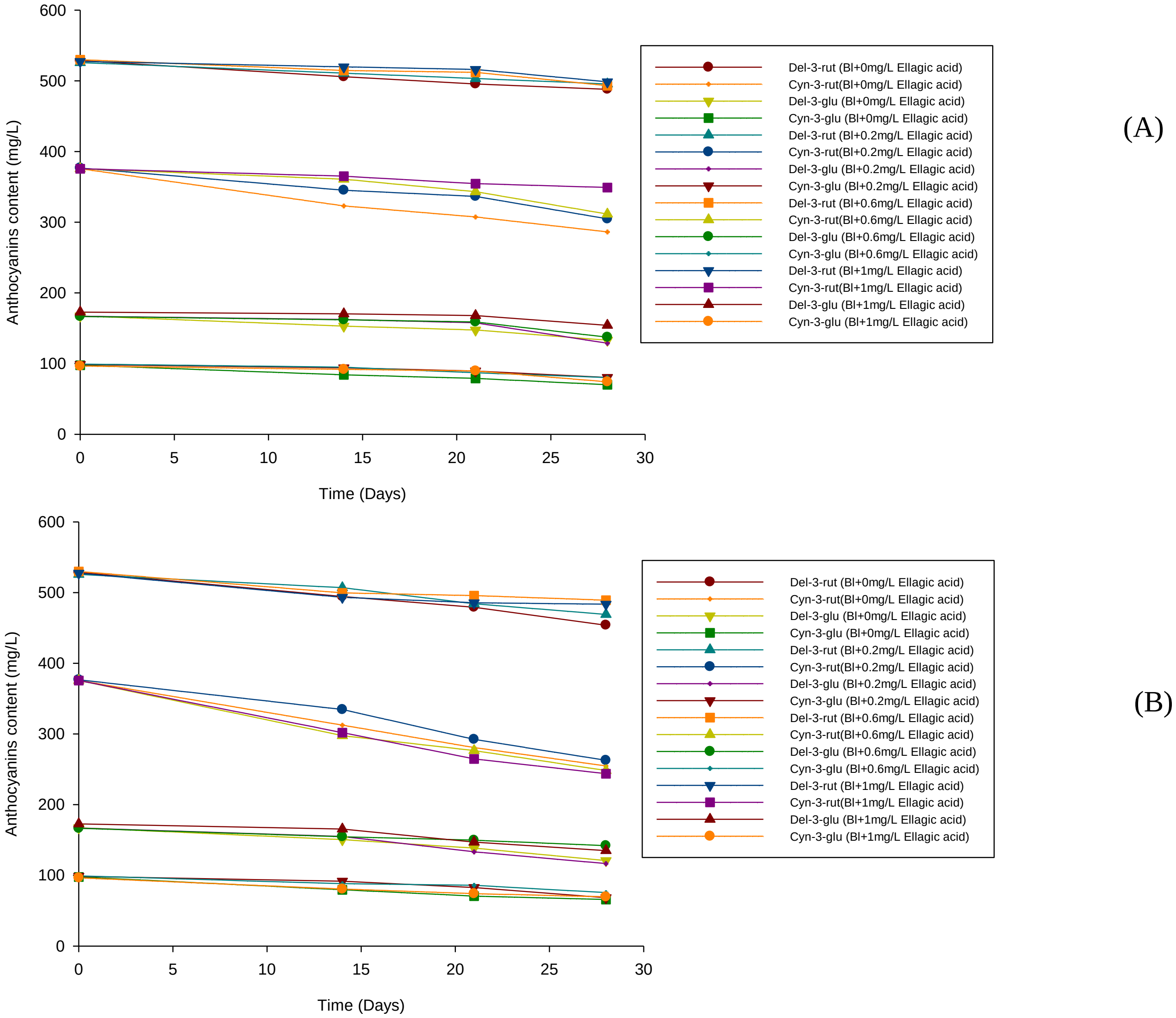


Figure 5.10 Changes in anthocyanins profile blackcurrant juice enhanced with 0, 0.2, 0.6 and 1 mg/L of kaemferol, quercetin and ellagic acid stored at (A) 5°C and (B) 20°C (Bl = Blackcurrant juice) (mean ± SD, n=2)

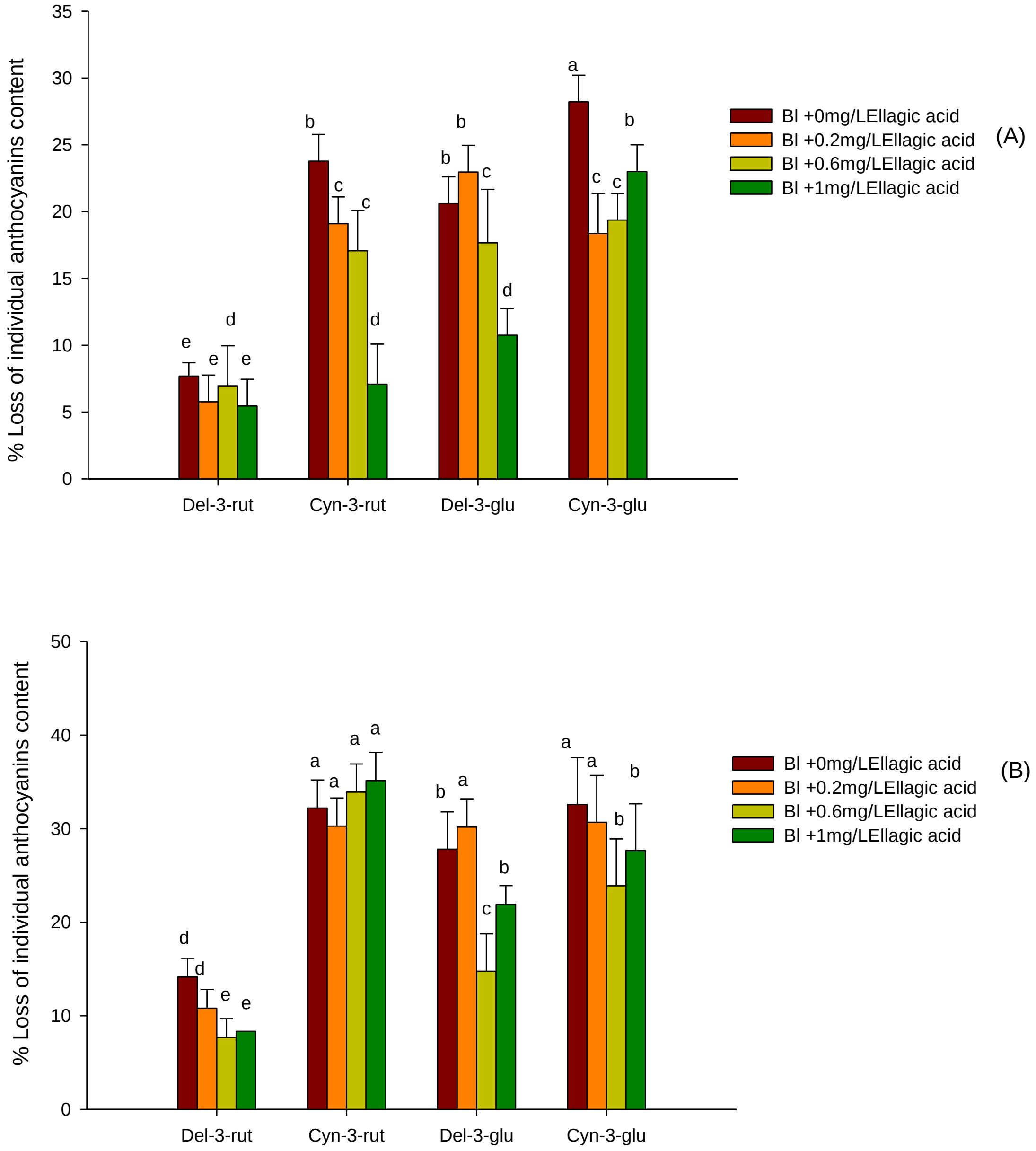


Figure 5.11 % Loss of individual anthocyanins content determined by HPLC of blackcurrant enhanced with 0, 0.2, 0.6 and 1mg/L concentrations of kaemferol, quercetin and ellagic acid stored at 5°C (A) and 20°C (B) (Bl = Blackcurrant juice). Values marked by the same letter are not significantly different.

Table 5.2 Degradation of anthocyanins of blackcurrant juice enhanced with 0, 0.2, 0.6 and 1 mg/L of ellagic acid, quercetin and ellagic acid stored at 5^{o}C and 20^{o}C, first order reaction rate constants.

Phenolic acids	Phenolics concentration added (mg/L)	Storage Temperature (^{o}C)	Rate constant (k) (day^{-1})	R^2	Rate constant (k) (day^{-1})	R^2	Rate constant (k) (day^{-1})	R^2	Rate constant (k) (day^{-1})	R^2
			Del-3-rut		Cyn-3-rut		Del-3-glu		Cyn-3-glu	
Kaemferol	0	5	0.039	0.923	0.011	0.993	0.008	0.986	0.011	0.989
	0.2		0.004	0.978	0.012	0.998	0.009	0.971	0.010	0.998
	0.6		0.004	0.998	0.014	0.981	0.006	0.949	0.013	1.000
	1		0.003	0.989	0.014	0.981	0.008	0.989	0.011	0.976
	0	20	0.051	0.935	0.013	0.933	0.014	0.981	0.014	0.989
	0.2		0.006	0.968	0.015	0.990	0.010	0.958	0.012	0.911
	0.6		0.005	0.973	0.017	0.990	0.011	0.983	0.015	0.947
	1		0.005	0.992	0.015	0.972	0.008	0.996	0.013	0.982
Quercetin	0	5	0.970	0.987	0.015	0.941	0.010	0.935	0.014	0.986
	0.2		0.004	0.927	0.016	0.844	0.008	0.992	0.013	0.977
	0.6		0.004	0.945	0.017	0.858	0.011	0.988	0.015	0.975
	1		0.005	0.940	0.015	0.938	0.010	0.981	0.013	0.983
	0	20	0.007	0.005	0.027	0.909	0.013	0.991	0.015	0.942
	0.2		0.007	0.873	0.019	0.947	0.010	0.969	0.014	0.937
	0.6		0.006	0.927	0.026	0.885	0.012	0.997	0.015	0.929
	1		0.007	0.937	0.023	0.897	0.008	0.942	0.013	0.924
Ellagic acid	0	5	0.003	0.995	0.010	0.995	0.008	0.950	0.011	0.984
	0.2		0.002	1.000	0.007	0.943	0.008	0.661	0.007	0.871
	0.6		0.002	0.909	0.006	0.867	0.006	0.689	0.008	0.908
	1		0.002	0.828	0.003	0.979	0.004	0.685	0.008	0.724
	0	20	0.005	0.985	0.014	0.999	0.011	0.947	0.014	0.995
	0.2		0.004	0.962	0.013	0.966	0.013	0.904	0.012	0.853
	0.6		0.003	0.935	0.015	0.994	0.006	0.992	0.009	0.944
	1		0.003	0.912	0.016	0.996	0.009	0.886	0.012	0.995

5.1.7 Changes in total phenolic acids content

The total phenolic content of blackcurrant juice slightly increased immediately after the addition of 0.2, 0.6 and 1 mg/L of kaemferol, quercetin and ellagic acid (Figure 5.12), as expected, and confirmed by mass balance calculations (Appendices 41&42).

During the first 15 days of storage, the total phenolic content of all blackcurrant juices at the two storage temperatures decreased quickly and after that, the rate of loss declined slowly until the end of storage. At the end of 28 days storage, the total phenolic content of all models of blackcurrant juices enhanced and not enhanced with phenolic acids decreased at all storage temperatures, and there was also significant difference in total phenolic content loss between the two storage temperatures for each juice ($P \leq 0.05$) (Figure 5.12). Blackcurrant juice enhanced with quercetin and ellagic acid gave a small decrease trend in total phenolics content during storage ($P \leq 0.05$) (Figure 5.13).

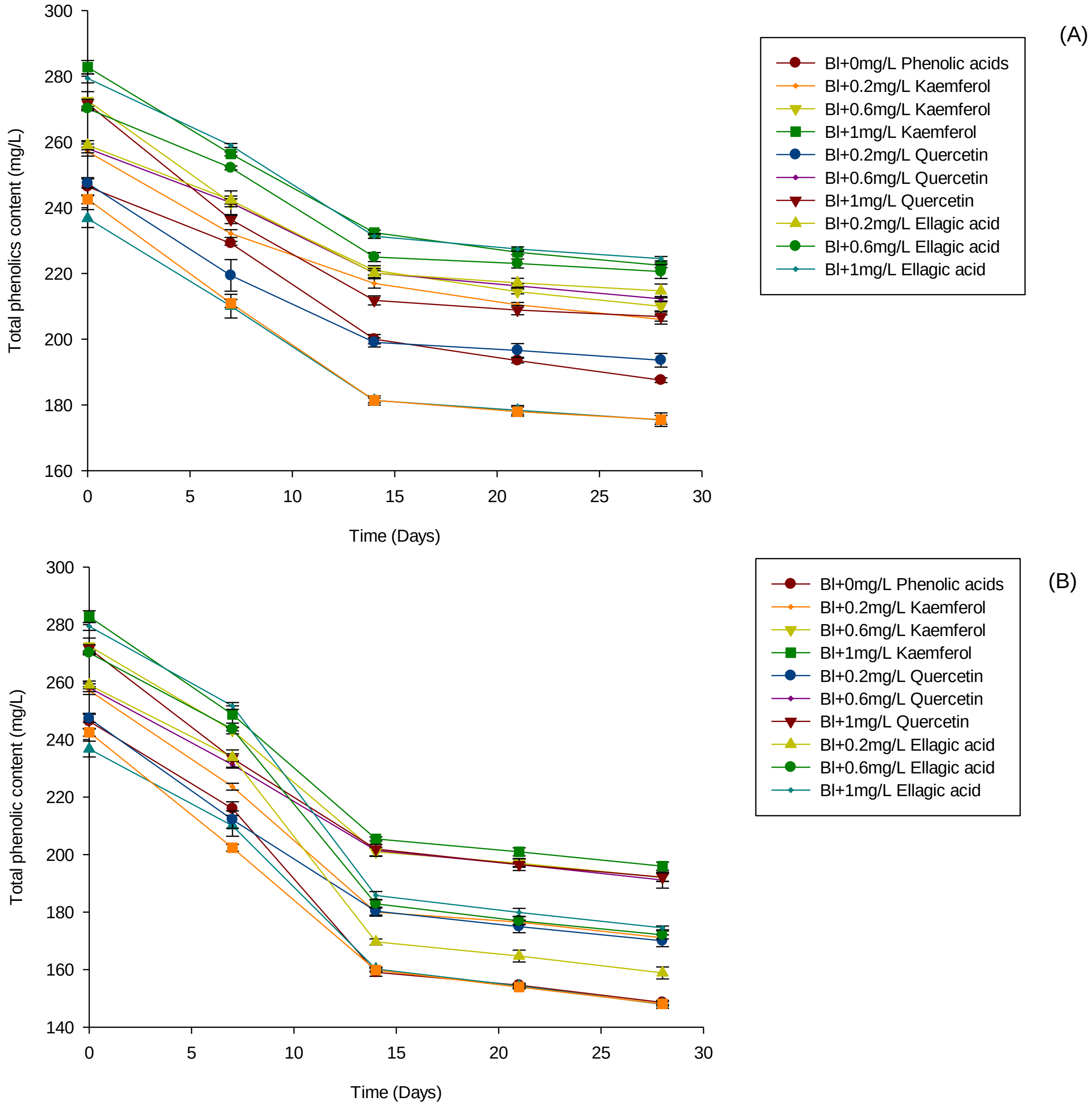


Figure 5.12 Change in total phenolics content of blackcurrant juice enhanced with 0, 0.2, 0.6 and 1 mg/L of kameferol, quercetin and ellagic acid stored at (A) 5^{o}C and (B) 20^{o}C (Bl = Blackcurrant juice) (mean ± SD, n=2)

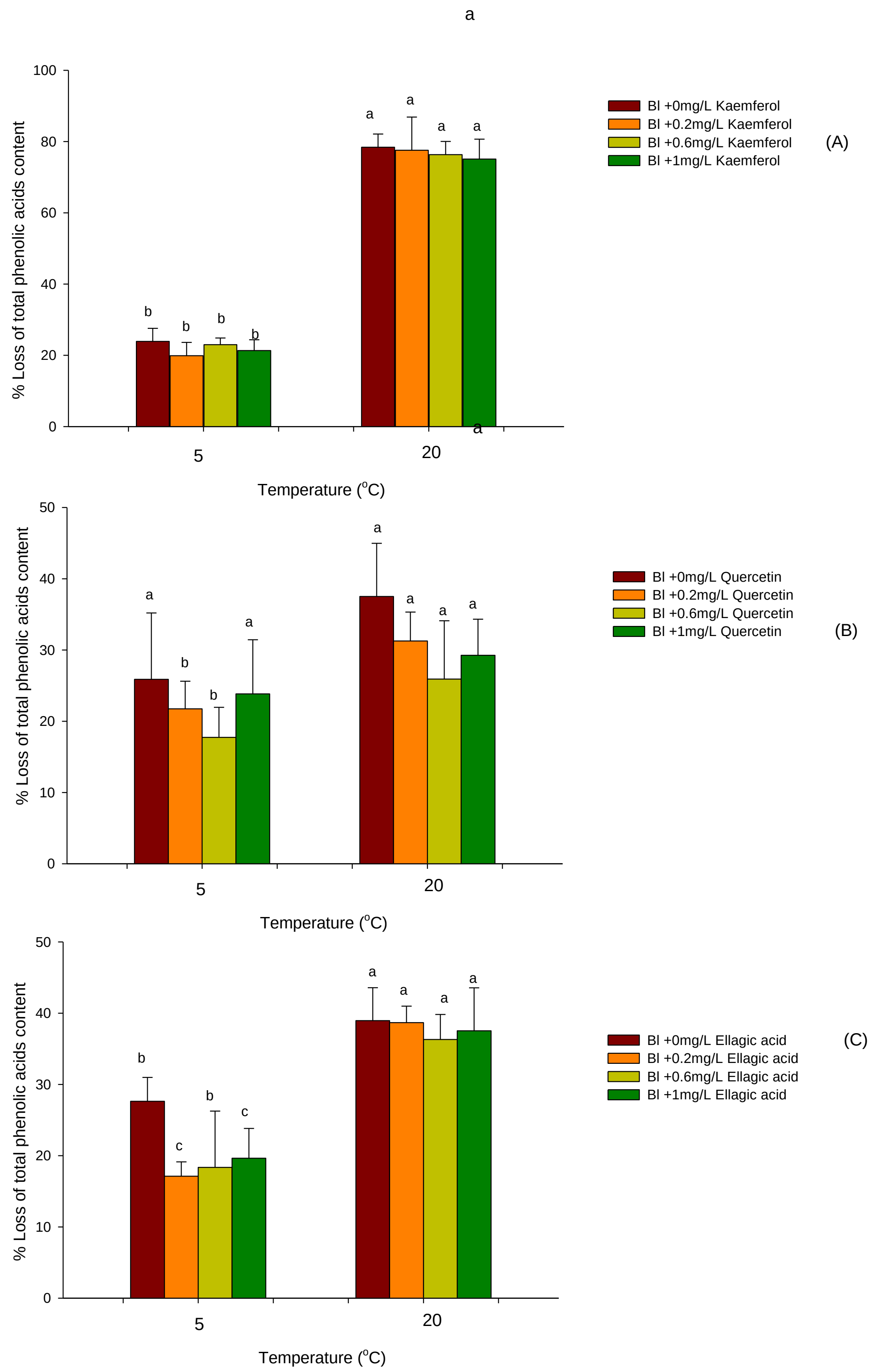


Figure 5.13 % Loss of total phenolics acids content determined by spectrophotometric technique of blackcurrant enhanced with 0, 0.2, 0.6 and 1mg/L concentrations of kaemferol (A), quercetin (B) and ellagic acid (C) stored at 5°C and 20°C (Bl = Blackcurrant juice). Values marked by the same letter are not significantly different.

5.1.8 Changes in individual phenolics profile during storage

After the addition of kaemferol, quercetin and ellagic acid into blackcurrant juice, the concentrations of individual phenolics found in blackcurrant juice slightly decreased, but it was not found to be significant ($P>0.05$). As a calculation check of the individual phenolic content by mass balance calculation found that the concentration of individual phenolic acids of all enhanced blackcurrant juices calculated was comparable to actually measured (Appendices 41&42). A decrease in the concentration of individual phenolics may be a result of degradation of phenolic acids in blackcurrant juice. The results in Section 4.1 showed that four major phenolic acids were found in blackcurrant juice were p-coumaric, caffeic acid, benzoic acid and quercetin.

During 28 days of storage, the four main phenolics in blackcurrant juice were not stable at both two temperatures, although kaemferol, quercetin and ellagic acid were added into blackcurrant juice ($P\leq0.05$) (Figure 5.14 and Appendix 27-29). At 5^{o}C, it was found that quercetin and caffeic acid were more stable at 5^{o}C than p-coumaric, benzoic acid and ellagic acid in blackcurrant juice. However, with higher storage temperature, 20^{o}C, all individual phenolics acids decreased in concentrations during storage. The reduction in phenolic acids over the storage period in blackcurrant juice enhanced with ellagic acid are shown in Figure 5.15.

Similar results were found in blackcurrant juice enhanced with kaemferol and quercetin (Appendix 27-29). All individual phenolic acids were not stable throughout 28 days of storage at both 5 and 20^{o}C; however, at lower temperature, the phenolic acids were relatively more stable. Quercetin and caffeic acid were more stable at refrigerated temperature than benzoic acid in blackcurrant juice.

Storage temperatures affected the stability of phenolic acids and anthocyanins in blackcurrant juice. Additions of kaemferol, quercetin and ellagic acid in blackcurrant juice did not improve stabilities of individual phenolics in blackcurrant juice during storage.

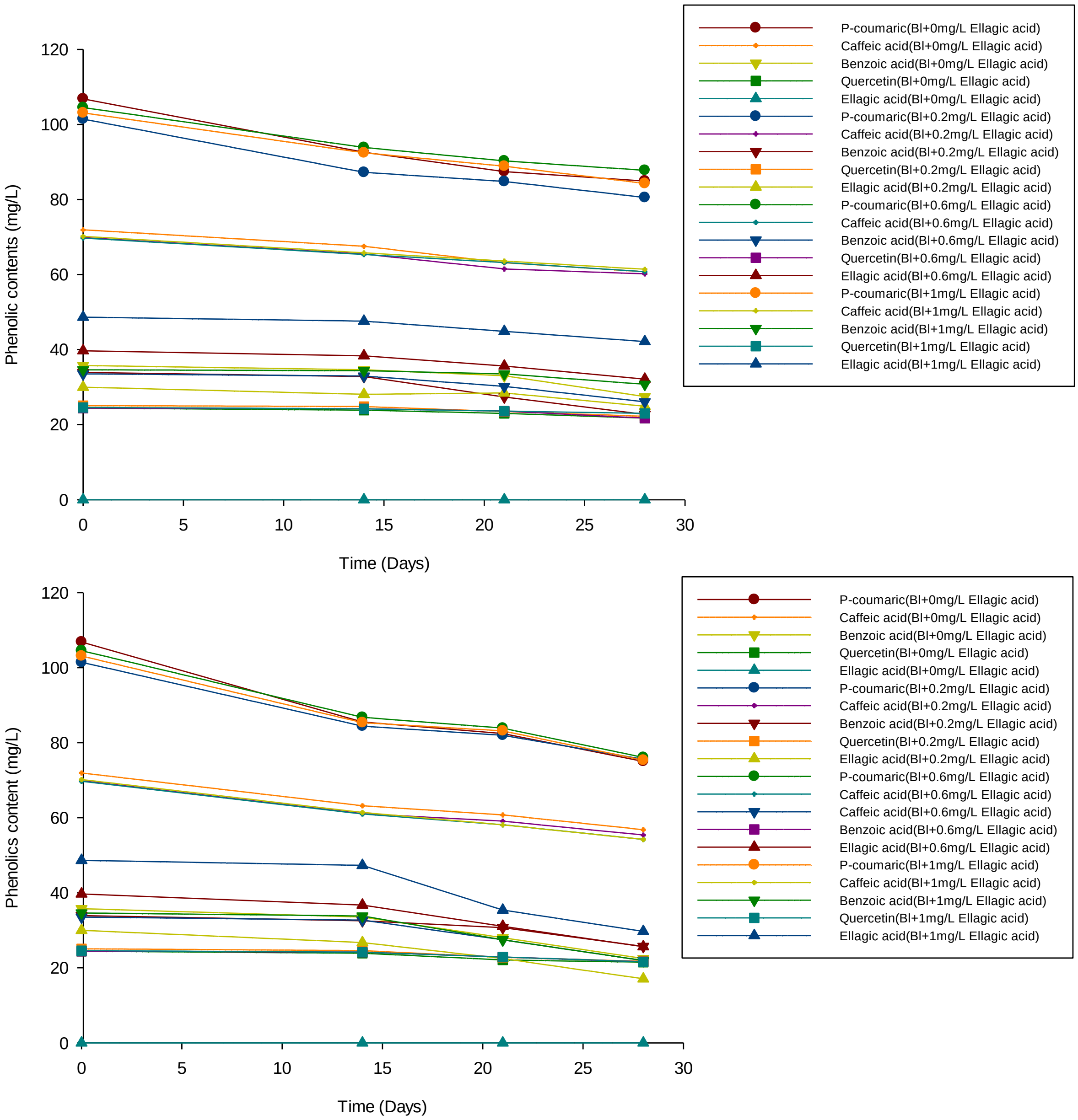


Figure 5.14 Changes in phenolics profile of enhanced with 0, 0.2, 0.6 and 1 mg/L of ellagic acid stored at (A) 5°C and (B) 20°C (Bl = Blackcurrant juice) (mean ± SD, n=2)

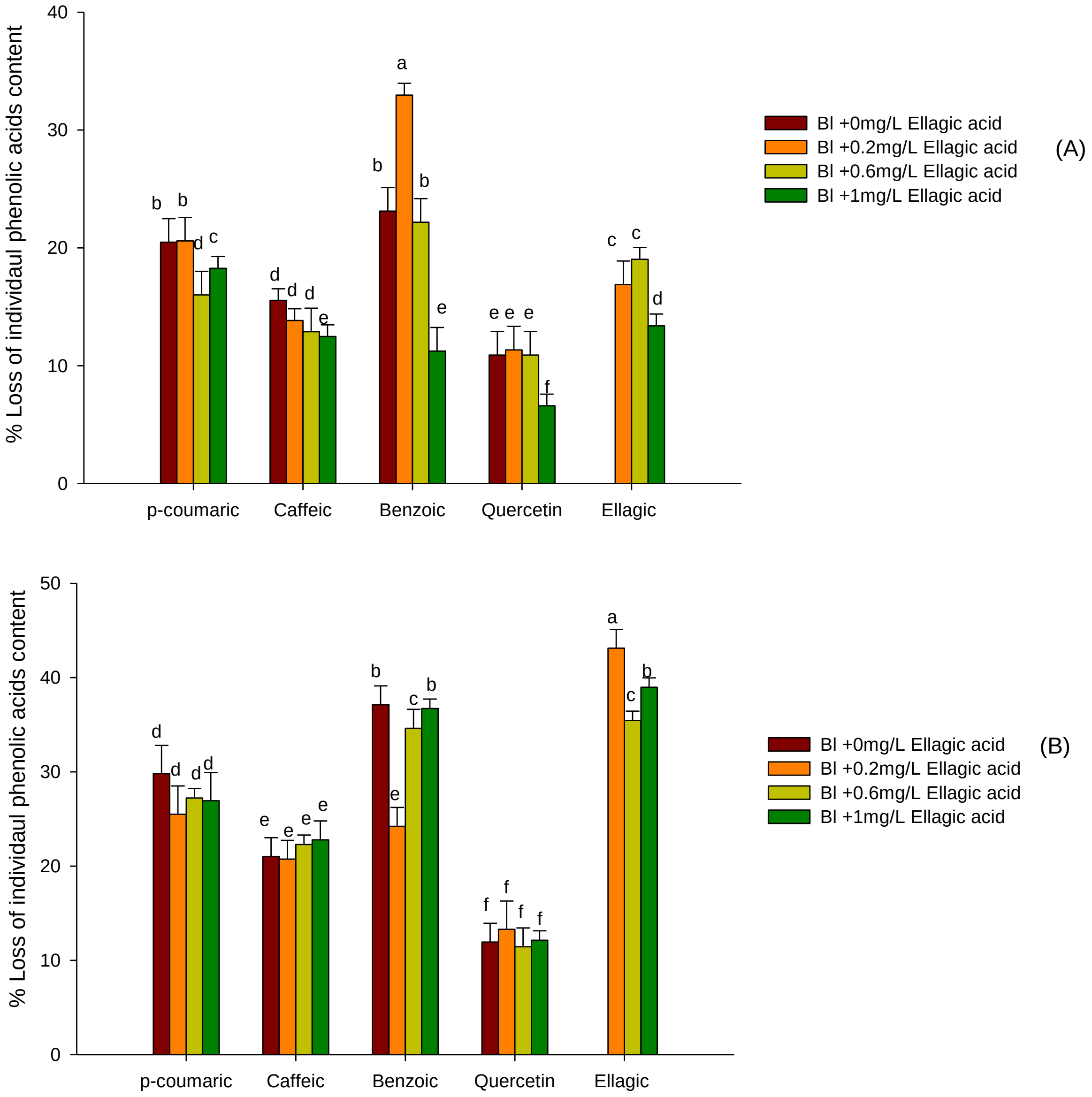


Figure 5.15 % Loss of individual phenolic acids content determined by HPLC of blackcurrant enhanced with 0, 0.2, 0.6 and 1mg/L concentrations of ellagic acid stored at 5°C (A) and 20°C (B) (Bl = Blackcurrant juice). Values marked by the same letter are not significantly different.

5.2 Discussion

The phenolic acids used in this study were kaemferol, quercetin and ellagic acid. The results showed that only ellagic acid induced the hyperchromic effect in blackcurrant juice. This may be due to intermolecular copigmentation between ellagic acid and blackcurrant anthocyanins as the colour was stabilised and the degradation of anthocynains was reduced, and no new anthocyanin compounds were detected with HPLC.

In an acidic environment, there are four anthocyanin structures present in equilibrium: the flavylium cation, quinoidal base, cabinol pseudobase, and chalcone (Hrazdina, 1974) (Figure 5.17). As the flavylium cation and the quinonoidal base are almost planar, with efficiently delocalized π-electrons, this makes the interactions between copigments and the anthocyanin much easier leading to overlapping arrangement of the two molecules, protecting the coloured flavylium cation from the nucleophilic attack of the water molecule (Williams & Hrazdina, 1979; Delgado-Vargas & Peredes-Lopes, 2003). An example of the transformation of anthocyanidin (delphinidin) present in blackcurrant juice at different pH levels is shown in Figure 5.16). The ellagic acid might be binding with blackcurrant anthocyanins via intermolecular copigmentation or anthocyanins themselves. At the low pH values of juice, copigmentation primary involves anthocyanins in their flavylium state. This shifts the free anthocyanins equilibrium toward the coloured flavylium state, further enhancing colour and tending to shift the colour toward purple (Boulton, 2001).

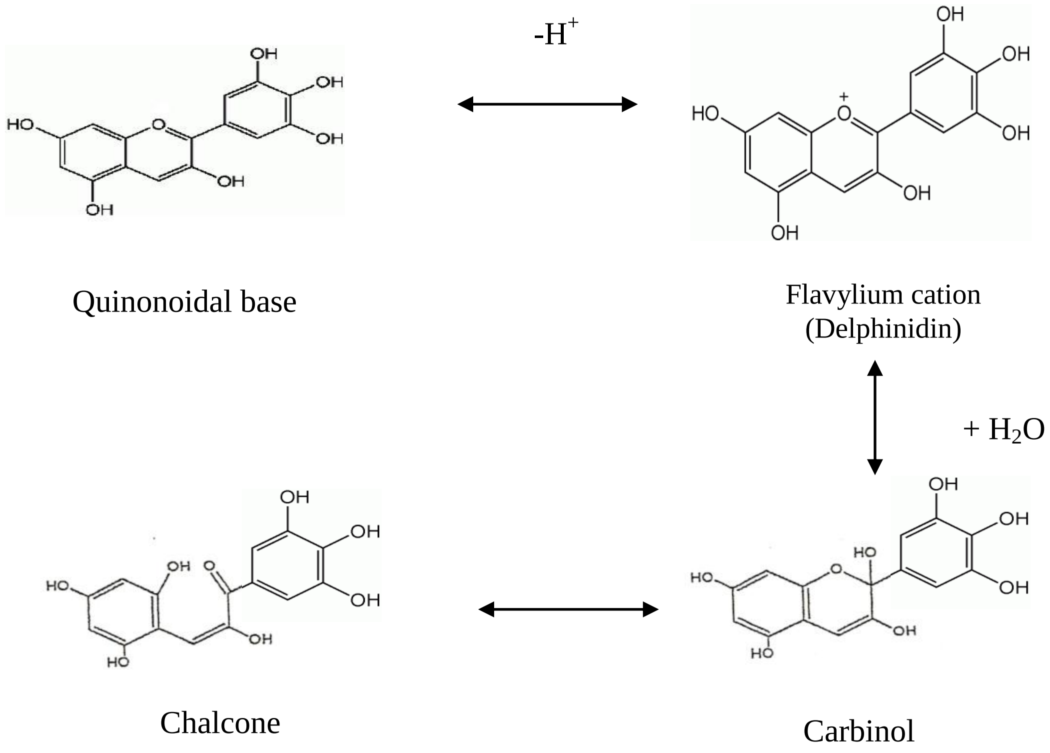


Figure 5.16 An example of structural forms of delphinidin present in blackcurrant juice at different pH levels

There are many factors that may affect the stability of the blackcurrant juice colour such as pH of the solution, the structure of anthocyanin, the structure of copigment, temperature and, concentration of the copigment (Dangles et al., 1993).

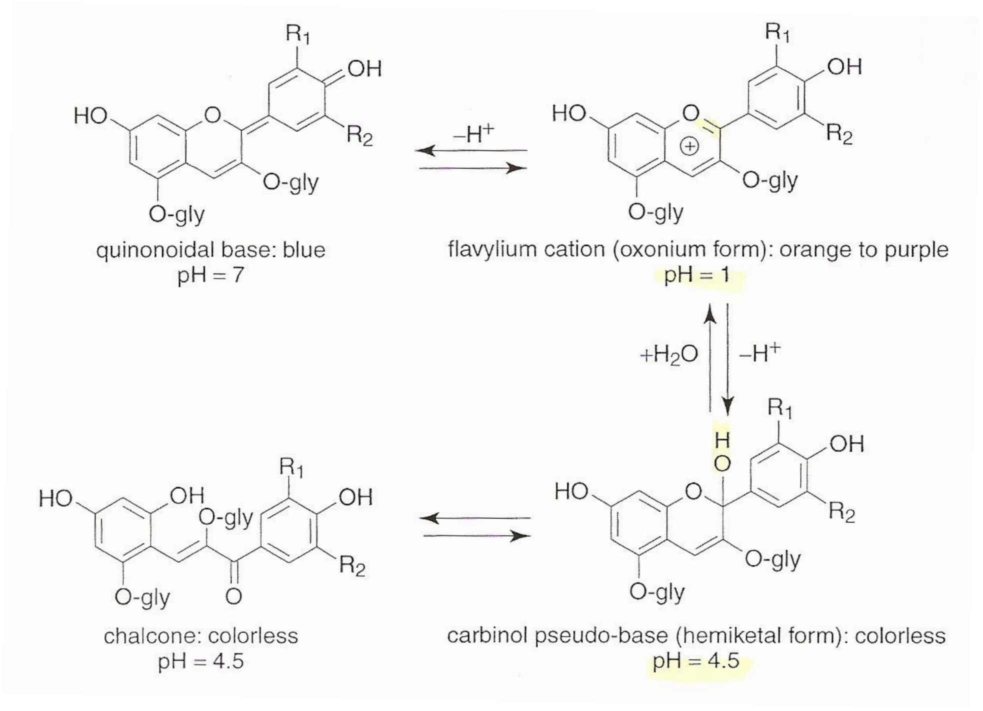


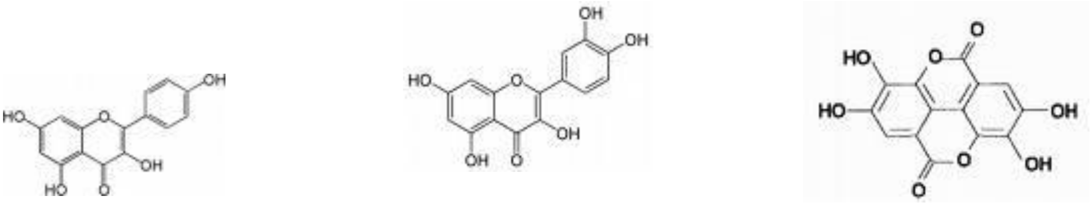
Figure 5.17 Structural forms of anthocyanins present at different pH levels (Wrolstad, 2005a)

- pH

The different pH values can affect the stabilisation of the blackcurrant juice colour during storage. As discussed before, anthocyanins are more stable in acidic solutions than neutral or alkaline solution (Mazza & Miniati, 1993). The results showed that after the addition of kaemferol, quercetin and ellagic acid into blackcurrant juice, the pH values of the blackcurrant juice enhanced with all three phenolic acids were 3.1, 3.0 and 3.5, respectively. William & Hrazdina (1979) stated that for 3-monoglucosides, maximum effect of colour improvement was observed to occur at pH 3.5-4.2; however, the maximum effect for 3,5-diglucosides and 3-acylglucoside-5-glucosides occurred at pH 3.1. Therefore, the pH of blackcurrant juice enhanced with ellagic acid, at pH 3.5, may be more suitable for delphinidin-3-rutinoside or other 3-monoglucosides to form copigmentation complexes with ellagic acid via intermolecular copigmentation.

- Structure of phenolic acids

Waterhouse (2002) stated that the number of hydroxyl groups in the flavonoid copigment affects the magnitude of copigmentation. More hydroxyl groups present on the copigment, the stronger the copigmentation and complex formation. In this study, three phenolic acids were used as copigments in blackcurrant juice. Kaemferol, quercetin and ellagic acid contain 4, 5 and 4 hydroxyl groups, respectively (Figure 5.18). Therefore, according to Waterhouse (2002), quercetin should be the most efficient phenolic acid among the three for copigmentation. However, the results showed that copigmentation occurs when ellagic acid was added into blackcurrant juice only. In this study, the number of hydroxyl groups is not a reason for copigmentation in blackcurrant juice.



Kaemferol Quercetin Ellagic acid

Figure 5.18 Structure of phenolic acids found in blackcurrant juice

Asen et al. (1972) and Brouillard et al. (1989) also reported that the main mechanistic driving forces for the intermolecular copigmentation are hydrogen bonding and hydrophobic interactions. Electrostatic interactions can be a possible means for the intermolecular copigmentation (Clifford, 2000).

- Temperature

Temperature also is another factor, which has a role in destabilising the anthocyanin molecular structure. Temperature increase and pH values between 2 and 4.5 encourages the loss of the glycosyl moieties of the anthocyanins by hydrolysis of the glysosidic bond (Ahmed et al., 2004; Turker et al., 2004). As the aglycones are less stable than their glycosidic forms, this results in a loss of anthocyanin colour. Another reason is that the hydrolysation of the pyrilium ring results in production of chalcones, which are responsible for brown colour. Laleh et al (2006) reported that increasing temperatures (5, 15, 25 and 35°C) are able to spoil the anthocyanin molecule. Also, Palamidis and Markakis (1974) have studied the effect of temperature on the stability of anthocyanin in soft drinks and have shown that an increase in the storage temperature greatly accelerates the destruction of pigments in soft drinks. Similar results were found in our study that storage blackcurrant juice enhanced with ellagic acid at 5°C was more stable in colour stability than at 20 °C. Figure 5.19 shows the thermal degradation of anthocyanin at pH 3.7 (Rein, 2005).

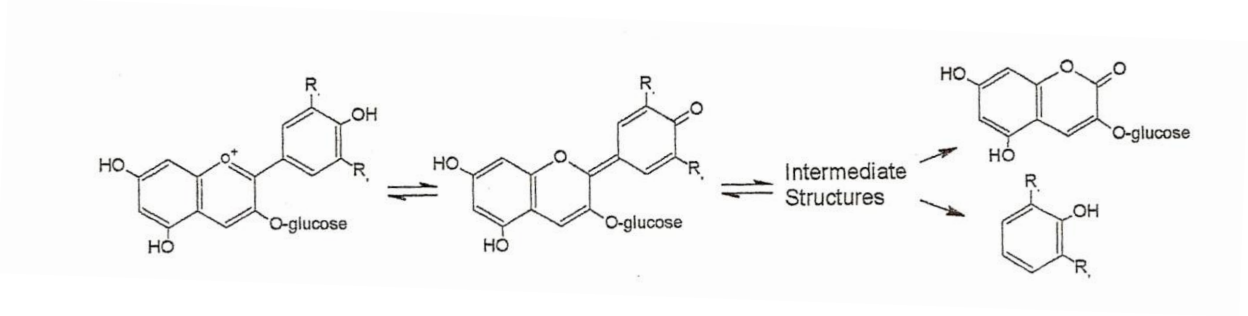


Figure 5.19 Thermal degradation of anthocyanin at pH 3.7 (Rein, 2005).

- Structure of anthocyanins

Mazzaracchio et al. (2004) reported that the structure of aglycone in anthocyanins also seems to promote copigmentation. The high number of hydroxyl groups found in the B ring leads the anthocyanins molecules to being more accessible to water and easily complexed with other compounds. According to this study, blackcurrant juice contained four major anthocyanins (cyanidin-3-rutinoside, cyanidin-3-glucoside, delphinidin-3-rutinoside and delphinidin-3-glucoside). Delphinidin-3-monoglucosides were found only in blackcurrant and pomegranate juices, but not detected in cranberry and boysenberry juices. However, pomegranate juice contained only a small amount of delphinidin monoglucosides compared to blackcurrant juice. There are a higher number of hydroxyl groups found in the B ring of delphinidin monoglucosides (hydroxyls =2), when compared other cyanidins (hydroxyls =1) and pelargonidins (hydroxyls =0) (Figure 2.1). This means that blackcurrant juice that contained two types of delphinidin monoglucosides as the main anthocyanins had a higher number of hydroxyl groups than other juices.

Among the three berry juices, blackcurrant and pomegranate juices containing delphinidin monoglucosides may be more advantagous to complexing with other phenolics in boysenberry juice (Ellagic acid). However, as previously discussed, addition of boysenberry juice only improved the colour stability of blackcurrant juice through intermolecular copigmentation. There is a very low concentration of delphinidin-3-glucoside found in pomegranate juice (174.62 mg/L) compared to blackcurrant juice, hence any copigmentaion effect due to boysenberry juice were not detectable with the methods used in this study.

CHAPTER 6

INTERACTIONS BETWEEN BOYSENBERRY JUICE AND ELLAGIC ACID

6.1 The effect of the addition of boysenberry juice and ellagic acid in blackcurrant juice colour stability (Study 4)

As shown in Section 4.2, the addition of boysenberry juice in blackcurrant juice was found to improve the colour stability of blackcurrant juice at refrigerated temperature (5 °C). Also, the phenolic acid (ellagic acid) had an effect on the colour stability of blackcurrant juice (Section 4.3). These results are postulated to be due to intermolecular copigmentation between the phenolic acid and some anthocyanins in blackcurrant juice (Rein, 2005). However, the intermolecular copigmentation may not be the only reason for the colour stability of blackcurrant juice, therefore, in this study the objective was to investigate if anthocyanins themselves can have an effect on colour stability via intramolecular copigmentation. Boysenberry juice at concentrations of 0, 1, 2 and 3% (w/v) and ellagic acid at concentrations of 0, 0.2, 0.6 and 1 mg/L were added together into blackcurrant juice at different levels (Table 6.1) and all juices then were stored at 5^{o}C. Stepwise multiple regression was applied to find a meaningful subset of independent variables which explains each dependent variable efficiently. To see which factors were more effective for each parameter, the R-squared change was used to compare regression models with different numbers of independents. Table 6.1 represents the molar concentrations of ellagic acid in boysenberry juice and in pure ellagic acid added into blackcurrant juice.

Table 6.1 Summary of concentration of ellagic acid added into blackcurrant juice from boysenberry juice and pure ellagic acid

Treatment	Ellagic acid (mg/L)	Molar concentrations of ellagic acid (mol/L)	Boysenberry juice (% w/v)	Concentrations of ellagic acid (mg/L)	Molar concentrations of ellagic acid (mol/L)	Total molar concentrations of ellagic acid (mol/L)
1	0	0	0%	0	0	0
2	0	0	1%	1.27	4.2E-06	4.2E-06
3	0	0	2%	2.54	8.4E-06	8.4E-06
4	0	0	3%	3.81	1.3E-05	1.3E-05
5	0.2	6.6E-07	0%	0	0	6.6E-07
6	0.2	6.6E-07	1%	1.27	4.2E-06	4.9E-06
7	0.2	6.6E-07	2%	2.54	8.4E-06	9.1E-06
8	0.2	6.6E-07	3%	3.81	1.3E-05	1.4E-05
9	0.6	2.0E-06	0%	0	0	2.0E-06
10	0.6	2.0E-06	1%	1.27	4.2E-06	6.2E-06
11	0.6	2.0E-06	2%	2.54	8.4E-06	1.0E-05
12	0.6	2.0E-06	3%	3.81	1.3E-05	3.3E-06
13	1	3.3E-06	0%	0	0	3.3E-06
14	1	3.3E-06	1%	1.27	4.2E-06	7.5E-06
15	1	3.3E-06	2%	2.54	8.4E-06	1.2E-06
16	1	3.3E-06	3%	3.81	1.3E-05	1.6E-06

6.1.1 Changes in total anthocyanin content

As shown in Figure 6.1 the addition of boysenberry juice at 1, 2 and 3% (w/v) concentrations and ellagic acid at concentrations of 0, 0.2, 0.6 and 1 mg/L alone in blackcurrant juice were found to keep the pigment stable at 5°C during 28 days of storage, compared to non-enhanced blackcurrant juice. It can be seen from Table 6.2 that there was a significant difference between total anthocyanin content of blackcurrant juice enhanced with different concentrations of boysenberry juice. The variance caused by the interaction between the samples was much larger when compared to the variance that appears within each group (Sum of Squares, Table 6.2).

Table 6.3 showed that the addition of boysenberry juice had an effect on total anthocyanin content of the blackcurrant juice during storage ($P \leq 0.05$). A similar result was found in blackcurrant juice enhanced with ellagic acid. Analysis of variance result shows that ellagic acid also had an influence on colour stability of blackcurrant juice ($P \leq 0.05$) (Table 6.4&6.5).

Table 6.2 Summary of results from ANOVA of total anthocyanin content of blackcurrant juice enhanced with boysenberry juice at different concentrations

		Sum of Squares	df	Mean Square
Boysenberry 0%(w/v)	Between Groups	14.045	1	14.045
	Within Groups	0.000	0	.
	Total	14.045	1	
Boysenberry 1%(w/v)	Between Groups	222.605	1	222.605
	Within Groups	0.000	0	.
	Total	222.605	1	
Boysenberry 2%(w/v)	Between Groups	222.605	1	222.605
	Within Groups	0.000	0	.
	Total	222.605	1	
Boysenberry 3%(w/v)	Between Groups	13.520	1	13.520
	Within Groups	0.000	0	.
	Total	13.520	1	

Table 6.3 Summary of results from ANOVA of overall effect of boysenberry juice on colour stability of blackcurrant juice

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	21706.044	3	7235.348	61.216	0.001
Within Groups	472.775	4	118.194		
Total	22178.819	7			

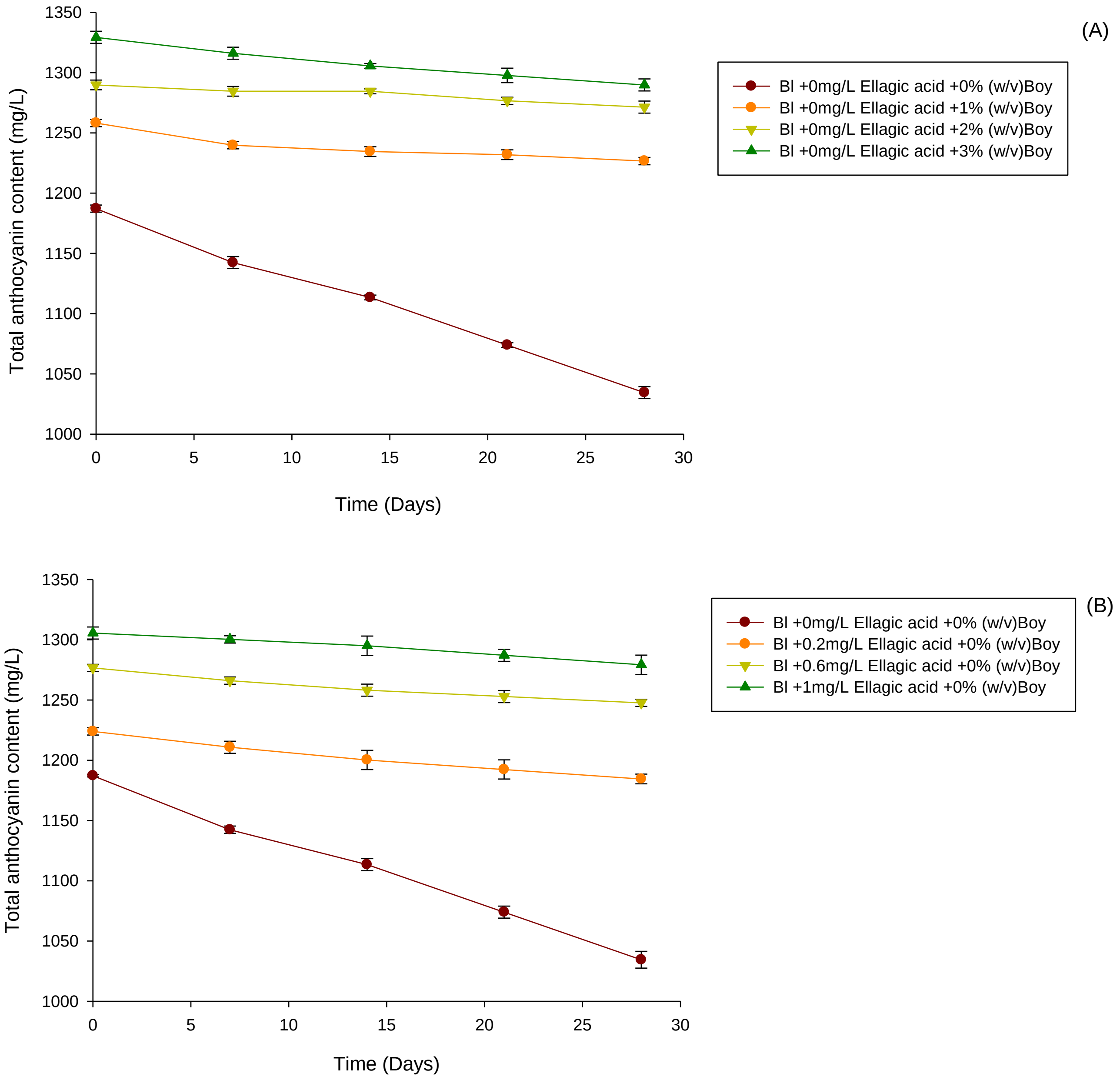


Figure 6.1 Change in total anthocyanin content of blackcurrant juice enhanced with 0, 1, 2 and 3% (w/v) boysenberry juice (A) and 0, 0.2, 0.6 and 1 mg/L of ellagic acid (B) stored at 5°C (Bl = Blackcurrant juice, Boy = Boysenberry juice)

Table 6.4 Summary of results from ANOVA of total anthocyanin content of blackcurrant juice enhanced with ellagic acid at different concentrations

		Sum of Squares	Df	Mean Square
Ellagic acid 0mg/L	Between Groups	14.045	1	14.045
	Within Groups	0.000	0	.
	Total	14.045	1	
Ellagic acid 0.2mg/L	Between Groups	14.045	1	14.045
	Within Groups	0.000	0	.
	Total	14.045	1	
Ellagic acid 0.6mg/L	Between Groups	14.045	1	14.045
	Within Groups	0.000	0	.
	Total	14.045	1	
Ellagic acid 1mg/L	Between Groups	220.500	1	220.500
	Within Groups	0.000	0	.
	Total	220.500	1	

Table 6.5 Summary of results from ANOVA of overall effect of ellagic acid on total anthocyanin content of blackcurrant juice

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	16828.764	3	5609.588	85.435	0.000
Within Groups	262.635	4	65.659		
Total	17091.399	7			

When both ellagic acid and boysenberry juice were added separately or together to blackcurrant juice, there was a significant increase in total anthocyanin content of blackcurrant juice ($P \leq 0.05$) (Figure 6.2). As both factors had an effect on colour stability of blackcurrant juice, to investigate which one had more effect on the improvement of colour of blackcurrant juice, Stepwise Multiple Regression was applied. Results presented in Table 6.6 show, that ellagic acid had a larger correlation with the total anthocyanin content of blackcurrant juice, R = 0.527, $p \leq 0.05$, compared to boysenberry juice, R = 0.495, $p \leq 0.05$. This means that ellagic acid at the concentrations added was more effective for stabilising the colour of blackcurrant juice than boysenberry juice at the concentrations added. About 28 % (R Square

Change) of the variation in the total anthocyanin content can be explained by the regression model with ellagic acid (Table 6.7).

Table 6.6 The correlation matrix of the effect of ellagic acid and boysenberry juice on total anthocyanin content of blackcurrant juice

		Total anthocyanin content	Ellagic acid	Boysenberry juice
Pearson Correlation	Total anthocyanin content	**1.000**	**0.527**	**0.495**
	Ellagic acid	0.527	1.000	0.000
	Boysenberry juice	0.495	0.000	1.000
Sig. (1-tailed)	Total anthocyanin content	.	0.000	0.000
	Ellagic acid	0.000	.	0.500
	Boysenberry juice	0.000	0.500	.
N	Total anthocyanin content	160	160	160
	Ellagic acid	160	160	160
	Boysenberry juice	160	160	160

Table 6.7 The summary model of effect of ellagic acid and boysenberry juice on total anthocyanin content of blackcurrant juice

Model	Change Statistics				
	R Square Change	F Change	df1	df2	Sig. F Change
1	**0.277(a)**	60.671	1	158	0.000
2	0.245(b)	80.790	1	157	0.000

a Predictors: (Constant), concofEllagic

b Predictors: (Constant), concofEllagic, ConofBoy

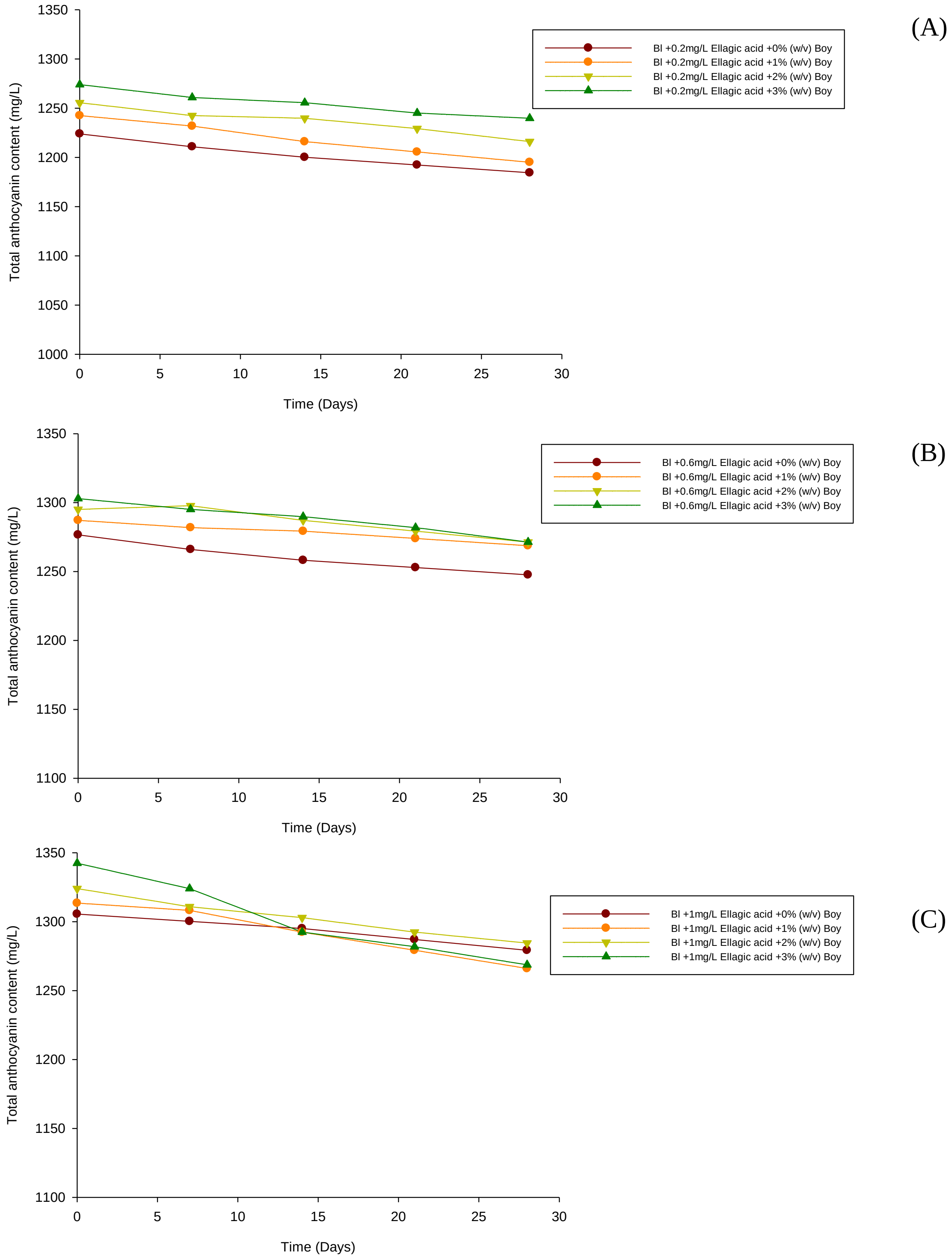


Figure 6.2 Change in total anthocyanin content of blackcurrant juice enhanced with 0, 1, 2 and 3% (w/v) boysenberry juice and 0.2, 0.6 and 1 mg/L of ellagic acid stored at 5^{o}C (Bl = Blackcurrant juice, Boy = Boysenberry juice)

6.1.2 Hyperchromic shift

In terms of changes in absorbance (hyperchromic shift) of blackcurrant juices during storage, the results were similar to section 6.1.1; there was a slight decrease over the storage time in hyperchromic shift of blackcurrant juice enhanced with boysenberry juice (1, 2 and 3% (w/v)) and ellagic acid (0.2, 0.6 and 1 mg/L) together. There was no significant difference in hyperchromic shift between all blackcurrant juices ($P<0.05$).

It was found that boysenberry juice improved the colour of blackcurrant juice immediately after the addition (Figure 6.3). The addition of 0, 1, 2 and 3% (w/v) concentrations of boysenberry juice in blackcurrant increased colour intensity at the start of the study from 1.1 for non enhanced blackcurrant juice to 1.2, 1.3 and 1.5, respectively. Tables 6.8 & 6.9 show that boysenberry juice had an impact on improvement of colour in blackcurrant juice and there was a significant difference in the hyperchromic shift of blackcurrant juice at different concentrations ($P\leq0.05$). Also, the between group variation and the within group variation (Sum of squares) are the same with 0.00, this means the means of each sample were not different to each other (Table 6.8).

Ellagic acid also was found to maintain the colour stability of blackcurrant juice at 5^{o}C storage temperature (Figure 6.3). In the beginning of study, the colour intensity of plain blackcurrant juice was 1.1 and of the blackcurrant juices enhanced with 0.2, 0.6 and 1 mg/L of ellagic acid they increased to 1.3, 1.5 and 1.6, respectively (Figure 6.3). This was confirmed by the One Way Analysis of Variance (Table 6.10 &11) that ellagic acid had an influence on colour intensity of blackcurrant juice during 28 days of storage and there was a significant difference between hyperchromic shifts at different concentrations ($P\leq0.05$).

Table 6.12 shows that ellagic acid had a larger correlation with hyperchromic shift of blackcurrant juice, $R = 0.754$, $p \leq 0.05$, compared to boysenberry juice, $R = 0.370$, $p \leq 0.05$. This means that ellagic acid at the concentrations added was more effective for maintaining the colour stability of blackcurrant juice than boysenberry juice at the concentrations added. About 57 % (R Square change) of the variation in the total anthocyanin content can be explained by the regression model with ellagic acid (Table 6.13).

In conclusion, total anthocyanin content and hyperchromic shift results (Section 6.1.1& 6.1.2) confirmed that ellagic acid had the main influence on colour stability of blackcurrant juice through intermolecular copigmentation,while anthocyanins copigmentation did not show any changes in colour of blackcurrant juice during storage.

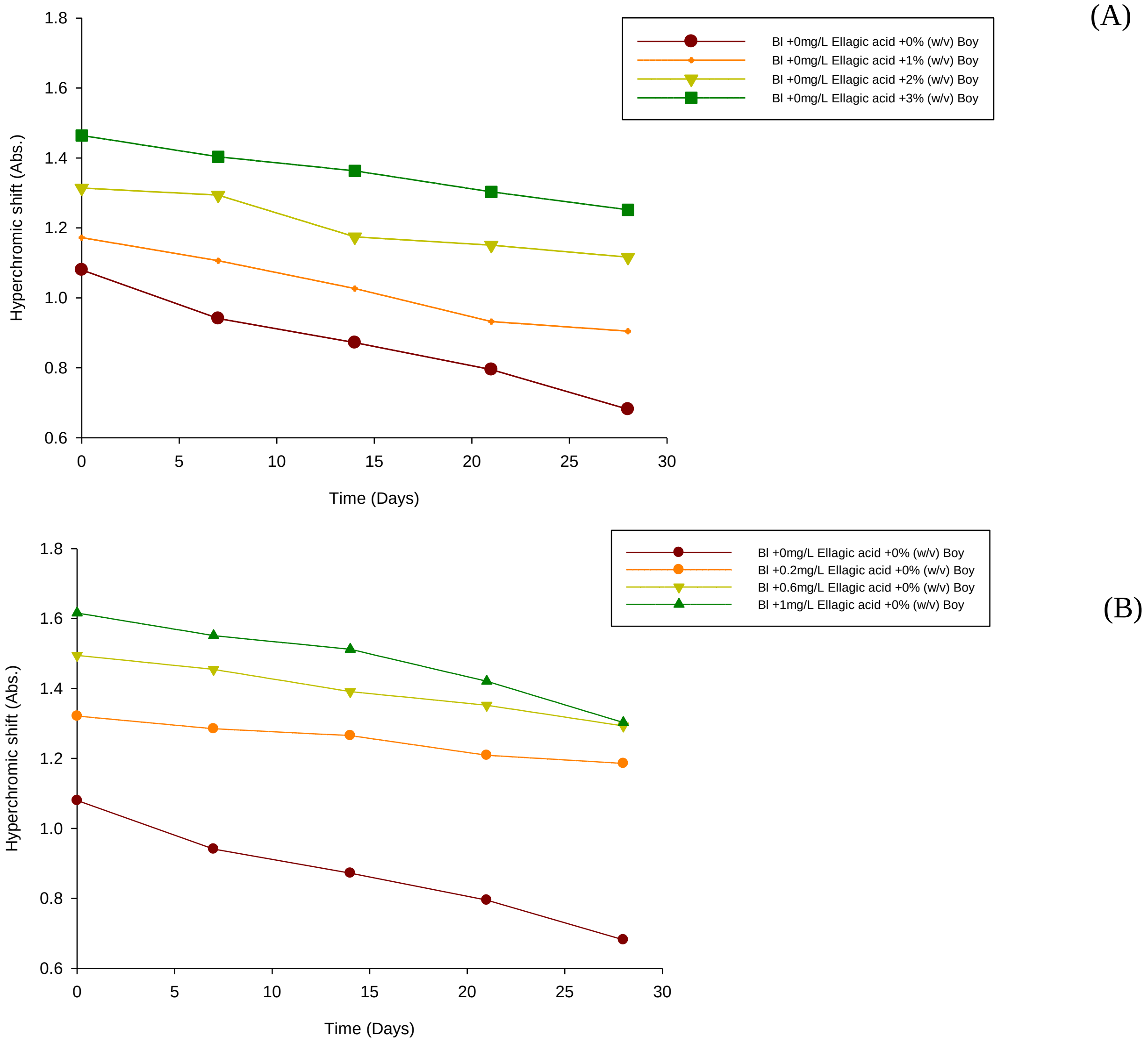


Figure 6.3 Change in hyperchromic shift of blackcurrant juice enhanced with 0, 1, 2 and 3% (w/v) boysenberry juice (A) and 0, 0.2, 0.6 and 1 mg/L of ellagic acid (B) stored at 5°C (Bl = Blackcurrant juice, Boy = Boysenberry juice)

Table 6.8 Summary of results from ANOVA of hyperchromic shift of blackcurrant juice enhanced with boysenberry juice at different concentrations

		Sum of Squares	df	Mean Square	F	Sig.
Boysenberry 0%(w/v)	Between Groups	0.000	1	0.000	.	.
	Within Groups	0.000	0	.		
	Total	0.000	1			
Boysenberry 1%(w/v)	Between Groups	0.000	1	0.000	.	.
	Within Groups	0.000	0	.		
	Total	0.000	1			
Boysenberry 2%(w/v)	Between Groups	0.000	1	0.000	.	.
	Within Groups	0.000	0	.		
	Total	0.000	1			
Boysenberry 3%(w/v)	Between Groups	0.000	1	0.000	.	.
	Within Groups	0.000	0	.		
	Total	0.000	1			

Table 6.9 Summary of results from ANOVA of effect of boysenberry juice on hyperchromic shift of blackcurrant juice

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.169	3	0.056	11286.800	0.000
Within Groups	0.000	4	0.000		
Total	0.169	7			

Table 6.10 Summary of results from ANOVA of hyperchromic shift of blackcurrant juice enhanced with ellagic acid at different concentrations

		Sum of Squares	df	Mean Square
Ellagic acid 0mg/L	Between Groups	0.000	1	0.000
	Within Groups	0.000	0	.
	Total	0.000	1	
Ellagic acid 0.2mg/L	Between Groups	0.000	1	0.000
	Within Groups	0.000	0	.
	Total	0.000	1	
Ellagic acid 0.6mg/L	Between Groups	0.000	1	0.000
	Within Groups	0.000	0	.
	Total	0.000	1	
Ellagic acid 1mg/L	Between Groups	0.000	1	0.000
	Within Groups	0.000	0	.
	Total	0.000	1	

Table 6.11 Summary of results from ANOVA of effect of ellagic acid on hyperchromic shift of blackcurrant juice

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.324	3	0.108	48009.259	0.000
Within Groups	0.000	4	0.000		
Total	0.324	7			

Table 6.12 The correlation matrix of the effect of ellagic acid and boysenberry juice on hyperchromic shift of blackcurrant juice

		Hyperchromic	Ellagic acid	Boysenberry
Pearson Correlation	Hyperchromic	**1.000**	**0.754**	**0.370**
	Ellagic acid	0.754	1.000	0.000
	Boysenberry	0.370	0.000	1.000
Sig. (1-tailed)	Hyperchromic	.	0.000	0.000
	Ellagic acid	0.000	.	0.500
	Boysenberry	0.000	0.500	.
N	Hyperchromic	160	160	160
	Ellagic acid	160	160	160
	Boysenberry	160	160	160

Table 6.13 The summary model of effect of ellagic acid and boysenberry juice on hyperchromic shift of blackcurrant juice

Model	Change Statistics				
	R Square Change	F Change	df1	df2	Sig. F Change
1	**0.568(a)**	208.051	1	158	0.000
2	0.137(b)	92.878	1	157	0.000

a Predictors: (Constant), concofEllagic

b Predictors: (Constant), concofEllagic, ConofBoy

6.1.3 Changes in individual anthocyanin profile during storage

In was found in Section 4.1, blackcurrant juice contained four individual anthocyanins: delphinidin-3-rutinoside, cyanidin-3-rutinoside, delphinidin-3-glucoside and cyanidin-3-glucoside. After addition of boysenberry juice and ellagic acid into blackcurrant juice, the concentration of these four anthocyanins decreased throughout 28 days of storage to a similar extent.

Without the addition of boysenberry juice and ellagic acid in blackcurrant juice, delphinidin-3-rutinoside, cyanidin-3-rutinoside, delphinidin-3-glucoside and cyanidin-3-glucoside gave the highest values of % Loss of anthocyanin content by 16.1, 5.9, 20.8 and 20.9%, respectively (Table 6.14). When boysenberry juice at concentrations of 1, 2 and 3% (w/v) was added individually in blackcurrant juice, there was a small decrease in % Loss of individual anthocyanins contents in blackcurrant juice.

When boysenberry juice (1, 2 and 3% (w/v)) and ellagic acid (0.2, 0.6 and 1 mg/L) were added together in blackcurrant juice, the concentration of individual anthocyanins content did not considerable change. As can be seen in Table 6.14 that % Loss of individual anthocyanin contents ranged between 1.5 and 8%.

Individual anthocyanins were hence found to be more stable during storage when boysenberry juice or ellagic acid were added to blackcurrant juice. There was no significant difference in individual anthocyanins contents when ellagic acid and boysenberry juice were added alone, or when they were added to blackcurrant juice together (P>0.05).

6.1.4 Changes in individual phenolic acids profile during storage

In Section 4.1, it was found that blackcurrant juice contained p-coumaric, caffeic acid, benzoic acid and quercetin as the major phenolics. At the beginning of storage, without addition of ellagic acid in blackcurrant juice, blackcurrant juice contained only those four phenolics. When ellagic acid was added alone into blackcurrant juice, the ellagic acid was identified as a new peak by HPLC (Table 6.15). It was found that the concentration of p-coumaric, caffeic acid, benzoic acid and quercetin in blackcurrant juice slightly decreased immediately after the addition of ellagic (0, 0.2, 0.6 and 1 mg/L) and boysenberry juice (0, 1, 2 and 3% (w/v)) (Table 6.15). During 28 days of storage at 5°C, the individual phenolic contents decreased gradually with similar % Loss. Ellagic acid was lost the most compared to other phenolic acids in blackcurrant juice. The concentration of ellagic acid and boysenberry juice in blackcurrant did not seem to have any effect on concentration of phenolic acids in blackcurrant juice ($P>0.05$). It was also found that the concentration of individual phenolic acids in blackcurrant juice did not significantly change when ellagic acid and boysenberry juice were added alone or together ($P>0.05$) (Table 6.15).

Table 6.14 %Loss of individual anthocyanins contents when blackcurrant juice was enhanced with 0, 1, 2 and 3% (w/v) boysenberry juice and 0, 0.2, 0.6 and 1 mg/L of ellagic acid stored at 5^{o}C

Time(days)	Blackcurrant juice (10^{o}Brix)								Time(days)	Blackcurrant juice (10^{o}Brix)							
	0 mg/L Ellagic acid									0.2 mg/L Ellagic acid							
	0%(w/v) Boysenberry juice				1%(w/v) Boysenberry juice					0%(w/v) Boysenberry juice				1%(w/v) Boysenberry juice			
	Del-3-rut	cyn-3-rut	Del-3-glu	cyn-3-glu	Del-3-rut	cyn-3-rut	Del-3-glu	cyn-3-glu		Del-3-rut	cyn-3-rut	Del-3-glu	cyn-3-glu	Del-3-rut	cyn-3-rut	Del-3-glu	cyn-3-glu
0	517.6 ± 26	359.8 ± 12	162.6 ± 10	92.3 ± 8	527.1 ±15	417.7 ± 10	169.0 ± 8	120.9 ± 8	0	525.8 ± 10	376.6 ± 13	183.9 ±11	109.8 ±12	557.6 ±22	408.0 ±8	169.5 ±16	105.1 ±10
28	484.1 ± 22	338.7 ±10	128.8 ± 27	73.0 ±10	512.0 ± 28	407.2 ±12	156.9 ± 18	118.6 ±12	28	514.4 ± 28	355.6 ±17	176.7 ±11	105.4 ±9	542.4 ±26	386.9 ±10	164.7 ±16	98.4 ±10
% Loss	16.1 ±1.2	5.9 ± 0.7	20.8 ± 1.8	20.9 ± 2.0	2.9 ± 0.1	2.5 ± 0.3	7.1 ± 1.3	1.9 ± 0.3	% Loss	2.2 ± 1.0	5.6 ± 1.4	3.9 ±1.2	4.1 ±1.6	2.7 ±0.6	5.2 ±1.9	2.8 ±0.4	6.4 ±1.5
Time(days)	2%(w/v) Boysenberry juice				3%(w/v) Boysenberry juice					2%(w/v) Boysenberry juice				3%(w/v) Boysenberry juice			
	Del-3-rut	cyn-3-rut	Del-3-glu	cyn-3-glu	Del-3-rut	cyn-3-rut	Del-3-glu	cyn-3-glu		Del-3-rut	cyn-3-rut	Del-3-glu	cyn-3-glu	Del-3-rut	cyn-3-rut	Del-3-glu	cyn-3-glu
0	513.6 ± 24	457.8 ± 8	170.4 ± 13	133.4 ± 12	524.2 ± 16	484.6 ± 13	165.6 ± 15	140.0 ± 14	0	557.2 ±20	394.3 ±16	176.4 ±14	119.2 ±15	551.9 ±19	428.7 ±18	169.2 ±9	123.1 ±16
28	513.6 ± 20	436.7 ±12	168.0 ± 14	126.7 ± 7	522.9 ± 15	463.5 ± 10	160.8 ± 20	133.3 ± 16	28	542.0 ±15	362.7 ±14	169.1 ±14	112.4 ±20	540.5 ±17	407.6 ±15	166.8 ±12	116.4 ±10
% Loss	3.6 ± 0.6	4.6 ± 0.3	1.4 ± 0.5	5.1 ± 1.2	2.1 ± 0.7	4.3 ± 0.3	2.9 ± 0.7	4.8 ± 0.6	% Loss	2.7 ±1.0	8.0 ±1.5	4.1 ±2.5	5.7 ±1.1	2.1 ±1.4	4.9 ±2.0	1.4 ±0.5	5.5 ±1.4
Time(days)	Blackcurrant juice (10^{o}Brix)								Time(days)	Blackcurrant juice (10^{o}Brix)							
	0.6 mg/L Ellagic acid									1 mg/L Ellagic acid							
	0%(w/v) Boysenberry juice				1%(w/v) Boysenberry juice					0%(w/v) Boysenberry juice				1%(w/v) Boysenberry juice			
	Del-3-rut	cyn-3-rut	Del-3-glu	cyn-3-glu	Del-3-ruti	cyn-3-ruti	Del-3-glu	cyn-3-glu		Del-3-rut	cyn-3-rut	Del-3-glu	cyn-3-glu	Del-3-rut	cyn-3-rut	Del-3-glu	cyn-3-glu
0	542.3 ± 30	366.3 ± 10	192.9 ± 16	114.1 ± 11	564.0 ± 20	383.9 ± 16	171.6 ± 12	109.2 ±11	0	563.6±20	384.3±18	205.1±18	123.0±10	563.2±19	382.8±15	190.7±16	109.9±14
28	531.0 ± 33	345.3 ± 12	183.2 ± 31	109.6 ± 14	556.4 ± 20	373.3 ± 12	166.8 ± 12	102.5 ± 9	28	552.2±30	373.8±15	190.2±30	118.5±14	565.6±26	361.7±21	183.4±24	105.4±15
% Loss	2.1 ± 0.4	5.8 ± 1.8	5.0 ± 2.1	3.9 ± 1.0	1.3 ± 0.4	2.7 ± 0.2	2.8 ± 1.3	6.2 ± 0.7	% Loss	2.0±0.3	2.7±0.5	2.4±0.6	3.7±1.2	1.3±0.6	5.5±1.3	3.8±1.5	4.1±2.0
	2%(w/v) Boysenberry juice				3%(w/v) Boysenberry juice					2%(w/v) Boysenberry juice				3%(w/v) Boysenberry juice			
	Del-3-rut	cyn-3-rut	Del-3-glu	cyn-3-glu	Del-3-ruti	cyn-3-ruti	Del-3-glu	cyn-3-glu		Del-3-rut	cyn-3-rut	Del-3-glu	cyn-3-glu	Del-3-rut	cyn-3-rut	Del-3-glu	cyn-3-glu
0	564.6 ± 14	405.8 ±15	172.6 ±11	116.0 ± 10	563.5 ± 13	427.2 ± 15	173.4 ± 14	120.8 ± 11	0	552.6±20	407.9±12	179.7±15	122.4±15	556.6±16	428.3±21	184.8±10	121.1±12
28	559.7 ± 21	395.3 ±14	167.8 ±14	113.7 ± 11	556.0 ± 18	407.2 ± 10	168.6 ± 16	114.0 ± 9	28	558.9±22	380.5±16	172.5±15	115.7±14	560.5±20	396.7±17	177.6±25	114.3±20
% Loss	0.9 ± 0.4	2.6 ± 1.6	2.8 ± 0.8	1.9 ± 0.2	1.3 ± 0.5	4.7 ± 2.0	2.8 ± 1.2	5.6 ± 2.0	% Loss	2.4±1.0	6.7±0.7	4.0±1.1	5.5±1.4	2.8±0.6	7.4±1.3	3.9±0.5	5.6±1.1

Table 6.15 %Loss of individual phenolics contents when blackcurrant juice was enhanced with 0, 1, 2 and 3% (w/v) boysenberry juice and 0, 0.2, 0.6 and 1 mg/L of ellagic acid stored at 5^{o}C

Time(days)	Blackcurrant juice (10^{o}Brix)										Time(days)	Blackcurrant juice (10^{o}Brix)									
	0 mg/L Ellagic acid											0.2 mg/L Ellagic acid									
	0%(w/v) Boysenberry juice					1%(w/v) Boysenberry juice						0%(w/v) Boysenberry juice					1%(w/v) Boysenberry juice				
	p-coumaric	Caffeic	Benzoic	Quercetin	Ellagic	p-coumaric	Caffeic	Benzoic	Quercetin	Ellagic		p-coumaric	Caffeic	Benzoic	Quercetin	Ellagic	p-coumaric	Caffeic	Benzoic	Quercetin	Ellagic
0	112.5 ± 12	78.5± 8	36.8± 11	24.8± 10	ND	101.7± 13	73.9± 12	34.9± 6	27.4± 8	ND	0	108.1± 16	77.0± 15	35.0± 11	24.7± 13	25.6± 9	102.1± 11	72.3± 15	35.6± 12	28.1± 6	26.7± 11
28	87.6± 16	67.5± 10	33.5± 14	23.0± 10	ND	87.5± 9	60.4± 14	30.2± 10	23.7± 11	ND	28	86.8± 12	63.9± 15	29.5± 10	23.5± 12	20.2± 5	87.9± 12	61.4± 19	30.0± 8	26.9± 10	20.7± 7
% Loss	22.1± 2	13.9± 1.7	9.0± 2.1	7.5± 1.0	ND	14.0± 1.5	18.3± 2.0	13.5± 1.2	13.6± 1.4	ND	% Loss	19.7± 1.5	17.0± 1.8	15.8± 2.0	5.1± 1.4	21.1± 1.1	13.9± 2.0	15.1± 2.4	15.6± 1.2	4.4± 1.5	22.4± 2.0
	2%(w/v) Boysenberry juice					3%(w/v) Boysenberry juice						2%(w/v) Boysenberry juice					3%(w/v) Boysenberry juice				
	p-coumaric	Caffeic	Benzoic	Quercetin	Ellagic	p-coumaric	Caffeic	Benzoic	Quercetin	Ellagic		p-coumaric	Caffeic	Benzoic	Quercetin	Ellagic	p-coumaric	Caffeic	Benzoic	Quercetin	Ellagic
0	95.7± 10	73.3± 12	35.4± 8	27.5± 7	ND	94.6± 10	70.8± 14	35.1± 11	27.9± 12	ND	0	103.3± 10	71.6± 12	35.9± 8	30.3± 5	28.0± 13	101.8± 11	71.0± 9	34.6± 8	33.2± 15	28.1± 8
28	88.6± 17	62.0± 14	31.3± 12	25.1± 10	ND	85.7± 15	61.2± 11	31.5± 18	25.4± 10	ND	28	89.1± 7	58.4± 11	30.3± 8	27.0± 7	21.5± 14	87.6± 10	60.0± 10	29.1± 12	27.6± 11	22.7± 7
% Loss	7.4± 2	15.5± 1.2	11.7± 2.2	9.0± 1.0	ND	9.4± 1.4	13.6± 1.5	10.3± 2.6	9.0± 1.8	ND	% Loss	13.7± 1.4	18.3± 2.1	15.5± 0.8	10.9± 1.1	23.3± 2.5	13.9± 2.0	15.4± 1.2	16.0± 1.8	16.9± 2.2	19.3± 1.0
Time(days)	Blackcurrant juice (10^{o}Brix)										Time(days)	Blackcurrant juice (10^{o}Brix)									
	0.6 mg/L Ellagic acid											1 mg/L Ellagic acid									
	0%(w/v) Boysenberry juice					1%(w/v) Boysenberry juice						0%(w/v) Boysenberry juice					1%(w/v) Boysenberry juice				
	p-coumaric	Caffeic	Benzoic	Quercetin	Ellagic	p-coumaric	Caffeic	Benzoic	Quercetin	Ellagic		p-coumaric	Caffeic	Benzoic	Quercetin	Ellagic	p-coumaric	Caffeic	Benzoic	Quercetin	Ellagic
0	100.6± 14	76.5± 15	34.1± 8	24.8± 12	33.2± 9	98.2± 11	73.6± 15	35.4± 9	25.0± 8	34.4± 7	0	99.5± 12	77.8± 15	33.8± 8	24.5± 11	45.1± 13	97.9± 9	75.8± 16	33.2± 10	26.1± 12	45.6± 9
28	86.4± 10	59.0± 17	28.5± 8	23.8± 7	22.3± 6	84.0± 13	56.1± 10	29.8± 17	23.8± 9	26.2± 5	28	78.2± 11	55.9± 14	28.2± 12	23.3± 10	23.4± 9	76.6± 9	53.9± 12	27.6± 8	23.6± 10	33.8± 8
% Loss	14.1± 1.7	22.9± 2.4	16.3± 2.1	3.8± 0.5	32.7± 2.7	14.5± 2.2	23.8± 1.9	15.7± 1.0	5.0± 1.7	23.7± 2.1	% Loss	21.4± 2.0	28.1± 2.1	16.5± 1.7	5.1± 1.5	48.1± 2.1	21.8± 1.0	28.9± 2.0	16.8± 1.7	9.5± 1.4	25.9± 1.0
	2%(w/v) Boysenberry juice					3%(w/v) Boysenberry juice						2%(w/v) Boysenberry juice					3%(w/v) Boysenberry juice				
	p-coumaric	Caffeic	Benzoic	Quercetin	Ellagic	p-coumaric	Caffeic	Benzoic	Quercetin	Ellagic		p-coumaric	Caffeic	Benzoic	Quercetin	Ellagic	p-coumaric	Caffeic	Benzoic	Quercetin	Ellagic
0	97.8± 10	72.7± 15	35.3± 13	25.9± 15	35.1± 4	97.1± 19	71.3± 16	36.1± 8	26.7± 6	36.8± 6	0	97.8± 8	75.3± 7	32.2± 12	27.0± 9	46.4± 14	98.8± 10	73.8± 8	33.8± 12	28.5± 7	48.2± 10
28	83.6± 16	61.7± 11	29.8± 12	23.4± 15	24.3± 7	79.3± 12	56.0± 10	30.5± 5	23.0± 4	25.9± 10	28	80.1± 12	61.8± 7	27.2± 15	23.3± 6	30.1± 12	77.5± 13	56.3± 15	28.2± 14	27.2± 11	32.0± 14
% Loss	14.5± 2	15.1± 1.8	15.7± 2.0	9.7± 2.2	30.9± 0.6	18.3± 2.0	21.5± 1.7	15.4± 0.4	14.0± 0.2	29.5± 2.1	% Loss	18.2± 1.7	18.0± 0.9	15.5± 1.1	13.8± 0.7	35.1± 2.1	21.6± 2.2	23.7± 1.8	16.4± 2.0	4.4± 2.2	33.7± 2.0

6.2 Discussion

This step confirms that the addition of boysenberry juice and ellagic acid alone or together in blackcurrant juice was found to improve colour stability of blackcurrant juice (total anthocyanin content and hyperchromic shift) during storage ($P \leq 0.05$). As discussed in Section 5.2, intermolecular copigmentation can be affected by many factors such as pH of solution, temperature, structure of anthocyanin and phenolics, and concentration of copigments. Optimum pH at 3.5, low storage temperature, high concentrations of delphinidin monoglucosides resulting in a high number of hydroxyl groups in anthocyanins, were important reasons why boysenberry juice can form a complex with ellagic acid via intermolecular copigmentation but in other juices.

The concentration of copigments was also a significant issue inducing copigmentation between anthocyanins and copigments. As seen in Table 6.16, blackcurrant juice enhanced with 1, 2 and 3% (w/v) concentrations of boysenberry juice gave a molar ellagic acid/anthocyanin ratio of 2:1000. This molar ratio was similar to blackcurrant juice enhanced with 1 mg/L concentrations of pure ellagic acid (Table 6.16). Therefore, to reach a maximum effect of ellagic acid on copigmentation at a molar ellagic acid/anthocyanin ratio of 2:1000, 1, 2 or 3% (w/v) concentrations of boysenberry juice or 1 mg/L concentration of pure ellagic acid should be added into blackcurrant juice. This study shows the effect of phenolic acids at very low concentrations compared to other researchers such as 100:1 (Rein, 2005) and copigment/pigment of 2:1 (Shikov et al., 2008)

Statistically results showed that among boysenberry juice and ellagic acid added to blackcurrant juice, there was an interaction between the two when they were added into blackcurrant juice ($P \leq 0.05$), but this point was too complex to discuss in this study.

It was also found that the effect on colour stabilisation and colour enhancement of boysenberry juice on blackcurrant juice was not as significant as the corresponding amount of ellagic acid. Ellagic acid pure standard added was more effective on maintaining the colour stability of blackcurrant juice than boysenberry juice. Although ellagic acid pure standard used in this study and in boysenberry juice have the same chemical structure and properties, a difference in degree of purification of ellagic acid in boysenberry juice (natural sources) and pure standard (96%) may be a reason for improved effectiveness of ellagic acid on the colour stability in blackcurrant juice.

Table 6.16 Molar ratio of contentration of copigment and anthocyanins calculated when boysenberry juice were added with blackcurrant, cranberry and pomegranate juices, and also blackcurrant juice enhanced with phenolic acids

Study 2	Molar ratio (Ellagic acid in boysenberry juice: anthocyanin concentrations in berry juices)
Blackcurrant	0
Blackcurrant + 1% Boysenberry	2:1000
Blackcurrant + 2% Boysenberry	2:1000
Blackcurrant + 3% Boysenberry	2:1000
cranberry	0
cranberry + 1% Boysenberry	2:1000
cranberry+ 2% Boysenberry	2:1000
cranberry+ 3% Boysenberry	2:1000
Pomegranate	0
Pomegranate + 1% Boysenberry	2:1000
Pomegranate+ 2% Boysenberry	2:1000
Pomegranate+ 3% Boysenberry	2:1000
Study 3	Molar ratio (phenolics concentrations: anthocyanin concentrations in blackcurrant juice)
Blackcurrant + 0.2mg/L Ellagic acid	3:10000
Blackcurrant + 0.6mg/L Ellagic acid	1:1000
Blackcurrant + 1mg/L Ellagic acid	2:1000
Blackcurrant + 0.2mg/L Kaemferol	3:10000
Blackcurrant + 0.6mg/L Kaemferol	1:1000
Blackcurrant + 1mg/L Kaemferol	2:1000
Blackcurrant + 0.2mg/L Quercetin	3:10000
Blackcurrant + 0.6mg/L Quercetin	1:1000
Blackcurrant + 1mg/L Quercetin	2:1000

CHAPTER 7

OVERALL DISCUSSIONS

The stability of the colour of berry products has been studied based on the effects of processing methods (Skede et al., 2000), packaging material and preservative addition (Thakur & Arya, 2007), the addition of sugar (Wrolstad et al., 1990) and the addition of natural anthocyanin extracts (Rein, 2005). But, no studies focused on colour enhancement by the addition of natural boysenberry juice into other berry juices.

The non-enhanced blackcurrant, cranberry and pomegranate juices lost their anthocyanin pigments and redness quickly during storage. With the addition of boysenberry juice to the three juices, only boysenberry juice improved the blackcurrant by enhancing the anthocyanin colour intensity and stabilising the colour during storage at 5°C. The effects of phenolics on anthocyanin colour in model systems (Delgado-Vargas & Peredes-Lopes, 2003) and in blood orange juice (Maccarone et al., 2006) has been studied before. After the addition of kaemferol, quercetin and ellagic acid to blackcurrant juice, only ellagic aicd was found to improve the juice colour of blackcurrant juice. This may be due to intermolecular copigmentation between ellagic acid and blackcurrant anthocyanins as the colour was stabilised and the degradation of anthocynains was reduced, and no new anthocyanin compounds were detected with HPLC. It was also found that the effects on colour stabilisation and colour enhancement of boysenberry juice on blackcurrant juice was not as significant as the corresponding addition of ellagic acid. Though the addition of boysenberry juice to stabilize colour in blackcurrant juice is a more natural additive one that consumers are more likely to accept.

There were many factors that may affect the stability of the blackcurrant juice colour such as pH of the solution, the structure of anthocyanin, the structure of copigments and anthocyanins, temperature and, concentration of copigment (Dangles & Brouillard, 1993). Cranberry and pomegranate juices contained less anthocyanins and phenolic acids than blackcurrant juice. This concentration factor might be a reason for copigmentation reactions during storage (Kahkonen et al., 2001; Prior et al., 2001).

Also, the different pH values can have an effect on the stabilisation of the blackcurrant juice colour during storage. The pH was little higher in blackcurrant juice as compared to cranberry and pomegranate juices. After addition of boysenberry juice, the pH value of all blackcurrant, cranberry and pomegranate juices were increased. HPLC data, showed that most anthocyanins found in blackcurrant, cranberry and pomegranate juices were 3-monoglucosides which have a maximum effect of copigmentation at pH 3.5-4.2 (William & Hrazdina, 1979). Therefore, blackcurrant juice enhanced with boysenberry juice resulted in a juice with a more optimum pH value for copigmentation compared to other two juices.

After addition of kaemferol, quercetin and ellagic acid into blackcurrant juice, the pH values of the blackcurrant juice enhanced with all three phenolic acids were increased; however, only ellagic acid that reach the optimum pH range (3.5-4.2) for monoglucoside copigmentation (William & Hrazdina, 1979). As a result, ellagi acid may form copigmentation complexes with delphinidin-3-rutinoside or other 3-monoglucosides in blackcurrant juice.

Temperature also is another factor, which has a role in destabilising the anthocyanin molecular structure. It was found that an increase in the storage temperature greatly accerelates the destruction of anthocyanin pigments in blackcurrant juice. The number of hydroxyl groups in the flavonoid copigment also impacts the degree of copigmentation and complex formation (Waterhouse, 2002). Kaemferol, quercetin and ellagic acid contain 4, 5 and 4 hydroxyl groups, respectively. Quercetin containing 5 hydroxyl groups should be the most efficiently phenolic acid among the three for copigmentation in blackcurrant juice as copigmentation occurs when ellagic acid was added into blackcurrant juice only.

The structure of anthocyanins also seems to promote copigmentation. The high number of hydroxyl groups found in the B ring gave the anthocyanins molecules more accessible to water and easily complexed with other compounds. Blackcurrant juice that contained two types of delphinidin monoglucosides as the main anthocyanins had a higher number of hydroxyl groups than other juices resulting in stronger copigmentation.

A maximum effect of ellagic acid on copigmentation in this study was found at a molar ellagic acid/anthocyanin ratio of 2:1000 when 1, 2 or 3% (w/v) concentrations of boysenberry juice, or 1 mg/L concentration of pure ellagic acid was added into blackcurrant juice. This study shows effect of phenolic acids at very low concentrations (2:1000) compared to other researchers such as 100:1 (Rein, 2005) and copigment/pigment of 2:1 (Shikov et al., 2008). This reflected the effectiveness of copigmentation between anthocyanins and ellagic acid as very low concentrations of ellagic acid can give a positive effect of copigmentation resulting in maintaining colour stability of blackcurrant juice during storage. The presence of ellagic acid in boysenberry juice may be enough to induce the copigmentation observed and as mentioned above is a more natural copigment to add to stabilise the colour in blackcurrant juice.

CHAPTER 8

CONCLUSIONS

In this study, it was found that boysenberry juice improved the colour of blackcurrant, cranberry and pomegranate juices immediately after addition; however, only blackcurrant juice colour was stable during storage at 5°C. There was no influence on the stability of total anthocyanins in pomegranate or cranberry juices when boysenberry juice was added. Of the three juices, pomegranate had the highest rate of degradation. The total anthocyanin content of blackcurrant juice enhanced with boysenberry juice was more stable than for cranberry and pomegranate juices.

The addition of ellagic acid improved the colour of the blackcurrant juice by stabilising and enhancing its colour at 5°C; however, the colour intensity of blackcurrant enhanced with kaemferol and quercetin decreased with storage. The copigmentation interactions between the anthocyanins themselves were not found to have a significant effect on colour stability of blackcurrant juice. The reactions observed with the blackcurrant juices and added phenolic acids differed significantly by their mechanisms and manifestations. Intermolecular copigmentation reactions are most likely responsible for the colour enhancement by the conjugated ellagic acid, which protected blackcurrant juice anthocyanins.

In conclusion, ellagic acid as found in boysenberry juice formed intermolecular copigmentation with blackcurrant juice anthocyanins, so this resulted in stabilised juice colour during storage; however, the effect was found when the juice was stored at 5°C only. It was also found that the effects on colour stabilisation and colour enhancement of boysenberry juice in blackcurrant juice was not as significant as the equivalent level of ellagic acid that was present in the added boysenberry juice.

CHAPTER 9

RECOMMENDATIONS

- In this study, the effect of ellagic acid on colour stability in blackcurrant juice was investigated. The experimental results showed that the intermolecular copigmentation between ellagic acid and blackcurrant juice anthocyanins was the main reason on colour stability; however, the chemically mechanism between them has not been studied yet. For further research, reactions of pure anthocyanins present in blackcurrant juice with ellagic acid could be studied.

- Monoglucoside anthocyanins in blackcurrant juice may be complexed with ellagic acid to stabilise the colour of blackcurrant juice. To optimise this copigmentation result, pH of juices solution should be adjusted to 3.5 before adding ellagic acid.

- This study looked at methods to maintain colour stability during storage. It would be important to investigate the impact of processing conditions (e.g. pastuerisation, packaging) the ability of boysenberry juice and ellagic acid to maintain the colour of the juice.

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APPENDICES

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Appendix 1 Total anthocyanin content of blackcurrant, cranberry and pomegranate juice enhanced with 0, 1, 2 and 3% (w/v) concentrations boysenberry juice at various temperatures (Study 2) * Bl = Blackcurrant juice; C = cranberry juice;
P = pomegranate juice; Boy = boysenberry juice

Temperature (^{o}C)	Time (days)	Total anthocyanin content (mg/L)			
		Bl +0%boy	Bl +1%boy	Bl +2%boy	Bl +3%boy
5	0	1308.2 ± 63.3	1329.2 ± 3.7	1360.8 ± 63.3	1379.2 ± 74.4
	3	1305.5 ± 44.7	1331.8 ± 29.8	1352.9 ± 0.0	1384.5 ± 59.6
	7	1289.7 ± 14.9	1339.7 ± 18.6	1355.5 ± 40.9	1381.8 ± 26.1
	14	1268.7 ± 0.0	1355.5 ± 11.2	1352.9 ± 7.4	1408.2 ± 3.7
	21	1247.6 ± 7.4	1334.5 ± 3.7	1342.4 ± 7.4	1379.2 ± 14.9
	28	1229.2 ± 18.6	1321.3 ± 0.0	1331.8 ± 7.4	1374.0 ± 7.4
	35	1055.5 ± 3.7	1297.6 ± 33.5	1305.5 ± 29.8	1321.3 ± 7.4
20	0	1308.2 ± 63.3	1329.2 ± 3.7	1360.8 ± 63.3	1379.2 ± 74.4
	3	1271.3 ± 55.8	1323.9 ± 5.6	1350.3 ± 18.6	1352.9 ± 29.8
	7	1297.6 ± 40.9	1302.9 ± 55.8	1329.2 ± 40.9	1318.7 ± 18.6
	14	1237.6 ± 0.7	1300.3 ± 7.4	1318.7 ± 3.7	1297.6 ± 3.7
	21	1213.4 ± 40.9	1279.2 ± 7.4	1302.9 ± 3.7	1281.8 ± 3.7
	28	1181.8 ± 3.7	1242.3 ± 14.9	1268.7 ± 7.4	1250.2 ± 3.7
35	0	1308.2 ± 63.3	1329.2 ± 3.7	1360.8 ± 63.3	1379.2 ± 74.4
	3	1097.6 ± 18.6	1226.6 ± 74.4	1260.8 ± 48.4	1237.1 ± 148.9
	7	873.9 ± 44.7	1039.7 ± 11.2	1079.7 ± 23.1	989.7 ± 22.3
	14	829.1 ± 11.2	1018.6 ± 18.6	1044.9 ± 3.7	952.8 ± 7.4
	21	702.8 ± 3.7	981.8 ± 11.2	1002.8 ± 3.7	902.8 ± 3.7
	28	647.5 ± 0.0	939.7 ± 3.7	960.7 ± 3.7	852.8 ± 14.9

Temperature (^{o}C)	Time (days)	Total anthocyanin content (mg/L)			
		C+0% Boy	C+1% Boy	C+2% Boy	C+3% Boy
5	0	304.1 ± 5.44	317.1 ± 2.69	335.1± 2.55	352.2 ± 5.59
	3	293.3 ± 4.67	311 ± 2.47	323.4 ± 2.97	351 ± 1.56
	7	280.1 ± 0.42	300.5 ± 0.42	318.6 ± 0.42	350.1± 1.70
	14	253.9 ± 1.70	270.4 ± 2.12	290.9 ± 0.42	323.1 ± 4.24
	21	229.5 ±4.67	258.4 ± 3.82	280.4 ± 0.85	305.3 ± 4.67
	28	211.8 ± 3.39	239.7 ± 0.42	260.8 ± 1.27	281 ± 0.85
20	0	304.1 ± 5.44	317.1 ± 2.69	335.1 ± 2.55	352.2 ± 5.59
	3	277.9 ± 4.31	302 ± 3.39	308 ± 1.70	346.2 ± 0.21
	7	259.9 ± 6.79	281 ± 2.55	299.6 ± 4.24	324.6 ± 0.42
	14	229.5 ± 2.97	254.5 ± 0.85	274.3 ± 1.70	291.8 ± 1.70
	21	211.5 ± 2.12	231.9 ± 0.42	249.7 ± 4.24	273.7 ± 6.86
	28	185.9 ± 3.39	207.6 ± 5.16	227.7 ± 2.12	245.8 ± 2.12
35	0	304.1 ± 5.44	317.1 ± 2.69	335.1 ± 2.55	352.2 ± 5.59
	3	271.6 ± 1.27	290.6 ± 0.00	301.4 ± 5.09	345 ± 5.52
	7	225 ± 4.24	258.1 ± 4.24	290.9 ± 1.27	333.6 ± 2.97
	14	194.3 ± 3.39	224.4 ± 6.79	253.9 ± 12.73	298.1 ± 10.61
	21	149.8 ± 1.70	191.3 ± 1.70	210.6 ± 1.70	261.4 ± 2.12
	28	123.3 ± 0.85	161.5 ± 1.27	174.8 ± 0.42	219.6 ± 3.39

Appendix 1 Total anthocyanin content of blackcurrant, cranberry and pomegranate juice enhanced with 0, 1, 2 and 3% (w/v) concentrations boysenberry juice at various temperatures (Study 2) * Bl = Blackcurrant juice; C = cranberry juice; P = pomegranate juice; Boy = boysenberry juice (Cont.)

Temperature (^{o}C)	Time (days)	Total anthocyanin content (mg/L)			
		P+0% Boy	P+1% Boy	P+2% Boy	P+3% Boy
5	0	90.4 ± 0.0	96.3 ± 1.1	107.1 ± 0.3	112.3 ± 0.1
	3	89.8 ± 0.8	93.8 ± 0.7	100.8 ± 0.1	105.8 ± 0.6
	7	84.5 ± 0.8	90.2 ± 0.9	95.4 ± 0.8	98.4 ± 0.1
	14	59.5 ± 1.6	72 ± 1.6	80.2 ± 0.6	95.7 ± 0.8
	21	50.3 ± 0.3	62.2 ± 0.1	59.9 ± 0.1	70.6 ± 0.3
	28	30.8 ± 0.4	45.3 ± 0.0	40.9 ± 0.2	55.3 ± 0.6
20	0	90.4 ± 0.0	96.3 ± 1.1	107.1 ± 0.3	112.3 ± 0.1
	3	83.3 ± 0.6	80.8 ± 1.1	91.3 ± 1.5	95.2 ± 0.6
	7	64.7 ± 0.1	70.5 ± 0.2	70.9 ± 1.4	79.7 ± 1.0
	14	45.6 ± 0.2	60.5 ± 1.5	61.1 ± 1.8	71.1 ± 0.8
	21	40.8 ± 0.4	50.2 ± 1.5	45.3 ± 0.4	60.8 ± 0.2
	28	15 ± 0.1	25.3 ± 0.1	20.2 ± 0.1	35.1 ± 0.6
35	0	90.4 ± 0.0	96.3 ± 1.1	107.1 ± 0.3	112.3 ± 0.1
	3	78.4 ± 0.1	86.2 ± 0.6	91.4 ± 0.5	100.8 ± 0.2
	7	62.6 ± 0.5	67.2 ± 0.4	75.3 ± 1.0	81.8 ± 0.2
	14	40.8 ± 0.5	54.5 ± 1.1	65.7 ± 1.3	73 ± 2.5
	21	30.7 ± 0.0	39.6 ± 0.8	46 ± 0.8	55.5 ± 0.3
	28	4 ± 0.1	4.7 ± 0.1	5.4 ± 0.4	9.9 ± 0.1

Appendix 2 Total anthocyanin content of blackcurrant juice enhanced with 0, 0.2, 0.6 and 1 mg/L of kaemferol, quercetin and ellagic acid at various temperatures (Study 3)

* Bl = Blackcurrant juice

Temperature (^{o}C)	Time (days)	Total anthocyanin content (mg/L)			
		Bl+0mg/L	Bl+0.2mg/L	Bl+0.6mg/L	Bl+1mg/L
		Kaemferol	Kaemferol	Kaemferol	Kaemferol
5	0	1139.7 ± 3.7	1145.0 ± 3.7	1150.2 ± 11.2	1134.4 ± 3.7
	7	1102.8 ± 3.7	1108.1 ± 3.7	1113.4 ± 3.7	1102.8 ± 3.7
	14	1063.4 ± 0.0	1050.2 ± 3.7	1073.9 ± 22.3	1066.0 ± 11.2
	21	1008.1 ± 3.7	1013.4 ± 18.6	1018.6 ± 26.0	1010.7 ± 22.3
	28	960.7 ± 3.7	950.2 ± 3.7	963.3 ± 7.4	942.3 ± 22.3
20	0	1139.7 ± 3.7	1145.0 ± 3.7	1150.2 ± 11.2	1134.4 ± 3.7
	7	1071.3 ± 3.7	1052.8 ± 7.4	1079.2 ± 14.9	1060.7 ± 3.7
	14	1021.3 ± 0.0	1010.7 ± 14.9	1026.5 ± 0.0	1034.4 ± 3.7
	21	960.7 ± 3.7	937.0 ± 0.0	973.9 ± 7.4	979.1 ± 14.9
	28	892.3 ± 3.7	879.1 ± 14.8	908.1 ± 3.7	884.4 ± 7.4

Temperature (^{o}C)	Time (days)	Total anthocyanin content (mg/L)			
		Bl+0mg/L Quercetin	Bl+0.2mg/L Quercetin	Bl+0.6mg/L Quercetin	Bl+1mg/L Quercetin
5	0	1147.6 ± 0.0	1142.3 ± 22.3	1152.9 ± 37.3	1150.2 ± 48.4
	7	1123.9 ± 3.7	1113.4 ± 3.7	1118.6 ± 3.7	1110.7 ± 0.0
	14	1073.9 ± 0.0	1079.2 ± 7.4	1087.1 ± 3.7	1081.8 ± 3.7
	21	1008.1 ± 3.7	984.4 ± 7.5	1013.4 ± 3.7	1005.5 ± 7.4
	28	971.2 ± 3.7	963.3 ± 7.4	973.9 ± 7.4	952.8 ± 14.8
20	0	1147.6 ± 0.0	1142.3 ± 22.3	1152.9 ± 37.3	1150.2 ± 48.4
	7	1079.2 ± 7.4	1084.4 ± 7.4	1097.6 ± 3.7	1102.8 ± 3.7
	14	1023.9 ± 3.7	1031.8 ± 0.0	1037.0 ± 7.4	1052.8 ± 7.4
	21	963.3 ± 18.6	971.2 ± 7.4	952.8 ± 3.7	976.5 ± 3.7
	28	884.4 ± 7.4	892.3 ± 3.7	873.9 ± 0.0	897.5 ± 3.7

Temperature (^{o}C)	Time (days)	Total anthocyanin content (mg/L)			
		Bl+0mg/L Ellagic acid	Bl+0.2mg/L Ellagic acid	Bl+0.6mg/L Ellagic acid	Bl+1mg/L Ellagic acid
5	0	1137.1 ± 44.7	1158.1 ± 37.2	1202.9 ± 48.4	1255.5 ± 48.4
	7	1102.8 ± 3.7	1150.2 ± 3.7	1192.3 ± 3.7	1247.6 ± 0.0
	14	1071.3 ± 3.7	1131.8 ± 14.8	1166.0 ± 3.7	1226.6 ± 0.0
	21	1021.3 ± 0.0	1118.6 ± 3.7	1150.2 ± 3.7	1221.3 ± 0.0
	28	971.2 ± 3.7	1116.0 ± 14.8	1142.3 ± 7.4	1200.2 ± 0.0
20	0	1137.1 ± 44.7	1158.1 ± 37.2	1202.9 ± 48.4	1255.5 ± 48.4
	7	1068.6 ± 0.0	1118.6 ± 3.7	1171.3 ± 3.7	1218.7 ± 3.7
	14	1018.6 ± 3.7	1081.8 ± 3.7	1147.6 ± 0.0	1187.1 ± 18.6
	21	963.3 ± 7.4	1018.6 ± 3.7	1108.1 ± 3.7	1113.4 ± 3.7
	28	902.8 ± 3.7	989.7 ± 7.4	1050.2 ± 18.7	1066.0 ± 3.7

Appendix 3 Total anthocyanin content of blackcurrant juice enhanced with 0, 0.2, 0.6 and 1 mg/L of ellagic acid and 0, 1, 2 and 3% (w/v) concentrations boysenberry juice at 5^{o}C (Study 4)

Time(days)	Total anthocyanin content (mg/L)							
	Blackcurrant juice (10^{o}Brix)							
	0 mg/L Ellagic acid				0.2 mg/L Ellagic acid			
	Boysenberry juice (%w/v)				Boysenberry juice (%w/v)			
	0%	1%	2%	3%	0%	1%	2%	3%
0	1187.1 ± 3.7	1258.1 ± 14.9	1289.7 ± 14.9	1329.2 ± 3.7	1223.9 ± 3.7	1242.3 ± 14.9	1255.5 ± 3.7	1273.9 ± 0.0
7	1142.3 ± 14.9	1239.7 ± 3.7	1284.5 ± 0.0	1316.0 ± 7.4	1210.8 ± 7.4	1231.8 ± 7.4	1242.3 ± 7.4	1260.8 ± 3.7
14	1113.4 ± 3.7	1234.5 ± 3.7	1284.5 ± 7.4	1305.5 ± 0.0	1200.2 ± 22.3	1216.0 ± 0.0	1239.7 ± 3.7	1255.5 ± 3.7
21	1073.9 ± 0.0	1231.8 ± 0.0	1276.6 ± 3.7	1297.6 ± 3.7	1192.3 ± 18.6	1205.5 ± 0.0	1229.2 ± 3.7	1245.0 ± 3.7
28	1034.4 ± 3.7	1226.6 ± 7.4	1271.3 ± 3.7	1289.7 ± 7.4	1184.4 ± 14.9	1195.0 ± 0.0	1216.0 ± 7.4	1239.7 ± 3.7
Time(days)	0.6 mg/L Ellagic acid				1 mg/L Ellagic acid			
	Boysenberry juice (%w/v)				Boysenberry juice (%w/v)			
	0%	1%	2%	3%	0%	1%	2%	3%
0	1276.6 ± 3.7	1287.1 ± 3.7	1295.0 ± 14.9	1302.9 ± 11.2	1305.5 ± 14.9	1313.4 ± 3.7	1323.9 ± 18.6	1342.4 ± 7.4
7	1266.0 ± 3.7	1281.8 ± 3.7	1297.6 ± 3.7	1295.0 ± 7.4	1300.3 ± 7.4	1308.2 ± 11.2	1310.8 ± 0.0	1323.9 ± 11.2
14	1258.1 ± 0.0	1279.2 ± 0.0	1287.1 ± 3.7	1289.7 ± 7.4	1295.0 ± 7.4	1292.4 ± 3.7	1302.9 ± 3.7	1292.4 ± 11.2
21	1252.9 ± 0.0	1273.9 ± 0.0	1279.2 ± 0.0	1281.8 ± 3.7	1287.1 ± 3.7	1279.2 ± 7.4	1292.4 ± 3.7	1281.8 ± 11.2
28	1247.6 ± 0.0	1268.7 ± 7.4	1271.3 ± 3.7	1271.3 ± 3.7	1279.2 ± 7.4	1266.0 ± 11.2	1284.5 ± 0.0	1268.7 ± 14.9

Appendix 4 Colour density and polymeric colour of blackcurrant juice enhanced with 0, 1, 2 and 3% (w/v) concentrations boysenberry juice at various temperatures (Study 2) * Bl = Blackcurrant juice; Boy = boysenberry juice

Temperature (^{o}C)	Time (days)	Colour density				Polymeric colour			
		Bl +0%boy	Bl +1%boy	Bl +2%boy	Bl +3%boy	Bl +0%boy	Bl +1%boy	Bl +2%boy	Bl +3%boy
5	0	85.1 ± 2.0	89.8 ± 3.6	92.4 ± 0.4	97.9 ± 1.1	13.7 ± 0.4	17.8 ± 0.6	20.3 ± 1.7	23.1 ± 0.6
	3	83.4 ± 0.5	91.5 ± 0.9	90.0 ± 0.9	98.3 ± 1.3	14.3 ± 0.1	18.3 ± 0.5	19.4 ± 0.9	23.1 ± 0.1
	7	80.4 ± 0.3	92.3 ± 0.4	90.4 ± 0.1	98.6 ± 0.0	15.1 ± 0.4	18.4 ± 0.5	18.0 ± 1.1	22.8 ± 0.1
	14	74.8 ± 0.6	91.0 ± 0.2	91.4 ± 0.1	106.0 ± 0.4	17.3 ± 0.5	17.2 ± 0.3	17.1 ± 0.0	21.4 ± 0.3
	21	65.2 ± 0.3	85.6 ± 0.0	83.1 ± 0.1	95.8 ± 0.2	18.6 ± 0.1	18.4 ± 0.2	18.7 ± 0.2	23.3 ± 0.2
	28	50.9 ± 0.4	84.8 ± 0.2	80.2 ± 0.3	92.1 ± 0.0	19.1 ± 0.1	18.5 ± 0.2	18.6 ± 0.1	24.4 ± 0.6
20	0	85.1 ± 2.0	89.8 ± 3.6	92.4 ± 0.4	97.9 ± 1.1	13.7 ± 0.4	17.8 ± 0.6	20.3 ± 1.7	23.1 ± 0.6
	3	78.1 ± 0.9	87.5 ± 1.9	90.0 ± 0.9	95.1 ± 0.2	14.4 ± 0.4	17.4 ± 0.4	21.6 ± 0.4	22.3 ± 0.0
	7	79.4 ± 0.5	85.4 ± 0.2	85.3 ± 0.3	90.4 ± 0.5	15.4 ± 0.7	18.3 ± 0.2	21.4 ± 0.2	22.3 ± 0.0
	14	68.5 ± 0.9	85.9 ± 0.1	80.3 ± 0.4	92.3 ± 0.4	18.3 ± 0.1	18.1 ± 0.0	21.9 ± 0.3	20.9 ± 0.8
	21	58.5 ± 0.2	85.4 ± 0.2	70.2 ± 0.3	83.3 ± 0.4	19.3 ± 0.3	18.3 ± 0.2	22.6 ± 0.1	22.7 ± 0.1
	28	50.6 ± 0.2	75.4 ± 0.6	60.6 ± 1.5	72.2 ± 0.4	20.3 ± 0.1	19.2 ± 0.3	23.6 ± 0.3	23.5 ± 0.0
35	0	85.1 ± 2.0	89.8 ± 3.6	92.4 ± 0.4	97.9 ± 1.1	13.7 ± 0.4	17.8 ± 0.6	20.3 ± 1.7	23.1 ± 0.6
	3	75.4 ± 0.5	85.0 ± 0.7	88.3 ± 0.3	95.7 ± 0.4	17.4 ± 0.6	17.3 ± 0.6	22.6 ± 0.2	22.3 ± 0.0
	7	70.4 ± 0.2	84.1 ± 0.4	82.4 ± 0.3	91.8 ± 0.3	20.4 ± 0.5	22.4 ± 1.9	24.9 ± 0.2	24.3 ± 0.1
	14	65.3 ± 0.3	80.4 ± 1.5	78.2 ± 0.1	93.9 ± 1.1	22.4 ± 0.2	23.6 ± 0.4	23.9 ± 0.1	24.3 ± 0.1
	21	55.9 ± 0.8	70.3 ± 0.2	69.9 ± 0.4	80.0 ± 0.5	23.7 ± 0.1	25.2 ± 0.1	25.7 ± 0.1	25.8 ± 0.6
	28	40.5 ± 0.5	61.7 ± 1.3	50.7 ± 0.1	70.5 ± 0.2	25.9 ± 0.3	28.0 ± 0.4	28.4 ± 0.0	28.9 ± 1.0

Appendix 5 Colour density and polymeric colour of cranberry juice enhanced with 0, 1, 2 and 3% (w/v) concentrations boysenberry juice at various temperatures (Study 2) * C = cranberry juice; Boy = boysenberry juice

Temperature (^{o}C)	Time (days)	Colour density				Polymeric colour			
		C+0% Boy	C+1% Boy	C+2% Boy	C+3% Boy	C+0% Boy	C+1% Boy	C+2% Boy	C+3% Boy
5	0	27.2 ± 0.7	29.7 ± 0.2	35.6 ± 0.1	39.1 ± 1.1	7.4 ± 0.2	6.9 ± 0.8	6.2 ± 0.0	5.1 ± 0.1
	3	26.6 ± 0.3	29.2 ± 0.1	34.3 ± 0.0	40.0 ± 0.4	7.5 ± 0.0	7.0 ± 0.0	7.0 ± 0.2	5.1 ± 0.2
	7	25.0 ± 0.0	28.1 ± 0.4	32.0 ± 0.1	39.1 ± 0.4	8.1 ± 0.0	7.6 ± 0.1	6.6 ± 0.2	5.2 ± 0.3
	14	22.5 ± 0.0	26.3 ± 0.0	29.1 ± 0.1	30.4 ± 0.0	9.1 ± 0.2	8.5 ± 0.1	6.2 ± 0.0	4.6 ± 0.1
	21	20.3 ± 0.4	25.2 ± 0.3	28.5 ± 0.3	28.0 ± 0.2	10.2 ± 0.1	9.1 ± 0.1	7.5 ± 0.0	6.6 ± 0.1
	28	18.7 ± 0.1	24.3 ± 0.2	26.3 ± 0.2	25.6 ± 0.2	11.4 ± 0.5	10.5 ± 0.1	8.1 ± 0.1	7.2 ± 0.1
20	0	27.2 ± 0.7	29.7 ± 0.2	35.6 ± 0.1	39.1 ± 1.1	7.4 ± 0.2	6.9 ± 0.8	6.2 ± 0.0	5.1 ± 0.1
	3	25.8 ± 0.0	28.2 ± 0.1	35.2 ± 0.1	37.6 ± 0.1	7.1 ± 0.1	6.7 ± 0.3	7.1 ± 0.1	5.3 ± 0.0
	7	23.2 ± 0.5	27.2 ± 0.0	31.4 ± 0.0	36.1 ± 0.3	6.4 ± 0.0	7.2 ± 0.1	6.0 ± 0.2	4.6 ± 0.0
	14	21.1 ± 0.1	25.5 ± 0.5	27.4 ± 0.2	34.4 ± 0.3	7.6 ± 0.0	8.2 ± 0.0	7.5 ± 0.0	5.5 ± 0.1
	21	18.6 ± 0.3	22.7 ± 0.1	25.6 ± 0.1	33.0 ± 0.2	8.2 ± 0.0	8.9 ± 0.1	8.5 ± 0.0	6.3 ± 0.0
	28	16.7 ± 0.1	20.4 ± 0.2	22.2 ± 0.1	31.3 ± 0.0	9.1 ± 0.0	9.4 ± 0.2	9.7 ± 0.1	7.3 ± 0.1
35	0	27.2 ± 0.7	29.7 ± 0.2	35.6 ± 0.1	39.1 ± 1.1	7.4 ± 0.2	6.9 ± 0.8	6.2 ± 0.0	5.1 ± 0.1
	3	25.2 ± 0.4	27.8 ± 0.2	34.4 ± 0.1	36.0 ± 0.3	7.0 ± 0.2	6.3 ± 0.3	6.7 ± 0.1	4.9 ± 0.1
	7	22.1 ± 0.2	26.1 ± 0.1	30.3 ± 0.3	34.2 ± 0.2	6.3 ± 0.0	7.2 ± 0.1	5.6 ± 0.2	4.4 ± 0.0
	14	20.3 ± 0.2	24.3 ± 0.2	28.4 ± 0.2	33.7 ± 0.1	7.5 ± 0.1	8.0 ± 0.3	5.5 ± 0.1	6.2 ± 0.1
	21	17.3 ± 0.2	21.0 ± 0.2	25.4 ± 0.1	31.3 ± 0.1	8.5 ± 0.1	9.3 ± 0.0	6.7 ± 0.1	7.2 ± 0.5
	28	15.4 ± 0.2	18.3 ± 0.1	23.1 ± 0.1	18.4 ± 0.3	9.6 ± 0.1	10.4 ± 0.3	7.7 ± 0.2	8.2 ± 0.1

Appendix 6 Colour density and polymeric colour of pomegranate juice enhanced with 0, 1, 2 and 3% (w/v) concentrations boysenberry juice at various temperatures (Study 2) * P = pomegranate juice; Boy = boysenberry juice

Temp. (^{o}C)	Time (days)	Colour density				Polymeric colour			
		P+0% Boy	P+1% Boy	P+2% Boy	P+3% Boy	P+0% Boy	P+1% Boy	P+2% Boy	P+3% Boy
5	0	9.7 ± 0.0	10.7 ± 0.1	11.3 ± 0.1	12.1 ± 0.0	1.9 ± 0.0	1.5 ± 0.0	1.4 ± 0.0	1.1 ± 0.0
	3	9.1 ± 0.0	9.0 ± 0.1	11.0 ± 0.1	11.3 ± 0.0	1.9 ± 0.1	1.7 ± 0.1	1.6 ± 0.0	1.5 ± 0.1
	7	8.1 ± 0.0	8.5 ± 0.0	9.0 ± 0.0	11.3 ± 0.0	2.3 ± 0.0	2.0 ± 0.1	1.7 ± 0.0	1.7 ± 0.0
	14	7.0 ± 0.0	7.5 ± 0.0	8.1 ± 0.0	10.2 ± 0.2	3.5 ± 0.0	3.1 ± 0.0	3.6 ± 0.0	3.0 ± 0.0
	21	6.1 ± 0.0	6.6 ± 0.0	8.1 ± 0.1	10.1 ± 0.1	4.1 ± 0.0	4.5 ± 0.0	4.5 ± 0.0	4.0 ± 0.0
	28	5.2 ± 0.0	5.5 ± 0.0	7.0 ± 0.1	9.1 ± 0.0	5.6 ± 0.1	5.3 ± 0.1	5.1 ± 0.1	4.6 ± 0.0
20	0	9.7 ± 0.0	10.7 ± 0.1	11.3 ± 0.1	12.1 ± 0.0	1.9 ± 0.0	1.5 ± 0.0	1.4 ± 0.0	1.1 ± 0.0
	3	8.1 ± 0.0	8.9 ± 0.2	9.5 ± 0.0	9.5 ± 0.0	1.9 ± 0.1	2.0 ± 0.0	1.8 ± 0.0	1.8 ± 0.0
	7	7.1 ± 0.1	7.1 ± 0.0	7.5 ± 0.1	8.0 ± 0.1	3.0 ± 0.0	2.5 ± 0.1	2.5 ± 0.0	2.0 ± 0.1
	14	6.2 ± 0.1	6.1 ± 0.1	6.6 ± 0.1	7.1 ± 0.1	4.1 ± 0.1	4.0 ± 0.0	4.0 ± 0.0	4.1 ± 0.1
	21	6.2 ± 0.1	6.5 ± 0.0	7.1 ± 0.1	7.1 ± 0.0	4.9 ± 0.0	4.8 ± 0.0	4.6 ± 0.0	5.1 ± 0.0
	28	5.2 ± 0.1	5.0 ± 0.0	6.1 ± 0.1	6.1 ± 0.0	6.0 ± 0.0	6.1 ± 0.1	5.5 ± 0.1	6.6 ± 0.0
35	0	9.7 ± 0.0	10.7 ± 0.1	11.3 ± 0.1	12.1 ± 0.0	1.9 ± 0.0	1.5 ± 0.0	1.4 ± 0.0	1.1 ± 0.0
	3	7.1 ± 0.0	7.5 ± 0.0	8.5 ± 0.1	9.1 ± 0.0	2.0 ± 0.0	2.1 ± 0.0	1.8 ± 0.0	1.8 ± 0.0
	7	6.1 ± 0.0	6.5 ± 0.0	8.1 ± 0.0	8.1 ± 0.1	3.5 ± 0.0	3.1 ± 0.1	2.0 ± 0.0	2.2 ± 0.1
	14	5.1 ± 0.1	6.1 ± 0.0	7.0 ± 0.0	8.1 ± 0.1	4.6 ± 0.0	4.1 ± 0.0	5.0 ± 0.1	4.1 ± 0.1
	21	5.5 ± 0.0	6.1 ± 0.0	6.6 ± 0.1	7.6 ± 0.1	5.1 ± 0.0	4.9 ± 0.0	5.3 ± 0.1	4.6 ± 0.0
	28	4.5 ± 0.0	5.5 ± 0.0	5.1 ± 0.1	6.1 ± 0.1	6.0 ± 0.0	5.1 ± 0.0	6.4 ± 0.1	5.5 ± 0.0

Appendix 7 Colour density and polymeric colour of blackcurrant juice enhanced with 0, 0.2, 0.6 and 1 mg/L of kaemferol at various temperatures (Study 3)

* Bl = Blackcurrant juice

Temp. (^{o}C)	Time (days)	Colour density			
		Bl+0mg/L Kaemferol	Bl+0.2mg/L Kaemferol	Bl+0.6mg/L Kaemferol	Bl+1mg/L Kaemferol
5	0	79.7 ± 0.3	81.1 ± 0.1	81.8 ± 1.2	81.6 ± 0.7
	7	75.7 ± 0.1	76.4 ± 0.1	75.6 ± 0.4	76.3 ± 0.2
	14	70.2 ± 0.3	71.7 ± 0.3	70.1 ± 0.0	71.5 ± 0.2
	21	64.6 ± 0.2	69.9 ± 0.0	65.3 ± 0.1	69.0 ± 0.2
	28	60.3 ± 0.7	64.6 ± 0.4	60.0 ± 0.4	66.1 ± 0.2
20	0	79.7 ± 0.3	81.1 ± 0.1	81.8 ± 1.2	81.6 ± 0.7
	7	70.3 ± 0.3	71.6 ± 1.1	70.6 ± 0.4	72.3 ± 0.2
	14	60.9 ± 0.6	62.7 ± 0.3	61.1 ± 0.3	61.6 ± 0.6
	21	49.7 ± 0.6	50.2 ± 0.3	53.6 ± 1.5	52.2 ± 0.7
	28	40.9 ± 0.7	40.8 ± 0.3	45.8 ± 0.2	44.7 ± 1.5

Temp. (^{o}C)	Time (days)	Polymeric colour			
		Bl+0mg/L Kaemferol	Bl+0.2mg/L Kaemferol	Bl+0.6mg/L Kaemferol	Bl+1mg/L Kaemferol
5	0	12.4 ± 0.4	13.7 ± 0.1	13.1 ± 0.4	13.7 ± 0.4
	7	13.7 ± 0.3	14.3 ± 0.1	14.6 ± 0.0	14.4 ± 0.0
	14	14.3 ± 0.4	15.1 ± 0.1	15.4 ± 0.2	15.6 ± 0.3
	21	15.3 ± 0.1	16.1 ± 0.0	15.9 ± 0.1	16.1 ± 0.3
	28	16.1 ± 0.1	16.9 ± 0.2	16.6 ± 0.2	16.7 ± 0.3
20	0	12.4 ± 0.4	13.7 ± 0.1	13.1 ± 0.4	13.7 ± 0.4
	7	14.7 ± 0.1	15.1 ± 0.2	15.4 ± 0.7	14.6 ± 0.4
	14	15.1 ± 0.4	16.2 ± 0.1	16.3 ± 0.1	15.0 ± 0.0
	21	16.6 ± 0.2	17.2 ± 0.3	17.1 ± 0.1	16.9 ± 0.0
	28	17.3 ± 0.2	18.0 ± 0.0	18.2 ± 0.1	17.8 ± 0.2

Appendix 8 Colour density and polymeric colour of blackcurrant juice enhanced with 0, 0.2, 0.6 and 1 mg/L of quercetin at various temperatures (Study 3)

* Bl = Blackcurrant juice

Temp. (^{o}C)	Time (days)	Colour density			
		Bl+0mg/L Quercetin	Bl+0.2mg/L Quercetin	Bl+0.6mg/L Quercetin	Bl+1mg/L Quercetin
5	0	82.3 ± 0.1	82.4 ± 0.4	82.3 ± 0.3	83.4 ± 0.0
	7	76.2 ± 0.6	77.4 ± 0.4	76.8 ± 0.1	76.3 ± 0.1
	14	75.6 ± 0.1	78.0 ± 0.2	75.3 ± 0.3	76.2 ± 0.3
	21	71.8 ± 0.1	73.0 ± 0.2	70.3 ± 0.3	72.4 ± 0.3
	28	66.2 ± 1.0	67.4 ± 0.7	70.3 ± 0.3	64.9 ± 0.3
20	0	82.3 ± 0.1	82.4 ± 0.4	82.3 ± 0.3	83.4 ± 0.0
	7	73.1 ± 0.0	73.3 ± 0.3	72.4 ± 0.4	72.3 ± 0.2
	14	60.8 ± 0.0	61.3 ± 0.2	60.5 ± 0.2	59.0 ± 0.2
	21	52.0 ± 0.0	54.3 ± 0.5	51.8 ± 0.2	50.3 ± 0.2
	28	40.8 ± 0.0	46.8 ± 0.5	42.4 ± 1.1	40.6 ± 0.4

Temp. (^{o}C)	Time (days)	Polymeric colour			
		Bl+0mg/L Quercetin	Bl+0.2mg/L Quercetin	Bl+0.6mg/L Quercetin	Bl+1mg/L Quercetin
5	0	12.4 ± 0.4	13.8 ± 0.5	13.2 ± 0.6	13.6 ± 0.5
	7	14.4 ± 0.4	14.2 ± 0.1	14.4 ± 0.3	14.1 ± 0.2
	14	15.3 ± 0.0	15.1 ± 0.0	15.6 ± 0.2	15.1 ± 0.1
	21	15.4 ± 0.1	15.8 ± 0.0	16.2 ± 0.3	15.7 ± 0.1
	28	15.1 ± 0.0	15.3 ± 0.0	16.2 ± 0.3	15.6 ± 0.1
20	0	12.4 ± 0.4	13.8 ± 0.5	13.2 ± 0.6	13.6 ± 0.5
	7	15.4 ± 0.2	15.2 ± 0.1	15.6 ± 0.1	15.3 ± 0.1
	14	16.1 ± 0.1	16.4 ± 0.1	16.6 ± 0.2	16.2 ± 0.1
	21	15.2 ± 0.3	15.3 ± 0.1	15.8 ± 0.1	15.1 ± 0.0
	28	15.9 ± 0.1	16.4 ± 0.1	16.8 ± 0.0	16.4 ± 0.0

Appendix 9 Colour density and polymeric colour of blackcurrant juice enhanced with 0, 0.2, 0.6 and 1 mg/L of ellagic acid at various temperatures (Study 3)

* Bl = Blackcurrant juice

Temp. (^{o}C)	Time (days)	Colour density			
		Bl+0mg/L Ellagic acid	Bl+0.2mg/L Ellagic acid	Bl+0.6mg/L Ellagic acid	Bl+1mg/L Ellagic acid
5	0	81.9 ± 0.6	88.6 ± 0.1	95.5 ± 2.3	101.6 ± 1.1
	7	78.4 ± 0.1	88.1 ± 0.1	94.8 ± 0.1	100.5 ± 0.4
	14	76.5 ± 0.2	86.6 ± 0.1	93.6 ± 0.0	98.1 ± 0.0
	21	72.8 ± 0.2	81.6 ± 0.1	90.1 ± 0.3	94.4 ± 0.0
	28	67.6 ± 0.1	77.8 ± 0.1	86.3 ± 0.3	89.4 ± 0.0
20	0	81.9 ± 0.6	88.6 ± 0.1	95.5 ± 2.3	101.6 ± 1.1
	7	73.4 ± 0.4	83.4 ± 0.5	90.1 ± 0.7	96.1 ± 0.3
	14	70.4 ± 0.4	78.3 ± 0.2	84.9 ± 0.1	92.2 ± 0.3
	21	64.6 ± 0.2	72.1 ± 0.2	77.4 ± 0.1	83.4 ± 0.3
	28	56.5 ± 1.1	65.1 ± 0.1	69.1 ± 0.6	74.1 ± 0.6

Temp. (^{o}C)	Time (days)	Polymeric colour			
		Bl+0mg/L Ellagic acid	Bl+0.2mg/L Ellagic acid	Bl+0.6mg/L Ellagic acid	Bl+1mg/L Ellagic acid
5	0	12.4 ± 0.4	18.4 ± 0.3	20.9 ± 0.8	23.4 ± 0.1
	7	14.3 ± 0.4	18.9 ± 0.1	21.7 ± 0.3	23.8 ± 0.2
	14	15.0 ± 0.0	20.4 ± 0.2	22.2 ± 0.1	24.3 ± 0.1
	21	15.4 ± 0.1	20.8 ± 0.0	22.7 ± 0.1	24.8 ± 0.2
	28	15.8 ± 0.1	21.3 ± 0.1	23.2 ± 0.3	25.1 ± 0.1
20	0	12.4 ± 0.4	18.4 ± 0.3	20.9 ± 0.8	23.4 ± 0.1
	7	14.6 ± 0.1	19.6 ± 0.1	21.6 ± 0.1	24.9 ± 0.4
	14	15.2 ± 0.8	20.4 ± 0.4	22.8 ± 0.1	25.1 ± 0.0
	21	15.9 ± 0.0	21.1 ± 0.2	23.7 ± 0.1	26.1 ± 0.2
	28	16.7 ± 0.1	22.0 ± 0.2	24.4 ± 0.2	26.7 ± 0.1

Appendix 10 Hyperchromic and bathochromic shift of blackcurrant juice enhanced with 0, 1, 2 and 3% (w/v) concentrations boysenberry juice at various temperatures (Study 2) * Bl = Blackcurrant juice; Boy = boysenberry juice

Temperature (^{o}C)	Time (days)	Hyperchromic shift (Abs.)				Bathochromic shift (λ_{max})			
		Bl +0%boy	Bl +1%boy	Bl +2%boy	Bl +3%boy	Bl +0%boy	Bl +1%boy	Bl +2%boy	Bl +3%boy
5	0	1.1 ± 0.0	1.1 ± 0.0	1.3 ± 0.0	1.3 ± 0.0	500 ± 0.0	502 ± 0.7	500 ± 0.0	501 ± 0.7
	3	1.0 ± 0.0	1.2 ± 0.0	1.3 ± 0.0	1.3 ± 0.0	500 ± 0.0	502 ± 0.0	500 ± 0.0	501 ± 0.0
	7	0.9 ± 0.0	1.2 ± 0.0	1.2 ± 0.0	1.4 ± 0.0	501 ± 0.7	501 ± 0.0	502 ± 0.7	502 ± 0.0
	14	0.9 ± 0.0	1.3 ± 0.0	1.3 ± 0.0	1.4 ± 0.0	502 ± 0.0	503 ± 0.0	502 ± 0.0	503 ± 0.0
	21	0.7 ± 0.0	1.2 ± 0.0	1.3 ± 0.0	1.4 ± 0.0	501 ± 0.7	502 ± 0.0	501 ± 0.0	502 ± 0.0
	28	0.6 ± 0.0	1.2 ± 0.0	1.3 ± 0.0	1.4 ± 0.0	502 ± 0.0	502 ± 0.0	503 ± 0.0	502 ± 0.0
	35	0.5 ± 0.0	1.2 ± 0.0	1.2 ± 0.0	1.3 ± 0.0	502 ± 0.0	502 ± 0.0	503 ± 0.0	502 ± 0.0
	42	0.4 ± 0.0	1.1 ± 0.0	1.2 ± 0.0	1.3 ± 0.0	502 ± 0.0	502 ± 0.0	503 ± 0.0	502 ± 0.0
20	0	1.1 ± 0.0	1.1 ± 0.0	1.3 ± 0.0	1.3 ± 0.0	500 ± 0.0	502 ± 0.7	500 ± 0.0	501 ± 0.7
	3	0.9 ± 0.0	1.2 ± 0.0	1.3 ± 0.0	1.3 ± 0.0	501 ± 0.0	501 ± 0.0	500 ± 0.7	502 ± 0.0
	7	0.9 ± 0.0	1.0 ± 0.0	1.2 ± 0.0	1.2 ± 0.0	501 ± 0.0	501 ± 0.7	500 ± 0.0	502 ± 0.7
	14	0.8 ± 0.0	0.9 ± 0.0	1.1 ± 0.0	1.2 ± 0.0	502 ± 0.7	502 ± 0.7	501 ± 0.0	502 ± 0.0
	21	0.7 ± 0.0	0.9 ± 0.0	1.0 ± 0.0	1.1 ± 0.1	501 ± 0.0	500 ± 0.0	501 ± 0.0	502 ± 0.0
	28	0.7 ± 0.0	0.8 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	503 ± 0.7	502 ± 0.7	502 ± 0.0	501 ± 0.0
35	0	1.1 ± 0.0	1.1 ± 0.0	1.3 ± 0.0	1.3 ± 0.0	500 ± 0.0	502 ± 0.7	500 ± 0.0	501 ± 0.7
	3	0.9 ± 0.0	0.9 ± 0.0	1.2 ± 0.0	1.2 ± 0.0	501 ± 0.0	500 ± 0.0	502 ± 0.7	502 ± 0.0
	7	0.8 ± 0.0	0.9 ± 0.0	1.2 ± 0.0	1.2 ± 0.0	501 ± 0.0	501 ± 1.4	502 ± 0.0	501 ± 0.7
	14	0.7 ± 0.0	0.7 ± 0.0	1.1 ± 0.0	1.0 ± 0.0	502 ± 0.0	503 ± 0.0	502 ± 0.0	501 ± 0.0
	21	0.6 ± 0.0	0.7 ± 0.0	1.1 ± 0.0	1.0 ± 0.0	501 ± 0.0	502 ± 0.0	502 ± 0.0	501 ± 0.0
	28	0.5 ± 0.0	0.6 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	502 ± 0.0	503 ± 0.0	502 ± 0.0	501 ± 0.0

Appendix 11 Hyperchromic and bathochromic shift of cranberry juice enhanced with 0, 1, 2 and 3% (w/v) concentrations boysenberry juice at various temperatures (Study 2) * C = cranberry juice; Boy = boysenberry juice

Temperature (^{o}C)	Time (days)	Hyperchromic shift (Abs.)				Bathochromic shift (λ_{max})			
		C+0% Boy	C+1% Boy	C+2% Boy	C+3% Boy	C+0% Boy	C+1% Boy	C+2% Boy	C+3% Boy
5	0	0.6 ± 0.0	0.6 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	501 ± 0.0	501 ± 0.7	501 ± 0.0	500 ± 0.0
	3	0.6 ± 0.0	0.6 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	501 ± 0.7	500 ± 0.0	501 ± 0.0	501 ± 0.7
	7	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.7 ± 0.0	501 ± 0.0	502 ± 0.7	502 ± 0.0	501 ± 0.0
	14	0.6 ± 0.0	0.5 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	501 ± 0.0	501 ± 0.0	501 ± 0.0	502 ± 0.7
	21	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	500 ± 0.0	501 ± 0.0	502 ± 0.0	501 ± 0.0
	28	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	501 ± 0.0	502 ± 0.7	502 ± 0.0	502 ± 0.0
20	0	0.6 ± 0.0	0.6 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	501 ± 0.0	501 ± 0.7	501 ± 0.0	500 ± 0.0
	3	0.6 ± 0.0	0.6 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	500 ± 0.0	501 ± 0.0	502 ± 0.0	501 ± 0.7
	7	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.7 ± 0.0	500 ± 0.0	502 ± 0.0	502 ± 0.0	501 ± 0.0
	14	0.5 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	501 ± 0.0	502 ± 0.7	502 ± 0.7	502 ± 0.0
	21	0.4 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	501 ± 0.0	502 ± 0.7	501 ± 0.0	500 ± 0.0
	28	0.3 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	501 ± 0.0	501 ± 0.0	500 ± 0.0	502 ± 0.0
35	0	0.6 ± 0.0	0.6 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	501 ± 0.0	501 ± 0.7	501 ± 0.0	500 ± 0.0
	3	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	502 ± 0.0	501 ± 0.7	502 ± 0.7	501 ± 0.0
	7	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	501 ± 0.0	500 ± 0.0	501 ± 1.4	501 ± 0.7
	14	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	502 ± 0.7	502 ± 0.0	502 ± 0.0	502 ± 0.7
	21	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	502 ± 0.7	501 ± 0.7	501 ± 0.0	501 ± 0.0
	28	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	501 ± 0.0	500 ± 0.0	501 ± 1.4	501 ± 0.7

Appendix 12 Hyperchromic and bathochromic shift of pomegranate juice enhanced with 0, 1, 2 and 3% (w/v) concentrations boysenberry juice at various temperatures (Study 2) * P = pomegranate juice; Boy = boysenberry juice

Temperature (^{o}C)	Time (days)	Hyperchromic shift (Abs.)				Bathochromic shift (λ_{max})			
		P+0% Boy	P+1% Boy	P+2% Boy	P+3% Boy	P+0% Boy	P+1% Boy	P+2% Boy	P+3% Boy
5	0	0.4 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	501 ± 0.7	502 ± 0.0	501 ± 0.7	501 ± 0.0
	3	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	500 ± 0.0	502 ± 0.7	501 ± 0.0	501 ± 0.7
	7	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	502 ± 0.0	502 ± 0.0	502 ± 0.7	503 ± 0.7
	14	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	502 ± 0.7	501 ± 0.0	502 ± 0.0	502 ± 0.0
	21	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	501 ± 0.7	502 ± 0.7	501 ± 0.0	501 ± 0.7
	28	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	501 ± 0.0	501 ± 0.7	501 ± 0.0	500 ± 0.0
20	0	0.4 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	501 ± 0.7	502 ± 0.0	501 ± 0.7	501 ± 0.0
	3	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	502 ± 0.0	500 ± 0.0	501 ± 0.7	503 ± 0.7
	7	0.3 ± 0.0	0.3 ± 0.1	0.3 ± 0.0	0.3 ± 0.0	503 ± 0.0	502 ± 0.0	501 ± 0.0	503 ± 0.7
	14	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	503 ± 0.0	502 ± 0.7	502 ± 0.0	502 ± 0.0
	21	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	502 ± 0.0	500 ± 0.0	501 ± 0.7	503 ± 0.7
	28	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	501 ± 0.0	501 ± 0.7	501 ± 0.0	500 ± 0.0
35	0	0.4 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	501 ± 0.7	502 ± 0.0	501 ± 0.7	501 ± 0.0
	3	0.3 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	503 ± 0.0	501 ± 0.7	502 ± 0.0	502 ± 0.0
	7	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	503 ± 0.7	500 ± 0.0	503 ± 0.7	503 ± 0.0
	14	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	503 ± 0.0	501 ± 0.0	502 ± 0.0	503 ± 0.0
	21	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	503 ± 0.0	501 ± 0.7	502 ± 0.0	502 ± 0.0
	28	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	501 ± 0.0	501 ± 0.7	501 ± 0.0	500 ± 0.0

Appendix 13 Hyperchromic and bathochromic shift of blackcurrant juice enhanced with 0, 0.2, 0.6 and 1 mg/L of kaemferol, quercetin and ellagic acid at various temperatures (Study 3) * Bl = blackcurrant juice

Temp. (°C)	Time (days)	Hyperchromic shift (Abs.)				Bathochromic shift (λ_{max})			
		Bl+0mg/L Kaemferol	Bl+0.2mg/L Kaemferol	Bl+0.6mg/L Kaemferol	Bl+1mg/L Kaemferol	Bl+0mg/L Kaemferol	Bl+0.2mg/L Kaemferol	Bl+0.6mg/L Kaemferol	Bl+1mg/L Kaemferol
5	0	1.1 ± 0.0	1.0 ± 0.0	1.1 ± 0.0	1.0 ± 0.0	500 ± 0.0	502 ± 0.0	500 ±0.7	501 ± 0.0
	7	1.0 ± 0.0	0.9 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	501 ± 0.0	501 ± 0.0	501 ± 0.7	500 ± 0.0
	14	0.9 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	501 ± 0.0	502 ± 0.7	501 ± 0.0	501 ± 0.7
	21	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	500 ± 0.0	502 ± 0.0	500 ± 0.7	501 ± 0.0
	28	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	501 ± 0.0	502 ± 0.7	501 ± 0.0	501 ± 0.7
20	0	1.1 ± 0.0	1.0 ± 0.0	1.1 ± 0.0	1.0 ± 0.0	500 ± 0.0	502 ± 0.0	500 ± 0.7	501 ± 0.0
	7	0.9 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	500 ± 0.0	501 ± 0.0	501 ± 0.0	501 ± 0.7
	14	0.8 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.8 ± 0.0	501 ± 0.0	502 ± 0.0	501 ± 0.0	501 ± 0.0
	21	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	500 ± 0.0	502 ± 0.0	500 ± 0.7	501 ± 0.0
	28	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	501 ± 0.0	502 ± 0.0	501 ± 0.0	501 ± 0.0

Temp. (°C)	Time (days)	Hyperchromic shift (Abs.)				Bathochromic shift (λ_{max})			
		Bl+0mg/L Quercetin	Bl+0.2mg/L Quercetin	Bl+0.6mg/L Quercetin	Bl+1mg/L Quercetin	Bl+0mg/L Quercetin	Bl+0.2mg/L Quercetin	Bl+0.6mg/L Quercetin	Bl+1mg/L Quercetin
5	0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	501 ± 0.0	501 ± 0.0	501 ± 0.0	500 ± 0.0
	7	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	500 ± 0.0	501 ± 0.0	501 ± 0.0	501 ± 0.7
	14	0.9 ± 0.0	0.9 ± 0.0	0.8 ± 0.0	0.8 ± 0.0	501 ± 0.0	502 ± 0.0	501 ± 0.0	501 ± 0.7
	21	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	500 ± 0.7	502 ± 0.7	500 ± 0.0	501 ± 0.0
	28	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	500 ± 0.7	502 ± 0.7	500 ± 0.7	501 ± 0.0
20	0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	501 ± 0.0	501 ± 0.0	501 ± 0.0	500 ± 0.0
	7	0.9 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	501 ± 0.7	500 ± 0.7	501 ± 0.0	501 ± 0.0
	14	0.8 ± 0.0	0.8 ± 0.0	0.8 ± 0.0	0.8 ± 0.0	501 ± 0.0	502 ± 0.0	501 ± 0.0	501 ± 0.7
	21	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	500 ± 0.7	502 ± 0.7	500 ± 0.7	501 ± 0.7
	28	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	500 ± 0.0	502 ± 0.0	500 ± 0.0	501 ± 0.0

Temp. (°C)	Time (days)	Hyperchromic shift (Abs.)				Bathochromic shift (λ_{max})			
		Bl+0mg/L Ellagic acid	Bl+0.2mg/L Ellagic acid	Bl+0.6mg/L Ellagic acid	Bl+1mg/L Ellagic acid	Bl+0mg/L Ellagic acid	Bl+0.2mg/L Ellagic acid	Bl+0.6mg/L Ellagic acid	Bl+1mg/L Ellagic acid
5	0	1.1 ± 0.0	1.3 ± 0.0	1.4 ± 0.0	1.6 ± 0.0	500 ± 0.0	502 ± 0.7	500 ± 0.0	501 ± 0.7
	7	1.0 ± 0.0	1.3 ± 0.0	1.4 ± 0.0	1.6 ± 0.0	501 ± 0.7	502 ± 0.0	501 ± 0.7	501 ± 0.0
	14	0.9 ± 0.0	1.2 ± 0.0	1.4 ± 0.0	1.5 ± 0.0	501 ± 0.0	501 ± 0.7	501 ± 0.0	500 ± 0.0
	21	0.7 ± 0.0	1.1 ± 0.0	1.3 ± 0.0	1.4 ± 0.0	500 ± 0.7	502 ± 0.0	501 ± 0.7	502 ± 0.7
	28	0.6 ± 0.0	1.1 ± 0.0	1.3 ± 0.0	1.4 ± 0.0	501 ± 0.0	502 ± 0.0	501 ± 0.7	502 ± 0.7
20	0	1.1 ± 0.0	1.3 ± 0.0	1.4 ± 0.0	1.6 ± 0.0	500 ± 0.0	502 ± 0.7	500 ± 0.0	501 ± 0.7
	7	0.9 ± 0.0	1.1 ± 0.0	1.3 ± 0.0	1.4 ± 0.0	501 ± 0.0	502 ± 0.0	501 ± 0.0	501 ± 0.0
	14	0.8 ± 0.0	1.0 ± 0.0	1.2 ± 0.0	1.3 ± 0.0	501 ± 0.0	501 ± 0.7	501 ± 0.0	500 ± 0.0
	21	0.6 ± 0.0	0.8 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	500 ± 0.0	501 ± 0.0	501 ± 0.0	501 ± 0.7
	28	0.4 ± 0.0	0.8 ± 0.0	0.8 ± 0.0	0.8 ± 0.0	501 ± 0.0	502 ± 0.0	502 ± 0.7	502 ± 0.0

Appendix 14 Hyperchromic shift of blackcurrant juice enhanced with 0, 0.2, 0.6 and 1 mg/L of ellagic acid and and 0, 1, 2 and 3% (w/v) concentrations boysenberry juice at 5°C (Study 4)

Time(days)	Hyperchromic shift (Abs.)							
	Blackcurrant juice (10°Brix)							
	0 mg/L Ellagic acid				0.2 mg/L Ellagic acid			
	Boysenberry juice (%w/v)				Boysenberry juice (%w/v)			
	0%	1%	2%	3%	0%	1%	2%	3%
0	1.1 ± 0.0	1.2 ± 0.0	1.3 ± 0.0	1.5 ± 0.0	1.3 ± 0.0	1.4 ± 0.0	1.4 ± 0.0	1.4 ± 0.0
7	0.9 ± 0.0	1.1 ± 0.0	1.3 ± 0.0	1.4 ± 0.0	1.3 ± 0.0	1.3 ± 0.0	1.3 ± 0.1	1.4 ± 0.0
14	0.9 ± 0.0	1.0 ± 0.0	1.2 ± 0.0	1.4 ± 0.0	1.3 ± 0.0	1.3 ± 0.1	1.3 ± 0.0	1.4 ± 0.0
21	0.8 ± 0.0	0.9 ± 0.0	1.2 ± 0.0	1.3 ± 0.0	1.2 ± 0.0	1.2 ± 0.0	1.3 ± 0.0	1.4 ± 0.0
28	0.7 ± 0.0	0.9 ± 0.0	1.1 ± 0.0	1.3 ± 0.0	1.2 ± 0.0	1.2 ± 0.0	1.2 ± 0.0	1.3 ± 0.0
Time(days)	0.6 mg/L Ellagic acid				1 mg/L Ellagic acid			
	Boysenberry juice (%w/v)				Boysenberry juice (%w/v)			
	0%	1%	2%	3%	0%	1%	2%	3%
0	1.5 ± 0.0	1.5 ± 0.0	1.6 ± 0.0	1.6 ± 0.0	1.6 ± 0.0	1.7 ± 0.0	1.6 ± 0.1	1.7 ± 0.0
7	1.5 ± 0.0	1.5 ± 0.0	1.5 ± 0.0	1.5 ± 0.0	1.6 ± 0.0	1.6 ± 0.0	1.6 ± 0.0	1.7 ± 0.0
14	1.4 ± 0.0	1.5 ± 0.0	1.5 ± 0.0	1.5 ± 0.0	1.5 ± 0.0	1.6 ± 0.0	1.6 ± 0.0	1.7 ± 0.0
21	1.4 ± 0.0	1.4 ± 0.0	1.4 ± 0.0	1.5 ± 0.0	1.4 ± 0.0	1.5 ± 0.0	1.5 ± 0.0	1.6 ± 0.0
28	1.3 ± 0.0	1.4 ± 0.0	1.4 ± 0.0	1.4 ± 0.0	1.3 ± 0.1	1.4 ± 0.0	1.4 ± 0.0	1.5 ± 0.0

Appendix 15 Total phenolic content of blackcurrant juice, cranberry and pomegranate juice enhanced with 0, 1, 2 and 3% (w/v) concentrations boysenberry juice at various temperatures (Study 2) * Bl = Blackcurrant juice; C = cranberry juice; P = pomegranate juice; Boy = boysenberry juice

Temperature (^{o}C)	Time (days)	Total phenolic acids (mg/L)			
		Bl +0%boy	Bl +1%boy	Bl +2%boy	Bl +3%boy
5	0	264.3 ± 22.6	244.0 ± 4.8	234.8 ± 2.7	234.3 ± 4.8
	3	261.5 ± 5.4	237.2 ± 2.4	227.0 ± 6.0	235.5 ± 1.2
	7	255.5 ± 4.9	223.6 ± 5.6	220.1 ± 3.5	216.7 ± 3.5
	14	229.6 ± 8.0	202.3 ± 2.9	209.5 ± 2.9	200.7 ± 3.6
	21	212.1 ± 3.5	189.2 ± 1.4	186.7 ± 2.1	183.8 ± 3.5
	28	201.5 ± 2.7	169.3 ± 1.3	175.0 ± 2.7	169.3 ± 2.7
20	0	264.3 ± 22.6	244.0 ± 4.8	234.8 ± 2.7	234.3 ± 4.8
	3	251.7 ± 4.8	231.7 ± 3.0	234.3 ± 10.2	230.0 ± 4.2
	7	248.6 ± 2.1	220.1 ± 4.2	215.2 ± 4.2	213.8 ± 0.7
	14	217.7 ± 4.4	202.8 ± 5.1	197.6 ± 3.6	191.4 ± 8.0
	21	200.9 ± 2.8	181.3 ± 2.8	178.4 ± 1.4	177.9 ± 0.7
	28	159.4 ± 2.0	157.5 ± 2.0	159.9 ± 1.3	154.6 ± 2.0
35	0	264.3 ± 22.6	244.0 ± 4.8	234.8 ± 2.7	234.3 ± 4.8
	3	245.3 ± 5.4	226.6 ± 3.0	229.2 ± 2.1	221.5 ± 2.8
	7	239.3 ± 4.2	217.2 ± 0.7	214.2 ± 6.3	209.3 ± 4.9
	14	210.5 ± 1.5	195.1 ± 1.5	193.5 ± 6.6	188.4 ± 3.6
	21	194.5 ± 2.1	175.0 ± 0.7	170.6 ± 1.4	173.0 ± 0.7
	28	177.4 ± 3.3	150.9 ± 2.0	149.9 ± 0.7	153.2 ± 2.7
Temperature (^{o}C)	Time (days)	Total phenolic acids (mg/L)			
		C+0% Boy	C+1% Boy	C+2% Boy	C+3% Boy
5	0	145.4 ± 1.5	147.9 ± 2.1	155.6 ± 2.1	162.9 ± 2.1
	3	131.7 ± 0.8	148.1 ± 3.0	153.2 ± 3.0	168.1 ± 0.3
	7	113.2 ± 0.8	144.1 ± 1.9	145.5 ± 1.6	164.5 ± 1.9
	14	103.0 ± 3.5	124.8 ± 1.8	131.9 ± 1.8	150.5 ± 1.5
	21	90.6 ± 0.8	105.4 ± 0.6	109.9 ± 0.3	137.4 ± 2.5
	28	81.0 ± 0.6	100.7 ± 0.6	111.1 ± 1.8	130.6 ± 0.3
20	0	145.4 ± 1.5	147.9 ± 2.1	155.6 ± 2.1	162.9 ± 2.1
	3	124.7 ± 4.2	143.4 ± 1.9	146.2 ± 1.9	163.8 ± 0.8
	7	113.7 ± 1.6	132.2 ± 0.0	141.7 ± 1.6	152.8 ± 1.9
	14	104.5 ± 1.5	114.5 ± 2.1	120.8 ± 1.5	135.0 ± 3.2
	21	88.5 ± 1.1	101.6 ± 0.8	104.8 ± 0.8	110.9 ± 1.1
	28	70.1 ± 0.6	85.6 ± 0.6	86.7 ± 0.9	95.2 ± 0.6
35	0	145.4 ± 1.5	147.9 ± 2.1	155.6 ± 2.1	162.9 ± 2.1
	3	120.7 ± 1.4	140.3 ± 13.0	144.8 ± 0.0	143.7 ± 0.8
	7	101.5 ± 2.4	123.9 ±1.1	136.0 ± 0.5	146.6 ± 1.6
	14	103.5 ± 4.1	111.6 ± 2.1	117.0 ± 2.1	130.2 ± 2.4
	21	80.2 ± 1.1	90.0 ± 0.6	101.4 ± 1.1	111.7 ± 0.6
	28	60.7 ± 0.9	71.0 ± 0.6	81.2 ± 0.9	89.4 ± 1.2

Appendix 15 Total phenolic content of blackcurrant juice, cranberry and pomegranate juice enhanced with 0, 1, 2 and 3% (w/v) concentrations boysenberry juice at various temperatures (Study 2) * Bl = Blackcurrant juice; C = cranberry juice;
P = pomegranate juice; Boy = boysenberry juice (Cont.)

Temperature (°C)	Time (days)	Total phenolic acids (mg/L)			
		P+0% Boy	P+1% Boy	P+2% Boy	P+3% Boy
5	0	16.6 ± 0.3	18.2 ± 0.4	19.1 ± 0.3	19.6 ± 0.4
	3	18.2 ± 0.4	16.3 ± 0.2	21.0 ± 0.5	19.8 ± 0.2
	7	14.8 ± 0.1	15.4 ± 0.4	20.5 ± 0.4	19.2 ± 0.6
	14	13.8 ± 0.4	14.3 ± 0.4	19.5 ± 0.3	18.2 ± 0.5
	21	12.3 ± 0.2	13.6 ± 0.2	18.1 ± 0.2	18.7 ± 0.1
	28	11.1 ± 0.2	12.4 ± 0.1	17.9 ± 0.2	16.3 ± 0.3
20	0	16.6 ± 0.3	18.2 ± 0.4	19.1 ± 0.3	19.6 ± 0.4
	3	16.9 ± 0.5	15.6 ± 0.4	18.4 ± 1.9	19.8 ± 0.0
	7	10.2 ± 0.3	14.7 ± 1.3	19.3 ± 0.6	17.8 ± 0.6
	14	9.4 ± 1.0	13.2 ± 0.5	18.7 ± 0.7	16.5 ± 0.7
	21	8.4 ± 0.4	12.3 ± 0.2	17.9 ± 0.3	15.5 ± 0.2
	28	7.4 ± 0.3	11.4 ± 0.3	16.5 ± 0.2	15.8 ± 0.1
35	0	16.6 ± 0.3	18.2 ± 0.4	19.1 ± 0.3	19.6 ± 0.4
	3	15.3 ± 0.4	14.7 ± 0.2	17.6 ± 0.4	17.5 ± 0.6
	7	13.0 ± 0.4	13.6 ± 0.5	16.2 ± 0.7	14.4 ± 0.9
	14	12.2 ± 0.4	12.4 ± 0.2	15.4 ± 0.3	13.5 ± 0.5
	21	11.5 ± 0.1	12.0 ± 0.1	13.2 ± 0.2	11.9 ± 0.2
	28	10.7 ± 0.2	10.3 ± 0.1	12.4 ± 0.1	10.8 ± 0.1

Appendix 16 Total phenolic content of blackcurrant juice enhanced with 0, 0.2, 0.6 and 1 mg/L of kaemferol, quercetin and ellagic acid at various temperatures (Study 3)

* Bl = Blackcurrant juice

Temperature (^{o}C)	Time (days)	Total phenolic acids content (mg/L)			
		Bl+0mg/L Kaemferol	Bl+0.2mg/L Kaemferol	Bl+0.6mg/L Kaemferol	Bl+1mg/L Kaemferol
5	0	246.4 ± 2.7	257.1 ± 1.4	272.6 ± 2.7	282.8 ± 2.1
	7	229.2 ± 0.6	232.1 ± 1.2	241.5 ± 3.6	256.4 ± 0.6
	14	200.0 ± 1.4	217.0 ± 1.4	221.0 ± 1.4	232.4 ± 0.7
	21	193.5 ± 0.7	210.5 ± 0.7	214.5 ± 0.7	226.5 ± 0.7
	28	187.5 ± 0.7	206.0 ± 1.4	210.0 ± 1.4	222.5 ± 0.7
20	0	246.4 ± 2.7	257.1 ± 1.4	272.6 ± 2.7	282.8 ± 2.1
	7	216.0 ± 2.4	223.6 ± 1.2	243.2 ± 1.2	248.7 ± 3.0
	14	159.1 ± 1.4	180.0 ± 1.4	201.0 ± 1.4	205.5 ± 0.7
	21	154.6 ± 0.7	176.6 ± 0.7	197.0 ± 1.4	201.0 ± 1.4
	28	148.6 ± 0.7	171.1 ± 0.0	192.0 ± 1.4	196.0 ± 1.4

Temperature (^{o}C)	Time (days)	Total phenolic acids content (mg/L)			
		Bl+0mg/L Quercetin	Bl+0.2mg/L Quercetin	Bl+0.6mg/L Quercetin	Bl+1mg/L Quercetin
5	0	236.7 ± 2.7	247.4 ± 1.4	258.0 ± 1.4	271.6 ± 1.4
	7	210.0 ± 3.6	219.4 ± 4.8	241.5 ± 1.2	236.4 ± 1.2
	14	181.3 ± 1.4	199.0 ± 1.4	220.1 ± 0.7	211.8 ± 1.4
	21	178.4 ± 1.4	196.6 ± 2.1	216.2 ± 0.7	208.8 ± 1.4
	28	175.4 ± 1.4	193.6 ± 2.1	212.3 ± 0.7	206.9 ± 1.4
20	0	236.7 ± 2.7	247.4 ± 1.4	258.0 ± 1.4	271.6 ± 1.4
	7	210.0 ± 3.6	212.2 ± 3.0	231.3 ± 1.2	233.4 ± 3.0
	14	160.2 ± 0.7	180.4 ± 1.4	201.5 ± 2.1	202.0 ± 0.0
	21	154.3 ± 0.7	175.0 ± 2.1	196.6 ± 2.1	196.6 ± 0.7
	28	147.9 ± 1.4	170.0 ± 2.1	191.2 ± 2.8	192.1 ± 1.4

Temperature (^{o}C)	Time (days)	Total phenolic acids content (mg/L)			
		Bl+0mg/L Ellagic acid	Bl+0.2mg/L Ellagic acid	Bl+0.6mg/L Ellagic acid	Bl+1mg/L Ellagic acid
5	0	242.5 ± 1.4	259.0 ± 1.4	270.2 ± 0.7	279.4 ± 1.4
	7	210.9 ± 1.2	242.4 ± 1.2	252.1 ± 0.6	258.9 ± 0.6
	14	181.4 ± 0.7	220.1 ± 1.4	225.0 ± 1.4	231.3 ± 0.7
	21	178.0 ± 1.4	217.1 ± 1.4	223.0 ± 1.4	227.4 ± 0.7
	28	175.5 ± 2.1	214.7 ± 2.1	220.6 ± 2.1	224.5 ± 0.7
20	0	242.5 ± 1.4	259.0 ± 1.4	270.2 ± 0.7	279.4 ± 1.4
	7	202.4 ± 1.2	233.8 ± 1.2	243.6 ± 0.6	251.7 ± 1.2
	14	159.8 ± 0.7	169.6 ± 1.0	182.9 ± 1.4	185.8 ± 1.4
	21	154.0 ± 0.7	164.7 ± 2.1	177.0 ± 1.4	179.9 ± 1.4
	28	148.1 ± 0.7	158.9 ± 2.1	172.1 ± 1.4	174.5 ± 0.7

Appendix 17 Summary of anthocyanins profile for blackcurrant juice enhanced with 0, 1, 2 and 3% (w/v) concentrations boysenberry juice at various temperatures (Study 2) * Bl = Blackcurrant juice; Boy = boysenberry juice; Del-3-ruti = Delphinidin-3-rutinoside; cyn-3-ruti = cyanidin-3-rutinoside; del-3-glu = delphinidin-3-glucoside; cyn-3-glu = cyanidin-3-glucoside

Temperature (^{o}C)	Time (days)	Anthocyanin content (mg/L)							
		Bl+0% Boy				Bl+1% Boy			
		Del-3-ruti	Cyn-3-ruti	Del-3-glu	Cyn-3-glu	Del-3-ruti	Cyn-3-ruti	Del-3-glu	Cyn-3-glu
5	0	562.7 ± 9.1	418.8 ± 18.3	174.6 ± 1.0	104.3 ± 0.5	557.5± 8.5	449.3 ± 1.3	176.2 ± 0.7	109.6 ± 0.2
	7	534.9 ± 4.3	408.7 ± 4.7	160.3 ± 0.8	98.5 ± 2.4	540.7 ± 0.9	426.9 ± 6.0	161.5 ± 4.4	99.7 ± 1.1
	14	515.8 ± 3.2	398.5 ± 9.8	153.2 ± 2.4	92.6 ± 5.4	535.9 ± 6.5	413.9 ± 5.5	152.6 ± 2.3	79.7 ± 1.9
	21	509.9 ± 0.4	392.1 ± 7.4	141.2 ± 0.2	87.3 ± 0.3	515.0 ± 1.2	392.5 ± 3.0	145.8 ± 0.4	76.4 ± 0.7
	28	503.8 ± 0.4	382.6 ± 1.2	135.6 ± 0.4	82.8 ± 0.2	504.9 ± 1.5	386.9 ± 1.0	139.3 ± 5.1	72.2 ± 0.4
20	0	562.7 ± 9.1	418.8 ± 18.3	174.6 ± 1.0	104.3± 0.5	557.5 ± 8.5	449.3 ± 1.3	176.2 ± 0.7	109.6± 0.2
	7	511.0 ± 2.6	377.2 ± 7.7	153.1 ± 2.6	85.1 ± 2.8	514.1 ± 6.7	401.4 ± 14.6	153.8 ± 1.5	89.0 ± 3.3
	14	500.1 ± 5.0	365.3 ± 4.2	140.6 ± 1.4	74.3 ± 5.2	514.5 ± 1.4	389.4 ± 2.3	141.4 ± 1.6	81.2 ± 1.6
	21	480.2 ± 1.7	344.4 ± 5.2	124.0 ± 1.1	68.1 ± 0.4	501.2 ± 1.2	364.1 ± 0.9	131.5 ± 0.7	70.5 ± 0.5
	28	472.2 ± 1.1	328.5 ± 2.3	119.0 ± 0.7	66.5 ± 0.1	494.7 ± 0.4	343.0 ± 0.9	127.6 ± 0.7	67.4 ± 0.7
35	0	562.7 ± 9.1	418.8 ± 18.3	174.6 ± 1.0	104.3± 0.5	557.5 ± 8.5	449.3 ± 1.3	176.2 ± 0.7	109.6± 0.2
	7	407.1 ± 5.7	219.0 ± 22.6	107.3 ± 6.0	70.6 ± 1.6	430.2 ± 4.2	295.1 ± 8.5	114.7 ± 9.5	85.6 ± 2.4
	14	383.1 ± 3.9	170.8 ± 1.3	96.8 ± 0.4	62.6 ± 0.7	411.6 ± 1.0	277.1 ± 10.6	104.3 ± 3.0	76.6 ± 2.4
	21	362.0 ± 4.3	145.7 ± 3.3	78.5 ± 1.1	54.5 ± 1.3	392.8 ± 0.8	250.0 ± 4.2	94.2 ± 1.5	68.9 ± 0.4
	28	352.5 ± 1.6	124.7 ± 3.3	68.9 ± 1.1	50.2 ± 0.3	381.4 ± 4.5	231.0 ± 7.2	91.8 ± 4.9	65.5 ± 1.8

Appendix 17 Summary of anthocyanins profile for blackcurrant juice enhanced with 0, 1, 2 and 3% (w/v) concentrations boysenberry juice at various temperatures (Study 2) * Bl = Blackcurrant juice; Boy = boysenberry juice; Del-3-ruti = Delphinidin-3-rutinoside; cyn-3-ruti = cyanidin-3-rutinoside; del-3-glu = delphinidin-3-glucoside; cyn-3-glu = cyanidin-3-glucoside (Cont.)

Temperature (^{o}C)	Time (days)	Anthocyanin content (mg/L)							
		Bl+2% Boy				Bl+3% Boy			
		Del-3-ruti	Cyn-3-ruti	Del-3-glu	Cyn-3-glu	Del-3-ruti	Cyn-3-ruti	Del-3-glu	Cyn-3-glu
5	0	551.6 ± 6.3	468.3 ± 7.8	175.2 ± 2.4	117.7 ± 1.1	553.2 ± 6.1	479.3 ± 34.8	177.7 ± 0.1	128.8 ± 3.5
	7	542.9 ± 2.8	457.8 ± 7.1	167.9 ± 1.1	113.9 ± 6.5	550.8 ± 4.4	468.4 ± 10.4	173.6 ± 2.5	118.0 ± 0.4
	14	532.3 ± 3.4	441.8 ± 5.8	150.1 ± 2.8	100.1 ± 1.6	550.8 ± 4.4	468.4 ± 10.4	173.6 ± 2.5	118.0 ± 0.4
	21	521.6 ± 0.0	414.3 ± 2.3	134.7 ± 0.3	90.2 ± 0.1	537.3 ± 3.1	451.5 ± 4.6	160.8 ± 1.9	109.1 ± 0.4
	28	510.6 ± 0.7	396.6 ± 5.8	127.9 ± 2.1	83.6 ± 1.5	522.5 ± 2.8	438.8 ± 4.6	155.8 ± 1.1	104.6 ± 0.4
20	0	551.6 ± 6.3	468.3 ± 7.8	175.2 ± 2.4	117.7± 1.1	553.2 ± 6.1	479.3 ± 34.8	177.7 ± 0.1	128.8 ± 3.5
	7	522.3 ± 3.9	415.5 ± 7.6	155.8 ± 4.5	98.1 ± 3.3	532.1 ± 11.1	445.8 ± 12.1	164.7 ± 4.2	111.7 ± 3.2
	14	510.4 ± 1.1	399.8 ± 5.7	135.3 ± 0.0	85.6 ± 2.7	521.9 ± 2.6	423.7 ± 4.3	148.2 ± 0.5	94.5 ± 1.9
	21	499.3 ± 1.6	366.2 ± 3.1	129.9 ± 1.1	70.0 ± 0.2	507.2 ± 1.2	398.7 ± 6.3	132.6 ± 1.7	80.2 ± 0.6
	28	490.9 ± 0.5	347.3 ± 2.9	122.0 ± 0.7	66.4 ± 0.5	481.4 ± 13.9	335.4 ± 6.3	106.1 ± 5.1	68.1 ± 0.1
35	0	551.6 ± 6.3	468.3 ± 7.8	175.2 ± 2.4	117.7± 1.1	553.2 ± 6.1	479.3 ± 34.8	177.7 ± 0.1	128.8 ± 3.5
	7	460.9 ± 3.9	320.1 ± 13.0	139.4 ± 1.8	89.2 ± 2.9	495.4 ± 34.1	404.0 ± 18.1	148.6 ± 5.1	96.1 ± 6.0
	14	430.4 ± 4.2	294.2 ± 4.2	113.1 ± 1.7	80.4 ± 3.4	473.4 ± 3.0	381.1 ± 5.4	123.9 ± 3.9	84.2 ± 0.5
	21	410.3 ± 1.7	277.5 ± 6.1	103.1 ± 1.5	69.2 ± 1.0	463.3 ± 1.8	357.4 ± 5.2	115.4 ± 2.0	72.4 ± 1.3
	28	402.7 ± 1.7	256.4 ± 6.1	98.3 ± 5.3	68.3 ± 0.3	456.5 ± 0.7	336.4 ± 5.2	103.3 ± 1.4	67.3 ± 0.4

Appendix 18 Summary of anthocyanins profile for cranberry juice enhanced with 0, 1, 2 and 3% (w/v) concentrations boysenberry juice at various temperatures (Study 2) * C = cranberry juice; Boy = boysenberry juice; Pn-3-gal = peonidin-3-galactoside; cyn-3-gal = cyanidin-3-galactoside; cyn-3-ara = cyanidin-3-arabinoside; pn-3-ara = peonidin-3-arabinoside

Temperature (^{o}C)	Time (days)	Anthocyanin content (mg/L)							
		C+0% Boy				C+1% Boy			
		Pn-3-gal	Cyn-3-gal	Cyn-3-ara	Pn-3-ara	Pn-3-gal	Cyn-3-gal	Cyn-3-ara	Pn-3-ara
5	0	119.3 ± 1.0	86.2 ± 0.2	43.4 ± 0.2	34.71 ± 0.2	118.2 ± 0.4	89.2 ± 5.6	44.4 ± 1.2	34.93 ± 0.5
	7	98.4 ± 2.8	78.1 ± 0.3	39.5 ± 0.3	32.97 ± 0.8	105.5 ± 1.7	83.2 ± 2.9	42.9 ± 0.3	33.84 ± 0.8
	14	89.8 ± 0.5	70.2 ± 0.6	40.5 ± 0.4	30.57 ± 0.5	101.6 ± 1.5	71.1 ± 0.4	41.0 ± 0.6	31.22 ± 0.4
	21	80.5 ± 0.3	63.1 ± 0.2	36.2 ± 0.0	29.26 ± 0.1	85.2 ± 1.6	63.0 ± 0.3	37.0 ± 0.2	29.48 ± 0.2
	28	56.8 ± 0.3	32.1 ± 0.2	77.9 ± 0.4	28.17 ± 0.2	57.0 ± 1.6	32.2 ± 1.4	78.1 ± 0.5	28.39 ± 0.5
20	0	119.3 ± 1.0	86.2 ± 0.2	43.4 ± 0.2	34.71 ± 0.2	118.2 ± 0.4	89.2 ± 5.6	44.4 ± 1.2	34.93 ± 0.5
	7	89.8 ± 0.1	69.7 ± 0.8	39.1 ± 0.3	30.35 ± 0.2	94.2 ± 3.9	79.3 ± 2.6	41.7 ± 1.4	30.01 ± 0.1
	14	80.4 ± 0.1	62.1 ± 0.6	31.2 ± 0.9	28.39 ± 0.1	80.9 ± 0.3	76.1 ± 0.9	40.8 ± 0.4	29.26 ± 0.5
	21	72.9 ± 0.7	54.3 ± 0.7	26.4 ± 0.2	25.99 ± 0.2	70.2 ± 0.1	69.6 ± 0.4	34.9 ± 0.4	27.52 ± 0.5
	28	64.5 ± 0.8	45.6 ± 0.4	22.0 ± 0.2	24.25 ± 0.2	58.9 ± 0.6	62.8 ± 1.3	30.5 ± 0.5	25.12 ± 0.2
35	0	119.3 ± 1.0	86.2 ± 0.2	43.4 ± 0.2	34.71 ± 0.2	118.2 ± 0.4	89.2 ± 5.6	44.4 ± 0.5	34.93 ± 0.5
	7	80.5 ± 0.2	65.3 ± 0.3	35.2 ± 0.6	28.39 ± 0.5	84.6 ± 1.5	72.0 ± 0.9	39.0 ± 1.2	29.92 ± 0.4
	14	71.3 ± 2.5	55.7 ± 2.0	30.8 ± 0.7	26.21 ± 0.1	75.5 ± 1.0	64.8 ± 0.9	30.5 ± 0.1	27.30 ± 0.2
	21	44.3 ± 0.5	21.6 ± 0.8	65.2 ± 0.8	24.03 ± 0.1	56.0 ± 0.9	23.4 ± 0.8	75.7 ± 0.4	24.25 ± 0.2
	28	53.3 ± 1.0	36.4 ± 0.8	17.0 ± 0.5	21.41 ± 0.1	57.2 ± 0.1	48.0 ± 0.8	19.1 ± 0.3	22.07 ± 0.2

Appendix 18 Summary of anthocyanins profile for cranberry juice enhanced with 0, 1, 2 and 3% (w/v) concentrations boysenberry juice at various temperatures (Study 2) * C = cranberry juice; Boy = boysenberry juice; Pn-3-gal = peonidin-3-galactoside; cyn-3-gal = cyanidin-3-galactoside; cyn-3-ara = cyanidin-3-arabinoside; pn-3-ara = peonidin-3-arabinoside (Cont.)

Temperature (^{o}C)	Time (days)	Anthocyanin content (mg/L)							
		C+2% Boy				C+3% Boy			
		Pn-3-gal	Cyn-3-gal	Cyn-3-ara	Pn-3-ara	Pn-3-gal	Cyn-3-gal	Cyn-3-ara	Pn-3-ara
5	0	117.6 ± 0.3	92.0 ± 7.1	44.3 ± 0.9	35.37 ± 0.1	118.3 ± 0.9	98.2 ± 1.5	43.7 ± 0.1	35.15 ± 0.2
	7	107.4 ± 4.2	89.3 ± 2.4	43.6 ± 0.0	34.28 ± 0.4	113.1 ± 0.2	90.8 ± 0.8	43.2 ± 0.0	34.06 ± 0.7
	14	101.8 ± 3.4	80.7 ± 0.7	42.2 ± 0.7	32.31 ± 0.1	106.1 ± 0.8	84.0 ± 0.6	39.2 ± 0.1	31.01 ± 0.1
	21	87.9 ± 2.2	73.7 ± 0.2	35.8 ± 0.1	30.35 ± 0.4	94.0 ± 2.6	75.2 ± 0.3	36.5 ± 0.4	30.57 ± 0.1
	28	64.9 ± 1.5	33.6 ± 0.9	176.6 ± 0.5	29.48 ± 0.2	80.5 ± 1.1	67.3 ± 0.3	32.1 ± 0.4	28.82 ± 0.1
20	0	117.6 ± 0.3	92.0 ± 7.1	44.3 ± 0.9	35.37 ± 0.1	118.3 ± 0.9	98.2 ± 1.5	43.7 ± 0.1	35.15 ± 0.2
	7	100.1 ± 1.0	82.4 ± 1.7	43.6 ± 0.0	31.22 ± 0.2	106.8 ± 2.2	88.4 ± 1.8	42.9 ± 0.4	31.44 ± 0.1
	14	94.5 ± 2.3	76.6 ± 0.6	43.2 ± 0.3	28.82 ± 0.1	100.9 ± 1.6	81.5 ± 2.8	39.8 ± 1.2	29.04 ± 0.2
	21	84.7 ± 1.4	71.1 ± 1.0	35.8 ± 1.2	27.30 ± 0.2	93.5 ± 1.9	74.1 ± 0.2	33.6 ± 0.1	26.64 ± 0.5
	28	78.8 ± 0.7	63.1 ± 1.0	30.6 ± 0.0	24.46 ± 0.5	81.3 ± 0.5	67.7 ± 0.2	29.5 ± 0.2	24.68 ± 0.2
35	0	117.6 ± 0.3	92.0 ± 7.1	44.3 ± 0.9	35.37 ± 0.1	118.3 ± 0.9	98.2 ± 1.5	43.7 ± 0.1	35.15 ± 0.2
	7	90.5 ± 1.2	78.2 ± 1.8	40.7 ± 0.4	28.82 ± 0.1	99.4 ± 2.0	82.7 ± 0.5	41.0 ± 0.6	29.04 ± 0.2
	14	86.9 ± 2.6	70.3 ± 1.6	35.6 ± 1.5	27.08 ± 0.1	95.1 ± 2.4	74.6 ± 2.0	35.8 ± 0.6	26.43 ± 0.4
	21	62.0 ± 0.7	28.4 ± 1.5	80.9 ± 0.5	25.12 ± 0.2	64.0 ± 0.9	28.6 ± 0.7	72.9 ± 1.5	24.46 ± 0.5
	28	68.0 ± 1.2	53.6 ± 0.9	23.2 ± 0.8	21.19 ± 0.4	73.6 ± 0.9	56.0 ± 0.7	23.6 ± 0.5	20.54 ± 0.5

Appendix 19 Summary of anthocyanins profile for pomegranate juice enhanced with 0, 1, 2 and 3% (w/v) concentrations boysenberry juice at various temperatures (Study 2) * P = pomegranate juice; Boy = boysenberry juice; cyn-3-glu = cyanidin-3-glucoside; Dp-3, 5-diglu = Delphinidin-3, 5- diglucoside; Pg-3-glu = Pelargonidin-3-glucoside; Dp-3-glu = Delphinidin-3-glucoside

Temperature (°C)	Time (days)	Anthocyanin content (mg/L)							
		P+0% Boy				P+1% Boy			
		Cyn-3-glu	Dp-3,5-diglu	Pg-3-glu	Dp-3-glu	Cyn-3-glu	Dp-3,5-diglu	Pg-3-glu	Dp-3-glu
5	0	30.5 ± 0.1	21.5 ± 0.4	14.3 ± 0.4	10.6 ± 2.0	30.6 ± 0.1	22.5 ± 0.1	14.1 ± 0.1	10.3 ± 0.8
	7	28.6 ± 0.1	17.0 ± 0.0	11.2 ± 0.6	8.4 ± 2.6	29.5 ± 0.5	20.1 ± 0.1	12.0 ± 0.2	9.4 ± 0.9
	14	28.0 ± 0.2	15.7 ± 0.1	10.4 ± 0.2	7.7 ± 0.8	27.0 ± 0.1	19.2 ± 0.8	11.7 ± 0.1	7.9 ± 0.6
	21	26.4 ± 0.1	13.6 ± 0.1	8.8 ± 0.2	5.9 ± 0.6	26.0 ± 0.0	17.5 ± 0.2	11.3 ± 1.1	5.9 ± 0.4
	28	25.5 ± 0.1	11.5 ± 0.1	6.8 ± 0.2	4.4 ± 0.1	24.2 ± 0.1	13.5 ± 0.5	8.7 ± 0.4	4.2 ± 0.0
20	0	30.5 ± 0.1	21.5 ± 0.4	14.3 ± 0.4	10.6 ± 2.0	30.6 ± 0.1	22.5 ± 0.1	14.1 ± 0.1	10.3 ± 0.8
	7	26.8 ± 0.0	14.8 ± 0.9	7.3 ± 0.1	5.4 ± 0.4	28.3 ± 0.0	16.3 ± 0.2	9.3 ± 1.6	6.8 ± 0.5
	14	25.9 ± 0.0	13.2 ± 0.2	6.3 ± 0.0	5.4 ± 0.2	27.8 ± 0.0	15.3 ± 0.0	8.0 ± 0.1	5.0 ± 0.1
	21	24.5 ± 0.1	11.2 ± 0.7	4.8 ± 0.1	4.3 ± 0.8	25.8 ± 0.0	11.6 ± 0.3	6.3 ± 0.1	4.3 ± 0.5
	28	24.0 ± 0.1	10.4 ± 0.3	3.7 ± 0.1	3.8 ± 0.1	24.0 ± 0.0	8.9 ± 0.3	5.3 ± 0.1	4.1 ± 0.0
35	0	30.5 ± 0.1	21.5 ± 0.4	14.3 ± 0.4	10.6 ± 2.0	30.6 ± 0.1	22.5 ± 0.1	14.1 ± 0.1	10.3 ± 0.8
	7	26.1 ± 0.0	13.4 ± 0.4	6.6 ± 0.2	5.2 ± 0.1	27.8 ± 0.0	15.3 ± 0.1	8.3 ± 0.1	6.3 ± 0.3
	14	24.9 ± 0.1	12.3 ± 0.5	6.4 ± 0.1	5.0 ± 0.1	26.8 ± 0.1	14.0 ± 0.3	7.4 ± 0.0	4.9 ± 0.0
	21	24.1 ± 0.0	9.2 ± 1.4	4.6 ± 0.4	3.6 ± 0.6	24.2 ± 0.0	11.3 ± 0.9	6.0 ± 0.2	4.0 ± 0.3
	28	24.0 ± 0.0	7.6 ± 0.9	3.5 ± 0.4	3.1 ± 0.0	24.0 ± 0.0	8.3 ± 0.3	4.9 ± 0.2	3.3 ± 0.4

Appendix 19 Summary of anthocyanins profile for pomegranate juice enhanced with 0, 1, 2 and 3% (w/v) concentrations boysenberry juice at various temperatures (Study 2) * P = pomegranate juice; Boy = boysenberry juice; cyn-3-glu = cyanidin-3-glucoside; Dp-3, 5-diglu = Delphinidin-3, 5- diglucoside; Pg-3-glu = Pelargonidin-3-glucoside; Dp-3-glu = Delphinidin-3-glucoside (Cont.)

Temperature (^{o}C)	Time (days)	Anthocyanin content (mg/L)							
		P+2% Boy				P+3% Boy			
		Cyn-3-glu	Dp-3,5-diglu	Pg-3-glu	Dp-3-glu	Cyn-3-glu	Dp-3,5-diglu	Pg-3-glu	Dp-3-glu
5	0	30.6 ± 0.0	22.2 ± 0.1	14.4 ± 0.3	8.9 ± 0.3	30.5 ± 0.1	22.1 ± 0.3	14.5 ± 0.4	11.3 ± 1.3
	7	30.0 ± 0.0	21.8 ± 0.1	13.5 ± 0.2	8.4 ± 0.1	30.4 ± 0.1	22.1 ± 0.3	14.5 ± 0.4	11.3 ± 1.3
	14	28.4 ± 0.3	20.9 ± 0.7	12.3 ± 0.6	7.7 ± 1.1	28.3 ± 0.0	21.0 ± 1.1	13.1 ± 0.1	9.5 ± 0.8
	21	26.9 ± 0.2	18.0 ± 1.4	10.8 ± 0.3	6.2 ± 0.8	27.3 ± 0.0	18.3 ± 0.6	12.0 ± 0.3	7.6 ± 0.4
	28	25.2 ± 0.1	16.7 ± 0.5	7.2 ± 0.3	4.5 ± 0.2	26.0 ± 0.0	17.2 ± 0.9	10.4 ± 0.4	5.6 ± 0.4
20	0	30.6 ± 0.0	22.2 ± 0.1	14.4 ± 0.3	8.9 ± 0.3	30.5 ± 0.1	22.1 ± 0.3	14.5 ± 0.4	11.3 ± 1.3
	7	29.4 ± 1.4	18.1 ± 0.2	9.1 ± 0.6	7.0 ± 0.4	29.1 ± 0.1	19.8 ± 1.2	9.0 ± 0.4	8.3 ± 1.7
	14	28.5 ± 0.3	17.7 ± 0.3	8.4 ± 0.1	6.1 ± 0.3	28.5 ± 0.3	17.9 ± 0.7	8.5 ± 0.2	7.2 ± 0.3
	21	26.2 ± 0.1	14.7 ± 0.5	5.6 ± 0.4	4.3 ± 0.3	26.2 ± 0.1	15.2 ± 0.9	6.1 ± 0.1	5.7 ± 0.2
	28	25.0 ± 0.0	9.4 ± 0.5	4.7 ± 0.3	4.0 ± 0.0	9.9 ± 0.0	9.9 ± 0.9	5.0 ± 0.1	5.2 ± 0.3
35	0	30.6 ± 0.0	22.2 ± 0.1	14.4 ± 0.3	8.9 ± 0.3	30.5 ± 0.1	22.1 ± 0.3	14.5 ± 0.4	11.3 ± 1.3
	7	26.3 ± 2.9	18.0 ± 0.6	8.8 ± 0.5	6.6 ± 0.5	28.6 ± 0.4	16.9 ± 0.1	8.7 ± 0.3	8.1 ± 1.5
	14	26.8 ± 0.0	15.0 ± 0.2	7.0 ± 0.6	5.6 ± 0.2	26.8 ± 0.0	14.9 ± 0.3	7.1 ± 0.4	6.7 ± 0.7
	21	24.2 ± 0.0	13.2 ± 0.7	6.0 ± 0.2	4.6 ± 0.3	24.2 ± 0.0	12.1 ± 0.1	6.5 ± 0.1	6.0 ± 0.3
	28	24.0 ± 0.0	8.6 ± 0.5	4.9 ± 0.2	3.8 ± 0.3	24.0 ± 0.0	10.0 ± 0.1	5.4 ± 0.1	4.8 ± 0.4

Appendix 20 Summary of anthocyanins profile for blackcurrant juice enhanced with 0, 0.2, 0.6 and 1 mg/L of kaemferol at various temperatures (Study 3) * Bl = Blackcurrant juice; Del-3-ruti = Delphinidin-3-rutinoside; cyn-3-ruti = cyanidin-3-rutinoside; del-3-glu = delphinidin-3-glucoside; cyn-3-glu = cyanidin-3-glucoside

Temperature (^oC)	Time (days)	Anthocyanin content (mg/L)							
		Bl+0mg/L kaemferol				Bl+0.2mg/L kaemferol			
		Del-3-ruti	Cyn-3-ruti	Del-3-glu	Cyn-3-glu	Del-3-ruti	Cyn-3-ruti	Del-3-glu	Cyn-3-glu
5	0	539.9 ± 9.1	387.2 ± 3.4	172.2 ± 2.4	99.8 ± 0.5	532.6 ± 0.9	384.0 ± 7.8	172.8 ± 1.0	97.4 ± 2.1
	14	505.8 ± 3.8	323.9 ± 3.4	150.5 ± 1.0	83.4 ± 1.1	498.5 ± 6.3	320.8 ± 7.8	147.5 ± 3.4	84.0 ± 2.1
	21	488.0 ± 0.0	305.2 ± 0.0	141.6 ± 0.0	78.1 ± 0.0	486.4 ± 0.0	294.2 ± 0.0	140.2 ± 0.0	77.8 ± 0.0
	28	480.4 ± 0.0	284.2 ± 0.0	136.8 ± 0.0	73.6 ± 0.0	478.9 ± 0.0	273.1 ± 0.0	135.4 ± 0.0	73.3 ± 0.0
20	0	539.9 ± 9.1	387.2 ± 3.4	172.2 ± 2.4	99.8 ± 0.5	532.6 ± 0.9	384.0 ± 7.8	172.8 ± 1.0	97.4 ± 2.1
	14	498.2 ± 3.8	302.9 ± 3.4	145.7 ± 1.0	77.5 ± 0.9	494.7 ± 11.7	299.7 ± 7.8	142.6 ± 3.4	75.0 ± 2.1
	21	472.8 ± 0.0	305.2 ± 0.0	124.7 ± 3.4	68.9 ± 0.3	463.7 ± 0.0	273.1 ± 0.0	135.4 ± 0.0	71.9 ± 0.0
	28	463.3 ± 0.5	263.1 ± 0.0	117.5 ± 0.0	68.7 ± 0.0	458.0 ± 7.0	252.1 ± 0.0	130.6 ± 0.0	69.7 ± 0.0
Temperature (^oC)	Time (days)	Bl+0.6mg/L kaemferol				Bl+1mg/L kaemferol			
		Del-3-ruti	Cyn-3-ruti	Del-3-glu	Cyn-3-glu	Del-3-ruti	Cyn-3-ruti	Del-3-glu	Cyn-3-glu
5	0	534.7 ± 2.2	386.1 ± 1.3	173.8 ± 4.1	100.6 ± 0.2	534.5 ± 1.7	387.2 ± 3.3	172.8 ± 1.0	97.4 ± 2.1
	14	508.2 ± 7.6	322.9 ± 1.3	154.5 ± 2.7	82.9 ± 0.2	508.0 ± 7.1	324.0 ± 3.3	155.9 ± 4.4	81.7 ± 5.3
	21	495.2 ± 0.0	300.9 ± 0.0	149.2 ± 3.4	76.0 ± 3.2	497.8 ± 0.0	300.5 ± 0.0	144.6 ± 0.0	74.2 ± 3.2
	28	480.0 ± 0.0	258.7 ± 0.0	146.8 ± 0.0	69.3 ± 0.0	490.2 ± 0.0	258.4 ± 0.0	139.7 ± 0.0	71.9 ± 0.0
20	0	534.7 ± 2.2	386.1 ± 1.3	173.8 ± 4.1	100.6 ± 0.2	534.5 ± 1.7	387.2 ± 3.3	172.8 ± 1.0	97.4 ± 2.1
	14	493.0 ± 3.1	301.8 ± 1.3	147.3 ± 0.7	78.1 ± 0.2	500.4 ± 7.1	302.9 ± 3.3	153.5 ± 1.0	79.5 ± 2.1
	21	487.6 ± 0.0	279.8 ± 0.0	142.0 ± 0.0	69.3 ± 0.0	482.6 ± 0.0	268.9 ± 14.9	147.0 ± 3.4	71.9 ± 0.0
	28	464.9 ± 0.0	237.7 ± 0.0	127.5 ± 0.0	68.4 ± 0.0	459.9 ± 0.0	258.4 ± 0.0	139.7 ± 0.0	68.8 ± 0.0

Appendix 21 Summary of anthocyanins profile for blackcurrant juice enhanced with 0, 0.2, 0.6 and 1 mg/L of quercetin at various temperatures (Study 3) * Bl = Blackcurrant juice; Del-3-ruti = Delphinidin-3-rutinoside; cyn-3-ruti = cyanidin-3-rutinoside; del-3-glu = delphinidin-3-glucoside; cyn-3-glu = cyanidin-3-glucoside

Temperature (^{o}C)	Time (days)	Anthocyanin content (mg/L)							
		Bl+0mg/L Quercetin				Bl+0.2mg/L Quercetin			
		Del-3-ruti	Cyn-3-ruti	Del-3-glu	Cyn-3-glu	Del-3-ruti	Cyn-3-ruti	Del-3-glu	Cyn-3-glu
5	0	536.2 ± 3.8	377.7 ± 4.1	166.2 ± 2.7	98.9 ± 0.7	526.6 ± 1.2	370.3 ± 6.3	168.9 ± 0.4	97.2 ± 1.7
	14	505.8 ± 3.8	335.5 ± 4.1	151.7 ± 2.7	84.5 ± 0.5	511.4 ± 1.2	338.7 ± 8.6	149.6 ± 0.3	83.7 ± 1.8
	21	487.2 ± 1.1	290.5 ± 0.0	142.6 ± 3.4	75.9 ± 0.0	484.8 ± 4.2	296.6 ± 8.6	140.0 ± 0.4	77.0 ± 1.4
	28	465.2 ± 0.0	248.3 ± 0.0	125.7 ± 0.0	66.9 ± 0.0	467.1 ± 0.5	228.9 ± 14.9	134.9 ± 0.0	67.0 ± 0.0
20	0	536.2 ± 3.8	377.7 ± 4.1	166.2 ± 2.7	98.9 ± 0.7	526.6 ± 1.2	370.3 ± 6.3	168.9 ± 0.4	97.2 ± 1.7
	14	498.2 ± 3.8	314.5 ± 4.1	142.1 ± 2.7	73.5 ± 0.2	496.2 ± 1.2	317.6 ± 8.6	142.4 ± 3.1	74.7 ± 1.7
	21	457.6 ± 0.0	206.2 ± 0.0	130.5 ± 0.0	68.7 ± 0.0	478.1 ± 5.4	250.0 ± 14.9	134.9 ± 0.0	67.0 ± 0.0
	28	442.4 ± 0.0	185.1 ± 0.0	116.0 ± 0.0	65.5 ± 0.0	425.0 ± 16.1	218.4 ± 29.8	130.1 ± 6.8	66.3 ± 1.0
Temperature (^{o}C)	Time (days)	Bl+0.6mg/L Quercetin				Bl+1mg/L Quercetin			
		Del-3-ruti	Cyn-3-ruti	Del-3-glu	Cyn-3-glu	Del-3-ruti	Cyn-3-ruti	Del-3-glu	Cyn-3-glu
5	0	529.8 ± 1.5	369.2 ± 4.7	171.4 ± 0.7	98.4 ± 3.0	530.7 ± 3.6	375.6 ± 7.8	170.4 ± 2.4	95.2 ± 1.1
	14	514.6 ± 1.5	337.6 ± 10.2	152.1 ± 0.7	82.6 ± 0.2	511.8 ± 1.7	333.4 ± 7.8	153.5 ± 1.0	81.7 ± 1.1
	21	492.9 ± 0.0	288.3 ± 0.0	137.2 ± 0.0	69.3 ± 0.0	480.7 ± 2.7	285.8 ± 0.0	142.6 ± 2.7	71.2 ± 1.0
	28	477.8 ± 0.0	225.0 ± 0.0	127.5 ± 0.0	65.2 ± 0.0	467.5 ± 0.0	243.6 ± 0.0	130.1 ± 0.0	67.4 ± 0.0
20	0	529.8 ± 1.5	369.2 ± 4.7	171.4 ± 0.7	98.4 ± 3.0	530.7 ± 3.6	375.6 ± 7.8	170.4 ± 2.4	95.2 ± 1.1
	14	499.4 ± 1.5	327.1 ± 4.7	142.5 ± 0.7	73.7 ± 0.2	496.6 ± 1.7	333.4 ± 7.8	143.9 ± 1.0	75.0 ± 2.1
	21	485.4 ± 0.0	225.0 ± 0.0	132.3 ± 0.0	67.5 ± 0.0	471.3 ± 5.4	243.6 ± 0.0	139.7 ± 0.0	67.2 ± 0.3
	28	447.4 ± 0.0	182.9 ± 0.0	122.7 ± 0.0	66.1 ± 0.0	429.5 ± 10.7	201.5 ± 0.0	134.9 ± 0.0	67.4 ± 0.0

Appendix 22 Summary of anthocyanins profile for blackcurrant juice enhanced with 0, 0.2, 0.6 and 1 mg/L of ellagic acid at various temperatures (Study 3) * Bl = Blackcurrant juice; Del-3-ruti = Delphinidin-3-rutinoside; cyn-3-ruti = cyanidin-3-rutinoside; del-3-glu = delphinidin-3-glucoside; cyn-3-glu = cyanidin-3-glucoside

Temperature (^{o}C)	Time (days)	Anthocyanin content (mg/L)							
		Bl+0mg/L Ellagic acid				Bl+0.2mg/L Ellagic acid			
		Del-3-ruti	Cyn-3-ruti	Del-3-glu	Cyn-3-glu	Del-3-ruti	Cyn-3-ruti	Del-3-glu	Cyn-3-glu
5	0	528.6 ± 3.8	375.6 ± 7.1	167.4 ± 1.0	97.5 ± 0.7	525.8 ± 0.1	376.6 ± 2.6	167.0 ± 0.3	98.6 ± 0.2
	14	505.8 ± 3.8	322.9 ± 7.8	152.9 ± 1.0	84.0 ± 0.7	510.6 ± 0.1	345.0 ± 17.6	162.2 ± 0.3	94.1 ± 0.2
	21	495.5 ± 0.0	307.4 ± 0.0	147.4 ± 0.0	79.0 ± 0.0	503.1 ± 0.0	336.4 ± 0.0	157.6 ± 0.0	89.5 ± 0.0
	28	488.0 ± 0.0	286.3 ± 0.0	132.9 ± 0.0	70.0 ± 0.0	495.5 ± 0.0	304.8 ± 14.9	128.7 ± 0.0	80.5 ± 0.0
20	0	528.6 ± 3.8	375.6 ± 7.1	167.4 ± 1.0	97.5 ± 0.7	525.8 ± 0.1	376.6 ± 2.6	167.0 ± 0.3	98.6 ± 0.2
	14	494.4 ± 3.8	312.4 ± 7.1	150.5 ± 4.4	79.5 ± 7.1	506.8 ± 5.2	334.5 ± 2.6	154.9 ± 9.9	91.8 ± 3.3
	21	479.3 ± 0.0	280.7 ± 7.8	138.5 ± 1.0	70.5 ± 0.7	484.1 ± 5.5	292.4 ± 2.7	133.2 ± 6.5	82.8 ± 16.1
	28	453.8 ± 0.0	254.7 ± 14.9	120.9 ± 3.4	65.8 ± 0.3	469.0 ± 5.4	262.6 ± 14.9	116.6 ± 3.4	68.3 ± 0.0
Temperature (^{o}C)	Time (days)	Bl+0.6mg/L Ellagic acid				Bl+1mg/L Ellagic acid			
		Del-3-ruti	Cyn-3-ruti	Del-3-glu	Cyn-3-glu	Del-3-ruti	Cyn-3-ruti	Del-3-glu	Cyn-3-glu
5	0	529.8 ± 1.5	375.6 ± 4.3	166.6 ± 0.7	99.3 ± 1.7	527.3 ± 2.3	375.6 ± 7.8	172.8 ± 1.0	96.3 ± 0.5
	14	514.6 ± 1.5	360.8 ± 4.7	161.7 ± 0.7	94.8 ± 1.7	519.7 ± 8.5	365.1 ± 22.7	170.4 ± 2.4	91.8 ± 5.9
	21	511.9 ± 5.4	343.0 ± 0.0	158.9 ± 3.4	86.8 ± 3.2	515.9 ± 13.8	354.5 ± 37.6	168.0 ± 5.8	89.6 ± 9.1
	28	492.9 ± 0.0	311.4 ± 14.9	137.2 ± 0.0	80.1 ± 0.0	498.6 ± 0.0	349.0 ± 29.8	154.2 ± 0.0	74.2 ± 0.0
20	0	529.8 ± 1.5	375.6 ± 4.3	166.6 ± 0.7	99.3 ± 1.7	527.3 ± 2.3	375.6 ± 7.8	172.8 ± 1.0	96.3 ± 0.5
	14	499.4 ± 1.5	297.6 ± 4.7	154.5 ± 4.1	88.0 ± 1.4	493.2 ± 7.6	301.8 ± 7.1	165.5 ± 2.4	80.6 ± 2.7
	21	495.7 ± 3.8	276.5 ± 25.1	149.7 ± 4.1	85.8 ± 1.8	485.5 ± 7.7	264.7 ± 0.0	147.0 ± 3.4	74.2 ± 0.0
	28	489.1 ± 5.4	248.2 ± 14.9	142.0 ± 0.0	75.6 ± 0.0	483.4 ± 0.0	243.6 ± 0.0	134.9 ± 0.0	69.7 ± 0.0

Appendix 23 Summary of anthocyanins profile for blackcurrant juice enhanced with 0, 0.2, 0.6 and 1 mg/L of ellagic acid and 0, 1, 2 and 3%(w/v) concentrations boysenberry juice at 5^{o}C (Study 4) * Del-3-ruti = Delphinidin-3-rutinoside; cyn-3-ruti = cyanidin-3-rutinoside; del-3-glu = delphinidin-3-glucoside; cyn-3-glu = cyanidin-3-glucoside

Time(days)	Anthocyanin content (mg/L)							
	Blackcurrant juice (10^{o}Brix)							
	0 mg/L Ellagic acid							
	0%(w/v) Boysenberry juice				1%(w/v) Boysenberry juice			
	Del-3-ruti	cyn-3-ruti	Del-3-glu	cyn-3-glu	Del-3-ruti	cyn-3-ruti	Del-3-glu	cyn-3-glu
0	517.6 ± 1.0	359.8 ± 14.5	162.6 ± 1.0	92.3 ± 1.7	527.1 ± 2.2	417.7 ± 16.2	169.0 ± 4.1	120.9 ± 3.0
28	434.1 ± 9.7	338.7 ± 45.1	128.8 ± 7.8	73.0 ± 2.7	512.0 ± 8.5	407.2 ± 1.3	156.9 ± 6.2	118.6 ± 6.5
Time(days)	2%(w/v) Boysenberry juice				3%(w/v) Boysenberry juice			
	Del-3-ruti	cyn-3-ruti	Del-3-glu	cyn-3-glu	Del-3-ruti	cyn-3-ruti	Del-3-glu	cyn-3-glu
0	532.6 ± 9.8	457.8 ± 7.1	170.4 ± 2.4	133.4 ± 2.1	534.2 ± 11.5	484.6 ± 2.5	165.6 ± 1.9	140.0 ± 0.3
28	513.6 ± 6.3	436.7 ± 22.7	168.0 ± 5.8	126.7 ± 5.2	522.9 ± 4.6	463.5 ± 2.5	160.8 ± 1.9	133.3 ± 3.5
Time(days)	Blackcurrant juice (10^{o}Brix)							
	0.2 mg/L Ellagic acid							
	0%(w/v) Boysenberry juice				1%(w/v) Boysenberry juice			
	Del-3-ruti	cyn-3-ruti	Del-3-glu	cyn-3-glu	Del-3-ruti	cyn-3-ruti	Del-3-glu	cyn-3-glu
0	525.8 ± 0.1	376.6 ± 2.6	183.9 ± 3.0	109.8 ± 3.1	557.6 ± 13.7	408.0 ± 1.9	169.5 ± 5.1	105.1 ± 0.2
28	514.4 ± 5.2	355.6 ± 2.6	176.7 ± 6.5	105.4 ± 3.1	542.4 ± 2.9	386.9 ± 1.9	164.7 ± 5.1	98.4 ± 3.4
	2%(w/v) Boysenberry juice				3%(w/v) Boysenberry juice			
	Del-3-ruti	cyn-3-ruti	Del-3-glu	cyn-3-glu	Del-3-ruti	cyn-3-ruti	Del-3-glu	cyn-3-glu
0	557.2 ± 7.4	394.3 ± 10.3	176.4 ± 0.4	119.2 ± 7.3	551.9 ± 5.5	428.7 ± 1.5	169.2 ± 2.2	123.1 ± 0.2
28	542.0 ± 3.3	362.7 ± 4.6	169.1 ± 3.8	112.4 ± 2.2	540.5 ± 0.2	407.6 ± 31.3	166.8 ± 1.2	116.4 ± 3.4

Appendix 23 Summary of anthocyanins profile for blackcurrant juice enhanced with 0, 0.2, 0.6 and 1 mg/L of ellagic acid and 0, 1, 2 and 3%(w/v) concentrations boysenberry juice at 5°C (Study 4) * Del-3-ruti = Delphinidin-3-rutinoside; cyn-3-ruti = cyanidin-3-rutinoside; del-3-glu = delphinidin-3-glucoside; cyn-3-glu = cyanidin-3-glucoside (Cont.)

Time(days)	Blackcurrant juice (10°Brix)							
	0.6 mg/L Ellagic acid							
	0%(w/v) Boysenberry juice				1%(w/v) Boysenberry juice			
	Del-3-ruti	cyn-3-ruti	Del-3-glu	cyn-3-glu	Del-3-ruti	cyn-3-ruti	Del-3-glu	cyn-3-glu
0	542.3 ± 2.1	366.3 ± 12.0	192.9 ± 2.8	114.1 ± 3.6	564.0 ± 0.9	383.9 ± 6.1	171.6 ± 2.0	109.2 ± 5.4
28	531.0 ± 3.3	345.3 ± 12.0	183.2 ± 2.8	109.6 ± 10.0	556.4 ± 0.9	373.3 ± 21.0	166.8 ± 2.0	102.5 ± 4.1
	2%(w/v) Boysenberry juice				3%(w/v) Boysenberry juice			
	Del-3-ruti	cyn-3-ruti	Del-3-glu	cyn-3-glu	Del-3-ruti	cyn-3-ruti	Del-3-glu	cyn-3-glu
0	564.6 ± 2.0	405.8 ± 7.4	172.6 ± 4.7	116.0 ± 0.2	563.5 ± 9.1	427.2 ± 9.2	173.4 ± 2.5	120.8 ± 3.5
28	559.7 ± 1.7	395.3 ± 7.5	167.8 ± 4.7	113.7 ± 3.4	556.0 ± 9.1	407.2 ± 7.7	168.6 ± 4.3	114.0 ± 0.4

Time(days)	Blackcurrant juice (10°Brix)							
	1 mg/L Ellagic acid							
	0%(w/v) Boysenberry juice				1%(w/v) Boysenberry juice			
	Del-3-ruti	cyn-3-ruti	Del-3-glu	cyn-3-glu	Del-3-ruti	cyn-3-ruti	Del-3-glu	cyn-3-glu
0	563.6 ± 0.6	384.3 ± 17.4	205.1 ± 0.4	123.0 ± 2.3	573.2 ± 2.0	382.8 ± 11.4	190.7 ± 0.2	109.9 ± 0.5
28	552.2 ± 16.7	373.8 ± 32.3	200.2 ± 0.4	118.5 ± 4.0	565.6 ± 2.0	361.7 ± 11.4	183.4 ± 3.6	105.4 ± 0.5
	2%(w/v) Boysenberry juice				3%(w/v) Boysenberry juice			
	Del-3-ruti	cyn-3-ruti	Del-3-glu	cyn-3-glu	Del-3-ruti	cyn-3-ruti	Del-3-glu	cyn-3-glu
0	572.6 ± 1.1	407.9 ± 10.3	179.7 ± 5.8	122.4 ± 2.6	576.6 ± 7.6	428.3 ± 22.7	184.8 ± 4.4	121.1 ± 2.7
28	558.9 ± 1.0	380.5 ± 1.3	172.5 ± 2.4	115.7 ± 0.6	560.5 ± 1.7	396.7 ± 37.6	177.6 ± 1.0	114.3 ± 0.5

Appendix 24 Summary of phenolics profile for blackcurrant juice enhanced with 0, 1, 2 and 3% (w/v) concentrations boysenberry juice at various temperatures (Study 2) * Bl = Blackcurrant juice; Boy = boysenberry juice

Temperature (^{o}C)	Time (days)	Phenolic acids content (mg/L)							
		Bl+0% Boy				Bl+1% Boy			
		p-coumaric	Caffeic	Benzoic	Quercetin	p-coumaric	Caffeic	Benzoic	Quercetin
5	0	113.6 ± 0.7	74.3 ± 2.5	37.4 ± 0.9	25.3 ± 0.1	99.6 ± 0.4	64.5 ± 2.3	36.2 ± 1.9	27.3 ± 0.1
	7	104.4 ± 2.2	70.8 ± 1.0	35.3 ± 0.2	24.7 ± 0.2	93.4 ± 2.4	62.0 ± 1.1	34.6 ± 0.0	26.9 ± 0.1
	14	94.9 ± 0.0	65.9 ± 1.3	32.6 ± 0.7	20.8 ± 0.4	90.3 ± 0.6	55.5 ± 0.4	30.5 ± 0.4	23.9 ± 0.1
	21	90.6 ± 2.1	61.2 ± 1.0	28.2 ± 0.1	19.4 ± 0.0	85.7 ± 0.9	50.0 ± 1.4	27.0 ± 0.7	22.8 ± 0.3
	28	82.4 ± 0.6	59.9 ± 0.3	22.9 ± 0.3	18.1 ± 0.0	81.0 ± 0.6	42.6 ± 0.5	22.0 ± 0.1	20.3 ± 0.3
20	0	113.6 ± 0.7	74.3 ± 2.5	37.4 ± 0.9	25.3 ± 0.1	99.6 ± 0.4	64.5 ± 2.3	36.2 ± 1.9	27.3 ± 0.1
	7	99.6 ± 1.1	67.3 ± 3.3	33.8 ± 0.4	24.0 ± 0.1	86.4 ± 1.3	60.3 ± 0.4	30.3 ± 0.4	25.3 ± 0.1
	14	90.9 ± 1.6	60.6 ± 0.2	30.9 ± 0.2	20.4 ± 0.3	86.0 ± 0.6	53.8 ± 0.6	25.7 ± 0.9	23.3 ± 0.4
	21	84.3 ± 1.1	55.4 ± 1.2	26.0 ± 0.4	18.5 ± 0.7	75.6 ± 2.9	47.6 ± 0.7	21.4 ± 0.1	21.2 ± 0.3
	28	75.8 ± 0.9	50.2 ± 0.1	24.3 ± 0.4	17.0 ± 0.0	71.0 ± 0.6	38.8 ± 0.7	19.2 ± 0.1	18.7 ± 0.3
35	0	113.6 ± 0.7	74.3 ± 2.5	37.4 ± 0.9	25.3 ± 0.1	99.6 ± 0.4	64.5 ± 2.3	36.2 ± 1.9	27.3 ± 0.1
	7	93.7 ± 2.4	61.8 ± 1.5	30.9 ± 0.2	23.3 ± 0.2	83.4 ± 0.7	59.3 ± 0.7	27.5 ± 0.4	25.3 ± 0.1
	14	84.5 ± 1.5	55.8 ± 0.6	25.7 ± 1.0	19.6 ± 0.2	81.0 ± 0.6	55.7 ± 0.3	24.3 ± 0.3	20.9 ± 0.8
	21	73.3 ± 2.1	48.9 ± 1.9	23.6 ± 0.3	18.9 ± 0.0	72.6 ± 0.2	49.7 ± 0.2	21.2 ± 0.1	19.2 ± 0.0
	28	66.9 ± 1.1	41.9 ± 0.5	21.3 ± 0.1	17.7 ± 0.0	64.8 ± 0.8	44.9 ± 0.4	19.5 ± 0.1	17.8 ± 0.2

Appendix 24 Summary of phenolics profile for blackcurrant juice enhanced with 0, 1, 2 and 3% (w/v) concentrations boysenberry juice at various temperatures (Study 2) * Bl = Blackcurrant juice; Boy = boysenberry juice (Cont.)

Temperature (^{o}C)	Time (days)	Phenolic acids content (mg/L)							
		Bl+2% Boy				Bl+3% Boy			
		p-coumaric	Caffeic	Benzoic	Quercetin	p-coumaric	Caffeic	Benzoic	Quercetin
5	0	92.2 ± 0.6	63.3 ± 0.7	35.7 ± 1.1	27.8 ± 0.0	88.9 ± 2.0	62.7 ± 1.3	34.9 ± 0.7	28.1± 0.1
	7	90.9 ± 1.2	61.4 ± 0.8	34.7 ± 0.4	27.6 ± 0.1	86.4 ± 0.5	61.2 ± 0.3	32.7 ± 0.7	27.6 ± 0.1
	14	88.1 ± 0.5	55.8 ± 0.1	30.2 ± 0.3	25.3 ± 0.0	84.5 ± 0.2	58.4 ± 1.3	30.3 ± 0.4	25.3 ± 0.1
	21	81.8 ± 0.3	50.0 ± 1.0	26.2 ± 0.6	23.3 ± 0.3	75.2 ± 0.2	53.3 ± 0.9	27.4 ± 0.6	23.9 ± 0.5
	28	74.7 ± 0.3	44.5 ± 0.7	21.8 ± 0.2	21.7 ± 0.0	68.1 ± 0.2	49.0 ± 0.9	22.4 ± 0.2	23.0 ± 0.1
20	0	92.2 ± 0.6	63.3 ± 0.7	35.7 ± 1.1	27.8 ± 0.0	88.9 ± 2.0	62.7 ± 1.3	34.9 ± 0.7	28.1 ± 0.1
	7	84.5 ± 0.5	59.6 ± 0.3	33.1 ± 0.4	24.4 ± 0.2	85.0 ± 0.5	59.7 ± 0.6	31.6 ± 0.9	25.3 ± 0.2
	14	80.9 ± 0.5	59.5 ± 0.3	28.3 ± 0.2	22.5 ± 0.7	83.0 ± 0.5	56.0 ± 0.2	28.2 ± 0.4	23.7 ± 0.9
	21	76.1 ± 1.3	52.8 ± 0.5	25.4 ± 0.3	21.7 ± 0.0	75.4 ± 0.7	53.2 ± 0.1	25.2 ± 0.4	20.9 ± 0.7
	28	68.3 ± 0.3	48.4 ± 0.5	22.9 ± 0.1	19.0 ± 0.1	67.6 ± 1.8	48.8 ± 0.1	22.7 ± 0.0	18.4 ± 0.2
35	0	92.2 ± 0.6	63.3 ± 0.7	35.7 ± 1.1	27.8 ± 0.0	88.9 ± 2.0	62.7 ± 1.3	34.9 ± 0.7	28.1 ± 0.1
	7	82.0 ± 0.2	57.5 ± 0.1	32.7 ± 0.1	23.6 ± 0.2	82.1 ± 0.5	57.7 ± 0.2	28.2 ± 0.7	25.0 ± 0.3
	14	75.4 ± 0.8	53.2 ± 0.1	25.2 ± 0.3	20.9 ± 0.7	80.5 ± 1.1	54.2 ± 0.9	25.6 ± 1.6	22.9 ± 0.3
	21	67.1 ± 0.2	46.8 ± 0.8	20.5 ± 0.5	18.9 ± 0.1	71.5 ± 1.1	50.0 ± 1.0	21.1 ± 0.4	19.1 ± 0.3
	28	60.0 ± 0.2	38.3 ± 1.1	18.6 ± 0.2	17.2 ± 0.2	63.7 ± 0.1	43.0 ± 1.5	19.7 ± 0.0	17.8 ± 0.2

Appendix 25 Summary of phenolics profile for cranberry juice enhanced with 0, 1, 2 and 3% (w/v) concentrations boysenberry juice at various temperatures (Study 2) * C = cranberry juice; Boy = boysenberry juice

Temperature (^{o}C)	Time (days)	Phenolic acids content (mg/L)							
		C+0% Boy				C+1% Boy			
		Benzoic	Quercetin	Myricetin	p-coumaric	Benzoic	Quercetin	Myricetin	p-coumaric
5	0	55.8 ± 0.6	42.9 ± 0.8	22.8 ± 1.4	14.4 ± 0.1	55.8 ± 0.1	45.5 ± 0.4	22.7 ± 0.5	14.5 ± 0.1
	7	47.7 ± 2.1	38.6 ± 0.2	16.1 ± 0.6	12.1 ± 0.0	50.5 ± 2.0	44.5 ± 1.1	21.5 ± 0.3	13.0 ± 0.2
	14	40.9 ± 0.5	30.9 ± 0.9	14.9 ± 0.3	10.8 ± 0.2	45.3 ± 0.7	41.9 ± 0.6	17.9 ± 0.3	11.6 ± 0.0
	21	39.0 ± 1.2	28.9 ± 0.7	13.0 ± 0.4	9.9 ± 0.1	42.8 ± 0.9	38.6 ± 0.6	16.4 ± 0.8	10.0 ± 0.0
	28	34.3 ± 1.4	22.7 ± 0.9	9.8 ± 0.4	9.5 ± 0.1	40.0 ± 0.2	37.1 ± 1.4	13.5 ± 0.4	9.6 ± 0.0
20	0	55.8 ± 0.6	42.9 ± 0.8	22.8 ± 1.4	14.4 ± 0.1	55.8 ± 0.1	45.5 ± 0.4	22.7 ± 0.5	14.5 ± 0.1
	7	40.9 ± 0.4	35.0 ± 0.9	14.6 ± 0.8	11.6 ± 0.0	50.5 ± 0.3	44.5 ± 0.6	21.5 ± 0.7	13.0 ± 0.4
	14	34.9 ± 1.6	30.6 ± 0.8	12.3 ± 0.1	8.7 ± 0.0	39.6 ± 0.4	35.0 ± 1.0	14.0 ± 1.1	10.1 ± 0.3
	21	31.4 ± 1.4	28.7 ± 0.2	10.3 ± 0.2	8.3 ± 0.0	38.5 ± 0.7	34.0 ± 0.7	12.6 ± 0.2	8.1 ± 0.0
	28	27.3 ± 0.2	22.1 ± 0.9	9.3 ± 0.6	8.1 ± 0.0	35.5 ± 0.3	30.7 ± 1.2	10.4 ± 1.4	8.0 ± 0.0
35	0	55.8 ± 0.6	42.9 ± 0.8	22.8 ± 1.4	14.4 ± 0.1	55.8 ± 0.1	45.5 ± 0.4	22.7 ± 0.5	14.5 ± 0.1
	7	40.9 ± 0.2	35.0 ± 0.4	14.6 ± 0.8	11.6 ± 0.1	44.0 ± 0.7	38.7 ± 0.2	15.6 ± 1.1	10.9 ± 0.2
	14	37.2 ± 0.8	28.4 ± 1.3	10.0 ± 0.6	8.1 ± 0.0	38.5 ± 0.7	34.4 ± 1.2	13.6 ± 1.4	8.1 ± 0.0
	21	32.5 ± 0.3	23.5 ± 1.1	8.1 ± 0.7	8.0 ± 0.0	35.0 ± 1.0	30.2 ± 1.7	12.0 ± 0.5	8.0 ± 0.0
	28	28.8 ± 0.4	16.9 ± 0.1	6.3 ± 0.5	8.0 ± 0.0	31.7 ± 1.0	23.6 ± 0.6	8.8 ± 0.3	8.0 ± 0.0

Appendix 25 Summary of phenolics profile for cranberry juice enhanced with 0, 1, 2 and 3% (w/v) concentrations boysenberry juice at various temperatures (Study 2) * C = cranberry juice; Boy = boysenberry juice (Cont.)

Temperature (^{o}C)	Time (days)	Phenolic acids content (mg/L)							
		C+2% Boy				C+3% Boy			
		Benzoic	Quercetin	Myricetin	p-coumaric	Benzoic	Quercetin	Myricetin	p-coumaric
5	0	56.2 ± 0.2	50.1 ± 0.1	23.3 ± 0.2	14.6 ± 0.1	55.5 ± 0.2	54.2 ± 0.5	22.9 ± 0.2	14.7 ± 0.1
	7	55.2 ± 0.0	43.6 ± 0.2	21.7 ± 0.2	12.8 ± 0.3	52.1 ± 0.9	53.0 ± 1.0	21.4 ± 0.4	14.4 ± 0.2
	14	50.1 ± 0.6	42.3 ± 0.3	18.4 ± 0.4	11.5 ± 0.1	48.3 ± 0.6	47.6 ± 0.1	18.4 ± 0.2	13.3 ± 0.2
	21	49.3 ± 0.8	40.1 ± 0.6	16.6 ± 0.1	10.1 ± 0.1	46.1 ± 0.7	45.8 ± 1.6	16.9 ± 0.5	12.0 ± 0.0
	28	46.4 ± 0.3	33.8 ± 1.1	14.2 ± 0.5	9.7 ± 0.1	43.4 ± 0.0	41.2 ± 0.2	12.3 ± 0.6	11.6 ± 0.0
20	0	56.2 ± 0.2	50.1 ± 0.1	23.3 ± 0.2	14.6 ± 0.1	55.5 ± 0.2	54.2 ± 0.5	22.9 ± 0.2	14.7 ± 0.1
	7	55.2 ± 0.4	43.6 ± 0.8	21.7 ± 0.6	12.8 ± 0.1	52.1 ± 1.2	53.0 ± 0.9	21.4 ± 0.8	14.4 ± 0.5
	14	50.2 ± 1.2	40.1 ± 0.6	16.6 ± 0.1	10.4 ± 0.1	47.8 ± 1.4	45.5 ± 1.0	17.4 ± 0.3	12.0 ± 0.0
	21	46.7 ± 1.5	37.0 ± 1.8	13.7 ± 1.3	9.7 ± 0.1	44.9 ± 1.0	38.9 ± 0.8	14.6 ± 0.2	10.0 ± 0.0
	28	44.1 ± 2.2	31.1 ± 0.3	9.2 ± 0.2	9.3 ± 0.1	43.1 ± 1.3	31.5 ± 0.8	10.1 ± 0.9	9.6 ± 0.0
35	0	56.2 ± 0.2	50.1 ± 0.1	23.3 ± 0.2	14.6 ± 0.1	55.5 ± 0.2	54.2 ± 0.5	22.9 ± 0.2	14.7 ± 0.1
	7	49.4 ± 0.8	38.0 ± 2.8	15.1 ± 1.9	10.7 ± 0.1	43.7 ± 1.7	44.5 ± 0.6	16.8 ± 0.2	11.9 ± 0.0
	14	47.9 ± 1.3	37.7 ± 0.2	14.5 ± 0.3	9.9 ± 0.1	43.4 ± 1.1	43.4 ± 0.1	15.8 ± 1.3	11.8 ± 0.1
	21	40.8 ± 0.7	34.4 ± 0.7	12.0 ± 0.3	8.8 ± 0.0	42.2 ± 0.4	38.9 ± 0.8	14.3 ± 0.9	9.9 ± 0.1
	28	35.9 ± 0.7	27.4 ± 0.2	9.9 ± 0.4	8.4 ± 0.1	39.3 ± 0.5	31.9 ± 0.2	11.1 ± 1.4	9.1 ± 0.1

Appendix 26 Summary of phenolics profile for pomegranate juice enhanced with 0, 1, 2 and 3% (w/v) concentrations boysenberry juice at various temperatures (Study 2) * P = pomegranate juice; Boy = boysenberry juice

Temperature (^{o}C)	Time (days)	Phenolic acids content (mg/L)							
		P+0% Boy		P+1% Boy		P+2% Boy		P+3% Boy	
		Ellagic	Gallic	Ellagic	Gallic	Ellagic	Gallic	Ellagic	Gallic
5	0	9.3 ± 0.1	6.4 ± 0.0	10.3 ± 0.1	6.5 ± 0.1	11.4 ± 0.3	6.4 ± 0.0	12.0 ± 0.2	6.4 ± 0.0
	7	7.7 ± 0.3	5.6 ± 0.1	7.8 ± 0.1	6.4 ± 0.0	10.6 ± 0.3	6.6 ± 0.1	11.8 ± 0.1	5.6 ± 0.0
	14	6.9 ± 0.2	5.1 ± 0.2	7.4 ± 0.3	6.0 ± 0.3	10.1 ± 0.1	6.0 ± 0.0	11.5 ± 0.7	5.4 ± 0.3
	21	5.8 ± 0.1	4.9 ± 0.1	6.7 ± 0.1	4.9 ± 0.2	8.5 ± 0.3	5.4 ± 0.1	10.2 ± 0.8	4.9 ± 0.1
	28	4.7 ± 0.1	3.7 ± 0.1	5.2 ± 0.1	4.5 ± 0.2	6.2 ± 0.1	4.6 ± 0.1	8.2 ± 0.4	4.5 ± 0.1
20	0	9.3 ± 0.1	6.4 ± 0.0	10.3 ± 0.1	6.5 ± 0.1	11.4 ± 0.3	6.4 ± 0.0	12.0 ± 0.2	6.4 ± 0.0
	7	5.9 ± 0.2	4.6 ± 0.2	7.0 ± 0.2	4.5 ± 2.1	10.2 ± 0.4	6.0 ± 0.1	10.1 ± 0.2	7.0 ± 0.3
	14	4.6 ± 0.1	4.3 ± 0.1	4.5 ± 0.1	4.3 ± 0.1	9.7 ± 0.9	5.2 ± 0.2	9.3 ± 0.3	6.6 ± 0.3
	21	4.1 ± 0.2	3.3 ± 0.0	3.4 ± 0.3	3.7 ± 0.1	8.8 ± 0.5	4.8 ± 0.1	8.6 ± 0.5	5.9 ± 0.1
	28	3.6 ± 0.2	3.2 ± 0.0	3.1 ± 0.0	3.1 ± 0.0	6.4 ± 0.1	4.6 ± 0.2	7.0 ± 0.4	5.7 ± 0.1
35	0	9.3 ± 0.1	6.4 ± 0.0	10.3 ± 0.1	6.5 ± 0.1	11.4 ± 0.3	6.4 ± 0.0	12.0 ± 0.2	6.4 ± 0.0
	7	4.6 ± 0.3	4.3 ± 0.0	5.1 ± 0.3	4.4 ± 0.0	8.9 ± 0.2	5.2 ± 0.0	8.9 ± 0.6	6.7 ± 0.1
	14	3.3 ± 0.1	3.7 ± 0.1	4.5 ± 0.5	3.3 ± 0.0	6.7 ± 0.2	4.9 ± 0.2	7.7 ± 0.5	6.3 ± 0.2
	21	3.0 ± 0.1	3.2 ± 0.1	3.6 ± 0.2	3.1 ± 0.0	4.5 ± 0.2	4.3 ± 0.1	7.0 ± 0.7	4.9 ± 0.2
	28	2.1 ± 0.0	3.0 ± 0.0	2.3 ± 0.0	3.0 ± 0.0	3.4 ± 0.2	3.6 ± 0.1	5.1 ± 0.3	4.1 ± 0.2

Appendix 27 Summary of phenolics profile for blackcurrant juice enhanced with 0, 0.2, 0.6 and 1 mg/L of kaemferol at various temperatures (Study 3) * Bl = Blackcurrant juice

Temperature (^{o}C)	Time (days)	Phenolic acids content (mg/L)									
		Bl+0mg/L kaemferol					Bl+0.2mg/L kaemferol				
		p-coumaric	Caffeic	Benzoic	Quercetin	Kaemferol	p-coumaric	Caffeic	Benzoic	Quercetin	Kaemferol
5	0	113.2 ± 0.2	73.4 ± 1.2	36.3 ± 0.1	24.6 ± 0.3	-	109.7 ± 4.8	69.7 ± 0.3	34.9 ± 0.5	24.5 ± 0.1	26.9 ± 0.3
	14	99.0 ± 0.2	66.9 ± 1.9	33.5 ± 0.1	23.1 ± 0.1	-	99.0 ± 0.2	65.3 ± 0.3	33.8 ± 0.5	23.0 ± 0.0	24.2 ± 0.3
	21	92.0 ± 0.0	61.2 ± 0.0	33.0 ± 0.0	23.0 ± 0.0	-	88.5 ± 5.0	61.2 ± 0.0	27.8 ± 0.4	22.5 ± 0.3	22.9 ± 0.0
	28	87.4 ± 1.5	58.5 ± 0.0	29.7 ± 0.0	21.7 ± 0.0	-	86.4 ± 2.0	57.7 ± 0.0	24.7 ± 0.0	21.5 ± 0.0	20.2 ± 0.0
20	0	113.2 ± 0.2	73.4 ± 1.2	36.3 ± 0.1	24.6 ± 0.3	-	109.7 ± 4.8	69.7 ± 0.3	34.9 ± 0.5	24.5 ± 0.1	26.9 ± 0.3
	14	84.8 ± 0.2	62.5 ± 1.9	31.8 ± 0.1	22.2 ± 0.3	-	84.8 ± 0.2	60.3 ± 0.0	33.2 ± 0.5	22.9 ± 0.1	23.7 ± 0.4
	21	79.3 ± 0.0	56.4 ± 0.0	28.0 ± 0.0	21.7 ± 0.0	-	77.8 ± 0.0	56.4 ± 0.0	28.0 ± 0.0	21.7 ± 0.0	21.8 ± 0.0
	28	75.7 ± 0.0	52.0 ± 0.0	22.5 ± 0.0	19.9 ± 0.9	-	75.0 ± 0.0	53.3 ± 0.0	27.5 ± 0.0	20.5 ± 0.0	19.7 ± 0.0
Temperature (^{o}C)	Time (days)	Bl+0.6mg/L kaemferol					Bl+1mg/L kaemferol				
		p-coumaric	Caffeic	Benzoic	Quercetin	Kaemferol	p-coumaric	Caffeic	Benzoic	Quercetin	Kaemferol
5	0	105.0 ± 3.6	64.8 ± 1.5	34.8 ± 0.6	24.9 ± 0.6	43.8 ± 0.3	103.1 ± 2.0	64.0 ± 0.6	34.8 ± 0.3	24.7 ± 0.4	54.6 ± 0.3
	14	94.3 ± 1.4	62.6 ± 1.6	33.1 ± 0.2	24.5 ± 0.2	36.4 ± 0.1	97.8 ± 0.6	60.3 ± 0.2	33.7 ± 0.5	24.2 ± 0.1	52.7 ± 0.1
	21	86.2 ± 0.0	59.7 ± 0.0	32.4 ± 0.0	24.2 ± 0.0	36.3 ± 0.0	91.8 ± 0.0	59.6 ± 0.0	32.8 ± 0.0	24.0 ± 0.2	50.3 ± 0.4
	28	87.3 ± 0.5	58.9 ± 0.0	30.4 ± 0.4	23.1 ± 0.2	32.5 ± 0.0	84.7 ± 0.0	58.7 ± 0.0	29.5 ± 0.0	22.7 ± 0.0	45.2 ± 0.0
20	0	105.0 ± 3.6	64.8 ± 1.5	34.8 ± 0.6	24.9 ± 0.6	43.8 ± 0.3	103.1± 2.0	64.0 ± 0.6	34.8 ± 0.3	24.7 ± 0.4	54.6 ± 0.3
	14	87.2 ± 1.4	62.6 ± 1.6	32.8 ± 0.2	24.3 ± 0.1	35.9 ± 0.1	88.9 ± 2.0	59.7 ± 0.6	32.9 ± 0.1	24.3 ± 0.0	52.7 ± 0.1
	21	79.1 ± 0.0	57.1 ± 0.0	27.4 ± 0.0	23.0 ± 0.0	31.1 ± 0.0	80.4 ± 0.0	56.6 ± 0.0	27.3 ± 0.0	23.1 ± 0.0	47.3 ± 0.0
	28	77.7 ± 0.0	53.6 ± 0.0	25.1 ± 0.0	21.7 ± 0.0	28.6 ± 0.4	78.3 ± 1.0	54.8 ± 0.6	25.6 ± 0.8	22.7 ± 0.0	44.1 ± 0.0

Appendix 28 Summary of phenolics profile for blackcurrant juice enhanced with 0, 0.2, 0.6 and 1 mg/L of quercetin at various temperatures (Study 3) * Bl = Blackcurrant juice

Temperature (^{o}C)	Time (days)	Phenolic acids content (mg/L)							
		Bl+0mg/L Quercetin				Bl+0.2mg/L Quercetin			
		p-coumaric	Caffeic	Benzoic	Quercetin	p-coumaric	Caffeic	Benzoic	Quercetin
5	0	106.1 ± 0.2	71.2 ± 1.9	34.9 ± 0.3	24.6 ± 0.2	108.6 ± 1.3	70.4 ± 0.6	34.7 ± 0.3	32.8 ± 0.6
	14	91.9 ± 0.2	66.9 ± 1.9	33.8 ± 0.3	24.0 ± 0.1	101.5 ± 1.3	63.8 ± 3.7	33.8 ± 0.1	30.9 ± 0.3
	21	87.1 ± 1.0	62.5 ± 0.0	33.0 ± 0.0	23.5 ± 0.0	97.9 ± 6.3	61.6 ± 0.6	33.0 ± 0.5	29.6 ± 0.3
	28	84.9 ± 0.0	59.6 ± 0.3	30.8 ± 0.0	22.6 ± 0.0	94.2 ± 5.0	59.9 ± 0.0	31.7 ± 0.0	28.0 ± 0.0
20	0	106.1 ± 0.2	71.2 ± 1.9	34.9 ± 0.3	24.6 ± 0.2	108.6 ± 1.3	70.4 ± 0.6	34.7 ± 0.3	32.8 ± 0.6
	14	84.8 ± 0.2	62.5 ± 1.9	33.0 ± 0.1	23.9 ± 0.1	94.4 ± 1.3	61.6 ± 0.6	32.7 ± 0.1	29.6 ± 0.3
	21	81.0 ± 0.5	59.0 ± 0.6	28.9 ± 0.4	22.3 ± 0.1	79.3 ± 0.0	58.1 ± 0.0	29.5 ± 0.0	27.3 ± 0.0
	28	76.4 ± 2.0	54.2 ± 0.0	23.0 ± 0.0	21.0 ± 0.0	70.9 ± 1.3	52.9 ± 0.6	22.3 ± 0.8	25.2 ± 0.4
Temperature (^{o}C)	Time (days)	Bl+0.6mg/L Quercetin				Bl+1mg/L Quercetin			
		p-coumaric	Caffeic	Benzoic	Quercetin	p-coumaric	Caffeic	Benzoic	Quercetin
5	0	106.5 ± 0.3	70.2 ± 0.9	34.4 ± 0.7	43.2 ± 0.5	106.8 ± 0.2	70.6 ± 0.3	34.1 ± 0.5	57.6 ± 2.2
	14	102.9 ± 5.3	68.0 ± 4.0	33.3 ± 0.7	42.6 ± 0.4	99.7 ± 0.2	66.2 ± 0.3	33.6 ± 0.3	56.3 ± 3.9
	21	95.8 ± 5.3	65.8 ± 7.1	32.5 ± 1.9	42.0 ± 1.3	96.2 ± 4.8	64.0 ± 3.4	32.7 ± 0.1	52.6 ± 0.4
	28	89.2 ± 1.0	61.8 ± 0.3	30.0 ± 0.0	38.6 ± 0.1	91.0 ± 0.5	60.7 ± 0.0	31.1 ± 0.0	51.0 ± 1.8
20	0	106.5 ± 0.3	70.2 ± 0.9	34.4 ± 0.7	43.2 ± 0.5	106.8 ± 0.2	70.6 ± 0.3	34.1 ± 0.5	57.6 ± 2.2
	14	88.7 ± 4.7	61.4 ± 0.9	32.5 ± 0.3	40.7 ± 0.5	92.6 ± 0.2	61.8 ± 0.3	32.7 ± 0.1	53.2 ± 0.5
	21	84.9 ± 0.0	58.5 ± 0.0	27.3 ± 0.0	35.1 ± 4.9	85.6 ± 0.0	57.2 ± 0.0	30.0 ± 0.0	48.6 ± 0.0
	28	77.8 ± 0.0	54.2 ± 3.1	24.5 ± 0.0	30.5 ± 0.9	82.1 ± 5.0	55.0 ± 3.1	27.3 ± 3.9	42.3 ± 8.8

Appendix 29 Summary of phenolics profile for blackcurrant juice enhanced with 0, 0.2, 0.6 and 1 mg/L of ellagic acid at various temperatures (Study 3) * Bl = Blackcurrant juice

Temperature (^{o}C)	Time (days)	Phenolic acids content (mg/L)									
		Bl+0mg/L Ellagic acid					Bl+0.2mg/L Ellagic acid				
		p-coumaric	Caffeic	Benzoic	Quercetin	Ellagic	p-coumaric	Caffeic	Benzoic	Quercetin	Ellagic
5	0	106.8 ± 1.2	71.9 ± 0.9	35.7 ± 0.1	24.4 ± 0.1	-	101.4 ± 1.4	69.8 ± 0.6	33.9 ± 0.6	25.1 ± 0.3	30.0 ± 0.5
	14	92.6 ± 1.2	67.5 ± 1.0	34.6 ± 0.1	23.8 ± 0.4	-	87.2 ± 1.4	65.5 ± 0.6	32.8 ± 0.2	24.8 ± 0.1	28.1 ± 0.5
	21	87.4 ± 1.5	63.1 ± 0.9	33.0 ± 0.0	23.0 ± 0.0	-	84.8 ± 0.0	61.5 ± 0.0	27.4 ± 0.0	23.5 ± 0.0	28.4 ± 0.0
	28	84.9 ± 0.0	60.7 ± 0.0	27.5 ± 0.0	21.7 ± 0.0	-	80.5 ± 5.0	60.2 ± 0.0	22.7 ± 0.3	22.2 ± 0.0	24.9 ± 0.4
20	0	106.8 ± 1.2	71.9 ± 0.9	35.7 ± 0.1	24.4 ± 0.1	-	101.4 ± 1.4	69.8 ± 0.6	33.9 ± 0.6	25.1 ± 0.3	30.0 ± 0.7
	14	85.5 ± 1.2	63.2 ± 0.9	33.5 ± 0.1	23.9 ± 0.1	-	84.4 ± 0.6	61.1 ± 0.6	32.5 ± 0.2	24.6 ± 0.0	26.7 ± 0.1
	21	82.5 ± 0.5	60.7 ± 0.0	28.0 ± 0.0	22.1 ± 0.9	-	82.0 ± 1.0	59.1 ± 0.9	30.7 ± 0.8	22.8 ± 0.3	22.5 ± 0.0
	28	75.0 ± 0.0	56.8 ± 0.0	22.5 ± 0.0	21.5 ± 0.0	-	75.6 ± 0.0	55.4 ± 0.0	25.7 ± 0.0	21.7 ± 0.0	17.1 ± 0.0
Temperature (^{o}C)	Time (days)	Bl+0.6mg/L Ellagic acid					Bl+1mg/L Ellagic acid				
		p-coumaric	Caffeic	Benzoic	Quercetin	Ellagic	p-coumaric	Caffeic	Benzoic	Quercetin	Ellagic
5	0	104.5 ± 2.1	69.7 ± 0.3	33.5 ± 0.9	24.4 ± 0.1	39.7 ± 0.7	103.1 ± 4.1	70.2 ± 0.9	34.6 ± 0.9	24.6 ± 0.2	48.6 ± 0.3
	14	93.8 ± 3.0	65.3 ± 0.3	33.0 ± 0.1	24.2 ± 0.0	38.3 ± 1.1	92.4 ± 1.0	65.8 ± 1.0	34.3 ± 0.5	24.2 ± 0.0	47.6 ± 0.4
	21	90.3 ± 2.1	63.2 ± 2.8	30.2 ± 3.8	23.6 ± 0.8	35.6 ± 4.9	88.9 ± 4.1	63.6 ± 4.0	33.5 ± 0.7	23.6 ± 0.8	44.8 ± 3.4
	28	87.8 ± 0.5	60.7 ± 0.0	26.1 ± 0.4	21.7 ± 0.0	32.1 ± 0.0	84.3 ± 0.6	61.4 ± 0.9	30.7 ± 4.6	23.0 ± 0.0	42.1 ± 0.4
20	0	104.5 ± 2.1	69.7 ± 0.3	33.5 ± 0.9	24.4 ± 0.1	39.7 ± 0.7	103.1 ± 4.1	70.2 ± 0.9	34.6 ± 0.9	24.6 ± 0.2	48.6 ± 0.3
	14	86.7 ± 3.0	61.0 ± 0.3	32.7 ± 0.5	24.1 ± 0.1	36.7 ± 0.3	85.3 ± 1.0	61.4 ± 0.9	33.8 ± 0.3	24.1 ± 0.1	47.3 ± 0.1
	21	83.9 ± 0.0	58.1 ± 0.6	27.5 ± 0.0	22.9 ± 0.0	31.1 ± 0.0	83.1 ± 1.0	58.1 ± 0.6	27.5 ± 0.0	22.9 ± 0.0	35.4 ± 0.8
	28	76.0 ± 1.0	54.2 ± 0.0	21.9 ± 0.0	21.6 ± 0.0	25.6 ± 0.0	75.3 ± 0.0	54.2 ± 0.0	21.9 ± 0.0	21.6 ± 0.0	29.7 ± 1.1

Appendix 30 Summary of phenolics profile for blackcurrant juice enhanced with 0, 0.2, 0.6 and 1 mg/L of ellagic acid and 0, 1, 2 and 3%(w/v) concentrations boysenberry juice at 5^{o}C (Study 4)

Time(days)	Phenolic acids content (mg/L)									
	Blackcurrant juice (10^{o}Brix)									
	0 mg/L Ellagic acid									
	0%(w/v) Boysenberry juice					1%(w/v) Boysenberry juice				
	p-coumaric	Caffeic	Benzoic	Quercetin	Ellagic	p-coumaric	Caffeic	Benzoic	Quercetin	Ellagic
0	112.5 ± 0.8	78.5 ± 4.0	36.8 ± 0.1	24.8 ± 0.0	-	101.7 ± 0.3	73.9 ± 0.1	34.9 ± 0.1	27.4 ± 0.0	-
28	87.6 ± 4.2	67.5 ± 1.0	33.5 ± 0.1	23.0 ± 0.0	-	87.5 ± 0.2	60.4 ± 0.7	30.2 ± 4.2	23.7 ± 0.0	-
	2%(w/v) Boysenberry juice					3%(w/v) Boysenberry juice				
	p-coumaric	Caffeic	Benzoic	Quercetin	Ellagic	p-coumaric	Caffeic	Benzoic	Quercetin	Ellagic
0	95.7 ± 0.6	73.3 ± 0.6	35.4 ± 0.7	27.5 ± 0.2	-	94.6 ± 1.0	70.8 ± 0.4	35.1 ± 0.4	27.9 ± 0.0	-
28	88.6 ± 0.6	62.0 ± 3.1	31.3 ± 2.7	25.1 ± 0.2	-	85.7 ± 1.6	61.2 ± 1.6	31.5 ± 2.4	25.4 ± 0.0	-
Time(days)	Blackcurrant juice (10^{o}Brix)									
	0.2 mg/L Ellagic acid									
	0%(w/v) Boysenberry juice					1%(w/v) Boysenberry juice				
	p-coumaric	Caffeic	Benzoic	Quercetin	Ellagic	p-coumaric	Caffeic	Benzoic	Quercetin	Ellagic
0	108.1 ± 0.8	77.0 ± 0.3	35.0 ± 1.0	24.7 ± 0.0	25.6 ± 0.1	102.1 ± 0.6	72.3 ± 0.9	35.6 ± 0.3	28.1 ± 0.1	26.7 ± 0.7
28	86.8 ± 0.8	63.9 ± 5.9	29.5 ± 0.9	23.5 ± 0.0	20.2 ± 0.1	87.9 ± 0.6	61.4 ± 4.0	30.0 ± 0.3	26.9 ± 0.1	20.7 ± 0.1
	2%(w/v) Boysenberry juice					3%(w/v) Boysenberry juice				
	p-coumaric	Caffeic	Benzoic	Quercetin	Ellagic	p-coumaric	Caffeic	Benzoic	Quercetin	Ellagic
0	103.3 ± 0.8	71.6 ± 0.4	35.9 ± 0.4	30.3 ± 0.2	28.0 ± 0.2	101.8 ± 0.8	71.0 ± 0.4	34.6 ± 0.4	33.2 ± 0.3	28.1 ± 0.0
28	89.1 ± 0.8	58.4 ± 0.4	30.3 ± 0.4	27.0 ± 0.4	21.5 ± 1.4	87.6 ± 0.8	60.0 ± 3.5	29.1 ± 0.4	27.6 ± 0.5	22.7 ± 0.0

Appendix 30 Summary of phenolics profile for blackcurrant juice enhanced with 0, 0.2, 0.6 and 1 mg/L of ellagic acid and 0, 1, 2 and 3%(w/v) concentrations boysenberry juice at 5^{o}C (Study 4) (Cont.)

Time(days)	Blackcurrant juice (10^{o}Brix)									
	0.6 mg/L Ellagic acid									
	0%(w/v) Boysenberry juice					1%(w/v) Boysenberry juice				
	p-coumaric	Caffeic	Benzoic	Quercetin	Ellagic	p-coumaric	Caffeic	Benzoic	Quercetin	Ellagic
0	100.6 ± 0.4	76.5 ± 0.6	34.1 ± 0.1	24.8 ± 0.1	33.2 ± 0.7	98.2 ± 1.0	73.6 ± 0.3	35.4 ± 0.3	25.0 ± 0.1	34.4 ± 0.7
28	86.4 ± 0.5	59.0 ± 0.6	28.5 ± 0.1	23.8 ± 0.5	22.3 ± 0.7	84.0 ± 1.0	56.1 ± 0.3	29.8 ± 0.3	23.8 ± 0.1	26.2 ± 3.1
	2%(w/v) Boysenberry juice					3%(w/v) Boysenberry juice				
	p-coumaric	Caffeic	Benzoic	Quercetin	Ellagic	p-coumaric	Caffeic	Benzoic	Quercetin	Ellagic
0	97.8 ± 0.4	72.7 ± 0.4	35.3 ± 0.2	25.9 ± 0.3	35.1 ± 0.4	97.1 ± 0.6	71.3 ± 1.5	36.1 ± 0.1	26.7 ± 0.0	36.8 ± 1.1
28	83.6 ± 0.4	61.7 ± 2.7	29.8 ± 0.2	23.4 ± 0.3	24.3 ± 0.4	79.3 ± 4.4	56.0 ± 1.6	30.5 ± 0.1	23.0 ± 0.0	25.9 ± 1.1
Time(days)	Blackcurrant juice (10^{o}Brix)									
	1 mg/L Ellagic acid									
	0%(w/v) Boysenberry juice					1%(w/v) Boysenberry juice				
	p-coumaric	Caffeic	Benzoic	Quercetin	Ellagic	p-coumaric	Caffeic	Benzoic	Quercetin	Ellagic
0	99.5 ± 0.9	77.8 ± 0.6	33.8 ± 0.3	24.5 ± 0.1	45.1 ± 0.7	97.9 ± 0.5	75.8 ± 1.4	33.2 ± 0.4	26.1 ± 0.2	45.6 ± 0.0
28	78.2 ± 1.0	55.9 ± 0.6	28.2 ± 0.3	23.3 ± 0.1	23.4 ± 0.7	76.6 ± 0.5	53.9 ± 1.4	27.6 ± 0.4	23.6 ± 0.2	33.8 ± 1.4
	2%(w/v) Boysenberry juice					3%(w/v) Boysenberry juice				
	p-coumaric	Caffeic	Benzoic	Quercetin	Ellagic	p-coumaric	Caffeic	Benzoic	Quercetin	Ellagic
0	97.8 ± 1.5	75.3 ± 1.5	32.2 ± 0.7	27.0 ± 0.3	46.4 ± 0.4	98.8 ± 0.5	73.8 ± 0.2	33.8 ± 1.0	28.5 ± 0.1	48.2 ± 0.8
28	80.1 ± 3.5	61.8 ± 2.1	27.2 ± 0.0	23.3 ± 0.3	30.1 ± 0.4	77.5 ± 0.5	56.3 ± 0.2	28.2 ± 1.0	27.2 ± 0.1	32.0 ± 0.8

Appendix 31 % Loss of individual anthocyanin concentrations in cranberry juice enhanced with 0, 1, 2, and 3% (w/v) concentrations of boysenberry juice (Study 1)

Temperature (^oC)	Individual anthocyanins	Anthocyanin content (mg/L)	Concentrations of boysenberry juice (% (w/v))			
			0	1	2	3
5	Pn-3-gal	Initial content	119.3	118.2	117.6	118.3
		Final content	56.8	57.0	64.9	80.5
		% Loss	52.4	51.8	44.8	32.0
	Cyn-3-gal	Initial content	86.2	89.2	92.0	98.2
		Final content	32.1	32.2	33.6	67.3
		% Loss	62.8	63.9	63.5	31.5
	Cyn-3-ara	Initial content	43.4	44.4	44.3	43.7
		Final content	32.08	32.21	33.62	32.1
		% Loss	26.1	27.5	24.1	26.6
	Pn-3-ara	Initial content	34.71	34.93	35.37	35.15
		Final content	28.17	28.39	29.48	28.82
		% Loss	18.8	18.7	16.6	18.0
20	Pn-3-gal	Initial content	119.3	118.2	117.6	118.3
		Final content	64.5	58.9	78.8	81.3
		% Loss	46.0	50.2	33.0	31.3
	Cyn-3-gal	Initial content	86.2	89.2	92.0	98.2
		Final content	45.6	62.8	63.1	67.7
		% Loss	47.1	29.7	31.4	31.1
	Cyn-3-ara	Initial content	43.4	44.4	44.3	43.7
		Final content	22.0	30.5	30.6	29.5
		% Loss	49.3	31.3	30.9	32.6
	Pn-3-ara	Initial content	34.71	34.93	35.37	35.15
		Final content	24.25	25.12	24.46	24.68
		% Loss	30.2	28.1	30.8	29.8
35	Pn-3-gal	Initial content	119.3	118.2	117.6	118.3
		Final content	53.3	57.2	68.0	73.6
		% Loss	55.4	51.6	42.2	37.8
	Cyn-3-gal	Initial content	86.2	89.2	92.0	98.2
		Final content	36.4	48.0	53.6	56.0
		% Loss	57.8	46.2	41.7	43.0
	Cyn-3-ara	Initial content	43.4	44.4	44.3	43.7
		Final content	17.0	19.1	23.2	23.6
		% Loss	60.9	57.1	47.6	46.0
	Pn-3-ara	Initial content	34.71	34.93	35.37	35.15
		Final content	21.41	22.07	21.19	20.54
		% Loss	38.3	36.8	40.1	41.6

Appendix 32 % Loss of individual anthocyanin concentrations in pomegranate juice enhanced with 0, 1, 2, and 3% (w/v) concentrations of boysenberry juice (Study 1)

Temperature (^{o}C)	Individual anthocyanins	Anthocyanin content (mg/L)	Concentrations of boysenberry juice (% (w/v))			
			0	1	2	3
5	Cyn-3-glu	Initial content	30.5	30.6	30.6	30.5
		Final content	25.5	24.2	25.2	26.0
		% Loss	16.4	20.7	17.6	14.7
	Dp-3,5-diglu	Initial content	21.5	22.5	22.2	22.1
		Final content	11.5	13.5	16.7	17.2
		% Loss	46.6	40.0	24.9	22.1
	Pg-3-glu	Initial content	14.3	14.1	14.4	14.5
		Final content	6.8	8.7	7.2	10.4
		% Loss	52.8	38.2	50.2	28.0
	Dp-3-glu	Initial content	10.6	10.3	8.9	11.3
		Final content	4.4	4.2	4.5	5.6
		% Loss	58.1	59.3	49.5	49.9
20	Cyn-3-glu	Initial content	30.5	30.6	30.6	30.5
		Final content	24.0	24.0	25.0	9.9
		% Loss	21.2	21.5	18.2	67.6
	Dp-3,5-diglu	Initial content	21.5	22.5	22.2	22.1
		Final content	10.4	8.9	9.4	9.9
		% Loss	51.8	60.3	57.8	55.3
	Pg-3-glu	Initial content	14.3	14.1	14.4	14.5
		Final content	3.7	5.3	4.7	5.0
		% Loss	73.9	62.4	67.4	65.3
	Dp-3-glu	Initial content	10.6	10.3	8.9	11.3
		Final content	3.8	4.1	4.0	5.2
		% Loss	64.4	60.2	54.8	53.5
35	Cyn-3-glu	Initial content	30.5	30.6	30.6	30.5
		Final content	24.0	24.0	24.0	24.0
		% Loss	21.2	21.5	21.5	21.2
	Dp-3,5-diglu	Initial content	21.5	22.5	22.2	22.1
		Final content	7.6	8.3	8.6	10.0
		% Loss	64.5	63.0	61.1	54.9
	Pg-3-glu	Initial content	14.3	14.1	14.4	14.5
		Final content	3.5	4.9	4.9	5.4
		% Loss	75.4	65.1	65.6	62.5
	Dp-3-glu	Initial content	10.6	10.3	8.9	11.3
		Final content	3.1	3.3	3.8	4.8
		% Loss	71.0	67.9	57.5	57.8

Appendix 33 % Loss of individual phenolics acid in cranberry juice enhanced with 0, 1, 2, and 3% (w/v) concentrations of boysenberry juice (Study 1)

Temperature (°C)	Individual Phenolics	Phenolics content (mg/L)	Concentrations of boysenberry juice (% (w/v))			
			0	1	2	3
5	Benzoic acid	Initial content	55.8	55.8	56.2	55.5
		Final content	34.3	40.0	46.4	43.4
		% Loss	38.5	28.3	17.5	21.9
	Quercetin	Initial content	42.9	45.5	50.1	54.2
		Final content	22.7	37.1	33.8	41.2
		% Loss	47.1	18.5	32.6	24.0
	Myricetin	Initial content	22.8	22.7	23.3	22.9
		Final content	9.8	13.5	14.2	12.3
		% Loss	57.2	40.8	38.9	46.1
	p-coumaric	Initial content	14.4	14.5	14.6	14.7
		Final content	9.5	9.6	9.7	11.6
		% Loss	34.3	33.9	33.3	20.9
20	Benzoic acid	Initial content	55.8	55.8	56.2	55.5
		Final content	27.3	35.5	44.1	43.1
		% Loss	51.0	36.4	21.6	22.3
	Quercetin	Initial content	42.9	45.5	50.1	54.2
		Final content	22.1	30.7	31.1	31.5
		% Loss	48.5	32.7	38.0	41.8
	Myricetin	Initial content	22.8	22.7	23.3	22.9
		Final content	9.3	10.4	9.2	10.1
		% Loss	59.3	54.1	60.6	55.9
	p-coumaric	Initial content	14.4	14.5	14.6	14.7
		Final content	8.1	8.0	9.3	9.6
		% Loss	43.8	44.6	36.3	34.7
35	Benzoic acid	Initial content	55.8	55.8	56.2	55.5
		Final content	28.8	31.7	35.9	39.3
		% Loss	48.4	43.1	36.2	29.2
	Quercetin	Initial content	42.9	45.5	50.1	54.2
		Final content	16.9	23.6	27.4	31.9
		% Loss	60.6	48.1	45.4	41.1
	Myricetin	Initial content	22.8	22.7	23.3	22.9
		Final content	6.3	8.8	9.9	11.1
		% Loss	72.4	61.4	57.7	51.5
	p-coumaric	Initial content	14.4	14.5	14.6	14.7
		Final content	8.0	8.0	8.4	9.1
		% Loss	44.8	44.9	42.2	38.2

Appendix 34 % Loss of individual phenolics acid in pomegranate juice enhanced with 0, 1, 2, and 3% (w/v) concentrations of boysenberry juice (Study 1)

Temperature (°C)	Individual Phenolics	Phenolics content (mg/L)	Concentrations of boysenberry juice (% (w/v))			
			0	1	2	3
5	Ellagic acid	Initial content	9.3	10.3	11.4	12.0
		Final content	4.7	5.2	6.2	8.2
		% Loss	49.8	49.9	45.5	31.3
	Gallic acid	Initial content	6.4	6.5	6.4	6.4
		Final content	3.7	4.5	4.6	4.5
		% Loss	42.4	30.3	28.8	30.3
20	Ellagic acid	Initial content	9.3	10.3	11.4	12.0
		Final content	3.6	3.1	6.4	7.0
		% Loss	61.8	70.0	43.6	41.4
	Gallic acid	Initial content	6.4	6.5	6.4	6.4
		Final content	3.2	3.1	4.6	5.7
		% Loss	50.4	52.2	29.1	10.8
35	Ellagic acid	Initial content	9.3	10.3	11.4	12.0
		Final content	2.1	2.3	3.4	5.1
		% Loss	77.1	77.3	70.3	57.7
	Gallic acid	Initial content	6.4	6.5	6.4	6.4
		Final content	3.0	3.0	3.6	4.1
		% Loss	52.7	53.8	43.3	35.9

Appendix 35 Analysis of effect of factors on total anthocyanin content, total phenolic acids, colour desity, hyperchromic and bathochromic shift for blackcurrant, cranberry and pomegranate juice enhanced with boysenberry juice (Study 2)

Blackcurrant juice was enhanced with boysenberry juice at concentrations of 0, 1, 2 and 3% (w/v) and stored at 5, 20 and 35 ^{o}C for 28 days

Factors	Total anthocyanin content	Total phenolic acids	Colour density	Polymeric colour	Hyperchromic shift	Bathochromic shift
	P value	P value	P value	P value	P value	P value
Concentrations of boysenberry juice	0.020	0.000	0.001	0.000	0.000	0.696
Storage temperatures	0.000	0.061	0.044	0.031	0.036	0.111
Days	0.006	0.000	0.009	0.000	0.027	0.200

Cranberry juice was enhanced with boysenberry juice at concentrations of 0, 1, 2 and 3% (w/v) and stored at 5, 20 and 35 ^{o}C for 28 days

Factors	Total anthocyanin content	Total phenolic acids	Colour density	Polymeric colour	Hyperchromic shift	Bathochromic shift
	P value	P value	P value	P value	P value	P value
Concentrations of boysenberry juice	0.023	0.000	0.000	0.009	0.001	0.201
Storage temperatures	0.015	0.055	0.028	0.043	0.025	0.059
Days	0.000	0.000	0.000	0.001	0.000	0.089

Pomegranate juice was enhanced with boysenberry juice at concentrations of 0, 1, 2 and 3% (w/v) and stored at 5, 20 and 35 ^{o}C for 28 days

Factors	Total anthocyanin content	Total phenolic acids	Colour density	Polymeric colour	Hyperchromic shift	Bathochromic shift
	P value	P value	P value	P value	P value	P value
Concentrations of boysenberry juice	0.002	0.001	0.001	0.049	0.065	0.072
Storage temperatures	0.033	0.057	0.011	0.053	0.040	0.060
Days	0.000	0.000	0.000	0.000	0.000	0.094

Appendix 36 Analysis of effect of factors on Individual anthocyanins and phenolic acids for blackcurrant, cranberry and pomegranate juice enhanced with boysenberry juice (Study 2)

Blackcurrant juice was enhanced with boysenberry juice at concentrations of 0, 1, 2 and 3% (w/v) and stored at 5, 20 and 35 ^{o}C for 28 days								
	Anthocyanins				**Phenolic acids**			
Factors	Del-3-rut	Cyn-3-rut	Del-3-glu	Cyn-3-glu	p-coumaric	Caffeic	Benzoic acid	Quercetin
	P value	P value	P value	P value	P value	P value	P value	P value
Concentrations of boysenberry juice	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.001
Storage temperatures	0.039	0.042	0.010	0.024	0.073	0.025	0.031	0.051
Days	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Cranberry juice was enhanced with boysenberry juice at concentrations of 0, 1, 2 and 3% (w/v) and stored at 5, 20 and 35 ^{o}C for 28 days								
	Anthocyanins				**Phenolic acids**			
Factors	Cyn-3-gal	Pn-3-gal	Cyn-3-ara		Benzoic acid	Quercetin	Myrecetin	p-coumaric
	P value	P value	P value		P value	P value	P value	P value
Concentrations of boysenberry juice	0.000	0.000	0.001		0.000	0.000	0.003	0.000
Storage temperatures	0.921	0.720	0.028		0.016	0.042	0.040	0.010
Days	0.000	0.001	0.000		0.000	0.000	0.000	0.000
Cranberry juice was enhanced with boysenberry juice at concentrations of 0, 1, 2 and 3% (w/v) and stored at 5, 20 and 35 ^{o}C for 28 days								
	Anthocyanins				**Phenolic acids**			
Factors	Cyn-3-glu	Dp-3,5-diglu	Pg-3-glu	Dp-3-glu	Ellagic acid	Gallic		
	P value	P value	P value	P value	P value	P value		
Concentrations of boysenberry juice	0.070	0.000	0.000	0.000	0.000	0.000		
Storage temperatures	0.597	0.679	0.853	0.822	0.037	0.029		
Days	0.000	0.000	0.000	0.000	0.000	0.000		

Appendix 37 Analysis of effect of factors on total anthocyanin content, total phenolic acids, colour desity, hyperchromic and bathochromic shift for blackcurrant juice enhanced with kaemferol, quercetin and ellagic acid (Study 3)

Blackcurrant juice was enhanced with kaemferol at concentrations of 0, 0.2, 0.6 and 1 mg/L and stored at 5 and 20 ^{o}C for 28 days						
Factors	Total anthocyanin content	Total phenolic acids	Colour density	Polymeric colour	Hyperchromic shift	Bathochromic shift
	P value	P value	P value	P value	P value	P value
Concentrations of boysenberry juice	0.539	0.004	0.243	0.061	0.000	0.864
Storage temperatures	0.006	0.049	0.0 10	0.023	0.046	0.856
Days	0.000	0.000	0.000	0.000	0.000	0.205
Blackcurrant juice was enhanced with Quercetin at concentrations of 0, 0.2, 0.6 and 1 mg/L and stored at 5 and 20 ^{o}C for 28 days						
Factors	Total anthocyanin content	Total phenolic acids	Colour density	Polymeric colour	Hyperchromic shift	Bathochromic shift
	P value	P value	P value	P value	P value	P value
Concentrations of boysenberry juice	0.056	0.000	0.000	0.094	0.000	0.000
Storage temperatures	0.015	0.037	0.032	0.039	0.010	0.927
Days	0.000	0.000	0.000	0.000	0.000	0.010
Blackcurrant juice was enhanced with Ellagic acid at concentrations of 0, 0.2, 0.6 and 1 mg/L and stored at 5 and 20 ^{o}C for 28 days						
Factors	Total anthocyanin content	Total phenolic acids	Colour density	Polymeric colour	Hyperchromic shift	Bathochromic shift
	P value	P value	P value	P value	P value	P value
Concentrations of boysenberry juice	0.000	0.073	0.001	0.000	0.014	0.440
Storage temperatures	0.028	0.043	0.026	0.020	0.032	0.994
Days	0.003	0.000	0.011	0.019	0.001	0.547

Appendix 38 Analysis of effect of factors on individual anthocyanins and phneolic acids for blackcurrant juice enhanced with kaemferol, quercetin and ellagic acid (Study 3)

Blackcurrant juice was enhanced with kaemferol at concentrations of 0, 0.2, 0.6 and 1 mg/L and stored at 5 and 20 oC for 28 days									
	Anthocyanins				Phenolic acids				
Factors	Del-3-rut	Cyn-3-rut	Del-3-glu	Cyn-3-glu	p-coumaric	Caffeic	Benzoic acid	Quercetin	Kaemferol
	P value	P value	P value	P value	P value	P value	P value	P value	P value
Concentrations of boysenberry juice	0.060	0.054	0.063	0.065	0.000	0.000	0.000	0.001	0.000
Storage temperatures	0.049	0.021	0.053	0.034	0.64	0.099	0.012	0.869	0.531
Days	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001
Blackcurrant juice was enhanced with Quercetin at concentrations of 0, 0.2, 0.6 and 1 mg/L and stored at 5 and 20 oC for 28 days									
	Anthocyanins				**Phenolic acids**				
Factors	Del-3-rut	Cyn-3-rut	Del-3-glu	Cyn-3-glu	p-coumaric	Caffeic	Benzoic acid	Quercetin	
	P value	P value	P value	P value	P value	P value	P value	P value	
Concentrations of boysenberry juice	0.051	0.050	0.067	0.060	0.001	0.000	0.002	0.000	
Storage temperatures	0.041	0.024	0.013	0.412	0.493	0.216	0.216	0.808	
Days	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
Blackcurrant juice was enhanced with Ellagic acid at concentrations of 0, 0.2, 0.6 and 1 mg/L and stored at 5 and 20 oC for 28 days									
	Anthocyanins				**Phenolic acids**				
Factors	Del-3-rut	Cyn-3-rut	Del-3-glu	Cyn-3-glu	p-coumaric	Caffeic	Benzoic acid	Quercetin	Ellagic acid
	P value	P value	P value	P value	P value	P value	P value	P value	P value
Concentrations of boysenberry juice	0.070	0.060	0.065	0.062	0.010	0.000	0.005	0.024	0.000
Storage temperatures	0.032	0.035	0.036	0.026	0.516	0.582	0.357	0.008	0.413
Days	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001

Appendix 39 Actual measurement of total anthocyanin and individual anthocyanin content (mg/L) of berry juices enhanced with boysenberry juice and phenolic acids at different concentrations

Study 2	Total anthocyanin content	Delphinidin-3-rutinoside	Cyanidin-3-rutinoside	Delphinidin-3-glucoside	Cyanidin-3-glucoside
Blackcurrant	1308.2	562.7	418.8	174.6	104.3
Blackcurrant + 1% Boysenberry	1329.2	557.5	449.3	176.2	109.6
Blackcurrant + 2% Boysenberry	1360.8	551.6	468.3	175.2	117.7
Blackcurrant + 3% Boysenberry	1379.2	553.2	479.3	177.7	128.8
	Total anthocyanin content	Peonidin-3-galactoside	Cyanidin-3-galactoside	Cyanidin-3-arabinoside	Peonidin-3-arabinoside
Cranberry	304.1	119.3	86.2	43.4	34.71
Cranberry + 1% Boysenberry	317.1	118.2	89.2	44.4	34.93
Cranberry+ 2% Boysenberry	335.1	117.6	92	44.3	35.37
Cranberry + 3% Boysenberry	352.2	118.3	98.2	43.7	35.15
	Total anthocyanin content	Cyanidin-3-glucoside	Delphinidin-3,5-diglucoside	Pelargonidin-3-glucoside	Delphinidin-3-glucoside
Pomegranate	90.4	30.5	21.5	14.3	10.6
Pomegranate + 1% Boysenberry	96.3	30.6	22.5	14.1	10.3
Pomegranate+ 2% Boysenberry	107.1	30.6	22.2	14.4	8.9
Pomegranate + 3% Boysenberry	112.3	30.5	22.1	14.5	11.3
Study 3	Total anthocyanin content	Delphinidin-3-rutinoside	Cyanidin-3-rutinoside	Delphinidin-3-glucoside	Cyanidin-3-glucoside
Blackcurrant	1139.7	539.9	387.2	172.2	99.8
Blackcurrant + 0.2mg/L Ellagic acid	1158.1	525.8	376.6	167	98.6
Blackcurrant + 0.6mg/L Ellagic acid	1202.9	529.8	375.6	166.6	99.3
Blackcurrant + 1mg/L Ellagic acid	1255.5	527.3	375.6	172.8	96.3
Blackcurrant + 0.2mg/L Kaemferol	1145	532.6	384	172.8	97.4
Blackcurrant + 0.6mg/L Kaemferol	1150.2	534.7	386.1	173.8	100.6
Blackcurrant + 1mg/L Kaemferol	1134.4	534.5	387.2	172.8	97.4
Blackcurrant + 0.2mg/L Quercetin	1142.3	526.6	370.3	168.9	97.2
Blackcurrant + 0.6mg/L Quercetin	1152.9	529.8	369.2	171.4	98.4
Blackcurrant + 1mg/L Quercetin	1150.2	530.7	375.6	170.4	95.2

Appendix 40 Mass balance calculations of total anthocyanin and individual anthocyanin content of berry juices enhanced with boysenberry juice and phenolic acids at different concentrations

Study 2	Total anthocyanin content	Delphinidin-3-rutinoside	Cyanidin-3-rutinoside	Delphinidin-3-glucoside	Cyanidin-3-glucoside
Blackcurrant + 1% Boysenberry	1255.4	557.1	414.7	172.9	107.3
Blackcurrant + 2% Boysenberry	1252.8	551.4	410.7	171.1	110.3
Blackcurrant + 3% Boysenberry	1250.3	545.8	408.8	169.4	113.3
	Total anthocyanin content	Peonidin-3-galactoside	Cyanidin-3-galactoside	Cyanidin-3-arabinoside	Peonidin-3-arabinoside
Cranberry + 1% Boysenberry	289.2	118.1	85.3	43.0	34.4
Cranberry+ 2% Boysenberry	296.4	116.9	84.4	42.6	34.0
Cranberry + 3% Boysenberry	303.5	115.7	83.6	42.1	33.7
	Total anthocyanin content	Cyanidin-3-glucoside	Delphinidin-3,5-diglucoside	Pelargonidin-3-glucoside	Delphinidin-3-glucoside
Pomegranate + 1% Boysenberry	108.5	34.2	21.3	14.2	10.5
Pomegranate+ 2% Boysenberry	117.5	37.9	21.1	14.0	10.4
Pomegranate + 3% Boysenberry	126.5	41.7	20.8	13.9	10.3
Study 3	Total anthocyanin content	Delphinidin-3-rutinoside	Cyanidin-3-rutinoside	Delphinidin-3-glucoside	Cyanidin-3-glucoside
Blackcurrant + 0.2mg/L Ellagic acid	1258.0	562.7	418.8	174.6	104.3
Blackcurrant + 0.6mg/L Ellagic acid	1258.0	562.7	418.8	174.6	104.3
Blackcurrant + 1mg/L Ellagic acid	1258.0	562.7	418.8	174.6	104.3
Blackcurrant + 0.2mg/L Kaemferol	1258.0	562.7	418.8	174.6	104.3
Blackcurrant + 0.6mg/L Kaemferol	1258.0	562.7	418.8	174.6	104.3
Blackcurrant + 1mg/L Kaemferol	1258.0	562.7	418.8	174.6	104.3
Blackcurrant + 0.2mg/L Quercetin	1258.0	562.7	418.8	174.6	104.3
Blackcurrant + 0.6mg/L Quercetin	1258.0	562.7	418.8	174.6	104.3
Blackcurrant + 1mg/L Quercetin	1258.0	562.7	418.8	174.6	104.3

Appendix 41 Actual measurement of total phenolics and individual phenolic content (mg/L) of berry juices enhanced boysenberry juice and phenolic acids at different concentrations

Study 2	Total phenolic content	p-coumaric	caffeic	benzoic	quercetin	
Blackcurrant	264.3	113.6	74.3	37.4	25.3	
Blackcurrant + 1% Boysenberry	244	99.6	64.5	36.2	27.3	
Blackcurrant + 2% Boysenberry	234.8	92.2	63.3	35.7	27.8	
Blackcurrant + 3% Boysenberry	234.3	88.9	62.7	34.9	28.1	
	Total phenolic content	Benzoic	Quercetin	Myricetin	p-coumaric	
Cranberry	145.4	55.8	42.9	22.8	14.4	
Cranberry + 1% Boysenberry	147.9	55.8	45.5	22.7	14.5	
Cranberry+ 2% Boysenberry	155.6	56.2	50.1	23.3	14.6	
Cranberry + 3% Boysenberry	162.9	55.5	54.2	22.9	14.7	
	Total phenolic content	Ellagic	Gallic			
Pomegranate	16.6	9.3	6.4			
Pomegranate + 1% Boysenberry	18.2	10.3	6.5			
Pomegranate+ 2% Boysenberry	19.1	11.4	6.4			
Pomegranate + 3% Boysenberry	19.6	12	6.4			
Study 3	Total phenolic content	p-coumaric	Caffeic	Benzoic	Quercetin	Ellagic
Blackcurrant + 0.2mg/L Ellagic acid	259	101.4	69.8	33.9	25.1	30
Blackcurrant + 0.6mg/L Ellagic acid	270.2	104.5	69.7	33.5	24.4	39.7
Blackcurrant + 1mg/L Ellagic acid	279.4	103.1	70.2	34.6	24.6	48.6
	Total phenolic content	p-coumaric	Caffeic	Benzoic	Quercetin	Kaemferol
Blackcurrant + 0.2mg/L Kaemferol	257.1	109.7	69.7	34.9	24.5	26.9
Blackcurrant + 0.6mg/L Kaemferol	272.6	105	64.8	34.8	24.9	43.8
Blackcurrant + 1mg/L Kaemferol	282.8	103.1	64	34.8	24.7	54.6
	Total phenolic content	p-coumaric	Caffeic	Benzoic	Quercetin	
Blackcurrant + 0.2mg/L Quercetin	247.4	108.6	70.4	34.7	32.8	
Blackcurrant + 0.6mg/L Quercetin	258	106.5	70.2	34.4	43.2	
Blackcurrant + 1mg/L Quercetin	271.6	106.8	70.6	34.1	57.6	

Appendix 42 Mass balance calculations of total phenolics and individual phenolic content (mg/L)of berry juices enhanced boysenberry juice and phenolic acids at different concentrations

Study 2	Total phenolic content	p-coumaric	caffeic	benzoic	quercetin	
Blackcurrant + 1% Boysenberry	253.4	112.4	73.6	37.0	25.0	
Blackcurrant + 2% Boysenberry	256.0	111.3	72.8	36.7	24.8	
Blackcurrant + 3% Boysenberry	258.7	110.2	72.1	36.3	24.5	
	Total phenolic content	Benzoic	Quercetin	Myricetin	p-coumaric	
Cranberry + 1% Boysenberry	139.6	55.2	44.4	22.5	14.3	
Cranberry+ 2% Boysenberry	143.4	54.7	45.8	22.3	14.1	
Cranberry + 3% Boysenberry	147.2	54.1	47.3	22.1	14.0	
	Total phenolic content	Ellagic	Gallic			
Pomegranate + 1% Boysenberry	20.6	9.2	7.6			
Pomegranate+ 2% Boysenberry	25.6	9.1	8.8			
Pomegranate + 3% Boysenberry	30.6	9.0	10.0			
Study 3	Total phenolic content	p-coumaric	Caffeic	Benzoic	Quercetin	Ellagic
Blackcurrant + 0.2mg/L Ellagic acid	250.7	113.6	74.3	37.4	25.3	0.0
Blackcurrant + 0.6mg/L Ellagic acid	250.7	113.6	74.3	37.4	25.3	0.0
Blackcurrant + 1mg/L Ellagic acid	250.7	113.6	74.3	37.4	25.3	0.0
	Total phenolic content	p-coumaric	Caffeic	Benzoic	Quercetin	Kaemferol
Blackcurrant + 0.2mg/L Kaemferol	250.7	113.6	74.3	37.4	25.3	0.0
Blackcurrant + 0.6mg/L Kaemferol	250.7	113.6	74.3	37.4	25.3	0.0
Blackcurrant + 1mg/L Kaemferol	250.7	113.6	74.3	37.4	25.3	0.0
	Total phenolic content	p-coumaric	Caffeic	Benzoic	Quercetin	
Blackcurrant + 0.2mg/L Quercetin	250.7	113.6	74.3	37.4	25.3	
Blackcurrant + 0.6mg/L Quercetin	250.7	113.6	74.3	37.4	25.3	
Blackcurrant + 1mg/L Quercetin	250.7	113.6	74.3	37.4	25.3	