Phytochemical variation during blueberry juice processing

A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Biotechnology at Massey University, New Zealand.

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ABSTRACT

Blueberry is regarded as a 'super fruit' by many consumers and believed to offer health benefits for humans. It is well known for its high antioxidant levels and for the diversity of its anthocyanins. Blueberries can be eaten fresh but are very perishable, so are commonly kept frozen and available all year round. Frozen blueberries are suitable for a range of products including juice. During juicing, there are likely to be changes in phytochemical constituents arising from the various processing steps. These changes lead to variable composition of the finished juice and uncertain impacts on the 'health value' of the product. Therefore, this study focused on evaluating three major phytochemicals (anthocyanins, chlorogenic acid (CGA), and procyanidin B2) throughout juice processing in order to model compositional change.

Blueberry juice processing involves a series of unit operations: thawing, blanching, mincing, enzyme treatment, separation of juice from pomace, pasteurisation, and bottling. Enzymatic degradation occurs during thawing of blueberries as they still contain 'live' oxidases. Prolonged thawing at warm temperatures would therefore be particularly bad for phytochemical degradation. If these oxidases are destroyed by blanching, thermal degradation also occurs but was found to be less aggressive than polyphenoloxidase (PPO) activity. Blanching at high temperature (≥ 70 °C) for 3 min eliminated PPO and significantly increased the phytochemical concentration in the juice but it induced pectin gel formation which reduced juice recovery. Depectinisation is essential after berry blanching to dissolve pectin gel and to avoid juice volume penalty. Significant losses of phytochemicals were also observed during pressing of the berries into juice, due to physical associations between the

phytochemicals and the berry matrix, and entrapment. Blanching at 90 °C for 3 min followed by pectinase enzyme treatment at 50 °C for 2 h was the best way to deliver high phytochemical concentration in the juice with high juice volume recovery and acceptable viscosity. There is a risk that juices with high phytochemical concentration will seem bitter or astringent. This was found not to be the case in sensory trials, with consumers consistently preferring the high-phytochemical juices; it seems sugars in the juice masked any adverse perceptions.

Because of the complexity of blueberry juice processing, the processing model developed in this study was simplified into three components: a defrost model, a recovery model and a thermal model. In short, the defrost model was used for the whole berry phase during thawing when PPO was still active; the recovery model accounted for losses into the pomace; and the thermal model covered the subsequent liquid phase. These processing models were able to predict anthocyanin and CGA changes throughout processing (particularly in blanched products) but procyanidin B2 behaviour was not predictable.

This modelling approach provides the ability to predict variations in composition arising from changes in the juicing process and offers manufacturers the opportunity to produce consistent blueberry juice with a high phytochemical concentration.

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In loving memory of my mother

You are so loved and missed....

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

AOAC	Association of Official Analytical Chemists
BI	browning index
C	concentration
C_o	initial phytochemical concentration
CGA	chlorogenic acid
DW	dry weight
E_a	activation energy
FCP	free choice profiling
FW	fresh weight
GRG	generalized reduced gradient algorithm
h	hour
H_2O_2	hydrogen peroxide
HPLC	high performance liquid chromatography
IDF	insoluble dietary fiber
k	rate constant
k_o	frequency factor
MAF	Ministry of Agriculture and Fisheries (up till 1995) / Ministry of Agriculture and Forestry
min	minute
NTU	Nephelometric Turbidity Units
ORAC	Oxygen radical absorbance capacity
PCA	principal component analysis
PET	polyethylene terephthalate
PFR	NZ Institute for Plant and Food Research Ltd
POD	peroxidase
PPO	polyphenoloxidase
PVPP	polyvinylpolypyrrolidone
R	universal gas constant
R^2	regression coefficient
s	second
SDF	soluble dietary fiber
SSE	sum of squares error
t	time
T	temperature
$t_{1/2}$	half-life
TA	titratable acidity
TDF	total dietary fiber
T_{ref}	arbitrary reference temperature
TSS	total soluble solids
T_t	temperature at time
β	thermal history

1. Research background, literature review and objective

1.1. Research background

It is a commonly held belief among consumers that the consumption of fresh fruits or vegetables is more beneficial than consuming processed products. Exploiting this consumer belief however, is difficult for the blueberry industry as blueberries have a short growing season and the fresh fruit has a short shelf life. Blueberries are harvested during the summer months and have a short natural postharvest lifespan, so the warm summer temperatures make blueberries extremely perishable. A potential solution to the shelf life issue is the adoption of freezing technology which enables blueberries to be available all year round. Freezing blueberries, in addition to enabling the public to consume whole blueberries throughout the year, gives processing companies the opportunity, due to the economies of scale when processing large volumes, to manufacture a wide range of products in an economically feasible manner. Thus freezing opens up more opportunities to deliver blueberry-derived health benefits for consumers through a diverse product range. Juice is one of these options and it also offers the industry a greater return compared to frozen berries.

Blueberries are considered as a 'super fruit' due to their high antioxidant capacity which is associated with a level of high phenolic compounds (Szajdek & Borowska, 2008). The antioxidant activity of fresh blueberries is typically around 2400 units ORAC activity / 100 g berries and this is among the highest activity of any fruit or vegetable (Su & Chien, 2007). The phenolic compounds in blueberries include anthocyanins, flavonols, hydroxycinnamic acids, flavones, flavanonols, and flavan-3-ols (Cho et al., 2005). All these compounds are believed to play an important role

in health promotion including antioxidant, anticarcinogenic, and antihypertension effects. The reader is referred to the article by Kalt & Dufour, (1997) for a more comprehensive review of blueberry health benefits.

Due to disruption of the berry microstructure, detrimental changes to phytochemicals are more likely during juice processing, compared to fresh and intact whole frozen blueberries. Therefore, it is a challenge to deliver those phytochemicals in a more stable form in the juice. The juice manufacturing process, which involves various unit operations, can result in extensive losses of phytochemicals in the final products. Phytochemical loss may be the direct result of processing (such as thermal degradation or oxidation processes) or the degradation may also occur due to degradation enzymes such as polyphenoloxidase (PPO). Further losses of phytochemicals in the juice also occur during storage - particularly over time at higher temperatures.

Phytochemical changes in the final juice product may also have an impact on the sensory quality and thus consumer acceptance. Polyphenol-rich beverages are usually associated with astringent and bitter characteristics that may affect consumer acceptance. The key factors influencing sensory quality and consumer acceptability during different processing methods remain uncertain and need to be explored to make sure the product is ready for the market.

1.2. Blueberry and blueberry industry

Blueberry belongs to the *Ericaceae* family and sub-family *Vacciniaceae*. Blueberries are found in many areas of the world, but have gained greatest acceptance in the

United States of America (USA) and Canada, perhaps because of the abundance of Lowbush blueberry (*Vaccinium angustifolium*) growing in the wild in this region. There are several blueberry species and hybrids found in the marketplace (Table 1-1). However, only the first four have commercial importance and are widely discussed in the literature.

Table 1-1: Types of blueberry (Hortideas, 2010)

Common name	Species name
Highbush	*V. corymbosum*
Southern Highbush	*V. corymbosum* hybrid
Rabbiteye	*V. ashei*
Lowbush	*V. angustifolium*
Half highbush	*V. corymbosum* introgressed by *V. angustifolium*
Constables's blueberry	*V. constablei*
Ornamental blueberry	*V. simulatum* and unknown species

The various species are all sold as 'blueberries', despite their diverse genetic origin. It is not always clear which one is referred to in publications, although to a blueberry grower they look and taste dissimilar and biochemically they differ in composition. This has led to considerable lack of clarity in the literature.

Blueberries are regarded as having several health benefits for humans. They are well known for their high antioxidant levels, and are widely reported as having the most diverse array of anthocyanins of any commonly-consumed fruit (Cao, 1996; Prior et al., 1998; Häkkinen et al., 1999; Moyer et al., 2002).

Blueberries contain phenolic compounds, including anthocyanins, flavonols, chlorogenic acid (CGA), and procyanidins (Cho et al., 2005), which are believed to be associated with such benefits as a reduction in coronary heart disease, improvement in brain health and memory, improvement in eyesight, the treatment of urinary tract infections, and a reduction in the risk of cancer (Kalt & Dufour, 1997; Skrede et al., 2000; Huntley, 2009). Blueberries may be eaten raw or processed into juice or wine and other items; and may be frozen or cooked before consumption. However, it is not clear to what extent the health benefits (and the phytochemicals that are believed to be responsible) are retained after these various forms of processing.

In New Zealand, the blueberry industry was established in the 1970s, when the Ministry of Agriculture and Fisheries (MAF) imported blueberry cultivars from the USA. After ten years evaluating the potential of blueberry as a commodity crop, MAF decided to begin a selection programme, in order to increase fruit size and quality, improve post-harvest performance, and extend the fruiting season of the blueberry. Early season Highbush cultivars ('Puru', 'Nui' and 'Reka') were released in 1988 and late season Rabbiteye cultivars ('Maru' and 'Rahi') in 1991. These cultivars have also been released to nurseries in Europe, Japan, and USA (under licence) with the royalties flowing back to NZ Institute for Plant and Food Research Ltd (PFR; New Zealand Horticulture Facts & Figures: Fruit, flowers & vegetables, 2004).

Currently there are about 60 blueberry growers in New Zealand with approximately 400 hectares planted. The majority of blueberries were planted in the upper North

Island especially the Waikato region and new areas are being planted in the South Island (Blueberry New Zealand: fruit for thought, 2014). Blueberry exports commenced during the 1980s and reached $2 M in 1985. The domestic and export value increased from year to year and recent statistics show that, in 2013, domestic sales for fresh and frozen blueberries were $10.4 M and $4 M respectively. The export market is worth $22.7 M and $3 M for fresh and frozen blueberry respectively. Total industry value is therefore ca. $40 M with the main export markets for New Zealand fresh blueberries being Australia, USA, and Japan (Fresh Facts, 2014).

1.3. Potential health benefits of blueberries

The health benefits of blueberries became widely accepted after several studies found they had the highest antioxidant activity among the fruits and vegetables evaluated (Gao & Mazza, 1994; Wang et al., 1997; Cao et al., 1998; Prior et al., 1998; Wu et al., 2004; Wu et al., 2006). Antioxidants are chemicals that may prevent or delay some types of cell damage by inhibiting oxidation of the cells. In biological systems, lipid oxidation can produce toxic compounds and this initiates other harmful reactions (Su & Chien, 2007). Phenolic compounds can act as antioxidants in many potential ways such as free radical-scavenging, oxygen radical absorbance, and chelating of metal ions (Halliwell et al., 1995).

There is widespread belief that high antioxidant activity in fruits and vegetables can increase the health benefits and this debate is ongoing. However, the current understanding is that there is a more specific relationship between particular ‘antioxidants’ with particular benefits and these effects may not depend on

antioxidant activity *per se* (Heyes, 2013). This is because the antioxidants we eat are poorly absorbed into the bloodstream and we have an efficient antioxidant system in our blood already (Manach et al., 2005; Stevenson & Hurst, 2007). Direct health benefits may come from tiny quantities of particular phytochemicals triggering changes in gene expression and upregulating the body's natural defence mechanism (Munday et al., 2008). Indirect health benefits from antioxidant consumption may also occur via modification of the gut flora, which plays an important part in our overall health (Scharlau et al., 2009).

There are many studies on the phenolic content and antioxidant activity of blueberries (Prior et al., 1998; Kalt et al., 2000; Moyer et al., 2002; Sellappan et al., 2002; Carlson, 2003; Zheng et al., 2003; Cho et al., 2005; Schmidt et al., 2005; Brambilla et al., 2008; Brownmiller et al., 2008; Castrejón et al., 2008; Koca & Karadeniz, 2009; Howard et al., 2010; Eichholz et al., 2011). Most have studied the correlation between antioxidant activity and the concentration of phenolic compounds, and have found that antioxidant activities of blueberry are highly correlated with phenolic compounds. These findings sparked numerous investigations into the health benefits of blueberries which led to *in vivo* and *in vitro* studies. Because of the diversity in blueberries of phytochemicals that are biochemically 'antioxidants', it is possible that there is a link between particular classes of phytochemical and particular health benefits in particular conditions. However, as a general rule, whenever a particular compound is proposed as a 'bioactive' compound and subjected to clinical testing in the form of a supplement, it does not perform as well as a diet rich in fruit and vegetables. This implies that other fruit components may be necessary to obtain the full health benefit i.e. the health

benefit may not be solely due to the isolated bioactive compound. Studies on blueberry supplementation in diets have been associated with: improvements in features of metabolic syndrome; reducing the risk of certain cancers; improving visual acuity; improving brain health and memory; weight management; improving gut health and muscle repair. These are summarised and tabulated in Table 1-2.

Table 1-2: Summary of the potential health benefits of blueberry

Potential health benefit	Reference	Summary of finding
Metabolic syndrome	Bolling (2009)	Rats with a blueberry-enriched diet had less abdominal fat, lower triglycerides, lower cholesterol, and improved fasting glucose and insulin sensitivity.
	Basu et al. (2010)	Consumption of freeze-dried blueberry beverage had no effect on inflammatory biomarkers but it improved features of metabolic syndrome, especially in relation to cardiovascular risk.
	Pranprawit (2014)	Addition of blueberry to rat diet improved management of some markers of metabolic syndrome, especially insulin sensitivity and glucose tolerance.
Cancer	Bomser et al. (1996)	Lowbush blueberry procyanidins induced enzymes that protect against cancer and reduce rapid tumour growth.
	Vinson et al. (2001)	High levels of antioxidants in blueberry may contribute to anti-carcinogenic properties by inhibiting reactive oxygen species, which otherwise may increase the risk of cancer.
	Schmidt et al. (2005)	Procyanidins from both Highbush and Lowbush blueberry inhibited the androgen-dependent growth of prostate cancer cells.

Table 1-2 (continued): Summary of the potential health benefits of blueberry

Potential health benefit	Reference	Summary of finding
Cancer	Matchett et al. (2006)	Lowbush blueberry procyanidins inhibited metastasis regulators, matrix metalloproteinases in DU145 human prostate cancer cell lines.
	Yi et al. (2006)	Phenolic compounds in Rabbiteye blueberry ('Briteblue', 'Tifblue' and 'Powderblue') could inhibit HepG2 liver cancer cell population growth, and induce apoptosis.
Eye health	Kajimoto (1999)	Consumption of blueberry extracts may improve weak eyesight symptom and offer significant protection against retinal degeneration and development of age related eye problems such as cataracts.
Brain health and memory	Andres-Lacueva et al. (2005)	After blueberry consumption, anthocyanins have been identified in specific cerebral sites, including the hippocampus and neocortex, which are essential regions for cognitive function: and anthocyanin distribution in the hippocampus has been related to increasing neuronal signalling in that structure
	Willis et al. (2005)	Blueberry extracts can improve neuronal deficit in aged animals. The finding suggests that compounds in blueberry may have a significant effect on the development and organisation of the intraocular hippocampal, one of the important brain regions.
	Krikorian et al. (2010).	Consumption of wild blueberry juice, commercially prepared from Lowbush blueberry (*V. angustifoilum*), may improve the memory of older adults. The study suggests that moderate-term blueberry juice supplementation may have some benefits on neuro-cognition and it establishes a basis for more comprehensive human trials, in order to study the preventive potential of juice consumption and neuronal mechanisms

Table 1-2 (continued): Summary of the potential health benefits of blueberry

Potential health benefit	Reference	Summary of finding
Gut health	Molan et al. (2009)	Addition or administration of blueberry water extract could modify the bacteria profile in humans by increasing the numbers of beneficial bacteria (*Lactobacillus rhamnosus* and *Bifidobacterium breve)* and thereby improving gut health.
Muscle repair	Galvano et al. (2004), McAnulty et al. (2004)	Blueberry supplementation may be beneficial for athletes, who are exercising in hot environments, through decreased lipid hydroperoxidase in plasma.
	Connolly et al. (2006)	Antioxidants can inhibit the biological changes that occur in muscle during fatigue and slow the decline in force output. Therefore, a dietary supplementation of antioxidants from blueberry is an alternative and attractive choice for athletes or health practitioners, who are engaged in intensive or excessive training programmes.
	Hurst et al. (2010)	Anthocyanins in blueberry and the pure individual glycosides (malvidin galactoside and/or malvidin glucoside), were the active compounds which alleviated muscle damage caused by oxidative stress.
	McLeay et al. (2012)	Consumption of blueberry smoothies may improve muscle performance and recovery in human subjects but this early research needs confirmation with an improved study design and larger sample size.
Weight management	Molan et al. (2008)	Blueberries may be a good satiety inducer and weight management modulator. Rats given premeal of blueberry extracts showed reduction of food intake compared to control rats and the decrease in food intake was mainly because of satiating effect not a stomach distension effect.

1.4. Phytochemical composition of blueberry

Phytochemicals are chemical compounds found naturally in plants. They can be classified into seven major classes (Figure 1-1) according to their chemical structure (Erdman et al., 2007). Phenolics are the largest class of phytochemicals and consist of phenolic acids, flavonoids, tannins, coumarins, and stilbenes. Among these subclasses, flavonoids are the most diverse compounds and include flavonols, flavones, flavan-3-ols, flavanones, anthocyanidins, and isoflavones.

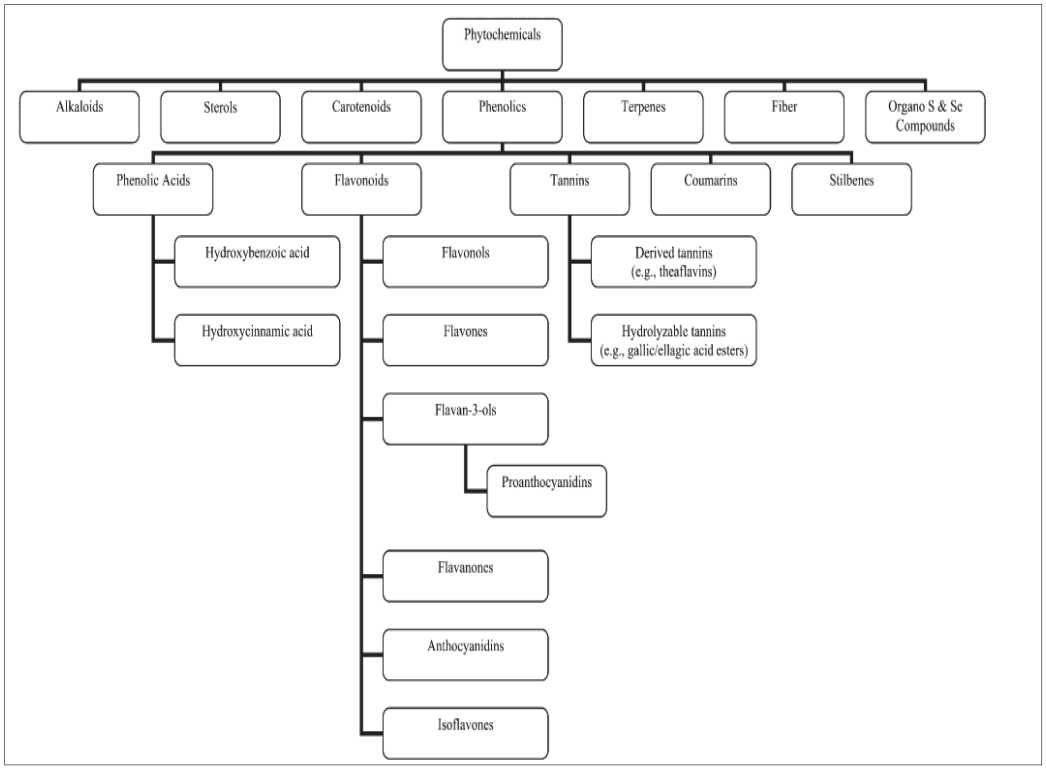


Figure 1-1: Phytochemical classes (Erdman et al., 2007).

The phytochemical profile and composition of blueberry is well documented (Castrejón et al., 2008) but, as stated earlier, the term 'blueberry' refers to a commercial entity which includes at least five different species. Generally, blueberries are known to contain high levels of phenolic compounds, including

anthocyanins, flavonols, CGA, and procyanidins, which have high biological activity and may provide health benefits as antioxidants, in addition to preventing disease (Cho et al., 2005; Howard & Hager, 2007).

Many researchers have reported that there are significant differences in the phytochemical profile of blueberry varieties: not only in the main cultivated blueberry, the Northern and Southern Highbush blueberries, but also the Lowbush (or wild blueberry) and Rabbiteye blueberry (Prior et al., 1998; Häkkinen et al., 1999; Taruscio et al., 2004). Concentrations of phytochemicals in blueberry are influenced by many factors, including environmental conditions, the degree of ripeness, cultivar, cultivation site, processing, and storage of the fruit (Beattie et al., 2005). There are several studies on the concentrations of phytochemicals in the main blueberry types. Koca & Karadeniz (2009) reported that, in general, total anthocyanin and total phenolic concentrations of Lowbush blueberry were higher than those found in Highbush blueberry. This finding is consistent with earlier reports by Kalt et al. (1999). Other studies have indicated that Rabbiteye varieties have higher concentrations of total phenolics and anthocyanins than Southern Highbush blueberry varieties (Howard & Hager, 2007). According to Prior et al. (1998) the Lowbush blueberry has higher concentrations of total phenolics and anthocyanins than Rabbiteye, Southern Highbush, or Highbush blueberry. The concentrations of total phenolics, total anthocyanins, total procyanidin, and total flavonols in Highbush, Lowbush, Rabbiteye and Southern Highbush blueberry have been reported by Howard & Hager (2007) (Table 1-3).

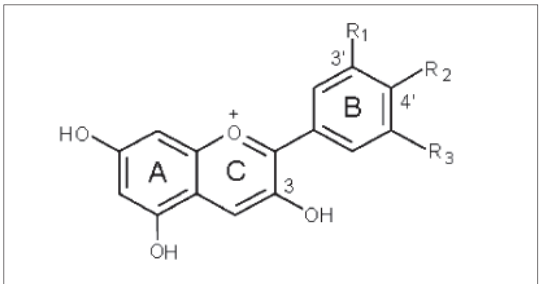
Table 1-3: Phytochemical content of different types of blueberry. Adapted from Howard & Hager, (2007).

mg/100 g FW	Total phenolic	Total anthocyanin	Total procyanidin	Total flavonols
Highbush	106-435	20-269	180	9-40
Lowbush	295-495	91-260	332	na
Rabbiteye	231-961	13-515	na	3-17
Southern Highbush	116-586	35-823	na	17-33

na=not available

1.4.1. Anthocyanins

Anthocyanins are the major compound in blueberry responsible for the red, blue and purple colours of the berry and are predominantly present in the vacuoles of berry skin. Anthocyanins are water soluble compounds and have the same basic anthocyanidin structure (Figure 1-2) but vary in the number and position of hydroxyl and methoxyl groups, the identity, number and position at which sugars are attached, and the extent of sugar acylation and the acylating agent (Wu et al., 2006).



ANTHOCYANIDIN	R_1	R_2	R_3
Pelargonidin (Pg)	H	OH	H
Cyanidin (Cy)	OH	OH	H
Delphinidin (Dp)	OH	OH	OH
Peonidin (Pn)	OMe	OH	H
Petunidin (Pt)	OMe	OH	OH
Malvidin (Mv)	OMe	OH	OMe

Figure 1-2: Chemical structures of six commonly anthocyanins (Wu et al., 2006).

Although making a comparison of the phenolic content of different blueberry types from the literature is difficult, because of the various analytical methods used (Häkkinen et al., 1999), approximately 281 anthocyanins have been characterised by HPLC-MS, as shown in Table 1-4.

Blueberry contains monoglycosides (glucoside, galactoside, and arabinoside) of delphinidin, cyanidin, petunidin, peonidin, and malvidin. The anthocyanin composition and content of blueberry is influenced by genetics, with some generalisations beginning to emerge. Southern Highbush genotypes contain high levels of galactosides and arabinosides and low levels of glucosides, whilst the Northern Highbush genotype contains similar levels of the three glycosides, indicating that synthesis of the transferases involved in attachment of specific sugar moieties is genetically coordinated (Zhao, 2007). The effect of genetics on the composition of acylated anthocyanins has been reported (Gao & Mazza, 1995). They found that 7 of 10 Lowbush blueberry genotypes did not vary significantly in anthocyanin composition, but three genotypes had practically no acylated anthocyanins, suggesting that the synthesis of transferases involved in the attachment of acyl groups is also under genetic control.

The percent distribution of anthocyanidins was shown to vary amongst commercially important blueberry species, with cyanidin, delphinidin, malvidin, peonidin, and petunidin showing 14, 38, 24, 7, and 16% respectively in the Lowbush blueberry: and 6, 41, 32, 1, and 19% respectively, in the Highbush blueberry. Acetylated anthocyanins are prevalent in the Lowbush blueberry, but generally not in cultivated blueberry (Prior et al., 1998).

Table 1-4: Identified anthocyanins in blueberry from various sources. Adapted from Howard & Hager (2007).

Anthocyanins	m/z [M+]	Reference				
		Fragments	Wu et al. (2006)	Cho et al. (2005)	Gao & Mazza (1994)	Skrede et al. (2000)
Delphinidin 3-galactoside	465	303	√	√	√	√
Delphinidin 3-glucoside	465	303	√	√	√	√
Cyanidin 3-galactoside	449	287	√	√	√	√
Delphinidin 3-arabinoside	435	303	√	√	√	√
Cyanidin 3-glucoside	449	287	√	√	√	√
Petunidin 3-galactoside	479	317	√	√	√	√
Cyanidin 3-arabinoside	419	287	√	√	√	
Petunidin 3-glucoside	479	317	√	√	√	√
Peonidin 3-galactoside	463	301	√	√	√	√
Petunidin 3-arabinoside	449	317	√	√	√	√
Peonidin 3-glucoside	463	301	√	√		
Malvidin 3-galactoside	493	331	√	√	√	√
Peonidin 3-arabinoside	433	301			√	
Malvidin 3-glucoside	493	331	√	√	√	√
Malvidin 3-arabinoside	463	331	√	√	√	√

Table 1-4 (continued): Identified anthocyanins in blueberry from various sources. Adapted from Howard & Hager (2007).

Anthocyanins	m/z [M+]	Reference				
		Fragments	Wu et al. (2006)	Cho et al. (2005)	Gao & Mazza (1994)	Skrede et al. (2000)
Petunidin pentoside	449	317	√			
Cyanidin 3-(malonyl)glucoside	535	287	√			
Cyanidin 3-(6”-acetyl)galactoside	491	287	√			
Malvidin acetyl hexoside	535	331	√			
Delphinidin 3-(malonyl)glucoside	551	303	√			
Malvidin 3-(malonyl)glucoside	579	331	√			
Delphinidin 3-(6”-acetyl)glucoside	507	303	√	√		
Peonidin 3-(6”-acetyl)galactoside	505	301	√			
Cyanidin 3-(6”-acetyl)glucoside	491	287	√	√		
Malvidin 3-(6”-acetyl)galactoside	535	331	√	√		
Petunidin 3-(6”-acetyl)glucoside	521	317	√	√		
Peonidin 3-(6”-acetyl)glucoside	505	301	√	√		
Malvidin 3-(6”-acetyl)glucoside	535	331	√	√		√

A more comprehensive study on anthocyanin composition in blueberry differentiated a large number of species in the *Vaccinium* genus (Scalzo et al., (2008). They found that ornamental blueberries (*V. simulatum* and unknown species) had the highest concentrations of total anthocyanins, followed by Rabbiteye (*V. ashei*), Southern Highbush (*V. corymbosum* hybrid), and Northen Highbush (*V. corymbosum*) with 3093, 2102, 1524, and 1453 µg/g fruit respectively.

1.4.2. Flavonols

Blueberry flavonols are located predominantly in the skin, with a small amount found in the seed and none detected in the flesh. Types of flavonols are distinguished by the number of hydroxyl group on the B ring (Figure 1-3). Blueberries contain a large number of flavonols compared to other berries (Howard & Hager, 2007).

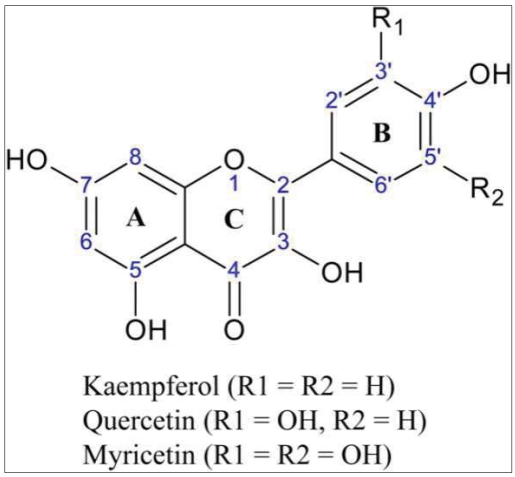


Figure 1-3: Chemical structure of three main flavonols.

Cho et al. (2005) reported that 17 flavonols had been identified using HPLC/ESI-MS (Table 1-5). In general, the content of quercetin is higher than myricetin or kaempferol in blueberry (Hakkinen et al., 1999). A similar finding by Bilyk &

Sapers (1986) suggests that ripe Highbush blueberry varieties contain mainly quercetin, with no kaempferol or myricetin.

The amounts of quercetin in lyophilised samples of 'Northblue' are more than 100 mg per 100 g seedless dry weight and it had the highest levels of flavonols found in blueberry (Hakkinen et al., 1999). Beattie et al. (2005) and Howard & Hager (2007) suggested that quercetin 3-galactoside is the most predominant flavonol in blueberry.

Table 1-5: Identified flavonols from blueberries (Cho et al., 2005).

Flavonol glucoside	m/z	
	M−	Fragments
Myricetin 3-hexoside	479	317 [M − hexose], (myricetin)
Quercetin 3-rutinoside	609	463 [M − rhamnose], 301 [463-glucose], (quercetin)
Quercetin 3-galactoside	463	301 [M − galactose], (quercetin)
Quercetin 3-methoxyhexoside	493	463 [M − methoxy], 301 [463-hexose], (quercetin)
Quercetin 3-glucoside	463	301 [M − glucose], (quercetin)
Quercetin 3-pentoside	433	301 [M − pentose], (quercetin)
Quercetin 3-glucuronide	477	301 [M − glucuronic acid], (quercetin)
Quercetin 3-*O*-[6__-(3-hydroxy-3-methylglutaroyl)]-*β*-galactoside	607	463 [M − hydroxymethylglutaric acid], 301 [463-galactose], (quercetin)
Quercetin 3-glucosylpentoside	595	433 [M − glucose], 301 [433-pentose], (quercetin)
Quercetin 3-caffeoylgalactoside	623	463 [M − caffeic acid], 301 [463-galactose], (quercetin)
Quercetin 3-caffeoylglucoside	623	463 [M − caffeic acid], 301 [463-glucose], (quercetin)
Quercetin 3-oxalylpentoside	505	433 [M − oxalic acid], 301 [433-pentose], (quercetin)
Quercetin 3-rhamnoside	447	301 [M − rhamnose], (quercetin)
Quercetin 3-dimethoxyrhamnoside	507	477 [M − methoxy], 447 [477-methoxy], 301 [447-rhamnose], (quercetin)
Quercetin 3-acetylgalactoside	505	463 [M − acetic acid], 301 [463-galactose], (quercetin)
Quercetin 3-acetylglucoside	505	463 [M − acetic acid], 301 [463-glucose], (quercetin)
Quercetin	301	(Quercetin)

1.4.3. Chlorogenic acid (CGA)

CGA (5-O-caffeoylquinic acid) (Figure 1-4) is the predominant hydroxycinnamic acid ester found in blueberry and it is the single most abundant polyphenolic acid ester found in the fruit. Apart from CGA, specific hydroxybenzoic and hydroxycinnamic acid derivatives that have been identified in blueberry include neochlorogenic acid (3-caffeoyquinic acid), 5-p-coumaroylquinic, 5-feruolyquinic acid, and the β-D-glucosides of caffeic, p-coumaric, ferulic, p-hydroxybenzoic, protocatechuic, and gallic acids (Howard & Hager, 2007). Stoehr & Herrmann (1975) found that caffeic acid is the main phenolic acid in blueberry, but this finding contradicts the data reported by Hakkinen et al. (1999), which indicated that caffeic acid content was low and ferulic acid was the main phenolic compound in blueberry.

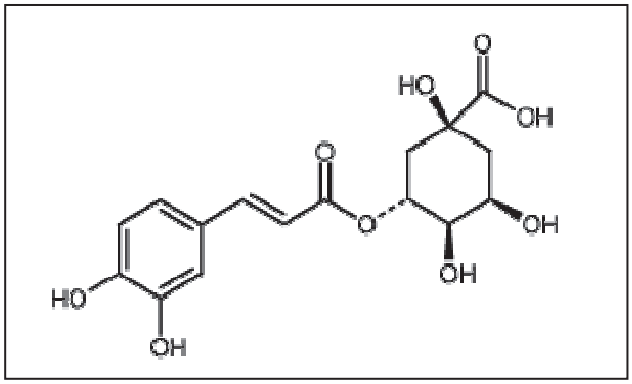


Figure 1-4: Structure of chlorogenic acid.

1.4.4. Procyanidin

Proanthocyanidins are also present in blueberry (Prior et al., 2001; Moyer et al., 2002; Taruscio et al., 2004) and the major class of proanthocyanidins found is procyanidins, which is composed exclusively of epi (catechin) units. A study by Prior et al. (2001) confirmed that a series of oligomers found in blueberries consisted

of (epi) catechin units, which were exclusively and singly linked (B1, monomer) through to B8 (octamer) (Figure 1-5).

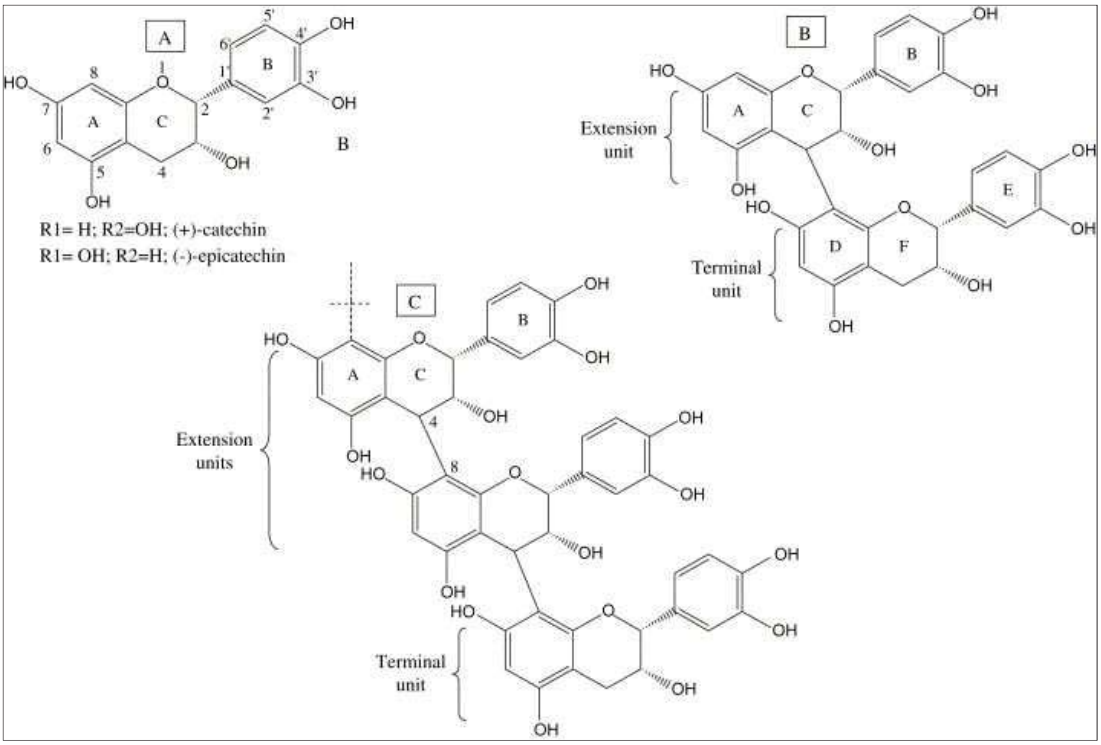


Figure 1-5: General structure of flavan-3-ol unit. A) (+)catechin and (-)epicatechin monomers, B) procyanidin B2 dimer, and C) procyanidin oligomers with C4-C8 linkage (Contreras-Domínguez et al., 2006).

The total proanthocyanidin content of Highbush and Lowbush is 180 and 332 mg/100 g FW, respectively. Polymeric flavan-3-ols (a degree of polymerisation greater than 10), the predominant proanthocyanidin in Lowbush blueberry, were characterised by Gu et al. (2002), who reported a range in the degree of polymerization from 20 to 114, with epicatechin accounting for 100% of the extension units, and catechin and epicatechin accounting for 67 and 33% of the terminal units, respectively. Epicatechin is the major flavan-3-ol present in blueberry, at a concentration of 1 mg/100 g FW.

1.4.5. Other compounds

Ellagitannins and ellagic acid derivatives have not been identified in blueberry. This is consistent with the low concentration of total ellagic acid found, as reported by Hakkinen et al. (1999), with only 0.9 – 2.3% in the fruit acid hydrolysate. Blueberry also lacks the genetic capacity to synthesize ellagitannins and ellagic acid derivatives (Howard & Hager, 2007).

According to Mazur et al. (2000) as reported by Howard & Hager (2007), blueberry contains 0.84 mg/100 g dry weight (DW) of secoisolariciresinol, a type of lignan, which is associated with the prevention of cancers, osteoporosis, and coronary heart disease. Sterols have also been found in blueberry. Piironen et al. (2000) found that sitosterol was the predominant sterol with 22.2 mg/100 g fresh weight (FW). Sterols were present at low concentrations with 1.7 mg/100 g FW, whilst the content of plant sterol in blueberry was reported as 26.4 mg/100 g FW. Another class of phytochemical contained in blueberry is stilbenes. Pterostilbene and piceatannol have been found in Rabbiteye and Highbush, which contained 9.9 to 52.0 mg/ 100 g DW and 18.6 to 42.2 mg/100 g DW, respectively.

Blueberry is a unique and special fruit because of its diversity of phytochemicals, especially phenolic compounds which include anthocyanins, flavonols, CGA, and procyanidin. All these compound have high biological activity and they may provide health benefits as discussed previously. The key phytochemicals likely to have a bioactive role in blueberry are anthocyanins, CGA and procyanidin because of the diversity of anthocyanins and the high concentration of all three groups.

1.5. Degradation of phytochemicals

Phytochemicals in blueberries have been shown to be associated with health benefits. It is a huge challenge to retain those phytochemicals in processed blueberries or blueberry products. Phytochemicals in processed blueberry products are likely to change or be lost during processing because of factors such as pH, temperature, oxygen, and degradative enzymes. This section will discuss factors that influence the degradation of the key phytochemicals (anthocyanins, CGA and procyanidin) in blueberries.

1.5.1. Degradation of anthocyanins

There are a few studies describing degradation of anthocyanins and these found that anthocyanins are greatly affected by thermal and degradative enzymes. However, the exact mechanisms for both types of degradation have not been fully elucidated.

Fennema (1996) proposed three possible pathways for thermal degradation of anthocyanins, with coumarin 3,5-diglycoside as a common degradation product for anthocyanin (Figure 1-6). In the first pathway, the flavylium cation is first transformed to the quinonoidal base, then to several intermediates, and finally to the coumarin derivative and a compound corresponding to the B-ring. The second pathway suggests that the flavylium cation is first transformed to the colourless carbinol base, then to the chalcone and finally to brown degradation products; and in the last pathway, the flavylium cation is first transformed to the colourless carbinol base, then to chalcone and degradation products of chalcone, and finally to brown degradation products.

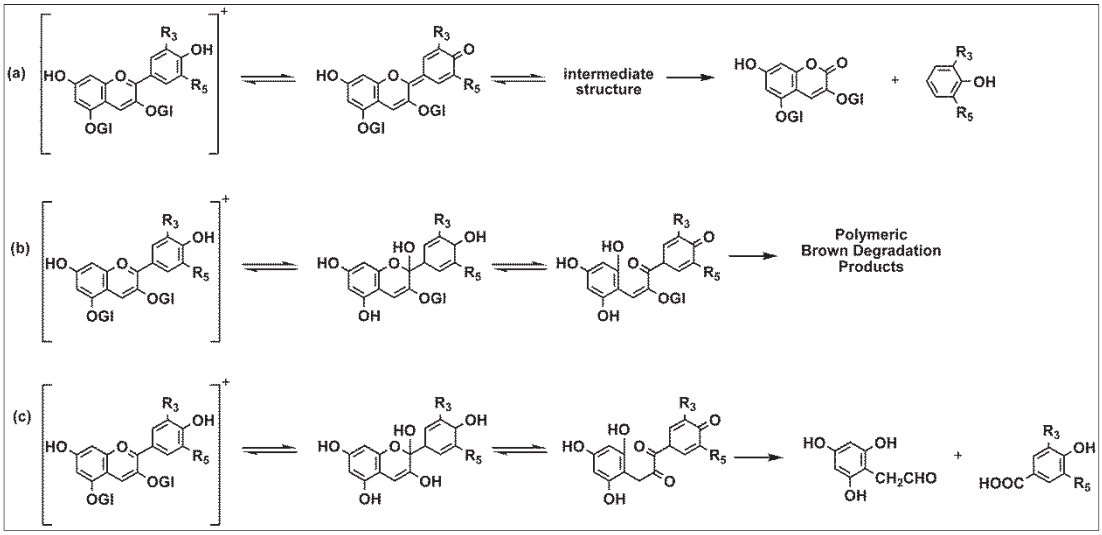


Figure 1-6: Mechanism of thermal degradation of anthocyanin as proposed by Fennema (1996).

Oren-Shamir (2009) proposed three alternative pathways to describe enzymatic degradation of anthocyanins and tissue browning in fruit extracts (Figure 1-7). Degradative enzymes such as peroxidase (POD) and polyphenoloxidase (PPO) in the presence of oxygen and o-diphenol are able to degrade anthocyanins eventually resulting in browning of tissues. POD is dependent on H_2O_2 while PPO is dependent on O_2 for its activity. The first pathway is coupled oxidation, i.e. reduction of quinones of phenolic compounds to the original phenolic compounds in parallel to oxidation of anthocyanin to anthocyanin quinone. The second pathway is degradation in two steps: de-glycosylation with anthocyanase (β-glucosidase), and then oxidation with polyphenoloxidase or peroxidase; and the last pathway is direct oxidation of anthocyanin by peroxidase.

Stability of anthocyanin is also affected by the pH and diversity of anthocyanin structure. Anthocyanins are more stable under acidic conditions. Hydroxylation of the compounds is inversely proportional to stability of anthocyanins while

methylation of the compounds is directly proportional to the stability of anthocyanin. Pelargonidin, cyanidin, or delphinidin aglycone are less stable than petunidin or malvidin aglycones. The increased stability of petunidin and malvidin is because at least one of their reactive hydroxyl groups is blocked by methylation (Figure 1-2) (Fennema, 1996).

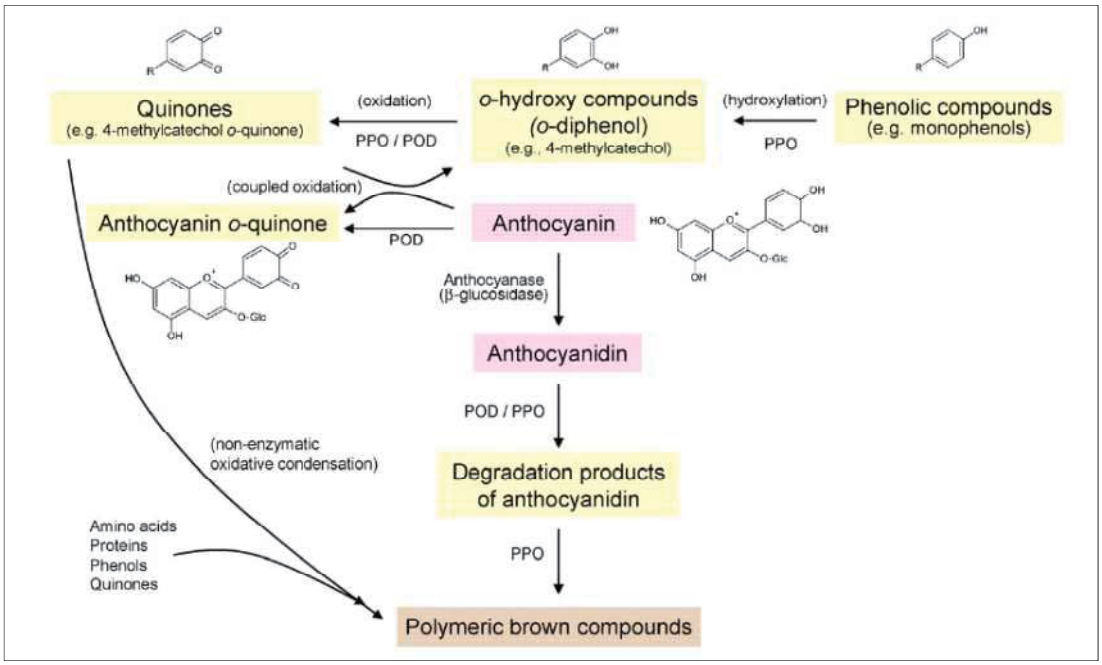


Figure 1-7: Mechanism of enzymatic degradation of anthocyanins and tissue browning in fruit extracts proposed by Oren-Shamir (2009).

1.5.2. Degradation of CGA

Degradation of CGA is not well documented or understood. CGA is stable at acid pH, and not readily destroyed by mild heat (temperature range of 36 – 40 °C) or during storage of juice (Friedman & Jürgens, 2000; Birt, 2011; Narita & Inouye, 2013). CGA is more stable at lower pH. It is suggested that stability of CGA in acid pH is due to the esterification of carboxyl group of caffeic acid with quinic acid to form CGA (Friedman & Jürgens, 2000).

Birt (2011) and Narita & Inouye (2013) found that degradation of CGA during juice storage was minimal at mild elevated temperatures (25 and 37 °C respectively). At higher temperatures about 50% of CGA was degraded in *Chrysanthemum coronarium* L. after 5 min of boiling (Chuda et al., 1998). They suggested that the presence of 'another compound' was responsible for the degradation, and further study by Martinez & Duvnjak (2006) confirmed this compound is PPO. CGA as a substrate reacted with PPO which resulted in 98% of CGA degradation in 3 h of enzymatic treatment. The optimum condition for CGA degradation was at a temperature of 45 °C and pH of 4.3. Although boiling would have inactivated PPO, it would first have led to breakdown of cells during tissue heating, providing a brief period when PPO would have access to CGA before it was itself inactivated. The stability of CGA in juice presumably relates to a negligible PPO content in the juice, but this requires verification.

1.5.3. Degradation of procyanidin

Procyanidin is a thermally labile compound. A study conducted by Dallas et al. (1995) on the degradation of procyanidin in red wine during maturation showed that degradation of each of the procyanidins studied appears to follow a first order kinetic reaction at a rate which increases with temperature. Khanal et al. (2010) found that the concentration of procyanidin in blueberry and grape pomace decreased significantly when heated to 60 – 125 °C but was not affected when heated to 40 °C for up to 72 h. They further concluded that it is 'safe' to dry blueberry pomace at 40 °C for up to 3 days without any negative effect on procyanidin content.

However, for blueberry juice and purée, degradation of procyanidin does occur at low temperatures during storage. Almost all the procyanidin in blueberry juice and purée was lost from the juice during 6 months of storage at 25 °C (Brownmiller et al., 2009) and total loss of procyanidin has been observed in apple juice concentrate during storage at 25 °C for 9 months (Spanos et al., 1990). It is not clear whether this represents true degradation or whether procyanidin is simply becoming undetectable through complexing with soluble and insoluble fiber in the sample (Le Bourvellec et al., 2004; Pinelo et al., 2006).

Although thermal degradation of procyanidin has been described as above, Haslam (1980) observed that a condensation reaction between anthocyanin and procyanidin may occur which results in the precipitation or formation of procyanidin or more complex procyanidins in the solution during storage. Procyanidin may also be broken into its oligomers by breaking of C-C bonds or it may be transformed to other complex procyanidins by C-C bonds forming. The mechanisms of degradation and/or formation of procyanidin, and the relationship with fiber, are still unclear and need further investigation.

1.6. Juice processing and storage

1.6.1. Blueberry juice processing

Basic fruit juice processing involves crushing, maceration, and pressing, in order to release the juice. In most studies, enzymes and heat treatment were applied to improve the extractability and pressability of the homogenate: and to increase the quality and overall juice yield (Fuleki & Hope, 1964; Kalt et al., 2000; Leavens,

2006). Blueberry juice can be processed either hot or cold, and the crushing of the blueberry is the first step in both processes. Normally, crushed blueberry is heated by using a jacketed steam kettle, at temperatures between 47 and 90 °C, before pressing in the hot process. In an alternative cold processing technique, the crushed blueberry is pressed at room temperature (approximately 22 °C), and it may then be pasteurised at 90 °C for 30 s (Carlson, 2003).

Detailed descriptions of unit operations and specific processing conditions of blueberry juice processing were reviewed by Stewart (2005). The general unit operations involved in processing fruit to juice, purée, or pulp product are the same for blueberries, currants, and gooseberries (Figure 1-8).

In general, at the industrial scale, frozen blueberries are used for juice processing because the fresh product has a very short shelf life (Moulton, 1992). Frozen berries require thawing and milling (Skrede, 1996). Commercial enzymes are added after milling and the berries heated to the optimal temperatures for the added enzymes, and held for one to two hours (Downes, 1995), before pressing and pasteurising. However, blueberry contains more mucilaginous material than most berries and this can cause problems with juice preparation (Albrigo et al., 1980).

The freezing and thawing process may help to release pigments and other compounds from the cells because it leads to cell rupture, but, according to Stewart (2005), partially thawed blueberry is better for milling, as this helps with cell wall breakdown and extraction of polyphenolics and other compounds. Milling is a

process that reduces the size of the blueberry and it can be used to help in yield recovery and to increase extraction efficiency (Moulton, 1992).

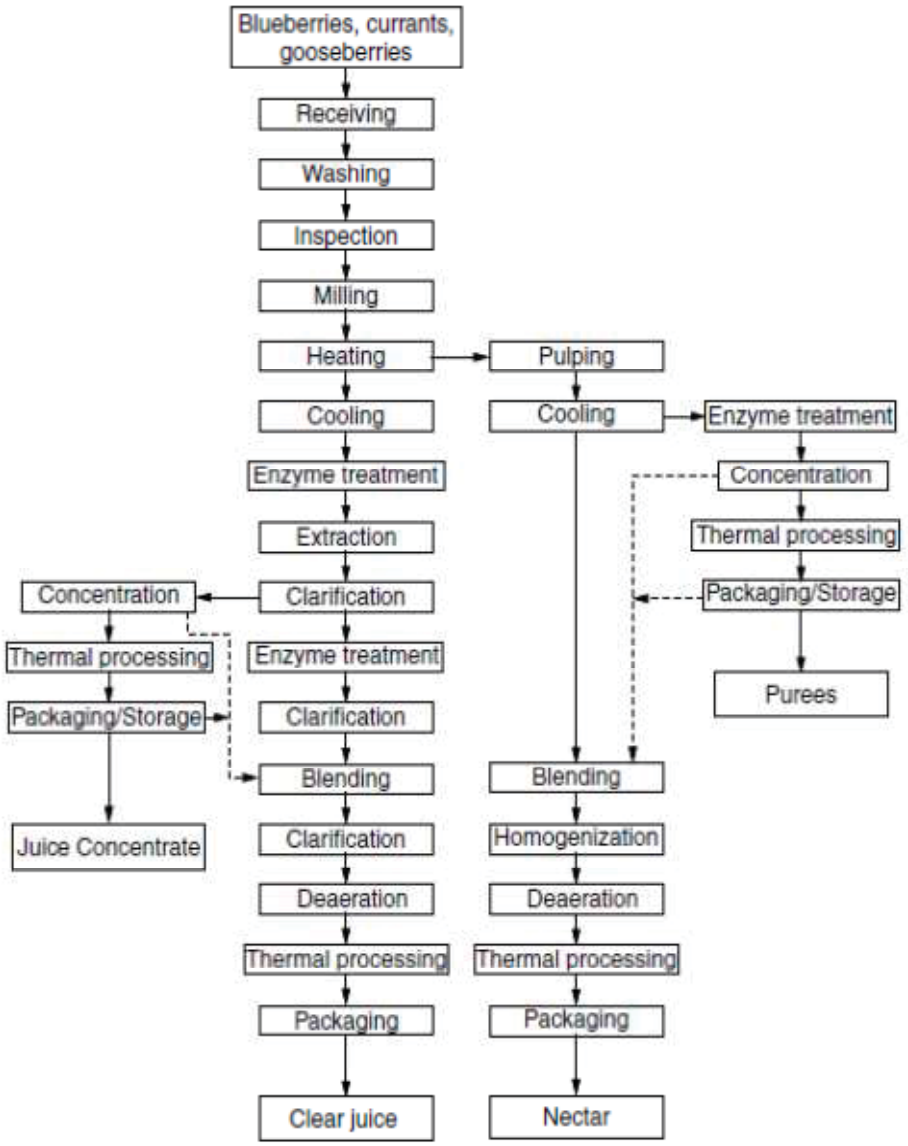


Figure 1-8: General unit operations involved in processing of blueberry, currant and gooseberry, to juice, purée or pulp products (Stewart, 2005).

Enzymes can be applied after milling, with optimal heat treatment. Two types of enzymes are used: pectinolytic enzymes and/or maceration enzymes. Both enzymes are specialised for breaking down the cell structures and pectinolytic enzymes are also ideal for dissolving pectin. The optimal heat that is applied will aid in cell rupture and increase the reaction rates of the enzymes. Therefore, enzymes facilitate release of juice from the cells and improve pressability (Downes, 1995). The application of heat in the juice processing at this stage also helps in the extraction of pigments from the skin and flesh of the fruit.

The pasteurisation process is the final process in juice processing, in which heat is applied in order to destroy pathogenic micro-organisms. It also inactivates unwanted enzymes that cause instability in the juice (Stewart, 2005).

Table 1-6 summarises the unit operations involved in blueberry juice processing, as documented by selected researchers. Each researcher applied different processing techniques, equipment, starting material, and enzymes (at various concentrations), from household scale to industrial scale, which is a more challenging area to be explored for comparison and optimisation. It is also important to note that commercial juice companies in New Zealand vary in their practices (see Chapter 7).

Table 1-6: Unit operations involved in blueberry juice processing and percent of compound recovery in the juice after pasteurisation.

Reference	Samples	Unit operations								Recovery of compounds (%) after pasteurisation			
		Pre-treatment					Juicing		Pasteurisation				
		Thawing	Blanching	Crushing	SO_2	Enzymes	Extraction equipment	Pressing MPa		anthocyanins	CGA	Flavonol glycosides	Procyanidins
Skrede et al. (2000)	26 kg	5 °C	-	Comminuting machine	-	Rapidase, 2 h, 43 °C	Bag press	5.0	HTST Heat Exhanger; 90 °C for 1 min	32	53	35	43
Lee et al. (2002)	20 kg	Heated to 32 °C	Steam kettle; 2 min, 95 °C	Stephan vertical cutter mixer	-	Pectinex smash, 27 °C	Bag press	5.0	HTST Heat Exhanger; 90 °C for 90 s	22	38	nd	nd
	20 kg	Heated to 32 °C		Stephan vertical cutter mixer	-	Pectinex smash, 27 °C	Bag press	5.0	HTST Heat Exhanger; 90 °C for 90 sec	12	35	nd	nd
	20 kg	Heated to 32°C		Stephan vertical cutter mixer	50 ppm	Pectinex smash, 27 °C	Bag press	5.0	HTST Heat Exhanger; 90 °C for 90 s	23	39	nd	nd
Rossi et al. (2003)	5 kg Frozen	5 °C, 12 h	-	Commercial mini-processor	-	Cytolase, 1 hr at room temperature	Bag press	0.88 MPa	HTST Heat Exhanger; 90 °C for 1 min	12	nd	nd	nd
	5 kg frozen	5 °C, 12 h	Steam blanching tunnel; 3 min	Commercial mini-processor	-	Cytolase, 1 hr at room temperature	Bag press	0.88 MPa	HTST Heat Exhanger; 90 °C for 1 min	23	nd	nd	nd
Leavans (2006)	unknown	yes		Hand-operated pulper	-	Rapidase, 3 °C, overnight	Basket bladder press	High Hydrostatic Pressure (HPP) 400 MPa	-	nd	nd	nd	nd
	unknown	yes		Hand-operated pulper	-	Rapidase, 3 °C, overnight	Basket bladder press	-	75 °C for 30 s	nd	nd	nd	nd

nd = not determined

Table 1-6 (continue): Unit operation involved in blueberry juice processing and percent of compound recovery in the juice after pasteurisation.

Reference	Samples	Unit operations								Recovery of compounds (%) after pasteurisation			
		Pre-treatment					Juicing		Pasteurisation				
		Thawing	Blanching	Crushing	SO_2	Enzymes	Extraction equipment	Pressing MPa		anthocyanins	CGA	Flavonol glycosides	Procyanidins
Sivastava et al. (2007)	2.1 kg	5 °C, 12 h	Boiling water; 3 min, 100 °C	Household blender		Pectinase, 1 h room temp	Centrifuge 9740 g for 20 min at 10 °C.	-	Jacketed vessel; 85 °C for 2 min	30	nd	nd	nd
Brambilla et al. (2008)	1 kg Highbush blueberry		Steam blanching tunnel; 3 min, 85 °C	Commercial hand blender	-	Pectinase, 1 h, room temperature	Filter beg press	-	90 °C for 1 min	38 - 46	nd	nd	nd
Brownmiller et al. (2008)		-	steam kettle; 95 °C for 3 min	bladder press		Pectinex Smash, 1 h at 40 °C	-	-	Steam box; Until temp reached 90 °C.	72	nd	nd	nd
Sablani et al. (2010)	Fresh	-	-	-	-	-	wooden block juice	-	Steel pan on a hot plate; 90 °C for 2 min	7 -10	nd	nd	nd
	Fresh	-	steam blanched; 95 °C for 2 min	-	-	-	wooden block juice		Steel pan on a hot plate; 90 °C for 2 min	40 - 55	nd	nd	nd
Birt (2011)	500 g	20 °C, 24 h	-	home food processer	-	Celluzyme, 1 h at 30°C	bench-top screw press		steam jacketed kettle; 90 °C for 1 min	7	26	nd	nd
	500 g	-	Steam blanched pan: 85 °C for 5 min	home food processer	-	Celluzyme, 1 h at 30 °C	bench-top screw press		steam jacketed kettle; 90 °C for 1 min	43	38	nd	nd

nd = not determined

1.6.2. Losses during juice processing

The juice manufacturing process, which involves various unit operations, heating temperatures, and duration, has often resulted in extensive losses of phytochemicals in the final products. The literature on various processing methods and how they affect the quality of final products is limited. It becomes more difficult to make a comparison, when nearly every researcher has employed different processing techniques and used different equipment and variables.

A study by Skrede et al. (2000) using a mimicking process (similar to industrial practices) recovered only 32% of the anthocyanin pigments in single-strength Highbush blueberry juice (after pasteurisation). A further 18% of the anthocyanins were recovered in the press-cake residue, after pressing of the pulp. This means that approximately 50% of the anthocyanins were lost during juice processing i.e. in the milling, enzyme maceration, and pressing unit operations. The most stable anthocyanins identified during juice processing were malvidin glycosides; the delphinidin glycosides were the least stable. In the same study, the recovery of CGA, flavonol glucosides, and procyanidins in the juice, was 53, 35, and 43%, respectively. In contrast to the anthocyanins, the pressed cake residue contained only minor amounts of these phenolics, indicating that those polyphenolic compounds were more easily released from the blueberry fruit and skin tissue than the anthocyanins; but were subject to the same significant degradation.

Lee et al. (2002) conducted a study to evaluate the loss of anthocyanin and CGA with two different treatments: blanching at 95 °C for 2 min and SO_2 treatment. Both treatments were used to inhibit the activity of PPO and increase anthocyanin

extraction. Their results show that there was no significant difference between these two treatments and the control. Rossi et al. (2003) also studied the effect of blanching in blueberry juice processing and found a higher recovery of anthocyanin in steam blanched juice for 3 min, compared to unblanched juice.

Thermal processing appears to be one of the most important factors for the retention of polyphenolics, especially anthocyanin, in juice processing. The majority of studies report a high recovery of anthocyanins through the application of blanching steps (Table 1-7). Blanching increased anthocyanin recovery from juice (Rossi et al., 2003). During clarification, there were significant losses of anthocyanin in 'no blanch' juice with only 5% of anthocyanin recovered from the juice compared to about 20% recovery from 'blanch' juice (Lee et al., 2002).

Table 1-7: Selected reports on anthocyanin recovery in percentage (%) at each juice processing step.

Reference	Anthocyanin recovery (%)				
	Blanching	Pomace	Pressed juice	Clarified juice	Pasteurised juice
Rossi et al. (2003)	Yes		-	-	23
	No		-	-	12
Lee et al. (2002)	Yes	42	20	20	22
	No	55	4	5	13
Skrede et al. (2000)	No	18	28	-	32
Brownmiller et al. (2008)	Yes	15	80	46	41
	Yes	15	80	-	72

After pasteurisation of non-clarified juice, the concentration of anthocyanin in the juice was slightly increased (by 2 – 8%) compared to the initial pressed juice and it

was concluded that pasteurisation inactivated enzymes which may destroy anthocyanins (Skrede et al., 2000; Brownmiller et al., 2008). The study by Brownmiller et al. (2008) shows that there are huge losses of anthocyanin in 'clarified and pasteurised' juice compared to 'non-clarified and pasteurised' juice with about 41 and 72% of anthocyanin recovered in the juice respectively. The loss is probably because anthocyanin may remain in the sediment during clarification.

Blanching destroys PPO and so reduces deterioration of anthocyanins and helps to soften plant cell membranes and wall pectin (Van Buggenhout et al., 2009), thus releasing more anthocyanins from the tissue. Blanching is good for releasing phytochemicals but at low temperature (≤ 50 °C) it will increase PPO activity and encourage thermal degradation. During blanching, anthocyanins diffused from the pigmented epidermis to the core of berries, while the anthocyanins in the vacuole of the epicarp cell leaked out through softened tissue (Brambilla et al., 2011) (Figure 1-9).

Kader et al. (1997) suggested that the loss of polyphenolics is caused by polyphenoloxidase (PPO). Inactivation of PPO will eliminate this mechanism for degradation. This can be done by applying heat treatment such as blanching but, since anthocyanins are heat sensitive, the use of blanching may further increase anthocyanin degradation in the blueberry. Siddiq et al. (1992) suggested that heat inactivation of PPO should be avoided, when dealing with anthocyanin-containing juice products for the same reasons. It is a huge challenge to retain anthocyanin and other polyphenolic compounds in blueberry juice, although thermal processing shows a promising recovery of the compounds, as discussed previously. Although

non-thermal processing can be used as an alternative to thermal processing, the breakdown or destruction of the skin is needed, in order to release or extract anthocyanin, which is found only in the skin (Lee et al., 2002).

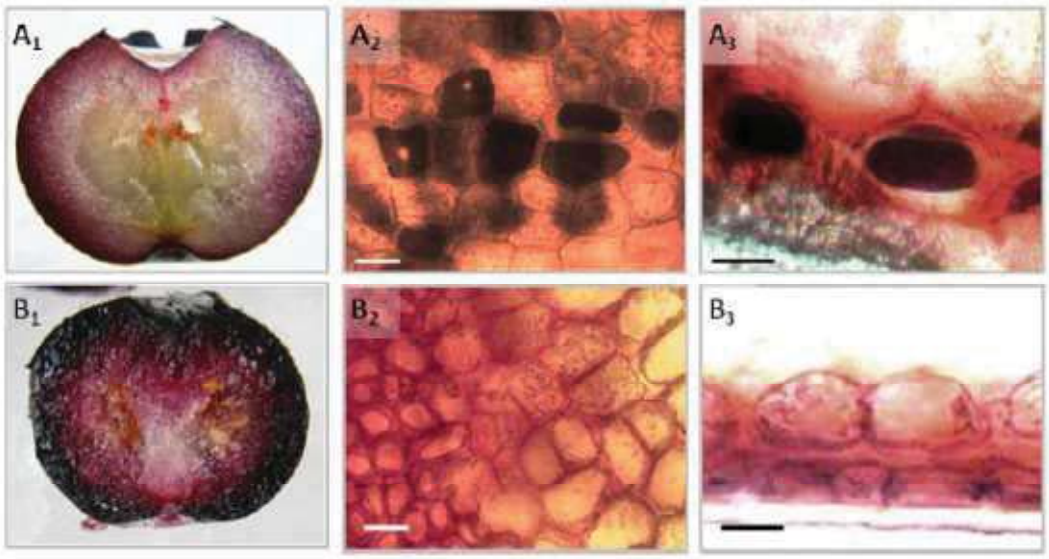


Figure 1-9: Effect of steam blanching on pigment diffusion in blueberry (A. Not blanched; B. Blanched). 1- berries longitudinal section. 2-Epidermis panoramic views. 3-Epidermal tissue cross-section (Bars=30 µm) (Brambilla et al., 2011).

1.6.3. Effect of storage

Anthocyanins play an important role in the colour quality of blueberry juice. Degradation of anthocyanin will make the colour of the juice unattractive for consumer sensory acceptance (Patras et al., 2010). Usually, blueberry juice will be packed in glass or in polyethylene terephthalate (PET) bottles, for a certain time, before entering the market.

Srivastava et al. (2007) reported the effect of storage conditions on blueberry extract packed in glass bottles. They found that, after 60 days of storage at 35 °C, no

anthocyanins were detected in the blueberry extract. They also investigated the effect of storage at different temperatures (-20, 6, 23, and 35 °C). No significant loss of anthocyanin was observed at -20 °C storage (up to 30 days), but a significant loss was observed from 15 to 30 days, at 6, 23, and 35 °C of storage. They concluded that anthocyanins and other phenolic acids in blueberry extract are less influenced by cold storage and frozen conditions.

Birt (2011) had similar results, which confirmed that, after six months of storage, juice stored at a cold temperature (5 °C) contained higher anthocyanin concentrations than juice stored at a high temperature (25 °C) and the degradation of anthocyanin can be observed over the time of storage. The degradation occurring during storage was purely thermal degradation because the PPO in the juice was inactivated by pasteurisation. Birt (2011) further concluded that glass bottles and dark storage are better for anthocyanin retention, compared to plastic materials and light exposure.

Ochoa et al. (1999) suggested that anthocyanins were extensively polymerised during storage and loss of total monomeric anthocyanins would increase polymeric colour values. Anthocyanins and other phenolic compounds are easily oxidised and thus they are susceptible to oxidative degradation during various steps of processing and storage (Patras et al., 2010).

Although anthocyanins and other phenolic compounds in the juice were degraded, the antioxidant capacity of the juice remained stable over time during storage (Hager et al., 2008), probably because of other antioxidant compounds such as CGA

which are more stable than anthocyanins. There is also the possibility that the compounds in the juice were hydrolysed and/or condensed to form other compounds during storage; for example condensation of anthocyanin to tannin (Satanina, 2011).

1.7. Sensory evaluation

Degradation of phytochemicals in the juice during processing can be managed if the mechanism underlying each of the processes is fully understood. This will increase the phytochemicals in the juice. However, it is important for the phytochemical-rich juice to be accepted by the consumer. There are risks that a phytochemical-rich juice may contribute to the astringency and bitterness of the juice.

Bett-Garber & Lea (2013) conducted a study to produce a flavour lexicon of freshly pressed and processed blueberry juice from 20 blueberry products. This study aimed to provide a reference for flavour characters in freshly pressed and bottled blueberry juices. They developed 32 attributes of aroma/flavour, taste, and mouthfeel to describe blueberry juice and found that freshly pressed juice had a different flavour profile compared to processed juice. Freshly pressed juice was high in blueberry, strawberry, floral, sweet aroma, and sweet taste while processed juice was high in cranberry, molasses/dark corn syrup, canned tomato, fermented, processed berry juice, sour aroma, and pungency aroma (Bett-Garber & Lea, 2013).

Flavour changes during blueberry juice processing are expected. It is suggested that flavour changes are associated with the changes of phytochemical content during juice processing (Brownmiller et al., 2008; Brownmiller et al., 2009; Howard et al., 2012). Polyphenols are an important class of phytochemicals that influence sensory

properties and contribute to bitterness and astringency of a beverage (Lesschaeve & Noble, 2005). Increasing the phytochemical content in foods and beverages could have an adverse impact on the consumer (Drewnowski & Gomez-Carneros, 2000). However, the unacceptable taste (i.e. bitterness and astringency) of the juice can be effectively masked by the addition of sweetener to improve acceptability of the juice by the consumer (Muir et al., 1998; Jaeger et al., 2009). Leavans (2006) also found that thermally processed blueberry which contains high phytochemical concentrations is preferred by consumers. However, the relationship between increased phytochemicals and consumer preference, and the mechanisms involved still need further investigation.

The relationship of chemical composition, in a drink manufactured from *Hibiscus sabdariffa* L., to sensory profile and consumer acceptance was studied by Bechoff et al., (2014). They found that chemical (especially anthocyanin) composition and content in the drink significantly correlated to the sensory attributes especially appearance, odour and taste. The acceptability of the drink was also correlated with polyphenol content which was associated with the bitter taste of the drink. They concluded that chemical composition and concentration of the drink may influence consumer acceptance.

Laaksonen et al. (2013) studied the sensory quality and compositional characteristics of blackcurrant juice prepared with and without enzyme-aided pressing. They found that phenolic concentration was higher in enzyme-aided pressed juice compared to non-enzyme juice, and mouth-drying astringency and bitterness intensity was also higher in enzyme-aided juice compared to non-enzyme juice which had a more

pleasant sensory profile. This result led to the conclusion that mouth-drying astringency and bitterness intensity was associated with flavonol glycosides and hydroxycinnamic acid in the juice.

A study of consumer acceptance on blackberry, blueberry, and concord juice blends shows that most of the juice and juice blends were well-liked by the consumer. However, when they were informed about the potential health benefits of anthocyanins, and the anthocyanin content of the juices, they were more likely to buy juice with highest anthocyanin content. This suggests that informing consumers of the benefit of the juice could affect consumers' acceptance of a product (Lawless et al., 2012).

Clearly, there is a need to investigate consumer perceptions of the juice from different kinds of processing and of different known compositions. Even if there was some loss of acceptance in beverages with higher phytochemical content, there may be an opportunity to associate this in consumer minds with some perceived health benefit. In this way the taste could potentially have a marketing benefit and thus outweigh some loss of perceived sensory quality.

1.8. Aim and research objective

After reviewing the literature, it can be seen that there is an opportunity to develop a model for blueberry juice processing in terms of phytochemical composition. Developing a processing model is intended to provide a tool that can be used to reliably generate juice of a particular desired composition. As knowledge increases it seems highly likely that particular blueberry phytochemicals will be more strongly

associated with particular health benefits. Conceivably, it may therefore prove to be desirable (for different reasons) to generate a juice that is, for example, particularly high in antioxidants; or one that is enriched in anthocyanin; or one that is high in CGA; or one that is high in procyanidin. The intention of the project is to gather the data that would allow such outcomes to be achieved, by varying the choice of variety, the processing method, and the storage regime.

Therefore, the objectives of this research are as follows:

1. To identify and evaluate the losses of three important compounds (anthocyanins, CGA and procyanidin B2) in blueberry juice processing.
2. To investigate the mechanism of release of these compounds (anthocyanins, CGA and procyanidin B2) from berry matrix during juice processing.
3. To evaluate consumer acceptance and sensory characteristics of blueberry juices varying in phytochemical composition.
4. To develop a juice processing model to provide tools that can be used to predict variation in blueberry juice composition.

Process modelling can be used as a tool to meet customer expectations or to predict the likely effect of changing processing parameters on final product quality. A model that relates processing conditions to product quality is an important requirement for any optimisation process. By understanding the underlying mechanisms that operate at each stage of production, and which affect the phytochemical composition of the final juice, the developed processing model may be exploited in the design of improved industrial commercial production methods.

2. Materials and Methods

In this section of the thesis, the methodologies used for the core analyses in the study are described. Materials and methods that are specific to some experiments carried out during this work are described in the related chapters. Replication conducted in this work involves true replicates (at least three separate samples that received all the same treatments and then were analysed independently to give the true variation of the samples) unless mentioned specifically in the specific method.

2.1. Materials

In this study, three types of blueberry were used: 'Maru', a Rabbiteye variety (*V. ashei*) and 'Burlington' and 'Dixi', both Highbush (*V. corymbosum*). 'Maru' were donated by Blueberries New Zealand Inc (BBNZ) through PFR. 'Burlington' and 'Dixi' were donated by Mamaku Blue Winery Ltd. The blueberries were harvested at full blue colour (commercially mature stage) and stored at -20 °C before juice processing.

2.2. Phytochemical analysis

Phytochemicals in whole blueberries and blueberry juice were analysed by High Performance Liquid Chromatography (HPLC) following the method of Wang et al., (2000) with some modifications as reported by Birt (2011). Briefly, 1 mL of berry extract (Section 3.2.1) or juice (Section 3.2.2) was filtered through a 0.22 µm regenerated cellulose syringe filter into a HPLC vial. The HPLC system consisted of a Shimadzu CTO-20A (Shimadzu Corp., Kyoto, Japan) coupled with an auto-sampler SIL-20AC and a photodiode array (PDA) detector SPD-M20A. Phytochemicals were separated on a Phenomenex Luna C18 (2) 150 × 4.6 mm

(5 μm) reverse phase column fitted with a Phenomenex guard cartridge system (part no KJ0-4284) containing an AQ C18 4×3 mm safety cartridge (part no AJ0-4287) (Phenomenex, North Shore City, NZ). The solvent gradient was made with 5% (v/v) aqueous formic acid (solvent A) and 100% HPLC grade methanol (solvent B). The flow rate was 1.00 mL min^{-1}, with a linear gradient profile with the following proportions (v/v) of solvent B: 0.00 – 1.00 min, 14% B; 1 – 10.24 min, 14 – 17% B; 10.24 – 35.28 min, 17 – 23% B; 35.28 – 64.59 min, 23 – 47% B; and 64.59 – 66.59 min, 47 – 14% B. The total running time was 70 min. The column was operated at 25 °C in an air conditioned room at 22 ± 3 °C.

Anthocyanin, CGA, and procyanidin B2 identification was based on the retention time of standards and on the order of elution reported by other researchers for Highbush blueberry. Quantification of anthocyanins, CGA and procyanidin B2 was based on peak areas determined at 520, 350, and 280 nm respectively and compared to the absorbance of cyanidin 3-O-glucoside chloride, CGA, and procyanidin B2 concentrations at concentration of 1 – 1000 ppm. Hidden standard samples were used to ensure the HPLC's reproducibility.

2.3. Physico-chemical analysis

2.3.1. pH values

Juice pH was determined using a Thermo Scientific Orion 3 Star benchtop pH meter (Massachusetts, USA). Samples were brought to room temperature, before analysis.

2.3.2. Total soluble solids

Total soluble solids of the juice samples were measured with a Pocket Pal-1 refractometer (Atago, Japan). Routine calibration was done with distilled water. The total soluble solids are expressed as °Brix.

2.3.3. Titratable acidity

Titratable acidity, expressed as percentage of citric acid was determined using an auto titrator (TitroLine Easy, GmbH, Deutschland, Germany). The juice (1 mL) was diluted in 50 ml distilled water and titrated against 0.1 M NaOH to an end point of pH 8.2. Each solution of NaOH was standardised against 0.1 M HCl. The titratable acidity was calculated as % acid (wt/vol).

2.3.4. Turbidity

Turbidity is the amount of cloudiness in the juice and was measured using a Hach 2100P Turbidimeter, U.S.A. The units for turbidity (Nephelometric Turbidity Units, NTU) refer to the way light is scattered by suspended particles in the water; the greater the scattering, the higher the turbidity. The instrument was calibrated using StablCal® Stabilized Formazin Standards at ranges of <0.1 NTU, 20 NTU, 100 NTU and 800 NTU.

2.3.5. Viscosity

Rheological measurement was carried out as described by Nindo et al. (2005) with some modification. It was conducted using a controlled-stress Paar Physica MCR 301 Rheometer (Anton-Paar, GmbH, Germany) equipped with RheoPlus software. Initially a 20 mL sample of freshly prepared juice was put into the stationary

rheometer cup (diameter 28.9 mm; diameter of bob 26.7 mm; length of bob 40 mm) and measured at 20 °C using a thermostatic bath. The instrument was set to apply a shear rate from 1 to 500 s^{-1} and measurements were taken during acceleration and deceleration over 570 s. The data collected were validated with water. Water has viscosity of 0.001 Pa s at 20 °C that should not change when shear rate and shear stress are increased. However, in this study, viscosity of water appeared to increase when shear rate and shear stress were increased. Water was further validated by using double gap geometry and it was found that double gap geometry was the best geometry to measure viscosity of water or juice (non viscous liquid) compared to cup and bob geometry. Due to these issues, viscosity in this study was reported as a single viscosity at shear rate of 48.5 (s^{-1}) as suggested by Sharma & Sherman (1973) and Costell & Duran (2000).

2.4. Polyphenoloxidase assay

2.4.1. Enzyme extraction

Enzyme extraction was carried out as described by Terefe et al. (2010), with some modification. Frozen blueberries or prepared blueberry juice were mixed at a ratio of 1:2 with a pre-chilled extraction solution: i.e. 0.2 M sodium phosphate buffer (pH = 7.0) consisting of 4% (w/v) polyvinylpolypyrrolidone (PVPP) and 1% (v/v) Triton X-100. The mixture was homogenised using an Ultra turrax homogeniser (Ika Labortechnik, Malaysia) at 4 °C for 3 min and centrifuged (Thermo Scientific Sorvall Centrifuge, Massachusetts, U.S.A) at 10,000 *x g* and 4 °C for 10 min. The supernatant was used as the crude enzyme extract for the polyphenoloxidase (PPO) assay.

2.4.2. Enzyme assay

For the PPO assay, the reaction mixture consisted of 150 µL of enzyme extract and 1350 µL of 0.1 M catechol in a 0.2 M phosphate buffer (pH = 7.0) solution. The blank was prepared in the same way, except that 150 µL of 0.2 M phosphate buffer (pH = 7.0) was used instead of crude enzyme extract. The absorbance of the assay mixture was measured at 420 nm at room temperature using a UV-visible spectrophotometer (Thermo Scientific Helios Gamma Spectrophotometer, Massachusetts, U.S.A). One unit of PPO activity was defined as the amount of enzyme which caused an absorbance increase of 0.001 units per min.

2.5. Kinetic analysis

In general, disappearance of a compound under isothermal conditions can be modelled using a simple rate law

$$\frac{dC}{dt} = -kC \quad \text{Equation 2-1}$$

where t is the time (min); k is the rate constant (min^{-1}) and C is the concentration at time zero.

Integrating with respect to time for zero order reaction in nonlinear form gives:

$$\frac{C}{C_o} = -kt \quad \text{Equation 2-2}$$

for first-order reaction:

$$\frac{C}{C_o} = \exp(-kt) \quad \text{Equation 2-3}$$

and for a *n*-th order reaction (n≠1):

$$\frac{C}{C_o} = \left(1 + k't(n-1)\right)^{\frac{1}{1-n}} \text{ ... ...Equation 2-4}$$

where C_o is the concentration at time zero.

The rate constant, *k*, can be obtained by regression fitting the data to equations using the Solver™ function within Microsoft® Excel to minimise the sum of residual squares.

The half-life ($t_{½}$) (h) of the compound was expressed by the following equation:

$$t_{1/2} = -\ln\frac{0.5}{k} \text{ ... ...Equation 2-5}$$

Dependency of the degradation rate constant on temperature is represented by the Arrhenius equation

$$k = k_o \exp\left(-E_a/RT\right) \text{ ... ...Equation 2-6}$$

where *k* is the rate constant (min^{-1}); k_o is the frequency factor (min^{-1}); E_a is the activation energy (kJ mol^{-1}); *R* is the universal gas constant (8.314 J mol^{-1} K^{-1}); and T is the absolute temperature (Kelvin).

However, degradation of compounds in solid or semi solid food such as fruit or berry pomace is not isothermal, therefore kinetic modelling should include time-temperature history. Dolan (2003) proposed a one step kinetic model for determining kinetic parameters for non isothermal food process. The independent variables time

(t) and juice temperature (T) were combined into one variable, the thermal history (β), according to Equation 2-7

$$\boldsymbol{\beta = \int_0^t \exp\left[\frac{-E_a}{R}\left(\frac{1}{T_t} - \frac{1}{T_{ref}}\right)\right] dt} \quad \text{... ... ...Equation 2-7}$$

where T_{ref} is the arbitrary reference temperature and T_t is the temperature at time (t).

The reaction rate constant, k_{ref} at T_{ref} for a first-order reaction (n = 1) could be calculated according to Equation 2-8

$$\boldsymbol{\frac{C}{C_0} = \exp\left(-k_{ref}\,\beta\right)} \text{ ... ...Equation 2-8}$$

In the cases where deviations from simple first-order reactions are observed, the n^{th}-order reaction (n≠1) may be more appropriate and calculated as per Equation 2-9

$$\boldsymbol{\frac{C}{C_o} = \left[1 + (n-1)C_0^{n-1}k_{ref}\beta\right]^{1/(1-n)}} \text{ ... ...Equation 2-9}$$

The kinetic parameters (k_{ref} and E_a) were calculated by minimising the sum of squares error (SSE) between the experimental and calculated values for C/C_o by using Solver™ in Microsoft® Excel. A goodness of fit of the data was assessed with Regression coefficients (R^2). The higher the R^2 value, the better the adequacy of the model to describe the data.

2.6. Pilot plant blueberry juice processing

Blueberry juice processing was carried out in the Pilot Plant, School of Food and Nutrition, Massey University. Juice processing was summarised as in Figure 2-1 with sampling points indicated (A-H). About 14 – 15 kg of blueberries was used in

each trial. Approximately 400 g samples of Highbush blueberries were put into retort pouches to ensure that all the blueberries were evenly distributed in a single layer (to minimise heating time). Pouches were evacuated (10 mBar evacuated pressure, Multivac MC06-K20, New Zealand). The vacuum packed blueberries were then thawed in running tap water until they reached room temperature (~20 °C). Then they were blanched at 70 or 90 °C for 3 min in a preheated jacketed pan, and immediately cooled in iced water before crushing with a commercial electric meat mincer (TK-M12, China). For 'no blanch' control treatment, blueberries were thawed in two buckets at 4 °C overnight (15 h) before crushing. This mimics current commercial practice. The crushed berries from all treatments were then treated with 2 mL/100 kg Pectinex Ultra Clear® (Novozyme, Denmark), previously diluted 1/10 with distilled water and incubated at 50 °C for 2 h with occasional stirring. The treated berries were transferred to an organza bag (pore size ca. 280 x 310 μm) and pressed using a screw basket press to yield the juice. All the juice was pasteurised at 90 °C for 30 s using Alpha Laval Pasteuriser (Lund, Sweden), cooled to 4 °C and aseptically dispensed into 300 mL condiment glass bottle with twist cap (Arthur Holmes limited, New Zealand) in a laminar flow workstation. Each bottle was labelled by treatment, numbered, and filled with juice in a randomised order. Before each experimental run, the pasteuriser plant was pre-sterilised by circulating water at 120 °C outlet temperature for 1 h and by irradiating the juice collection area with UV light. The holding temperature was set at 90 °C and the flow rate at 0.833 L min^{-1} to obtain a holding time of 30 s. The bottled juices were stored in the dark at 25 °C for shelf life analysis. In this pilot plant processing trial, each trial was run only once. Therefore, confidence intervals for each trial were calculated based on analytical replicates; samples were taken twice at each sampling point.

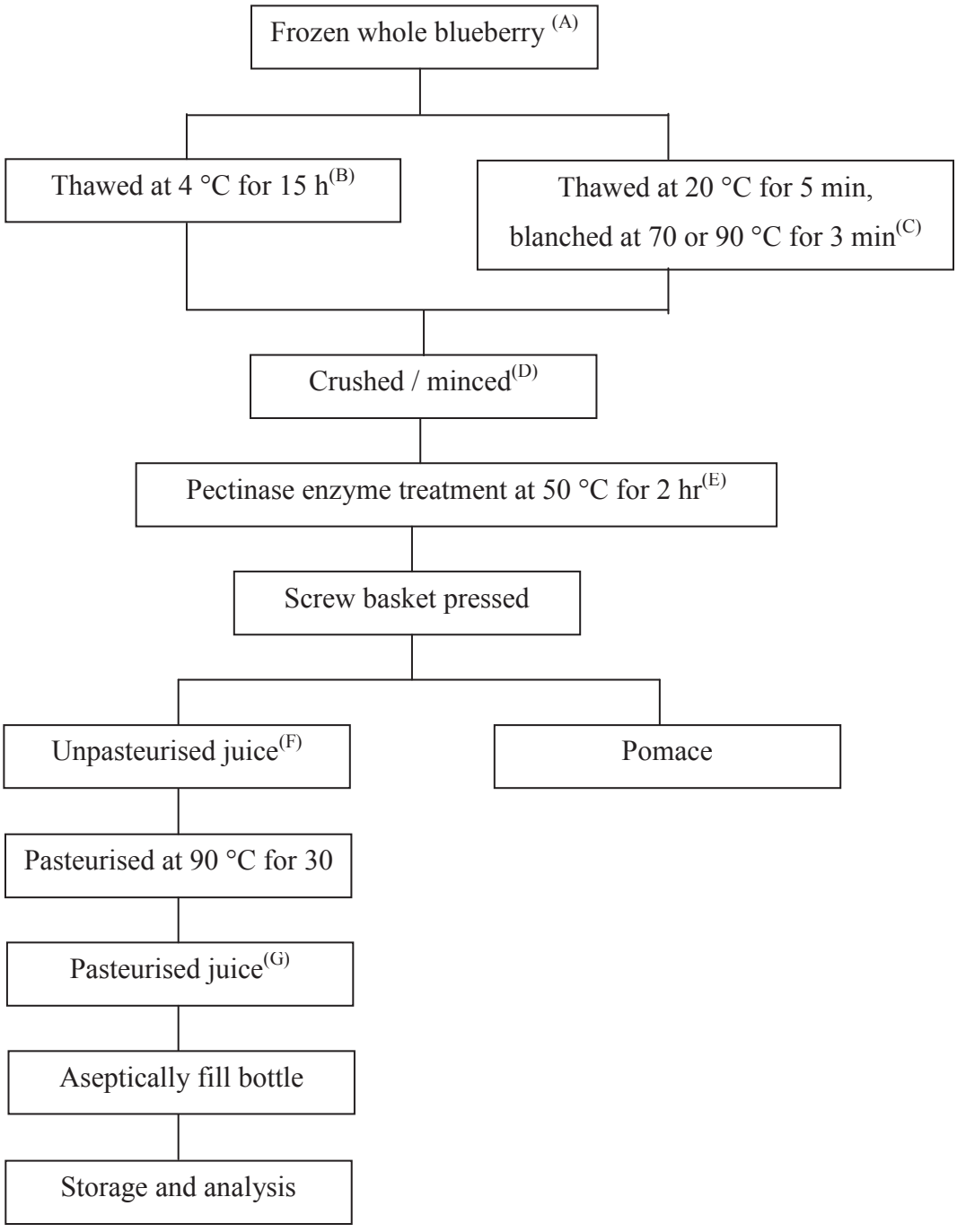


Figure 2-1: Process for blueberry juice processing indicating samples (A-G) that were collected for phytochemical analysis.

3. Blueberry and blueberry juice composition and thermal degradation of phytochemicals in blueberry juice

3.1. Introduction

Blueberries are known to contain high levels of phenolic compounds, including anthocyanins, flavonols, CGA, and procyanidin, which are reported to have high biological activity and may provide health benefits as antioxidants, in addition to preventing disease (Cho et al., 2005; Howard & Hager, 2007). Different compounds may behave differently during processing and it is not known how their relative concentrations will vary through the juicing and bottling process. A number of major factors are important for phytochemical losses: thermal degradation, enzymatic and non-enzymatic oxidation, and losses caused by incomplete extraction from the berry matrix.

Among these, temperature (heating) is likely to be one of the major factors affecting the degradation of phytochemicals, especially anthocyanins. The stability of anthocyanins and all pigments found in food decreases as temperature rises (Patras et al., 2010). Many authors have studied the influence of temperature on anthocyanin stability from difference sources and demonstrated that heating has a detrimental effect on anthocyanin content (Kırca & Cemeroğlu, 2003; Kechinski et al., 2010; Hillmann et al., 2011). However, thermal treatment is needed to preserve and extend the shelf-life of a product. Blanching or pasteurisation is commonly used to inactivate degrading enzymes and to kill spoilage organisms.

Material in this chapter is included in the paper: Kasim, F.K., Hurst, R.D., Carr, A.J. & Heyes, J.A. Effect of berry blanching on phytochemicals and physicochemical properties of blueberry juice. *Proceeding of FVHH 2013 International Symposium on Quality Management of Fruits and Vegetable for Human Health. August 5-8, 2013, Golden Tulip Sovereign Hotel, Bangkok, Thailand*. 151-157.

A considerable amount of literature has been published on thermal degradation of anthocyanins in various fruit and/or juice model systems. The studies reported that thermal degradation of anthocyanin followed a first-order reaction in blackberry (Wang & Xu, 2007; Harbourne et al., 2008; Jiménez et al., 2012), raspberry (Verbeyst et al., 2011), plum (Ahmed et al., 2004), blood orange juice (Cao et al., 2011), and black rice (Hou et al., 2011) to name a few. All these findings are in agreement with Kechinski et al., (2010) who studied thermal degradation kinetics of anthocyanins in blueberry juice during thermal treatment at 40 – 80 °C. Thermal degradation of other phenolic compounds in blueberry juice is much less well understood and is crucial to understanding concentration changes of phytochemicals during processing and storage.

The objectives of this chapter were to investigate the phytochemical composition, including three key phytochemicals (anthocyanins, CGA, and procyanidin B2) and polyphenoloxidase enzyme (PPO), of three common varieties of blueberry and four types of blueberry juice prepared with different processing conditions. The phytochemical changes in raw blueberry juice during thermal treatment and in pasteurised juice during long-term storage at elevated temperature were also investigated.

Raw juice of ‘Maru’ blueberry was used as a model system to determine the impact of thermal processing on phytochemical concentrations without the added complexity of the release of phytochemicals from the matrix, which will be assessed in Chapter 5.

3.2. Materials and methods

3.2.1. Blueberry extraction

A sample of 2 – 6 g of frozen blueberry was weighed and homogenised with an Ultra Turrax T25 Basic homogeniser (Ika Labortechnik, Malaysia) with 10 mL of solvent mix (acetone/methanol/water/formic acid [40:40:20:0.1]) in a 50 mL centrifuge tube. The centrifuge tubes were vortexed for 30 s and allowed to stand at room temperature for 5 min: and then centrifuged at 4000 *x g* for 10 min at 20 °C (Thermo Scientific Heraeus multifuge IS-R centrifuge, Massachusetts, U.S.A.). The supernatant was collected in a 100 mL rotary evaporator flask and this procedure was repeated several times, until the supernatant was clear. The solvent in the collected supernatant was then removed, by using a Rotavapor® R-215 with Vacuum controller V-850, operating at 50 °C and 72 mBar. The dried extract was subsequently re-solubilised in 20 mL of the starting eluent phase of HPLC analysis, comprising 5% formic acid/methanol (86:14). These procedures were repeated six times using different batches of blueberry, randomly sampling from the original sample box.

3.2.2. Blueberry juice preparation

Three batches of raw juice were prepared with frozen Rabbiteye blueberries (*V. ashei* cv. Maru). The frozen berries were crushed using a coffee grinder (Breville CG2B, Australia), thawed for 20 min in the dark at room temperature and centrifuged (4000 *x g* for 1 h at 15 °C) to yield the juice. The juice was stored at -20 °C until use. Three kinds of pasteurised juice (‘no blanch’, ‘blanched at 70 °C’ and ‘blanched at 90 °C’) were prepared as in Section 2.6.

3.2.3. Thermal degradation kinetics of phytochemicals in raw blueberry juice

The thermal degradation kinetics of the phytochemicals in blueberry juice (prepared in section 3.2.2) were studied at different temperatures (50, 60, 70, 80, and 90 °C) over a 4 h period. Containers of frozen raw blueberry juice were thawed under running tap water until completely thawed. Aliquots of 10 mL of juice were put into screw-capped test tubes and incubated in a thermostatically controlled shaking water bath. At different time intervals, samples were removed from the water bath and rapidly cooled by plunging them into an ice-water bath (Kechinski et al., 2010). The samples were stored at -20 °C until further analysis. Phytochemical concentration, PPO activity, and kinetic data analysis were measured as in Sections 2.2, 2.4 and 2.5 respectively.

3.2.4. Thermal degradation of phytochemicals in pasteurised blueberry juice during storage

Two bottles of each pasteurised blueberry juice (prepared in Section 2.6) for shelf life analysis were stored at 25 °C for 20 weeks. Phytochemical changes in the juice were assessed at 0, 4, 8, 12 and 20 weeks of storage. Phytochemicals and PPO activity were measured, and kinetic data analysis carried out as described in Sections 2.2, 2.4, and 2.5.

3.3. Results and discussion

3.3.1. Blueberry composition

Three types of blueberry were chosen as model varieties to be used in the juice processing trial: one variety from the Rabbiteye species (i.e. ‘Maru’) and the other

two varieties from the Highbush species (i.e. 'Dixi' and 'Burlington'). Blueberry cultivars are variable in size. 'Maru' is the largest fruit for Rabbiteye species (Blueberry New Zealand: Fruit for thought, 2014), while for Highbush species, 'Dixi' was one of the largest cultivars and 'Burlington' was considered as the smallest cultivar. In this study, 'Maru' and 'Dixi' were similar in size, and 'Burlington' was relatively small (Figure 3-1).

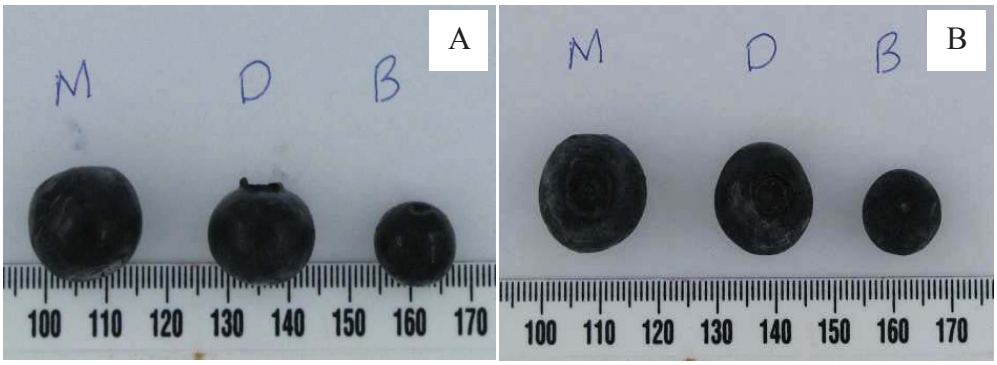


Figure 3-1: Size and shape of three types of blueberry.A) side view B) top view; where M = 'Maru', D = 'Dixi' and B = 'Burlington'. These were representative of the size ranges of each cultivar.

Initial concentrations of phytochemicals and PPO in these three common varieties of frozen blueberry extracted using solvent extraction are shown in Table 3-1. Phytochemical concentration varied among the varieties. 'Burlington' had a high concentration of anthocyanins and CGA, but was lowest in procyanidin B2. 'Maru' contained high anthocyanins and procyanidin B2, but was lowest in CGA. 'Dixi' contained high CGA and procyanidin B2, but was low in anthocyanins. However, PPO concentration was not significantly different ($P > 0.05$) amongst the three varieties.

Table 3-1: Anthocyanins, CGA, procyanidin B2, and PPO activity in three common varieties of frozen blueberry. Anthocyanin, CGA and procyanidin B2 were extracted using solvent extraction and PPO was extracted using sodium phosphate buffer solution.

	Anthocyanins (mg/kg FW)	CGA (mg/kg FW)	Procyanidin B2 (mg/kg FW)	PPO (Units/g FW)
'Burlington'	2018 ± 231^{a}	1243 ± 148^{a}	6 ± 3^{b}	335 ± 111^{a}
'Dixi'	1309 ± 126^{b}	1162 ± 156^{ab}	21 ± 7^{a}	359 ± 107^{a}
'Maru'	2362 ± 568^{a}	923 ± 323^{b}	25 ± 14^{a}	346 ± 91^{a}

Data represent means ± confidence intervals at 95% (N=6). Values that are followed by different letters within each column are significantly different ($P < 0.05$) using Tukey's Honest Significant Difference test.

The range of phytochemical concentrations in blueberries in this study was in agreement with published research (Table 3-2). Phytochemical concentrations depend on the berry variety, berry maturity, and growing conditions (Lata et al., 2005). Different extraction methods and solvents will also give differing concentrations from the same starting tissue. For example Pranprawit (2014) found that anthocyanin concentration in 'Dixi' extracted using 5% formic acid was 1247 mg/kg FW but only 1137 mg/kg FW of anthocyanin was found by Birt (2011) using a mixture of solvents consisting of acetone / methanol / water / formic acid at ratio of 40:40:20:0.1.

Anthocyanins, CGA, and procyanidin were the three compounds of interest in this study. Anthocyanins and CGA were the predominant compounds in blueberry (Gao & Mazza, 1994; Skrede et al., 2000; Taruscio et al., 2004). Procyanidin was selected because of its growing association with inflammation regulation and health benefits

Table 3-2: Range of phytochemical concentrations in Highbush and Rabbiteye blueberries as reported by several researchers.

Phytochemical	Highbush		Rabbiteye	
	Concentration (mg/kg FW)	Reference	Concentration (mg/kg FW)	Reference
Total anthocyanin	343 – 1260	Howard & Hager (2007)	105 – 6860	Howard & Hager (2007)
CGA	200 – 2690	Taruscio et al. (2004); Brownmiller et al. (2008); Rodriguez-Mateos et al. (2011)	130 – 5150	Wang et al. (2012)
Total procyanidin	1800	Howard & Hager (2007)	na	
Procyanidin B2	25 – 40	Gu et al. (2004); Brownmiller et al. (2009)	na	

na=not available

(Llópiz et al., 2004; Iglesias et al., 2012). In this study, we only measured the concentration of procyanidin B2.

Procyanidins are found in a series of oligomers of (epi) catechin units, from singly linked (B1, monomer) through to B8 (octamer). Interaction of the oligomers in the juice may occur because of sorption and/or desorption to and from fibre in the juice (Le Bourvellec et al., 2004). Due to limitations of the instrument used here only the dimer of procyanidin (procyanidin B2) could be measured.

3.3.2. Blueberry juice composition

During juicing, the phytochemical and PPO concentrations in the juice dropped significantly. Crushing the whole fresh/ frozen berries leads to rapid enzymatic and non-enzymatic destruction of phytochemicals. With solvent extraction of the berries, complete extraction of phytochemical from the berry matrix can be achieved. It gives the concentration of phytochemical in the original berries, while measurement of the juice gives how much phytochemical survives the process. It is important to know how much phytochemical is retained in the juice because juice is the product that can be consumed.

Results in Table 3-3 show that blanching increased phytochemical concentration in the final juice. Pasteurisation did not alter the phytochemical concentration but PPO activity in the juice was completely removed by pasteurisation.

Table 3-3: Comparison of anthocyanins, CGA, procyanidin B2, and PPO concentration in blueberry ('Dixi') juice with different processing conditions.

Juices	Anthocyanins (mg/L)	CGA (mg/L)	Procyanidin B2 (mg/L)	PPO (U/ml)
Raw juice ('No blanch'; not pasteurised)	375 ± 139^{c}	702 ± 165^{b}	15 ± 10^{b}	28 ± 7^{a}
'No blanch'; pasteurised	368 ± 192^{c}	741 ± 36^{b}	16 ± 3^{b}	0^{b}
Blanched at 70 °C; pasteurised	567 ± 118^{b}	972 ± 28^{a}	31 ± 4^{a}	0^{b}
Blanched at 90 °C; pasteurised	753 ± 77^{a}	965 ± 8^{a}	33 ± 5^{a}	0^{b}

Data represent means ± confidence intervals at 95% (N=2-3). Values that are followed by different letters within each column are significantly different ($P<0.05$) using Tukey's Honest Significant Difference test.

In general, phytochemical concentration was low in raw juice ('no blanch', not pasteurised) but the PPO degrading enzyme was still present in the raw juice and not the blanched juice. This suggests that phytochemical composition in the juices varied depending on the juice processing method applied especially heat treatment during blanching. Studies conducted by Rossi et al. (2003) and Brambilla et al. (2008) show that blanching greatly increased the phytochemical recovery in blueberry juice especially anthocyanins, CGA, and total cinnamates. Blanching destroyed the PPO enzyme and significantly reduced deterioration of the phytochemical concentration in blueberry. Blanching also helped to soften plant cell membranes and wall pectins, thus releasing more phytochemicals from the tissue (Van Buggenhout et al., 2009).

The presence of active PPO in the juice rapidly degraded some phytochemicals. Therefore, detailed analysis of the early effects of PPO was further studied, as the first few minutes of heating during blanching would stimulate PPO activity.

3.3.3. Thermal degradation kinetics of phytochemical in raw bluberry juice

When blueberry juice was heat treated and assessed periodically during heat treatment, anthocyanin degradation was temperature dependent (Figure 3-2 A). When transformed, data gave a linear Arrhenius plot (Figure 3-2 D) with an activation energy of 63.8 ± 7.9 $kJmol^{-1}$. Thermal degradation analysis of anthocyanins in blueberry juice corresponded to first-order kinetics, with the rate increasing with temperature. Calculated kinetics parameters are shown in Table 3-4.

Thermal degradation kinetics of CGA in blueberry juice showed that CGA concentration was stable at all the temperatures studied (Figure 3-2 B).

Procyanidin B2 responses to temperature in blueberry juice were more complex. Procyanidin B2 concentration slightly increased during incubation at 50 – 80 °C. At 90 °C, this increase was followed by a shallow decrease after 165 min (Figure 3-2 C).

Thermal degradation of anthocyanin has previously been reported to follow first-order kinetics. However, Buckow et al. (2010), reported that the degradation kinetics of anthocyanins in blueberry juice during high pressure-temperature processing (100 – 700 MPa, 40 – 121 °C) was best described by 1.4^{th}-order reaction, a deviation

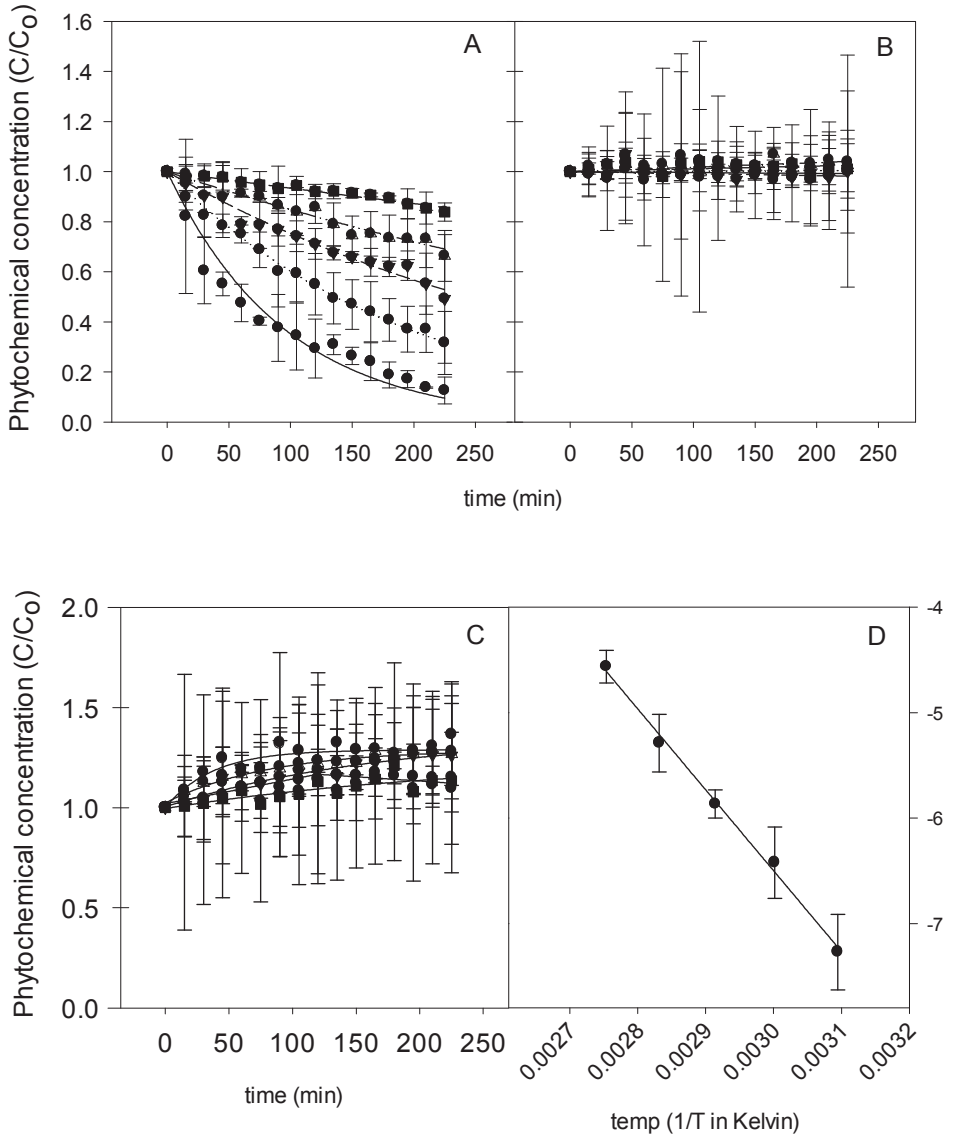


Figure 3-2: Thermal degradation of phytochemicals in raw blueberry juice at 50 (■), 60 (Δ), 70 (▼), 80 (○) and 90 °C (●). A) Anthocyanin degradation. Fitted line is predicted C/Co based on first order kinetics. B) CGA degradation. C) Procyanidin B2 degradation. Lines interpolating the experimental data points show the fit of exponential function from 50-80 °C and piecewise, two segment linear model for 90 °C. D) Thermal dependency of anthocyanin concentration of raw juice. Straight line is the predicted anthocyanin concentration based on Arrhenius equation. Data represent means of three replicates. Error bars indicate confidence interval at 95%.

Table 3-4: Kinetic parameters for thermal degradation of anthocyanin in raw blueberry juice.

Temperature (°C)	k (min^{-1})	$t_{1/2}$ (h)	R^2
50	0.0007 ± 0.0001	16.73 ± 2.73	0.949
60	0.0016 ± 0.0001	7.16 ± 1.06	0.954
70	0.0028 ± 0.0001	4.09 ± 0.25	0.975
80	0.0051 ± 0.0002	2.30 ± 0.28	0.994
90	0.0104 ± 0.0010	1.11 ± 0.08	0.950

Data represent means ± confidence intervals at 95% (N=3).

from simple first-order kinetics. The reaction was slightly faster initially than first-order reaction; but this result may have been affected by PPO enzyme activity at low pressure-temperature combinations, with thermal degradation taking priority at high pressure-temperature.

Albarici & Cruz Pessoa (2012) studied the effects of heat treatment of an acai drink, comparing the kinetic reaction of anthocyanin in a drink prepared from pasteurised and non-pasteurised pulp. They found that anthocyanin degradation of the juice from pasteurised pulp followed first order kinetics while juice from non-pasteurised pulp followed a second-order kinetic reaction. They suggested that pasteurisation can improve the preservation of anthocyanin in the pulp by inactivating the degrading enzymes and eliminating thermally-sensitive microorganisms.

There is limited literature available on thermal degradation kinetics of CGA. Degradation of CGA in this study was similar to that found by Birt (2011) who found that CGA concentration in blueberry juice was stable during storage at 5, 15, and 25 °C, over a 6 month period. This result is consistent with work by Narita &

Inouye (2013) which found that CGA was a stable compound and that thermal decomposition of CGA to caffeic acid occurs only minimally at 37 °C. Most studies focused on enzymatic degradation of CGA by PPO (Pomenta & Burns, 1971; Martinez & Duvnjak, 2006) and this will be further discussed in Chapter 4.

Procyanidin B2 reaction to thermal treatment was complex. Procyanidin B2 increases in this study may be due to the condensation of anthocyanins and other phenolic compounds in the juice. However, we interpret these results as indicating a release of procyanidin B2 from sediment or fibre, or polymerisation of monomeric procyanidin occuring in the juice during heating. The decrease of procyanidin B2 after 165 min at 90 °C may be due to thermal degradation. Since procyanidin B2 responses to temperature were complex, no further investigations were done to identify its kinetic degradation and it was not used for modelling.

Procyanidin B2 reactions in this study seem to be consistent with Dallas et al. (1995), who found that complex reactions occurred when they studied the interaction between anthocyanins, procyanidin B2, and acetaldehyde in a model wine solution. Condensations between anthocyanins and procyanidin B2 with or without an acetaldehyde bridge were the main reactions that occurred in the model wine solution. At the same time, degradation of anthocyanins, transformation of procyanidin B2, reaction between anthocyanins and acetaldehyde, and reaction between procyanidin B2 and acetaldehyde can also occur simultaneously, which makes degradation of procyanidin B2 highly complex. All these reactions were involved in the precipitation/formation of procyanidin or more complex procyanidins in the solution (Jurd, 1969; Haslam, 1980).

Generally, procyanidins are found in plant vacuoles where they are separated from other cellular components, and the concentration of free and bound dimer (procyanidin B2) are highest compared to other procyanidin oligomers. Concentration of bound procyanidin B2 was about 80% higher than free procyanidin B2 (Howard et al., 2011). When the vacuoles were ruptured, the procyanidin oligomer is released and may interact with other cellular components such as the cell wall through hydrogen bonding and hydrophobic interactions. These interactions results in covalent bonding of the procyanidin oligomer to the polysaccharide matrix (Pinelo et al., 2006). Procyanidins in particular have a strong affinity for cell wall material (Le Bourvellec et al., 2004) with higher molecular weight compounds having a greater affinity for binding than smaller compounds.

3.3.4. Thermal degradation of phytochemicals in pasteurised blueberry juice during storage

This study was done to monitor the stability of phytochemicals in pasteurised juice during long-term storage at elevated temperature. The juices were prepared by manipulating heat treatment during blanching. All the juices were pasteurised to inactivate the PPO enzyme and destroy harmful microorganisms in the juice.

Thermal degradation of anthocyanins, CGA, and procyanidin B2 in pasteurised blueberry juices over 20 weeks of storage at 25 °C in the dark corresponded to first-order kinetics (Figure 3-3: A – C respectively). After blanching and pasteurisation, juices had significant differences in composition, but a thermal degradation pattern was identified. There was no residual PPO in any treatment. Calculated kinetics parameters are shown in Table 3-5.

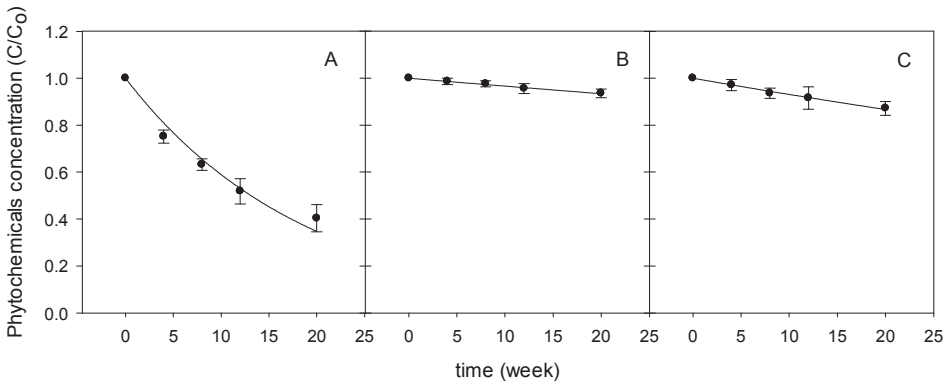


Figure 3-3: Degradation of phytochemicals in pasteurised juice during storage at 25 °C for 20 weeks. A: anthocyanin; B: CGA; C: procyanidin B2. Data represent means of six replicates. Error bars indicate confidence interval at 95%. Fitted line is predicted C/C_o based on first order kinetics reaction.

Table 3-5: Kinetics parameters for thermal degradation of phytochemicals in pasteurised blueberry juice during storage at 25 °C for 20 weeks.

Phytochemical	k (min^{-1})	$t_{1/2}$ (w)	R^2
Anthocyanins	0.0529 ± 0.0104	13.51 ± 0.89	0.967
CGA	0.0034 ± 0.0003	190.51 ± 32.46	0.944
Procyanidin B2	0.0072 ± 0.0007	104.75 ± 37.57	0.934

Data represent means ± confidence interval at 95% (N=6).

Thermal degradation of raw blueberry juice (Section 3.3.3) showed that degradation of anthocyanin occurs rapidly, CGA was stable and procyanidin B2 could apparently increase at higher temperatures. When pasteurised, bottled and stored in long-term storage these three phytochemicals decreased slowly over time. Anthocyanins were the least stable compounds, with 50% of the anthocyanin degraded after 13 – 14 weeks when stored at 25 °C. Results in this study were consistent with other research. Degradation of anthocyanin in pasteurised blueberry juice after 16 months of storage at 4 °C and 20 °C was slow with 40% and 80% of degradation

respectively (Satanina, 2011). The half-life of anthocyanins was much shorter at room temperature than in cold storage. This confirmed that pasteurisation effectively inactivated PPO and degradation of phytochemicals in pasteurised juice over long term storage was solely due to thermal degradation.

CGA was a stable compound. In this study, a very slow degradation of CGA was observed over a long storage time. Only 17% of CGA was degraded over one year of storage at 25 °C due to thermal degradation. The same observation was observed in chokeberry (Hellström et al., 2013) and blueberry juice (Birt, 2011). Over 96% and 80% of CGA remained in pasteurised chokeberry and blueberry juice after storage at room temperature for 9 weeks and 6 months respectively.

Procyanidin B2 can be considered a stable compound because degradation of procyanidin B2 in this study was quite slow. After one year of storage at 25 °C, only about 25 – 32% of procyanidin B2 disappears in the juice. This finding is consistent with Dallas et al. (1995) who reported that procyanidin B2 was the most stable compound compared to other procyanidin oligomers. However, degradation of each procyanidin oligomer still occurred during storage at low temperature (12 and 22 °C) and followed first order kinetic reaction. No PPO was detected in this juice, therefore degradation of procyanidin B2 due to PPO activities can be discounted. The losses of procyanidin B2 in blueberry juice may be due to polymerization reactions with anthocyanins, and interaction of procyanidin with cell-wall polysaccharide forming precipitates during storage (Brownmiller et al., 2008).

There is no doubt that long-term storage resulted in lower concentration of phytochemicals in the juice. Storing at room temperature should be avoided if good long-term preservation of phytochemicals is desired especially for anthocyanins which have a very poor stability during storage (Buckow et al., 2010; Gancel et al., 2011). Storing of the juice at ambient temperature for 3 months resulted in about 26% anthocyanin degradation while only 2% of anthocyanin degradation occurs when stored in refrigerated conditions for the same duration. Therefore, it is best to store the juice under refrigeration for a better quality of the juice.

3.4. Thermal model

The thermal model was used to describe thermal degradation that occurs during juice processing. In juice processing, thermal degradation occurs all the time. Therefore, it is important to keep 'holding time' short and 'holding temperature' as low as possible. Thermal degradation also occurs during pasteurisation and storage. To develop the thermal model for blueberry juice processing, the characteristics of the juice in each processing step need to be considered. The main additional parameter needing consideration is PPO activity in the juice.

Raw juice was prepared to demonstrate juice with PPO while pasteurised juice was prepared to represent juice with no PPO. PPO was fully inactivated during pasteurisation (Charles-Rodríguez et al., 2007; Buckow et al., 2010). Therefore, two thermal models are needed to explain and predict anthocyanin degradation in blueberry juice processing (Figure 3-4).

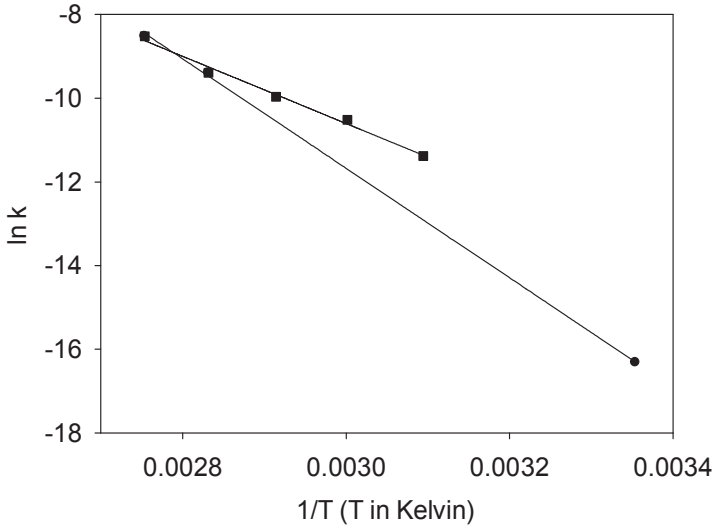


Figure 3-4: Thermal dependency of anthocyanins of raw juice (■) and pasteurised juice (●). Straight line is the predicted anthocyanin concentration based on an Arrhenius equation.

The first model is 'raw juice model' used to predict anthocyanin degradation when blanching was omitted during juice processing and PPO is still present in the juice. This model is the same model as presented in Section 3.3.3. It was described by Arrhenius equation as Equation 3.1 (R^2 = 0.9955). Confidence interval at 95% for the coefficient is presented in Table 3-6.

$$y = 13.50 - 8035\,x \quad \text{.....Equation 3-1}$$

Table 3-6: Confidence interval at 95% for the coefficient for Equation 3.1.

Coefficients	Confidence interval at 95%
13.50	4.14
8035	1419

Data represent means of three replicates

The second model is the 'pasteurised juice model' to predict anthocyanin degradation when blanching was applied during juice processing and no PPO was present in the juice. Due to insufficient data for thermal kinetics of pasteurised juice, this model was developed by using data extrapolated from pasteurised juice during storage and raw juice treated at 80 and 90 °C. The model is described by an Arrhenius equation as Equation 3.2 (R^2 = 0.9997). Confidence interval at 95% for the coefficient is presented in Table 3-7.

$$y = 27.60 - 13090\,x \quad \text{..... Equation 3-2}$$

Table 3-7: Confidence interval at 95% for the coefficient for Equation 3.2.

Coefficients	Confidence interval at 95%
27.60	8.66
13090	2895

Data represent means of three replicates

Thermal degradation kinetics of phytochemicals (Section 3.3.2) revealed that anthocyanins were thermally labile, CGA was thermally stable, and the reaction of procyanidin B2 was complex. This strong effect of temperature on anthocyanin concentration was a significant factor for consideration when developing a blueberry juice processing model.

3.5. Conclusion

Changes of phytochemicals, particularly anthocyanins, CGA, and procyanidin B2 in raw blueberry juice during thermal treatment and pasteurised juice during long-term storage have been successfully described. Raw juice was used as a model system to avoid complexity of releasing phytochemicals from the matrix and tissue homogenate. However, the reactions that occur may still be influenced by the residue of PPO in the juice. Therefore, pasteurised juice was used to demonstrate juice conditions without PPO. Based on the thermal degradation kinetics of the phytochemicals, anthocyanins were a significant factor for consideration when developing a blueberry juice processing model. CGA was thermostable and procyanidin B2 behaviour was complex. Two thermal models have been developed to predict anthocyanin degradation in blueberry juice processing. A raw juice model will be used for the early stages of juice processing when PPO is still active, while a pasteurised juice model will be applied to juices after blanching or pasteurisation.

4. Enzymatic degradation of phytochemicals in blueberry

4.1. Introduction

Browning reactions are a common phenomenon occurring in food especially during processing and storage, and also as a result of mechanical injury. Browning usually affects the sensory quality as it is associated with changes in flavour, colour, and softening (probably due to the action of pectic enzymes) as well as losses in nutritional properties (Vamos-Vigyazo et al., 1977; Martinez & Whitaker, 1995). There are two types of browning reaction: enzymatic and non-enzymatic (Eskin, 1990; deMan, 1999). In most juice processing, browning during thermal processing is due to both enzymatic and non-enzymatic browning reactions taking place during storage. Enzymatic browning is rapid but enzymes are destroyed during processing while non-enzymatic is slower but persists after removal of enzymes by processing (Babsky et al., 1986; Koca et al., 2003; Selen Burdurlu & Karadeniz, 2003).

Enzymatic reactions in fruits and vegetables occur due to the presence of endogenous degrading enzymes such as polyphenoloxidase (PPO) and peroxidase (POD) (Vamos-Vigyazo et al., 1977). Both enzymes are responsible either directly or indirectly for degrading anthocyanins and other polyphenols eventually leading to browning. Although both enzymes are involved in enzymatic degradation, in blueberry PPO is the main enzyme responsible for the reaction rather than POD (Kader et al., 1997). Therefore, only PPO will be investigated in more detail in this study.

Typical non-enzymatic browning involves caramelization, ascorbic acid degradation, and Maillard reactions (Clegg, 1964).

In blueberry juice processing, a blanching step has normally been introduced to increase recovery of phytochemicals (Skrede et al., 2000; Rossi et al., 2003). To our surprise, some major blueberry juice processors in New Zealand don't use blanching. Only one small juice producer that we know used blanching in their processing and the juice produced has a different texture and flavour. Blanching of the fruit before processing is believed to be one of the most efficient methods to inactivate PPO and prevent anthocyanin discoloration. Therefore, the objectives of this chapter are to quantify enzymatic degradation and deduce enzymatic activity in blueberry.

4.2. Material and methods

4.2.1. Browning in blueberry mash and juice

For blueberry mash, about 60 g of frozen blueberry ('Dixi') was weighed, frozen with liquid nitrogen and finely ground using a coffee grinder (Breville CG2B, Australia). An accurately weighed 10 g sample of the berry powder was put into each of several test tubes, mixed with 20 mL of distilled water and incubated at room temperature. At different time intervals, 5 mL of the solution was taken out, mixed with 10 mL of 95% ethanol and centrifuged at 800 *x g* at 4 °C for 10 min. The supernatants were measured spectrophotometrically at 420 and 520 nm.

Determination of the browning index of blueberry juice was carried out by diluting the juice with 95% ethanol (1:4 v/v) and then measuring spectrophotometrically at 420 and 520 nm. It is difficult to measure browning in highly coloured samples as the brown colour of the juice is masked by the red or blue colour. To overcome this problem, a browning index was defined to maximise absorbance changes, by taking

the ratio of 420 nm (where brown pigments absorb) and 520 nm (where anthocyanins absorb) (Marquez et al., 2013).

The thermal kinetics of browning index in raw and pasteurised juice were analysed as described in Sections 3.2.3 and 3.2.4 respectively.

4.2.2. Enzymatic degradation of whole berry

Accurately measured 4 – 6 g samples of frozen blueberry ('Dixi') were weighed, put into test tubes, and incubated at different temperatures (4, 20, 30, and 40 °C) in a thermostatically-controlled water bath overnight. At different time intervals, samples were removed and extracted by homogenising the berries in 10 mL of a pre-cooled solvent mixture of acetone/methanol/water/formic acid (40:40:20:0.1) using an Ultra Turrax T25 Basic homogeniser (Ika Labortechnik, Malaysia). Homogenates were centrifuged at 4000 *x g*, 20 °C for 5 min (Thermo Scientific Heraeus multifuge IS-R centrifuge, Massachusetts, U.S.A.). Supernatants were collected and this procedure was repeated three times to make sure all phytochemicals were extracted. The collected supernatants were stored at -20 °C for later HPLC analysis as described in Section 2.2.

4.2.3. Enzymatic degradation of mashed berry

Approximately 80 g of frozen blueberry sample ('Dixi') was frozen with liquid nitrogen and finely ground in a coffee grinder (Breville CG2B, Australia). An accurately weighed 4 – 5 g sample of berry mash was put into test tubes and incubated at different temperatures (4, 20, 30, and 40 °C) in a thermostatic water bath overnight. At different time intervals, samples were removed from the water

bath and immediately extracted with 10 mL of a pre-cooled solvent mixture of acetone/methanol/water/formic acid (40:40:20:0.1). The mixture was vortexed and centrifuged at 4000 *x g*, 20 °C for 5 min. Supernatants were collected and this procedure was repeated three times to make sure all phytochemicals were extracted. The collected supernatants were stored at -20 °C for later HPLC analysis as described in Section 2.2.

4.2.4. Berry blanching

Approximately 50 g of a single layer of frozen blueberries was vacuum packed in a retort pouch at 10 Bar. Blueberry pouches were thawed in a water-bath using tap water for 10 min and blanched at pre-determined water-bath temperatures (50, 60, 70, 80, and 90 °C) for 0, 1, 3, and 10 min. When sampled, pouches were immediately cooled in ice slurry for 10 min. The samples were then rolled using a rolling pin to crush the berries, removed from pouches and centrifuged (4000 *x g* for 20 min at 15 °C) to yield the juice. The juice was stored at -20 °C until further analysis. Phytochemical determination was done as per Section 2.2 and PPO analysis as per Section 2.4.

4.2.5. Kinetic analysis of PPO inactivation

Kinetic inactivation of PPO in blueberry can be described by a biphasic model (Equation 4-1) as reported by Terefe et al. (2010):

$$\frac{A}{A_o} = A_S \exp(-k_S t) + A_L \exp(-k_L t) \quad \text{... ... ...Equation 4-1}$$

where A_S and A_L are activities of the stable and labile fractions respectively and k_S and k_L are the inactivation rate constants of the stable and labile fractions, respectively.

The temperature dependence of the inactivation rate constant can be described by the Arrhenius equation given in Equation 4-2.

$$\boldsymbol{k = k_{ref}\exp\left(\frac{-E_a}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right)} \text{ ... .Equation 4-2}$$

where T and T_{ref} are the experimental and reference temperatures (K), E_a is the activation energy (J/mol), R is the universal gas constant (8.314 J/mol K) and k and k_{ref} are the inactivation rate constants (s^{-1}) at T and T_{ref} respectively.

Equation 4-1 and 4-2 may be combined to obtain the global biphasic model Equation 4-3.

$$\frac{A}{A_o} = A_S\exp\left[-k_{Sref}\exp\left(\frac{-E_{as}}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right)t\right] + A_L\exp\left[-k_{Lref}\exp\left(\frac{-E_{aL}}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right)t\right] \text{ ... ... Equation 4-3}$$

where k_{Sref} and k_{Lref} are the inactivation rate constants of the stable and labile fractions, respectively, at the reference temperature: and E_{as} and E_{aL} are the activation energies for the inactivation of the stable and labile fractions, respectively.

The kinetic parameters (k_{Sref}, k_{Lref}, E_{as} and E_{aL}) were calculated by minimising the sum of squares error (SSE) between the experimental and calculated values for A/A_o

using Solver™ in Microsoft® Excel. A goodness of fit of the data was assessed with regression coefficients (R^2).

4.3. Results and discussion

4.3.1. Browning in blueberry and blueberry juice

When frozen blueberries were crushed, the colour of the berry mash became brown. The colour was measured as a browning index which increased over time (Figure 4-1 A). Browning was also observed in raw blueberry juice when the juice was heated at 50 – 90 °C for 4 h (Figure 4-1 B) and in pasteurised juice stored at 25 °C for 5 months (Figure 4-1 C) respectively, but at a much slower rate than in the berry mash.

The kinetics of browning in blueberry mash, raw blueberry juice, and pasteurised blueberry juice was zero-order (Equation 2-2). Browning rate in blueberry mash at room temperature was rapid compared to raw blueberry juice treated at 50 – 90 °C for 4 h and was very low in pasteurised blueberry juice (Table 4-1). The high rates of browning in blueberry mash were therefore probably a result of enzymatic browning by PPO. PPO is predominantly located in the vacuole and when the cell wall and internal membranes are ruptured, PPO is released and reacts with CGA to degrade anthocyanin and form the brown colour (Kader et al., 1997). Browning rates in pasteurised juice were very low because the PPO was inactivated during blanching and pasteurisation as discussed in Section 3.3.3. The slow browning reaction occurring in pasteurised juice during storage was presumably solely non-enzymatic browning.

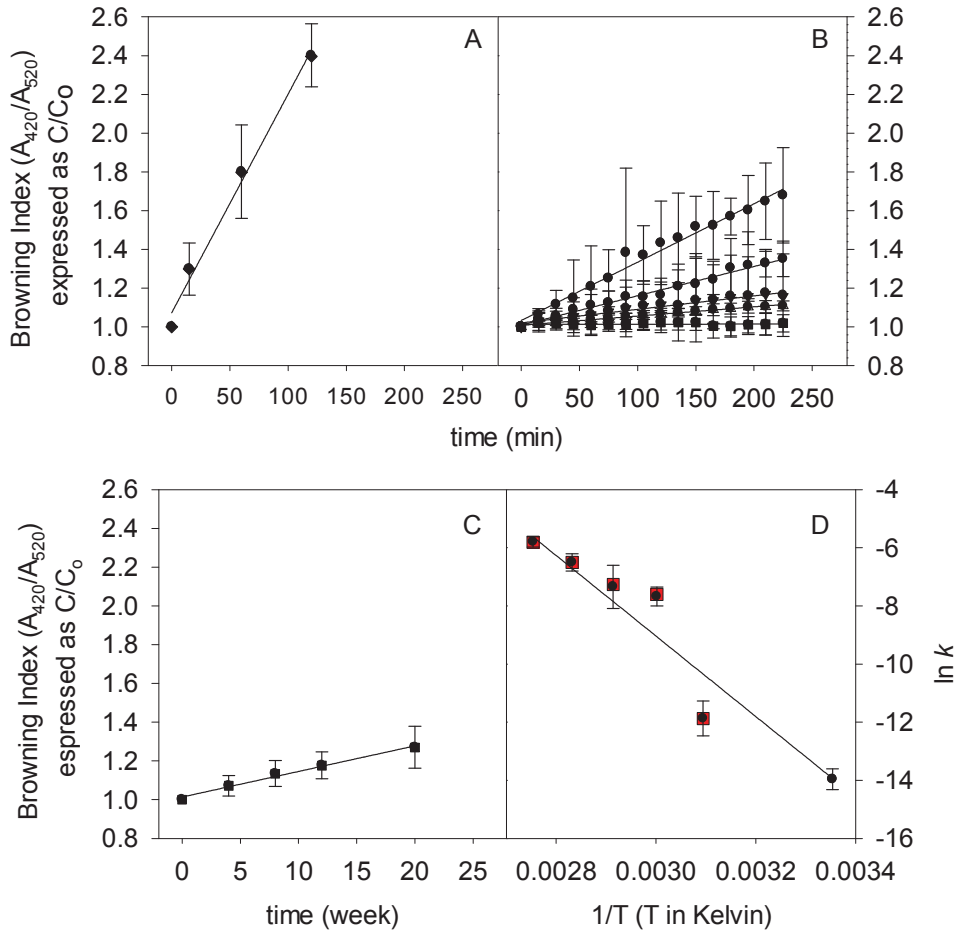


Figure 4-1: Kinetics of browning in A) mashed berries at room temperature (N=3); B) raw juice during thermal treatment at 50 (■), 60 (Δ), 70 (▼), 80 (○), and 90 °C (●) (N=3); C) pasteurised juice during long term storage at 25 °C (N=6); and D) Arrhenius plot for browning rates in raw (■) and pasteurised juice (●). Line interpolating the experimental data points shows the fit of linear regression. Error bars indicate confidence interval at 95%.

When transformed, the temperature-dependent rate constant of browning in raw blueberry juice gave a broken Arrhenius plot (represented with ■ symbol in Figure 4-1 D). This may result from both enzymatic and non-enzymatic browning reaction occurring in the same samples. To confirm this, data from pasteurised juice were used to predict browning at 25 °C. Browning after blanching at 80, 90 °C, and in pasteurised juice at 25 °C were re-plotted and gave a straight line (R^2 = 0.99) indicating 'true' non-enzymatic browning. Browning at 60 and 70 °C was a little

faster than would be expected, perhaps because PPO has not yet been inactivated at these temperatures. However, browning at 50 °C, was slower than expected with a shallow curve which resulted in a very bad fit with $R^2 = 0.005$ (Table 4-1), making this data point unreliable in estimating the activation energy (E_a). E_a calculated for the non-enzymatic browning reaction (in the temperature range 25 – 90 °C) in this study was 115 $kJmol^{-1}$.

Half-life ($t_{1/2}$) is a term normally used to refer to any period of time in which a quantity reduces by half. For browning it is opposite, $t_{1/2}$ is defined as time required for browning to increase by half. The rate constant (k) and $t_{1/2}$ values for mashed berries, raw blueberry juice, and pasteurised juice were tabulated in Table 4-1.

Table 4-1: Kinetic parameters of mashed berries at room temperature, raw blueberry juice during thermal treatment at 50 – 90 °C, and pasteurised blueberry juice during long-term storage at 25 °C.

Samples/treatments	k (min^{-1})	$t_{1/2}$ (h)	R^2
Blueberry mash	0.0113 ± 0.0048	1.04 ± 0.41	0.989
Raw blueberry juice			
50	0.000007 ± 0.000004	1688 ± 1036	0.005
60	0.0005 ± 0.0001	25.00 ± 8.28	0.989
70	0.0007 ± 0.0005	18.40 ± 12.89	0.964
80	0.0015 ± 0.0004	7.78 ± 2.39	0.985
90	0.0030 ± 0.001	3.82 ± 0.67	0.982
Pasteurised juice	0.0000013 ± 0.0000002	9951 ± 1752	0.988

Data represent means ± confidence interval at 95% (N=3 for mashed berries and raw juice; N=6 for pasteurised juice).

Coseteng & Lee (1987) listed several factors which influenced browning in apple such as enzyme activity, substrate level, presence of ascorbic acid, and other inhibitors or promoters. Among all these, they concluded that PPO activity and its substrate concentrations appeared to be the major factors involved in the browning reaction. This is in agreement with this study which demonstrates that PPO action is more aggressive in mashed berries than in juice. As discussed earlier, PPO activity was high in the mashed berries compared to the juice, and CGA as the substrate for the PPO was abundantly available in both blueberry and the juice. This result also suggested that PPO inactivation is necessary in blueberry juice processing to prevent browning and retain anthocyanins in the final juice.

4.3.2. Degradation of whole blueberries and blueberry mash during thawing

When whole frozen berries and mashed blueberries were thawed, an immediate browning was observed as a result of degradation and/or oxidation of phytochemicals, especially anthocyanins. In this study, solvent extractions were carried out in order to measure phytochemical degradation in both systems. Both thermal and enzymatic degradation occurred in both systems during thawing but optimum temperature for PPO activity varies with plant source from 30 – 45 °C (Yoruk & Marshall, 2003) and PPO will be inactivated at temperatures above 60 °C (Mizobutsi et al., 2010). Therefore, these experiments were designed at temperatures between 4 and 40 °C to study enzymatic degradation of phytochemicals in both systems. Thermal degradation between these temperatures was minimal ($t_{1/2}$ for anthocyanins degradation at 40 °C = 33.76 h).

Enzymatic degradation of anthocyanins and CGA in whole and mashed berries shows deviation from first-order kinetics. It was rapid in the first few hours and slower afterwards (Figure 4-2 A – D). It seems that enzymatic degradation of anthocyanins and CGA in whole and mashed berries followed second-order kinetics. However, degradation of compounds in solid or semi solid food such as fruit or berry pomace is not isothermal, therefore kinetic modelling should include the time-temperature history (Dolan, 2003).

The independent variable time (t) and temperature (T) were combined into one variable, the thermal history (β) as in Equation 4-4.

$$\boldsymbol{\beta = \int_0^t \exp\left[\frac{-E_a}{R}\left(\frac{1}{T_t} - \frac{1}{T_{ref}}\right)\right] dt} \quad \text{... ... ...Equation 4-4}$$

where T_{ref} is the arbitrary reference temperature (20 °C) and T_t is the temperature (T) of a berry at time (t). The measured C/C_o values could then be plotted as a function of β. The reaction rate constant (k_r) at T_t was determined and a set of C/C_o values calculated according to Equation 4-5.

$$\boldsymbol{\frac{C}{C_o} = \left[1 + (n-1)C_o^{n-1} k_{ref} \beta\right]^{1/(1-n)}} \quad \text{... ... ...Equation 4-5}$$

The kinetic parameters (k_t and E_a) were calculated by minimising the sum of squares error (SSE) between the experimental and calculated values for C/C_o using Solver in Microsoft® Excel.

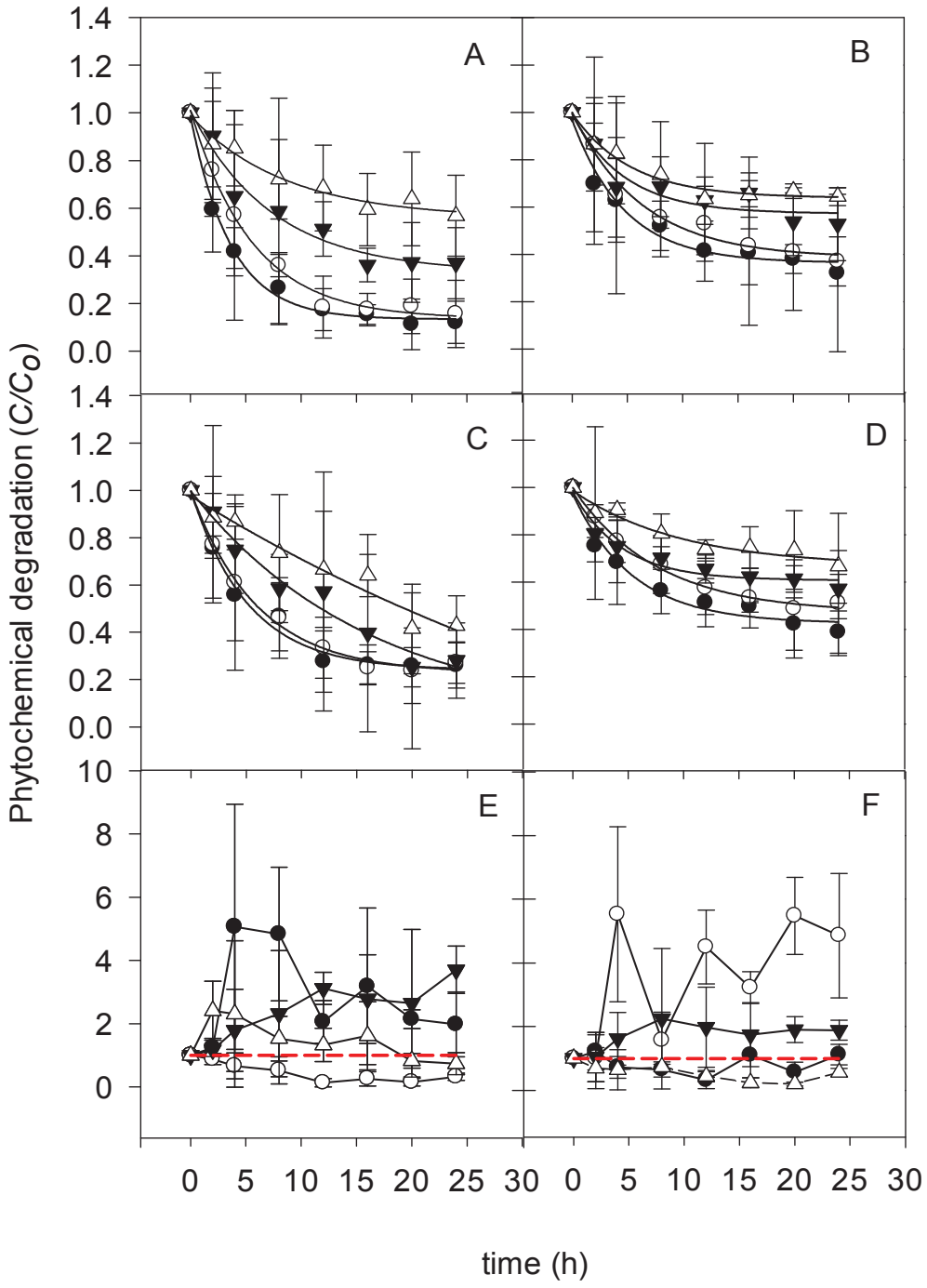


Figure 4-2: Effect of thawing on phytochemical degradation. Degradation of anthocyanins in whole (A) and mashed (B) berries; degradation of CGA in whole (C) and mashed (D) berries; and degradation of procyanidin B2 in whole (E) and mashed (F) berries. Where Δ = 4 °C, ▼ = 20 °C, ○= 30 °C, ● = 40 °C. Fitted line in A – D is the predicted C/C_o based on Equation 4-11. Error bars indicate confidence interval at 95%.

Although the model that has been generated is adequate for predicting anthocyanin and CGA loss during thawing in this work, the model is not universally applicable as the rate of temperature change (i.e. time temperature profile) during thawing is dependent on specific engineering design variables including the geometry of defrost equipment, the flowrate of the heating medium, and the geometry of the frozen blueberries themselves - specifically the surface area for heat transfer relative to the volume: a given mass of free flow frozen blueberries will have a higher surface area compared to an equivalent mass of blueberries frozen in a block.

However, once the blueberries have reached thermal equilibrium (Phase II) (Figure 4-3) then the degradation of anthocyanins and CGA at that temperature will only be dependent on the holding time i.e. degradation will now be independent of process design considerations. Therefore, it is possible to generate a model that is universally applicable to Phase II.

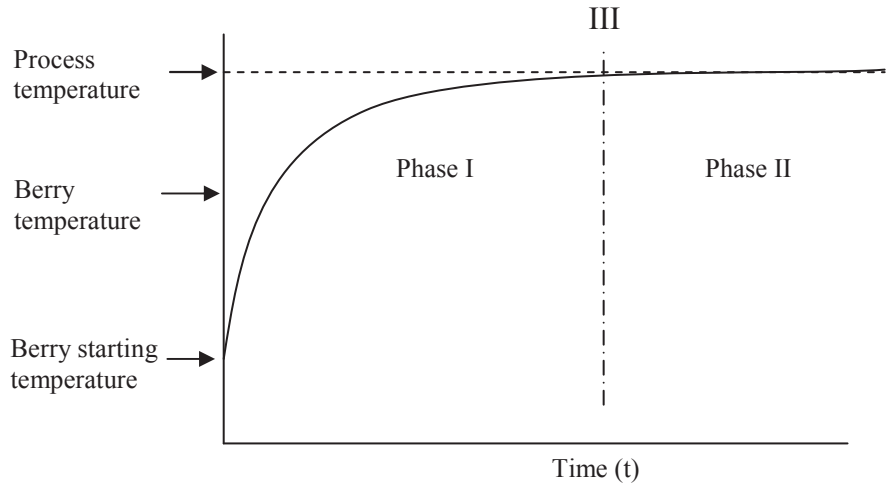


Figure 4-3: Schematic diagram of *β*-equivalent time of blueberry during thawing process. Phase I is the unsteady state where the temperature continuously changes; Phase II is the steady state where temperature is constant and Phase III is the transition to equilibrium and has been arbitrarily defined as β-equilibrium for this work.

The incremental changes in the β value for the collected experimental data sets were constant after a certain point in each of the experiments. The last 100 min of data, in which the data were constant under all experimental setups, was used to generate the Phase II equilibrium model. The incremental β change per s for anthocyanins and CGA in whole berry and mashed berry are shown in Table 4-2:

Table 4-2: Incremental β change per s for enzymatic degradation of anthocyanins and CGA in whole berries and mashed berry.

Temp (1/T (in Kelvin))	Incremental β change per s			
	Anthocyanin in whole berry	Anthocyanin in mashed berry	CGA in whole berry	CGA in mashed berry
0.00361	0.31	0.54	0.5439	0.54
0.00341	0.98	1.02	1.0207	1.02
0.00330	1.91	1.42	1.4122	1.43
0.00319	3.53	1.90	1.9587	1.91

Data represent means of three replicates.

The data were best fitted with the exponential model of the form shown in Equation 4-6 (anthocyanin in whole berry), Equation 4-7 (anthocyanin in mashed berry), Equation 4-8 (CGA in whole berry) and Equation 4-9 (CGA in mashed berry). The models appeared adequate with a correlation coefficient of 0.9989, 0.9988, 1.000 and 0.9988 respectively (Figure 4-4).

y = 423000000 * exp (-5830 1/T) .....Equation 4-6

y = 30200 * exp (-3000 1/T) .....Equation 4-7

y = 33300 * exp (-3050 1/T) .....Equation 4-8

y = 39800* exp (-3100 1/T) .....Equation 4-9

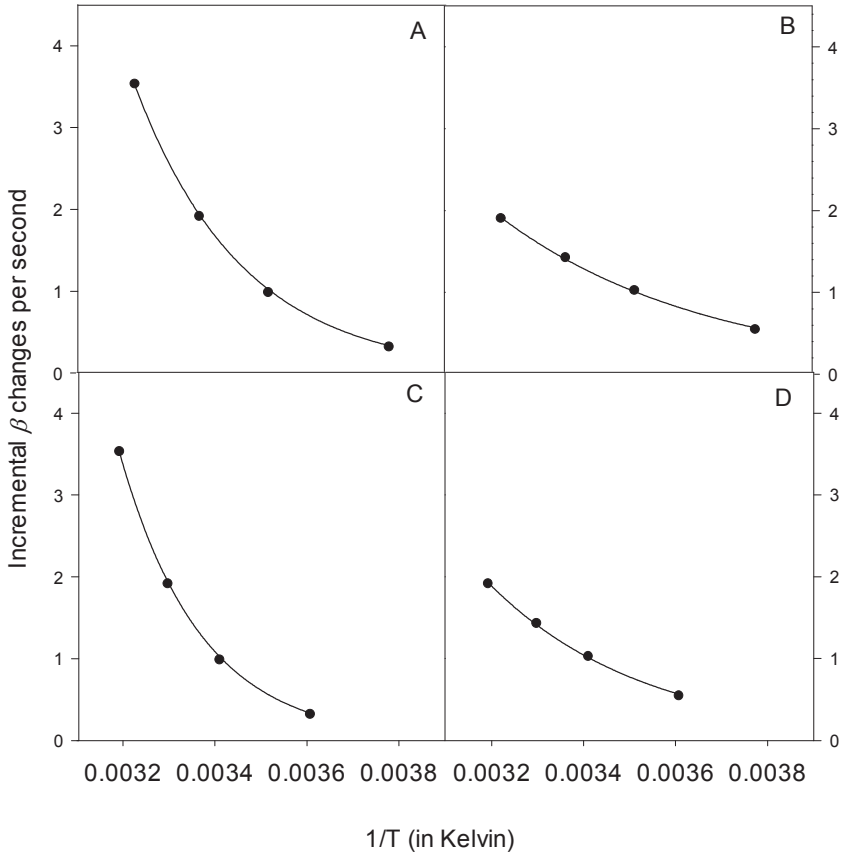


Figure 4-4: Relationship between the *instantaneous* β values and temperature for enzymatic degradation of anthocyanin in whole berries (A), anthocyanin in mashed berries (B), CGA in whole berries (C) and CGA in mashed berries (D). Data represent means of three replicates.

By substituting Equation 4-6 into Equation 4-5, a specific model which is capable of predicting anthocyanin degradation in whole berries can be formulated as in Equation 4-10.

$$\frac{C}{C_o} = \left[1 + (n-1)C_o^{n-1} k_{ref}\left(423000000 exp^{\frac{-5830}{T}}\right) t\right]^{1/(1-n)} \quad \text{..... Equation 4-10}$$

This model has been developed for determining the degradation of anthocyanins in whole blueberries. The industry is interested in determining the impact of processing on a range of phytochemicals, not only during the thawing of whole berries but also thawing of blueberry mash. Therefore, it was of interest to consider whether the approach taken to determine the specific model (Equation 4-10) could be used to apply the generalised form of the model (Equation 4-11) to other phytochemicals and to blueberry mash.

$$\frac{C}{C_o} = \left[1 + (n-1)C_o^{n-1}k_{ref}\left(ae^{\frac{-b}{T}}\right)t\right]^{1/(1-n)} \quad \text{..... Equation 4-11}$$

The derived values of *a* and b for predicting anthocyanin and CGA in whole berries and berry mash, and the confidence intervals at 95% for each coefficients, are presented in Table 4-3.

Table 4-3: Confidence interval at 95% for the coefficients for anthocyanin and CGA degradation in whole berries and berry mash during the defrost phase.

	Coefficient	Estimate of Coefficient	Confidence Interval at 95%
Anthocyanin in whole berries	*a*	423,000,000	34,200,000
	b	5,830	540
CGA in whole berries	*a*	39,800	6,900
	b	3,100	50
Anthocyanin in berry mash	*a*	30,200	6,450
	b	3,000	360
CGA in berry mash	*a*	33,300	9,200
	b	3,050	370

Data represent means of three replicates.

Degradation of anthocyanins in whole blueberry fruit has been observed with about 59% degradation occurring during storage at -18 °C for 6 months (Reque et al., 2014). Reque and co-workers suggested that during frozen storage, oxidation reactions caused by the action of enzymes and the presence of phenolic compounds could have led to anthocyanin degradation. It was strongly believed that anthocyanin degradation during thawing in this study is due to the same reasons. Oxidation and heat during thawing may damage the cell, liberating PPO enzyme which works really well in the presence of CGA and oxygen to accelerate anthocyanin degradation.

In this work significant losses of CGA were observed during thawing in both whole and mashed berries. CGA was shown in Section 3.3.3 to be thermo-stable. Therefore, the losses found here represent enzymatic degradation and are consistent with oxidation of CGA in the presence of PPO to form quinine products; and further reactions degrade the anthocyanin as suggested by Fennema (1996) (Figure 4-5). Degradation of anthocyanins and CGA during thawing occurs concurrently with a continuous supply of oxygen and is limited by PPO.

Degradation of anthocyanins and CGA in whole frozen berries during thawing seems to be more rapid compared to mashed berries as shown by *a* values (Table 4-4). Destruction of both phytochemicals in whole berries was rapid because during thawing, the berries were surrounded by air space and more exposed to oxygen. In mashed berries, degradation may have been slower because of reduced access to oxygen (Renard et al., 2001; Le Bourvellec & Renard, 2005). Another possibility is

that the pectin gel may reform when berries are crushed and heated and thus slow the diffusion of phytochemicals from the skin during thawing.

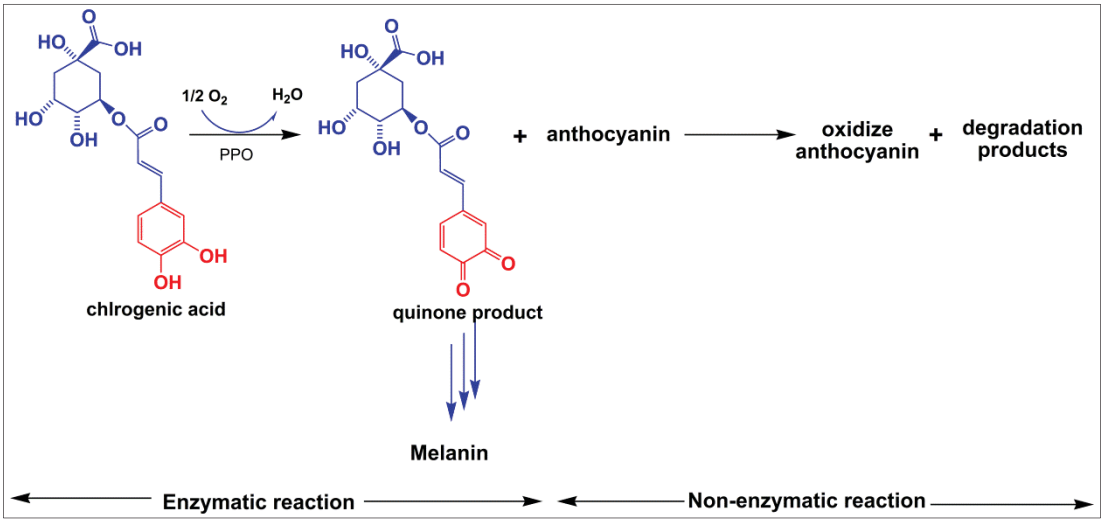


Figure 4-5: Mechanism of anthocyanins and CGA degradationas proposed by Fennema (1996).

Padayachee et al. (2012) studied the binding of polyphenols in plant cell walls and suggested that anthocyanins may have direct interaction with cellulose and/or pectin which acts to minimise diffusion of anthocyanins.

Degradation of procyanidin B2 in both whole and mashed berries was complex (Figure 4-2 E – F). The same phenomenon had been observed and discussed in Section 3.3.3. In short, sorption and desorption of procyanidin B2 on fiber, or polymerization and depolymerisation of procyanidin B2 may occur simultaneously during thawing, processing, and storage of berries and juices derived from them.

Table 4-4: Calculated kinetic parameters of anthocyanins and CGA in whole and mashed berries during the thawing process as per Equation 4-8.

	Temperature (°C)	k (s^{-1})	E_a (kJ mol^{-1})	R^2
Whole berry Anthocyanin	4	8.11E-09 ± 1.32E-09	48.1 ± 9.4	0.999
	20	2.53E-08 ± 4.12E-09		0.999
	30	4.85E-08 ± 7.89E-09		0.999
	40	8.92E-08 ± 1.45E-08		0.999
CGA in whole berry	4	1.52E-08 ± 2.29E-09	25.6 ± 3.1	0.999
	20	2.79E-08 ± 4.21E-09		0.999
	30	3.95E-08 ± 5.95E-09		0.999
	40	5.46E-08 ± 8.23E-09		0.999
Anthocyanin in berry mash	4	7.10E-09 ± 4.61E-10	25.6 ± 5.3	0.999
	20	1.30E-08 ± 8.47E-10		0.999
	30	1.84E-08 ± 1.84E-08		0.999
	40	2.55E-08 ± 1.66E-09		0.999
CGA in berry mash	4	6.63E-09 ± 1.20E-09	25.9 ± 2.8	0.999
	20	1.22E-08 ± 2.22E-09		0.999
	30	1.74E-08 ± 3.15E-09		0.999
	40	2.41E-08 ± 4.37E-09		0.999

Data represent means ± confidence interval (N=3).

4.3.3. Inactivation kinetics of PPO in blueberry juice

After berry blanching, the activity of PPO in the juice (Figure 4-6) decreased with temperature and time. PPO activity decreased rapidly in the first minute and then decreased continuously (but slowly) for up to 10 min. PPO was completely inactivated after 3 min blanching at temperatures ≥ 70 °C. Complete inactivation of PPO also can be seen after 10 min berry blanching at 60 °C.

Most studies report that kinetic inactivation of PPO follows a first order kinetic reaction, for example in strawberry (Chisari et al., 2007), table grape (Fortea et al., 2009), avocado (Soliva-Fortuny et al., 2002), apple (Yemenicioglu et al., 1997), and pineapple (Chutintrasri & Noomhorm, 2006). Anthon & Barret (2002) found that the PPO inactivation kinetics in potato were complex because their data could not easily be resolved into labile and resistant phases.

In this study however, thermal inactivation kinetics of PPO showed a deviation from first-order kinetics. Data were well described by a biphasic inactivation model (Equation 4-3) (Terefe et al., 2010). This may indicate the presence of two isoenzyme fractions of differing heat stability, one component being heat labile and the other being heat resistant, which are inactivated at different rates. The inactivation kinetic parameters estimated from the biphasic model of the data using 70 °C (343.15 K) as the reference temperature are presented in Table 4-5. The activation energies of enzyme inactivation were estimated to be 60 ± 7 kJ/mol and 1.4 ± 6.1E-06 kJ/mol for the stable and the labile isoenzymes respectively.

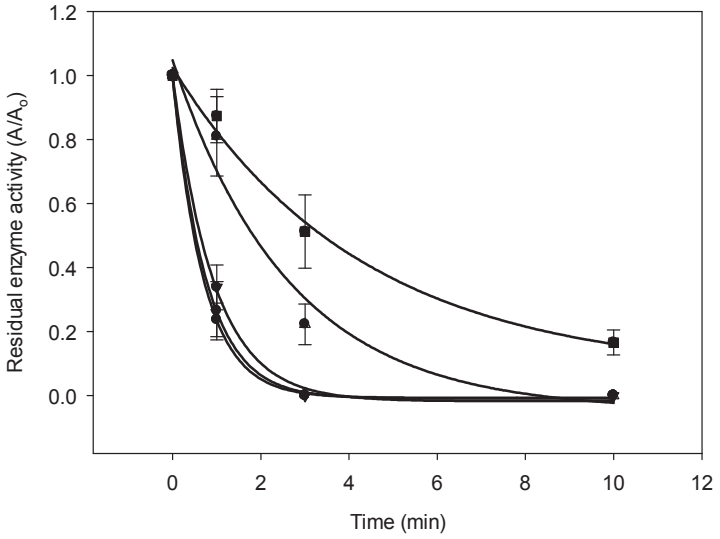


Figure 4-6: Thermal inactivation kinetics of PPO in blueberry juice after berry blanchingat 50 (■), 60 (Δ), 70 (▼), 80 (○), and 90 °C (●) for up to 10 min. Lines interpolating the experimental data points show the fits of a biphasic model. Data represent means of six replicates. Error bars indicate confidence interval at 95%.

Table 4-5: Estimated kinetics parameters for thermal inactivation of PPO in blueberry juice with T_{ref}=343.15.

Parameter	Stable fraction	Labile fraction
k (min^{-1})	1.07 ± 0.09	0.00002 ± 0.00001
Ea (kJ/mol)	60.77 ± 7.51	1.42 ± 6.13E-06
k_{ref}(min^{-1})	0.74 ± 0.26	5.20E-08 ± 1.19E-13
R^2=0.995		

Data represent means ± confidence intervals at 95% (N=6).

Biphasic inactivation kinetics were commonly observed for thermal inactivation of POD (Liing & Lund, 1978) and combined high pressure-thermal treatment of PPO (Terefe et al., 2010). There appears to be no study on the thermal inactivation kinetics of PPO in blueberry or blueberry juice, but a study conducted by Buckow et al. (2010) shows that pasteurising blueberry puree at 85 °C for 5 min is able to fully

inactivate PPO in the final juice. Fortea et al. (2009) reported that PPO in table grapes were completely inactivated with > 90% losses of relative activity after only 5 min of incubation at 78 °C. A reduction of more than 90% of PPO activity was found in cherry pulp (Gui et al., 2006) and garlic (Fante & Noreña, 2012) after blanching at 85 °C and 100 °C respectively for 4 min. According to Fante & Noreña (2012), a reduction of 90% in PPO activity after blanching is recommended to control undesirable changes caused by the enzyme and maintain optimum quality of product during storage. Short exposure to temperatures greater than 70 °C in the fruit tissue or model solution was adequate for partial or complete inactivation of PPO (Vamos-Vigyazo et al., 1977; Bello & Sule, 2012).

All these results are consistent with this study which shows that blanching at temperatures ≥ 70 °C for 3 min was able to inactivate PPO in order to minimise phytochemical and colour deterioration during further processing. This observation is also in agreement with the study conducted by Rossi et al. (2003) which reported that steam blanching treatment is effective in inactivating PPO in blueberry juice.

4.4. Defrost model

During the thawing process, both enzymatic and thermal degradation occur simultaneously. However, thermal degradation during thawing is minimal and negligible because berry thawing normally will be carried out at low temperature (between 4 – 10 °C). Therefore, the model developed in Section 4.3.2 (Equation 4-11) will be used as a global model for enzymatic degradation to predict losses of anthocyanin and CGA during thawing of whole berries. This model applies at temperatures below 50 °C which is the maximum temperature for PPO activity.

4.5. Conclusion

PPO enzyme is the main reason for the enzymatic degradation of phytochemicals in blueberry. PPO enzyme inactivation is important in blueberry juice processing to prevent browning and retain phytochemicals, especially anthocyanins, in the final juice. Enzymatic degradation of anthocyanin and CGA both in whole berries and in berry mash was rapid and followed a second-order kinetic reaction while for procyanidin B2, the degradation was complex and could not be adequately modelled. Berry blanching at temperatures ≥ 70 °C for 3 min is able to fully inactivate PPO in order to minimise phytochemical losses and colour deterioration during further processing.

5. Phytochemical recovery from berry matrix

5.1. Introduction

Global industrial practices in juice processing have used blanching or thermal treatment as one unit operation to control phytochemical deterioration especially anthocyanin breakdown caused by PPO. But to our knowledge, none of the major juice producers in New Zealand use blanching in their unit operations. Only one small juice producer applies blanching in their juice processing. This producer used a steam stripping technique to produce juice which is different from other juice producers.

Blanching is one of the most important units in fruit processing. It has been shown that blanching is effective in improving stability and recovery of phenolic compounds (Lee et al., 2002; Rossi et al., 2003; Brambilla et al., 2008; Giovanelli et al., 2012). Blanching acts by inactivating PPO activity (Kader et al., 1997), and softening the cell wall thus increasing juice extractability (Brambilla et al., 2008).

Juice processed with the addition of steam-blanching steps was found to have higher recovery of anthocyanins compared to juices from unblanched berries (Lee et al., 2002; Rossi et al., 2003). Inactivation of PPO by blanching eliminated the mechanism for anthocyanin destruction but anthocyanins are still heat sensitive compounds. Prolonged blanching might further reduce anthocyanins in the blueberry due to thermal degradation. Therefore, Siddiq et al. (1992) suggested that heat inactivation of PPO should be avoided, when dealing with anthocyanin-containing juice products. It is a huge challenge to maintain and retain anthocyanins and other polyphenolic compounds in blueberry juice, because thermal processing shows a

promising recovery of the compounds. Although non-thermal processing can be used as an alternative to thermal processing, the breakdown or destruction of the skin is needed, in order to release or extract anthocyanins, as anthocyanin is only found in the skin (Lee et al., 2002).

One drawback of blanching is it induces pectin gel formation which leads to the risk of high viscosity in the juice. To overcome this problem, pectinase and/ or cellulose enzyme treatment is needed when a blanching step is applied.

The main objectives of this chapter are

i) To evaluate the effect of blanching on phytochemical release in the juice
ii) To evaluate the effect of pectinase enzyme treatment on the juice volume, viscosity, and phytochemical recovery from the berry matrix.
iii) To develop a phytochemical recovery model based on the understanding of blanching and pectinase enzyme treatment effects.

5.2. Materials and methods

5.2.1. Berry blanching

Berry blanching was carried out as described in Section 4.2.4; viscosity measured as described in Section 2.3.5; and phytochemical determination carried out according to Section 2.2.

5.2.2. Pectinase enzyme screening

For screening purposes, seven commercially available pectinase enzymes were used and details of each enzyme are shown in Table 5-1. Enzymes varied in type, declared

Table 5-1: Description of seven pectinase enzymes used in the depectinisation process.

Product name (Company)	Declared enzyme	Application	Declared activities*	Recommended rate of commercial usage
Celluzyme LX (Zymus)	Pectinase, cellulase, hemicellulase and arabinase	Maceration of fruit and vegetable juice	180,000 AJDU/g	1-5 mL per 100 kg
Kleerase 100XL (Zymus)	Pectin esterase and depolymerase, cellulases, hemicellulases and arabinase	Maceration, depectinization and clarification of fruit and vegetable juice; 'without gelling concerns'	100,000 AJDU/g	2-4 mL per 100 kg
Lallzyme HC (Lallemand)	Polygalacturonase, pectin esterase and pectin lyase	Clarification of musts and wines	na	0.5-2 g per 100 L
Pectinex Ultra AFP (Novozyme)	Pectin lyase, polygalacturonase	Mash treatment to break down the cell wall, depectinization and clarification in juice processing	10,000PECTU/mL	na
Pectinex Ultra Clear (Novozyme)	Polygalacturonase	Maceration and depectinization in juice processing	7900 PGNU/mL	na
Rapidase X Press (DSM)	Polygalacturonase	Maceration enzyme to improve pressability of white grapes	na	1-3 g per 100 kg
Rapidase Excolor (DSM)	Polygalacturonase	Maceration enzyme for improved color extraction	na	2-3 g per 100 L

*Declared activities of the enzymes where AJDU = Apple juice depectinization units; PECTU = pectinase unit; PGNU=polygalacturonase unit and na = data not available.

activities, and recommended usage rate but were similar in method of application (maceration) and effects (improvement of pressability and colour extraction). The enzyme concentration used in the screening was standardized to 2 mL per 100 kg of berry. Thus, 100 µL of enzyme was diluted with 900 µL distilled water, and 10 µL of the final mixture were used for each 100 g berry.

For enzyme treatment, frozen berries were thawed overnight at 4 °C and then were finely ground using a handheld stick mixer (Kenwood HB714M Tri Blade Hand Blender, UK). A 100 ± 1 g sample of finely ground berry mash was weighed and mixed with previously prepared enzyme in 100 mL schott bottle, then incubated in a shaking waterbath at 50 °C for 4 h. At the end of enzyme treatment, the treated berries were immediately cooled in ice slurry to reduce the temperature to room temperature (20 °C), removed from the bottles and centrifuged (Thermo Scientific Heraeus multifuge IS-R centrifuge, Massachusetts, U.S.A.) at 4000 *x g*, 20 °C for 20 min to yield the juice. The juices were stored frozen at -20 °C until HPLC analysis as described in Section 2.2.

5.2.3. Pectinase enzyme treatment

Pectinex Ultra AFP was selected for determining the effect of enzyme treatment with different blanching conditions. Frozen blueberries were blanched at 70, 80, and 90 °C for 3 min as described previously in Section 4.2.4 (Chapter 4) and 'no blanch' berries were used as the control. Berry mash was prepared by rolling thawed berries in their plastic sleeve with a rolling pin. Berry mash was then treated with 5 µL of prepared enzyme and incubated in a shaking waterbath at 50 °C for 0, 1, 2, 3, and 4 h. At the end of enzyme treatment, the treated berries were immediately cooled in

ice slurry to reduce the temperature to room temperature (20 °C), removed from their pouches and centrifuged at 4000 *x g*, 20 °C for 20 min to yield the juice. The juice was stored at -20 °C until further phytochemical analysis as described in Section 2.2. Viscosity of the juice was measured as in Section 2.3.5.

5.2.4. Microscopy analysis

Juice prepared in Section 5.2.3, with and without enzyme treatment, and its pomace, were examined in a light microscope (Olympus BX53, Tokyo, Japan) with attached camera (Olympus XC50, Tokyo, Japan).

5.3. Results and discussion

5.3.1. Effect of berry blanching on phytochemical release, juice volume recovery, and viscosity of blueberry juice

Phytochemical release from the berry matrix can be expressed in two different units i.e. in mg/L juice and mg/kg berry. Phytochemical concentration in mg/L juice is a direct measurement of phytochemicals in the juice and concentration in mg/kg berry was corrected based on the juice density of 1.0833 kg/L and the juice yield (Birt, 2011). In this study, the term 'recovery' was defined as the concentration of phytochemicals in prepared juice, expressed in mg/kg of berry, as this measurement is the best measurement to describe the phytochemical release into the juice. The phytochemical concentration (mg/kg FW) in the juice as a result of blanching, and percentage juice volume recovery was plotted as a function of time for different blanching temperatures (Figure 5-1).

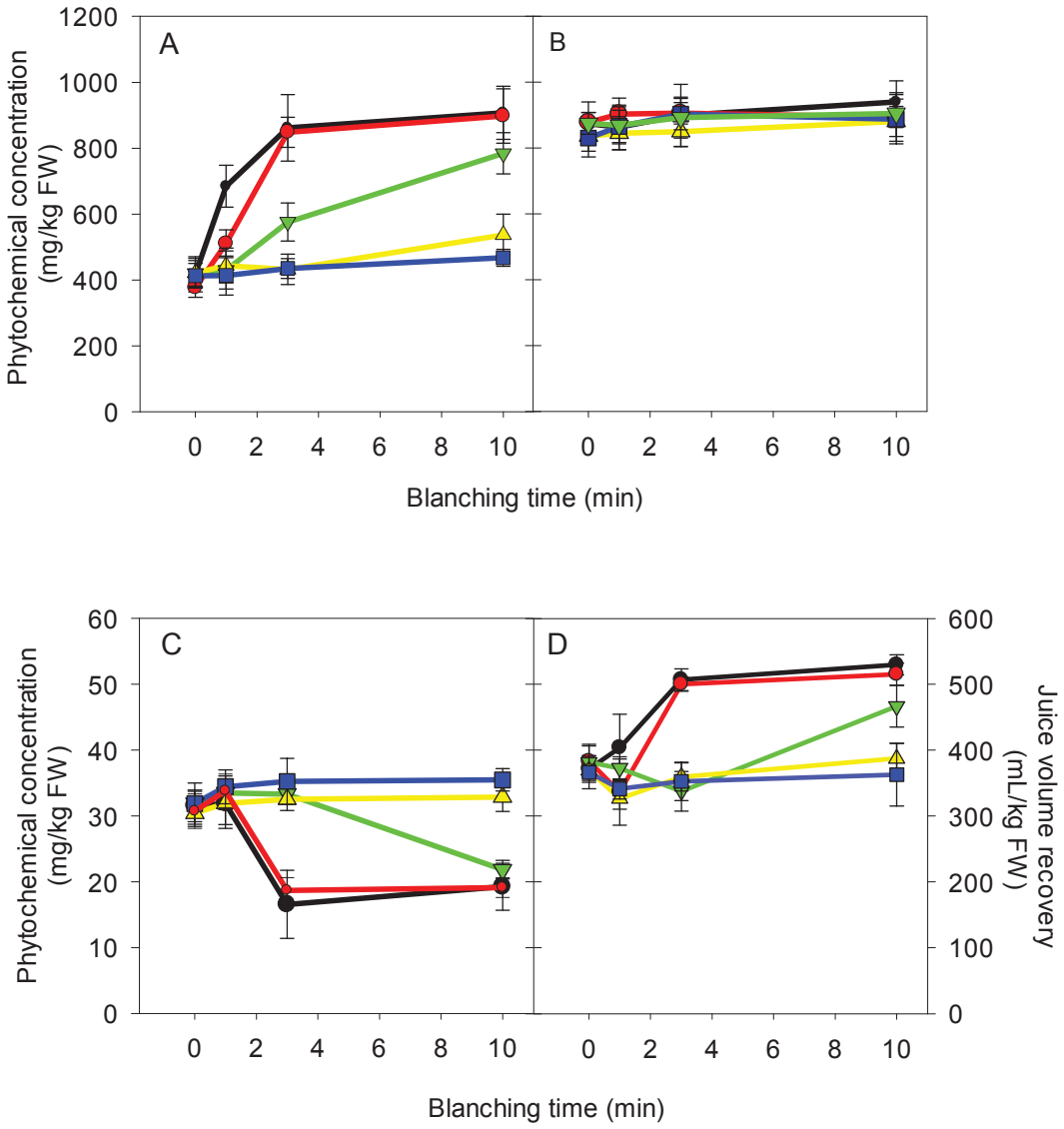


Figure 5-1: Effect of berry blanchingon A) anthocyanins, B) CGA, C) procyanidin B2 concentration and D) juice volume recovery in blueberry juice at 50 (■), 60 (▲), 70 (▼), 80 (●), and 90 °C (●). Data represent means ± confidence interval (N=6).

Anthocyanin concentration increased with blanching temperature but tended to reach a plateau of almost 70% of total recovery compared to its initial anthocyanin concentration in the berry (1300 mg/kg) over time (Figure 5-1 A). This suggests that significant amounts of anthocyanins (about 30%) still remain in berry pomace. A significant increase of anthocyanins due to blanching is related to both PPO inactivation and improved release of anthocyanins from epidermal tissues. As

discussed in Chapter 4, inactivation of PPO can be achieved with blanching treatment at temperature ≥ 70 °C for 3 min. Therefore, deterioration of anthocyanins was minimized when PPO was inactivated with a blanching treatment. Blanching also helps to soften cell walls and rupture plant cell membranes (Van Buggenhout et al., 2009) thus facilitating release of more anthocyanins from the tissue.

As in the thermal degradation study, the CGA concentration was not significantly affected by the blanching treatments, remaining stable at all temperatures studied (Figure 5-1 B).

The blanching effect on procyanidin B2 concentration was the inverse of that found for anthocyanins. Procyanidin B2 was stable at lower temperatures (50 – 60 °C) and slowly decreased at 70 °C, whereas, at 80 – 90 °C, procyanidin B2 decreased rapidly after 1 min and then remained stable for up to 10 min of further blanching treatment (Figure 5-1 C). It may be that enzymatic degradation of procyanidin B2 is initiated only when the berry matrix begins to be destroyed by heat, but stops once heat has inactivated the enzymes themselves. Alternatively, the loss of procyanidin B2 may be associated with pectin gel formation during blanching, which strengthens interactions between procyanidin B2 and cell wall material (Le Bourvellec et al., 2004).

Juice volume recovery seems to increase with blanching temperature (Figure 5-1 D). Blanching at low temperature (≤ 70 °C) for ≤ 3 min decreased the juice volume recovery and this decrease was associated with pectin gel formation. Juice blanched at low temperature (≤ 70 °C) formed 3 layers of juice which consist of pomace at the

bottom, gel in the middle and juice on the top layer in test tube after centrifugation. When juice was blanched at higher temperature (≥ 80 °C), all the gel was dispersed in the juice which increased the viscosity of the juice (Figure 5-2). The formation of pectin gel is the explanation for 'mucilaginous material' described by Albrigo (1980).

As mentioned above, one drawback of berry blanching is the induction of pectin gel formation, which increases the viscosity of the juice. Viscosity of the juice was observed to increase significantly with temperature when berries were blanched for 3 min (Figure 5-2). Viscosity affects consumer perception (Griffiths & Walkling-Ribeiro, 2014). It is also physically challenging for juice extraction. Despite that, blanching has been shown to help in releasing more phytochemicals into the juice especially anthocyanins. This finding suggests that if heat treatment is being applied during blueberry juice processing, some enzymatic treatments will also be required to recover high volumes of juice and to reduce viscosity of the finished juice.

Lee et al. (2002) reported that heat and SO_2 treatments of blueberry were effective in increasing anthocyanin recovery in the juice, but not recovery of other polyphenolics, information consistent with our findings. Similarly Rossi et al. (2003) found that heat treatment increased the recovery of anthocyanins, as well as increasing the cinnamate concentration in the juice. The other effect of heat is to inactivate PPO, thus preventing anthocyanin discoloration and producing more intense juice colour (Kader et al., 1997).

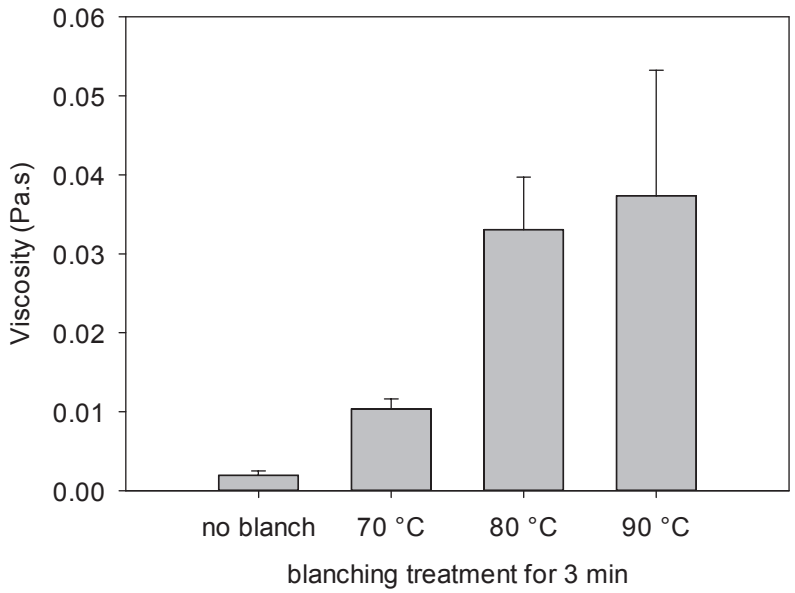


Figure 5-2: Effect of blanching treatment on viscosity of the juice before pectolytic enzyme addition. Viscosity was measured at shear rate of 48.5 (s^{-1}) at 20 °C. Data represent means ± confidence interval at 95% (N=3).

5.3.2. Effect of pectinase enzyme treatment on phytochemical release in the juice

5.3.2.1. Screening of commercial pectinase enzymes

It is important to note that in New Zealand, blanching is not a normal practice but the use of pectolytic enzymes is common practice in blueberry juice processing. In this study, seven commercially available enzymes were screened for their effect on juice volume and phytochemical concentration in blueberry juice without berry blanching to mimic the industrial practice. Generally, enzyme treatment increased the juice volume, anthocyanins, CGA, and procyanidin B2 concentration significantly

compared to control (without enzyme treatment) especially Pectinex Ultra AFP and Pectinex Ultra Clear (Table 5-2).

Table 5-2: Effect of commercial enzymes on the juice volume and phytochemical concentration in blueberry juice (expressed per kg of berries).

Pectinase enzymes	Juice volume (ml/kg)	Total anthocyanin (mg/kg)	CGA (mg/kg)	Procyanidin B2 (mg/kg)
Control	489 ± 107[a]	212 ± 67[a]	500 ± 115[a]	12.7 ± 9.5[a]
Celluzyme LX	647 ± 38	289 ± 41	615 ± 131	18.7 ± 6.5[a]
Kleerase 100XL	634 ± 23	293 ± 38	614 ± 102	19.0 ± 5.3
Lallzyme HC	625 ± 75	266 ± 28[a]	599 ± 105[a]	18.5 ± 6.4[a]
Pectinex Ultra AFP	658 ± 44	369 ± 72	603 ± 62	19.1 ± 4.8
Pectinex Ultra Clear	661 ± 48	383 ± 85	631 ± 107	19.0 ± 6.3
Rapidase Excolor	645 ± 2	264 ± 59[a]	600 ± 115[a]	19.3 ± 6.1
Rapidase X Press	618 ± 24	268 ± 19	607 ± 99	18.6 ± 5.8[a]

Data represent means ± confidence interval at 95% (N=3). Means not labelled with letter a are significantly different from control using Dunnett Test.

Commercial enzyme preparations used in this study contain pectinases, cellulases, and hemicellulases in various ratios, which act in different ways. The increase of juice volume and phytochemicals with enzyme treatment is due to the disruption of the cell wall which leads to degradation of pectin and significantly increases the juice volume and phytochemical concentration in the juice (Bates et al., 2001; Landbo & Meyer, 2004; Laaksonen et al., 2013).

Based on the juice volume and phytochemical recovery in this enzyme screening study, Pectinex Ultra AFP and Pectinex Ultra Clear were the best enzymes compared to control. Both enzymes were characterised by high activity of polygalacturonase,

but Pectinex Ultra AFP also contain pectin lyase activity. This suggests that polygalacturonase with or without pectin lyase may influence the phytochemical concentration in the juice and is an effective enzyme that may increase accessibility and facilitate diffusion of the compounds from berry matrix.

5.3.2.2. Effect of pectinase enzyme treatment on juice volume, viscosity, and phytochemical release into the juice

Blanching had a significant impact on anthocyanin release from the berry matrix (Figure 5-1) but induced pectin gel formation which increased viscosity of the juice (Figure 5-2) especially when blanched at 80 – 90 °C. This finding suggests that enzyme treatments are required when heat treatment is applied during blueberry juice processing to recover a high volume of juice and reduce viscosity of the finished juice. Pectinex Ultra AFP had been selected for determining the effect of enzyme treatment on the juice volume, viscosity, and phytochemical concentration in the juice after berry blanching for 3 min followed by enzyme treatment for up to 4 h (Figure 5-3).

Enzyme treatment shows a significant impact on the juice volume recovery. The juice volume was increased significantly after 1 h enzyme treatment, to a maximum at around 2 h of enzyme treatment (Figure 5-3 A).

Viscosity of all the juices was significantly reduced after 1 h enzyme treatment and then remained constant up to 4 h treatment (Figure 5-3 B). Enzyme treatment does not decrease viscosity of ‘no blanch’ juice. It seems that ‘no blanch’ juice has no cross linked pectin to cause viscosity increases.

As discussed in Section 5.3.1, anthocyanin concentration in the juice was increased in proportion to the blanching temperature. The use of pectinase enzyme helped in liberating more anthocyanins from the berry matrix into the juice especially for juices blanched at 80 and 90 °C. It reached a maximum during first 2 h of enzyme treatment and started to decrease slowly afterward (Figure 5-3 C). Anthocyanin concentration in 'no blanch' and blanched at 70 °C juice were not affected and remained almost stable during 4 h of enzyme treatment.

Enzyme treatment does not have a significant impact on CGA concentration in the juice during the incubation time (Figure 5-3 D). CGA concentrations in all the juices were stable throughout the enzyme treatment.

As for CGA concentration, procyanidin B2 concentration in the juice also seems to be stable for all treatments. A slight drop has been observed in treatments blanched at 70 and 80 °C but it was not significant (Figure 5-3 E).

Significant increase of the juice volume, decrease of viscosity, and increase of anthocyanin and CGA concentration was expected. This is because pectinase has the ability to hydrolyse pectin reducing the viscosity of the juice, improving the pressability of the pulp, and disintegrating the jelly structure. All these processes resulted in higher juice yield (Liew Abdullah et al., 2007; Mieszczakowska-Frąc et al., 2012).

Enzymatic treatment using pectinase is an effective way to reduce the pectin in the fruit juice. Pectin degradation by pectinase enzyme action leads to decrease of juice

viscosity and increases the juice yield (Liao et al., 2007; Mieszczakowska-Frąc et al., 2012). Pectinases are an integral part of fruit juice industries. They are used extensively for the extraction and clarification of fruit juice and also in wine making industry (Tapre & Jain, 2014).

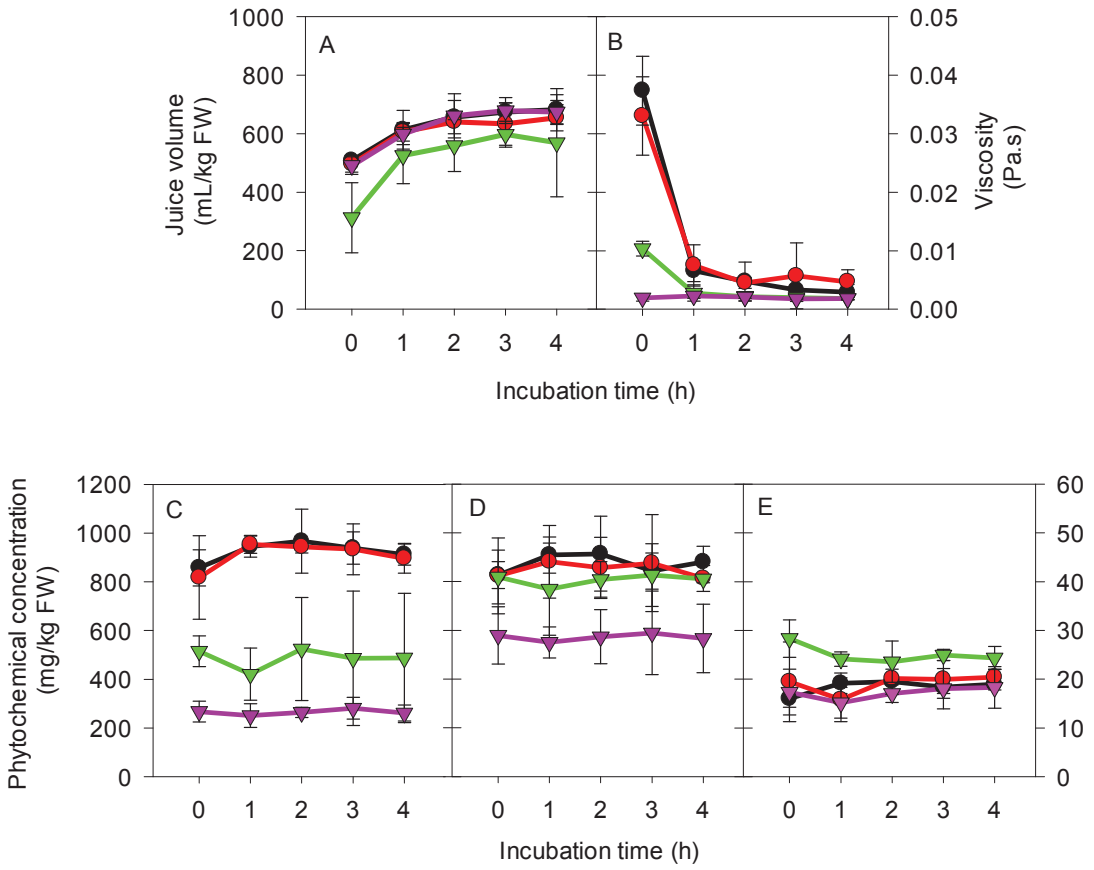


Figure 5-3: Effect of Pectinex Ultra AFP on A) juice volume, B) viscosity, C) anthocyanin, D) CGA and E) procyanidin B2 concentration of the juice after berry blanching at 70 (▼), 80 (●), and 90 °C (●) for 3 min and 'no blanch' (▼) followed by enzyme treatment up to 4 h. Data represent means ± confidence interval at 95% (N=3).

Enzyme-assisted processing also has been shown to increase the anthocyanin content in the juice. The enzymatic process makes use of enzymes to soften skins and tissue and release more phytochemicals (Tapre & Jain, 2014). Khandare et al. (2011) found significant increases of anthocyanin in black carrot treated with pectinase enzymes. Buchert et al. (2005) found similar results with increases of 13 – 41% and 18 – 29% of anthocyanin content in bilberry and blackcurrant juice respectively. Pectolytic enzyme treatment during juice clarification in pomegranate juice processing shows an increase of 2-fold of polyphenols and a decrease about 40% of turbidity (Rinaldi et al., 2013).

PPO is known as one of the degrading enzymes which is responsible for degrading anthocyanins and other polyphenols in blueberry. In this study, PPO effects were minimized by berry blanching. In short, blanching inactivated PPO and softened cell wall to release more anthocyanins, but at the same time induced pectin gel formation which increased the viscosity of the juice. Enzyme treatment is best overall combined with blanching. Pectinase action helps to increase juice volume, reduce the viscosity and liberate more anthocyanin and CGA. Enzyme treatment used without berry blanching was efficient in increasing the juice volume but had no benefit in phytochemical recovery. This suggests that in blueberry juice processing, the main action of pectinase enzyme was to hydrolyse pectin to increase juice volume and reduce viscosity of the juice. Pectinase needs to be used in blueberry juice processing, regardless of the starting material, to avoid a low juice volume yield. Pectinase also helped to liberate more anthocyanin and CGA into the juice.

As discussed earlier in Section 5.3.1, the decrease of procyanidin B2 may be because it became trapped in the pectin gel. Theoretically, with pectinase treatment, concentration of procyanidin B2 should increase but in this study, procyanidin B2 concentrations were almost stable. This phenomenon might be due to the tendency of procyanidin to stick to cell wall material and therefore remain mostly in the pomace. Alternatively, enzyme treatment dissolves pectin but does not remove it from the juice; so perhaps procyanidin B2 remains attached to soluble pectin in the juice and is precipitated during solvent extraction.

Enzyme concentrations also have significant impact on the juice quality. Although this study has demonstrated the value of blanching and enzyme treatment for the blueberry juicing industry, there are some aspects of optimization that have not been investigated in this project. For example, details of enzyme concentration and optimal temperature were simply not investigated, and would need to be studied before industrial use. Gao et al. (2014) found that optimum pectinase processing conditions for blueberry juice were at a temperature of 51.6 °C for 2.36 h with enzyme dosage of 0.05 mL/kg. The hydrolysis conditions seem to be very similar to this study in which maximum juice yield was achieved after 2 h with enzyme dosage of 0.02 mL/kg at 50 °C.

Enzyme treatment is considered to be complete once the viscosity of the juice has returned to its original level or less (Madden, 2000). Therefore, it was suggested that 2 h was the maximum time for enzyme treatment to obtain high juice volume, low viscosity, and high anthocyanin and CGA in the finished juice. Prolonged incubation

times were not recommended because they lead to thermal degradation of the phytochemicals, especially anthocyanins.

5.3.3. Effect of juicing techniques (centrifugation versus basket press) on phytochemical recovery

All the above experiments were done in small tubes using a centrifuge. It is important to verify that centrifugation is a reasonable mimic of pressing. Pressing is the most common juicing technique used by New Zealand blueberry juice producers. A small experiment was carried out to study the effect of juicing techniques (centrifugation vs. basket pressing) on phytochemical recovery in the finished juice.

Phytochemical recovery was calculated as the ratio of phytochemical concentration released into the juice compared to initial concentration of phytochemicals in the berry. There was no significant effect of the juicing techniques on the anthocyanin, CGA, or procyanidin B2 recovery of the final juice ($P>0.05$) (Table 5-3).

Table 5-3: Effect of juicing techniques on the recovery of anthocyanins, CGA, and procyanidin B2 in the juice.

Juicing technique	Phytochemical recovery *		
	Total anthocyanin	CGA	Procyanidin B2
Centrifugation	0.20 ± 0.04^{a}	0.49 ± 0.20^{a}	0.81 ± 0.11^{a}
Basket pressing	0.22 ± 0.02^{a}	0.51 ± 0.10^{a}	0.79 ± 0.09^{a}

Data represent means ± confidence interval at 95% (N=3). Values that are followed by different letters within each column are significantly different ($P<0.05$) using Tukey's Honest Significant test. * is the ratio of phytochemical concentration released in the juice compared to initial concentration of phytochemicals in the berry.

Juicing is a method of separation of the juice from its pomace. Both techniques, centrifugation and pressing have the same purpose i.e. to separate juice from its pomace. Centrifugation used the centrifugal force to separate the juice and pomace whereas in pressing, pressure is applied to the fruit to squeeze the juice. In the fruit juice industry, both juicing methods are widely used. For example, centrifugation works for juice extraction from the large juice sacs of orange (Stinco et al., 2013) and pressing is required for the tough root tissues of black carrots (Suzme et al., 2014). In this study, centrifugation and pressing resulted in similar anthocyanin, CGA, and procyanidin B2 recovery in the final juice obtained from blueberries. According to Bates et al. (2001), the centrifugation technique in apple juice manufacturing is rapid but less efficient than pressing compared to other fruits. This might be associated with the type of the flesh of the fruit. Apple flesh is hard and it is advisable to press the pulp again after centrifugation to have a better yield. Blueberries are soft and delicate and easy to juice but need enzyme treatment to eliminate pectin gel formation.

On the other hand, van der Sluis (2005) found that pressing did not affect the quercetin glycoside in apple juice production. The amount of quercetin glycoside present in the raw juice and pomace was equal to the amount present in the fresh apple or in the pulp. Pressing only caused a 'partitioning' of the compounds between pomace and the juice. In orange juice processing, an increase of 22% and 25% of phenolics compounds and vitamin C respectively were observed in juice prepared by commercial squeezing compared to domestic squeezing (hand processing technique). Commercial squeezing was a more powerful and vigorous process as it can be controlled for the maximum capability of the machine compared to the hand

processing technique which relies on human capability and energy. Vigorous juice extraction or pressing will increase the compounds in the finished orange juice (Gil-Izquierdo et al., 2002) because most of the phenolic compounds in orange are most abundant in the edible part of the fruit (Toma´s-Barbera´n & Clifford, 2000). This shows that further study is needed to compare the extractability or recovery of the compounds in blueberry juice under different extraction pressures because of the nature of anthocyanins which are available in the skin and the tendency of anthocyanins to remain in the pomace after pressing.

5.3.4. Microscopy analysis

Blueberry juice and its pomace prepared with different blanching temperatures, with and without enzyme treatment were imaged with light microscopy without any stains. The juice samples appeared more granular without blanching; presumably these relate to particles of gelled pectins entrapping insoluble fibres. The granules become smaller and dispersed more evenly in blueberry juice that was blanched at higher temperatures (Figure 5-4 A – C). It is believed that the dispersion of the gel in the juice with temperature increases the viscosity of blanched juice compared to 'no blanch' juice. Enzyme treatment will further disaggregate the pectin gel and reduce the viscosity of the juice to acceptable levels (Figure 5-4 D – F). However, no clear differences were observed at this macroscopic level between pomace with or without blanching and enzyme treatment (Figure 5-4 G – L).

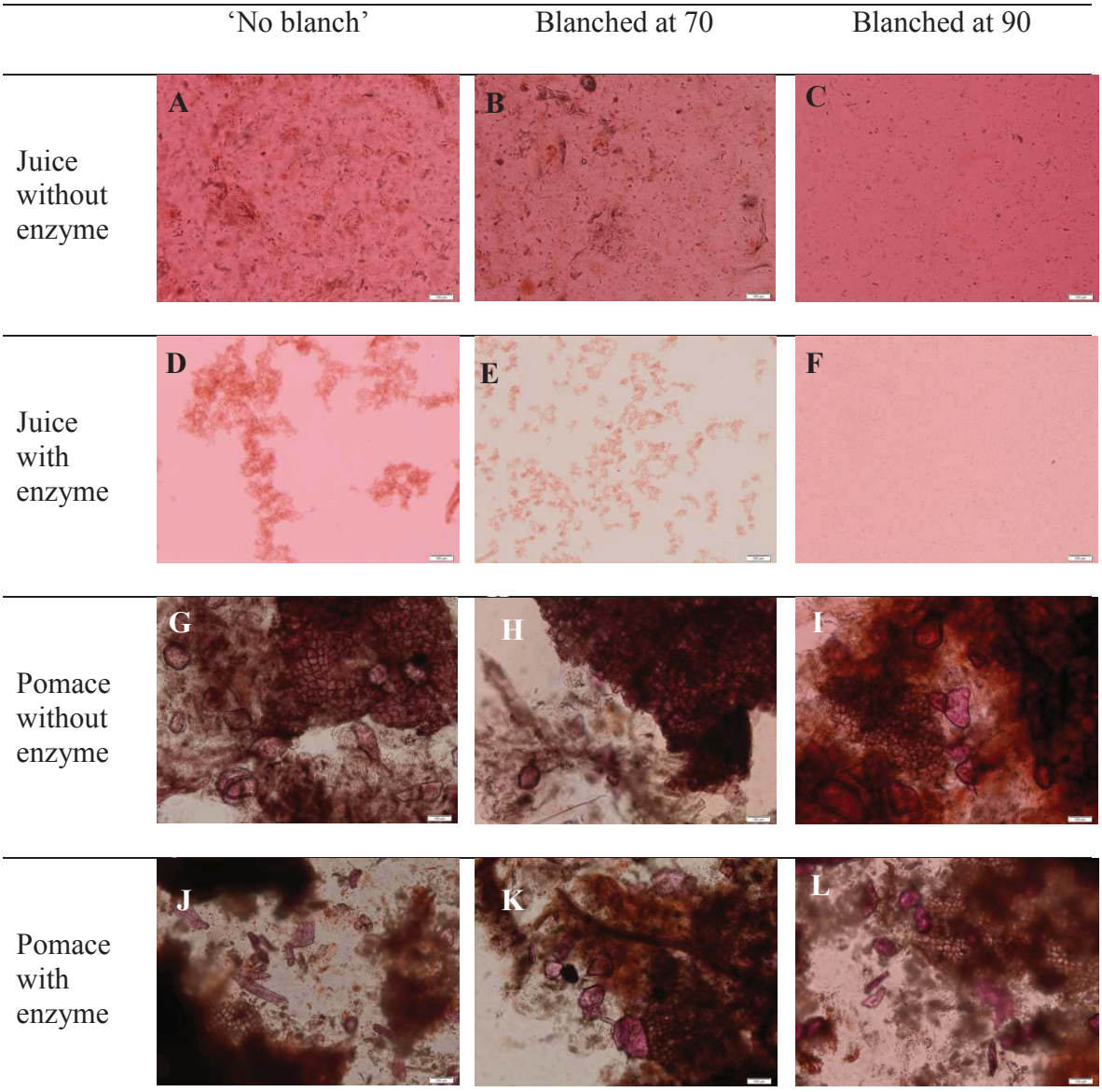


Figure 5-4: Microscopic analysis of blueberry juice and pomace with and without enzyme treatment (Bars = 100 µm).

5.4. Phytochemical recovery model

Two important factors that affect the phytochemicals released from the berry matrix into the juice were blanching temperature and enzyme treatment. These two processes however, need to be followed by the juicing process. Therefore, in order to model the phytochemical recovery in the juice, blanching, enzyme treatment, and juicing need to be considered as one factor.

The term 'phytochemical recovery factor' was defined as the ratio of phytochemical concentration in the juice after blanching, enzyme treatment, and pressing compared to initial concentration in the frozen berry (Figure 5-5). Because of the complexity of blanching, enzyme treatment steps, and pressing, for the sake of modelling they can be treated as single correction factors, dependent solely on blanching temperature and assuming a standard blanching time of 3 min and enzyme dosage of 2 mL per 100 kg. Figure 5-5 shows that there is no change to this correction factor if enzyme treatment is varied between 2 and 4 h.

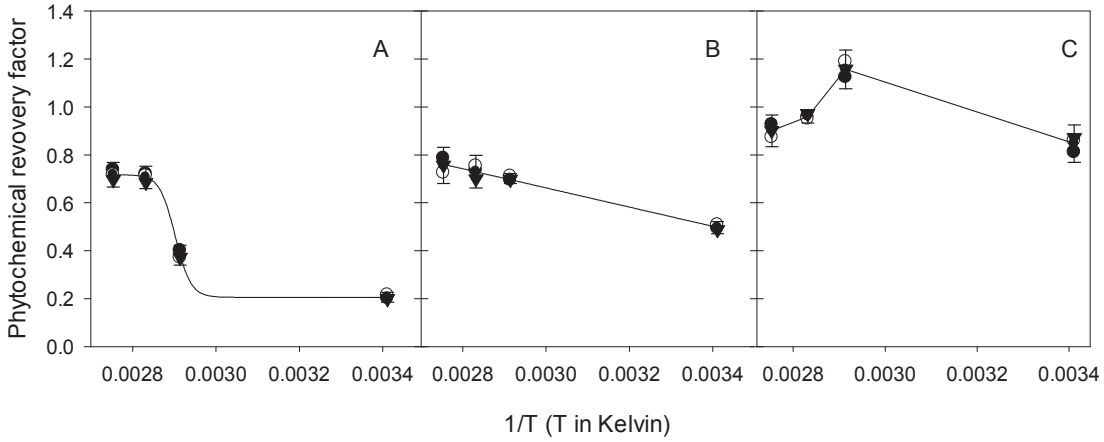


Figure 5-5: Phytochemical recovery model. A) Anthocyanins, B) CGA and C) procyanidin B2 in the juice compared to initial concentration in the frozen berry, where ● = 2, ○ = 3, and ▼= 4 h of treatment. Line in A and B are the predicted models as described in Equation 5-1 and 5-2 respectively. Data are means ± confidence interval at 95% (N=3).

The anthocyanin recovery correction factor was best fitted with a sigmoidal function model of the form described in Equation 5-1 and the confidence intervals at 95% for the coefficients are presented in Table 5-4.

$$y = 0.2055 + (0.512)/(1 + \exp\left(-\frac{x-0.0029}{-1.86E-05}\right) \text{ … … ….Equation 5-1}$$

Table 5-4: Confidence interval at 95% for the coefficient in Equation 5-1.

Coefficients	Confidence interval at 95%
0.2055	0.0225
0.512	0.0324
0.0029	0
1.86E-05	1.22E-05

Data represent means of three replicates.

This model appeared adequate (Figure 5-5 A), but the model has four coefficients and was developed with only four data points which means it is really just a description of what was observed. Further data need to be collected in future to generate a more reliable model; particularly between 20 and 70 °C.

The CGA recovery correction factor followed a linear regression model increasing with temperature of the form described in Equation 5-2 with R^2 = 0.971 (Figure 5-5 B). Confidence intervals for the coefficient are presented in Table 5-5.

$$y = 2.285 - 503x \quad \text{... ... . Equation 5-2}$$

Table 5-5: Confidence interval at 95% for the coefficient in Equation 5-2.

Coefficients	Confidence interval at 95%
2.285	0.147
503	49

Data represent means of three replicates.

For procyanidin B2, the recovery seemed to show no logical pattern. It had an initial increase from 'no blanch' to 'blanch at 70 °C' and then decreased with higher blanching temperatures (Figure 5-5 C). Therefore, procyanidin B2 was omitted from the juice processing model. The procyanidin B2 reaction was complex. It might be

polymerised to form trimers or tetramers or dissolve to monomer at high temperatures.

Data plotted in Figure 5-5 A and C seem to have interesting features between 20 and 70 °C, unfortunately in this study no data were collected between these two temperatures. This temperature range would be an interesting range to explore in the future. Rapid changes from 70 to 90 °C might be explained by PPO inactivation, softening tissue, and pectin solubilisation which released more anthocyanins at high temperature.

5.5. Conclusion

In blueberry juice processing, enzyme treatment is required when heat treatment (blanching) was applied. Blanching helps in releasing phytochemicals, especially anthocyanins and CGA, into the juice but also induced pectin gel formation which leads to increased viscosity of the juice. Enzyme treatment will further release anthocyanins and CGA from the berry matrix especially those trapped in the pectin gel. A complete enzyme treatment to give high juice volume, low viscosity, and an acceptable amount of phytochemicals in the juice can be achieved after 2 h enzyme incubation. Prolonged enzyme treatment for more than 4 h is not recommended because anthocyanin will be lost due to thermal degradation. Among the enzymes tested, Pectinex Ultra AFP and Pectinex Ultra Clear were the most efficient enzymes in ‘no blanch’ extraction and the assumption was made that they were also the best enzymes to be used in blanched extraction. Types of enzymes (pectinase and/or cellulase) used in blueberry juice processing did not significantly affect the juice volume or phytochemical recovery in the juice.

6. Pilot plant juice processing and modelling of phytochemical degradation during blueberry juice processing

6.1. Introduction

Normally blueberry juice processing starts from frozen berries and a series of unit operations have been defined to cover the process: thawing, blanching, mincing, enzyme treatment, separation of juice from pomace, pasteurisation, and bottling. Experiments conducted in Chapter 3, 4, and 5 were done at laboratory scale with a maximum of 50 g of berries in each of the experiments to understand the mechanism of phytochemical degradation and to develop a model to predict phytochemical degradation during juice processing. Based on these results, in order to model phytochemical degradation during blueberry juice processing, some of these unit operations can be combined. The unit operations involved have been simplified into three models: a defrost model, a recovery model, and a thermal model. In general, defrost model was used for whole berry phase during thawing; recovery model was used for intermediate phase between thawed whole berries and mash berry phase which involved blanching, mincing, enzyme treatment, and pressing; and thermal model was applied for the liquid phase i.e. after separation of juice from pomace (Figure 6.1). Pilot scale blueberry juice processing can be used to validate models that have been developed with laboratory scale data. Phytochemical data will be collected throughout juice processing and analysed accordingly.

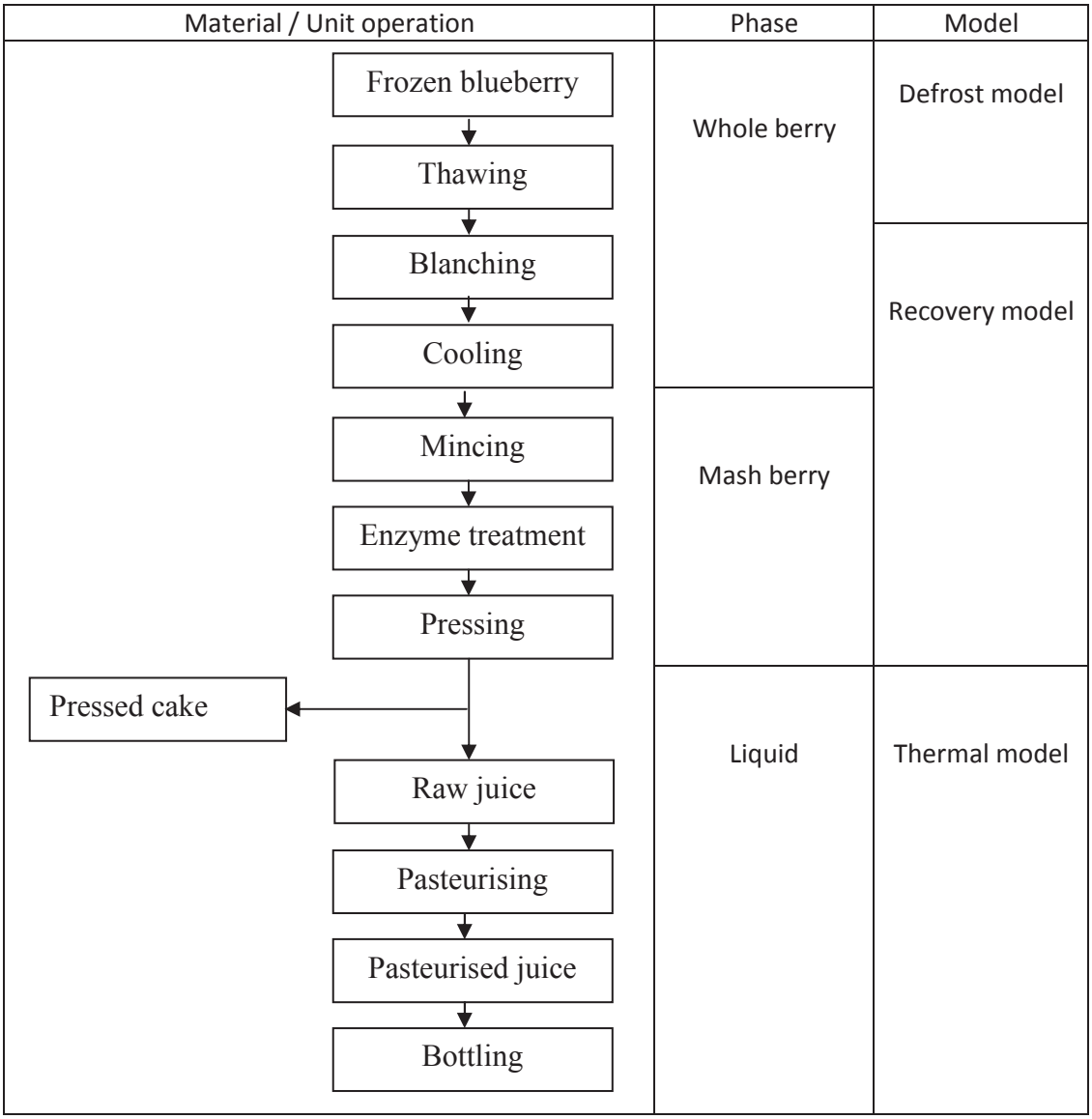


Figure 6-1: Overall unit operations of blueberry juice processing and models involved.

6.2. Materials and methods

Pilot plant blueberry juice processing was carried out as described in Section 2.6.

6.3. Results and discussion

6.3.1. Phytochemical recovery

Patterns of anthocyanin and CGA loss in the pilot plant trial confirm the patterns observed at laboratory scale (Table 6-1 and Table 6-2) but results were different for procyanidin B2 losses (Table 6-3).

The biggest loss of anthocyanins was observed during separation of juice and pomace with about 66 - 82% loss compared to the initial concentration in berries while the biggest losses of CGA and procyanidin B2 was during thawing of 'no blanch' berries with approximately 49 and 64% loss respectively. Anthocyanins in nature reside in the vacuole of small, tough cells in epidermal layers of the blueberry skin. This makes anthocyanins more difficult to extract than if they were in the large parenchyma cells of the fruit flesh, and during separation of juice and pomace most anthocyanins remain trapped in the pomace. CGA was known to be a substrate for anthocyanin degradation, and during thawing rapid degradation of CGA occurred in the presence of PPO. However, it was not clear how and why procyanidin B2 was lost during thawing; data were quite variable between reps, but it may have been lost through self-association or binding to pectin, and not been easy to measure in pomace (Padayachee et al., 2012).

Details of the mass and phytochemical balance for each of the pilot plant trial; 'no blanch', 'blanched at 70 °C' and 'blanched at 90 °C' are shown in Figure 6-2 to 6-4.

Table 6-1: Anthocyanin recovery at each step of pilot plant juice processing.

Unit Operation	'No blanch'*; pasteurised		'Blanched at 70 °C'**; pasteurised		'Blanched at 90 °C'**; pasteurised	
	mg	%	mg	%	mg	%
Frozen whole berry[(A)]	19,379 ± 1,734	100	19,397 ± 1,898	100	19,467 ± 4,009	100
Thawed berry[(B)]	15,570 ± 2,471	80	-		-	
Blanched berry[(C)]	-		19,649 ± 1,093	100	17,875 ± 2,281	92
Minced berry[(D)]	14,944 ± 423	77	19,449 ± 2,275	100	17,825 ± 4,672	92
Enzyme treated berry[(E)]	10,633 ± 213	55	17,455 ± 2,531	90	19,425 ± 3,692	100
Unpasteurised juice[(F)]	3,500 ± 713	18	5,242 ± 2,464	27	6,595 ± 1,302	34
Pasteurised juice[(H)]	3,669 ± 879	19	5,672 ± 2,025	29	7,744 ± 2,094	40

Data represent means ± confidence interval at 95%. (A-H) indicates sampling point at each of juice processing (refer Figure 2-1). *Initial quantity was in 15 kg of berries. ** Initial quantity was in 14 kg of berries.

Table 6-2: CGA recovery at each step of pilot plant juice processing.

Unit Operation	'No blanch'*; pasteurised		'Blanched at 70 °C'**; pasteurised		'Blanched at 90 °C'***; pasteurised	
	mg	%	mg	%	mg	%
Frozen whole berry[(A)]	16,174 ± 3,764	100	15,269 ± 1,306	100	15,222 ±1,647	100
Thawed berry[(B)]	8,263 ± 1,171	51	-		-	
Blanched berry[(C)]	-	-	11,989 ± 2,248	79	14,739 ± 2,772	97
Minced berry[(D)]	8,288 ± 940	51	12,082 ± 1,204	79	11,830 ± 1,554	78
Enzyme treated berry[(E)]	8,057 ± 365	50	11,067 ± 3,027	72	13,884 ± 1,073	91
Unpasteurised juice[(F)]	7,477 ± 786	46	9,733 ± 682	64	10,395 ± 1,551	68
Pasteurised juice[(H)]	7,125 ± 876	44	10,057 ± 1,538	66	10,034 ± 271	66

Data represent means ± confidence interval at 95%. (A-H) indicates sampling point at each of juice processing stages (refer Figure 2-1). *Initial quantity was in 15 kg of berries. **Initial quantity was in 14 kg of berries.

Table 6-3: Procyanidin B2 recovery at each step of pilot plant juice processing.

Unit Operation	'No blanch'*; pasteurised		'Blanched at 70 °C'**; pasteurised		'Blanched at 90 °C'**; pasteurised	
	mg	%	mg	%	mg	%
Frozen whole berry[(A)]	351 ± 652	100	432 ± 385	100	337 ± 169	100
Thawed berry[(B)]	100 ± 429	29	-		-	
Blanched berry[(C)]	-	-	252 ± 71	58	237 ± 134	70
Minced berry[(D)]	89 ± 188	25	295 ± 210	68	323 ± 171	96
Enzyme treated berry[(E)]	108 ± 281	31	304 ± 276	70	335 ± 85	99
Unpasteurised juice[(F)]	145 ± 40	41	327 ± 118	76	319 ± 622	95
Pasteurised juice[(H)]	153 ± 36	44	324 ± 132	75	351 ± 41	104

Data represent means ± confidence interval at 95%. (A-H) indicates sampling point at each juice processing stage (refer Figure 2-1). *Initial quantity was in 15 kg of berries. ** Initial quantity was in 14 kg of berries.

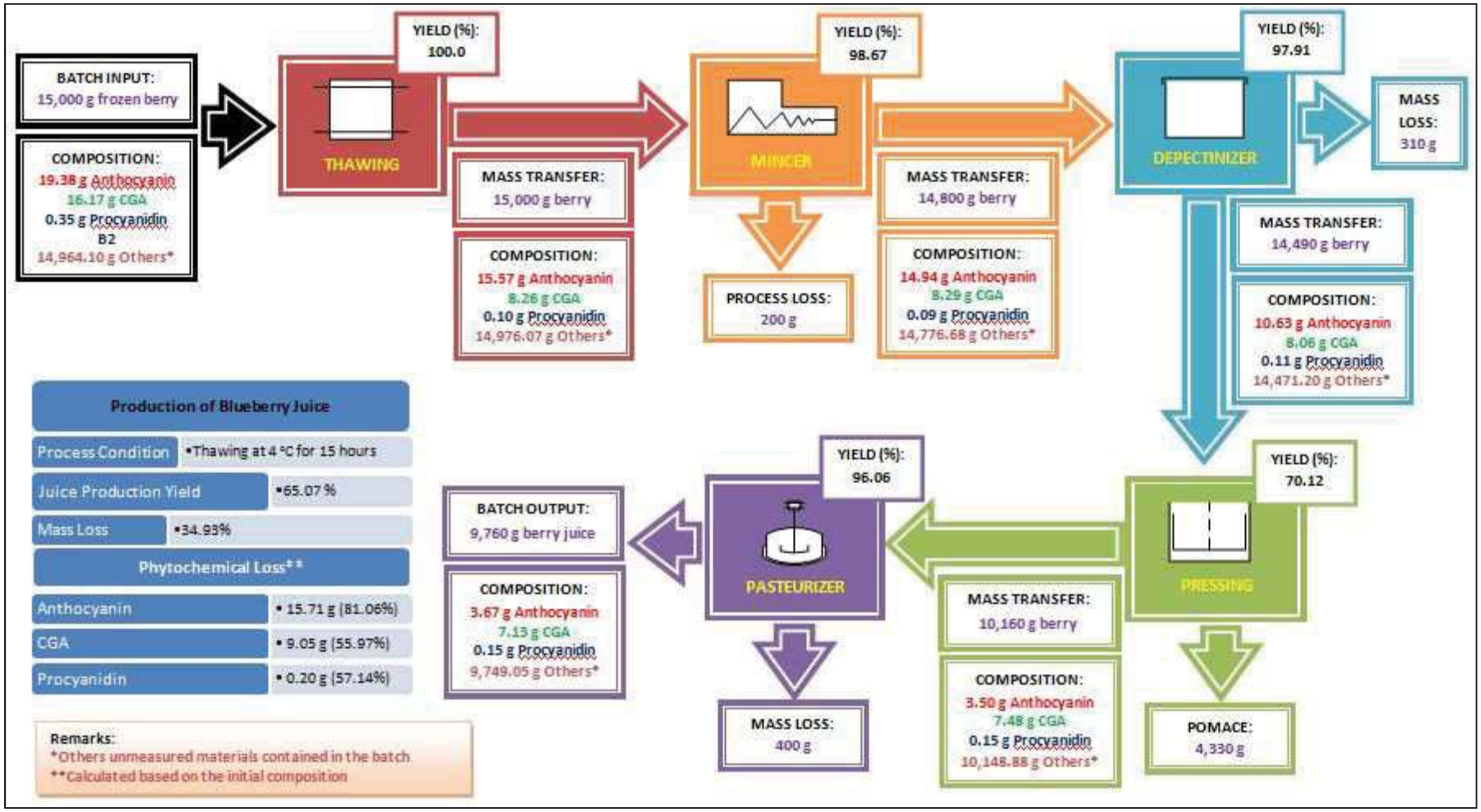


Figure 6-2: Overall mass and phytochemical balance in 'no blanch' juice processing.

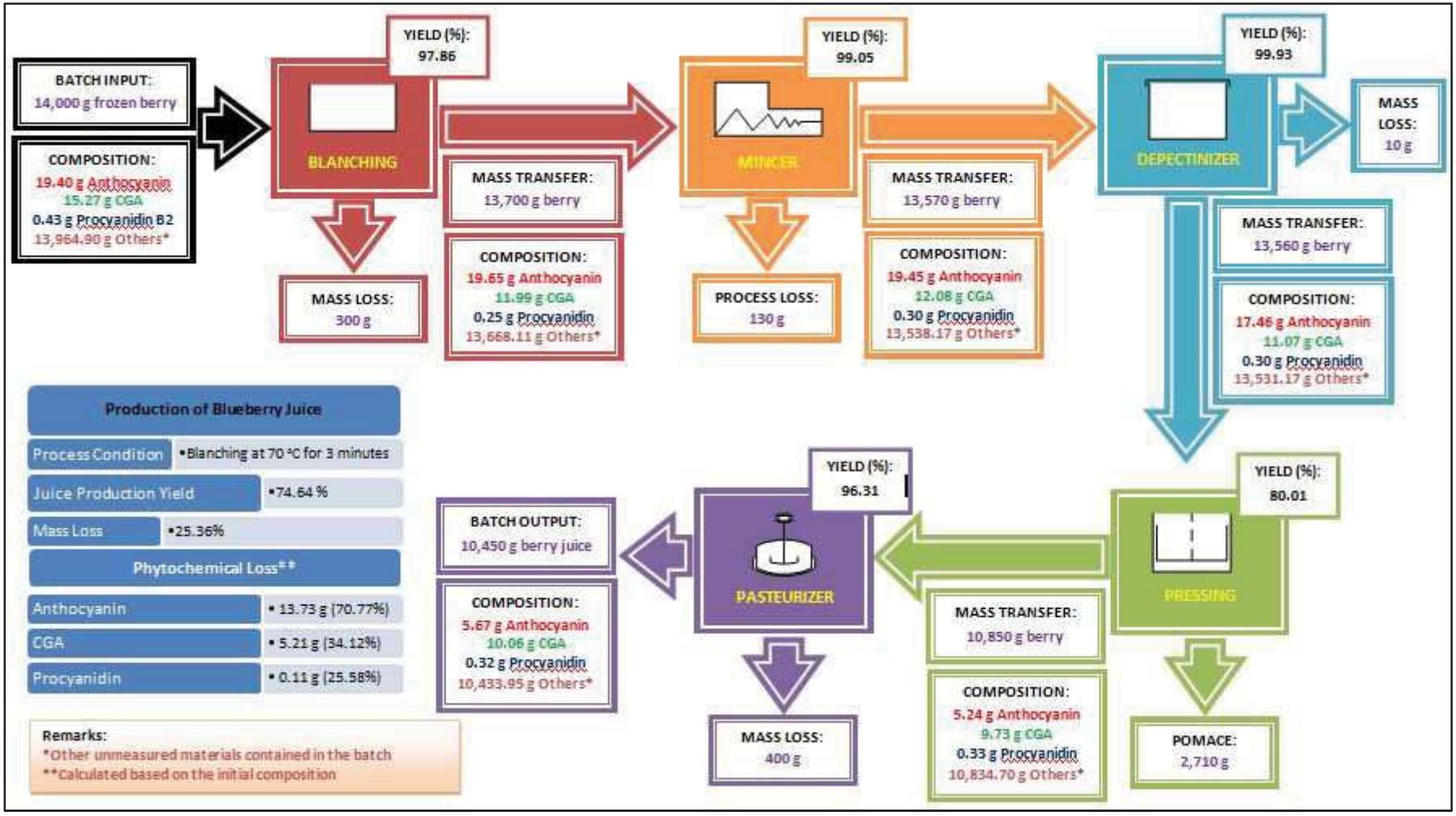


Figure 6-3: Overall mass and phytochemical balance in 'blanched at 70 °C' juice processing.

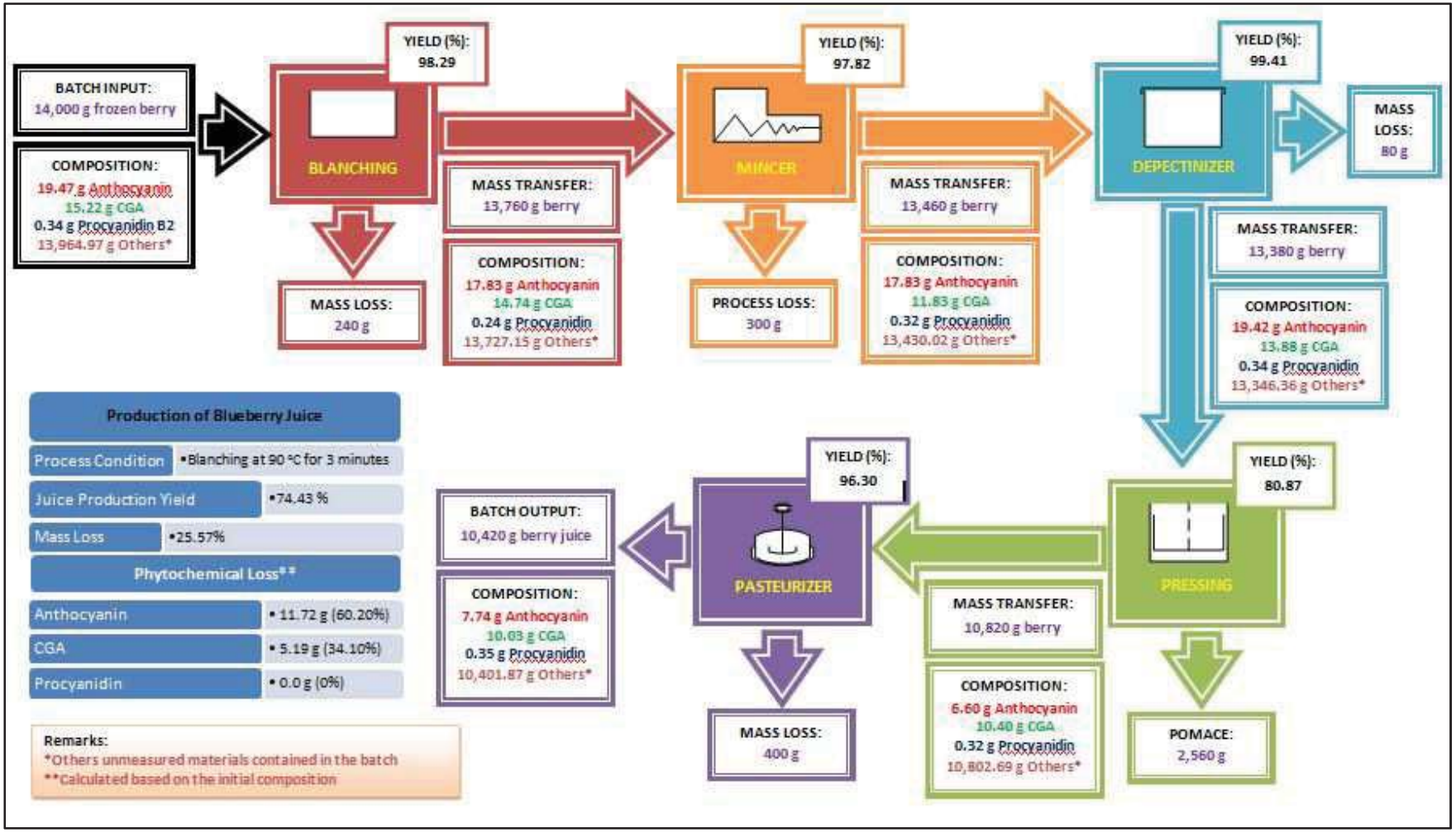


Figure 6-4: Overall mass and phytochemical balance in 'blanched at 90 °C' juice processing.

6.3.2. Juice processing model

The juice processing model combines all the three models; defrost model, recovery model, and thermal model. The developed juice processing model will only focus on anthocyanin and CGA degradation during blueberry juice processing. Procyanidin B2 has a complex behaviour and needs further study before it can be modelled.

The defrost model has been discussed in Section 4.3.2. Degradation in whole berries during thawing was modelled using a non-isothermal second-order kinetic reaction. The model was adequate to predict degradation of anthocyanins and CGA in whole berries during thawing. During thawing, two mechanisms of loss occur: enzymatic degradation by PPO enzyme and thermal degradation. At low temperatures (≤ 50 °C), PPO is very active compared to thermal degradation. Thermal degradation has less impact during thawing at temperatures ≤ 50 °C ($k = 0.0007 \pm 0.0001$ min^{-1}) and can be ignored. This model applies only during thawing at temperature ≤ 50 °C.

The recovery model has been discussed in Section 5.3.5. Because of the complexity of blanching, enzyme treatment steps, and pressing, for modelling they are treated as single correction factors, dependent solely on blanching temperature and assuming a standard blanching time of three min and enzyme dosage of 2 mg/100 kg. There is no change to this correction factor if enzyme treatment is varied between 2 and 4 h. Anthocyanin recovery was described by a sigmoidal function model and CGA recovery was described by linear regression.

The thermal model has been discussed in Section 3.4. For anthocyanins, two thermal models have been developed based on the availability of PPO enzyme in the juice.

The first model described juice with active PPO enzyme and second model described the juice without PPO enzyme. Both models were well described by an Arrhenius equation, whereas CGA was a thermostable compound.

Since blueberry juice processing is a continuous process, output from one model will be the input for the next model. For example, output from the defrost model will be the input for the recovery model and so on. The juice processing model for anthocyanin and CGA prediction is shown in Figure 6-5. The juice processing model for CGA prediction is simpler than that for anthocyanin. CGA is thermostable, therefore the overall processing model only involved the defrost and recovery models and could be used for all processing conditions.

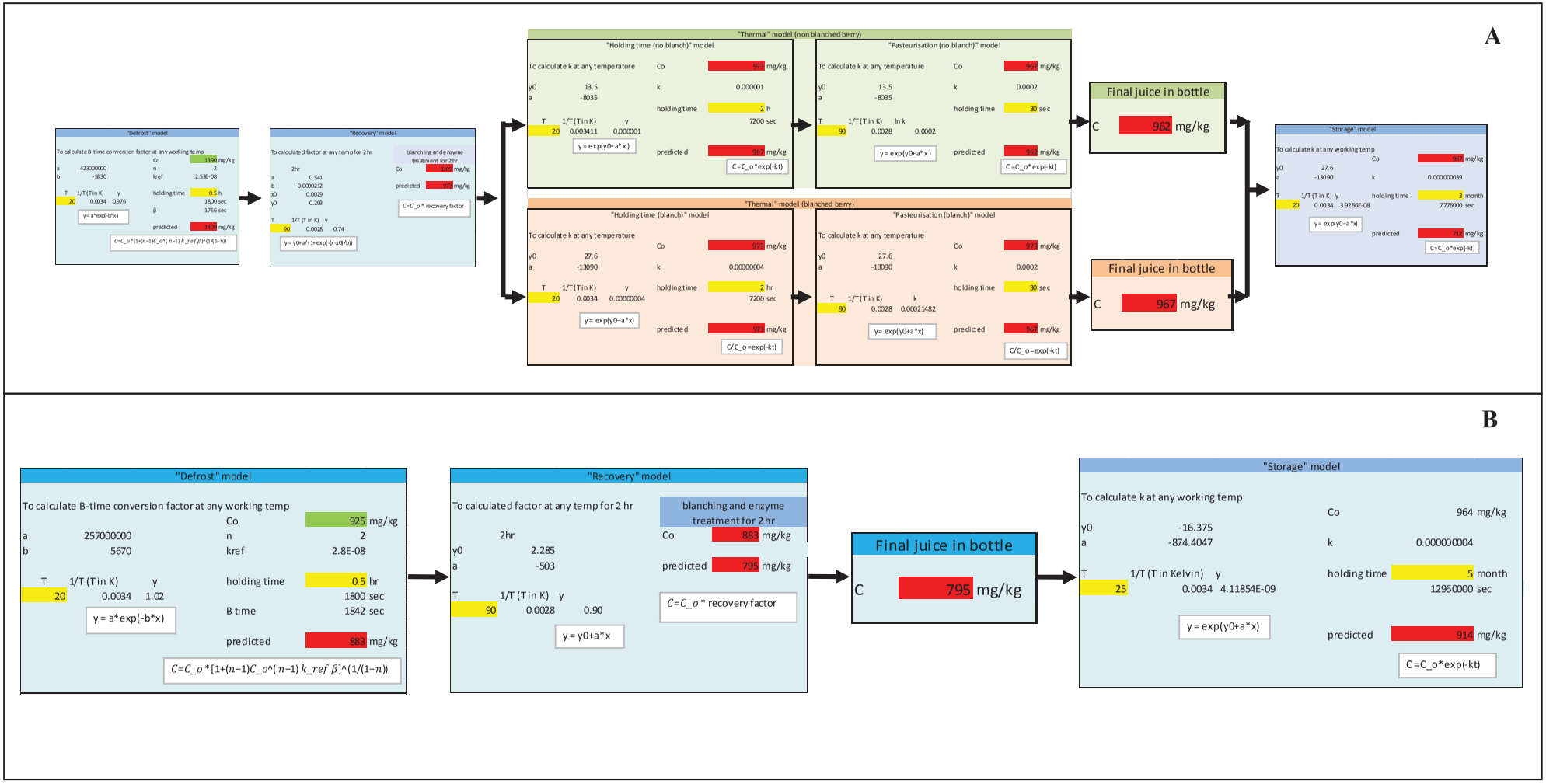


Figure 6-5: Juice processing model for A) anthocyanin concentration prediction in juice; B) CGA concentration prediction in juice. Example for berry 'blanched at 90 °C'.

6.3.3. Prediction and validation of the model

The developed juice processing models have been successfully used to predict phytochemical concentration in final juice. However, for the prediction and validation purpose, each part of the model will be run and discussed separately, and then compared to the overall juice processing model which links all the three models.

6.3.3.1. Defrost model

Table 6-4 presents the comparison of predicted and validation values of anthocyanin and CGA in the juice when a large batch (ca. 15 kg) of berries was processed in the FoodPilot. It shows that there is no significant difference between prediction and validation data for both anthocyanins and CGA during thawing. The developed model is adequate to predict anthocyanins and CGA degradation during thawing process.

Table 6-4: Prediction and validation values of anthocyanin and CGA in blueberry juice with different juice processing conditions during thawing using the defrost model.

Juice processing method	'No blanch' (thawed at 4 °C for 15 h)	'Blanched at 70 °C'; pasteurised	'Blanched at 90 °C'; pasteurised
Anthocyanin (mg)			
Prediction	13,125 ± 3,825[a]	-	-
Validation	15,570 ± 2,471[a]	-	-
CGA (mg)			
Prediction	9,450 ± 525[a]	-	-
Validation	8,263 ± 1,171[a]	-	-

Data are means ± confidence interval at 95%. (N=2-3). Values that are followed by different letters within each column are significantly different (P<0.05) using Tukey's Honest Significant Difference test.

6.3.3.2. Recovery model

Prediction and validation data for recovery model are tabulated in Table 6-5. The model had a good prediction for anthocyanin content in 'blanched at 70 °C', however, it underestimated anthocyanin in 'no blanch' juice and overestimated anthocyanin in 'blanched at 90 °C'. The model prediction for CGA also underestimated CGA in 'no blanch' juice but a very good prediction was achieved in 'blanched at 70 °C' and 'blanched at 90 °C' juice. The result shows that the recovery model should be reassessed to include more data points in the missing data between 20 and 70 °C as discussed in Section 5.4.

Table 6-5: Prediction and validation values of anthocyanin and CGA in blueberry juice with different juice processing conditions during blanching, cooling, mincing, enzyme treatment, and pressing using the recovery model.

Juice processing method	'No blanch' (thawed at 4 °C for 15 h)	'Blanched at 70 °C'; pasteurised	'Blanched at 90 °C'; pasteurised
Anthocyanin (mg)			
Prediction	2,164 ± 112[a]	5,292 ± 45[a]	10,295 ± 887[a]
Validation	3,500 ± 713[b]	5,242 ± 2,464[a]	6,595 ± 1,302[b]
CGA (mg)			
Prediction	4,095 ± 2,012[a]	8,512 ± 2,908[a]	11,396 ± 2,813[a]
Validation	7,477 ± 786[b]	9,733 ± 682[a]	10,395 ± 1,551[a]

Data are means ± confidence interval at 95%. (N=2-3). Values that are followed by different letters within each column are significantly different ($P<0.05$) using Tukey's Honest Significant Difference test.

6.3.3.3. Thermal model

Prediction and validation of thermal model (Table 6-6) also shows that there is no significant difference between prediction and validation for both anthocyanins and CGA during thermal treatment. The developed model is adequate to predict anthocyanins and CGA degradation during thermal treatment.

The same model has been used to predict anthocyanin and CGA in juice during long term storage at 25 °C (Table 6-7). The result shows that the model was adequate to predict both anthocyanin and CGA in the juice during long term storage. This shows that developed thermal model can be used to predict anthocyanin and CGA in the juice during processing.

Table 6-6: Prediction and validation values of anthocyanin and CGA in blueberry juice with different juice processing conditions during thermal treatment using the thermal model.

Juice processing method	'No blanch' (thawed at 4 °C for 15 h)	'Blanched at 70 °C'; pasteurised	'Blanched at 90 °C'; pasteurised
Anthocyanin (mg)			
Prediction	3,589 ± 1,820[a]	5,016 ± 1,087[a]	6,315± 2,094[a]
Validation	3,669 ± 1,713[a]	5,672 ± 2,464[a]	7,744 ± 1,302[a]
CGA (mg)			
Prediction	7,182 ± 2,012[a]	9,374 ± 2,908[a]	10,014 ± 2,813[a]
Validation	7,125 ± 876[a]	10,057 ± 1,538[a]	10,034 ± 271[a]

Data are means ± confidence interval at 95%. (N=2-3). Values that are followed by different letters within each column are significantly different (P<0.05) using Tukey's Honest Significant Difference test.

Table 6-7: Prediction and validation values of anthocyanin and CGA in blueberry juice with different juice processing conditions after 20 weeks of storage in the dark at 25 °C using the thermal model.

Juice processing method	'No blanch' (thawed at 4 °C for 15 h)	'Blanched at 70 °C'; pasteurised	'Blanched at 90 °C'; pasteurised
Anthocyanin (mg/L)			
Prediction	125 ± 70[a]	194 ± 44[a]	257 ± 25[a]
Validation	148 ± 76[a]	250 ± 61[a]	334 ± 148[a]
CGA (mg/L)			
Prediction	702 ± 19[a]	921 ± 18[a]	965 ± 26[a]
Validation	708 ± 46[a]	906 ± 20[a]	887 ± 100[a]

Data are means ± confidence interval at 95%. (N=2). Values that are followed by different letters within each column are significantly different (P<0.05) using Tukey's Honest Significant Difference test.

6.3.3.4. Overall juice processing model

Prediction and validation also had been done to assess the overall juice processing model (Table 6-8). In the overall model, all the three models were linked one to another; output from the previous model formed the input of the next model. Technically it would have been possible for the already noted 'poor' performance in one part of the model to still generate a reasonable approximation by the end of the process; but this was found not to be the case for the 'no blanch' juice. The overall model did however give a good prediction for both anthocyanin and CGA in 'blanched at 70 °C and 'blanched at 90 °C' juice.

The fact that prediction of both anthocyanin and CGA in 'no blanch' juice was underestimated when using overall model indicates that some constraints operating in the laboratory scale work are less serious at pilot plant scale; more of these phytochemicals partition to the juice than was predicted.

Table 6-8: Prediction and validation values of anthocyanin and CGA in blueberry juice with different juice processing conditions run using overall juice processing model which links all the three models.

Juice processing method	'No blanch' (thawed at 4 °C for 15 h)	'Blanched at 70 °C'; pasteurised	Blanched at 90 °C'; pasteurised
Anthocyanin (mg/L)			
Prediction	1,699 ± 248[a]	5,408 ± 2,058[a]	10,384 ± 3,907[a]
Validation	3,669 ± 879[b]	5,672 ± 2,025[a]	7,744 ± 2,094[a]
CGA (mg/L)			
Prediction	3,464 ± 508[a]	7,738 ± 2,325[a]	8,477 ± 2,446[a]
Validation	7,125 ± 876[b]	10,057±1,538[a]	10,034± 271[a]

Data are means ± confidence interval at 95%. (N=2). Values that are followed by different letters within each column are significantly different (P<0.05) using Tukey's Honest Significant Difference test.

Validation of each of the models shows that the thermal model is already reasonably well suited to be modified into a robust industrial model that identifies temperature gradients within a batch of mash and applies the model appropriately. However, the 'recovery' model is at present basically a complex correction factor for a number of different and overlapping processes that include the physical liberation of phytochemicals from the berry matrix; this 'recovery' component would ideally be developed further into a mechanistic model before it is applied industrially. Also, the initial defrost model is an oversimplification of the underlying mechanisms where enzymes and substrates first become able to diffuse together as compartmentation breaks down and would benefit from a more mechanistic approach.

Figure 6-6 simulated the blueberry juice processing model. Standard industrial blueberry juice processing (Figure 6-7 A) adapted from Birt (2011) shows that only 10% of anthocyanins remain in the juice after pasteurisation. This standard industrial processing took six days to complete and involved a long thawing process (3 days) that exposed anthocyanins to PPO degradation risk (although fortunately this was carried out in a cold room). A huge loss of anthocyanins was found during both thawing and separation of juice and pomace. Red and green dotted line in Figure 6-6 A indicates the prediction of anthocyanin and CGA using the overall processing model developed. The model predicted that there were great losses of both anthocyanins and CGA during standard industrial juice processing. From the predicted data, it shows that blanching approach would be better for the industry to increase the phytochemical content in the blueberry juice.

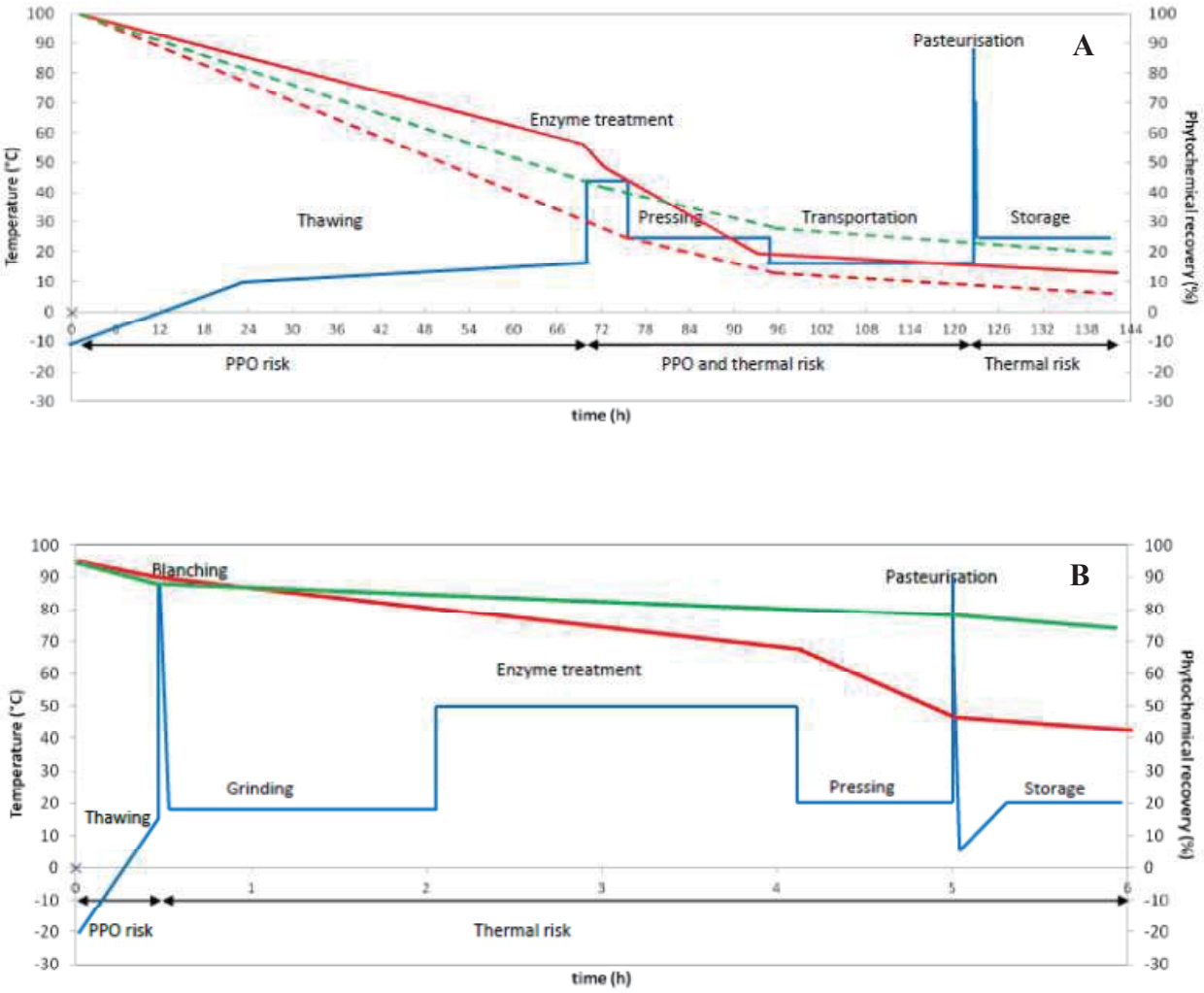


Figure 6-6: Simulated overall blueberry juice processing model. A: Standard industrial processing adapted from Birt (2011). B: Experimental blueberry juice processing model. In each case, blue line represents processing temperature profile, red line represents anthocyanin profile during processing and green line represents CGA profile during processing. Red and green dotted line in A indicates the prediction of anthocyanin and CGA using the overall processing model developed. Please note the very different time scales on A and B.

To minimise anthocyanin degradation during processing, PPO needs to be deactivated, particularly as PPO action is more aggressive than thermal degradation with respect to anthocyanins. The addition of a blanching step would deactivate PPO, reduce time taken for the thawing process and retain higher anthocyanin concentration in the juice as well as reducing the whole processing time (Figure 6-8 B). When blanching was applied, losses of anthocyanins during thawing were reduced but losses during separation of juice and pomace were still an issue. A combination of thermal treatment to inactivate PPO and fast thawing to reduce thermal degradation could result in a five-fold increase in anthocyanin concentration of the finished juice. About 80% of CGA remained in the final juice after pasteurisation.

6.4. Conclusion

Overall, the developed juice processing model can be used to predict anthocyanin and CGA content in final juice if blanching is used. The recovery model needs some further work to accurately predict anthocyanin and CGA recoveries during commercial processing. Manipulation of the processing conditions i.e. minimising the thawing process and maximising berry blanching at high temperature will greatly influence the recovery of anthocyanins and CGA in the final juice. It is also important to keep 'holding time' short and 'holding temperature' low as possible. The application of this model would allow industrial processors to estimate the impact of proposed modifications to their juicing process and could be used as appropriate input to calculate more energy-efficient ways of producing high-phytochemical juice.

7. Sensory evaluation

7.1. Introduction

In the beverage industry, especially for fruit and berry juices, attractive colour (Bates et al., 2001; Fernández-Vázquez et al., 2013) of the juices is the main criterion influencing the consumer perception. The consumer likes 'buying with their eyes' but flavour definitely will influence them to repeat purchase. Blueberry juice processing involves a series of unit operations: thawing, blanching, enzyme treatment, separation of the juice from pomace, pasteurisation, and bottling. All the processes may influence the phytochemical composition of the final juice (Brambilla et al., 2008; Brownmiller et al., 2008), thus affecting its colour, flavour, and other sensory qualities (Leavens 2006; Lawless et al., 2012; Barba et al., 2013). Polyphenol-rich beverages are usually associated with astringent and bitter characteristics which may affect the consumer acceptance as well (Jaeger et al., 2009; Bechoff et al., 2014). Consumers have always preferred more palatable beverages; even if it has high health benefits they still want it to taste good.

Results from previous chapters confirmed that phytochemical concentration in blueberry juice can be increased by modifying the processing conditions, especially blanching. However, consumer acceptability of juice with high phytochemical concentration remains questionable. We hypothesised that the increased phytochemical concentration of the blueberry juice is likely to affect the acceptance of the juice. Therefore, the objective of this study was to evaluate consumer acceptance and sensory characteristics of six blueberry juices, which included three commercial juices and three experimental juices, and their relation with the nutritional, physico-chemical, and phytochemical analysis.

7.2. Materials and methods

7.2.1. Juice preparation and experimental setup

Six (6) juices which included three (3) commercial juices and three (3) experimental juices were used in this study. Commercial blueberry juice samples were purchased from local juice companies and labelled as J1, J2, and J3. The juices contained only blueberry. Blends with other berries or fruits were not acceptable for this study. Information of the three commercial juices processing conditions are as follows (Table 7-1):

Table 7-1: Processing condition of the three commercial juices

Commercial juice	Processing condition
J1	Not heat treated; with pectinase enzyme treatment
J2	Steam tripping; without pectinase enzyme treatment
J3	Information not supplied

The experimental juices were produced with two extreme conditions i.e. blanching at 90 °C and 'no blanch'. Another juice was selected at an intermediate temperature (70 °C). The experimental juices were produced as described in Section 2.6.

Juices used for sensory evaluation were poured into one big jar from the original bottles before serving to ensure a homogeneous juice. Fresh bottles were used for each day of testing. Juices for nutritional, physico-chemical, and phytochemical analysis were sampled three times i.e. on each day that the sensory evaluation was conducted. Samples were kept frozen in -20 °C freezer until analysis.

7.2.2. Sensory evaluation

Two sensory evaluations were carried out for six blueberry juices using consumer based evaluation for acceptance evaluation, and free choice profiling (FCP) for descriptive evaluation. Acceptance evaluation measures liking or preference for a product from the consumer perspective and normally it involves large numbers of consumers (Stone & Sidel, 2004). Descriptive evaluation is for the description of both qualitative and quantitative sensory aspects of a product. In this study FCP was chosen to allow trained panellists to use their own attributes to describe and quantify the juice (Deliza et al., 2005).

7.2.2.1. Consumer based evaluation

One hundred and twenty (120) panellists, 41 male and 79 female, aged between 18 – 65 years old were randomly recruited from Massey University's staff and students. Sensory evaluation was conducted at SFN sensory lab equipped with 10 individual partitioned booths and was held in two sessions.

The panellists were asked to evaluate the overall liking, aroma, colour, sourness, sweetness, and thickness of the juices using a structured nine-point hedonic scale (1 = dislike extremely to 9 = like extremely) (Stone & Sidel, 2004) (Appendix I). Juices samples (15 mL each) were served in plastic cups and were coded using a random three-digit number. Samples were served individually, one after another, under normal lighting conditions at room temperature (25 °C) and a balanced complete block design was used to assign the presentation order. Drinking water was provided to clean and rinse the mouth between samples.

Data were analysed using Friedment's test from the R package using the R code of 'Tal Galili' (Galili, 2010).

7.2.2.2. Descriptive evaluation – free choice profiling (FCP)

Eight (8) trained panellists were recruited from the PFR (Palmerston North, New Zealand) panellists' list for their previous experience in sensory analysis methodology especially in juice sensory analysis. The panellists consisted of seven (7) female and one (1) male aged between 40 – 66 years old. The FCP was carried out in two sessions.

The first session was a vocabulary development session. The panellists were presented with all six juices simultaneously and they were instructed to generate their own list of attributes to describe the differences of the juice. They were instructed to taste the juices individually, rinse their mouth between samples and group the terms for appearance, aroma, flavour, mouthfeel, and aftertaste. After that, panellists were given an opportunity to discuss their attributes with other panellists and update their own attributes if necessary before finalizing their own score sheet of attributes to be used in the second session.

The second session was conducted on the following day in a sensory lab equipped with eight individual partitioned booths. In this session, panellists assessed six juice samples twice, with a 30 min gap between each evaluation. Panellists scored their own attributes using an unstructured line scale, ranging from 0 (none) to 10 (very high) to evaluate the differences of the samples. Drinking water and water crackers were provided to clean and rinse the mouth between samples. Juices samples

(30 mL) were coded using a random three-digit number and served individually, one after another, under normal lighting conditions at room temperature (25 °C) and a balanced complete block design was used to assign the presentation order. The data were analysed using FCP procedure from the R package SensoMineR (http://sensominer.free.fr/).

7.2.3. Nutritional, physico-chemical and phytochemical analysis

Nutritional analysis of the experimental juice was carried out by an accredited laboratory (Nutrition Laboratory, School of Food and Nutrition, Massey University, New Zealand). Protein content was analysed using total combustion method (AOAC 968.06) by LECO®, fat by soxtec extraction (AOAC 991.36), moisture content by convection oven at 105 °C (AOAC 930.15, 925.10), ash using a furnace at 550 °C (AOAC 942.05), and dietary fibre by an enzymatic-gravimetric method (AOAC 991.43). Energy was determined by calculation, minerals by plasma emission spectrometry (sub-contracted), carbohydrate by calculation, and sugar profile by using GC (sub-contracted). The nutritional values of the commercial juices were assumed to be as stated on the label. Physico-chemical and phytochemical analysis were carried out as described in Sections 2.3 and 2.2 respectively.

7.3. Results and discussion

7.3.1. Sensory evaluation

7.3.1.1. Consumer based evaluation

Mean scores of consumer acceptance of six blueberry juices are summarized in Table 7-2. A high mean score indicates that the juices were well-liked by consumers. All the six blueberry juices except J1 and J2 juices were acceptable or liked by the

Table 7-2: Mean acceptability scores of consumer acceptance attributes of six blueberry juice.

	Experimental juice			Commercial juice		
	'No blanch'	'Blanched at 70 °C'	'Blanched at 90 °C'	J1	J2	J3
Overall liking	6.75 ± 0.30^{a}	7.18 ± 0.26^{a}	6.97 ± 0.28^{a}	5.17 ± 0.33^{c}	4.48 ± 0.38^{d}	5.75 ± 0.33^{b}
Aroma	6.70 ± 0.32^{a}	6.93 ± 0.28^{a}	6.94 ± 0.29^{a}	5.86 ± 0.33^{b}	5.78 ± 0.37^{b}	6.00 ± 0.34^{b}
Colour	7.33 ± 0.23^{a}	7.49 ± 0.23^{a}	7.38 ± 0.24^{a}	6.62 ± 0.27^{b}	6.68 ± 0.31^{b}	6.78 ± 0.28^{b}
Sourness	6.43 ± 0.30^{a}	6.81 ± 0.28^{a}	6.77 ± 0.29^{a}	4.79 ± 0.34^{c}	4.33 ± 0.39^{c}	5.47 ± 0.36^{b}
Sweetness	6.58 ± 0.33^{a}	6.83 ± 0.31^{a}	6.82 ± 0.32^{a}	5.12 ± 0.31^{c}	4.57 ± 0.35^{c}	5.78 ± 0.35^{b}
Thickness	6.68 ± 0.29^{a}	6.96 ± 0.26^{a}	6.78 ± 0.28^{a}	5.88 ± 0.30^{b}	5.71 ± 0.35^{b}	5.90 ± 0.32^{b}

Data represent means ± confidence interval at 95% (N=120). Values that are followed by different letters within each row are significantly different ($P<0.05$) using Friedmen's test.

consumer because the mean scores were greater than a score of 5 (neither like nor dislike) (Knuckles et al., 1997). J1 juice was less-liked for the sourness and J2 was less-liked for overall liking, sourness, and sweetness. Further observation shows that consumer acceptability of experimental juices was higher compared to commercial juices. All experimental juice scored ≥ 6.75 whereas no commercial juice scored above 5.75. These differences were statistically significant (P > 0.05, Table 7-2). All experimental juices ('no blanch', blanched at 70, and blanched at 90) were statistically indistinguishable (all attributes) (Table 7-2).

Based on the overall liking acceptability, the order of consumer acceptance of the juices was: blanch at 70 ≥ blanch at 90 ≥ 'no blanch' > J3 > J1 > J2. Lawless et al. (2012) indicated that the overall liking score of blueberry juice in their study was 5.47. Therefore, experimental juices in this study had higher acceptability compared to the blueberry juice described by them.

The mean score of consumer acceptance attributes was also mapped using principal component analysis (PCA) to distinguish among the juices. The score plot (Figure 7-1) shows that 96% of total variation is explained by the first component. The first component corresponds to the type of the juice which separates experimental juice from commercial juice. Second component, which accounted for 3.4% of total variation of the data further separates the three commercial juices based on how they were manufactured. J2 was produced by steam stripping technique without any pectinase enzyme treatment and J1 was produced without heat treatment but treated with pectinase enzyme. There is no information supplied for J3.

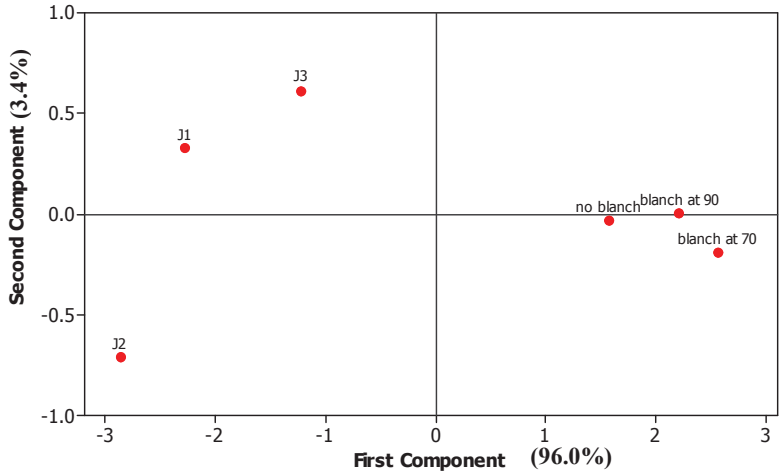


Figure 7-1: Score plot of mean acceptability scores of consumer acceptance attributes of six blueberry juices.

All the consumer attributes (overall liking, aroma, colour, sourness, sweetness, and thickness) have similar strong vectors (Figure 7-2) for first component emphasising that the experimental juices are more acceptable in every attribute and none of the attributes have a particularly large influence on the distribution. Consumer acceptability of 'no blanch' juice in this study was the same as blanched juices. 'No blanch' juice can be considered heat extracted juice because it has undergone heat treatment at 50 °C for 2 hr during enzyme incubation. It seems clear that processing juice at moderate (45 – 50 °C) or high (>70 °C) temperature does not lead to adverse consumer reaction. Commercial juices in this trial and in Leavens trial (Leavens, 2006) did less well than freshly prepared heat treated juices. Commercial juice may had been stored for some time and degraded over time. This phenomenon was expected to occur as has been observed in Section 3.3.3.

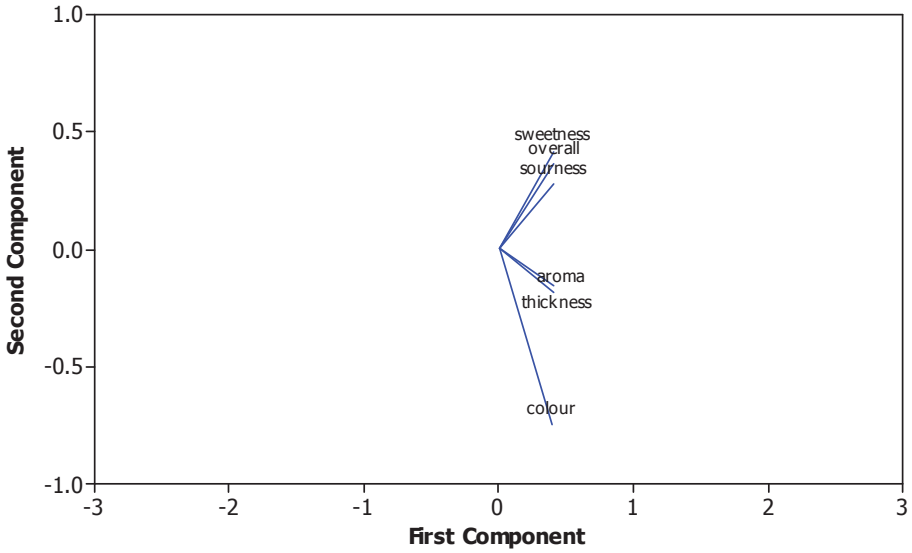


Figure 7-2: Correlation of consumer acceptance attributes of six blueberry juices for first component vs. second component.

7.3.1.2. Descriptive evaluation – free choice profiling (FCP)

Free choice profiling (FCP) of the six blueberry juice samples generated between 11 and 25 descriptors from each of 8 panellists. A total of 69 descriptors was generated by the panellists, and descriptors were categorised into five classes i.e. appearance, aroma, flavour, mouthfeel, and aftertaste. Table 7-3 tabulates how often each descriptor was used by the panellists. Among all the descriptors, nine (9) were the most frequent terms generated by the panellists and had been used at least by half of the panellists. The terms are sour and sweet to describe flavour (8 times), astringent to describe mouthfeel (7 times), bitter to describe flavour (5 times). Fermented and fruity to describe flavour, fermented and fruity to describe aroma, and purple to describe appearance were used 4 times by the panellists.

Table 7-3: Descriptors used by the panellists in free choice profiling.

Appearance	Frequencies used	Aroma	Frequencies used	Flavour	Frequencies used	Mouthfeel	Frequencies used	Aftertaste	Frequencies used
Purple	4	Fermented	4	Sour	8	Astringent	7	Sweet	2
Frothy	2	Fruity	4	Sweet	8	Dry	3	Acidic	1
Rich	2	Berry	3	Bitter	5	Bitter	2	Bitter	1
Thickness	2	Woody	3	Fermented	4	Acidic	1	Catches throat	1
watery	2	Acidic	2	Fruity	4	Apple juice	1	Sour	1
Frosty	1	Apple	2	Woody	3	Citric	1	Throat burn	1
Opacity	1	Grape	2	Apple	2	Metallic	1		
Plum	1	Overall	2	blueberry	2	Mouth watering	1		
		Sweet	2	Metallic	2	Prunes	1		
		Blackcurrant	1	Overall	2	Smooth	1		
		Blueberry	1	Tart	2	sweet	1		
		Cherry	1	Acidic	1	Thickness	1		
		Earthy	1	Berry	1	Thin	1		
		Floral	1	Cherry	1	Throat burn	1		
		Musty	1	Citric	1	Tingling	1		
		Raspberry	1	Grape	1	Tongue coating	1		
		Wine	1	Plastic	1				
				Prunes	1				
				Raspberry (Icing)	1				
				Stale	1				
				Watery	1				
				Wine	1				

Consensus configuration plus confidence intervals from FCP procedure for the juices were plotted against first dimension vs. second dimension of the analysis (Figure 7-3 A) and first dimension vs. third dimension of the analysis (Figure 7-3 B). The first three dimensions accounted for 83.8% of variation and were considered in the analysis. FCP is another form of multivariate analysis that, like PCA, clusters similar products in different dimensions. Dimensions 1, 2, and 3 accounted for 43.5, 22.5, and 17.8% of total variation respectively.

The first dimension corresponds to the types of juice and separates commercial juices (J1-J3) from the experimental samples. The second dimension further separates commercial juices on how the juices were produced and the third dimension distinguishes between blanched or 'no blanch' juice. These results show that FCP is able to distinguish commercial juices from experimental juices and also distinguish between blanched and 'no blanch' juice.

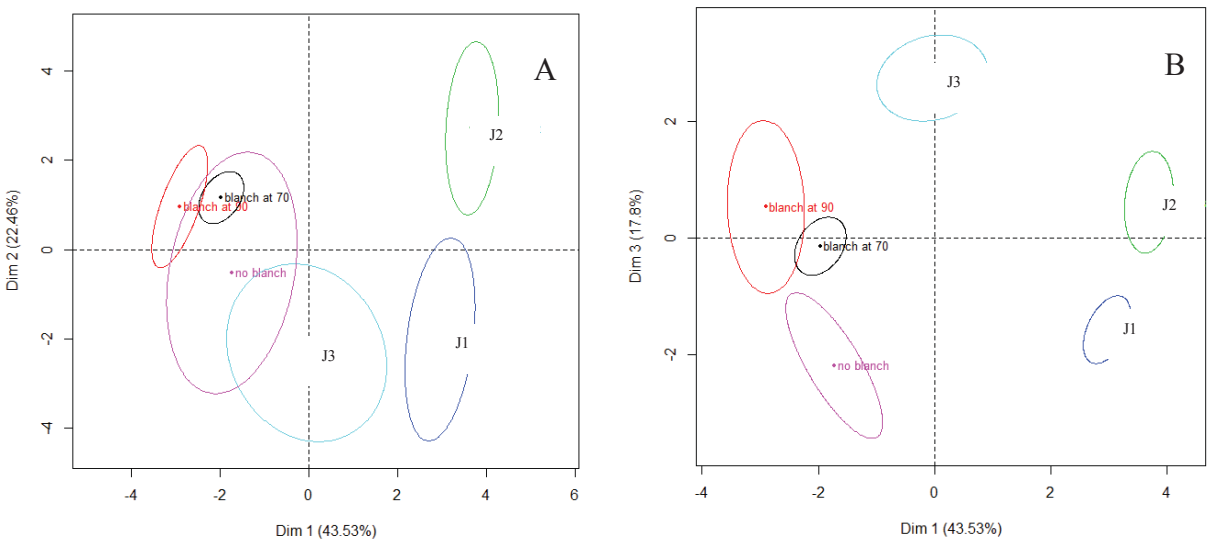


Figure 7-3: Confidence ellipses for the free choice profiling; A: Confidence ellipses for dimension 1 versus dimension 2; B: Confidence ellipses for dimension 1 versus dimension 3.

Correlations of the attributes generated by the panellists with the first two dimensions (PC1 horizontally, PC2 vertically) for each of the classes are shown in Figure 7-4. PC1 is associated with flavour, mouthfeel, and aftertaste differences. Samples to the left (blanched and 'no blanch') are sweeter, have smooth mouthfeel and sweet aftertaste while samples to the right (J1 and J2) are more sour, tart, metallic, and bitter and have more astringent mouthfeel and also have bitter and sour aftertastes. PC2 is associated with appearance and aroma. Samples towards the top (J2 and blanched juices) appear thicker, more plummy or purple, those towards the bottom (J1 and J3) appear watery and may have higher apple and other fruit aromas and flavours.

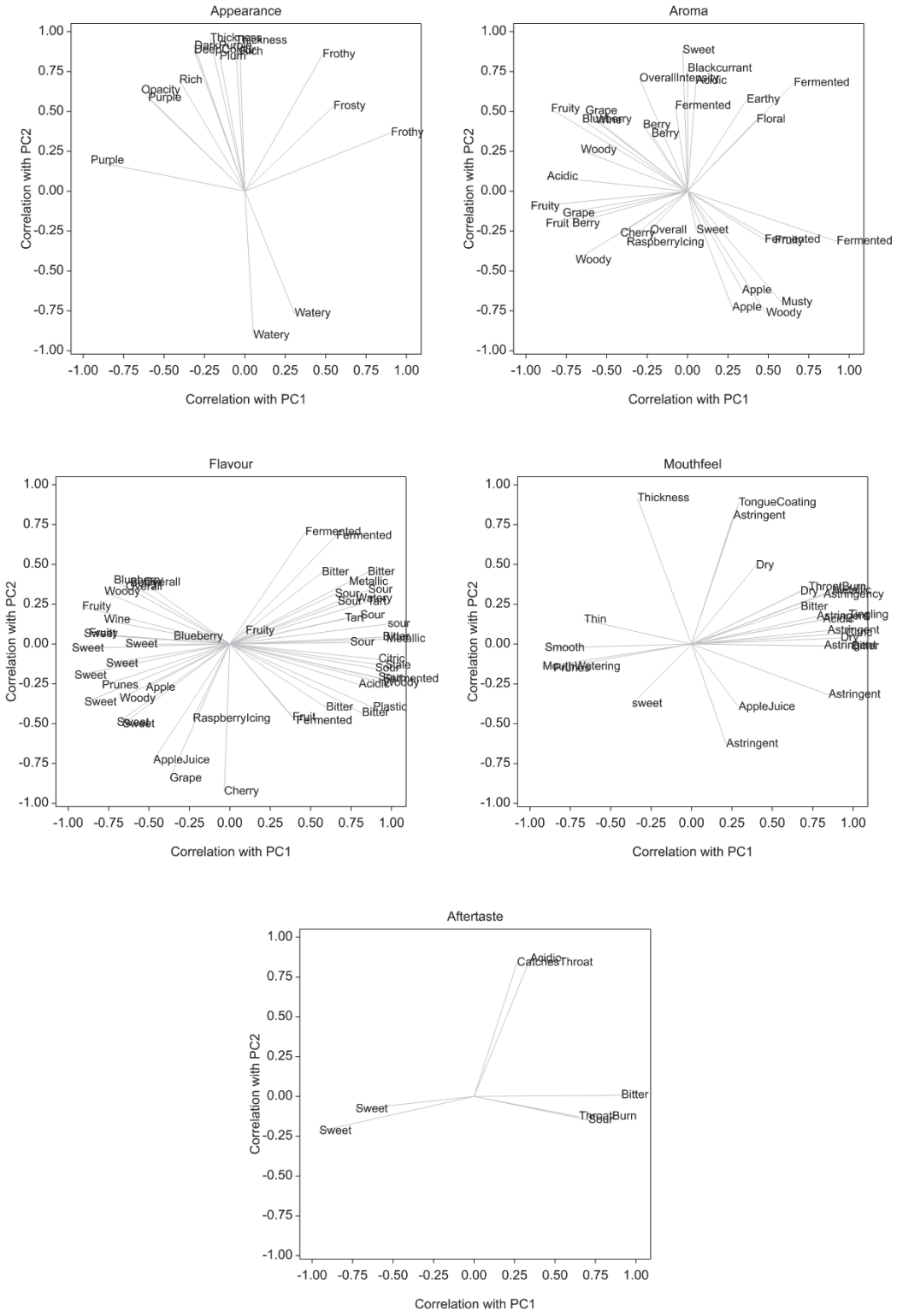


Figure 7-4: Correlation of the free choice profiling attributes PC1 vs. PC2.

7.3.2. Nutritional analysis

In this study, nutritional analysis of the blueberry juices was carried out only for the experimental juices ('no blanch', blanched at 70, and blanched at 90 °C juices). For the commercial juice, nutritional analysis was based on the information stated on the label. For that reasons, statistical analysis was only carried out for the experimental juices. Results (Table 7-4) show that blanching treatment does not alter the nutritional values of the juices statistically ($P < 0.05$). This suggested that varying blanching temperature of the berry did not much affect the nutritional composition of the final juice.

Blanching may change the nutritional properties in certain vegetables and fruits. For example, dietary fiber content in some vegetables has been reported to either increase, decrease or remain unchanged after blanching (Puupponen-Pimiä et al., 2003). The changes were due to the washing out of the soluble component in processed material or leakage into the processing water during blanching. In our experiment, the blanching process was done in a pouch, therefore, it was a closed system and no leakage into the processing water occurred. This explains why the nutritional values of the juices were not affected by the blanching process.

For commercial juices, comparisons can only be made between J2 and J3 juice because J1 juice didn't have the nutritional information on the label. Generally, J2 juice contained higher fat, protein, and sodium but lower carbohydrate and total sugar compared to J3 juice. Energy of both juices was similar. The difference in nutritional composition of commercial juice and experimental juice cannot be tested statistically. However, the nutritional compositions in the commercial juices seem to

Table 7-4: Nutritional analysis of the six blueberry juices.

	Experimental juice			Commercial juice		
	'No blanch'	'Blanched at 70 °C'	'Blanched at 90°C'	J1*	J2*	J3*
Fat (%)	0.02 ± 0.02[a]	0.04 ± 0.01 [a]	0.01 ± 0.01 [a]	-	0.4	0.1
Protein (%)	0.69 ± 0.22 [a]	0.43 ± 0.23 [a]	0.39 ± 0.21 [a]	-	0.7	0.1
Moisture (%)	85.59 ± 0.79 [a]	84.77 ± 0.45 [a]	84.87 ± 3.15 [a]	-	-	-
Ash (%)	0.19 ± 0.04 [a]	0.19 ± 0.01 [a]	0.20 ± 0.04 [a]	-	-	-
Carbohydrate (%)	13.52 ± 4.18 [a]	14.42 ± 0.32 [a]	14.27 ± 3.58 [a]	-	12	14
Energy (kJ/100 g)	239 ± 51 [a]	254 ± 3.04 [a]	250 ± 57 [a]	-	224	244
Potassium (mg/kg)	935 ± 64 [a]	940 ± 0.00 [a]	945 ± 1191[a]	-	-	890
Sodium (mg/kg)	13.00 ± 0.00 [a]	11.50 ± 6.35 [a]	11.50 ± 6.35 [a]	-	60	46
TDF (g/100 mL)	0.21 ± 0.06 [a]	0.15 ± 0.03 [a]	0.25 ± 0.01 [a]	-	-	-
IDF (g/100 mL)	0.07 ± 0.04 [a]	0.05 ± 0.04 [a]	0.06 ± 0.04 [a]	-		-
SDF (g/100 mL)	0.15 ± 0.02 [a]	0.14 ± 0.03 [a]	0.15 ± 0.03 [a]	-	-	-
Dry matter (%)	14.61 ± 2.53 [b]	15.23 ± 0.45 [a]	15.13 ± 3.15 [a]	-	-	-

Data represent mean ± confidence interval at 95% (N=2). Values that are followed by different letters within each row are significantly different ($P<0.05$) using Tukey's Honest Significant Different test. TDF = total dietary fiber; IDF = insoluble dietary fiber; SDF = soluble dietary fiber. (*) Data were physically checked from the information stated on the label. (-) Data not available.

Table 7-4 (cont): Nutritional analysis of the six blueberry juices.

	Experimental juice			Commercial juice		
	'No blanch'	'Blanched at 70 °C'	'Blanched at 90 °C'	J1*	J2*	J3*
Total sugar (%)	12.55 ± 0.64[a]	13.40 ± 1.27 [a]	13.20 ± 3.81 [a]	-	12	12
Sucrose (%)	<0.1	<0.1	<0.1	-	-	-
Lactose (anhydrous) (%)	<0.1	<0.1	<0.1	-	-	-
Maltose (%)	<0.1	<0.1	<0.1	-	-	-
Glucose (%)	6.60 ± 0.00 [a]	6.95 ± 0.64 [a]	6.85 ± 1.91 [a]	-	-	-
Fructose (%)	5.95 ± 0.64 [a]	6.45 ± 0.64 [a]	6.35± 1.91 [a]	-	-	-

Data represent mean ± confidence interval at 95% (N=2). Values that are followed by different letters within each row are significantly different ($P<0.05$) using Tukey's Honest Significant Different test. (*) Data were physically checked from the information stated on the label. (-) Data not available.

be in the same range as the experimental juices except for sodium content. J2 and J3 juice were high in sodium compared to the experimental juices. This may be explained by the cultivar differences and fertilizer usage by growers. Mineral fertilizers greatly affect the soil and plant growth, development and productivity (Siniagin, 1979). Another possible reason is that sodium ascorbate may have been added to the juice as an antioxidant or as an acid regulator but not listed on the label. Normally sodium ascorbate provides 131 mg of sodium per 1,000 mg of ascorbic acid (1,000 mg of sodium ascorbate contains 889 mg of ascorbic acid and 111 mg of sodium) (Stargrove et al., 2008).

Lack of information on the history of the commercial juice such as plantation management and practice, the variety used, and method of juice processing make it difficult to discuss more on this and it is also beyond the scope of this study.

7.3.3. Physico-chemical analysis

As in nutritional analysis, varying the berry blanching conditions did not affect the physico-chemical properties of the experimental juice much. The physico-chemical properties of commercial juices were also in the same range as experimental juices except for J2 juice. Obviously, J2 juice can be distinguished by containing the lowest TSS, and highest turbidity and viscosity compared to other juices (Table 7-5).

This can be explained by the juice processing conditions applied by the juice producer. J2 juice was produced using a steam stripping technique and they did not use any pectinase enzyme in their juicing process. As discussed in Chapter 5,

Table 7-5: Physico-chemical characteristic of experimental and commercial blueberry juice.

	Experimental juice			Commercial juice		
	'No blanch'	'Blanched at 70 °C'	'Blanched at 90 °C'	J1	J2	J3
pH	3.4 ± 0.6^{a}	3.3 ± 0.7^{a}	3.3 ± 0.1^{a}	3.1 ± 0.2^{ab}	2.9 ± 0.1^{b}	3.1 ± 0.2^{ab}
TSS (°Brix)	14.37 ± 0.14^{a}	15.47 ± 0.14^{a}	15.73 ± 0.14^{a}	12.47 ± 0.14^{b}	10.63 ± 0.29^{c}	14.57 ± 4.02^{a}
TA (% acid (wt/vol))	0.86 ± 0.07^{a}	0.78 ± 0.05^{a}	0.79 ± 0.06^{a}	0.79 ± 0.13^{a}	0.68 ± 0.10^{a}	0.86 ± 0.07^{a}
BI	0.84 ± 0.17^{bc}	0.67 ± 0.15^{c}	0.66 ± 0.08^{c}	1.05 ± 0.05^{a}	0.77 ± 0.10^{bc}	0.88 ± 0.33^{ab}
Turbidity (NTU)	154 ± 34^{b}	55 ± 10^{bc}	83 ± 17^{bc}	64 ± 14^{bc}	448 ± 34^{a}	23 ± 2^{c}
Apparent viscosity (Pa.s)	0.0025 ± 0.0006^{b}	0.0030 ± 0.0023^{b}	0.0027 ± 0.0017^{b}	0.0017 ± 0.0003^{b}	0.0080 ± 0.0035^{a}	0.0020 ± 0.0005^{b}

Data represent means ± confidence interval at 95% (N=3). Values that are followed by different letters within each row are significantly different ($P<0.05$) using Tukey's Honest Significant Difference Test. TSS = total soluble solid; TA = titratable acidity; BI = browning index

pectinase enzyme treatments are required when heat treatment is applied during blueberry juice processing to recover high volume of juice and reduce viscosity of finished juice.

7.3.4. Phytochemical analysis

In general, there were significant differences in phytochemical concentrations (anthocyanin, CGA and procyanidin B2) amongst all the juices studied (Figure 7-5). Phytochemical concentrations were higher in experimental juices compared to commercial juices except for CGA concentration which was highest in J2 juice (Figure 7-5 B). Phytochemical concentrations in experimental juices are proportional to blanching temperature. Further details were discussed in Chapter 5: Phytochemical recovery from berry matrix.

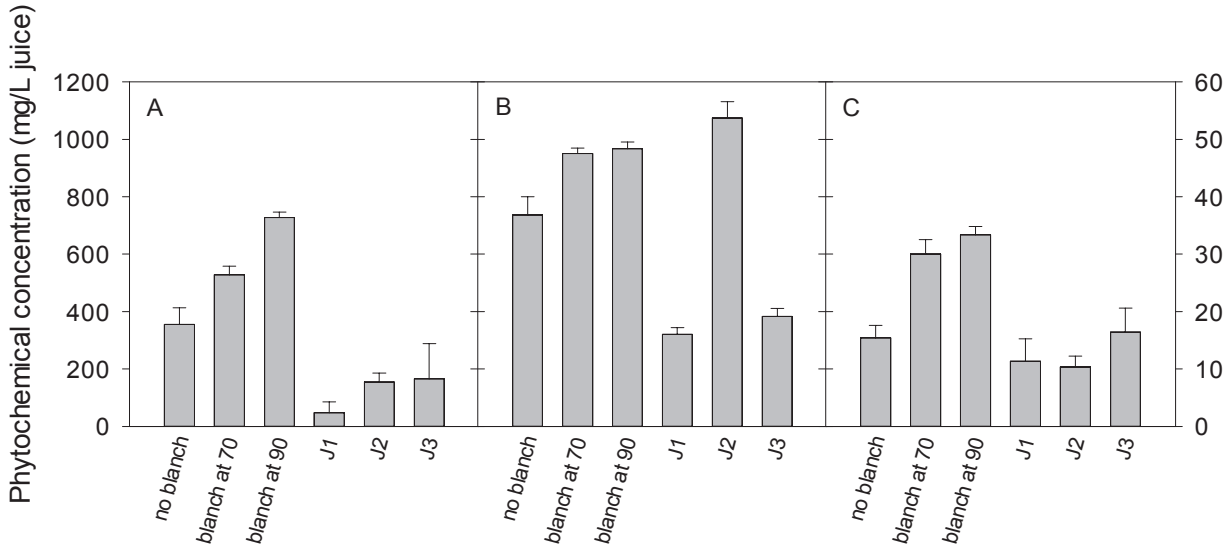


Figure 7-5: Phytochemical composition in the six blueberry juices; A) Anthocyanins; B) CGA; C) Procyanidin B2 concentration. Each bar represents mean ± confidence interval at 95% (N=3).

There are a few factors that influence the phytochemical concentration of both experimental and commercial juices. The first factor is associated with the age of the juices. Experimental juices were freshly prepared but there is no information about the commercial juices except for J3 juice which indicated a 'best before' date on their label. However, the age of the juice still cannot be estimated. Several researchers reported a decrease in phytochemical concentration of blueberry juice, especially anthocyanin, during storage. Therefore, the anthocyanin concentrations in commercial juice may have declined because of losses during storage (Brownmiller et al., 2008; Barba et al., 2012; Fracassetti et al., 2013).

Another factor was associated with the juice processing conditions applied by the juice producer. J1 juice was produced without blanching and used low heat treatment during processing. J2 juice was produced using a steam stripping technique. However, there is no information on juice processing method used by J3. J1 juice contained the lowest anthocyanin concentration of all the juices. As mentioned earlier, J1 juice was produced without blanching and was processed at low heat (about 40 °C). Therefore, the anthocyanin recovery was limited. This is in agreement with data presented in Chapter 5.

CGA concentration was high in J2 and comparable to experimental juice. The high concentration of CGA in J2 juice can be explained by the used of steam (high temperature) in the juice processing. Furthermore, CGA is a stable and water soluble compound (Shi et al., 2007) which makes it easy to extract during juicing.

Procyanidin B2 concentration in the juice was quite complicated to explain. It doesn't show any significant relationship to anthocyanin and CGA. It seems to be proportional to temperature but it was low in J2 juice. Interestingly, all the three phytochemicals studied were lower in all commercial juices compared to experimental juices except for CGA in J2.

7.3.5. Correlation between consumer attributes, FCP attributes, physico-chemical analyses and phytochemical concentration.

PCA was employed to describe relationships amongst consumer acceptance attributes, FCP attributes, physico-chemical analyses, and phytochemical concentration of the six blueberry juices studied. For FCP, only nine (9) attributes were used in the analysis; those that were used at least four (4) times by the panellists. Means of all the data were plotted against the first two components (Figure 7-6). The first component, accounting for 70.5% of total variation, separated experimental juices from commercial juice. The second component, accounting for 19.6% of total variation, separated blanched juices from 'no blanch' juice and J2 juice from J1 and J3 juice.

Correlation of the attributes generated from the data with first two dimensions (first dimension horizontally, second dimension vertically) for all the attributes tested are shown in Figure 7-7. The first component was associated with most of the attributes tested. Samples to the left (commercial juices) have fermented, bitter, and sour flavour, fermented aroma, and astringent mouthfeel. Samples to the right (experimental juices) had more sweet and fruity flavour, fruity aroma, were more liked by the consumer for all attributes tested, had higher anthocyanins and

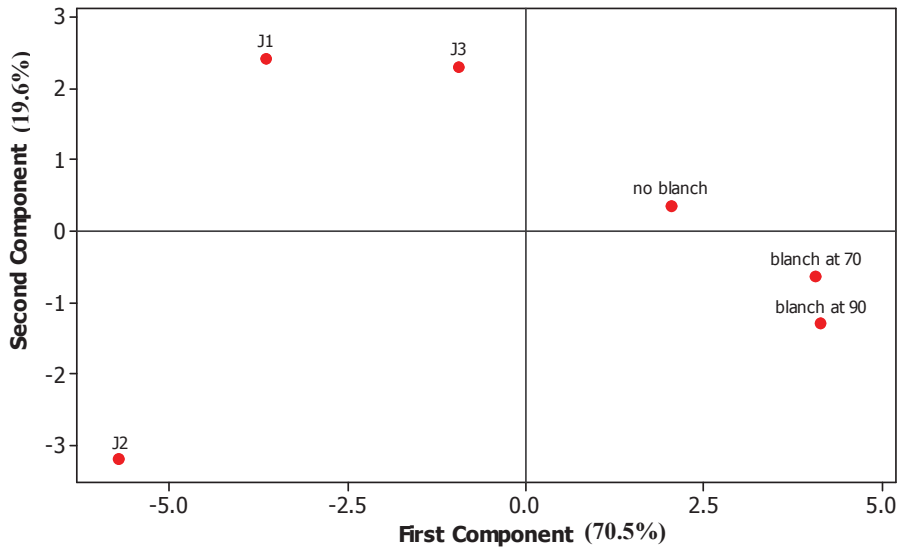


Figure 7-6: Principal component analysis of six blueberry juices for consumer attributes, Free Choice Profiling attributes, physico-chemical analyses, and phytochemical concentration.

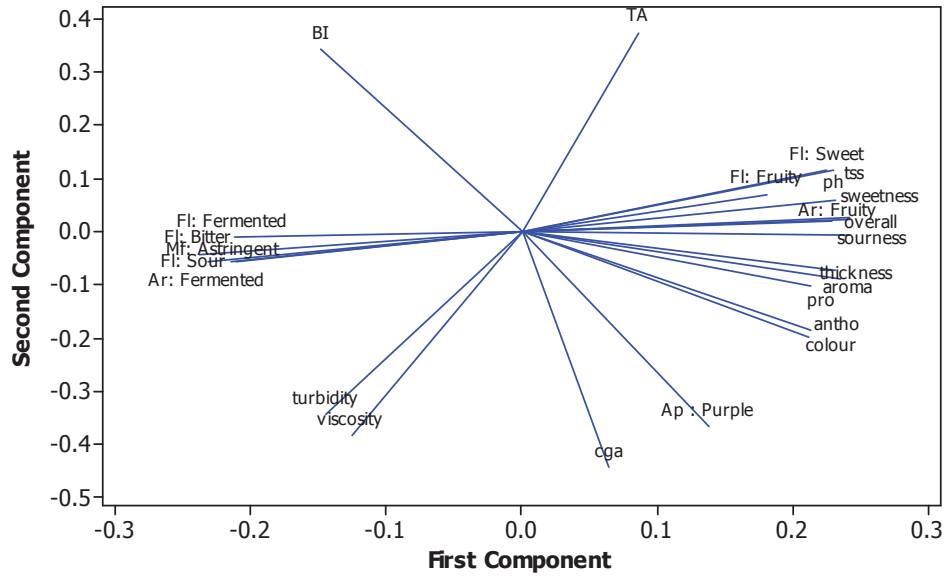


Figure 7-7: Correlation of the attributes generated by the panellist and objective data with the first two dimensions.

procyanidin B2 concentration, and higher TSS and pH values. The second component was associated with BI, TA, turbidity, viscosity, CGA concentration, and purple appearance. Samples towards the top (J1 and J2) have higher BI and TA, while samples towards bottom (J2) are more turbid and viscous.

Generally, phenolic compounds are thought to contribute to bitter and astringent taste/flavour of polyphenol-rich beverages (Jaeger et al., 2009; Lawless et al., 2012; Laaksonen et al., 2013). This has been confirmed by Bechoff et al. (2014) in a study conducted on a hibiscus drink. However, in this study, no correlation was found between anthocyanin or procyanidin B2 concentration and bitter and astringent flavour of the juice. This result is in agreement with a study conducted by Laaksonen et al. (2013) on blackcurrant juices. The bitterness and astringency taste/flavour of the blueberry juice may have been masked by the high content of sugar as shown by high TSS content. A study by Jaeger et al. (2009) found that the addition of sucrose to a beverage may achieve partial masking of bitterness. It seems that consumer acceptance of the beverages may increase with sucrose concentration but increasing sucrose concentration to a higher level may have insignificant effects on the consumer acceptance.

7.4. Conclusion

Both sensory evaluations conducted in this study show that experimental juices were significantly different to commercial juices. Consumer acceptability of the experimental juices was higher than commercial juices for all the attributes tested. FCP was able to distinguish between blanched and ‘no blanch’ juices. Correlation between both sensory evaluations and phytochemical concentration, and physico-

chemical analysis revealed that juices with higher phytochemical concentration were more liked by and more acceptable to the consumer. This study shows that the addition of a blanching step in blueberry juice processing may retain higher phytochemical levels in the juice and the juices were still well-liked by the consumer.

8. Overall discussion and future work

8.1. Introduction

The overall aim of this research was to develop a processing model which can be applied by the industry as a tool to predict blueberry juice composition. Phytochemical composition in the juice continues to change throughout processing and storage, but the precise nature of the changes, their underlying causes, and the impact on final juice composition were poorly understood. There is a huge demand from consumers for high value products that may be beneficial for human health. Therefore, it is a challenge to produce 'enriched blueberry juice' in New Zealand with a consistent compositional claim. Such a product would be not only beneficial for local consumers, it would also open up more opportunities for export to high-value health-conscious markets like Japan. There is likely to be economic value in delivering more consistent, high phytochemical juice, rather than relying on generic consumer willingness to pay a premium for something that just seems 'healthy'.

8.2. The findings

This study has quantified phytochemical losses at each unit operation throughout a laboratory scale blueberry juicing process. The losses were classified as either from oxidative damage i.e. enzymatic (Figure 4-2) or from non-enzymatic (Figure 3-2) degradation. In this study both of these degradative processes were shown to increase with temperature. However, at high temperatures, the PPO is inactivated (Figure 4-6); at which point only non-enzymatic degradation occurs (Figure 3-3). Other losses also occur during separation of juice and pomace (Chapter 5).

Patterns of anthocyanin and CGA loss in the pilot plant trial confirm the patterns observed at laboratory scale (Table 6-1 and Table 6-2) but results at the different scales were different for procyanidin B2 (Table 6-3).

Blanching of the berries helped in retaining almost all of the phytochemicals. The effect was significant for optimising anthocyanin and CGA retention with approximately 96 and 100% recovery from berries blanched at 90 °C. Blanching helped by inactivating PPO, thus eliminating enzymatic degradation of anthocyanin and CGA. Blanching also helped by softening berry skin and liberating more anthocyanin from the matrix, shown as a reduction in loss in the pomace (Van Buggenhout et al., 2009). This result is consistent with other authors who found that blanching increases anthocyanin recovery when non-blanched and blanched juices were compared in the same experiment (Lee et al., 2002; Rossi et al., 2003). Although not part of this work it should be noted that blueberry cultivars also differ significantly in overall concentrations and in relative proportions of different phytochemicals (Birt, 2011) which makes it possible to select particular cultivars for particular uses, if (for example) a high proportion of glucosylated or acylated anthocyanins were found to be beneficial for some health endpoint.

For procyanidin B2, a huge loss was observed during thawing and blanching. There is insufficient evidence from this work to derive a mechanism for this observation. However, it is likely that losses are due to thermal degradation or alternatively to extraction. The proposed latter cause is based on the work of Pinelo et al. (2006) who demonstrated that procyanidin B2 can bind to fiber and this, in a juicing operation, would result in a partitioning of procyanidin B2 into the pomace stream.

The current study indicated that increasing the blanching temperature may increase the yield of procyanidin B2 in the juice. However, blanching of berries in small lab-scale batches (Figure 5-1) showed reduction of procyanidin B2 when blanched at higher temperatures (80 and 90 °C).

Mincing and depectinisation led to significant losses of anthocyanins in 'no blanch' berries. However, after blanching, these operations led to very little loss of anthocyanins. Mincing and depectinisation were also found to result in minimal CGA losses. Interestingly both unit operations led to the restoration of the original procyanidin B2 concentration in blanched berries, particularly at the higher temperature tested (90 °C). Again, we can see that it is very important to deactivate PPO and thus minimise chemical losses, and to treat with pectinase enzymes which minimise physical losses of anthocyanin and CGA during separation processing by solubilising and breaking down pectin. Procyanidin B2 again showed a different behaviour, as losses were not recovered by pectinase enzyme treatment in small batches (Figure 5-3). The restoration of the procyanidin B2 after blanching and depectinisation observed in pilot plant trial is completely different when blueberry was prepared in small batches. These phenomena could occur if procyanidin B2 was either never degraded or it is degraded but the total concentration is maintained by the breakdown of longer chain procyanidins to form the two-unit procyanidin B2.

Unlike anthocyanin and CGA, procyanidin B2 behaviour during thermal treatment (Figure 3-2) and enzymatic degradation (Figure 4-2) was complex and data collected in this study were insufficient to be used in model development.

Pasteurisation is essential for the delivery of a food safe product and, in common with data from other researchers, had minimal impact on phytochemical concentration for any class of phytochemicals (Skrede et al., 2000; Lee et al., 2002; Brownmiller et al., 2008; Birt, 2011).

Despite the problems associated with modelling procyanidin B2, the results from thermal degradation aspects of this study can be used by manufacturers to 'tailor' the juice to produce high procyanidin B2 juice. By utilising high-heat blanching and longer holding time at high temperature a manufacturer can produce a juice with a higher procyanidin B2 content. The CGA content of the juice will be maintained but the high temperature processing will result in a lower anthocyanin concentration. This anthocyanin trade-off may be advantageous if higher procyanidin B2 were thought to be useful for some particular health endpoint.

Although the primary focus of this thesis was on optimising phytochemical concentration throughout juice processing the work included a preliminary study on the impact of phytochemical concentration on the sensory profile of the juice in recognition of the literature that suggested high concentrations of antioxidants can be associated with decreased consumer acceptability. The sensory profiling conducted here produced no data to suggest that a blueberry juice with high phytochemical levels will be unacceptable. Although statistically the differences between 70 and 90 °C blanched juices were not large, it looks as though 90 °C consistently contained the highest concentration of anthocyanin and in sensory tests this juice was indistinguishable from the juice blanched at 70 °C. Despite the evident risks of high temperatures degrading phytochemicals, this work has made it clear that blanching at

90 °C for 3 min coupled with enzyme treatment at 50 °C for 2 h is the best way to deliver a high phytochemical juice with a high consumer appeal. Detailed experimentation is still required on the best way to achieve these conditions at an industrial scale since blanching is dependent on the geometry and means of effecting heating throughout the blueberry process stream.

8.3. Industrial application and challenges

This study aimed to address problems faced by blueberry juice producers in New Zealand especially problems regarding low and inconsistent recovery of phytochemicals in the final juice. The problem with the current blueberry juice production method is that it is characterised by a long thawing time (albeit conducted at low temperatures) and a lack of blanching. Development of these processing models will definitely allow development of industrial protocols to deliver juices of higher and more consistent phytochemical concentrations. It also allows the industry to test the impact on phytochemical composition of proposed changes to a process without expensive experimentation.

Blanching is not a common practice by New Zealand's blueberry juice producers. However, results from this study indicate that the blanching step is required to maximise phytochemical recovery in the final juice. Therefore, blanching should be considered as an important unit operation in blueberry juice production. However, one drawback of blanching is the leaching out of juice to the water or condensed steam during defrosting (Jeremiah, 1996). In this study, frozen berries were put in a retort pouch for blanching. Therefore, the leached material was completely retained after blanching. To use retort pouches in large scale production is not practical so the

use of a minimal quantity of steam is recommended and losses will need to be minimised by design.

For large scale production, special equipment needs to be built to overcome the leaching problem. Another challenge is to immediately cool the blanched berries after blanching to avoid thermal degradation and 'cooked' flavour of the juice. A continuous blancher comprising (for example) high pressure steam injected into fruit moving down an auger and ending with a cooling step after blanching is required. The idea here is to steam-blanch free-flow frozen berries at 90 °C for 3 min as they move down an auger, then cool them to 50 °C by the time they reach a vat for pectinase enzyme treatment for the next 2 h. Accurate descriptions of heat or oxygen flow during blanching and cooling on the continuous blancher and in the vat needs to be further investigated and developed. It is very important to keep the time short and the temperature low in the period before PPO is inactivated.

Since blanching is not a normal practice carried out by blueberry juice producers in New Zealand, addition of blanching steps will increase their operational cost through the capital cost of purchasing the blancher itself and the redesigning of the production line, and associated plant utilities, to incorporate the blancher. Expensive engineering would be required for heating and cooling rapidly at an industrial scale. However, from a scientific point of view, this is the best and the safest way to enhance phytochemical concentration and add value to the juice. It is important for blueberry juice producers to invest in blanching technology in their production to produce high value-added juice with a consistent compositional claim that may permit them to open up new market opportunities.

Besides blanching, pectinase enzyme treatment is also an important unit operation in blueberry juice processing. Pectinase helps in dissolving pectin gel thereby liberating more phytochemicals, especially anthocyanins, from the pomace into the juice. Although blueberry juice can be processed without the use of pectinase if it is not blanched, it is still recommended to reduce the risk of pectin gel formation during pasteurisation (Ximenita, personal communication).

Considering all the issues discussed above, a potential production line for blueberry juice processing to maximise phytochemical recovery in the final juice is shown in Figure 8-1.

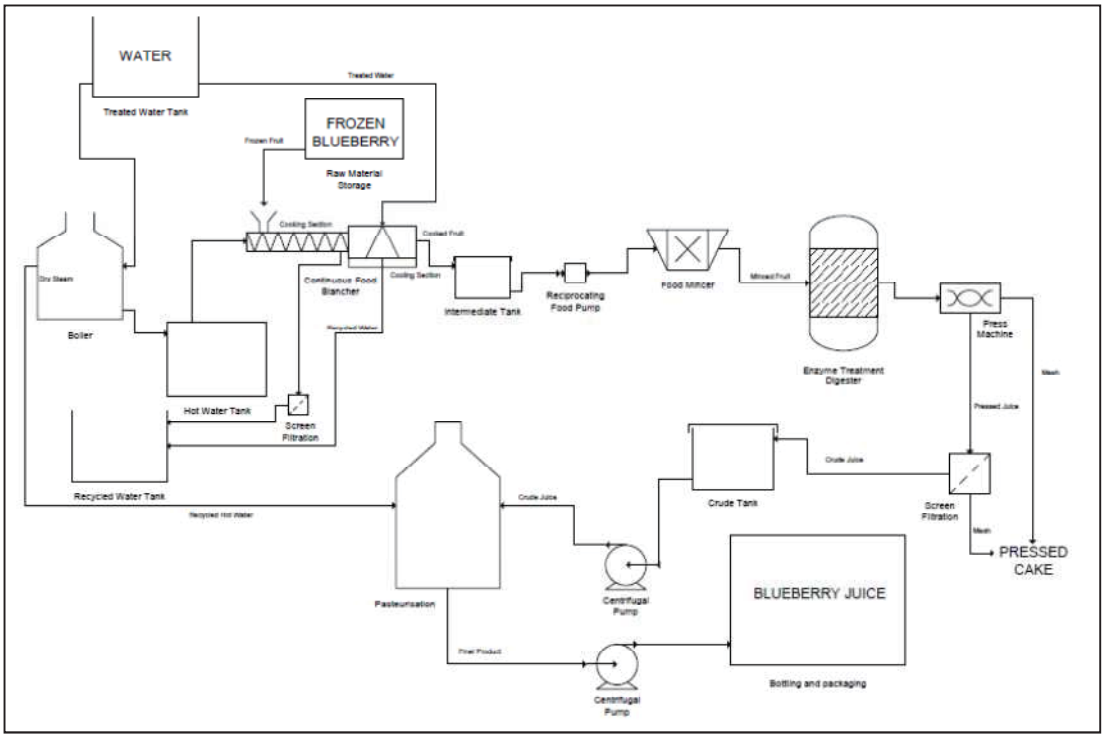


Figure 8-1: Proposed blueberry juice production line.

8.4. Future work

8.4.1. Mechanistic modelling

Processing models developed in this study were based on empirical models developed under specific experimental conditions. Such models strictly hold only for the conditions under which they were developed. The recovery model in particular was developed to summate the physical processes which liberate phytochemicals from the berry matrix during blanching, enzyme treatment and pressing. This model has a complex dependency on temperature and was essentially simplified into a temperature-dependent 'correction factor' rather than accounting separately for a number of different and overlapping processes. It appears that tissue disruption and pectin gelation induced during these processes influence the physical interaction between enzymes and phytochemicals more dramatically if the tissue is not blanched (in other words, under these conditions the model performed poorly); it would be good to investigate all of these underlying processes in a future mechanistic model. The final outcome would be a model suited to more generic application to industrial conditions based, for example, on detailed knowledge of the oxygen and temperature profiles of an industrial vessel and the ease of phytochemical and enzyme migration within the tissue.

8.4.2. Effects of temperature change on pectin gel formation

Although blueberry fruit has a relatively low pectin content, about 0.4% (Marlett & Cheung, 1997), this low pectin content has a high impact in blueberry juice processing especially during thermal processing. The use of temperature to soften the fruit skin in order to extract anthocyanins may trigger pectin gel formation in the

juice. Disruption or breakdown of cell walls releases pectin into the juice and thus increases juice viscosity. Therefore, the effects of temperature changes on the pectin gel behaviour in the fruit during juice processing and the mechanism involved should be evaluated.

Pectin gel formation is of most significance for mechanistic modelling: in the first place, the pectin gels will be *in situ* in the cell walls of ruptured tissue, therefore restricting diffusion in the cell wall space; thereafter solubilised pectin will be released into the juice where it is subject to breakdown by added pectinases. Released procyanidins may bind to and desorb from both soluble and insoluble fibre; and it is not at all clear what the dietary fate of phytochemicals bound to fibre would be; for example some authors (Manach et al., 2005; Scharlau et al., 2009) suggest they may be carried further into the gut and only be released once fibre is fermented in the large intestine, whereas other authors (Lafay & Gil-Izquierdo, 2008) suggest bound phytochemicals are more likely to pass right through the intestine and be eliminated.

8.4.3. Utilisation of blueberry pomace

Blueberry pomace is a significant by-product of the present juice processing industry, but still contains a high content of phytochemicals especially anthocyanins (Skrede et al., 2000) and total procyanidin (Howard et al., 2011). However, blueberry juice makers are finding a number of uses for the highly-coloured pomace. It can be sold as ground dried powder for use as a dietary supplement or to add into a hot drink; it has also been sold for ‘dog treats’. This nutritive by-product has potential applications in many fields. It can be used as a supplement in the

nutraceutical industry or as a colorant in the food industry. It also might be good idea to add finely-ground pomace to the final juice to alter the mouthfeel and increase anthocyanin and procyanidin concentration in the juice. Careful consideration of these alternatives would be required to evaluate the cost of producing novel products and the value recoverable from those products.

8.4.4. Effect of combining blueberry varieties for juice processing

This processing model and the validation of the model only focused on one variety of blueberries i.e. 'Dixi'. However, in many plantations, several varieties of blueberry are planted to extend the season as blueberry has a very short shelf life. A blend of blueberry varieties in juice processing should be conducted to further validate the developed processing model for wider industrial application. However, blueberry varieties are significantly different in terms of physical appearance and phytochemical composition. For example, 'Maru' and 'Dixi' are similar in size, but 'Burlington' is relatively small. Besides that, blueberry varieties may contain different concentrations of pectin. All these are very important parameters that need to be considered when assessing what blend of blueberry varieties to use.

8.4.5. Procyanidin extraction and analysis

Inconsistency of procyanidin B2 concentration in this study has not been fully explained. Inconsistency of procyanidin B2 concentration may be associated with heat treatment and its interaction with fiber. It seems that it would be beneficial to record concentrations of several oligomers of procyanidin; or perhaps total procyanidin; and monitor changes between pools of varying chain length. If data emerges that associate some particular procyanidin oligomers with particular health

benefits then detailed knowledge would be required to know how best to process berry tissue. HPLC equipped with a fluorescence detector can be used to measure individual procyanidins and enable this analysis.

8.5. Conclusion

From this study, a processing model has been developed as a tool to predict phytochemical concentration changes (especially anthocyanin and CGA) in blueberry juice throughout processing. These models will enable manufacturers to 'tailor' juice according to consumer demand and to produce juice high in anthocyanin, CGA, or procyanidin B2 by manipulating the processing conditions. However, the use of this model at industrial scale still requires further study. The understanding of oxygen diffusion into tissue / mash / juice is crucial and needs to be modelled before it can be used at the industrial scale. Oxygen will speed up PPO-driven deterioration. For example, degradation of phytochemicals is aggressive at 40 °C before blanching compared to thermal degradation which would be negligible at 40 °C after blanching. It is very important to work out how to achieve blanching quickly and remove the heat quickly in the industrial setting.

Thermal destruction of anthocyanin in blueberries has been fully investigated in this study and the knowledge can be extrapolated for use in production of other juices such as blackcurrant and other berries which have high anthocyanin content. High temperature thermal processing should be used to eliminate PPO but it needs to be done as quickly as possible and cooled to a safe temperature quickly to avoid thermal destruction. Extraction of anthocyanin into juice will be easier if the anthocyanin is located throughout the flesh as in cherry.

The experiments that have been done so far focus only on three phytochemicals of interest. From the insight of this experiment and the knowledge gained from this study, researchers could use this approach to investigate a series of other classes of phytochemicals in blueberry which may be important to health as long as their susceptibility to oxidative enzymes, thermal destruction and binding to fibre are known.

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Appendix

Appendix I

CONSUMER SENSORY EVALUATION FOR BLUEBERRY JUICE

All information provided will be used for academic purpose only.

Instruction:

i. You will be evaluated six (6) coded juice samples. You will be served one juice at a time.
ii. Before you taste each juice, please take a sip of water so that you remove any lingering tastes in your mouth.
iii. Evaluate the juice using the scale indicated below each attribute based on your degree of liking.
iv. Please tick at the box applicable to your liking and answer the question in the order they are asked.

Sample Code: _____

Please indicate by placing a mark in the box your liking on the following attributes of this juice

9-point hedonic scale	Overall liking	Aroma	Colour	Sourness	Sweetness	Thickness
Like extremely	☐	☐	☐	☐	☐	☐
Like Very Much	☐	☐	☐	☐	☐	☐
Like Moderately	☐	☐	☐	☐	☐	☐
Like Slightly	☐	☐	☐	☐	☐	☐
Neither Like nor Dislike	☐	☐	☐	☐	☐	☐
Dislike Slightly	☐	☐	☐	☐	☐	☐
Dislike Moderately	☐	☐	☐	☐	☐	☐
Dislike Very Much	☐	☐	☐	☐	☐	☐
Dislike Extremely	☐	☐	☐	☐	☐	☐

What, if anything, did you **LIKE** about this juice?

_____

_____

_____

What, if anything, did you **DISLIKE** about this juice?

_____

_____

_____

Once finished, please open the hatch to return the tray and you will be served with the next samples shortly.

GENERAL QUESTIONS

Please circle/complete the questions below:

Sex : Male / Female

Age :

How often do you drink pure fruit or vegetable juice?

- ☐ Never
- ☐ Daily
- ☐ Every 3 days
- ☐ Once weekly
- ☐ Once fortnightly
- ☐ Once Monthly

End of session. Open the hatch to return the tray.

THANK YOU FOR YOUR PARTICIPATION ☺

SENSORY EVALUATION OF BLUEBERRY JUICE – FREE CHOICE PROFILING METHOD

Session : Describing attributes **Name :**

Date : **Age :**

Gender :

Instruction

You have been presented with **six (6)** samples of blueberry juice. Take a sip from each individual juice presented in each sample in front of you, starting with the sample to your left and finishing with the sample to your right. Please cleanse your palate in between samples with water provided. Describe the difference you perceive between the juice by listing all the attributes/ characteristic you can think of in the box provided below.

SENSORY EVALUATION OF BLUEBERRY JUICE – FREE CHOICE PROFILING METHOD

Instruction:

i. You will be evaluated **six (6)** coded juice samples. You will be served one juice at a time.

ii. Before you taste each juice, please take a sip of water so that you remove any lingering tastes in your mouth.

iii. Please tick at the unstructured line scale applicable to the intensities of each attributes.

Name : **Age :** **Gender :**

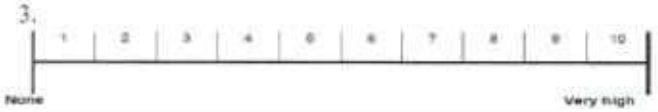
Please indicate by placing a mark on the intensities of the following attributes of this juice

Sample Code :

1.

2.

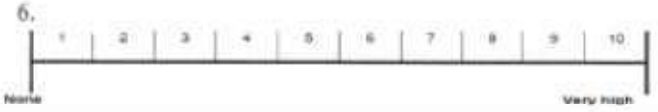
3.



4.

5.

6.



7.

8.

Appendix II

DRC 16

MASSEY UNIVERSITY
GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
TO DOCTORAL THESIS CONTAINING PUBLICATIONS

(To appear at the end of each thesis chapter/section/appendix submitted as an article/paper or collected as an appendix at the end of the thesis)

We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of Candidate: KHAIRUL KASIM

Name/Title of Principal Supervisor: JULIAN HEYES

Name of Published Research Output and full reference:

Kasim, F.K., Hurst, R.D., Carr, A.J. & Heyes, J.A. Effect of berry blanching on phytochemicals and physicochemical properties of blueberry juice. Proceeding of FVHH 2013 International Symposium on Quality Management of Fruits and Vegetable for Human Health. August 5-8, 2013, Golden Tulip Sovereign Hotel, Bangkok, Thailand. 151-157.

In which Chapter is the Published Work: Chapter 3 and 5

Please indicate either:

- The percentage of the Published Work that was contributed by the candidate: 80%

  and / or

- Describe the contribution that the candidate has made to the Published Work:

  Carried out all experimental work, data analysis, prepared figures; supervisors contributed to experimental design and manuscript completion.

Khairul Kasim — Digitally signed by Khairul Kasim DN: cn=Khairul Kasim, o=Massey University, ou, email=k5k_ari@yahoo.com, c=NZ Date: 2015.02.16 10:35:51 +08'00'

Candidate's Signature — 16/2/2015 — Date

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Principal Supervisor's signature — 4/3/2015 — Date

GRS Version 3– 16 September 2011