Changes in the Composition of Beetroot (*Beta vulgaris* L) During Their Growth Period.

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Abstract

Beetroot (*Beta vulgaris* L) is a root vegetable grown widely in the world. The beetroot contains high concentrations of red pigments predominantly betalains and have been reported to be beneficial for health. Beetroot have also been reported to assist with human performance as they are reported to contain high nitrate concentrations which assist with lowering oxygen consumption. Beetroot composition is affected by length of growth time, cultivar and environmental conditions. The aims of this project were to understand the effects of growth time on the accumulation of dry matter, sugars, total phenolic content, betacyanins and betaxanthins, nitrate and nitrite in beetroot.

Beetroot were harvested from two plantings, from a commercial grower, with 'Pablo' planted in September 2018 and three cultivars, 'Pablo', 'Monty' and 'Betty' planted in December 2018. Medium (size between 5.5 and 8.0 cm in diameter) and large (size >8.0 cm in diameter) 'Pablo' beetroot from the September planting were harvested weekly between 110 and 138 days of growth. Medium and large 'Pablo', 'Monty', and 'Betty' were harvested weekly from the December 2018 planting between 70 and 112 days of growth. Key quality and composition parameters of beetroot were monitored: size, weight, dry matter content, percentage of soluble solids, pH, titratable acidity, total phenolic content (TPC), sugar concentration, betacyanin and betaxanthin concentrations, nitrate and nitrite concentrations.

The results indicated that the dry matter of beetroot did not change significantly on most harvest dates for the three cultivars grown between day 70 and day 112. The TPC in juice gradually decreased by 42 to 30 % from day 70 to day 98 for the medium beetroot of three cultivars. Sucrose was the only soluble sugar identified in the beetroot juice samples. The sucrose concentration of the juice extracted from the three beetroot cultivars varied between 62.68 and 99.80 g/L over the 70 to 112 day growth period. For medium beetroot of all three cultivars, the betacyanin concentration in the juice fluctuated between 680 and 1544 mg/L while the betaxanthin concentration in juice gradually increased from 431 to 484 mg/L on day 70 to 565 to 763 mg/L on day 105. Overall, the nitrate content in 'Pablo' beetroot gradually decreased from 1656 to 618 mg/L for medium and from 2878 to 1002 mg/L for large beetroot between day 91 and day 138. The effect of covering beetroot was investigated but results obtained were inconclusive. After a 21-day postharvest storage in the dark at 4 ± 1°C the beetroot composition did not change.

When comparing ‘Pablo’, ‘Monty’ and ‘Betty’ grown between day 70 and day 112, the recommended cultivar and harvest time for obtaining high-nitrate beetroot juice was from large ‘Pablo’ grown for between 84 and 98 days. ‘Monty’ was the largest and heaviest beetroot cultivar, had the lowest titratable acidity and the highest dry matter content. The maximum total phenolic content was from medium ‘Monty’ beetroot grown between 70 and 84 days. The recommended cultivar and harvest time for obtaining high-betalain juice was medium ‘Monty’ harvested between 91 and 105 days of growth. ‘Betty’ was the smallest in the three cultivars, medium ‘Betty’ had higher mean nitrate content (1755 mg/L) than the other two medium cultivars, however, still lower than that from the large ‘Pablo’ beetroot (2195 mg/L).

In conclusion, changes in the composition for ‘Pablo’ beetroot from two plantings, ‘Monty’ and ‘Betty’ beetroot from one planting was monitored. After 21 days of postharvest dark storage (4 ± 1°C), the beetroot composition was generally stable. For a further study, the analyse of nitrate and amino acid content in beetroot taproot and leaves during their growth period are recommended to better understand the nitrogen cycle in beetroot. A high-performance-liquid-chromatography technique is recommended to identify and quantify the relative compounds participating in betalain biosynthesis.

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List of Abbreviations

ADI	Acceptable daily intake
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
ANOVA	Analysis of variance
ASP-AT/AAT	Aspartate aminotransferase
AS	Asparagine synthetase
ATP	Adenosine triphosphate
$C_6H_8O_7$	Citric acid
DM	Dry matter
ESI-MS	Electrospray mass spectrometry
Fe-GOGAT	Ferredoxin-dependent glutamate synthase
GAE	Gallic acid equivalent
GAD	Glutamate decarboxylase
GDH	Glutamate dehydrogenase
GOGAT	Glutamate synthase
GS	Glutamine synthetase
HPLC	High-performance liquid chromatography
KHP	Potassium hydrogen phthalate
MS	Mass spectrometry
Na_2CO_3	Sodium carbonate
NAD(P)H	Nicotinamide adenine dinucleotide phosphate
Na_2HPO_4	Disodium hydrogen phosphate
NaOH	Sodium hydroxide

NMR	Nuclear magnetic resonance
NO	Nitric oxide
NR	Nitrate reductase
NIR	Nitrite reductase
NOS	Nitric oxide synthase
ORAC	Oxygen radical absorbance capacity
% soluble solids	Percentage of soluble solids
P_i	inorganic phosphate
PP_i	inorganic pyrophosphate
RID	Refractive index detector
SEM	Standard error of mean
TA	Titratable acidity
TPC	Total phenolic content
UV-Vis	Ultraviolet visible
WHO	World Health Organization

Chapter 1 Introduction

Beetroot or red beet (*Beta vulgaris* L.*)* are part of *Chenopodiaceae* family which also includes spinach and pigweed (Kale, 2018*)*. Beetroot forms a large bulbous root that is normally harvested and is the main part of the plant eaten. Beetroot consumption has been reported to provide health benefits, including lowering blood pressure, and improving recovery time after exercise (Kale, 2018; Wylie et al., 2013). Beetroot has also been used as a natural food colorant (E162) as it contains high concentrations of red pigments (European Food Safety Authority, 2015). Beetroot are eaten raw and cooked, and common commercially available beetroot products in New Zealand include beetroot juice, pickled beetroot, and vacuumed packed baby beetroot. More recently beetroot has been used as a sports drink due to the high-nitrate content (Rienks, Vanderwoude, Maas, Blea, & Subudhi, 2015; Wylie et al., 2013).

Beetroot are well known for their high nitrate content in the whole plant, leaf and root (Santos et al., 2017; Mohamed, 2017). When consumed by humans nitrate in beetroot is converted to the bioactive compound nitric oxide (NO) which participates in numerous bioreactions in the human body, including inhibition of mitochondrial respiration which can lower oxygen consumption during exercise and improve recovery time (Brown, 1995; Lansley et al., 2011).

Beetroot also contains betalains, which include the red purple pigments, betacyanins, and the yellow pigments, betaxanthins (Cai, Sun, & Corke, 2005). The betalains in beetroot are known to be strong antioxidants, and they are the main source of the colorant named 'beetroot red' (E162) (Kale, 2018).

Beetroot are planted widely around the world in mild climates (Kale, 2018). Beetroot are generally planted in spring and harvested in summer (Kale, 2018; Michalik & Grzebelus, 1995). Not all beetroot cultivars are red. Like other root vegetables such as the carrot, beetroot can also be pink, orange, or white (Heber, 2017). Likewise, beetroot are not always round (spherical in shape), the cultivar 'Cylindra' red beetroot has a long-cylinder shape which is unusual compared to other cultivars (Heber, 2017).

Beetroot can normally be harvested after 40 to 80 days of growth depending on the cultivar and growing conditions (Albert, 2019). The nitrate and betalain content in beetroot varies with the length of the growth period and beetroot cultivar (Barba-Espin et al., 2018; Michalik & Grzebelus, 1995). Wruss et al. (2015) found the mean nitrate concentration in juice extracted from seven beetroot cultivars was 1970 mg/L with a large coefficient of variation (70%). Łukaszewska and Gawęda (2014) found the betalain content of beetroot also varied with cultivar and growth time. Hence, the beetroot cultivar and a minimum growth period are essential components for obtaining high-nitrate or high-betalain beetroot for commercial production.

The aim of this project was to determine the effect of growth period and postharvest storage time on dry matter, sugars, total phenolic content, betacyanins and betaxanthins, nitrate and nitrite in different beetroot cultivars. The objectives include:

- Determining how the levels of dry matter, total phenolic content, sugars, betacyanins and betaxanthins, nitrate and nitrite change with the different growth periods for medium and large 'Pablo' beetroot over two growing seasons and medium and large 'Monty' and 'Betty' beetroot over one growing season.

- Investigating the changes in other quality parameters including size, weight, percentage of soluble solids, pH and titratable acidity for medium and large 'Pablo' beetroot over two growing seasons and medium and large 'Monty' and 'Betty' over one growing season.

- Investigating the change in percentage of soluble solids, pH, titratable acidity, dry matter content, total phenolic content, sugar concentration, betacyanin and betaxanthin concentrations, nitrate and nitrite concentrations for large 'Pablo' beetroot during postharvest storage at 4 ± 1^{o}C.

Chapter 2 Literature Review

Beetroot have recently become more popular as its health benefits have been advertised more to the public. Beetroot consumption can reduce the risk of heart disease and lower blood pressure (Ware, 2017). Recent research has also shown that beetroot juice consumption can lower the oxygen consumption during either walking or intense exercise, and therefore humans recover faster from the exercise (Lansley et al., 2011; Rienks et al., 2015).

Beetroot (*Beta vulgaris* L) are generally round in shape, and most cultivars but not all have a rich purple colour and a unique "earthy" smell (Azeredo, 2009). In New Zealand, different beetroot (red beet) *Beta vulgaris* cultivars are available, including 'Pablo' the main cultivar plus 'Monty' and 'Betty'. Beetroot normally are planted in the spring and harvested after 40 to 80 days (Albert, 2019; Kale, 2018; Michalik & Grzebelus, 1995).

This chapter introduces five important compounds in beetroot: nitrate, nitrite, betalains, phenolics and sugars. Beetroot juice can be used in a sport drink as it contains high-concentrations of nitrate which can be converted to nitric oxide (NO) in the human body; NO helps humans to achieve a rapid recovery after exercise (Lansley et al., 2011; Wruss et al., 2015). The nitrogen-cycle and factors affecting the nitrogen-cycle in fresh beetroot are important and will be introduced in this chapter. Beetroot also contains antioxidants such as betalains and phenolics (Łukaszewska & Gawęda, 2014)

In common with other vegetables, beetroot contains proteins, carbohydrates, and sugars. However, they also contain compounds such as nitrate and nitrite (Bednar, Kies, & Carlson, 1991; Corleto, Singh, Jayaprakasha, & Patil, 2018), betalains (Kujala, Vienola, Klika, Loponen, & Pihlaja, 2002; Nizioł-Łukaszewska & Gawęda, 2014) and phenolics (Bazaria & Kumar, 2016; Wruss et al., 2015), which are not normally abundant in other vegetables. These compounds have been reported to be beneficial for human health, hence the increased interest in beetroot.

2.1 Nitrogen Metabolism and Nitrate Accumulation in Plants

Nitrate is important for a plant's growth. Nitrate is water-soluble and can be easily absorbed from soil and is the main starting material to produce amino acids and proteins (Chen et al., 2004). In nature, most nitrogen exits as nitrogen gas (N_2), which is converted to ammonia (NH_3) by nitrogen fixing bacteria in the soil (Marschner & Marschner, 2012; Neil et al., 2015; Taiz & Zeiger, 2010). Ammonia is then converted to ammonium ions (NH_4^+) with water in the soil, and NH_4^+ is then converted to nitrate ions (NO_3^-) by nitrifying-bacteria in the soil (Neil et al., 2015; Taiz & Zeiger, 2010). Both NH_4^+ and NO_3^- are actively absorbed by plants and provide the main inorganic nitrogen source for plant growth (Marschner & Marschner, 2012; Michalik & Grzebelus, 1995; Neil et al., 2015; Taiz, Zeiger, Møller, & Murphy, 2018).

Most nitrate absorbed from the soil is firstly stored in vacuoles in plant cells until it is needed and then moved to the cytosol where the nitrate is transformed to nitrite (NO_2^-) (Chen et al., 2004; Marschner & Marschner, 2012). When soil nitrate concentration is low, plants absorb the nitrate from the soil at a slow rate (Marschner & Marschner, 2012). When nitrate concentration in the outside environment is high (>0.5×10^{-3} mol/L in soil), plants increase the rate of absorption from the soil (Bosdriesz et al., 2018; Marschner & Marschner, 2012). The atmospheric temperature is also important, when the temperature is above 30°C, the nitrate absorption rate is decreased (Michalik & Grzebelus, 1995).

When the nitrate concentration in the plants is low, root cells can transform most nitrate to amino acids (Taiz & Zeiger, 2010). However, if the nitrate concentration in the plants is high, the nitrate is transported from the roots to the shoots (leaves) by xylem transpiration, and shoots can convert the nitrate to amino acids (Figure 2.1) (Chen et al., 2004; Marschner & Marschner, 2012; Neil et al., 2015; Taiz et al., 2018). The first reaction which transforms nitrate to nitrite is a reduction process as shown in Equation 2.1 (Oaks, 2011). The reduction efficiency (the ability to convert nitrate to nitrite) of shoots is normally higher than roots (Chen et al., 2004). This is because photosynthesis is normally concentrated in green leaves, providing more energy and carbon resource for nitrate reduction (Chen et al., 2004). Nitrate assimilation in plants primarily occurs in two phases: first nitrate assimilation takes place which is followed by ammonium assimilation (Taiz et al., 2018).

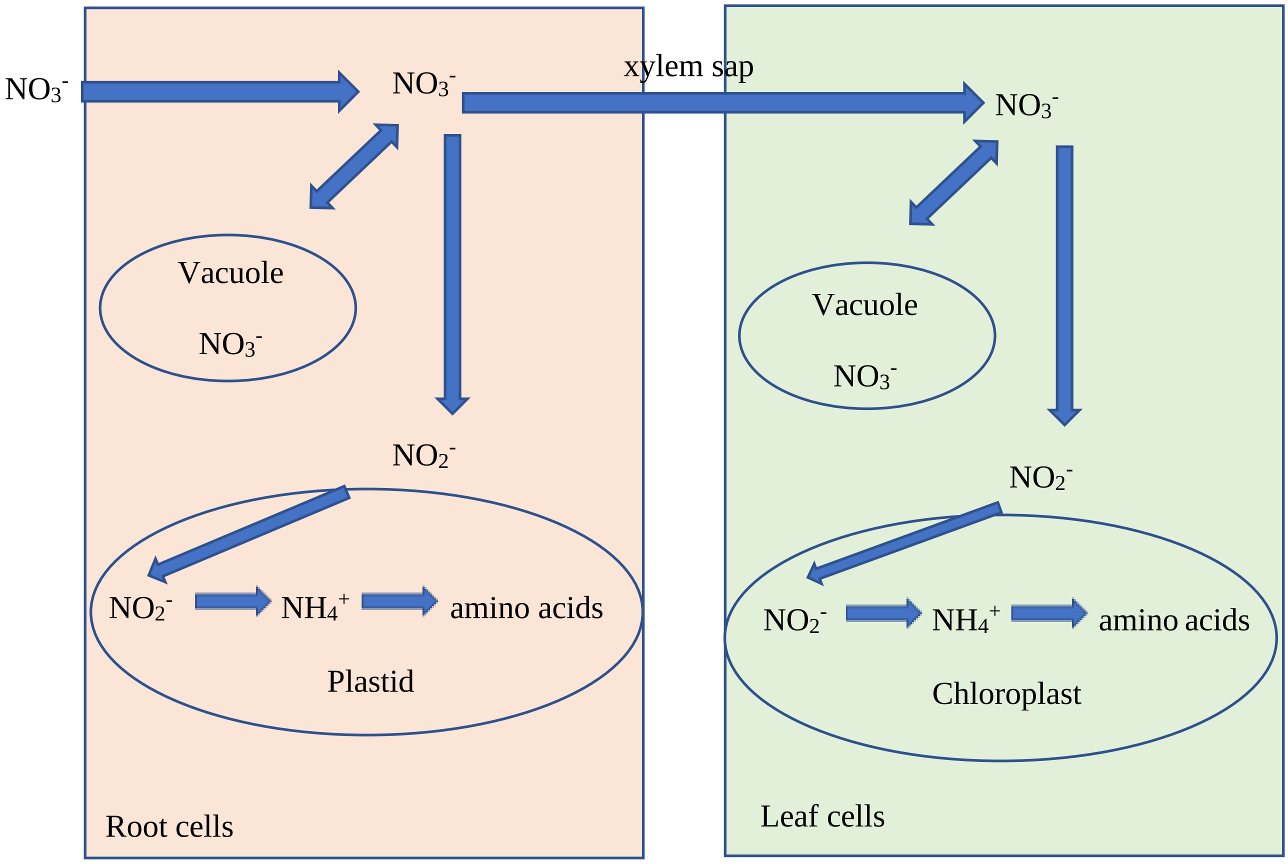


Figure 2.1 Nitrogen assimilations in plants. Figure is adapted from Marschner and Marschner (2012).

In summary, nitrate in soil is absorbed by root cells, some is stored in the vacuoles, some is converted to nitrite, ammonia and amino acids. As mentioned earlier, the extra nitrate can be transported to the leaves by xylem sap (transpiration) but some nitrate from the root can be stored in leaf cell vacuoles and some nitrate is utilised to build up amino acids in leaves (Marschner & Marschner, 2012).

2.1.1 Nitrate Assimilation in Plants

For nitrate assimilation, the first reaction occurs in the cytosol, where nitrate is converted to nitrite by the enzyme nitrate reductase (NR) under acidic conditions (Equation 2.1) (Chen et al., 2004; Mathews, 2013; Oaks, 2011; Solomonson & Barber, 1990; Taiz & Zeiger, 2010). This reduction reaction requires the donation of an electron by nicotinamide adenine dinucleotide phosphate (NADPH) (Solomonson & Barber, 1990). Higher light intensity and higher sugar content enhances the activation of NR, while darkness, magnesium, and glutamine inhibit the NR activity (Kaiser, Weiner, & Huber, 1999; Krapp et al., 1998; Lea, Leydecker, Quilleré, Meyer, & Lillo, 2006; Marschner & Marschner, 2012; Taiz & Zeiger, 2010).

Higher carbon dioxide concentration in the atmosphere inhibits nitrate assimilation (Bloom, Burger, Asensio, & Cousins, 2010). This is because the carbon cycle is more active than the nitrogen cycle, hence, the carbon cycle is more competitive and will use the reductant (NADPH) faster than the nitrogen-cycle (Bloom et al., 2010; Marschner & Marschner, 2012). Temperature has no significant effect on NR activity during the daytime, however, higher temperatures at night cause a faster NR enzyme loss, and leads to a higher nitrate content in plants (Nicholas, Harper, & Hageman, 1976)

In the second reaction of nitrate assimilation, nitrite from the previous nitrate reduction reaction (Equation 2.1) is converted to ammonium cations (NH_4^+) by the enzyme nitrite reductase (NIR) under acidic conditions in either the chloroplast or the plastid of the plant cells (Equation 2.2) (Taiz & Zeiger, 2010). Reduced ferredoxin (from photosynthesis) donates elections for this reduction reaction (Solomonson & Barber, 1990). Factors such as higher light intensity (provides more reduced ferredoxin from shoots) and higher soil nitrate concentration (more nitrite reduced from nitrate) induced NIR mRNA expression (Oaks, 2011; Taiz & Zeiger, 2010).

$$NO_3^- + NADH/NADPH \longrightarrow NO_2^- + NAD^+ /NADP^+$$

Equation 2.1

$$NO_2^- + \text{ferredoxin (reduced)} \xrightarrow{\text{NIR}} NH_4^+ + \text{ferredoxin (oxidised)}$$

Equation 2.2

2.1.2 Ammonium Assimilation in Plants

After the nitrate assimilation, nitrate absorbed from soil is converted to ammonium and ready for the next stage; ammonium assimilation, where two main enzymes are involved: glutamine synthetase (GS) and glutamine oxoglutarate aminotransferase (GOGAT) (Equation 2.3 and Equation 2.4) (Mathews, 2013; Oaks, 2011; Taiz & Zeiger, 2010). The GS reaction is catalysed by enzyme GS which converts glutamate and ammonium to glutamine as shown "GS" in Equation 2.3 (Marschner & Marschner, 2012; Oaks, 2011; Taiz & Zeiger, 2010). Then in the GOGAT reaction, glutamine is converted to glutamate catalysed by either enzyme ferredoxin-dependent glutamate synthase (Fe-GOGAT) or NAD(P)H-dependent glutamate synthase (NADH-GOGAT) as shown in Equation 2.4 (Marschner & Marschner, 2012; Oaks, 2011; Taiz & Zeiger, 2010). These GS and GOGAT pathways are favoured by plants when energy, light, and sugar content are high in the surrounding environment (Taiz & Zeiger, 2010). Therefore, more glutamine and glutamate are produced in plants when nutrients are abundant (Taiz & Zeiger, 2010). There are some other enzymes in ammonium assimilation, such as asparagine synthetase (AS) (Equation 2.5) (Oaks, 2011; Taiz & Zeiger, 2010), however, the reactions catalysed by GS and GOGAT predominate when nutrients are abundant (Miflin & Lea, 1976; Taiz & Zeiger, 2010).

$$\text{ammonium} + \text{glutamate} + \text{ATP} \xrightarrow{\text{GS}} \text{glutamine} + \text{ADP} + P_i$$

Equation 2.3

$$\text{glutamine} + \text{2-ogoglutarate} + \text{NAD(P)H} + H^+ \xrightarrow{\text{Fd-GOGAT}} \text{glutamate} + \text{NAD(P)}$$

OR

$$\text{glutamine} + \text{2-ogoglutarate} + \text{ferredoxin (re)} \xrightarrow{\text{NADH-GOGAT}} \text{glutamate} + \text{ferredoxin (ox)}$$

Equation 2.4

$$\text{glutamine} + \text{aspartate} + \text{ATP} \xrightarrow{\text{AS}} \text{asparagine} + \text{glutamate} + \text{AMP} + PP_i$$

Equation 2.5

For Equations 2.3 to 2.5, GS indicates enzyme glutamine synthetase, GOGAT indicates enzyme glutamine oxoglutarate aminotransferase, AS indicates enzyme asparagine synthetase. ATP indicates adenosine triphosphate, ADP indicates adenosine diphosphate, AMP indicates adenosine monophosphate, Pi indicates inorganic phosphate, PPi indicates inorganic pyrophosphate, ferredoxin (re) indicates reduced form of ferredoxin, ferredoxin (ox) indicates oxidised form of ferredoxin. The ATP, NADPH, glutamate, glutamine, 2-ogoglutarate, aspartate and ferredoxin (reduced) are from plant photosynthesis and respiration (Marschner & Marschner, 2012; Oaks, 2011; Taiz & Zeiger, 2010).

When energy and nutrients are not abundant plants utilise an alternative pathway (Taiz & Zeiger, 2010). Aspartate and glutamine are converted to asparagine and glutamate by the enzyme asparagine synthetase (AS) (Equation 2.5) (Oaks, 2011; Taiz & Zeiger, 2010). In this alternative pathway, more asparagine is generated, which is more stable than glutamate and glutamine, therefore, it is suitable when nutrients are not abundant and nitrogen concentration in soil is low (Goel & Singh, 2015; Marschner & Marschner, 2012; Taiz & Zeiger, 2010). The nitrate absorbed from the soil by plants is transformed into amino acids; plants can produce several amino acids depending on the environmental conditions (Taiz & Zeiger, 2010).

Nitrate accumulates in plants when the nitrate absorption rate from the soil is higher than the nitrate reduction rate in the plants, in contrast, nitrate content decreases when the nitrate absorption rate is lower than the nitrate reduction rate (Anjana Shahid & Iqbal, 2007; Cárdenas-Navarro, Adamowicz, & Robin, 1999; Maynard, Barker, Minotti, & Peck, 1976).

2.2 The Effects of Nitrate and Nitrite in Humans

2.2.1 The Nitrate-Nitrite-Nitric Oxide Cycle and Benefits of Nitric Oxide in Humans

Nitrate from food can be reduced to nitrite in the mouth by bacteria (Lundberg, Weitzberg, & Gladwin, 2008). The nitrate passes though mouth, stomach, intestine, blood vessels and tissues and finally absorbed by salivary glands (Figure 2.2) (Lundberg et al., 2008; Qu et al., 2016). Nitric oxide can be made in stomach, blood vessels and tissues, and can regulate some bioreactions such as mitochondrial respiration (Lundberg et al., 2008; Qu et al., 2016).

Several pathways can convert nitrite to nitric oxide in the human body. When oxygen and L-arginine are abundant, these two compounds can convert nitrite to nitric oxide (Lundberg et al., 2008). However, when oxygen is limited, for example, during physical exercise nitrite and deoxyhaemoglobin react to produce nitric oxide and methaemoglobin (Equation 2.6) (Lundberg, Feelisch, Björne, Jansson, & Weitzberg, 2006).

$$NO_2^- + \text{deoxyhaemoglobin } (Fe^{2+}) + H^+ \rightarrow NO + \text{methaemoglobin } (Fe^{3+}) + OH^-$$

Equation 2.6

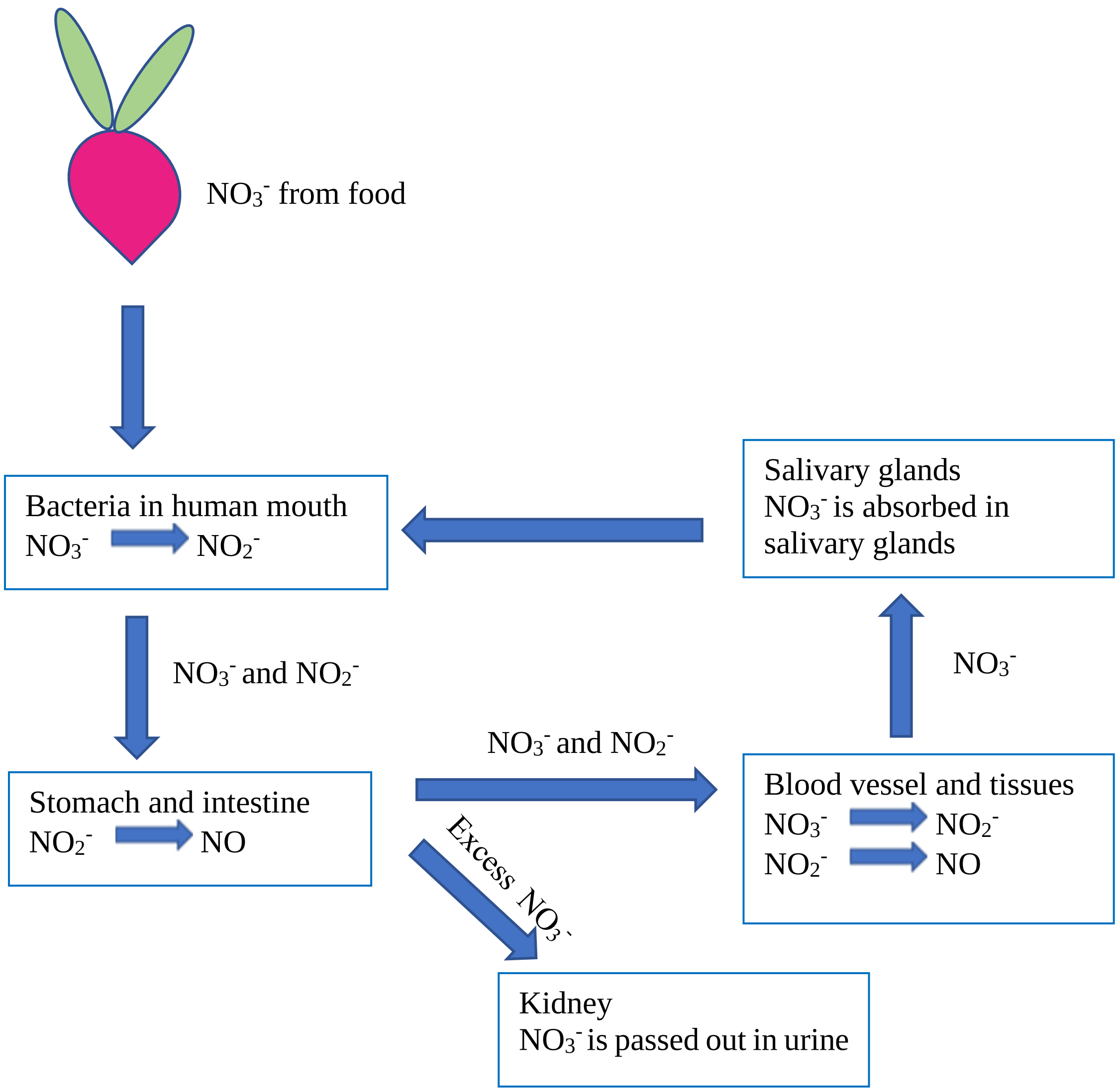


Figure 2.2 The nitrate-nitrite-nitric oxide pathway in humans. Figure is adapted from Lundberg et al. (2008); Qu et al. (2016).

Nitric oxide has many functions in the human body. Nitric oxide can reduce the oxygen consumption rate in mitochondria which means that there is less oxygen needed by the cells (Lundberg et al., 2008). Hord, Tang, and Bryan (2009) stated that nitrate is an essential nutrient and only bioactive and beneficial when it is reduced to nitric oxide, and nitric oxide keeps the human blood vascular system functions normally. During exercise consumption of high-nitrate drink such as beetroot juice, would result in the nitrate being converted to nitrite and subsequently nitric oxide, which lowers oxygen consumption and improves the recovery time (Qu et al., 2016). Nitric oxide has also been reported to assist with inhibition of some bacteria and stimulating the stomach to secrete more protective mucus (Lundberg et al., 2006).

2.2.2 Effects of Excess Nitrate on the Environment and Human Health

From an environmental perspective, if nitrate content in fertilizers is in excess of what plants can absorb, then due to its high water solubility the excess nitrate may leach from the soil with rainfall and finally flow into the lakes and rivers and cause water pollution (Chen et al., 2004). The high-nitrate water may cause eutrophication in lakes due to excess nutrients (such as nitrate) enabling bacteria to reproduce at a greater rate, and this in turn reduces the oxygen in the lakes for other creatures to survive (Mason, 2002). Hence eutrophication destroys the ecosystem balance in lakes and may leave the water unsafe for human consumption (Mason, 2002).

Although polluted high-nitrate water is suspected to cause some specific diseases such as methemoglobinemia of infants, an investigation report stated that most reports linking the high-nitrate water and infant methemoglobinemia utilized privately-owned wells rather than the public water supply system (Fewtrell, 2004). The reports linking the high-nitrate contained food or drink and methemoglobinemia ignored some other factors, for example, the bacteria in the water container which may cause the methemoglobinemia rather than the water itself (Bryan & Loscalzo, 2017; Fewtrell, 2004). Another study found that infants consumed 50 to 100 mg nitrate per day in their diet or water did not lead to methemoglobinemia (Cornblath & Hartmann, 1948). The previous hypothesis that the nitrate contained water or food may cause methemoglobinemia needs further research (Bryan & Loscalzo, 2017; McKnight, Duncan, Leifert, & Golden, 1999).

The acceptable daily intake (ADI) for nitrate and nitrite is 0 to 3.7 and 0 to 0.07 mg/kg body weight per day, respectively (Thomson, Nokes, & Cressey, 2007). Nitrite salts such as sodium nitrite is commonly used as an additive in cured meat to maintain flavours, colours, and increase shelf life (Hord et al., 2009; Thomson et al., 2007). Some consumers are concerned that the nitrite addition in food and drinks may cause cancers (World Health Organization International Agency For Research on Cancer, 2010).

According to a report by World Health Organization International Agency For Research on Cancer (2010), nitrate in food and drinks is unrelated to cancer while nitrite in food may be related with cancers in some random studies, however, more evidence is needed to make a conclusion if nitrite in food is safe or not. Interestingly, one study found that the previous conclusion which nitrite in red meat may cause cancer were incorrect in their conclusions as there are many other compounds in red meat (Cross et al., 2011). Cross et al. (2011) reported that it was probably the heterocyclic amines in the red meat which can increase the gastric cancer risks. Ma, Hu, Feng, and Wang (2018) explained why some investigations have related nitrite and cancer as some of them only focus on the nitrite in meat and ignored other compounds such as N-nitrosamine which is the product of nitrite and amides.

2.3 Nitrate and Nitrite in Beetroot and Some Beetroot Products

Raw beetroot nitrate content has been reported to vary between 352 and 2422 mg/kg for four cultivars ('Boltardy', 'Bolivar', 'Kosak' and 'Rote Kugel') harvested 240 days after planting (Feller & Fink, 2004). The nitrate concentration varied between 565 and 4626 mg/L in juice extracted from seven beetroot cultivars ('Mona Lisa', 'Moronia', 'Redval', 'Agyptische Plattrunde', Robuschka', 'Forono', and 'Bolivar') harvested 120 days after planting, with the mean nitrate concentration 1970 ± 1395 mg/L (Wruss et al., 2015). As shown in the previous studies (Feller & Fink, 2004; Wruss et al., 2015), the nitrate content variation among different beetroot cultivars was large.

Comparing with juice freshly extracted from raw beetroot, the boiled beetroot and commercial canned beetroot have a lower nitrate concentration (Lee, Shallenberger, Downing, Stoewsand, & Peck, 1971; Vasconcellos, Conte-Junior, Silva, Pierucci, Paschoalin, & Alvares, 2016). Vasconcellos et al. (2016) found beetroot lost 86 %

nitrate after boiling for 40 minutes. This nitrate loss is likely due to the high water solubility of nitrate (Bednar, Kies, & Carlson, 1991). A study found that the commercial canned beetroot nitrate content (250 mg/kg) was lower by 38 % than that in the juice extracted from fresh beetroot (400 mg/kg) (Lee et al., 1971). As beetroot processing factories utilize running water to peel the beetroot, the nitrate may be lost during this peeling process (Bednar et al., 1991).

In New Zealand, a study found the mean nitrate content of boiled beetroot was 635 mg/kg (fresh weight basis), while the mean nitrate content of canned beetroot was 763 mg/kg (Thomson et al., 2007). Thomson et al. (2007) also reported the nitrite content of canned beetroot as less than 5 mg/kg.

2.4 Factors Affecting Nitrate Concentration in Beetroot

Nitrate and nitrite content in plants may be affected by NR activity (Michalik & Grzebelus, 1995; Taiz & Zeiger, 2010), the enzyme which converts nitrate to nitrite (Campbell, 1999; Taiz & Zeiger, 2010). Inactivation of NR leads to a higher nitrate content and a lower nitrite content in plants (Michalik & Grzebelus, 1995).

Several factors affect nitrate accumulation in beetroot including rainfall, temperature, harvest time, and fertilizer application (Feller & Fink, 2004; Michalik & Grzebelus, 1995; Nizioł-Łukaszewska & Gawęda, 2014). Grzebelus and Baranski (2001) found the nitrate content in beetroot grown in a year with 30 % more rainfall was 60 % lower than that grown in a year with normal rainfall. The high temperatures may inhibit the plants nitrate absorption from the soil (≥30ºC), hence resulting in a lower nitrate content in the beetroot (Michalik & Grzebelus, 1995). The nitrate content in beetroot increases with application of nitrogen fertilizer (Feller & Fink, 2004; Michalik & Grzebelus, 1995; Ugrinovic, 1999). For the effect of harvest time, Michalik and Grzebelus (1995) found the nitrate content in beetroot harvested after 140 days of growth (2666 mg/kg) was higher than that after 100 days of growth (2202 mg/kg). However, Michalik and Grzebelus (1995) explained this was probably due to the extreme drought over the beetroot growth period, which inhibited the NR activity and lead to the higher nitrate content in beetroot.

Other factors, such as darkness and magnesium (Mg^{2+}) level in the soil can inhibit NR activity, and therefore increase nitrate concentration in beetroot (mentioned in Section 2.1.1) (Taiz & Zeiger, 2010). In addition, beetroot cultivar is important, even though the beetroot were planted and harvested on the same date in the same field, the nitrate concentration in juice extracted from different beetroot cultivars varied (Wruss et al., 2015). Wruss et al. (2015) found the highest nitrate concentration in extracted beetroot juice was from cultivar ‘Mono Lisa’ (4626 mg/L) while the lowest nitrate concentration was from cultivar ‘Robuschka’ (565 mg/L).

2.5 Betacyanins and Betaxanthins

Betalains are nitrogen-containing water-soluble pigments which mainly exist in red beetroot, although they are also found in red dragon fruits and prickly pears (Azeredo, 2009; Cai et al., 2005; Choo, 2015; Kujala, Loponen, & Pihlaja, 2001; Ravichandran et al., 2013). Although the colours of betalains and anthocyanins (flavonoids) are similar, their chemical structures are different (McWilliams, 2017; Nollet, 2000; Tanaka, Sasaki, & Ohmiya, 2008). Betalains contain red-purple betacyanins (e.g.betanin and isobetanin) and orange-yellow betaxanthins (e.g.vulgaxanthine I) (Azeredo, 2009; Cai et al., 2005; Castellar, Obón, Alacid, & Fernández-López, 2003; Kujala, Loponen, Klika, & Pihlaja, 2000; Nizioł-Łukaszewska & Gawęda, 2014; Ravichandran et al., 2013). The structures of betanin, isobetanin and vulgaxanthine I are shown in Figures 2.3 and 2.4 (Kujala et al., 2000; Wendel et al., 2015).

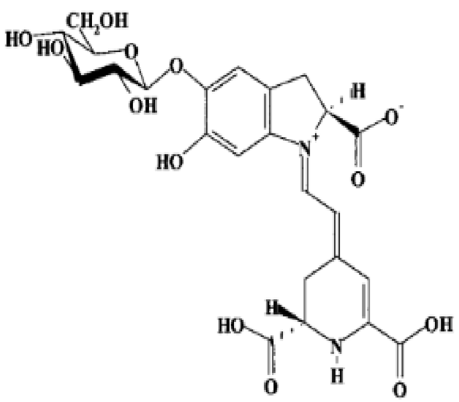


Figure 2.3 The chemical structure of betanin/isobetanin. Figure sourced from Kujala et al. (2000).

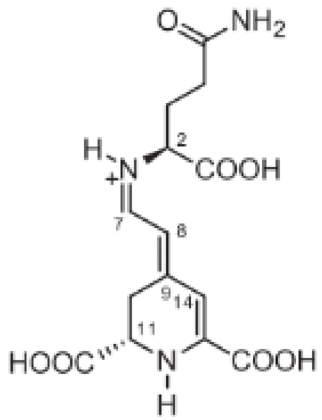


Figure 2.4 The chemical structure of vulgaxanthin I. Figure sourced from Wendel et al. (2015).

The betacyanins biosynthesis studies have agreed on the pathway shown in Figure 2.5 (Azeredo, 2009; Carle & Schweiggert, 2016; Hatlestad et al., 2012; Li, Meng, Zhu, & Li, 2019; Timoneda et al., 2018). As shown in Figure 2.5, the betacyanins in the plants are made with the betalamic acids and cyclo-DOPA-5-O-glycoside which both come from the amino acid tyrosine produced in plants (Cárdenas-Navarro, Adamowicz, & Robin, 1998; Carle & Schweiggert, 2016). It was found that the increase of kinetin which is a plant hormone and light intensity can increase the formation of both the betacyanins and betaxanthins in plants (Hussain, Sadiq, & Zia-Ul-Haq, 2018).

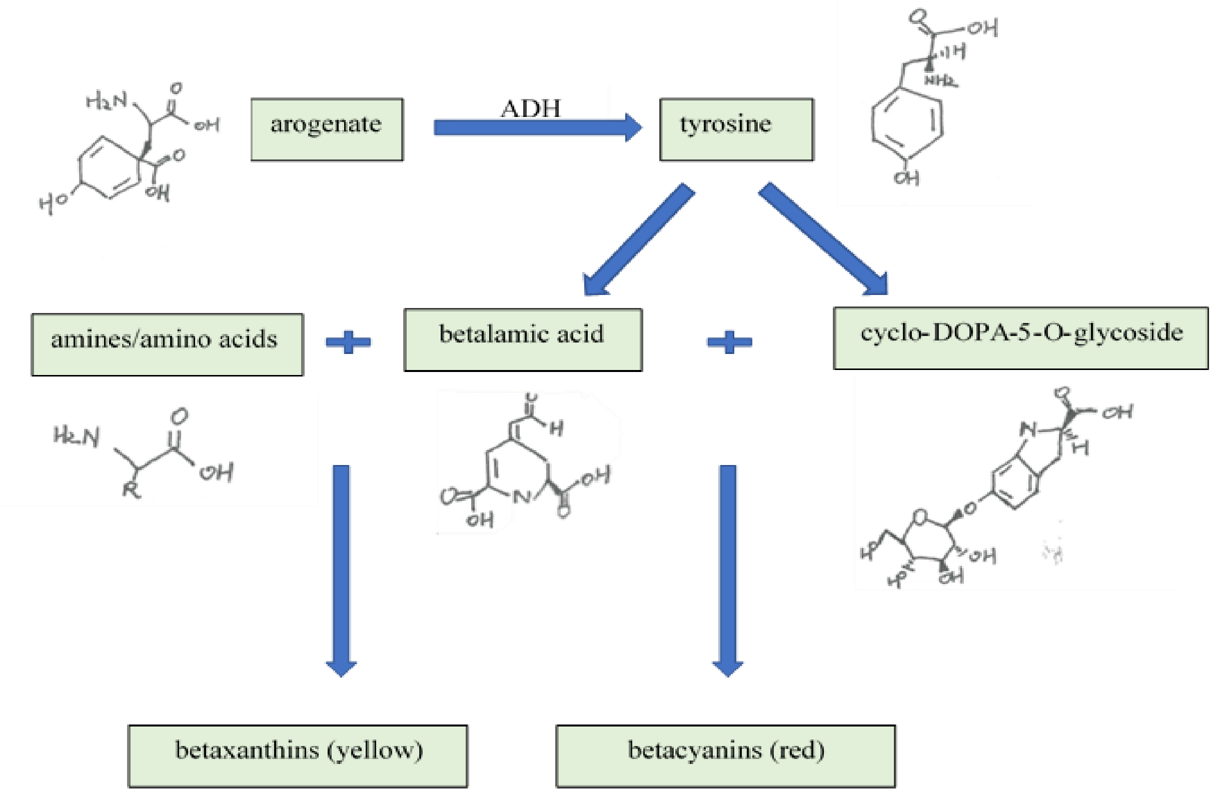


Figure 2.5 The biosynthesis of betacyanins and betaxantins of plants. Arogenate is an intermediate amino acid. ADH - arogenate dehydrogenase, an enzyme which converts arogenate to tyrosine. Tyrosine - an important amino acid in plants. Figure is adapted from Li et al. (2019); Timoneda et al. (2018).

Beetroot mainly contains red betanin (betacyanins) and yellow vulgaxanthine I (betaxanthins) (Figures 2.3 and 2.4) (Azeredo, 2009; Choo, 2015). Table 2.1 compares the colour, solubility, concentration (by spectrophotometer and HPLC) and stability between betacyanins and betaxanthins from previous studies (Bazaria & Kumar, 2016; Gasztonyi, Daood, Hájos, & Biacs, 2001; Herbach, Stintzing, & Carle, 2004; Kujala, Vienola, Klika, Loponen, & Pihlaja, 2002; Saper & Hornstein, 1979). Gasztonyi et al. (2001) found that the maximum betanin content is 0.5 g/kg found in *Beta vulgaris L* ‘Bonel’. Kujala et al. (2000) showed that betanin was highly concentrated on the outer layers of the beetroot.

Table 2.1 Colour, solubility, composition, concentration, stability of betacyanins and betaxanthins studies of beetroot.

	Cultivars	Betacyanins	Betaxanthins	References
Colour		Red	Yellow	
Solubility	-	water soluble	water soluble	Azeredo (2009)
Composition	‘Bonel’, ‘Nero’, ‘Favorit’, ‘Rubin’ and ‘Detroit’	betanin,isobetanindin, isobetanin, betanidin	vulgaxanthin I, vulgaxanthin II	Gasztonyi et al. (2001)
Concentration by Spectrophotometer	‘Rubra’	256.86 mg/L (538 nm)	148.42 mg/L (480 nm)	Bazaria and Kumar (2016)
Concentration by HPLC	‘Egyptische Platronde’, ‘Forono’, ‘Little Ball’ and ‘Rubia’	290 – 770 mg/kg	140 – 430 mg/kg	Kujala et al. (2002)
Stability at room temperature	‘Uniball’, ‘Slowbolt’, ‘Red’, ‘Bordo’, ‘Detroit Dark Red’, ‘Detroit Nero’ ‘Podzimniaja’, ‘Detroit Sluis’, ‘EarlyWonder’, ‘Little Ball’, ‘Choghundur’, ‘Lowa’, ‘Gladiator’, ‘Asmer Beethoven’, ‘Crveno’, ‘Polsko’, ‘Okragly Ciemnoczerwony’, ‘Spangsbjerg’ and ‘Rubidus’	more stable at 25°C, smaller pigment degradation rate constant	less stable at 25°C, larger pigment degradation rate constant	Saper and Hornstein (1979)
Heating stability	-	more stable after heating at 85°C for 8 hours	less stable after heating at 85°C for 8 hours	Herbach et al. (2004)

2.6 Factors affecting Betalain Content and Stability

Betalains are not abundant in nature and several factors influence the pigment content present in plants (Azeredo, 2009). The effects of growth time on beetroot betacyanins and betaxanthins were not consistent in previous studies (Michalik & Grzebelus, 1995; Shannon, 1970; Waston & Gabelman, 1982). For example, the study of Shannon (1970) found that betacyanin concentration in the extracted juice from either 'Detroit Dark Red' or 'Ruby Queen' beetroot decreased or increased between day 67 and 130 after planting. Similarly, the study of Waston and Gabelman (1983) found the betacyanin concentration in juice made from different cultivars of beetroot increased, decreased or remained constant during the harvesting time from day 55 to 97 after planting which depended on the cultivar.

The betaxanthin concentration in beetroot juice kept increasing from days 55 to 97 for 'Pacesetter', 'Redpack' and 'Detroit Dark Red' cultivars (Waston & Gabelman, 1982). Michalik and Grzebelus (1995) found there was no significant change in betaxanthins in beetroot juice from seven cultivars ('DZE 1 F1', 'DZE 2 F1', 'DZE3 F1', 'Czerwona K', 'Crosby', 'Okragly C' and 'Egipski') between day 100 and day 140 after planting. The difference between these two studies maybe due to the different harvest time, cultivars and beetroot growth environment (Stintzing, Herbach, Mosshammer, Kugler, & Carle, 2008).

Several factors such as pH, oxygen, water activity, light and temperature affect the stability of betalains in food (Azeredo, 2009; Carle & Schweiggert, 2016). Betalains are generally stable from pH 3 to 7, and are most stable at pH 5 (Azeredo, 2009; Wong & Siow, 2015; Woo, Ngou, Ngo, Soong, & Tang, 2011). Lower oxygen level in the atmosphere inhibits the degradation of betalains and therefore improves the stability of betalains (Huang & Elbe, 1987). Water activity also affects the stability of betalains, the betacyanin degradation rate constant increased from 5.21 to 83.55×10^{-3} per day when the water activity of beetroot power decreased from 0.32 to 0.75 (Cohen & Saguy, 1983). Exposure to light decreases the stability of betalains and hence accelerates degradation of betalains (Attoe & Elbe, 1981). Temperature also affects the stability of betalains, heating of small beetroot cubes (50 g) at 80ºC for 180 seconds resulted a 51 % reduction in betacyanins and a 33 % reduction in betaxanthins (Ravichandran et al., 2013).

2.7 Benefits of Betalains

Betalain pigments have several benefits in the food industry when betalains can be used as food additives to maintain the original food colour at low pH and they also have antimicrobial properties (Azeredo, 2009; Ravichandran et al., 2013). Fifteen microliters of the beetroot juice extracted from beetroot pomace (100 mg/ml) has been shown to be effective in inhibiting the growth of *Staphylococcus aureus* 266 and *Bacillus cereus* (Velićanski, Cvetković, Markov, Vulić, & Đilas, 2011). Betalains in fruits also provide antioxidant and anticancer potential with studies showing the phenolics and betalains present in prickly pear juice can lower cell viability of four cancer types (colon, prostate, herpatic and mammary cancer) (Azeredo, 2009; Chavez-Santoscoy, Gutierrez-Uribe, & Serna-Saldívar, 2009).

2.8 Phenolics in Fruits and Vegetables

Phenolic compounds are made up of a benzene ring with at least one alcohol group (-OH) (Bhattacharya, Sood, & Citovsky, 2010). Phenolics in beetroot primarily include gallic acid (21.8 mg/L), caffeic acid (4.82 mg/L) and syringic acid (1.85 mg/L) (Wruss et al., 2015). The structure of these three compounds are shown in Figure 2.6 (Phelps & Young, 1997). Phenolics are potent antioxidants which inhibit lipid oxidation (Shahidi & Ambigaipalan, 2015). Kujala et al. (2000) showed phenolics and the betacyanins were concentrated mostly in beetroot skin. Kujala et al (2000) also found after storage at -20°C for 9 months, the total phenolic content (TPC) of freeze-dried beetroot peels did not change significantly compared with the initial content.

For other tuberous vegetables, the distribution of phenolics was similar in potato, with the total phenolic content gradually decreased in order: from the skin, to outside section of potato and then to inside section of potato, with almost half amount of the total phenolic content was found in the skin of potato (Friedman, 1997).

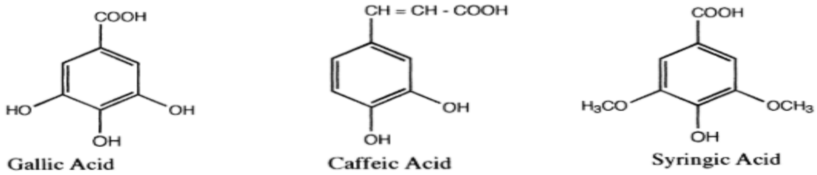


Figure 2.6 The structure of selected phenolic compound. Figure sourced from Phelps and Young (1997).

Considering the effect of growth period on the phenolic content, Reyes, Miller, and Cisneros-Zevallos (2004) reported that TPC in purple and red potatoes decreased by 19 to 29 % from day 88 to 154 after planting which varied with potato cultivars. After this period (day 88 to 109) the TPC in the potatoes remained relatively constant until day 156 after planting. For a beetroot study, Barba-Espin et al. (2018) found the TPC in juice extracted from 'Monty' and 'Belushi' cultivars decreased from approximately 500 GAE mg/kg to 1000 GAE mg/kg (GAE is gallic acid equivalent) respectively between three to eighteen weeks after planting.

2.9 Sugars in Beetroot

Sugars are critical for a plant's growth as they are the main energy source (Taiz & Zeiger, 2010). The three sugars found in beetroot are sucrose, glucose and fructose (Wruss et al., 2015). The average total sugar concentration of beetroot juice extracted from seven beetroot cultivars was 77.5 g/L ('Mona Lisa', 'Moronia', 'Redval', 'Agyptische Plattrunde', Robuschka', 'Forono', and 'Bolivar'), with sucrose being present at the highest concentration (73.5 g/L), and fructose being the lowest (1.51 g/L) (Wruss et al., 2015).

The effect of growth period on the beetroot sugar content varied with cultivars (Łukaszewska & Gawęda, 2014; Michalik & Grzebelus, 1995; Waston & Gabelman, 1982). Waston and Gabelman (1982) measured the percentage of soluble solids as an indication of the sucrose concentration in beetroot juice ('Detroit Dark Red', 'Firechief ', 'Garnet', 'Nero', 'Gladiator', 'Redpack' and 'Ruby Queen'), they found from day 68 to day 130 the soluble solids content increased, decreased or stayed constant during the growth time which depended on the cultivar. However, a more recent study showed that the sugars increased continuously during growth (from 42 to 77 days), reaching the maximum concentration at around 77 days after planting and decreased afterwards (from 77 to 105 days) (Nizioł-Łukaszewska & Gawęda, 2014).

2.10 The Effects of Growth Period on Beetroot Composition

Beetroot prefer warm growth conditions and are normally planted in spring (Kale, 2018). Depending on the different growth times, some beetroot quality parameters and composition may be different. Table 2.2 summarises published research on the effect of growth period on some beetroot quality parameters and composition. From Table 2.2, it appears that dry matter (DM) may decrease in beetroot after 100 days of growth. The soluble sugars in beetroot may increase initially, remain constant and then decrease with longer growth time (Montes-Lora, Rodríguez-Pulido, Cejudo-Bastante, & Heredia, 2018). The red pigment betacyanin content may increase, decrease or fluctuate while the betaxanthin content appears to gradually increase (Łukaszewska & Gawęda, 2014). The nitrate content in the beetroot may increase, decrease or remain the same between day 70 and day 140, which was strongly affected by the growing conditions, climate and environment (Michalik & Grzebelus, 1995; Tapio, Liisa, & Raili, 1992; Ugrinovic, 1999).

From Table 2.2, it appears that there are many beetroot cultivars (25 cultivars in total) in the summarised beetroot studies and most of the studies utilized more than one cultivar for their composition research (Feller & Fink, 2004; Łukaszewska & Gawęda, 2014; Shannon, 1970). The beetroot composition also varied with cultivar. For example, the betacyanin content varied between 86.8 mg/100g (in 'Redpack') and 135.5 mg/100 g (in 'Garnet') after 81 days' growth (Waston & Gabelman, 1982). The nitrate content varied between 1747 mg/kg (in 'Okragly') and 2874 mg/kg (in 'DZE 3 F1') for beetroot grown after 100 days' growth (Michalik & Grzebelus, 1995).

Table 2.2 Summary table on effects of growth period on beetroot composition and some key quality parameters.

Samples	Composition	Harvest details	Key results	References
Beetroot 'Detroit Dark Red', 'Ruby Queen', 'Mono King Explorer' and 'Firechief' **Beetroot were planted in New York, USA**	Betacyanins, percentage of soluble solids and hardness of beetroot	Three summer plantings were harvested from day 67 to day 130 after planting in autumn in the same year in the first year	• First year: The mean betacyanin concentration of extracted beetroot juice decreased significantly while percentage of soluble solids increased significantly from day 67 to day 130 after planting. Hardness of fresh beetroot increased significantly from day 67 to day 130 after planting.	Shannon (1970)
		One summer planting was harvested from day 77 to day 121 after planting in the autumn in the second year	• Second year: Similar results with the previous year, mean betacyanin concentrations of extracted beetroot juice decreased significantly while percentage of soluble solids increased significantly from day 77 to day 121 after planting. Hardness of fresh beetroot increased from day 77 to day 121.	
		One summer planting was harvested from day 68 to day 111 after planting in the autumn in the third year	• Third year: Mean betacyanin concentrations of extracted beetroot juice increased significantly while percentage of soluble solids also increased significantly from day 68 to day 111. Hardness of fresh beetroot increased significantly from day 68 to day 111.	

Beetroot **'Detroit Dark Red',** **'Firechief',** **'Gamet',** **'Nero',** **'Gladiator',** **'Redpack' and** **'Ruby Queen'** **Beetroot were planted in Wisconsin, USA**	Betacyanins, betaxanthins and percentage of soluble solids	One summer planting was harvested in two batches: early harvest (day 81 after planting) and late harvest (day 124 after planting) in the autumn in the first year Two summer plantings were harvested from day 55 to day 97 after planting in the autumn in the second year	• First year: The percentage of soluble solids in the juice from beetroot grown for 81 days were lower than that grown for 124 days. The mean betacyanin concentration in the juice from each beetroot cultivar may increase or decrease between day 81 and day 124 after planting. The mean betaxanthin concentration and percentage of soluble solids in the juice from each beetroot cultivar were higher at 124 days compared to 81 days • Second year: The mean betacyanin concentration in the juice may increase or decrease between 55 and 97 days of growing which depends on the cultivars. The mean betaxanthin concentration in the juice was higher in the beetroot at 97 days compared to 55 days.	Waston and Gabelman (1982)
Beetroot 'Little Ball SG' **Beetroot were planted in Jokioinen, Finland (60°49'N;23°28'E)**	Nitrate	Beetroot were planted in spring and harvested after 75, 85, 89, 97 and 102 days of growth	The nitrate content gradually decreased with longer growth time from day 75 to day 102.	Tapio et al. (1992)

Beetroot 'DZE 1 F1', 'DZE 2 F1', 'DZE3 F1', 'Czerwona K', 'Crosby', 'Okragly C', 'Egipski' **Beetroot were planted in Poland**	Dry matter, total soluble sugars, nitrate, betacyanins and betaxanthins	Beetroot were planted in spring and harvested after day 100,120 and 140 after planting	The mean dry matter (fresh weight) from seven cultivars of beetroot decreased significantly from 100 to 140 days of growth. There was no significant difference between the mean total soluble sugars content from seven cultivars of beetroot between day 100 and day 140. The mean nitrate concentration of the juice from seven cultivars of beetroot were significantly higher at day 140 compared with that at day 100 after planting. The mean betacyanin concentration in beetroot juice at day 140 was significantly lower than that at day 100 while there was no significantly difference between the mean betaxanthin concentration at day 100 and day 140.	Michalik and Grzebelus (1995)
Beetroot 'Bicores' and 'Pablo' **Beetroot were planted in Slovenia (46°09'N;14°35'E)**	Nitrate, betacyanins, betaxanthins, dry matter, oxalic acid, and ascorbic acid	The beetroot were planted in summer and harvested at day 118 and day 145 after planting	The nitrate concentration of two beetroot cultiavars at day 145 were similar with that at day 118 (not significant) while the dry matter, betacyanins, betaxanthins, oxalic acid and ascorbic acid concentrations at day 145 were all significantly lower than those at day 118.	Ugrinovic (1999)
Beetroot 'Bolivar', 'Boltardy' and 'Rote Kugel'	Nitrate and percentage of soluble solids	Three plantings in autumn (Aug, Sep and Oct plantings) and all of them harvested at 9	The average of percentage of soluble solids of beetroot juice from each cultivar varied between 10.1°Brix and 12.7°Brix. The average nitrate concentration in the juice from each cultivar varied between352 and 559 mg/kg for Aug planting, varied between 575 and 1350 mg/kg for the Sep	Feller and Fink (2004)

Beetroot were planted in Germany		months after planting (around 270 days)	planting and varied between 1949 and 2499 mg/kg for Oct planting, the nitrate concentration was higher in the beetroot juice from later planting (Oct planting).	
Beetroot ‘Boro’,‘Czerwona Kula’, ‘Nochowski’ and ‘Regulski Cylinder’ **Beetroot were planted in Poland**	Weight, diameter, dry matter, sugars, betacyanins and betaxanthins	Beetroot were planted in summer in the first and second year. Beetroot were harvested from day 42 to day 77 in the first year Beetroot were harvested from day 42 to day 105 after planting in the second year	• First year: Dry matter of the four cultivars of beetroot did not change significantly between day 42 and day 77 after planting. Sugar content of four cultivars of beetroot increased significantly at the end of harvest period (day 77) compared with that at day 42. Betacyanin and betaxanthin concentrations of beetroot juice were significantly higher at day 77 after planting when compared with that at day 42. • Second year: Dry matter of the four cultivars of beetroot increased significantly at day 105 after planting compared with that at day 42. Sugar content of four cultivars of beetroot increased significantly at the end of harvest period (day 105) compared with that at day 42. Betacyanin concentration of juice from four cultivars of beetroot were fluctuant from day 42 to day 105 after planting. Betaxanthin concentrations of juice from three cultivars besides ‘Regulski Cylinder’ increased significantly at day 91 compared to those at day 42.	Łukaszewska and Gawęda (2014)

Beetroot 'Detroit Dark Red' **Beetroot were planted in Japan**	Betacyanins and betaxanthins	Beetroot were planted in spring and harvested after 72, 81 and 106 days after planting in the same year	Betacyanin and betaxanthin results were similar, the concentrations of these two pigments of 'Detroit Dark Red' beetroot increased significantly at day 81 and then decreased significantly at day 106. The maximum betacyanin and betaxanthin concentration in beetroot juice were both found at day 81 after planting.	Watari, Ikeura, Tsuge, and Motoki (2017)

2.11 Methodology for the Determination of Nitrate and Nitrite Concentrations

The three main methods used to determine nitrate and nitrite concentrations in food or biological samples are spectrophotometric (e.g. ultraviolet–visible spectrophotometer), electrochemical (e.g. ion-selective electrodes) and chromatographic (e.g. high-performance liquid chromatography (HPLC)) (Moorcroft, Davis, & Compton, 2001). The spectrophotometric method is convenient and is widely available, however, chromatographic methods are more sensitive and accurate (Chou, Chung, And, & Hwang, 2003; Moorcroft et al., 2001). Table 2.3 summarises HPLC and other techniques which are used for the determination of nitrate and nitrite concentrations in food and other biological samples.

2.12 Methodology for the Determination of Betalain Concentration

Betalains are mainly determined by four methods: spectrophotometric, mass spectrometry (MS), HPLC and nuclear magnetic resonance (NMR) (Azeredo, 2009; Cai et al., 2005; Ravichandran et al., 2013; Stintzing, Conrad, Klaiber, Beifuss, & Carle, 2004; Stintzing, Schieber, & Carle, 2002). Spectrophotometric methods are easy, fast, quantitative and give total betalain content but are less accurate compared to HPLC, which can give the individual betalain content (Clifford et al., 2017; Stintzing, Schieber, & Carle, 2003). The use of NMR for betalains analysis is a new technique and needs further development compared with other techniques (Azeredo, 2009; Stintzing et al., 2004). Table 2.4 summarises some spectrophotometric and HPLC methods for the determination of betalain content in food.

2.13 Beetroot and Beetroot Juice Storage

Although beetroot are normally planted in spring, harvested and topped beetroot can be stored at 0°C for a maximum of 180 days (El-Ramady, Domokos-Szabolcsy, Abdalla, Taha, & Fári, 2015). Corleto, Singh, Jayaprakasha, and Patil (2018) tested the effect of storage on nitrate and nitrite concentrations in extracted beetroot juice at four different temperatures (-80°C, -20°C, 4°C and 25°C) for 32 days. Corleto et al. (2018) found at 25°C, from day 0 to day 1, the nitrate content in the beetroot decreased significantly from 4965.3 to 2167.3 µg/ml while the nitrite content increased from 0 to 904.12 µg/ml. At -80°C and -20°C, almost no change was found both for the nitrate and nitrite concentrations from day 0 to day 32. In general, nitrate and nitrite concentrations in beetroot juice stored at -80°C and -20°C were more stable than those stored at 4°C or 25°C for 32 days (Corleto et al., 2018). Betacyanin content in freeze-dried beetroot powder stored at -25°C for 9 months decreased from 38.7 to 30.7 mg/g dry weight, however, there was no significant change in total phenolic content during the nine-month storage (Kujala et al., 2000).

Table 2.3 HPLC and other equipment summary table for quantitative analysis of nitrate and nitrite in food and biological samples.

Samples	HPLC detectors	Columns	Mobile phase	Wavelengths (nm)	Flow rates (ml/min)	Injection volumes (µL)	References
Urine, saliva, plasma, gastric juice and milk	UV-visible detector	Cadmium and cation-exchange column	5% NH_4Cl, pH 9.0	645	0.32	30	Green et al. (1982)
Meat and vegetable	-	C18	0.005 M tertrabutylammonium hydrogen sulfate	214	3.0	10	Wootton, Kok, and Buckle (1985)
Vegetable juice	Photodiode array detector	C18, 5µm, 250 × 4.6 mm i.d.	20% methanol with 0.01 M octyammonium orthophosphate	230	0.5	20	Cheng and Tsang (1998)
Culture medium and biological samples	Fluorescence detector	C18 column, 5µm, 150 × 4.6 mm.i.d. with reversed phase C18 column, 40 µm, 50 × 4.6 mm.i.d.	50% methanol with 0.015 M sodium phosphate buffer, pH 7.5	375 to 415	1.3	15	Li, Meininger, and Wu (2000)
Human plasma	UV-Vis and an extra electrochemical detector for nitrite	10 µl, 150 × 3 mm, with 30 × 3 mm guard column	0.02 M $NaclO_4$, pH 3.9	212	0.6	*	Jedličková, Paluch, and Alušík (2002)
12 vegetables	UV-visible detector	C18, 5µm, 250 × 4.6 mm i.d.	30% methanol with 0.01 M octyammonium orthophosphate, pH 7.0	213	0.8	10	Chou et al. (2003)
Chicken and red meat	UV-Vis detector	Hermophenyl hexyl column, 3 µm, 150 × 4. 6 mm.i. d.	25% acetonitrile, pH 4.0	205	0.7	*	Abdulkair, Elzupir, and Alamer (2018)
Human saliva	Diode array detector	Phosphatidylcholine column, 10 µl, 4.6 × 150 mm.i.d.	NaCl solution (1-30 mM)	210	0.5	10	Małgorzata et al. (2019)

Samples	Other equipment	Equipment advantages	Equipment disadvantages	References
17 vegetables	Electron paramagnetic resonance spectrometry	Sensitive to specific ions, fast measurement	Expensive, heavy and complicated	Yordanov, Novakova, and Lubenova (2001)
11 vegetable powder	UV-Vis spectrophotometer at 410 nm	Easy measurement, not expensive	Time consuming	Parviz, Hassan, Saeed, Yousef, and Kazem (2012)
15 commercial fruit juice	UV-Vis spectrophotometer at 595.5 nm	Fast reaction time (100s), easy measurement, good detection limit (0.2 µg/ml)	Some ions (e.g. Fe^{2+} and Cu^{2+}) may affect the accuracy	Sobhanardakani, Farmany, Abbasi, Cheraghi, and Hushmandfar (2013)

Table 2.4 Summary table for quantitative analysis of betalains in plants.

Samples	Technique	HPLC columns	HPLC mobile phase and flow rates	References
Beetroot	HPLC	C18 column, 6µm, 240 × 4.6 mm.i.d.	1:5 methanol/0.01M $NaHPO_4$ at isocratic conditions, best separation result at pH 4.0	Gasztonyi et al. (2001)
Yellow beetroot and cactus pears	Electrospray mass spectrometry (ESI-MS) and HPLC	C18 column, 5µm, 250 × 3 mm.i.d. with C18 guard column, 4.0 × 3.0 mm.i.d.	Solvent A:0.2% trifluoroacetic acid and 10% HCOOH Solvent B:100% acetonitrile and 10% HCOOH at 1 ml/min	Stintzing et al. (2002)
Beetroot	HPLC	C18 column, 5µm, 250 × 4.0 mm.i.d. with a precolumn	Solvent A: acetonitrile Solvent B: formic acid and water (0.4:99.6) at 1 ml/min	Kujala et al. (2002)
Opuntia	Spectrophotometric and HPLC	C18 column, 5µm, 250 × 4.6 mm.i.d.	Solvent A:175 mM acetic acid with water Solvent B:175 mM acetic acid with acetonitrile at 1 ml/min	Castellar et al. (2003)
Dragon fruit	Nuclear Magnetic Resonance (NMR) and HPLC-NMR (^{1}H and ^{13}C of betalains can be both observed in NMR, normally only ^{1}H can be obtained in betalains compound in NMR)	C18 column, 5µm, 250 ×4.6 mm.i.d.	Solvent A:0.1% trifluoroacetic acid Solvent B: 60% acetonitrile at 0.8 ml/min	Stintzing et al. (2004)
Amaranth, wool flowers, gomphrena, and Iresine	ESI-MS and HPLC	C18 column, 5µm, 250 × 4.0 mm.i.d, C18 column, 5µm, 250 × 9.4 mm.i.d with C18 guard column, 5 µm, 4.0 × 4.0 mm.i.d.	betacyanins: Solvent A:1.5% H_3PO_4 with water; Solvent B: 1.5% H_3PO_4, 20% acetic acid, 25% acetonitrile with water at 1 ml/min betaxanthins: Solvent A:55 mM NaH_2PO_4 and 2.5 mM trimethylamine with pH 4.5; Solvent B:40% acetonitrile	Cai et al. (2005)
Prickly pears and beetroot	Spectrophotometer, HPLC and electrospray mass spectrometry (ESI-MS)	C18 column, 3.5µm, 150 × 4.6 mm.i.d.	Solvent A: water Solvent B: methanol at 1 ml/min	Castellanos-Santiago and Yahia (2008)
Beetroot	Spectrophotometer and HPLC	C18 column, 5µm, 250 × 4.0 mm.i.d.	Mobile phase: 0.2% formic acid with water and acetonitrile at 1 ml/min	Ravichandran et al. (2013)
Green/purple Joyweed leaves	HPLC with diode array detector and ESI-MS	C18 column, 5µm, 150 × 4.6 mm.i.d.	Solvent A: water Solvent B: acetonitrile at 0.5 ml/min	Deladino et al. (2017)

2.14 Conclusions

Nitrate as found in plants arrives there by active absorption from the soil and is converted to nitrite and then amino acids and proteins which is determined by climate, growing conditions and plant species. Nitrate aids the human by being converted to bioactive nitric oxide which can reduce the oxygen consumption and improve recovery time after exercise and keep the blood system functioning normally when humans do not exercise. Betalains are water-soluble nitrogen-containing pigments and provide red purple or orange yellow colour in plants. Beetroot are high in nitrate and betalains and have high nutritional values due to these compounds. Beetroot composition has been reported to depend on the growth time, climate and cultivar. Analysis of nitrate can be carried out by spectrophotometer and HPLC. Analysis of betalains can be carried out qualitatively and quantitatively by spectrophotometer, mass spectrometry (MS), HPLC and nuclear magnetic resonance (NMR).

Chapter 3 Materials and Methods

3.1 Experimental Design

Two plantings of beetroot sown in September and December 2018 were harvested weekly from January and February 2019 respectively. Half of the field of early sown (September planting) were covered under fleece. Medium and large beetroot were harvested from three random locations within the paddock to give three replicates, for each size, for each cultivar, on each sampling date. The experimental design was shown in Figure 3.1. In addition, one extra batch of large 'Pablo' beetroot grown for 91 days from the December planting were harvested and stored for three weeks to determine if the storage effected the beetroot composition.

Whole beetroot were tested for size, weight and dry matter content (DM) while beetroot juice extracted from the whole beetroot were tested for percentage of soluble solids (% soluble solids), pH, titratable acidity (TA), total phenolic content (TPC), sugar concentrations, betacyanin and betaxanthin concentrations, nitrate and nitrite concentrations.

The effect of harvest time, beetroot size, whether the beetroot was covered or not covered under fleece during the growth period, and the effects of cultivars on composition of the beetroot or juice were assessed.

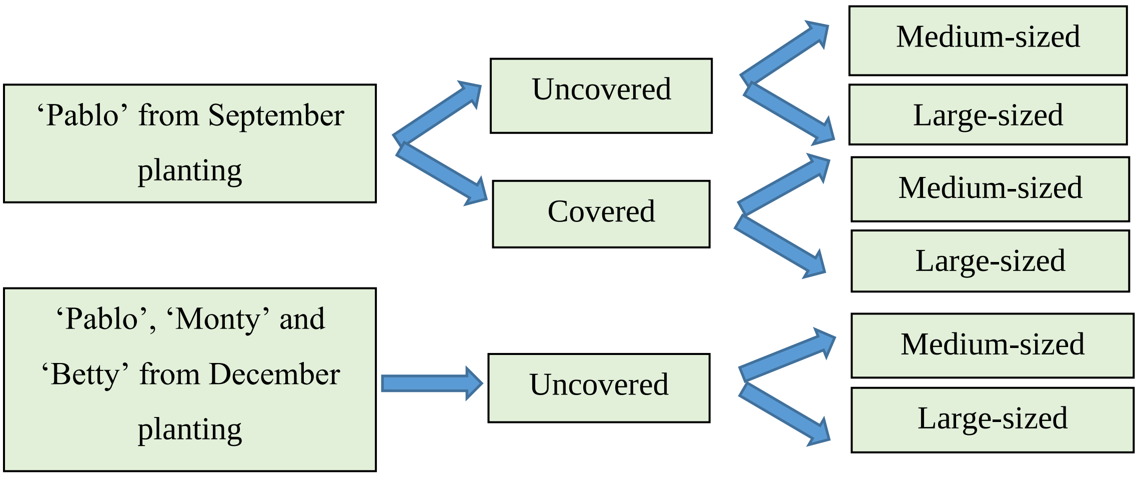


Figure 3.1 Diagram of the experimental design.

3.2 Materials and Equipment

3.2.1 Beetroot

Beetroot (*Beta vulgaris*) cultivars 'Pablo', 'Monty RZ F1'('Monty'), 'Betty RZ F1' ('Betty') were grown and harvested by Lovett Family Farms (Ashburton, South New Zealand). Beetroot were harvested from two planting dates, with one cultivar 'Pablo' planted on 19th September 2018 and three cultivars, 'Pablo', 'Monty' and 'Betty' planted on 16th December 2018. Harvesting for the September planning started on the 7th January 2019 (Table 3.1) and continued for four weeks (28 days). Harvesting for the December planting started on the 25th February 2019 and continued for six weeks (42 days) for cultivars Monty and Betty, and for seven weeks (49 days) for cultivar Pablo (Table 3.1).

From the preliminary nitrate concentration test of beetroot juice extracted from small (size < 5 cm), medium (size between 5.5 and 8.0 cm) and large (size > 8.0 cm) beetroot, the nitrate concentration of juice extracted from medium and large beetroot were three and four times higher than that from the small beetroot, respectively (Appendix A). Therefore, beetroot size may affect the beetroot composition. Based on the preliminary experiments of local beetroot size (Appendix B), the commercial grower was asked to harvest medium (size between5.5 and 8.0 cm in diameter) and large (size >8.0 cm in diameter) beetroot for the project. On each harvest date six medium and six large beetroot were harvested from three random locations within the paddock to provide three replicates for each size, with a total of 18 beets being harvested for each size of each cultivar. The beetroot planted in September 2018 had half of the paddock covered under fleece and the other half uncovered. The beetroot crop was completely covered (root and leaves) with an opaque cloth supplied by Cropsystems, United Kingdom. No fertilizers were applied for both plantings. Beetroot were harvested on a Monday and couriered unrefrigerated to Massey University, Albany campus overnight, to arrive at the School of Food & Advanced Technology within 48 hours of harvesting.

Table 3.1 Beetroot harvest plan for September and December plantings 2018.

September	Harvest days	Harvest weeks	Days after planting	Harvested samples *
	7-Jan	1	110	Medium not covered beetroot
				Large not covered beetroot
	14-Jan	2	117	No sample
	21-Jan	3	124	Medium covered/not covered beetroot
				Large covered/not covered beetroot
	28-Jan	4	131	Medium covered/not covered beetroot
				Large covered/not covered beetroot
	4-Feb	5	138	Medium not covered beetroot
				Large not covered beetroot
December	**Harvest days**	**Harvest weeks**	**Days after planting**	**Harvested samples ****
	25-Feb	1	70	Medium P/M/B
	27-Feb	1.3	72	Medium P/M/B
	4-Mar	2	77	Medium P/M/B
	11-Mar	3	84	Medium P/M/B
				Large P/M/B
	18-Mar	4	91	Medium P/M/B
				Large P/M/B
	25-Mar	5	98	Medium P/M/B
				Large P/M/B
	1-Apr	6	105	Medium P/M/B
				Large P/M/B
	8-Apr	7	112	Medium /Large P

* Only ‘Pablo’ beetroot were harvested for the September planting.

** P for ‘Pablo’, M for ‘Monty’, and B for ‘Betty’ for the December planting.

3.2.2 Juicing of Beetroot

Fresh beetroot were stored in the dark at 4 ± 1ºC, in cardboard boxes, prior to juicing. Juicing of the beetroot was conducted within 12 hours of the beetroot arriving at Massey University. Prior to juicing the beetroot were washed, any stems, leaves, dirt and fine roots were removed. The beetroot weighed to two decimal places with an electronic balance (UW6200H, Shimadzu, Philippines) and then the beet was cut vertically into halves for equatorial diameter measurement. The flow diagram in Figure 3.2 outlines the juicing process. The fresh beetroot harvested from the farm are shown in Figure 3.3. The beetroot were then quartered and juiced using a juicer (Atlas Pro Whole Slow Juicer, Biochef, Australia), as shown in Figure 3.4.

3.2.3 Storage of Whole Beetroot

A 21-day storage trial of whole large *Beta vulgaris* 'Pablo' was conducted to determine any composition changes during storage at 4 ± 1ºC. An extra batch of 54 large *Beta vulgaris* 'Pablo' grown for 91 days were harvested (18th March) for the storage trial. Stems and leaves were removed immediately after arrival at Massey University and before the beetroot were stored in the dark in a chiller at 4 ± 1ºC, in cardboard boxes. Three replicates (six beetroot per replicate) were taken from the chiller each week for the following 21 days. The fresh beetroot are normally recommended to be stored for up to14 days at between 3°C and 4°C for postharvest storage (Salunkhe & Kadam, 1998).

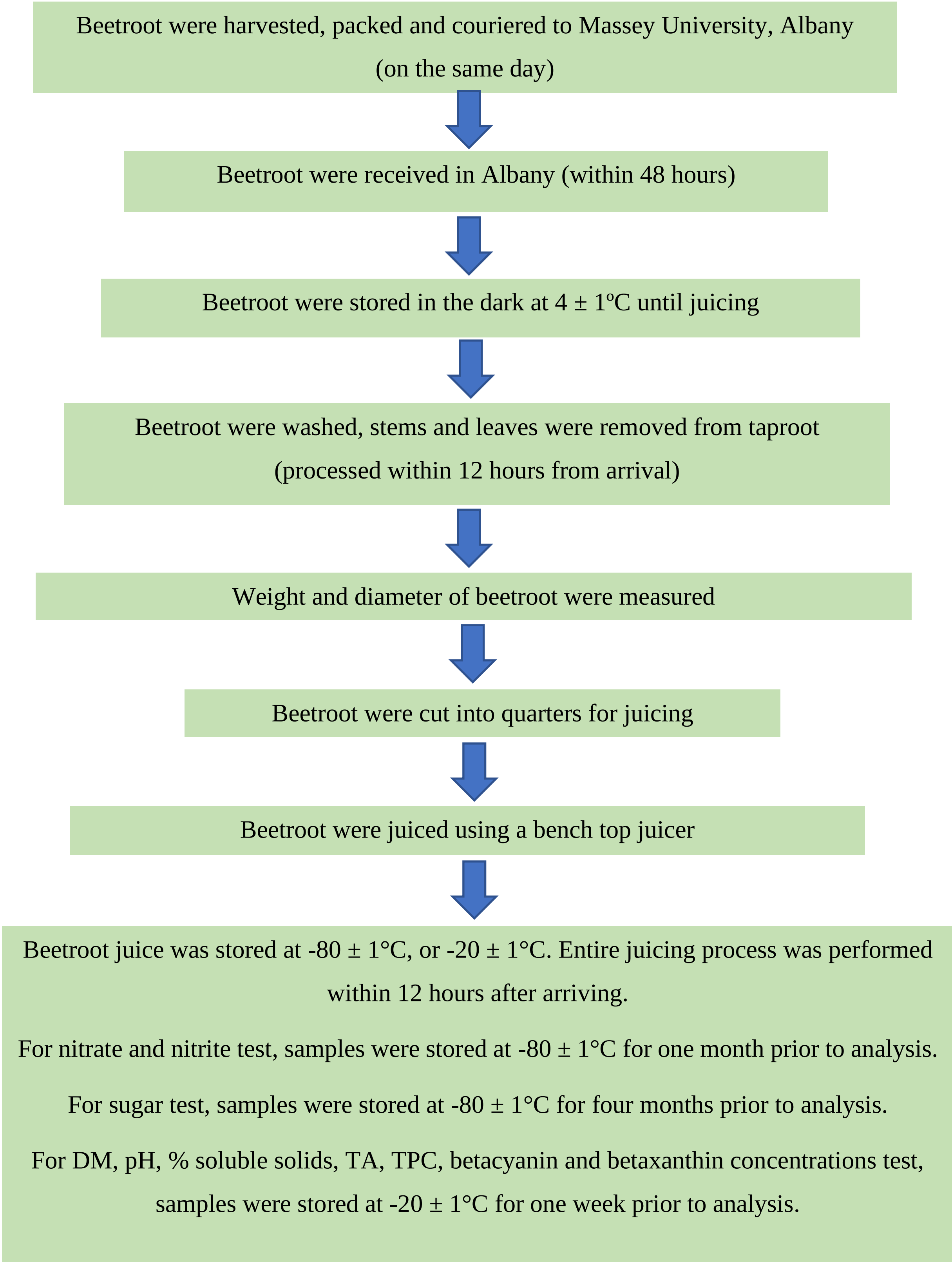


Figure 3.2 Flow diagram of beetroot juicing process.

Figure 3.3 Fresh beetroot from Lovett Family Farms, washed and ready for juicing.

Figure 3.4 The Juicer (Atlas Pro Whole Slow Juicer, Biochef, Australia).

3.3 Methods for Analysis of Beetroot and Beetroot Juice.

3.3.1 Introduction

The weight and diameter of each individual beetroot were measured prior to juicing. Beetroot used for dry matter determination were sealed in aluminium foil bags and frozen at -20 ± 1°C for one week prior to analysis. Beetroot juice for the following tests: pH, % soluble solids, TA, TPC, betacyanin and betaxanthin concentrations, was stored at -20 ± 1°C for one week prior to analysis. For sugar test, beetroot juice was stored at -80 ± 1°C for four months prior to analysis. For nitrate and nitrite test, beetroot juice was stored at -80 ± 1°C for one month prior to analysis.

3.3.2 Weight and Size of Whole Beetroot

The washed beetroot were weighed on a digital balance (UW6200H, Shimadzu, Philippines) to two decimal places. Beetroots were cut vertically from the centre in halves as shown in Figure 3.5 (blue arrow indicates the cutting direction, the black arrow indicates the equatorial diameter). The equatorial diameter (size) of both halves from one beetroot were measured with a ruler (mm).

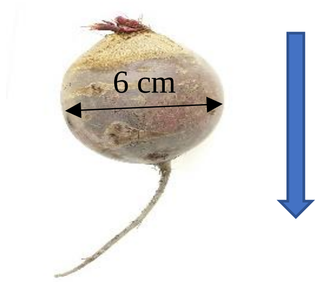


Figure 3.5 Equatorial diameter measurement.

3.3.3 Dry Matter of Beetroot Flesh

One beetroot half was cut vertically in half again to produce two quarters, and one quarter was placed in an aluminium foil bag (140mm × 200 mm). A total of six quarters were sealed in two aluminium bags for dry matter measurement for one composite sample for each replicate on each harvest/sampling day. Aluminium foil bags were heat-sealed with a constant heat foot sealer (ME-300 CFN, Mericer Corporation Ltd, Taiwan) and then stored at -20 ± 1°C before analysis.

The frozen quartered beetroot were thawed at room temperature (21 ± 1°C) for one hour prior to being cut into small cubes before being placed in the universal glass blender (PC-UM 1006, Profi Cook, Germany), and blended for at least one minute to obtain a homogenous puree as shown in Figure 3.6. The puree was placed into a clean dish in 150 g batches which was used for the triplicate tests for each location.

Dry aluminium pans were labelled and accurately weighed on an analytical balance (CP225d, Sartorius, USA) to four decimal places prior to ~30 grams of beetroot puree being weighed into each aluminium pan. The puree was placed in a laboratory oven (8150-150 Lt, Contherm Scientific Ltd, New Zealand) at 65 ± 1°C until a constant weight was achieved (Tyl & Sadler, 2017). Triplicate tests were carried out by testing one sample from each location with medium and large beetroot, from three locations of each cultivar harvested on each harvest date. The percentage of dry matter content was calculated according to Equation 3.1 (Tyl & Sadler, 2017):

$$\text{Dry matter content (\%)} = \frac{\text{Weight of dry flesh (g)}}{\text{Weight of fresh flesh (g)}} \times 100 \qquad \text{Equation 3.1}$$

Figure 3.6 Beetroot puree before being placed in the oven.

3.3.4 Percentage of Soluble Solids (ºBrix) of Beetroot Juice

Fifteen millilitres of beetroot juice was thawed at room temperature (21 ± 1ºC) for three hours prior to analysis of the percentage of soluble solids (ºBrix) using a digital refractometer Palette (PR 101, Atago Corporation Ltd, Japan). Milli-Q water was used to zero the refractometer which was cleaned with Milli-Q water between each sample.

Triplicate tests were carried out by testing one sample from each location with medium and large beetroot, from three locations of each cultivar harvested on each harvest date.

3.3.5 Titratable Acidity and pH of Beetroot Juice

3.3.5.1 Standardisation of 0.01 M Sodium Hydroxide

A 0.01 M sodium hydroxide solution (NaOH) (Thermofisher Scientific, UK) was prepared weekly and stored at 4 ± 1°C. Standardisation of the 0.01 M NaOH was carried out by titration with 0.8 ± 0.01 g potassium hydrogen phthalate (KHP) (analytical reagent grade, Thermofisher Scientific, UK). Three drops of 1 % phenolphthalein indicator was used to indicate the end of titration, when a faint pink colour remained for at least 10 seconds (Nielsen, 2017). The equation for normality of NaOH (mol/L) is shown in Equation 3.2 (Nielsen, 2017):

Normality of NaOH (mol/L)

= normality of KHP (mol/L)

$$= \frac{\text{mass of KHP (g)} \times 1000}{(\text{NaOH volume (ml)} \times \text{molecular weight } 204.22\text{ g/mol})} \qquad \text{Equation 3.2}$$

3.3.5.2 TA of Beetroot Juice

Fifteen millilitres of beetroot juice was thawed at room temperature (21 ± 1°C) for three hours prior to analysis. The pH of the beetroot juice was measured using a previously calibrated digital pH meter (PB-20 Sartorius, Germany) (pH 4.0, 7.0 and 10.0 buffers (Labserv, Thermofisher, NZ)). Ten millilitres of beetroot juice was titrated with 0.01 M NaOH until an end point of pH 8.1 was reached (AOAC International, 2005; Nielsen, 2010). The equation used to calculate TA is shown in Equation 3.3 (Nielsen, 2010). Triplicate tests were carried out by testing one sample from each location with medium and large beetroot, from three locations of each cultivar harvested on each harvest date. All the results were standardised to 10°Brix to allow comparison between the different samples, and the results are expressed as malic acid (g per 100 ml of juice) as this is the main organic acid in beetroot juice (Vasconcellos et al., 2016):

Malic acid g per 100 ml of sample =

$$\frac{\text{volume of NaOH (ml)} \times \text{normality of NaOH} \times 67(\text{equivalent weight of malic acid})}{(10\text{ ml of sample} \times 10\text{ unit factor})}$$

$$= \text{volume of NaOH} \times 0.67 \times \text{normality of NaOH} \qquad \text{Equation 3.3}$$

3.3.6 Spectrophotometric Quantification of Total Phenolic Content (TPC) in Beetroot Juice using the Folin-Ciocalteu Reagent

3.3.6.1 Standards and Sodium Carbonate (200 g/L) Preparation

Folin-Ciocalteu Reagent (2N) was purchased from Sigma-Aldrich, USA. TPC standards were prepared weekly using gallic acid (Sigma-Aldrich, China) (0, 0.2, 0.4, 0.6, 0.8 and 1.0 mg/ml), and stored at 4 ± 1°C for a maximum seven days. A 200 g/L sodium carbonate solution (Na_2CO_3) (analytical reagent grade, Thermofisher Scientific, Australia) was prepared weekly and stored at 4 ± 1°C.

3.3.6.2 Analysis of Beetroot Juice for Total Phenolic Content with the Folin-Ciocalteu Reagent

Ten millilitres of beetroot juice was thawed at room temperature (21 ± 1^{o}C) for three hours before being centrifuged (6-16KS, Sigma Zentrifugen, Germany) at 14560*g* for 10 minutes at 20^{o}C. TPC were measured using the Folin-Ciocalteu method with some modifications (Wrolstad, 2001). Twenty microliters supernatant of standards/beetroot juice (beetroot juice were diluted 1:1 with Milli-Q water), and 100 microliters Folin-Ciocalteu reagent was added, and the solutions were mixed by the vortex mixer (F20220176, VELP Scientifica, Europe) and allowed to stand at 21 ± 1^{o}C for 5 minutes. Three hundred microliters of 200 g/L Na_2CO_3 was then added and the test tubes were incubated at room temperature (21 ± 1^{o}C) in the dark for 120 minutes. For the standard curve, the standard solutions were also treated the same as the beetroot juice supernatant. The absorbance of the standards and beetroot juices were measured at 760 nm with a UV-VIS spectrophotometer (UV-1601, Shimadzu, Japan). The total phenolics concentration in the beetroot juice was expressed as mg/ml as gallic acid (GAE mg/ml). Triplicate tests were carried out by testing one sample from each location with medium and large beetroot, from three locations of each cultivar harvested on each harvest date All the results were standardised to 10°Brix to allow comparison between the different samples.

3.3.7 Quantification of Betalains in Beetroot Juice

A 0.2 M disodium hydrogen phosphate solution (Na_2HPO_4) (99.7%, Thermofisher Scientific, USA) and 0.1 M citric acid solution ($C_6H_8O_7$) (99.0%, Thermofisher Scientific, USA) were made weekly and stored at 4 ± 1^{o}C. Mcllvaine buffer (pH 6.5) was prepared weekly by mixing 14 ml 0.2 M disodium hydrogen phosphate and 6 ml 0.1 M citric acid and stored at 4 ± 1 °C (Stoll & Blanchard, 2009).

Ten millilitres of beetroot juice was thawed at room temperature (21 ± 1°C) for three hours before being centrifuged (6-16KS, Sigma Zentrifugen, Germany) at 14560*g* for 10 minutes at 20°C to remove any particulates. Thawed and centrifuged beetroot juice was analysed by the methods of Bazaria and Kumar (2016) and Netzel et al. (2005) with some modifications. One hundred microliters of beetroot juice supernatant was dissolved in 20 ml Mcllvaine buffer (pH 6.5) to give an absorbance between 0.8 and 1.0 at 538 nm (UV-1601, Shimadzu, Japan) for betacyanins and 480 nm for betaxanthins, and results were calculated using Equation 3.4 (Bazaria & Kumar, 2016; Netzel et al., 2005). Triplicate tests were carried out by testing one sample from each location with medium and large beetroot, from three locations of each cultivar harvested on each harvest date. All the results were standardised to 10°Brix to allow comparison between the different samples.

$$\text{Betacyanins/betaxanthins(mg/L)} = \frac{A \times DF \times MW \times 1000}{\varepsilon \times L} \qquad \text{Equation 3.4}$$

A=absorbance

L=1 cm of cuvette

ε = extinction coefficient = 60000 L/ (mol × cm) for betacyanins and 48000 L/ (mol × cm) for betaxanthins (Stintzing et al., 2008)

MW= 550 g/mol for betacyanins and 308 g/mol for betaxanthins

DF= dilution factor

3.3.8 Quantification of Individual Sugars in Beetroot Juices by HPLC

Fifteen millilitres of beetroot juice was thawed at room temperature (21 ± 1°C) for three hours before being centrifuged (6-16KS, Sigma Zentrifugen, Germany) at 14560*g* for 10 minutes at 20°C. Triplicate tests were carried out by testing one sample from each location with medium and large beetroot, from three locations of each cultivar harvested on each harvest date. All the results were standardised to 10°Brix to allow comparison between the different samples.

3.3.8.1 HPLC System

The details of the HPLC system, mobile phase, flow rate, injection volume, and retention time for glucose, sucrose and fructose are shown in Table 3.2. Chromatography data were integrated by Lab Solutions Software (Shimadzu, Japan). The mobile phase was filtered using a 0.22 µm filter (Merck Millipore Ltd, Ireland) and degassed in an Ultrasonic water bath (Super RK510, Bandelin Sonorex, Germany).

3.3.8.2 Preparation of Mixed Standards

Standards were made by diluting a prepared stock solution of 120 g/L sucrose (≥99.5% HPLC grade, Sigma-Aldrich, Switzerland), 12 g/L glucose (≥99.0%, Sigma-Aldrich, USA) and 6 g/L fructose (≥99.5%, Sigma-Aldrich, USA) to 20, 40, 60, 80, 100 and 120 g/L for sucrose, 2, 4, 6, 8, 10, 12 g/L for glucose and 1, 2, 3, 4, 5, 6 g/L for fructose. The standards were freshly made every seven days and stored in a 4 ± 1°C chiller. The standards were filtered through a 0.22 µm syringe filter (Thermofisher Scientific, New Zealand) before the injection.

3.3.8.3 Sample Preparation and Analysis for Individual Sugars

The supernatant of the thawed and centrifuged beetroot juice was filtered through a 0.22 µm syringe filter (Thermofisher Scientific, New Zealand) into a 2 ml glass HPLC vial for analysis. Triplicate tests were carried out by testing one sample from each location with medium and large beetroot, from three locations of each cultivar harvested on each harvest date. Peaks of beetroot juice were identified by comparison to the peaks of the standards, and the amounts of sugars were calculated from the peak areas by linear regression equations from the standard curves.

Table 3.2 The Shimadzu HPLC system for sucrose analysis.

Instrument	**Details**
Pump	Liquid chromatograph LC 20 AD, Shimadzu, Japan
Autosampler	Sil-30 AC, Shimadzu, Japan
Oven	Prominence column oven, Shimadzu, Japan
Column	Rezex-Monosacharide Ca^{2+} (300 × 7.8 mm), Phenomenex, New Zealand
Detector	RID-20A refractive index detector, Shimadzu, Japan
Mobile phase	Milli-Q water (filtered and degassed)
Injection volume	10 µL
Flow rate	0.6 ml/min at 80°C
Glucose retention time	11.00 minutes
Sucrose retention time	9.18 minutes
Fructose retention time	13.58 minutes

3.3.9 Quantification of Nitrate and Nitrite in Beetroot Juices by HPLC

The HPLC methodology for quantification of nitrate and nitrite in juices extracted from harvested beetroot was from Cheng and Tsang (1998), Chou et al. (2003) and Monton et al. (2016) with some modifications. Triplicate tests were carried out by testing one sample from each location with medium and large beetroot, from three locations of each cultivar harvested on each harvest date. All the results were standardised to 10°Brix to allow comparison between the different samples.

3.3.9.1 HPLC System

The details of the HPLC system, mobile phase, flow rate, injection volume, wavelength and retention time of nitrate/nitrite is shown in Table 3.3. Peak areas were integrated using Lab Solutions System software (Shimadzu, Japan). The mobile phase was prepared using Octylamine (99%, Sigma-Aldrich, USA) and orthophosphoric acid (85%, Fluka, Switzerland).

Table 3.3 The Shimadzu HPLC system for nitrate/nitrite analysis.

Instruments	**Details**
Degasser	DGU-20A5, Shimadzu, Japan
Pump	LC-20 AD, Shimadzu, Japan
Autosampler	SiL-20 AC HT, Shimadzu, Japan
Oven	CTO-20 AC, Shimadzu, Japan
Column	Grace smart RP 18, 5 µm, 120 A, 4.6 × 250 mm i.d., ThermoFisher, USA
Detector	SPD-M20A diode array detector and RF-20 A XS UV-Vis detector, Shimadzu, Japan
Mobile phase	0.01 M octylammonium orthophosphate pH 3 to 3.5
Injection volume	10 µL
Flow rate	0.8 ml/min at 20°C
Nitrite wavelength/retention time	193 nm/4.1 minutes
Nitrate wavelength /retention time	213 nm/6.7 minutes

3.3.9.2 Standards Preparation and Standard Curve

Nitrate/nitrite standards were made using sodium nitrate (≥99%, Sigma-Aldrich, Japan) and sodium nitrite (≥97%, Sigma-Aldrich, Japan) and Milli-Q water. Five standard concentrations (0.1, 1, 10, 50 and 100 µg/ml) were made weekly and stored at 4 ± 1°C.

3.3.9.3 Sample Preparation and Analysis of Beetroot Juices

One millilitre of beetroot juice was thawed at room temperature (21 ± 1°C) for one hour before being centrifuged (5424R, Eppendorf AG, Germany) at 14674*g* for 10 minutes at 20°C. The supernatant was initially filtered through a 0.45 µm syringe filter (Terumo, Australia) before dilution with 990 millilitre Milli-Q water and placement into a 2 ml glass HPLC vial. Peaks of beetroot juice were identified by comparison to the peaks of the standards, and the amounts of nitrate and nitrite were calculated from the peak areas by linear regression equations from the nitrate and nitrite standard curves.

3.4 Statistical Analysis

The DM, % soluble solids, TA, TPC, betacyanin and betaxanthin concentrations of beetroot and/or beetroot juice were determined by one test on triplicate samples from each field location. The sugars, nitrate and nitrite concentrations were determined by one sample in triplicate analyse from each field location, with three locations (replicates) were harvested for medium and large sized cultivar on each harvest date.

Each data point was shown as mean ± standard error of mean (SEM) (n=9). Analysis of variance (ANOVA) with Tukey Pairwise Comparisons and Tukey Simultaneous Tests were conducted to test for significant difference in mean weight, size, DM, % soluble solids, TA, TPC, sugars, betacyanin and betaxanthin concentrations, nitrate and nitrite concentrations on each harvest date ($\alpha = 0.05$). All statistical analyses were conducted using MINITAB 18 (Sydney, Australia).

CHAPTER 4 The Effect of Growth Period on Beetroot Composition

4.1 Introduction

The purpose of this chapter was to determine the effect of growth time on certain aspects of 'Pablo' beetroot quality: size, weight, DM, % of soluble solids, pH, TA, TPC, betacyanin and betaxanthin concentrations, sucrose concentration, nitrate and nitrite concentrations (Section 3.3.2 to 3.3.9). The results obtained in this chapter may provide some information on suitable harvest times to obtain high-nitrate/betacyanin/betaxanthin/TPC/sugar beetroot for juicing. Medium/large *Beta vulgaris* 'Pablo' planted in September and December 2018 were harvested as described in Section 3.1. To determine whether being covered under fleece would affect the qualities of the beetroot, two extra batches of covered medium and large beetroot were harvested from the September planting as described in Section 3.1. No fertilizers were applied to the beetroot for either of the two plantings.

4.2 Size of Whole Beetroot

Based on preliminary size (diameter) measurements of beetroot obtained from a local supermarket, the size of medium beetroot varied between 5.7 and 6.5 cm, and the size of large beetroot varied between 8.1 and 11.5 cm (Appendix B). This information was used to determine the specific size requirements for medium and large beetroot as described in Section 3.2.1. Figure 4.1 shows the size change of the medium and large 'Pablo' beetroot harvested from the September and December plantings. The mean size of medium beetroot from these two plantings was 6.2 ± 0.7 cm while that of large beetroot was 8.4 ± 0.9 cm. This confirmed that the size of the harvested/received beetroot generally met the size requirements described in Section 3.2.1.

The beetroot planted in September were received after 110 to 138 days of growth (Jan 2019) while those planted in December were received after 70 to 112 days of growth (Feb 2019). When comparing the size of beetroot from the two plantings, the mean size of medium beetroot after 110 days of growth from the September planting was larger than that after 112 days of growth from the December planting ($P<0.05$). No significant difference ($P\geq0.05$) was found with respect to size between large beetroot grown for 110 days (September planning) and 112 days (December planting). However, if the beetroot from the December planting were left in the ground for longer it was assumed, they would increase to a size similar to the September planting. Hence the data collected will provided information on ‘Pablo’ beetroot grown for 70 days to 138 days.

For beetroot planted in September, the mean size of beetroot harvested after 138 days of growth were significantly larger than those after 110 days of growth for both medium (+12 %) and large (+18 %) beetroot ($P<0.05$). This indicates that the longer the growth period, the larger size the beetroot reaches. For beetroot planted in December, which were harvested earlier, there was no significant difference in size between the beetroot harvested on the first day and the last day of harvest ($P\geq0.05$). The results show that to achieve beetroot ≥ 8 cm (large), the ‘Pablo’ cultivar needs to grow for approximately 100 days.

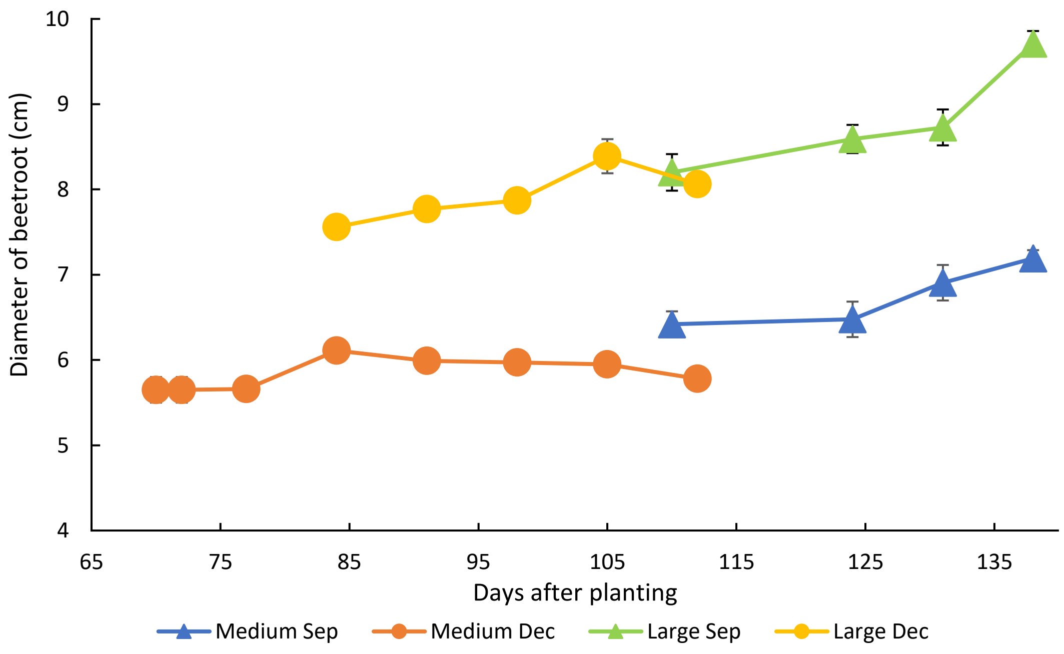


Figure 4.1 Diameter (cm) of medium and large *Beta vulgaris* ‘Pablo’, harvested on different days after planting (September and December 2018) (Each data point = Mean ± standard error of mean, n=18).

4.3 Weight of Whole Beetroot

The weight (mass) of the beetroot generally reflected the length of the growth period prior to harvesting, with beetroot harvested longer after planting being heavier (Figure 4.2). The effect of growth period on beetroot size (Figure 4.1) and weight (Figure 4.2) was similar. The mean weight of medium beetroot from the two plantings was 121 ± 45 g while that of large beetroot was 224 ± 58 g.

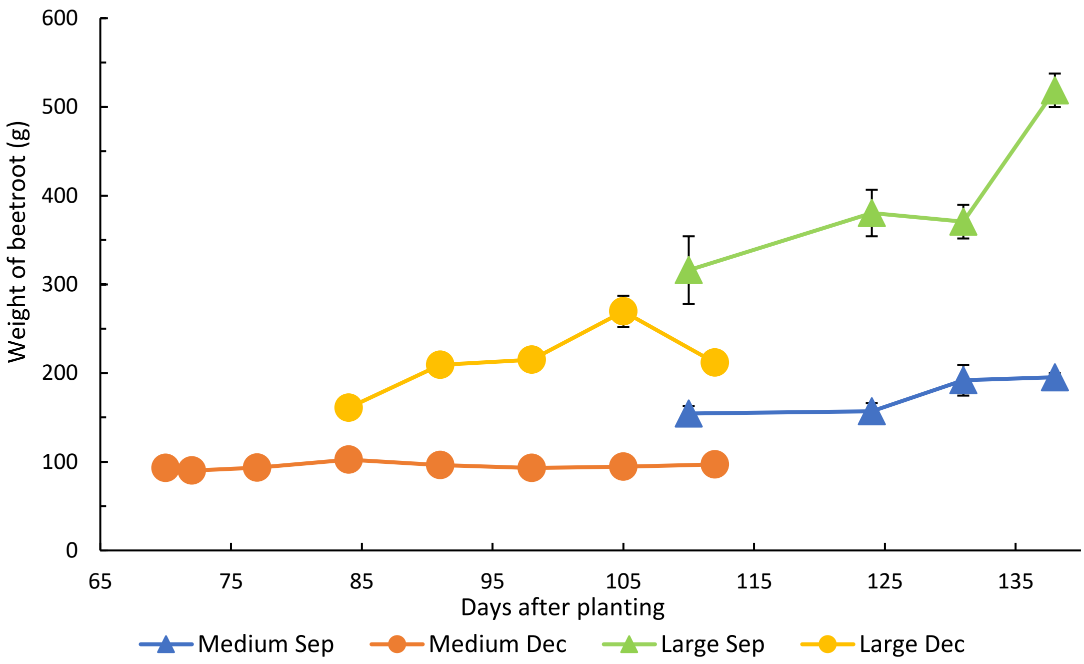


Figure 4.2 Weight (g) of medium and large *Beta vulgaris* 'Pablo', harvested on different days after planting (September and December 2018) (Each data point = Mean ± standard error of mean, n=18).

For the beetroot harvested from the September planting, there was a significant increase in mean weight of beetroot harvested after 138 days growth compared to 110 days, with medium beetroot increasing by 27 % and large beetroot increasing by 64 % ($P<0.05$). For beetroot planted in December, growth between 70 and 112 days, did not show any significant increase in weight ($P\geq0.05$), except for large beetroot harvested on day 105 were significantly heavier than those on days 84 and 112 ($P<0.05$).

The results show that the weight of beetroot after 110 days of growth (September planting, earlier planting) was significantly larger ($P<0.05$) than that after 112 days of growth (December planting, later planting) for both medium and large beetroot (Figure 4.2). As mentioned in Section 4.2, the size of beetroot from the September planting was significantly larger (P<0.05) than that from the December planting for medium beetroot. This indicated that the size and weight of beetroot from the September planting were significantly larger than those from the December planting as the beetroot had been grown for a longer time (Section 4.2).

4.4 Dry Matter of Beetroot Flesh

Dry matter (DM) is a measure of the amount of the total solids left after all moisture has been removed (Nielsen, 2017). The DM (%) ($^{w}/_{w}$ fresh weight) was determined from a pureed sample of fresh beetroot as described in Section 3.3.3. The DM of medium and large beetroot for the two separate plantings did not vary significantly between the first and last harvest dates within each planting during the sampling period (P≥0.05) (Figure 4.3). The mean DM of medium beetroot varied between 11.26 ± 0.29 and 12.98 ± 0.24 % for the September planting, and varied between 13.91 ± 0.28 and 16.41 ± 0.14 % for the December planting. The mean DM of large beetroot varied between 11.13 ± 0.27 and 12.08 ± 0.14 % for the September planting, and between 12.44 ± 0.33 and 14.61 ± 0.14 % for the December planting.

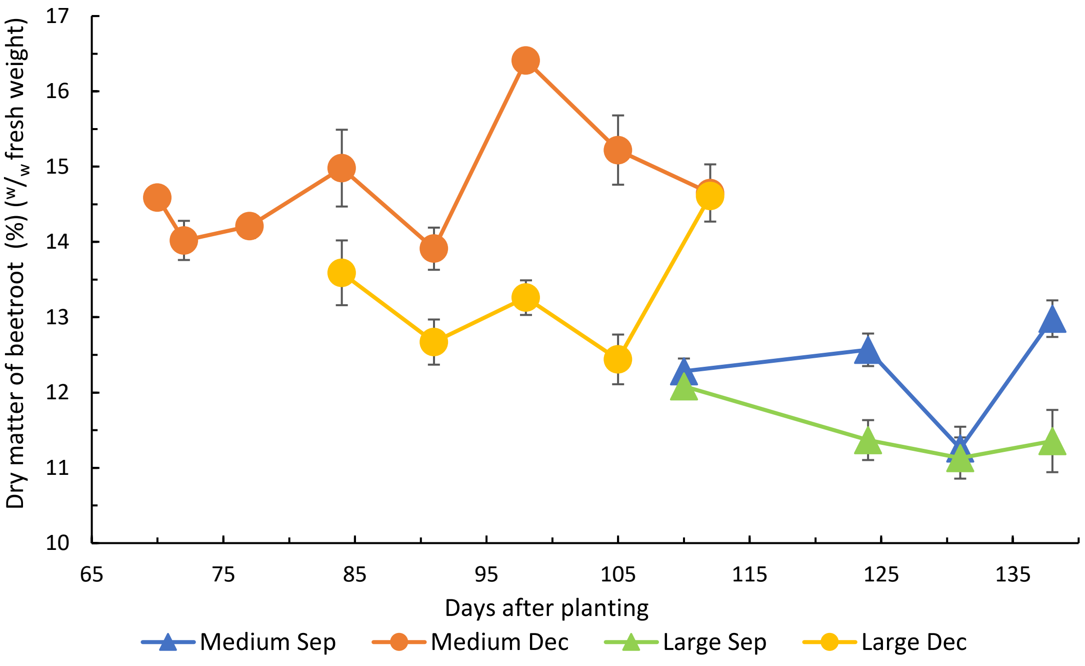


Figure 4.3 Dry matter content (%) ($^{w}/_{w}$ fresh weight) of medium and large *Beta vulgaris* 'Pablo' beetroot, harvested on different days after planting (September and December 2018) (Each data point = Mean ± standard error of mean, n=9).

As can be seen in Figure 4.3, the mean DM of medium beetroot harvested between 110 and 137 days of growth (September planting) was significantly lower than that found in medium beetroot harvested up to 112 days of growth (December planting) ($P<0.05$) while there was no significant difference in DM of the large beetroot for both plantings ($P\geq0.05$). The overall DM of medium beetroot from the September planting was significantly lower than that from the December planting ($P<0.05$) (Figure 4.3). Beetroot from the September planting were larger (Sections 4.2 and 4.3) than the December planting, but since they had an overall lower DM content, this could indicate that the beetroot took up more water during their growth period, either due to more irrigation or rainfall.

Furthermore, as can be seen in Figure 4.3, the medium beetroot for both plantings had significantly higher DM content than the large beetroot on most sampling days within each planting ($P<0.05$), except for days 84,110,112 and 131 when the difference was not significant ($P\geq0.05$).

4.5 Percentage of Soluble Solids (°Brix) of Beetroot Juice

The % soluble solids of juice extracted from beetroot harvested from the September planting varied between 9.9 ± 0.1 and 11.5 ± 0.1 ºBrix (Figure 4.4). The mean % soluble solids in juice from beetroot harvested after 138 days of growth increased significantly ($P<0.05$) for both medium (+13%) and large beetroot (+8%) compared to those after 110 days of growth. For the December planting, the % soluble solids in the beetroot juice varied between 9.2 ± 0.3 and 12.2 ± 0.6 ºBrix and was not significantly different between the beetroot harvested at the start and the end of the sampling period for either medium or large beetroot ($P\geq0.05$).

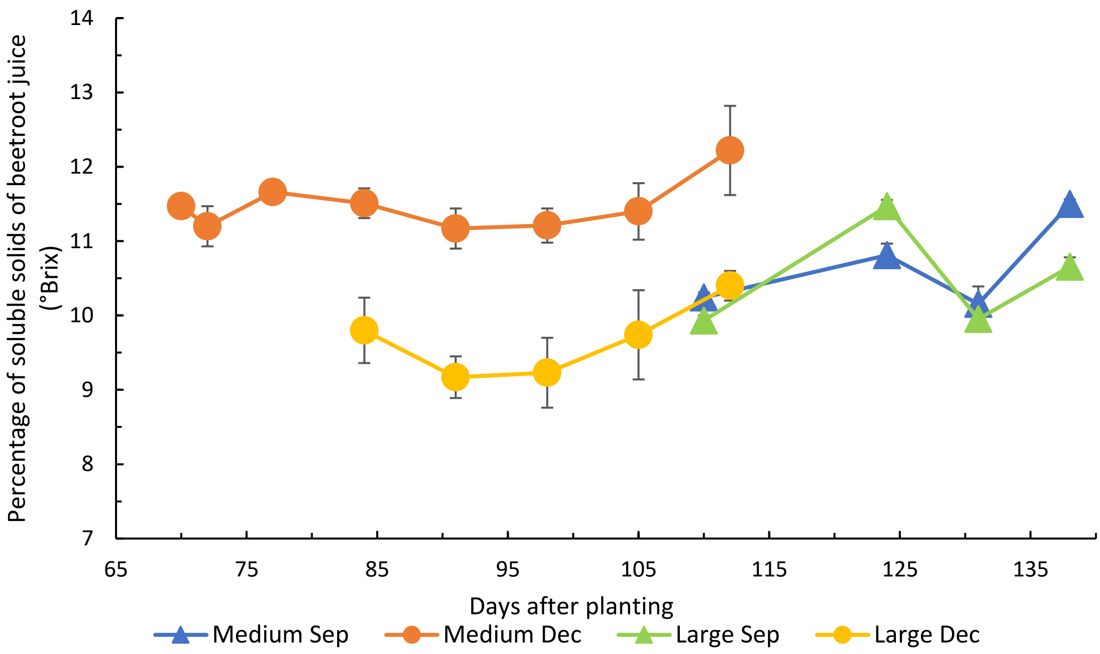


Figure 4.4 Percentage of soluble solids (°Brix) of juice extracted from medium and large *Beta vulgaris* 'Pablo' beetroot, harvested on different days after planting (September and December 2018) (Each data point = Mean ± standard error of mean, n=9).

4.6 pH of Beetroot Juice

For beetroot planted in September, the mean pH of the extracted beetroot juice fluctuated between 6.29 ± 0.01 and 6.41 ± 0.03 (Figure 4.5). For the September planting there was no significant difference in the pH of juice from beetroot harvested after 138 days of growth compared to that after 110 days of growth for either the medium or large beetroot ($P \geq 0.05$). For the December planting, the mean pH of beetroot juice varied between 6.19 ± 0.01 and 6.56 ± 0.00. The mean pH of juice from beetroot from the December planting after 112 days of growth was significantly less than that after 70 or 84 days of growth for medium and large beetroot, respectively ($P < 0.05$). It appears that the pH of the beetroot juice decreased with a longer length of growth, reaching a plateau after 112 days of growth (Figure 4.5).

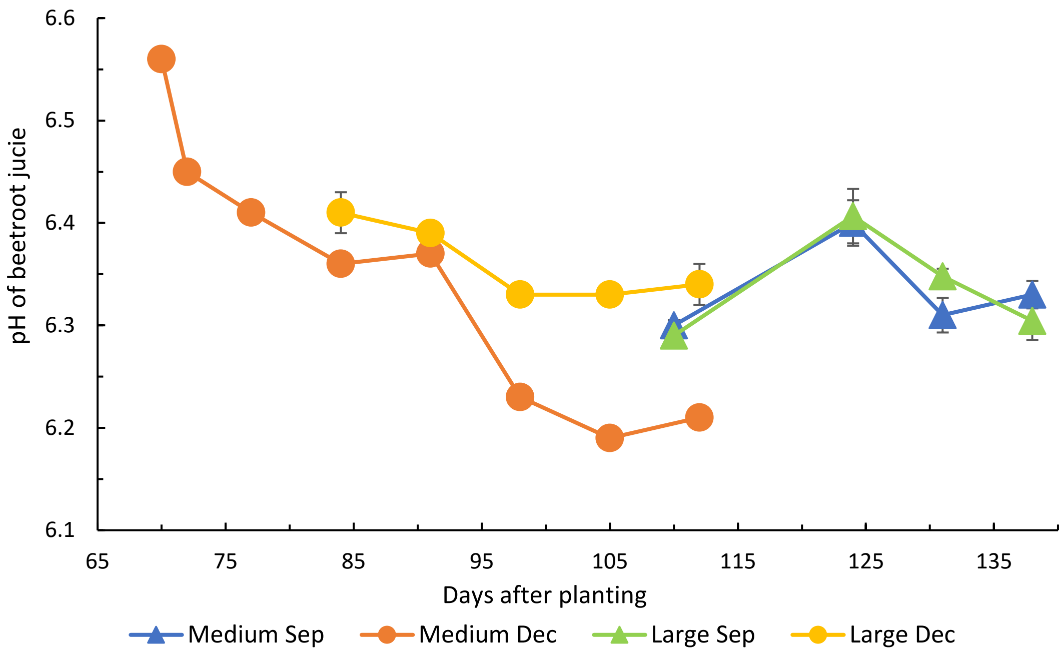


Figure 4.5 pH of juice extracted from medium and large *Beta vulgaris* 'Pablo' beetroot, harvested on different days after planting (September and December 2018) (Each data point = Mean ± standard error of mean, n=9).

4.7 Titratable Acidity (TA) of Beetroot Juice

The mean TA of juice from beetroot harvested from the September planting varied between 0.077 ± 0.004 and 0.104 ± 0.003 (%) ($^{w}/_{v}$ as malic acid) (Figure 4.6). The mean TA in juice from the beetroot harvested after 138 days of growth was significantly higher ($P<0.05$) than that after 110 days of growth for medium beetroot but not for large beetroot ($P\geq0.05$).

For the beetroot harvested from the December planting, the mean TA in beetroot juice varied between 0.064 ± 0.003 and 0.106 ± 0.006 (%) ($^{w}/_{v}$ as malic acid). The mean TA of juice for the medium beetroot increased significantly from 0.064 to 0.091 (%) ($^{w}/_{v}$ as malic acid) between 70 and 112 days of growth and for large beetroot increased from 0.080 to 0.096 (%) ($^{w}/_{v}$ as malic acid) between 84 and 112 days of growth ($P<0.05$) (Figure 4.6).

Furthermore, as mentioned in Section 4.2 and Section 4.3, if beetroot from the December planting had kept growing in the field, they may have reached a similar size to the beetroot harvested from the September planting. Therefore, the TA of beetroot juice may increase from day 70 to day 112 and then plateau.

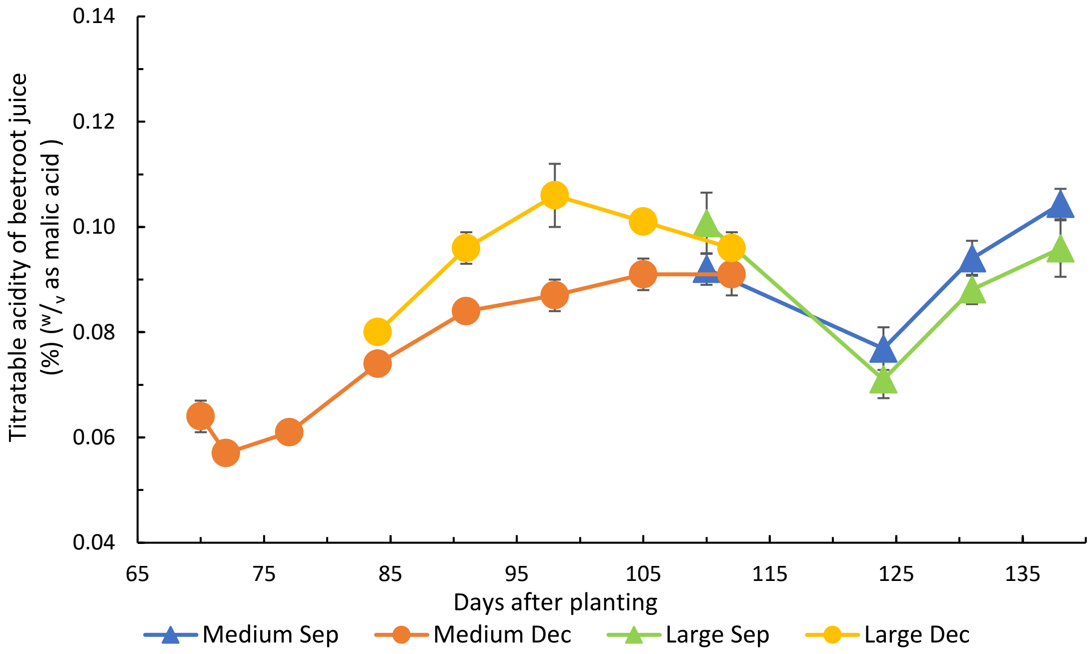


Figure 4.6 Titratable acidity (%) ($^{w}/_{v}$ as malic acid) of juice extracted from the medium and large *Beta vulgaris* 'Pablo' beetroot, harvested on different days after planting (September and December 2018) (Each data point = Mean ± standard error of mean, n=9). All the results were standardised to 10°Brix to allow comparison between the different samples.

4.8 Total Phenolic Content (TPC) in Beetroot Juice

For the beetroot from the September planting, the mean TPC varied between 0.66 ± 0.02 and 0.99 ± 0.05 GAE mg/ml (Figure 4.7), the mean TPC in juice from beetroot harvested after 138 days of growth was not significantly different to that from beetroot harvested after 110 days of growth for either medium or large beetroot (P≥0.05) (Figure 4.7).

For the beetroot from the December planting, the mean TPC in juice from medium beetroot decreased from 1.73 ± 0.07 to 1.17 ± 0.04 GAE mg/ml from day 70 to day 112. The mean TPC in juice from medium beetroot harvested at the start of the sampling period was significantly higher than most sampling dates except days 72, 77 and 91 (P<0.05). For the large beetroot, the mean TPC in beetroot juice harvested after 84 days of growth was significantly higher than that on days 91, 98 and 105 (P<0.05), but not day 112 (P≥0.05). It appeared that the TPC in beetroot decreased with increasing length of growth during the early harvest period (day 70 to 112) and then remained constant up to 138 days after planting. The recommended harvest time to obtain maximum TPC beetroot appears to be between 70 and 84 days after planting or earlier.

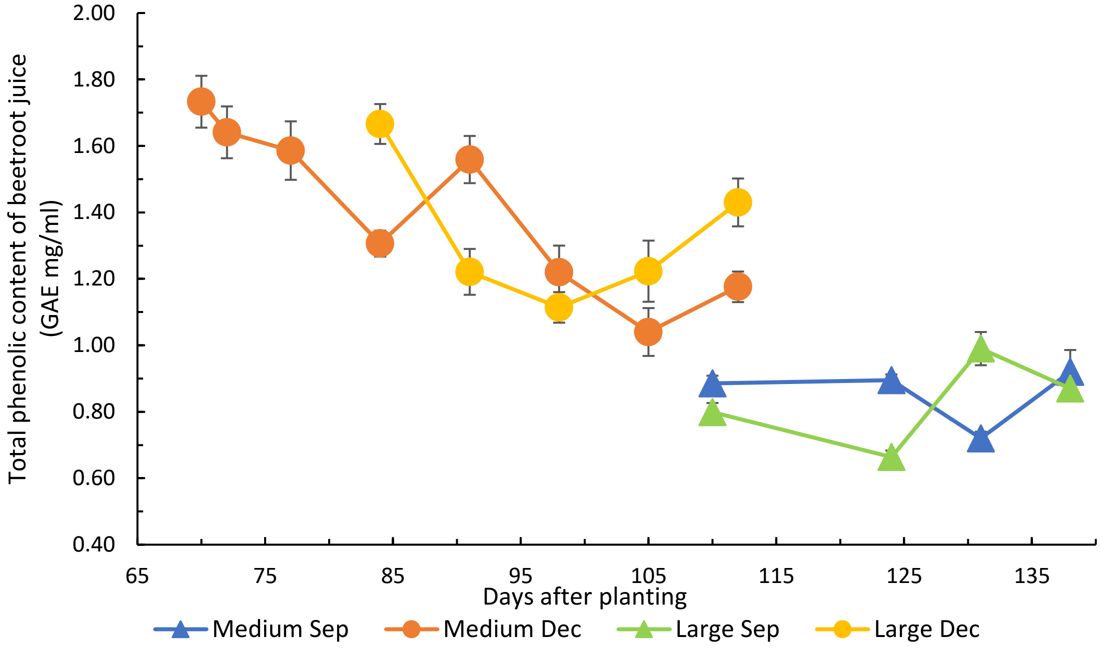


Figure 4.7 Total phenolic content (GAE mg/ml) of juice extracted from medium and large *Beta vulgaris* 'Pablo' beetroot, harvested on different days after planting (September and December 2018) (Each data point = Mean ± standard error of mean, n=9). All results were standardised to 10°Brix to allow comparison between the different samples.

The results show that the TPC of juice from both medium beetroot and large beetroot harvested after 110 days of growth (September planting) was significantly less than that harvested after 112 days of growth (December planting) ($P<0.05$). The overall TPC of beetroot from the September planting was lower than that from the December planting (Figure 4.7).

Beetroot from the September planting were larger, heavier (Section 4.2 and 4.3), and had a lower DM content than those from the December planting (Section 4.4). Beetroot from the September planting contained more water during their growth period (Section 4.4) and since the absolute TPC of beetroot may have remained the same, this would result in an overall lower concentration of the total phenolics in the beetroot from the later harvest dates of the September planting (Figure 4.7).

4.9 Individual Sugar Concentration in Beetroot Juice

The concentrations of sucrose, fructose and glucose were quantified in beetroot juice samples by HPLC as described in Section 3.3.9. Sucrose was the only sugar identified in the juice. Even though others (Wruss, 2005) have report the presence of fructose and glucose, these sugars were not found in the beetroot juice samples in the present study. The mean sucrose concentration in juice extracted from beetroot harvested from the September planting varied between 71.25 ± 2.71 and 83.27 ± 0.56 g/L (Figure 4.8). The mean sucrose concentration in juice extracted from beetroot from the December planting varied between 66.75 ± 1.69 and 90.76 ± 4.20 g/L. The mean sucrose concentration in beetroot juice from both medium and large beetroot did not show any significant difference ($P \geq 0.05$) between the first and the last harvest days for either of the two plantings.

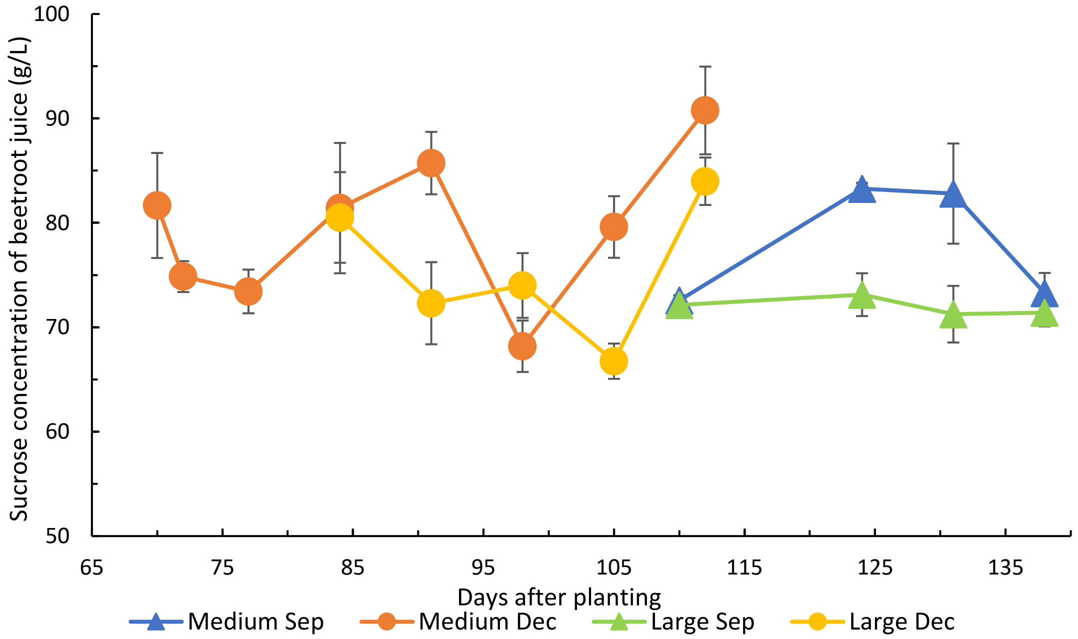


Figure 4.8 Sucrose concentration (g/L) in juice extracted from medium and large *Beta vulgaris* 'Pablo' beetroot, harvested on different days after planting (September and December 2018) (Each data point = Mean ± standard error of mean, n=9). All results were standardised to 10°Brix to allow comparison between the different samples.

4.10 Betacyanins in Beetroot Juice

In the present study, betacyanin concentrations of the beetroot juice were quantified as described in Section 3.3.7. The mean concentration of betacyanin in beetroot juice from the September planting varied between 622.96 ± 7.47 and 1077.40 ± 12.52 mg/L (Figure 4.9). The mean betacyanin concentration in juice from both medium and large beetroot harvested 124, 131 and 138 days after planting were all significantly lower ($P<0.05$) than that of beetroot harvested 110 days after planting. The betacyanin concentration of juice from beetroot harvested 138 days after planting was lower than that from beetroot harvested 110 days after planting by 351.86 mg/L and 383.25 mg/L for medium beetroot and large beetroot respectively (Figure 4.9).

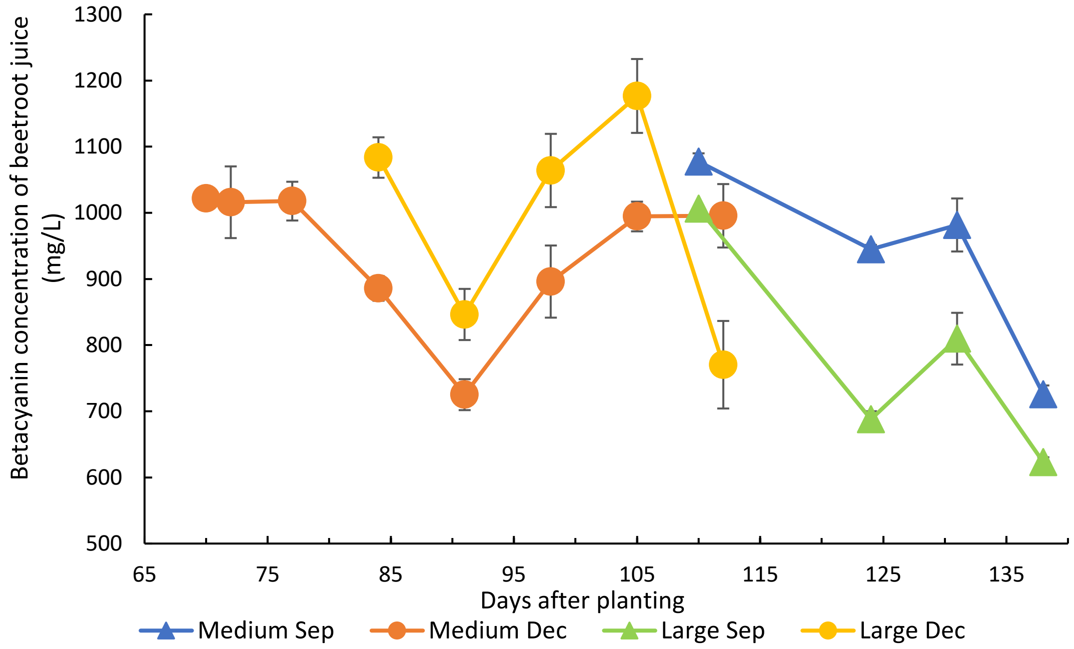


Figure 4.9 Betacyanin concentration (mg/L) in juice extracted from medium and large *Beta vulgaris* 'Pablo' beetroot, harvested on different days after planting (September and December 2018) (Each data point = Mean ± standard error of mean, n=9). All the results were standardised to 10°Brix to allow comparison between the different samples.

For beetroot harvested from the December planting, the mean betacyanin concentration in beetroot juice varied between 725.09 ± 23.4 and 1176.63 ± 55.84 mg/L (Figure 4.9). The data showed a large variation in betacyanin concentration within each planting. After ANOVA analysis, when each harvest point was compared to the first harvest date, no significant difference was found ($P\geq0.05$), except for medium beetroot harvested on day 91 and large beetroot on days 91 and 112, which were significantly lower than those on the first date of harvest ($P<0.05$).

The maximum concentration of the red pigment betacyanin was from juice extracted from large beetroot grown for 105 days (1176.63 mg/L). However, it was hard to draw a conclusion as to whether there is a trend for decreasing betacyanin levels for beetroot grown for a longer time due to the large variation observed in the present study.

4.11 Betaxanthins in Beetroot Juice

In the present study, betaxanthins were quantified as described in Section 3.3.7. The average concentration of betaxanthin in beetroot juice varied between 332.43 ± 3.63 and 584.55 ± 4.31 mg/L for the September planting (Figure 4.10 A). For medium beetroot, the mean betaxanthin concentration in juice from beetroot harvested after 138 days of growth was 153.42 mg/L lower than that from beetroot harvested after 110 days of growth. Similarly, for large beetroot, the betaxanthin concentration in juice from beetroot after 138 days of growing was 171.98 mg/L lower than that after 110 days of growth ($P<0.05$).

For beetroot planted in December, the average betaxanthin concentration in beetroot juice varied between 428.93 ± 12.84 and 674.75 ± 26.87 mg/L (Figure 4.10 A). The mean betaxanthin concentration in juice from medium beetroot harvested after 112 days of growth was 190.49 mg/L higher than that after 70 days of growth ($P<0.05$), however, no significant increase in betaxanthins was observed for the large beetroot over the same growth period ($P\geq0.05$).

The betaxanthin content in beetroot appeared to gradually increase from 70 to 112 days of growth and then decrease with continued growth up to 138 days. The maximum concentration of betaxanthin content was in beetroot grown for between 105 and 112 days (1176.63 ± 55.84 mg/L on day 105 for large beetroot) (Figure 4.10 A).

When comparing the ratio between the red and yellow pigments found in beetroot juice, the ratio of betacyanin/betaxanthin (red/yellow) (as shown in Figure 4.10 B) from the beetroot harvested at the end of the sampling period was significantly lower than that at the start of the harvest for large September-planted and medium and large December-planted beetroot ($P<0.05$). Although the red/yellow pigment ratio in juice from beetroot grown for 110 days (September planting) and 112 days (December planting) was different ($P<0.05$), this is probably due to the size and weight difference

(Section 4.2 and 4.3). The result shows that the red/yellow pigment ratio decreases with a longer growth time from 70 to 112 days of growth (Figure 4.10 B).

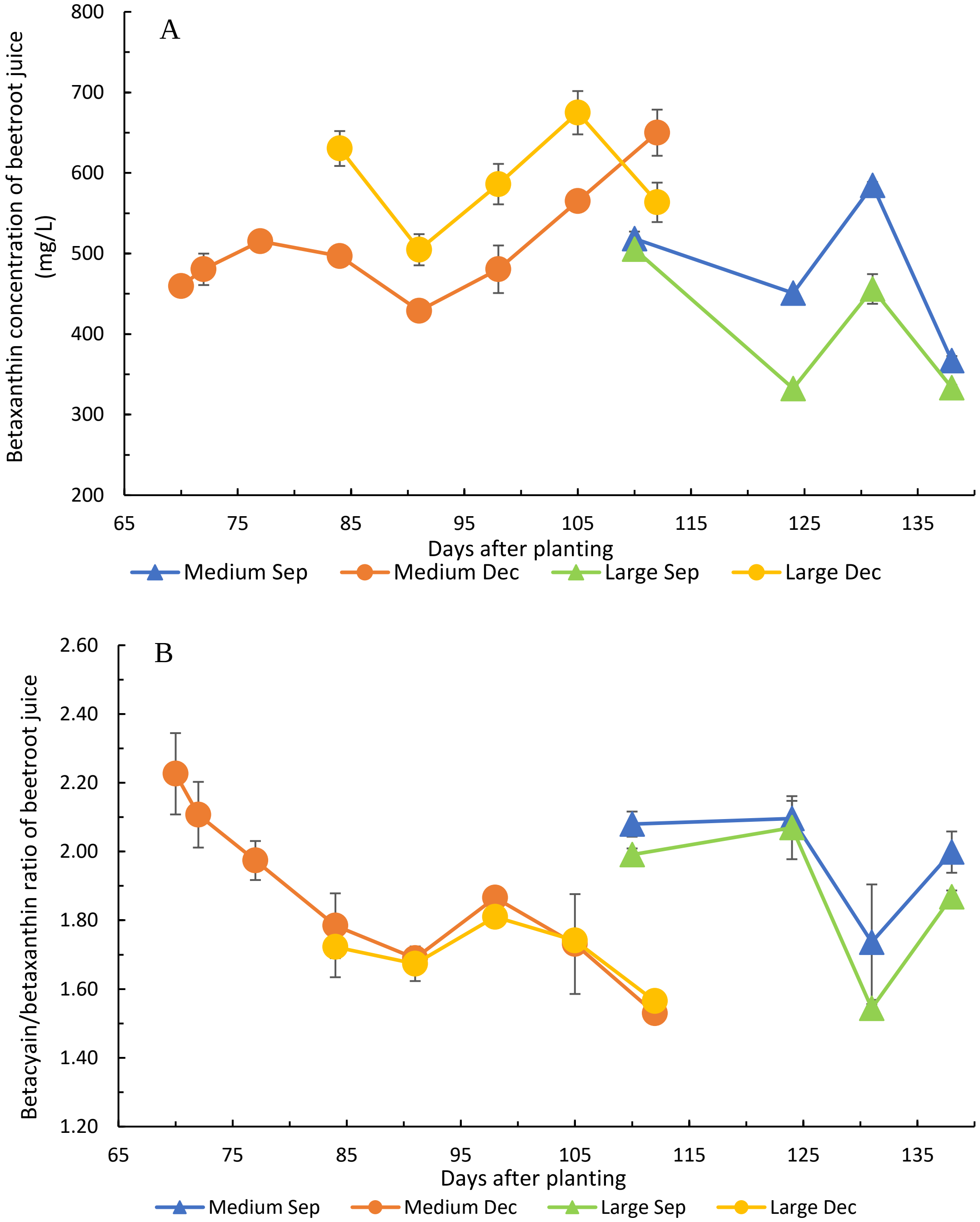


Figure 4.10 Betaxanthin concentration (mg/L) (A) and Betacyanin/betaxanthin ratio (B) in juice extracted from medium and large Beta vulgaris 'Pablo' beetroot, harvested on different days after planting (September and December 2018) (Each data point = Mean ± standard error of mean, n=9). All the results were standardised to 10°Brix to allow comparison between the different samples.

4.12 Nitrate and Nitrite Concentrations in Beetroot Juice

Nitrate and nitrite concentrations were quantified in the juices extracted from beetroot harvested after different growth times (described in Section 3.3.9) and the results are shown in Figures 4.11 A and B. The mean nitrate concentration in extracted juice from the beetroot planted in September varied between 618 ± 6 and 1123 ± 1 mg/L (Figure 4.11 A). The mean nitrate concentration in juice from medium beetroot grown for 138 days was significantly lower than that from beetroot harvested after 110 days growth ($P<0.05$), a reduction of 310 mg/L, while no significant difference in nitrate concentration was found for large beetroot ($P\geq0.05$) (Figure 4.11 B).

For beetroot received from the December planting, the mean nitrate concentration in extracted juice varied between 1200 ± 20 and 2878 ± 58 mg/L (Figure 4.11 A). There was a significant drop in nitrate concentration in beetroot juice from day 91 (1656 mg/L) to day 105 (1200 mg/L) for medium 'Pablo' beetroot ($P<0.05$). For the large beetroot harvested from the December planting the nitrate concentration generally decreased from 2214 mg/L on day 84 to 1613 mg/L on day 112, with an anomalous increase at day 91.

Although the nitrate concentrations in juice from the beetroot harvested from the September (day 110) planting were significantly lower ($P<0.05$) than that from the December planting (day 112) for both medium and large beetroot (Figure 4.11 A and B), as mentioned in Section 4.2 and 4.3, if the beetroot from the December planting were grown for longer, they may have reached a similar size to the September planting. Therefore, overall the nitrate content in beetroot may decrease with a longer growing period (day 91 to day 138) (Figure 4.11 A and B) ($P<0.05$).

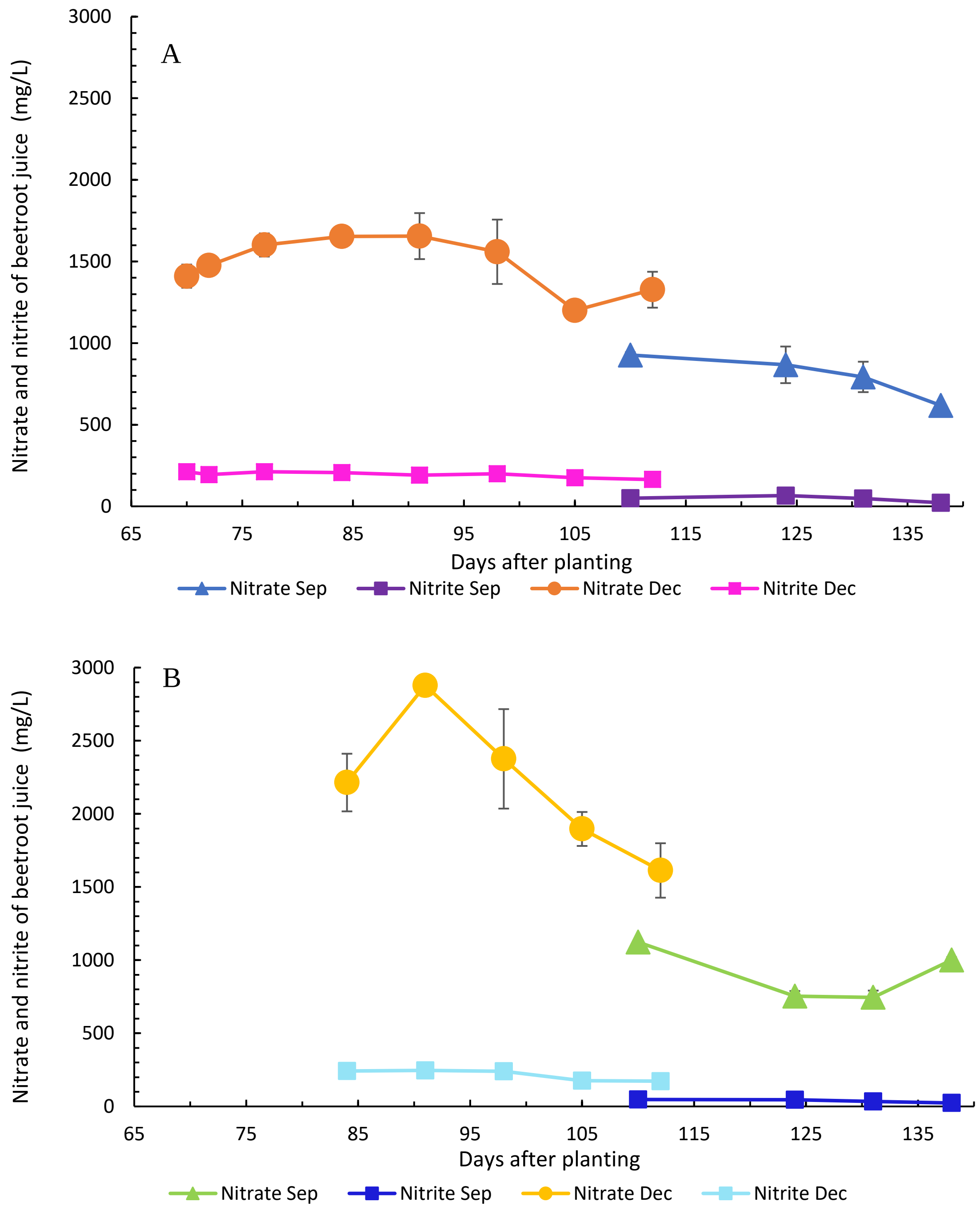


Figure 4.11 Nitrate and nitrite concentrations in juices extracted from medium (A) and large (B) *Beta vulgaris* 'Pablo' beetroot, harvested on different days after planting (September and December 2018) (Each data point = Mean ± standard error of mean, n=9). All the results were standardised to 10°Brix to allow comparison between the different samples.

For the medium beetroot harvested from the December planting, the mean nitrite concentration of juice gradually decreased from 211 mg/L on day 70 to 164 mg/L on day 112 ($P<0.05$). For the large beetroot, a significant drop in the nitrite concentration ($P<0.05$) in beetroot juice was found between day 84 (214 mg/L) and day 112 (151 mg/L). For the beetroot from the September planting, the nitrite concentration of beetroot juice from both medium and large beetroot also gradually dropped ($P<0.05$), from 50 mg/L on day 110 to 22 mg/L on day 138 for medium beetroot, and from 47 mg/L on day 110 to 23 mg/L on day 138 for large beetroot. Similarly, the nitrite concentration in beetroot harvested from the September planting (day 110) was significantly lower ($P<0.05$) than that from the December planting (day 112). Therefore, overall the nitrite content in beetroot decreased with a longer growth period (day 91 to day 138) (Figure 4.11 A and B) ($P<0.05$).

In summary, as the harvest time progressed the nitrate and nitrite levels decreased while the nitrite level also decreased with time. The optimum time to harvest ‘Pablo’ beetroot for high nitrate content in the extracted juice appeared to be between 84 and 98 days after planting.

4.13 Temperature and Rainfall During the Growth of the Beetroot

All the temperature and rainfall data were collected from the Lovett Family Farms paddock weather station. Figure 4.12 shows the daily air temperature at 6.00 am and 2.00 pm each day from October 2018 to April 2019, these times were chosen as they generally corresponded to the minimum and maximum temperatures each day. The weather data covers the growth period of the beetroot harvested from both the September (October to December) and December plantings (December to March). As can be seen in Figure 4.12, the temperature increased slowly from October 2018 to January 2019 and then decreased in April 2019. The highest temperature was 31.1°C in January 2019 at 2 pm while the lowest temperature was 2.8°C in April 2019 at 6.00 am.

Figure 4.13 shows the sum of the monthly rainfall during the beetroot growing period for the September and December plantings. The maximum precipitation occurred in November 2018 (165 mm) and then in April 2019 (195 mm). The precipitation in March 2019 was less than 1 mm. The total rainfall from October to December 2018 (262 mm) was 30 % higher than that from December 2018 to March 2019 (184 mm). This indicated that there was more rainfall during the growth period of beetroot harvested from the September planting than that from the December planting as mentioned in Section 4.4.

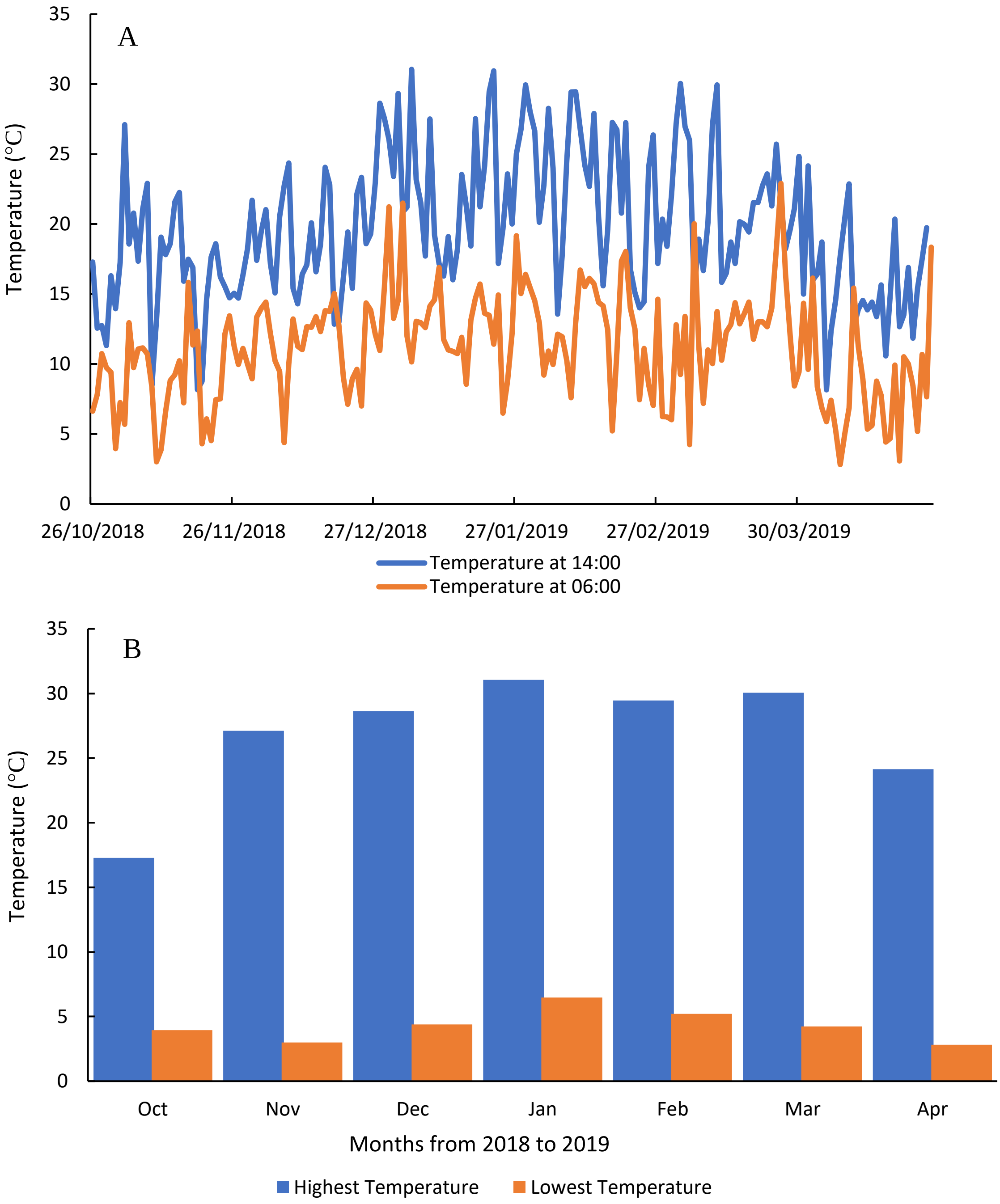


Figure 4.12 (A) Daily air temperature (°C) at 06:00 and 14:00 from October 2018 to April 2019 recorded at Lovett Family Farms. (B) Monthly highest and lowest temperature (°C) from October 2018 to April 2019 recorded at Lovett Family Farms, Ashburton, New Zealand.

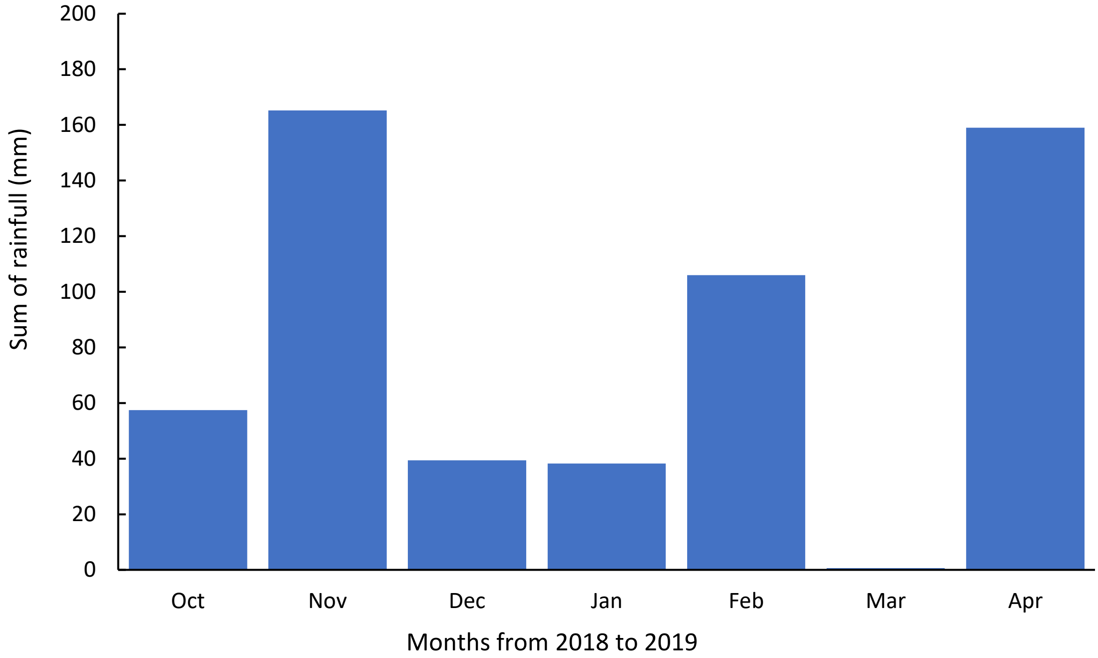


Figure 4.13 Sum of monthly rainfall (mm) from October 2018 to April 2019 recorded at Lovett Family Farms, Ashburton, New Zealand.

4.14 Effects of Growing under Fleece on Beetroot Composition

Lovett Family Farms planted additional 'Pablo' beetroot in the September planting and grew them with the beets covered by fleece to see if there were any significant differences between the beetroot grown under fleece and those which were not grown under fleece. The present study analysed size, weight, DM, % soluble solids, pH, TA, TPC, sucrose concentration, betacyanin and betaxanthin concentrations, nitrate and nitrite concentrations of the covered beetroot/beetroot juice (Section 3.3.2 to 3.3.9). The beetroot grown under fleece were planted at the same time as the uncovered beetroot in the September 2018 planting, and were received as described in Section 3.1.

As the farmer only harvested the beetroot grown under fleece on two harvest dates, days 124 and 131 after planting from the September planting for this project, the data collected for the present study to compare the quality difference between beetroot grown under fleece and not under fleece was not sufficient to draw any definite conclusions because only two data points were collected. More data points (different days after planting) would need to be collected in a further study to determine the effect of beetroot grown under fleece versus not under fleece on beetroot composition.

The results are shown in Table 4.1. For medium beetroot, both the sucrose and betacyanin concentrations in the juice extracted from beetroot grown under fleece were significantly lower ($P<0.05$) than those that were not grown under fleece on both harvest days (day 124 and day 131). Interestingly, for both medium and large beetroot the nitrate concentration in the juice extracted from beetroot grown under fleece were significantly higher ($P<0.05$) (~ two times higher) than those not grown under fleece (both harvest days: day 124 and day 131). The nitrite concentrations in the juice from medium beetroot grown for 124 and 131 days under fleece were significantly lower ($P<0.05$) than those not grown under fleece, but only on day 124 for large beetroot (Table 4.1). This may indicate that the beetroot grown under fleece had a higher nitrate content and lower nitrite content than those not grown under fleece, but this information needs to be confirmed in a further study.

Table 4.1 The size, weight, DM, % soluble solids, pH, TA, TPC, sucrose concentrations, betacyanin and betaxanthin concentrations, nitrate and nitrite concentrations of medium/large beetroot grown under fleece or not from the September planting.

	Day 124		**Day 131**	
Medium beetroot	**Not under fleece**	**Under fleece**	**Not under fleece**	**Under fleece**
Size (cm)	6.48 ± 0.21[a]	6.34 ± 0.14 [a]	6.91 ± 0.21 [a]	6.93 ± 0.17 [a]
Weight (g)	156.74 ± 9.44 [a]	162.58 ± 8.98 [a]	191.95 ± 17.36 [a]	185.70 ± 12.56 [a]
DM (%) (w/w fresh weight)	12.57 ± 0.22 [a]	11.02 ± 0.22 [b]	11.26 ± 0.29 [ab]	11.10 ± 0.50 [b]
Percentage of soluble solids (ºBrix)	10.8 ± 0.2 [a]	10.1 ± 0.0 [b]	10.2 ± 0.2 [b]	9.9 ± 0.1 [b]
pH	6.40 ± 0.02 [a]	6.28 ± 0.02 [b]	6.32 ± 0.02 [b]	6.29 ± 0.02 [b]
TA (%) (w/v as malic acid)	0.08 ± 0.00 [b]	0.08 ± 0.01 [b]	0.09 ± 0.00 [ab]	0.10 ± 0.00 [a]
TPC (GAE mg/mL)	0.99 ± 0.02 [b]	0.87 ± 0.04 [bc]	0.72 ± 0.02 [c]	1.59 ± 0.08 [a]
Sucrose (g/L)	83.27 ± 0.56 [a]	68.61 ± 1.14[b]	82.8 ± 4.8 [a]	66.62 ± 3.29 [b]
Betacyanins (mg/L)	945.03 ± 6.46[a]	835.67 ± 29.10[b]	981.63± 40.07[a]	703.87 ±16.11[c]
Betaxanthins (mg/L)	450.99 ± 2.92[b]	468.32 ± 19.92[b]	584.55 ± 4.31[a]	445.42 ± 2.12[b]
Nitrate (mg/L)	866.90 ± 112.14 [c]	1965.87 ± 12.06 [a]	792.82 ± 93.18 [c]	1597.92 ± 77.42 [b]
Nitrite (mg/L)	66.09 ± 2.53 [a]	37.18 ± 1.10 [c]	48.26 ± 3.72 [b]	26.69 ± 1.21 [c]
Large beetroot	**Not under fleece**	**Under fleece**	**Not under fleece**	**Under fleece**
Size (cm)	8.59 ± 0.17 [a]	8.68 ± 0.21 [a]	8.73 ± 0.21 [a]	8.37 ± 0.28 [a]
Weight (g)	380.39 ± 26.25 [a]	358.49 ± 26.13 [a]	370.66 ± 18.95 [a]	324.74 ± 25.65 [a]
DM (%) (w/w fresh weight)	11.37 ± 0.27 [a]	10.94 ± 0.26 [a]	11.13 ± 0.27 [a]	10.42 ± 0.80 [a]
Percentage of soluble solids (ºBrix)	11.5 ± 0.1 [a]	10.6 ± 0.1 [b]	10.0 ± 0.1 [c]	10.0 ± 0.2 [bc]
pH	6.41 ± 0.03 [a]	6.36 ± 0.02 [ab]	6.35 ± 0.01 [ab]	6.28 ± 0.03 [b]
TA (%) (w/v as malic acid)	0.07 ± 0.00 [c]	0.07 ± 0.00 [bc]	0.09 ± 0.00 [ab]	0.09 ± 0.00 [a]
TPC (GAE mg/mL)	0.66 ± 0.02 [b]	0.77 ± 0.02 [b]	0.99 ± 0.05 [a]	1.18 ± 0.10 [a]
Sucrose (g/L)	73.12 ± 2.05 [a]	58.46 ± 2.9 [b]	71.96 ± 1.61 [a]	68.37 ± 1.82 [a]
Betacyanins (mg/L)	687.83 ± 12.18 [a]	575.42 ± 17.10 [a]	588.41 ± 87.16 [a]	596.50 ± 9.03 [a]
Betaxanthins (mg/L)	332.43 ± 3.63 [c]	323.26 ± 10.24 [c]	455.98 ± 18.42 [a]	380.34 ± 11.02 [b]
Nitrate (mg/L)	752.80 ± 36.20 [b]	1707.64 ± 26.55 [a]	745.07 ± 46.96 [b]	1511.46 ± 162.90 [a]
Nitrite (mg/L)	45.63 ± 3.44 [a]	29.49 ± 1.62 [b]	33.43 ± 1.68 [b]	31.53 ± 1.75 [b]

Note: For medium or large beetroot, values in each row that do not share the same letter (superscripts) were significantly different ($P<0.05$). All the results of TA, TPC, sucrose, betacyanin, betaxanthin, nitrate and nitrite were standardised to 10°Brix to allow comparison between the different samples. Results are expressed as mean ± standard error of mean, n=9.

4.15 Effects of Postharvest Storage Time on Beetroot Composition

Three extra batches (18 × 3) of large *Beta vulgaris* 'Pablo' beetroot harvested on 91 days after planting from the December planting 2018 were stored at 4 ± 1°C for 21 days under dark conditions (Section 3.2.3). Day 91 was considered as the first day of storage (Day 0), with the following sampling dates being Day 7, 14 and 21 under storage. The following parameters for the beetroot were monitored: size, weight and DM, and for beetroot juice extracted after storage: % soluble solids, pH, TA, TPC, sucrose concentration, betacyanin and betaxanthin concentrations, nitrate and nitrite concentrations (Section 3.3.2 to 3.3.9). The results are shown in Table 4.2.

The results indicate that there was no significant difference in the weight for the four batches of beetroot for storage ($P \geq 0.05$). After the 21-day postharvest storage in the dark at 4 ± 1°C, the % soluble solids, TPC, sucrose content, betaxanthin content, nitrate and nitrite content of beetroot were not significantly different ($P \geq 0.05$). However, the pH of the beetroot juice decreased significantly ($P < 0.05$) during storage, while the TA of beetroot juice gradually increased, although this increase was not significant ($P \geq 0.05$). In general, the quality of the beetroot taproot did not change significantly throughout the 21-day storage period**.**

Table 4.2 The size, weight, DM, % soluble solids, pH, TA, TPC, sucrose concentration, betacyanin and betaxanthin concentrations, nitrate and nitrite concentrations of medium/large beetroot stored at 4 ± 1°C under dark.

	Day 0	**Day 7**	**Day 14**	**Day 21**
Size (cm)	7.77 ± 0.14 ^{b}	8.51 ± 0.27 ^{ab}	7.96 ± 0.11 ^{ab}	8.41 ± 0.19 ^{a}
Weight (g)	212.65 ± 11.77^{a}	276.30 ± 25.71 ^{a}	211.48 ± 13.51 ^{a}	265.17 ± 20.57 ^{a}
DM (%) (w/w fresh weight)	12.67 ± 0.30^{b}	12.96 ± 0.18^{ab}	13.73 ± 0.20^{a}	11.60 ± 0.13^{c}
Percentage of soluble solids (ºBrix)	9.2 ± 0.3 ^{bc}	8.8 ± 0.6 ^{c}	10.9 ± 0.4 ^{a}	10.3 ± 0.2 ^{ab}
pH	6.39 ± 0.01 ^{a}	6.31 ± 0.02 ^{b}	6.18 ± 0.01 ^{c}	6.14 ± 0.00 ^{c}
TA (%) (w/v as malic acid)	0.097 ± 0.003 ^{a}	0.102 ± 0.003 ^{a}	0.101 ± 0.002 ^{a}	0.106 ± 0.003 ^{a}
TPC (GAE mg/mL)	1.22 ± 0.07 ^{b}	1.95 ± 0.12 ^{a}	1.27 ± 0.07 ^{b}	1.56 ± 0.14 ^{ab}
Sucrose (g/L)	70.37 ± 3.82 ^{a}	69.85 ± 1.61 ^{a}	66.16 ± 4.41 ^{a}	63.48 ± 2.82 ^{a}
Betacyanins (mg/L)	846.32 ± 38.67 ^{c}	1266.67 ± 70.97 ^{a}	889.73± 80.63 ^{bc}	1071.87 ± 13.12 ^{ab}
Betaxanthins (mg/L)	504.67 ± 19.37 ^{b}	741.16 ± 45.57 ^{a}	519.77 ± 56.12 ^{b}	613.83 ± 14.64 ^{ab}
Nitrate (mg/L)	2878 ± 58 ^{a}	2302 ± 60 ^{b}	1673 ± 91 ^{c}	2697 ± 134 ^{a}
Nitrite (mg/L)	246 ± 9 ^{a}	213 ± 9 ^{ab}	182 ± 10 ^{b}	221 ± 8 ^{a}

Note: the value shared the same superscript letter across each row were not statistically significant (P≥0.05). All the results of TA, TPC, sucrose concentration, betacyanin and betaxanthin concentrations, nitrate and nitrite concentrations were standardised to 10°Brix to allow comparison between the different samples. Results are expressed as mean ± standard error of mean, n=9.

4.16 Discussion

4.16.1 Effects of Growth Period on Beetroot DM

The results indicated that for the December planting, in beetroot grown for between 70 and 112 days, the DM (%) ($^{w}/_{w}$ fresh weight) varied between 11 and 13 %. For the September planting, in beetroot grown for 110 to 138 days, the DM varied between 12 and 16 % (Section 4.4). As stated in Section 4.4 it is possible that the larger beetroot contained more moisture as they took up more water from the soil and as reported there was more rainfall between October and December 2018.

Barba-Espín et al. (2017) found there was a significant increase in DM (%) ($^{w}/_{w}$ fresh weight) between black carrot roots grown for seven weeks versus sixteen weeks (from 7 to 9 %), after which the DM remained constant up to thirty-five weeks of growth. Similar results have been found for four beetroot cultivars ('Boro F1', 'Czerwona Kula', 'Nochowski' and 'Regulski Cylinder') (Łukaszewska & Gawęda, 2014), which increased in DM (%) ($^{w}/_{w}$ fresh weight) from 10 to 16 % between six and fifteen weeks after planting (approximately 42 to 105 days of growth), possibly because these four cultivars were still able to accumulate total solids in the roots during this period. If grown for longer they may have stopped accumulating total solids in a similar way to the black carrot roots.

Judson, Mckenzie, Robinson, Nicgills, and Moorhead (2016) also found that the DM (%) ($^{w}/_{w}$ fresh weight) decreased as the beetroot weight increased. This decrease of DM may be explained in two ways: firstly, as the beetroot matures, the roots grow larger and deeper in the soil, enabling more moisture to be absorbed from the soil (Taiz & Zeiger, 2010). Secondly, as the beetroot grow bigger, the mature cells in the beetroot have larger central vacuoles which can store more absorbed moisture (Taiz & Zeiger, 2010). These two factors together (more efficient root hairs and larger vacuoles in the cells) may assist larger beetroot to store more moisture.

4.16.2 Effects of Growth Period on Beetroot TPC

The results showed that for the December planting, the TPC of juice extracted from the medium beetroot grown for up to 112 days of growth (approximately decreased by 0.557 GAE mg/ml) had less TPC than that harvested after 70 days of growth. For large beetroot the TPC decreased from 1.666 to 1.430 GAE mg/ml from 84 to 112 days of growth. For the September planting, after 110 to 138 days of growth, the TPC in beetroot juice for both medium and large beetroot remained relatively constant (Section 4.8). These results were similar to that for a potato study (Reyes et al., 2004), which reported that the reduction of TPC in purple/red potatoes from on day 88 to on day 109 was around 30 to 50 mg/100 g tissue depending on the cultivars. After this period the TPC in the potatoes remained relatively constant (until day 156).

When comparing with other higher phenolic containing plants such as berries, a study found the mean TPC of cranberry, blueberry and elderberry juices were 882, 1524 and 2205 GAE mg/L respectively (Granato, Karnopp, & Ruth, 2015). Comparing to the present study, the TPC of the beetroot juice from the two plantings varied between 0.66 and 1.73 GAE mg/ml which corresponded to 660 to 1730 GAE mg/L, which was similar to the TPC of cranberry and blueberry juice but lower than the elderberry juice.

4.16.3 Effects of Growth Period on Sugar Concentration in Beetroot

In the present study, the sucrose concentration of juice from beetroot grown for between 70 and 138 days varied between 66.75 and 90.76 g/L (Section 4.9). This is similar to the sucrose concentration of beetroot juice extracted from seven beetroot cultivars which varied between 56.3 and 86.9 g/L (Wruss et al., 2015). However, in contrast to this study, the Wruss et al. (2015) also reported the presence of fructose (1.26 to 4.04 g/L) and glucose (0.58 to 2.96 g/L) in the extracted beetroot juice, with the concentration being cultivar dependent.

There are limited published studies on the effect of growth time on beetroot sucrose content. One beetroot study which also utilized the ‘Pablo’ cultivar harvested one batch of beetroot and divided them into three stages based on weight: Stage I (weight less than 50 g), stage II (weight between 50 and 150 g), stage III (weight larger than 150 g) and measured the sucrose content in beetroot from each of these three stages (Montes-Lora et al., 2018). Their results showed that the sucrose in the beetroot increased from 20.32 g/100 g in stage I to 44.14 g/100 g in stage II, and then remained constant in stage III. In this research at day 70 the beetroot were on average 93.05 ± 5.74 g and similar to stage II in the Montes-Lora et al (2018) study, in which the sugar content did not increase significantly at this weight.

4.16.4 Effects of Growth Period on Beetroot Betacyanin and Betaxanthin Concentrations

In the present study, for beetroot grown between 70 and 138 days, the red pigment (betacyanin) in both medium and large beetroot fluctuated with growth time, while the yellow pigment (betaxanthin) in medium beetroot significantly increased from 459 to 650 mg/L from day 70 to 112 (Section 4.10 and 4.11).

Similar results have been found in another beetroot study, with the levels of the red pigment fluctuating significantly between six weeks (42 days) (0.57 to 1.03 mg/g fresh weight) and fifteen weeks of growth (105 days) (0.75 to 0.91 mg/g fresh weight) depending on the cultivar ('Boro F1', 'Czerwona Kula', 'Nochowski' and 'Regulski Cylinder') (Łukaszewska & Gawęda, 2014). They also found the yellow pigment in beetroot ('Boro F1', 'Czerwona Kula', and 'Regulski Cylinder') increased between six weeks after planting (42 days) (0.06 to 0.11 mg/g fresh weight) and fifteen weeks after planting (105 days) (0.29 to 0.31 mg/g fresh weight) again varying by cultivar (Łukaszewska & Gawęda, 2014).

In addition, the peak harvest period for betacyanins and betaxanthins was also important. In the current study, the concentrations of betacyanin and betaxanthin in beetroot juice reached a peak (1176 mg/L for betacyanins, 675 mg/L for betaxanthins) in large beetroot harvested 105 days after planting (Section 4.10 and 4.11). This is in agreement with another study of beetroot juice which reported peak betacyanin and betaxanthin concentrations of 990 mg/L and 590 mg/L respectively, in beetroot grown for 100 days (Michalik and Grzebelus,1995).

4.16.5 Effect of Growth Period on Beetroot Nitrate and Nitrite Content

The results presented in this chapter found a significant decreasing trend between the nitrate content in beetroot and length of growth period (91 to 138 days of growth). Based on the current results the recommended harvest time for obtaining the highest nitrate content in beetroot was between 85 and 98 days after planting (Section 4.12).

Similar to the present study, the nitrate content in carrots and cabbages have been reported to decrease with a longer growing times (Corre & Breimer, 1979; Geyer, 1978). Nitrate accumulation in plants depends on their nitrate absorption rate and the nitrate reduction rate of the plants (Sidhu et al., 2011; Taiz & Zeiger, 2010). The plants keep absorbing nitrate from the soil when they are at the early stages of growth, and the rate of nitrate absorption is greater than the rate of nitrate reduction in young plants, thus, nitrate accumulates in young plants (Corre & Breimer, 1979). However, once the plant has absorbed sufficient nitrate for growth, less is needed for the plant, and the nitrate absorption rate may decrease. The accumulated and stored nitrate may then be reduced to amino acids which are used for building proteins, and hence the nitrate content in the plant decreases (Corre & Breimer, 1979; Darwinkel, 1975; Nacry, Bouguyon, & Gojon, 2013; Sidhu et al., 2011).

The current results are in contrast to other studies which reported that the nitrate content in seven beetroot cultivars ('DZE 1 F1', 'DZE 2 F1', 'DZE3 F1', 'Czerwona K', 'Crosby', 'Okragly C', 'Egipski') increased significantly from 2202 to 2666 mg/kg between 100 and 140 days after planting (Michalik & Grzebelus, 1995). However, Michalik & Grzebelus (1995) explained this was likely due to the extremely dry and hot weather conditions (often above 30°C) during the growth period of the beetroot, as during hot weather NR activity is inhibited in the beetroot thus leading to a higher nitrate content (Michalik & Grzebelus, 1995) (Section 2.4). When comparing the weather conditions with the present study, as mentioned in Section 4.13, although the temperature gradually increased from October 2018 to January 2019 and then began to decrease, the day time temperature fluctuated between 10°C and 30°C and was lower than the temperature from the study of Michalik & Grzebelus (1995). The difference in weather conditions between the present study and the study of Michalik & Grzebelus (1995) may explain the contrasting results as nitrate in plants are strongly affected by weather (Michalik & Grzebelus, 1995; Tapio, Liisa, & Raili, 1992; Ugrinovic, 1999) (Section 2.10). No published research has been found with respect to the effects of harvest time on beetroot nitrite content.

4.16.6 Effects of Growing under Fleece on Beetroot Composition

The nitrate concentration of the juice from medium and large beetroot grown under fleece were higher (by 766 to 1099 mg/L) than that from the beetroot which were grown normally with no fleece covering. In contrast, the nitrite concentration of the juice from medium and large beetroot grown under fleece was less (by 2 to 29 mg/L) than that from the beetroot which were not under fleece during growth (Section 4.14). This difference is probably because darkness inactivates NR, which normally would convert nitrate to nitrite in the beetroot, thus resulting in a higher nitrate and a lower nitrite content in the beetroot grown under fleece (Riens & Heldt, 1992; Taiz & Zeiger, 2010) (Section 2.2.1). Riens and Heldt (1992) found that the NR activity (%) in the spinach leaves rapidly decreased by 15 % when the spinach leaves were exposed to the dark for two minutes.

4.16.7 Effects of Postharvest Storage on Beetroot Composition

In the present study, during the 21-day storage period in darkness at 4 ± 1°C, the % soluble solids, TPC, sucrose content, betaxanthin content, nitrate and nitrite content of beetroot were not significantly different ($P \geq 0.05$). Similar results were found from a carrot study by Lee, Yoo, and Patil (2011), who found the total sugars in carrots did not change significantly after a 28-day cold storage in the dark (4°C). Lee et al. (2014) also suggested that it is better to store commercial carrots for less than 14 days of cold storage (4°C) as the total carotenoid in carrots does not decrease during this period.

4.17 Conclusions

During the sampling period for beetroot collected for this research, the beetroot became larger and the TA of the beetroot juice increased as the growing period lengthened (70 to 112 days of growth) while the pH decreased at the same time. The TPC in beetroot dropped significantly between 70 and 112 days of growth and then remained constant up to 138 days of growth.

The red betacyanin pigments in beetroot fluctuated during growth (day 70 to day 138) while the yellow pigment increased during the early period of growth (up to day 112) and then slowly decreased. The nitrate content of the beetroot in this study was found to decrease with increasing periods of growth between day 91 and day 138. The peak for betacyanin and betaxanthin content was in beetroot grown for between 105 and 112 days of growth, while the peak for nitrate content is earlier in beetroot harvested between 84 and 98 days of growth.

CHAPTER 5

Chapter 5 Comparison of Three Cultivars of *Beta vulgaris* 'Pablo', 'Monty' and 'Betty'

5.1 Introduction

This chapter aims to compare the effect of growth time on certain beetroot quality parameters in three *Beta vulgaris* cultivars 'Pablo', 'Monty' and 'Betty' which were planted on 16th December 2018 at Lovett Family Farms, Ashburton, New Zealand. The beetroot quality parameters monitored included: size, weight, DM, % soluble solids, pH, TA, TPC, sucrose concentration, betacyanin and betaxanthin concentrations, nitrate and nitrite concentrations (Section 3.3.2 to 3.3.9).

For each cultivar, medium and large beetroot were harvested weekly between 10 and 16 weeks after planting (70 to 112 days after planting) and processed as described in Section 3.2. The results from this chapter may provide information on differences in quality or composition between each beetroot cultivar and length of the growth period. This data may provide some information in selecting the suitable cultivar and/or number of growing days required to yield beetroot with for example high-nitrate content, high betacyanin/betaxanthin content, high total phenolic content or high sugar content.

5.2 Size of Whole Beetroot

The beetroot were harvested between 70 and 112 days of growth for medium beetroot, and between 84 and 112 days of growth for large beetroot, as the beetroot did not meet the size requirements in the first two sampling weeks. No 'Monty' or 'Betty' beetroot were received on day 112 as Lovett Family Farms completed harvesting these cultivars before this sampling day. Figures 5.1 and Figure 5.2 show the medium beetroot grown for 84 days before and after cutting for size measurement.

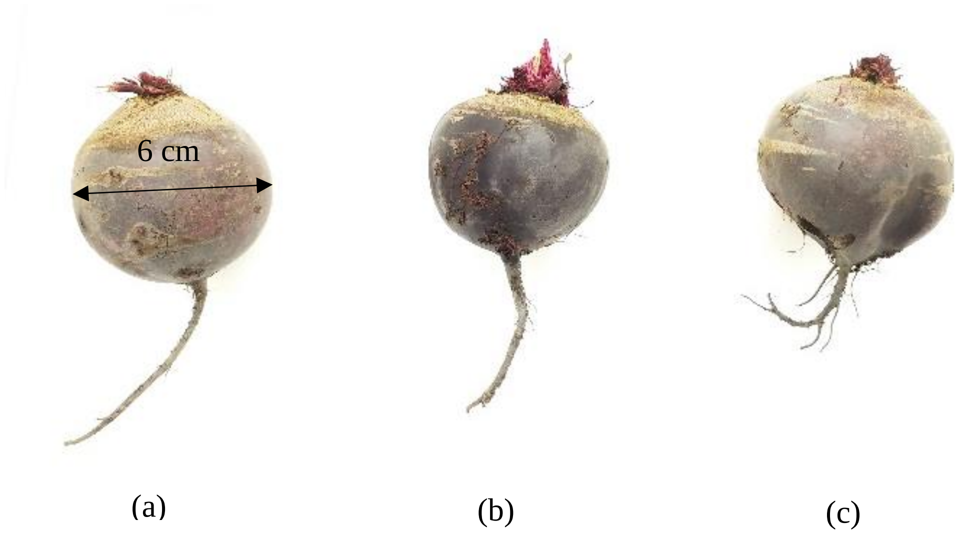


Figure 5.1 Fresh medium *Beta vulgaris* (a) ‘Pablo’ (b) ‘Monty’ and (c) ‘Betty’ harvested 84 days after planting (December 2018).

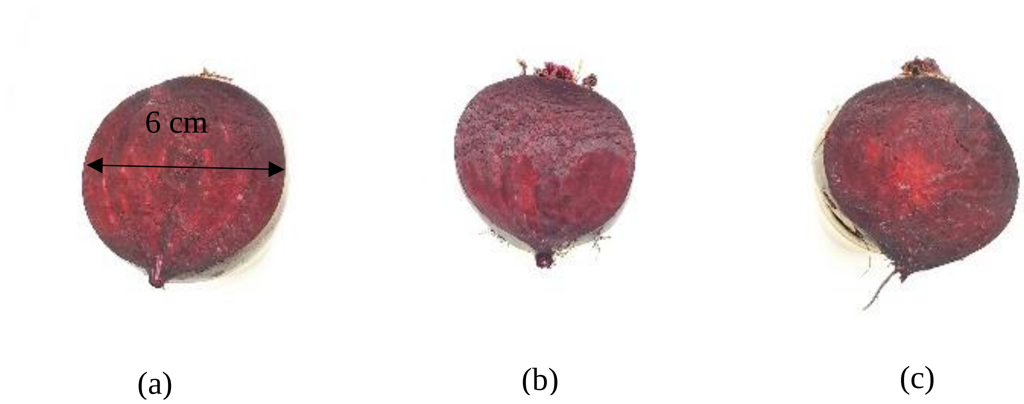


Figure 5.2 Fresh-cut medium *Beta vulgaris* (a) ‘Pablo’ (b) ‘Monty’ and (c) ‘Betty’ harvested 84 days after planting (December 2018).

The average size (diameter) of the beetroot harvested at the end of the sampling period was significantly larger than that at the start for medium ‘Betty’, large ‘Monty’ and large ‘Betty’($P<0.05$) (Figure 5.3). No significant difference in size was found between the initial and the end of the sampling period for medium ‘Pablo’, medium ‘Monty’ or large ‘Pablo’ ($P\geq0.05$) (Figure 5.3). For medium beetroot, no significant difference in size was observed between the three cultivars at the start or at the end of the sampling period ($P\geq0.05$). For large beetroot, large ‘Betty’ beetroot were significantly smaller than large ‘Monty’ at both the start and end of the sampling period (Figure 5.3) ($P<0.05$).

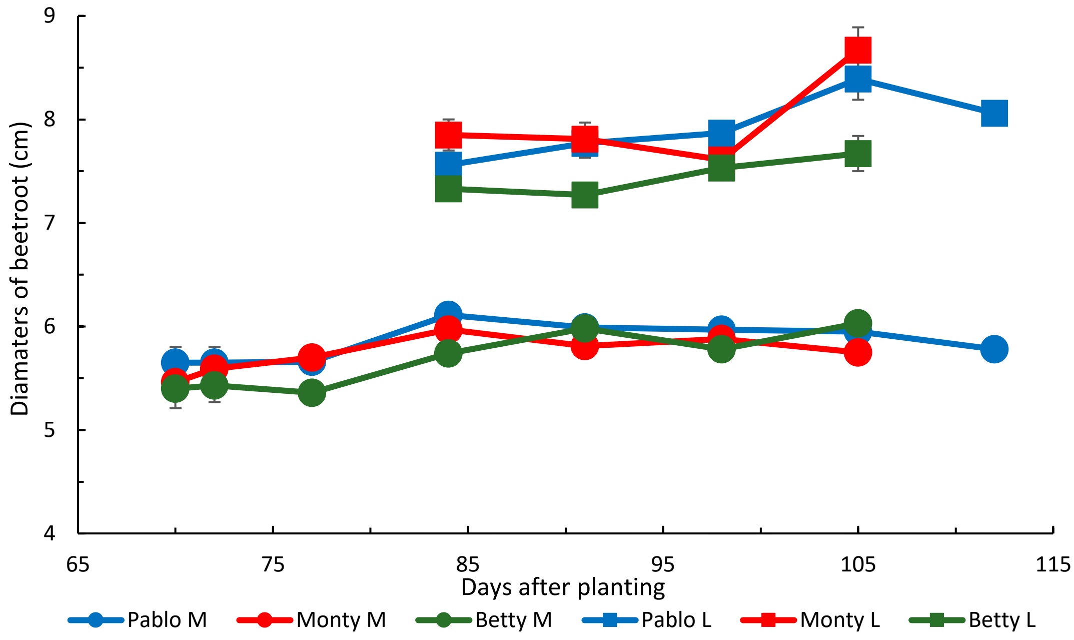


Figure 5.3 Diameter (cm) of medium (M) and large (L) *Beta vulgaris* 'Pablo', 'Monty' and 'Betty', harvested on different days after planting (December 2018) (Each data point = Mean ± standard error of mean, n=18).

5.3 Weight of Whole Beetroot

Medium 'Betty' beetroot harvested after 105 days of growth were significantly heavier (by 23 g) than the medium 'Betty' harvested after 70 days ($P<0.05$). No significant difference in weight was found for medium 'Pablo' and medium 'Monty' ($P\geq0.05$) harvested at the start and end of the sampling period. Large beetroot of all cultivars increased ($P<0.05$) in weight when harvested after 105 days of growth compared to after 84 days, with increases of 89 g, 82 g and 47 g, for 'Pablo', 'Monty' and 'Betty', respectively (Figure 5.4). This showed that the longer the growth time, the greater the weight of the beetroot.

When the weight of the three cultivars were compared with each other over the sampling period, there was no significant difference in weight for medium beetroot between the three cultivars, at the start or at the end of the sampling period ($P\geq0.05$) (Figure 5.4). For the large beetroot of each cultivar, 'Monty' beetroot harvested on day 84, 91 and 105 were significantly heavier by 90 g, 52 g and 120 g ($P<0.05$) than 'Betty' beetroot harvested on the day 84, 91 and 105, respectively.

The results presented for beetroot size (Figure 5.3) and weight (Figure 5.4) indicated that the 'Betty' cultivar was the smallest and lightest of the three cultivars. Furthermore, the 'Monty' beetroot was the heaviest of the three beetroot cultivars over the entire sampling period.

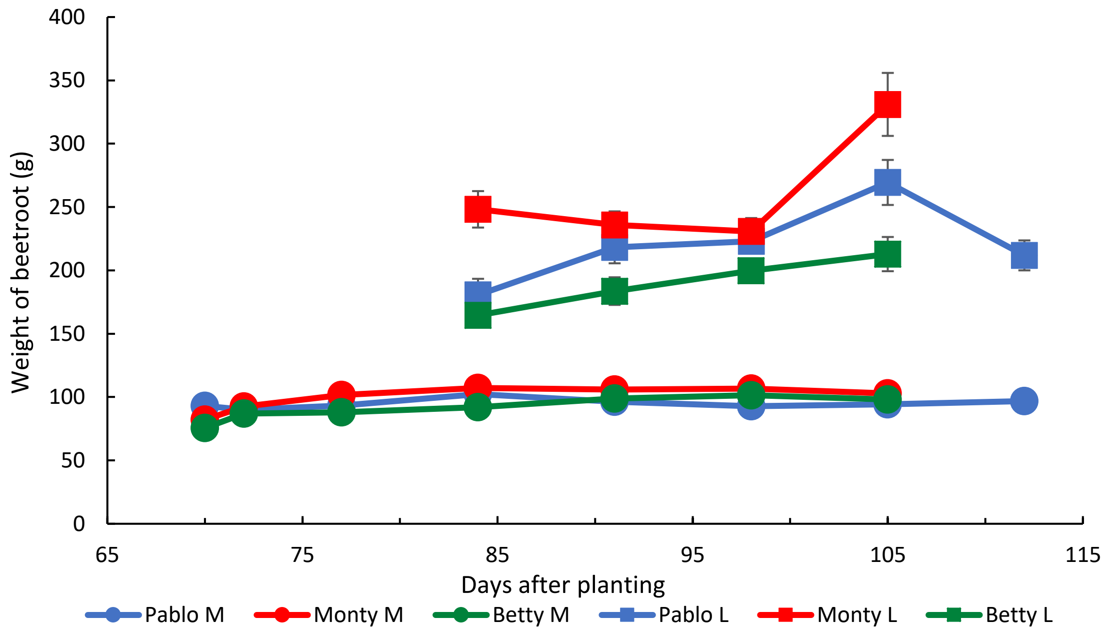
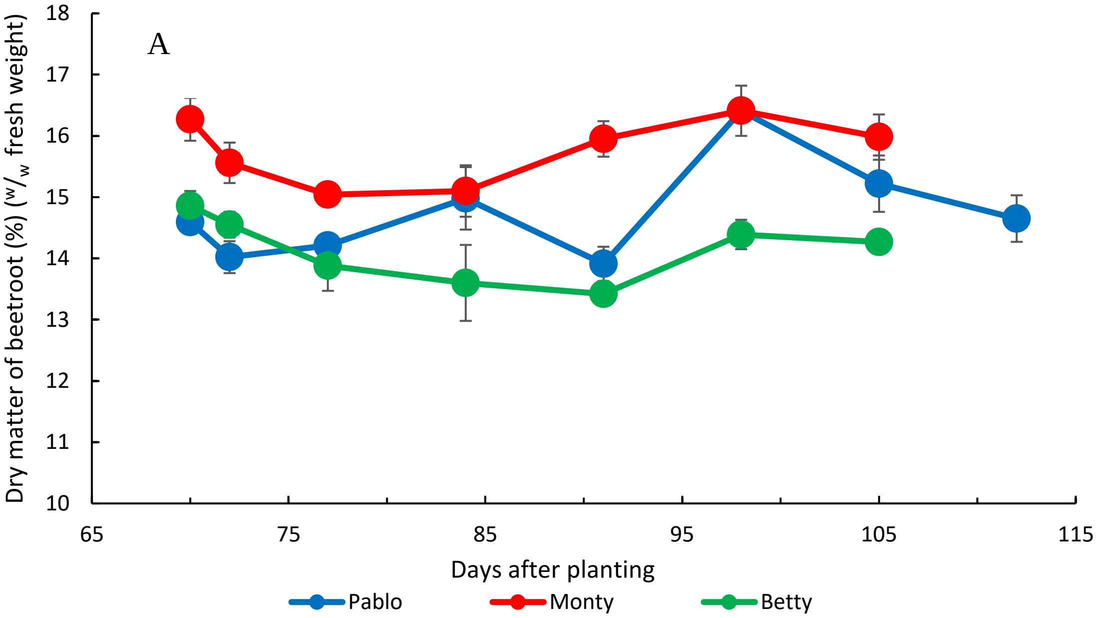


Figure 5.4 Weight (g) of medium (M) and large (L) *Beta vulgaris* 'Pablo', 'Monty' and 'Betty', harvested on different days after planting (December 2018) (Each data point = Mean ± standard error of mean, n=18).

5.4 Dry Matter of Beetroot Flesh

The DM (%) ($^{w}/_{w}$ fresh weight) of medium beetroot of each cultivar did not change significantly during the growth period (P≥0.05), except for the DM of medium 'Pablo' beetroot, where DM of beetroot on day 98 was significantly higher than those on days 72, 77, 91 and 112 (P<0.05), and for the medium 'Monty' beetroot, where DM of beetroot on day 98 was significantly higher than that on day 77 (P<0.05). For the large beetroot of each cultivar, the DM did not change significantly between day 84 and day 105 (P≥0.05), except for large 'Pablo' which had a significant higher DM on day 112 than on days 91, 98 and 105, for large 'Monty' and large 'Betty' which had a significantly higher DM on day 98 than those harvested on day 84 (P<0.05) (Figure 5.5). These results indicated that the DM in fresh beetroot was relatively constant during the growth period from day 70 to day 112, not only for ‘Pablo’ beetroot (Section 4.4), but also for the ‘Monty’ and ‘Betty’ cultivars.

The DM varied between 12.44 ± 0.33 and 16.41 ± 0.14 % for both medium and large ‘Pablo’ beetroot, 14.06 ± 0.57 and 16.41 ± 0.41 % for medium and large ‘Monty’ beetroot, and 11.59 ± 0.74 and 14.86 ± 0.24 % for medium and large ‘Betty’ beetroot. When comparing the DM between the three cultivars, the DM of fresh ‘Monty ‘was significantly higher than that of ‘Pablo’ and ‘Betty’ beetroot for both medium and large beetroot over the entire sampling period ($P<0.05$) (Figure 5.5). The average DM of medium ‘Pablo’, ‘Monty’ and ‘Betty’ were 14.75 %, 15.76 % and 14.14 % respectively. These results imply that the ‘Monty’ cultivar may contain more total solids in the taproot than the other two cultivars.



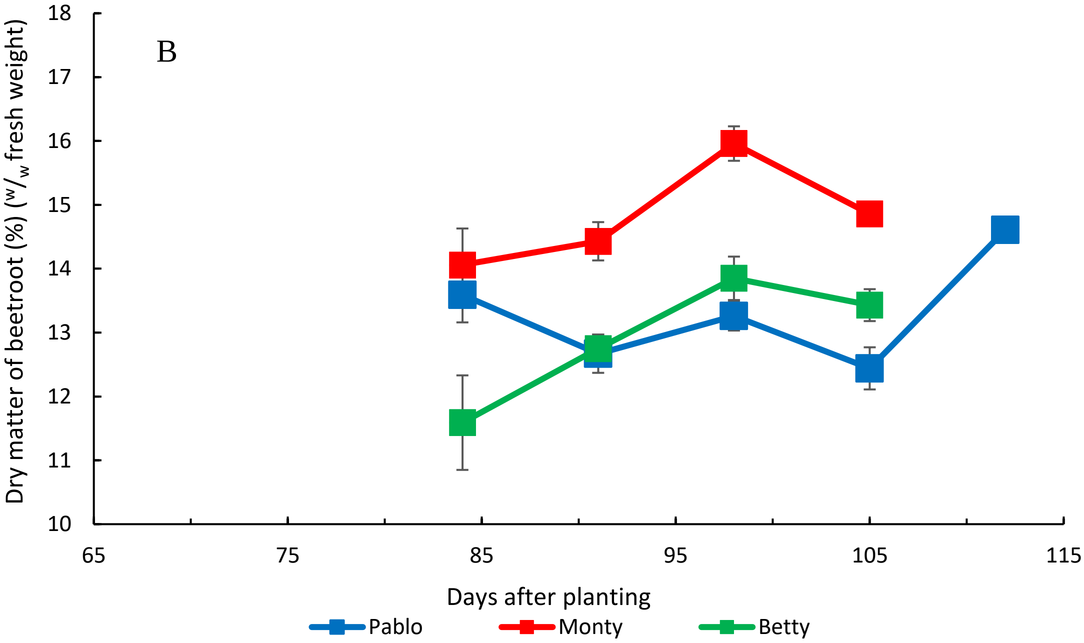


Figure 5.5 Dry matter content (%) ($^{w}/_{w}$ fresh weight) of medium (A) and large (B) *Beta vulgaris* 'Pablo', 'Monty' and 'Betty', harvested on different days after planting (December 2018 Planting) (Each data point = Mean ± standard error of mean, n=9).

5.5 Percentage of Soluble Solids (°Brix) of Beetroot Juice

For each cultivar and each size harvested, no significant difference in % soluble solids (°Brix) of the extracted beetroot juice was found between the first and the last date of the sampling period (P≥0.05) (Figure 5.6). The % soluble solids of the extracted juice from beetroot harvested on day 70 to day 112 varied between 9.2 ± 0.3 and 12.2 ± 0.6 °Brix for the two sizes of 'Pablo' beetroot, between 12.0 ± 0.1 and 13.8 ± 0.6 °Brix for 'Monty' beetroot and between 10.6 ± 0.5 and 11.8 ± 0.1 °Brix for 'Betty' beetroot.

The % soluble solids (°Brix) of extracted juice from the 'Monty' cultivar was significantly higher than that from the other two cultivars on most sampling days for both medium and large beetroot (P<0.05), with the exception of those harvested on day 77, where the % soluble solids (°Brix) of juice from medium beetroot from all three cultivars were the same (P≥0.05) (Figure 5.6 A and B). The % soluble solids (°Brix) of extracted juice from both medium and large 'Pablo' and 'Betty' beetroot were similar on most sampling days (P≥0.05), except on day 91 where the % soluble solids (°Brix) of juice from large 'Betty' beetroot was significantly higher than that from the large 'Pablo' beetroot (P<0.05). The higher % soluble solids of the juice extracted from 'Monty' beetroot supports the suggestion made in Section 5.4 that 'Monty' beetroot contained more total solids and less water in their taproot than the other two cultivars.

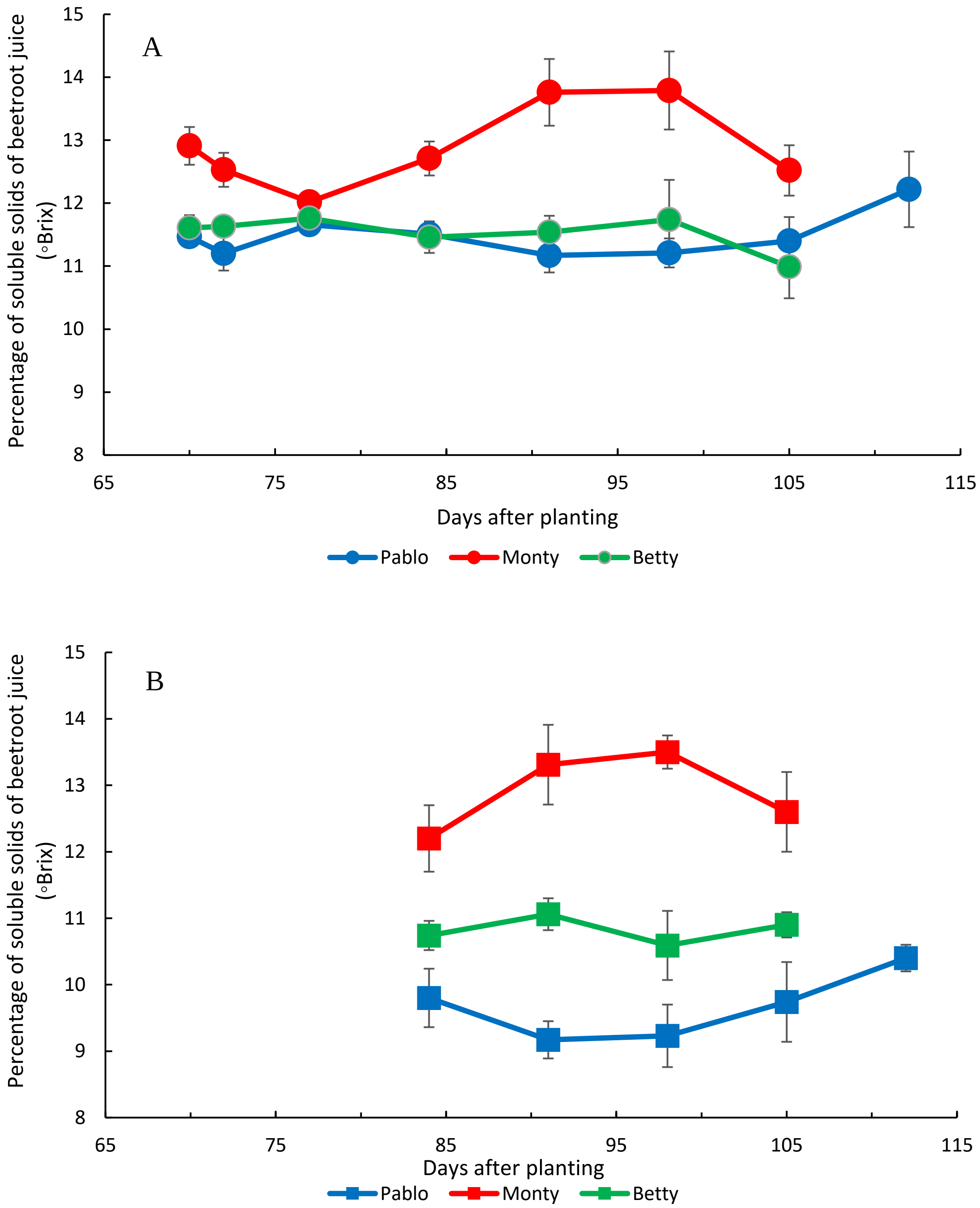


Figure 5.6 Percentage of soluble solids (ºBrix) of beetroot juice from medium (A) and large (B) *Beta vulgaris* ‘Pablo’, ‘Monty’ and ‘Betty’, harvested on different days after planting (December 2018 Planting) (Each data point = Mean ± standard error of mean, n=9).

5.6 pH of Beetroot Juice

The pH of the juice from ‘Pablo’, ‘Monty’ and ‘Betty’ varied between 6.2 ± 0.0 and 6.6 ± 0.0 during the entire sampling period, with the results being similar for the three cultivars (Figure 5.7). The pH of the beetroot juice slowly decreased with increasing growth period (between 70 and 112 days). For medium beetroot from each cultivar, the pH of extracted juice was significantly lower on the final sampling day for ‘Pablo’ (-0.35 units), ‘Monty’ (-0.27 units) and ‘Betty’ (-0.33 units) relative to the pH on the first sampling day (day 70) ($P<0.05$) (Figure 5.7). For large beetroot, there was a significant drop in the pH of the juice between the start and the end of the sampling period with ‘Pablo’ decreasing by 0.17 units and ‘Monty’ decreasing by 0.19 units ($P<0.05$). The pH of juice from large ‘Betty’ did not change significantly ($P\geq0.05$) between the start and end of the sampling period.

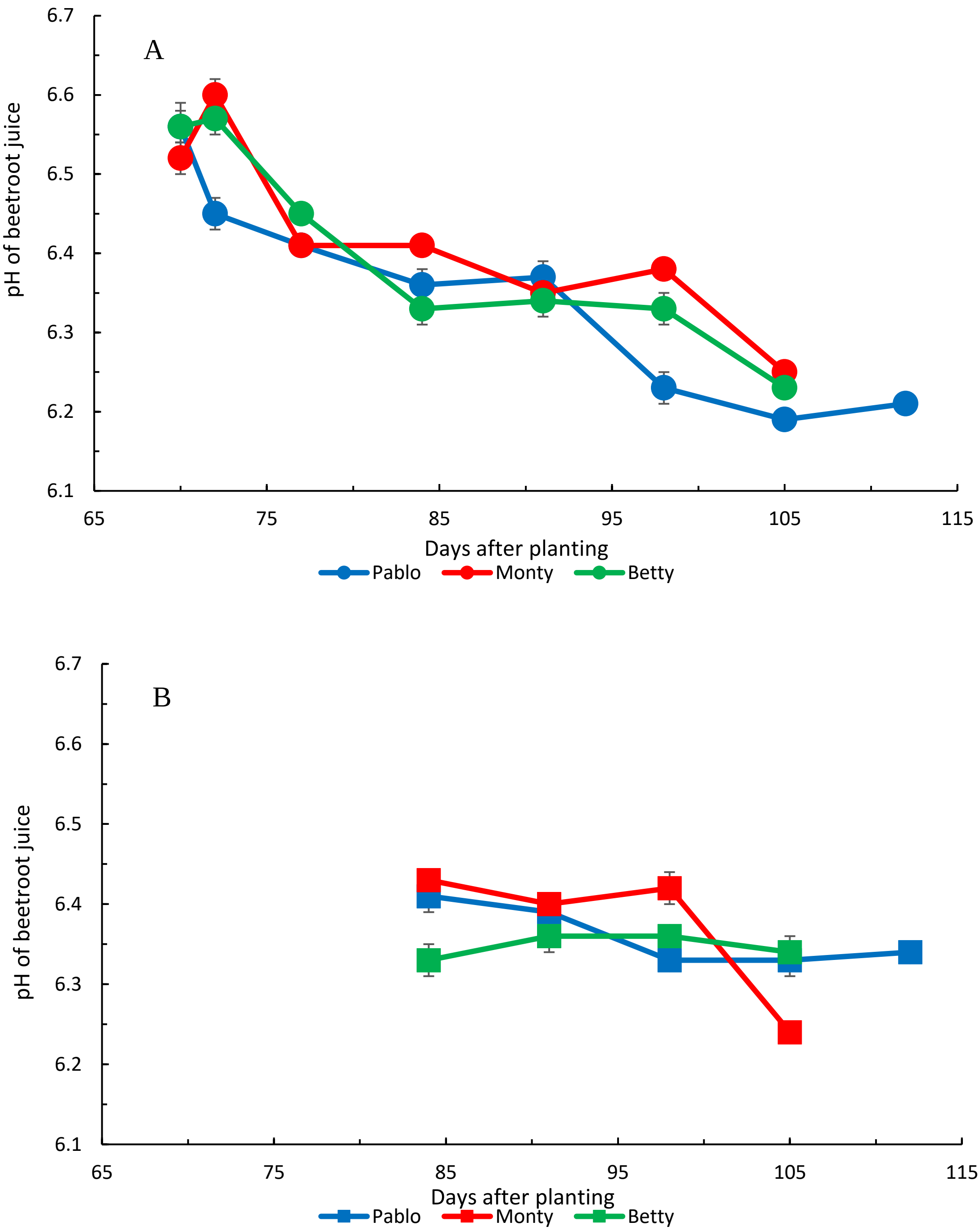


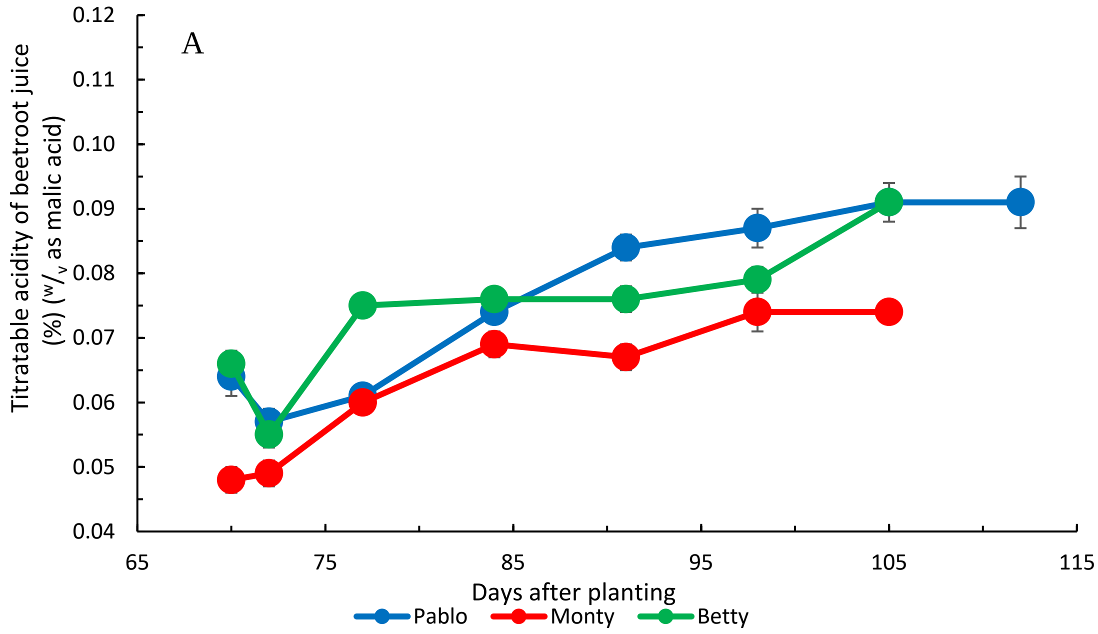
Figure 5.7 pH of juice from medium (A) and large (B) *Beta vulgaris* 'Pablo', 'Monty' and 'Betty', harvested on different days after planting (December 2018 Planting) (Each data point = Mean ± standard error of mean, n=9).

5.7 Titratable Acidity (TA) of Beetroot Juice

Over the harvesting period, the TA of beetroot juice from ‘Pablo’ varied between 0.057 ± 0.002 and 0.106 ± 0.006 (%) ($^{w}/_{v}$ as malic acid), while ‘Monty’ varied between 0.048 ± 0.002 and 0.074 ± 0.001 (%) ($^{w}/_{v}$ as malic acid), and ‘Betty’ varied between 0.055 ± 0.002 and 0.095 ± 0.002 (%) ($^{w}/_{v}$ as malic acid) (Figure 5.8). The TA of extracted juice slowly increased with increasing growth period for ‘Pablo’, ‘Monty ‘and ‘Betty’. This matched the pH drop of the juice for each cultivar observed in Section 5.6.

For medium beetroot of each cultivar, the average TA of extracted juice after 112 days of growth was significantly higher than those harvested after 70 days, increasing by 0.025 to 0.027 (%) ($^{w}/_{v}$ as malic acid) ($P<0.05$). Similar results were found for large beetroot of each cultivar, with a significant increase in TA of juice between the start and the end of the harvest period being found for both large ‘Pablo’ (increased by 0.016 (%) ($^{w}/_{v}$ as malic acid)) and large ‘Betty’ beetroot (increased by 0.015 (%) ($^{w}/_{v}$ as malic acid)) ($P<0.05$). In contrast, no significant difference in TA of juice was observed for large ‘Monty’ beetroot between the start and the end of the sampling period ($P\geq0.05$).

When comparing the TA of juice between the three cultivars, the TA of juice from ‘Monty’ beetroot was significantly lower than the other two cultivars for both medium and large beetroot throughout the entire sampling period ($P<0.05$) (Figure 5.8). The TA of juice from ‘Pablo’ and ‘Betty’ beetroot were similar over the entire sampling period for both medium and large beetroot ($P\geq0.05$). These results indicate that the ‘Monty’ beetroot may contain less acids (expressed as malic acid) than the other two cultivars, although the pH of juice extracted from the three cultivars were similar (Section 5.6).



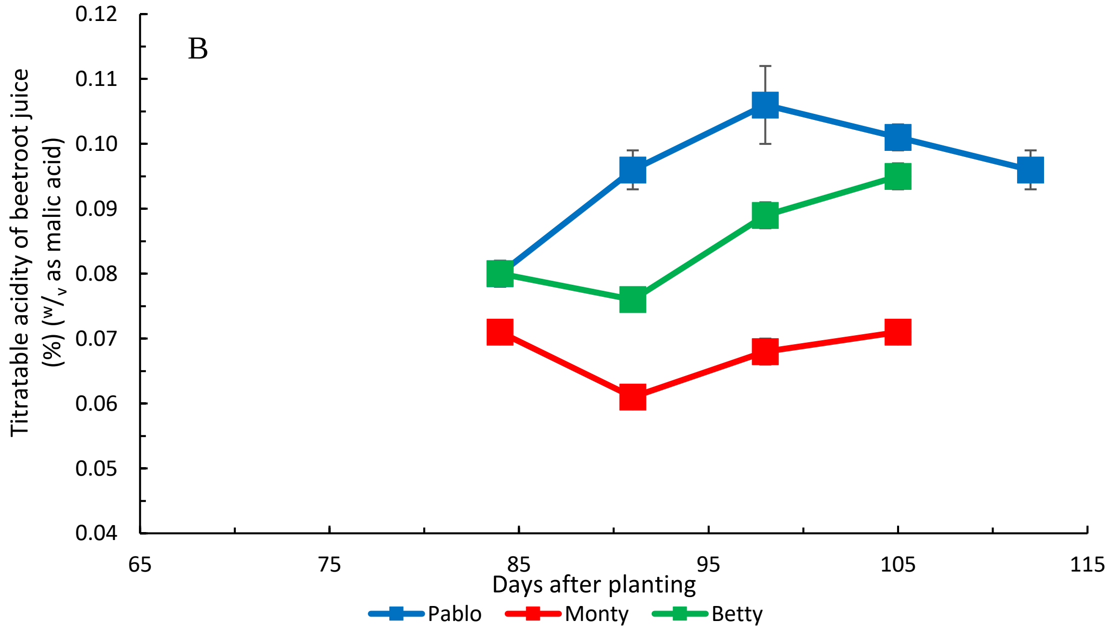


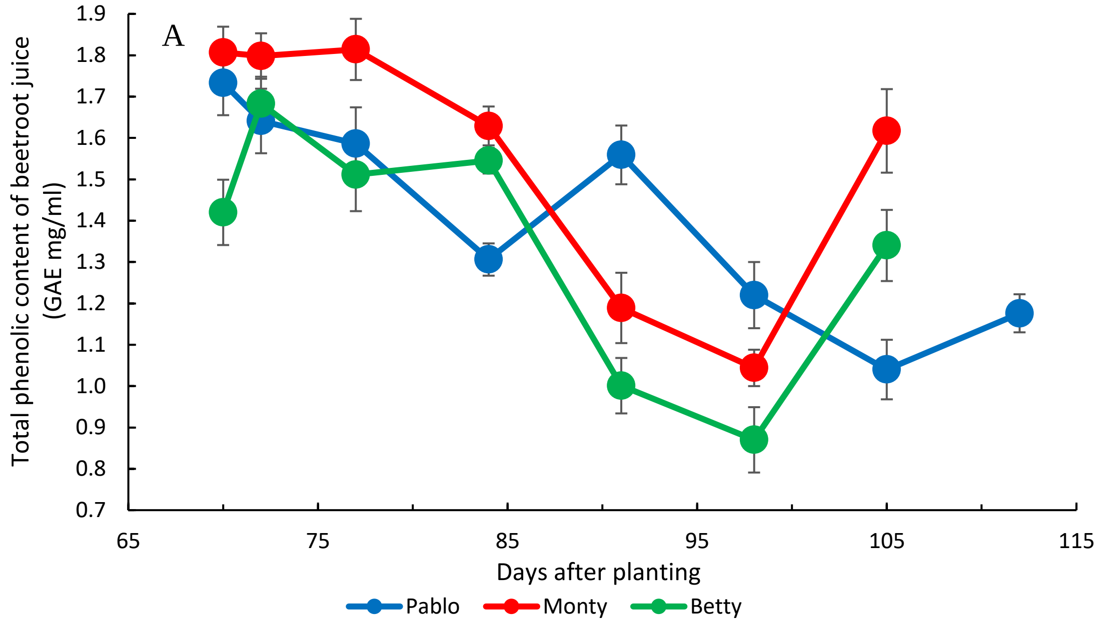
Figure 5.8 Titratable Acidity (%) ($^{w}/_{v}$ as malic acid) of beetroot juice from medium (A) and large (B) *Beta vulgaris* 'Pablo', 'Monty' and 'Betty', harvested on different days after planting (December 2018 Planting) (Each data point = Mean ± standard error of mean, n=9). All the results were standardised to 10°Brix to allow comparison between the different samples.

5.8 Total Phenolic Content of Beetroot Juice

For beetroot harvested from the December planting, the TPC found in the extracted juice from the 'Pablo' cultivar varied between 1.04 ± 0.07 and 1.73 ± 0.08 GAE mg/ml, while that from 'Monty' varied between 0.94 ± 0.11 and 1.81 ± 0.07 GAE mg/ml, and 'Betty' varied between 0.87 ± 0.08 and 1.68 ± 0.07 GAE mg/ml (Figure 5.9).

The average TPC of juice extracted from medium beetroot of each cultivar significantly ($P<0.05$) decreased from day 70 to day 98 after planting, with 'Pablo' decreasing by 30 %, 'Monty' decreasing by 42 % and 'Betty' decreasing by 39 %. A reduction in TPC was also found in juice from large beetroot, with the average TPC of juice from large beetroot grown for 105 days being significantly lower than that from beetroot harvested after 84 days of growth, with decreases of 27 % for both large 'Pablo' and large 'Monty' and a decrease of 34 % for large 'Betty' ($P<0.05$) (Figure 5.9). Hence the TPC of beetroot juice decreased with a longer growth time, up to 98 days for 'Pablo', 'Monty' and 'Betty'.

Based on this data, the most suitable cultivar and growth time to obtain the maximum TPC of beetroot juice was from the medium 'Monty' cultivar grown for 70 to 84 days (Figure 5.9).



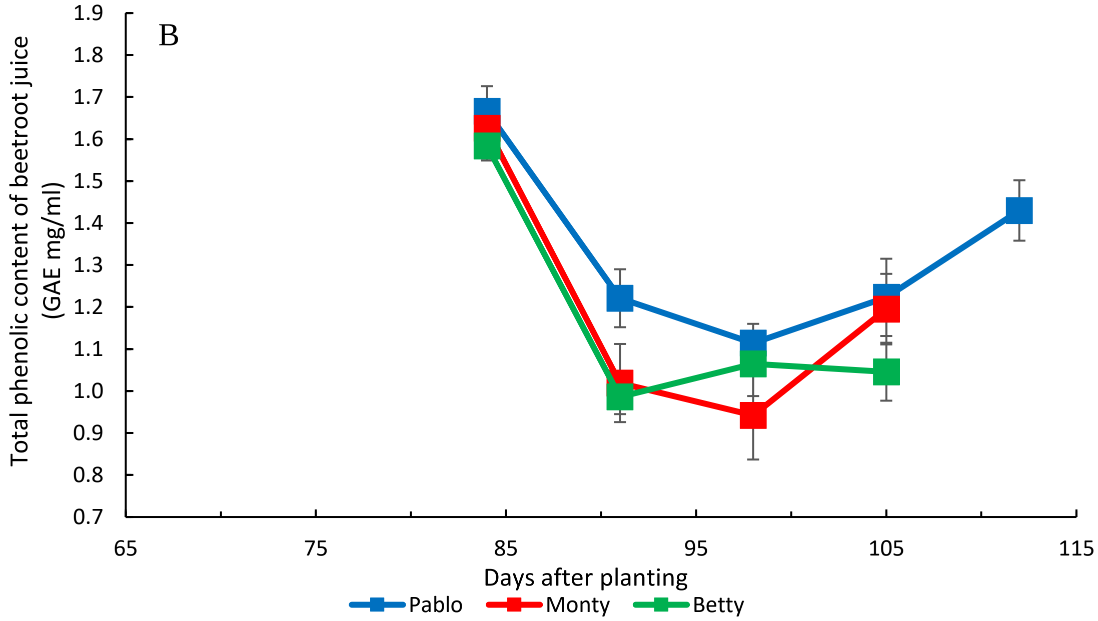


Figure 5.9 Total phenolic content (GAE mg/ml) of beetroot juice from medium (A) and large (B) *Beta vulgaris* 'Pablo', 'Monty' and 'Betty', harvested on different days after planting (December 2018 Planting) (Each data point = Mean ± standard error of mean, n=9). All the results were standardised to 10°Brix to allow comparison between the different samples.

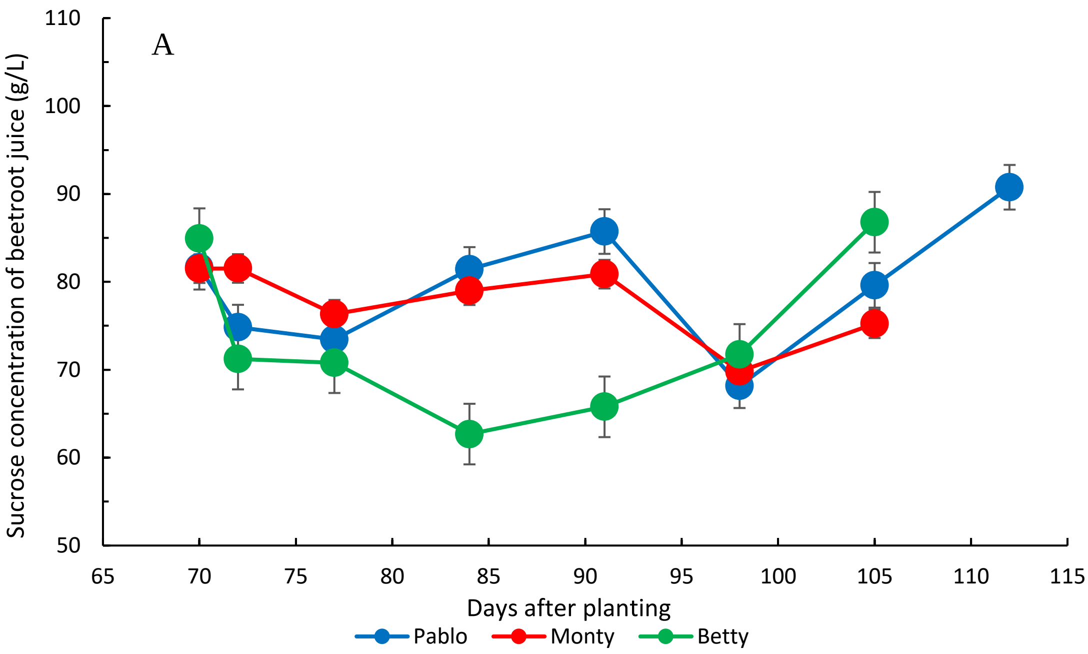
5.9 Individual Sugar Concentration in Beetroot Juice

In the present study, sucrose was the only sugar identified in the extracted beetroot juice by HPLC. The average sucrose concentration of the extracted beetroot juice from 'Pablo' varied between 66.75 ± 1.69 and 90.76 ± 4.13 g/L, while that from 'Monty' varied between 69.81 ± 5.26 and 99.80 ± 6.60 g/L and 'Betty' varied between 62.68 ± 2.61 and 86.95 ± 6.70 g/L (Figure 5.10).

For medium 'Pablo' beetroot, there was no significant difference in sucrose concentration in the extracted juice on most sampling dates ($P \geq 0.05$), with the exception of that from beetroot harvested on day 98, which had a significantly lower sucrose concentration than those on days 91 and 112 ($P<0.05$) (Figure 5.10). For medium 'Monty' beetroot, the sucrose concentrations of extracted beetroot juices were not significantly different during the entire sampling period ($P \geq 0.05$). For medium 'Betty' beetroot, the sucrose concentration of the extracted juice varied between 62.68 and 86.78 g/L, no significant difference was found on most sampling dates, except for day 70 and day 105 which were significantly higher than the other sampling dates ($P \geq 0.05$).

The sucrose concentration of juice from large 'Pablo' beetroot, grown for between 84 and 105 days was similar ($P \geq 0.05$), after which there was a significant increase in sucrose concentration in juice from beetroot harvested after 112 days ($P<0.05$). For large 'Monty' beetroot, the sucrose concentration gradually decreased from 99.80 to 77.80 g/L from day 84 to day 105 ($P<0.05$). For large 'Betty' beetroot, the sucrose concentration of extracted juice gradually increased from 76.11 to 86.95 g/L from day 84 to day 105, although this increase was not significant ($P \geq 0.05$) (Figure 5.10).

The highest sucrose concentration of the beetroot juice was from the large 'Monty' beetroot grown for between 84 and 91 days (Figure 5.10).



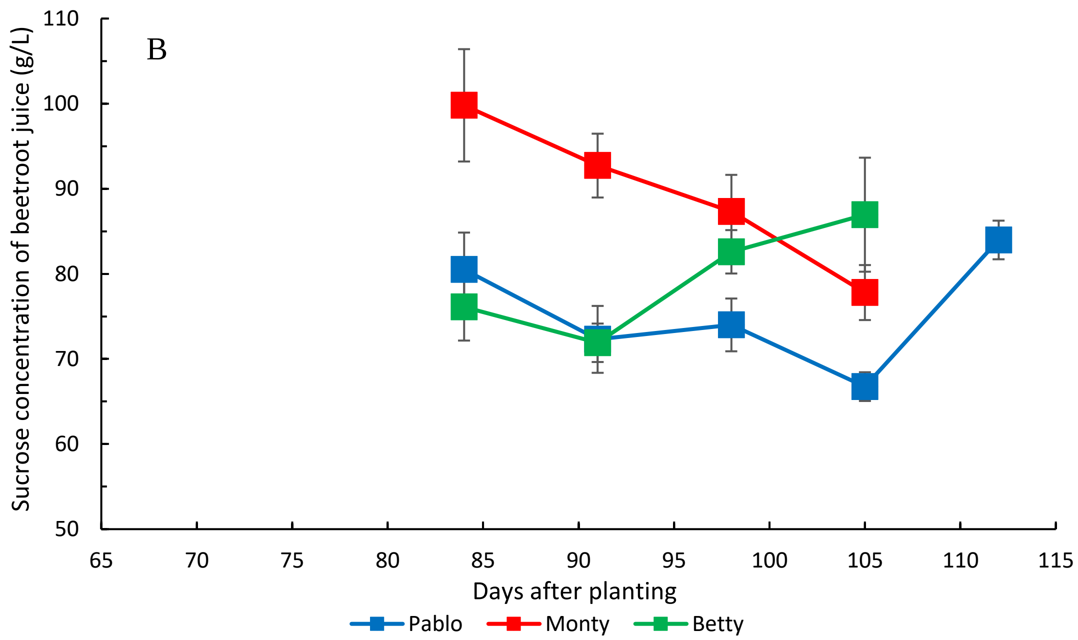


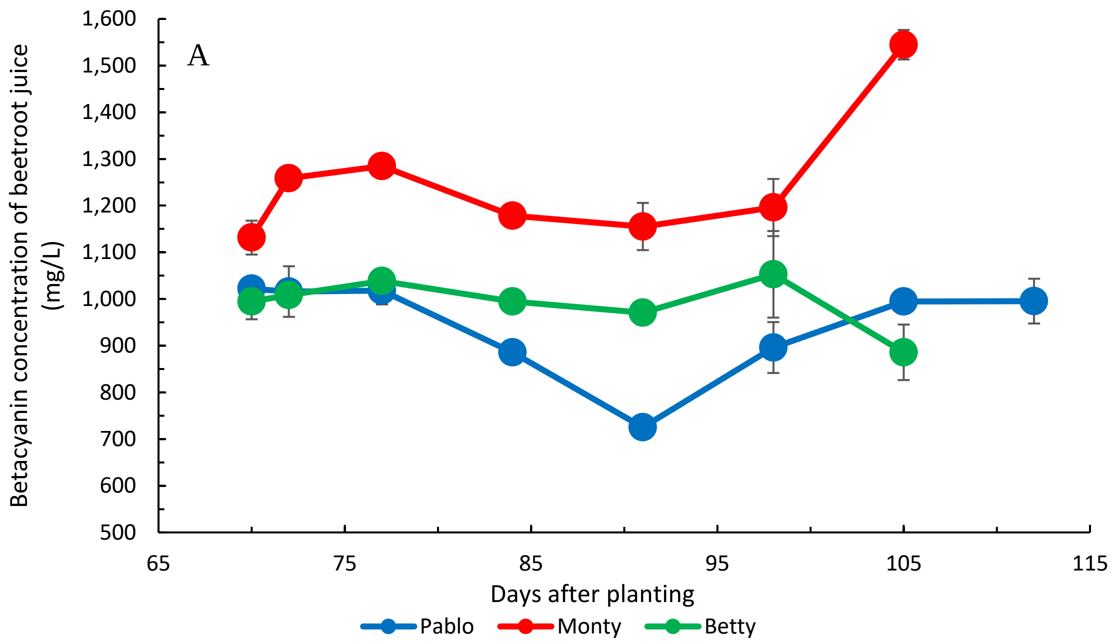
Figure 5.10 Sucrose concentration (g/L) of beetroot juice from medium (A) and large (B) *Beta vulgaris* 'Pablo', 'Monty' and 'Betty', harvested on different days after planting (December 2018 Planting) (Each data point = Mean ± standard error of mean, n=9). All the results were standardised to 10°Brix to allow comparison between the different samples.

5.10 Betacyanins in Beetroot Juice

The betacyanin concentration in juice from medium 'Pablo' beetroot, gradually decreased from 1021.83 to 725.09 mg/L from day 70 to day 91 ($P<0.05$), then gradually increased to 995.48 mg/L after 112 days of growth ($P<0.05$) (Figure 5.11). No significant difference in betacyanin concentration was found in juice from medium 'Pablo' harvested on day 70 and on day 112 ($P\geq0.05$). The betacyanin concentration of juice extracted from medium 'Monty' beetroot grown for between 70 and 98 days was similar ($P\geq0.05$), this was then followed by a significant increase on day 105 ($P<0.05$). For medium 'Betty' beetroot, the betacyanin concentration in the extracted beetroot juice remained constant between 70 and 105 days of growth ($P\geq0.05$).

For the large beetroot of each cultivar, the betacyanin concentration of the extracted juice fluctuated with growth time, the betacyanin concentration in juice from large 'Pablo' beetroot varied between 770.35 and 1176.60 mg/L, that from large 'Monty' beetroot varied between 903.25 and 1405.56 mg/L and large 'Betty' beetroot varied between 680.63 and 1091.42 mg/L.

When comparing the red pigment betacyanin concentration between each beetroot cultivar, the juice extracted from the 'Monty' cultivar contained significantly higher betacyanin concentrations on most sampling days than the other two cultivars ($P<0.05$). The exceptions were from large 'Monty' beetroot on day 84 and day 105 which were not significantly higher than the other two cultivars ($P\geq0.05$) (Figure 5.11). Hence, the recommended cultivar to obtain high betacyanin content was medium 'Monty' grown for between 98 and 105 days.



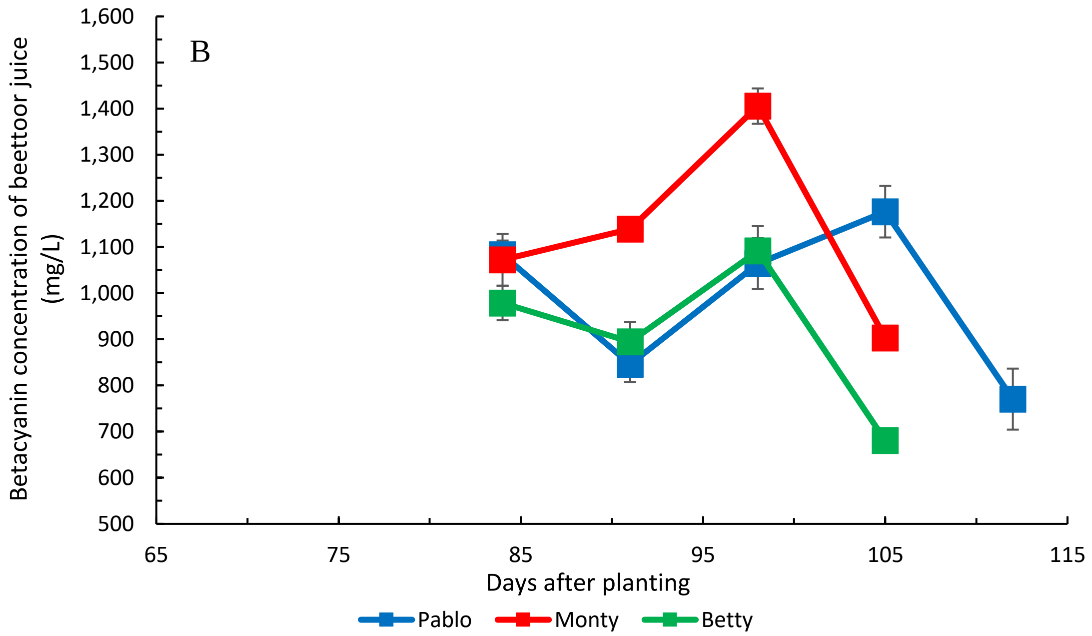


Figure 5.11 Betacyanin concentration (mg/L) of beetroot juice from medium (A) and large (B) *Beta vulgaris* 'Pablo', 'Monty' and 'Betty', harvested on different days after planting (December 2018 Planting) (Each data point = Mean ± standard error of mean, n=9). All the results were standardised to 10 °Brix to allow comparison between the different samples.

5.11 Betaxanthins in Beetroot Juice

For medium beetroot of each cultivar, the betaxanthin (yellow pigment) concentration of the juice extracted from beetroot harvested after 112 days of growth were all significantly higher ($P<0.05$) than those found on the first harvest day (70 days of growth) ('Pablo' increased by 190.49 mg/L, 'Monty 'increased by 278.53 mg/L and 'Betty' increased by 178.85 mg/L). The betaxanthin concentration in juice from medium 'Pablo', 'Monty' and 'Betty' beetroot increased significantly with growing time up to 112 days ($P<0.05$).

Similar to the betacyanins, the betaxanthin concentration of the extracted juice from the large beetroot of each cultivar, fluctuated with the growth time (Figure 5.12). The betaxanthin concentration in juice from large 'Pablo' beetroot varied between 504.67 and 674.75 mg/L, while that from large 'Monty' beetroot varied between 512.20 and 750.04 mg/L, and large 'Betty' beetroot varied between 461.66 and 613.86 mg/L.

When comparing the concentration of betaxanthins between the three beetroot cultivars, 'Monty' contained significantly higher concentrations of the yellow pigment in the taproot than the other two cultivars on most sampling dates ($P<0.05$). Exceptions were for the medium 'Monty' beetroot harvested on day 72 and day 98, and large 'Monty' beetroot harvested on day 84 and day 105 which were not significantly higher than the other two cultivars ($P\geq0.05$) (Figure 5.12). Similar to the results in Section 5.10, the recommended cultivar to obtain high betaxanthin concentrations was also the medium 'Monty' cultivar, in this case harvested over a slightly longer period of between 91 and 105 days of growth. For medium beetroot of each cultivar harvested, the red/yellow ratio of 'Pablo', 'Monty' and 'Betty' beetroot all dropped significantly at the end of sampling period (between 1.44 and 1.89) compared to those at the start (between 2.23 and 2.34) ($P<0.05$). For large beetroot, the red/yellow ratio of three beetroot cultivars varied between 1.47 and 1.88 between day 84 and day 112 (Figure 5.13).

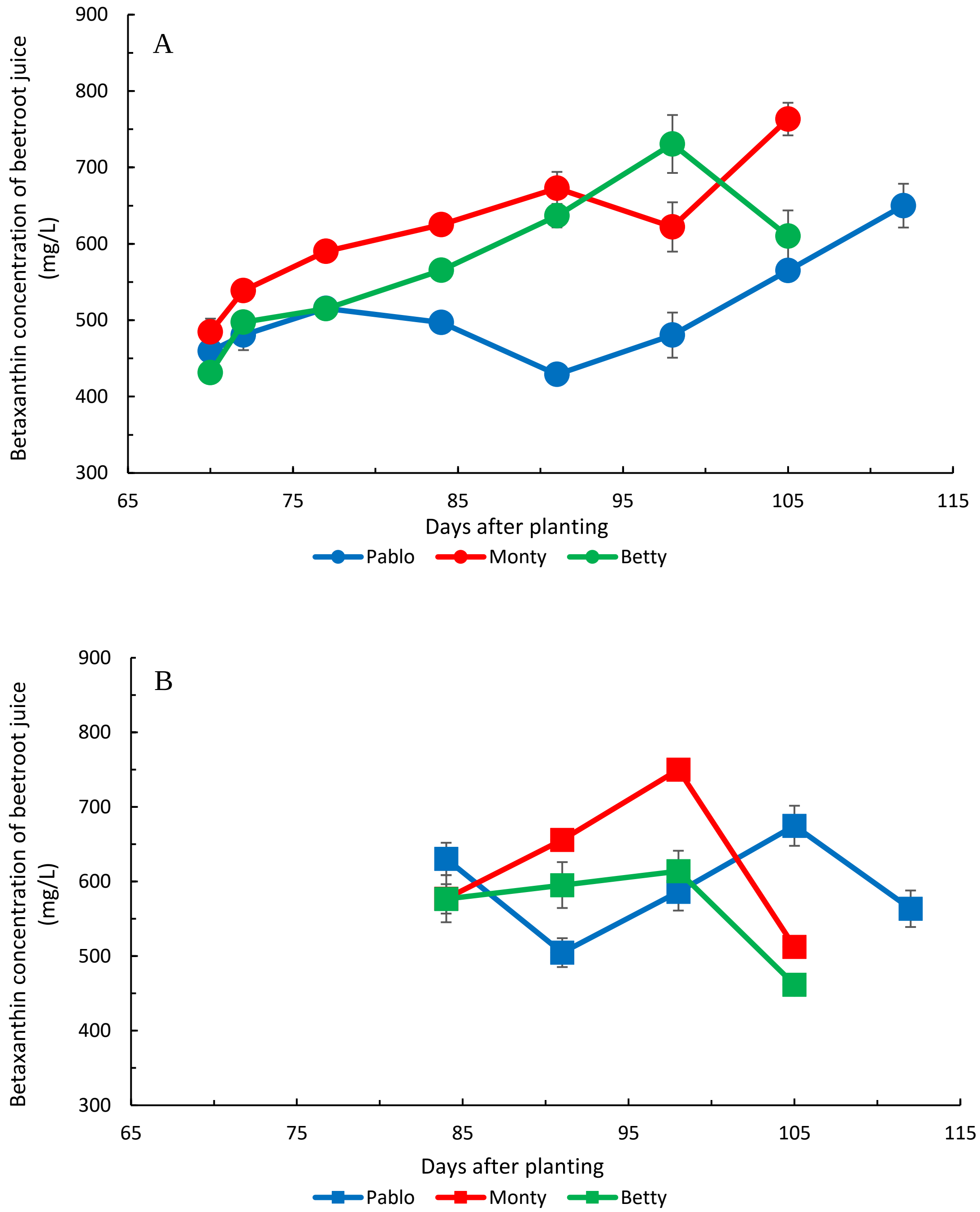


Figure 5.12 Betaxanthin concentration (mg/L) of beetroot juice from medium (A) and large (B) *Beta vulgaris* 'Pablo', 'Monty' and 'Betty', harvested on different days after planting (December 2018 Planting) (Each data point = Mean ± standard error of mean, n=9). All the results were standardised to 10°Brix to allow comparison between the different samples.

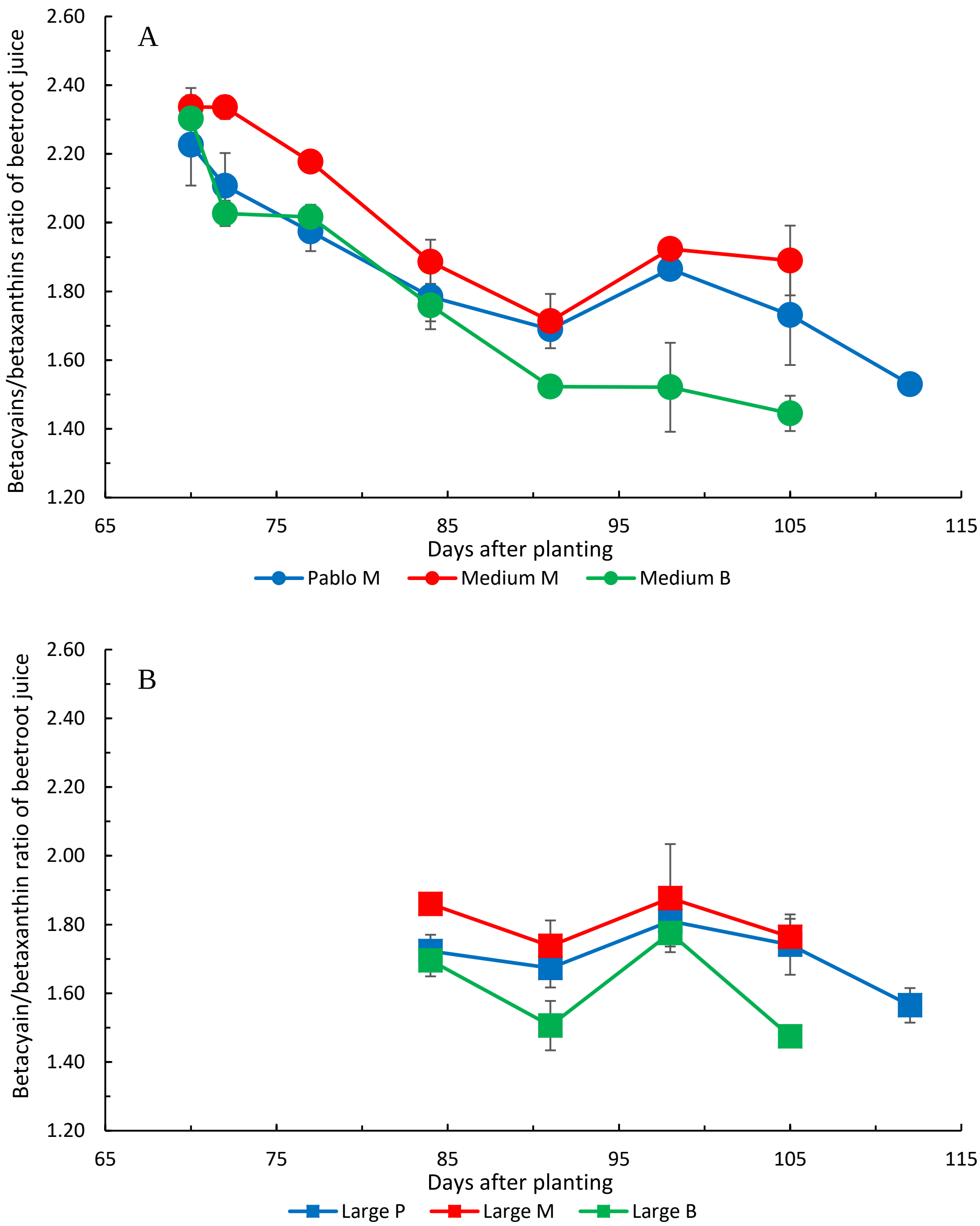


Figure 5.13 Betacyanin/betaxanthin ratio of beetroot juice from medium (A) and large (B) *Beta vulgaris* 'Pablo', 'Monty' and 'Betty', harvested on different days after planting (December 2018 Planting) (Each data point = Mean ± standard error of mean, n=9). All the results were standardised to 10°Brix to allow comparison between the different samples.

5.12 Nitrate and Nitrite Concentrations in Beetroot Juice

Over the entire harvesting period, the nitrate concentration of beetroot juice from 'Pablo' beetroot varied between 1200 ± 20 and 2878 ± 59 mg/L, while that from 'Monty' varied between 654 ± 22 and 1405 ± 38 mg/L and 'Betty' varied between 1371 ± 13 and 2080 ± 26 mg/L (Figure 5.14).

The nitrate concentration of extracted juice from medium 'Pablo' beetroot, did not change significantly between day 70 and day 91 ($P \geq 0.05$), however a significant drop in nitrate concentration was observed in beetroot juice from day 91 (1656 mg/L) to day 105 (1200 mg/L) ($P<0.05$). For medium 'Monty' beetroot, the nitrate concentration in the juice significantly dropped from day 77 (1405 mg/L) to day 105 (1097 mg/L). For juice extracted from medium 'Betty' beetroot, there was a significant increase in the nitrate concentration from 1377 to 2081 mg/L from day 70 to day 91 ($P<0.05$).

For large beetroot, the nitrate concentration of juice from the large 'Pablo' gradually decreased from 2878 mg/L on day 91 to 1613 mg/L on day 112 ($P<0.05$). No significant ($P \geq 0.05$) difference in nitrate concentration was observed between each harvest date for large 'Monty' beetroot. For large 'Betty' beetroot, the nitrate concentration of extracted juice was similar on most sampling dates ($P \geq 0.05$), except for that on day 98 which was significantly lower than those on day 84 and day 105 ($P<0.05$).

When comparing between cultivars, for medium beetroot, the nitrate concentration of the juice from medium 'Betty' was significantly ($P<0.05$) higher than that from medium 'Monty' from day 70 to day 105. With respect to the juice from the large beetroot, the concentration of nitrate from large 'Betty' and 'Pablo' were significantly higher than that from large 'Monty'($P<0.05$). Hence of the three cultivars, the 'Monty' appears to have had the lowest nitrate content in its taproot. The maximum nitrate concentration from all the beetroot juices was from large 'Pablo' grown for 91 days (2878 ± 58 mg/L).

When comparing the nitrite concentration of juice from beetroot harvested from day 70 to day 112, the nitrite concentration in juice from medium 'Pablo' significantly decreased from 211 mg/L on day 70 to 164 mg/L on day 112, while that from large 'Pablo' significantly dropped from 246 mg/L on day 91 to 173 mg/L on day 112

(P<0.05). No significant difference (P≥0.05) in nitrite concentration was observed for juice extracted from medium or large 'Monty' beetroot, on most sampling dates, except for large 'Monty' which had a significant lower nitrite on day 91 than that on day 84 (P<0.05). Similar to the increasing nitrate concentration in juice from the medium 'Betty' beetroot, there was also a significant increase in nitrite concentration of extracted juice from medium 'Betty' beetroot from 168 to 255 mg/L from day 70 to day 84 (P<0.05). For large 'Betty' beetroot, no significant difference (P≥0.05) in nitrite concentration was observed from extracted juice on most sampling dates, except that the nitrite concentration on day 91 was significantly lower than that on day 98.

In summary, large 'Pablo' beetroot harvested 84 to 98 days after planting resulted in the extracted juice with the highest nitrate content.

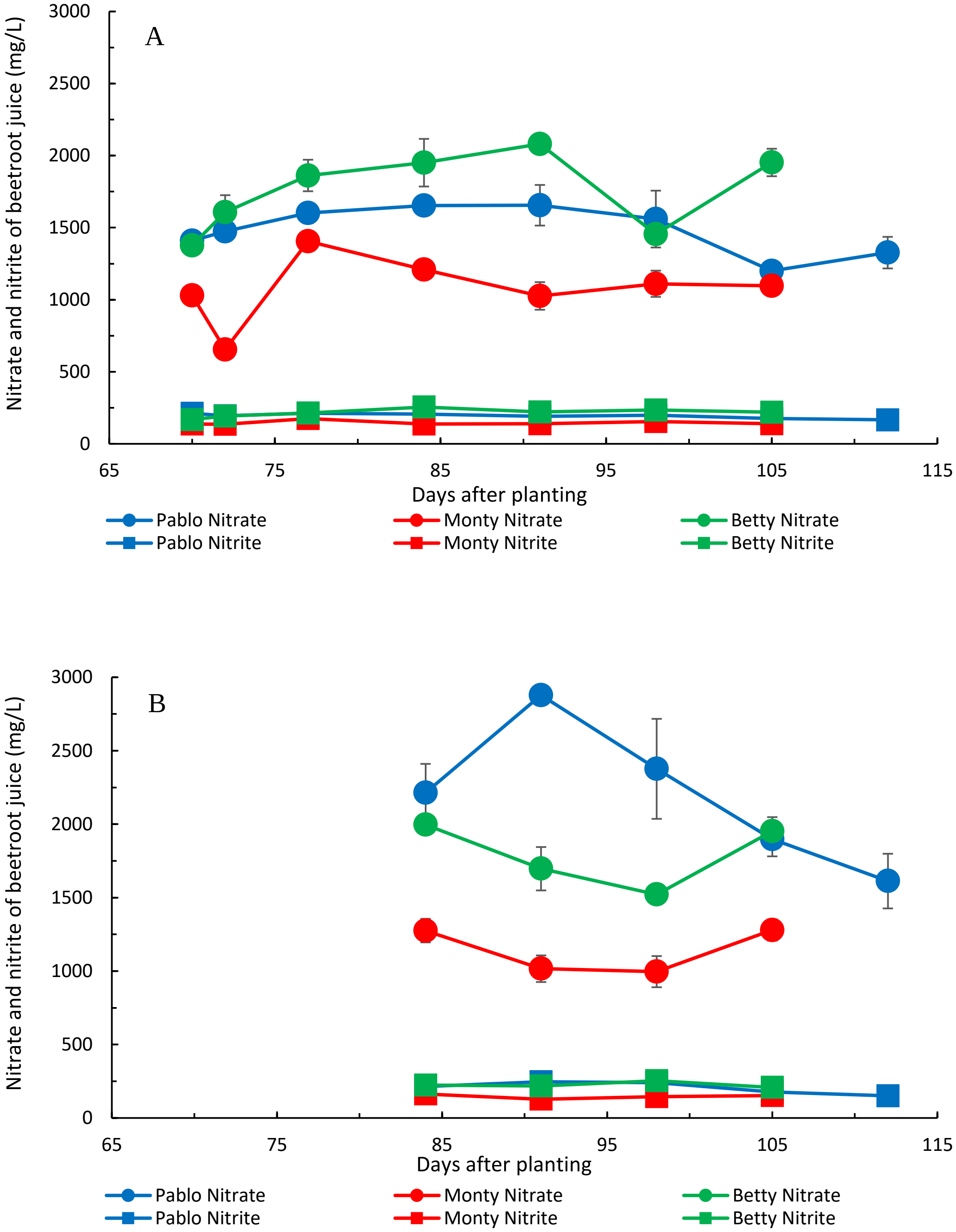


Figure 5.14 Nitrate and nitrite concentrations (mg/L) of beetroot juice from medium (A) and large (B) *Beta vulgaris* 'Pablo', 'Monty' and 'Betty', harvested on different days after planting (December 2018 planting) (Each data point = Mean ± standard error of mean, n=9). All the results were standardised to 10°Brix to allow comparison between the different samples.

5.13 Discussion of the Composition Differences between the Three Beetroot Cultivars

5.13.1 Effects of Growth Period on DM of *Beta vulgaris* 'Pablo', 'Monty' and 'Betty'

The DM of beetroot from the December planting, were relatively constant from day 70 to day 112 for medium (15 %) and large (13 %) 'Pablo', medium (16 %) and large (15 %) 'Monty', and medium (14 %) and large (13 %) 'Betty' (Section 5.4). However, the DM of 'Monty' beetroot (between 14 and 16 %) were significantly higher than the other two cultivars over the entire sampling period (Section 5.4).

As mentioned in Section 4.16.1, the reason that the DM results were constant over the growth period was probably because the accumulation of total solids in the beetroot had already leveled off prior to the first beetroot being harvested after 70 days of growth. Although limited studies on the DM content of different beetroot cultivars are available, the results observed in this study are similar to those previously reported for 'Pablo' beetroot grown for six months (approximate 180 days), with a mean DM at 13 %, 'Monty' and 'Belushi' beetroot grown for sixteen weeks (approximate 102 days), which varied between 12 and 14 %, and between 11 and 13 %, respectively (Bach, Mikkelsen, Kidmose, & Edelenbos, 2015; Barba-Espin et al., 2018).

5.13.2 Effects of Growth Period on TPC of *Beta vulgaris* 'Pablo', 'Monty' and 'Betty'

For the medium beetroot from the December planting, the TPC of 'Pablo', 'Monty' and Betty' decreased from 1.73 to 1.23 GAE mg/ml, 1.81 to 1.04 GAE mg/ml and 1.42 to 0.87 GAE mg/ml respectively from day 70 to day 98. For large beetroot, a similar decrease in TPC was also found for beetroot grown for between 84 and 98 days, with the TPC decreasing from 1.67 to 1.11 GAE mg/ml, 1.63 to 0.94 GAE mg/ml and 1.58 to 1.07 GAE mg/ml for 'Pablo', 'Monty' and 'Betty', respectively (Section 5.8).

In comparison to another 'Monty' beetroot study (Barba-Espin et al., 2018), in the present study, the TPC of juice from medium 'Monty' dropped by 0.763 GAE mg/ml from day 70 to day 98, which corresponded to a 221.27 GAE mg/kg reduction in whole beetroot based on a 29 % juice yield (v/w) (Appendix C). Barba-Espin et al. (2018), who found the TPC in whole beetroot from 'Monty' and 'Belushi' beetroot decreased approximately 500 GAE mg/kg and 1000 GAE mg/kg respectively in beetroot harvested between three weeks and eighteen weeks after planting (approximately 21 to 126 days of growth).

When comparing with other high-phenolic plants such as berries, the TPC of the juice from the three cultivars of beetroot varied between 0.87 to 1.81 GAE mg/ml in the present study (Section 5.8), which corresponded to 870 to 1810 mg/L, and is similar to the TPC of cranberry juice (882 mg/L) and blueberry juice (1524 mg/L) (Granato, Karnopp, & Ruth, 2015) (Section 4.16.2).

5.13.3 Effects of Growth Period on Sugar Content of *Beta vulgaris* 'Pablo', 'Monty' and 'Betty'

The sucrose concentration in juice extracted from beetroot harvested from the December planting, fluctuated during the sampling period, there was no significant difference in sucrose concentration at the start and end of sampling period for the three cultivars, except for large 'Monty' beetroot, where the mean sucrose concentration decreased from 99.80 to 77.80 g/L from day 84 to day 105 of growing (Section 5.9).

The sucrose content did not significantly increase or decrease on most harvest dates for beetroot grown between day 70 and day 112, probably because the sucrose accumulation had occurred prior to the initial harvest at 70 days growth (as discussed in Section 4.16.3). The decrease in sucrose concentration of the juice from large 'Monty' beetroot observed between day 84 and day 105 may have been due to the fact that the large 'Monty' grew faster than the medium 'Monty' and other cultivars, therefore, sucrose concentration may have started to decrease (Montes-Lora et al., 2018).

5.13.4 Effects of Growth Period on Betacyanin and Betaxanthin Concentrations of *Beta vulgaris* 'Pablo', 'Monty' and 'Betty'

In the present study, for the medium beetroot of three cultivars, it appears that while the betacyanin concentration fluctuated (varied between 680 and 1544 mg/L) over the sampling period, the betaxanthin content increased with increasing growth time, reaching 431 to 484 mg/L on day 70 and 565 to 763 mg/L on day 105. At the same time, the red/yellow pigment ratio decreased from day 70 to day 112 (Section 5.10 and 5.11).

Similar results were found by Łukaszewska and Gawęda (2014), who showed that the red pigment betacyanins of 'Boro F1', 'Czerwona Kula', 'Nochowsski', and 'Regulski Cylinder' beetroot varied between 340 and 1030 mg/kg from day 42 to day 105 after planting. The betaxanthin content of the four cultivars of beetroot gradually increased from 60 to 30 mg/kg on day 42 to 240 to 310 mg/kg on day 105. The red/yellow pigment ratio of the four cultivars also gradually decreased between 42 and 105 days of growth (Łukaszewska & Gawęda, 2014).

5.13.5 Effects of Growth Period on Nitrate and Nitrite Content of *Beta vulgaris* 'Pablo', 'Monty' and 'Betty'

Large 'Pablo' beetroot grown for 91 days had the highest nitrate content (2878 ± 58 mg/L) of the three cultivars harvested from the December planting. The nitrate concentration in juice varied between 654 and 2878 mg/L for the three cultivars over the sampling period. It appeared that the nitrate content in medium and large 'Pablo', and medium 'Monty' was starting to decrease while the nitrate content in medium 'Betty' was still increasing from days 70 to 91 (Section 5.12).

Nitrate accumulation depends on the relationship between the nitrate uptake rate from the soil and the nitrate reduction rate in plants (Cárdenas-Navarro et al., 1999; Sidhu et al., 2011; Taiz & Zeiger, 2010). When the nitrate absorption rate is higher than the nitrate reduction rate, nitrate accumulates in the plants, and if the absorption rate is lower than the reduction rate then the nitrate content in the plant decreases (Cárdenas-Navarro et al., 1999; Sidhu et al., 2011; Taiz & Zeiger, 2010). This relationship may explain why the nitrate content of medium and large 'Pablo', and medium 'Monty' decreased with increasing growth time. This decrease may have occurred because the nitrate uptake rate decreased with time, or the nitrate reduction rate of these plants may have increased with time as more amino acids and protein were produced to support beetroot growth (Cárdenas-Navarro, Adamowicz, & Robin, 1999; Sidhu et al., 2011). For the medium 'Betty' beetroot, the increase in nitrate content with growth time probably occurred as a result of the nitrate absorption rate being larger than the reduction rate, therefore, the nitrate content accumulated in the beetroot (Cárdenas-Navarro et al., 1999; Sidhu et al., 2011; Taiz & Zeiger, 2010).

5.13.6 Composition Differences between the Three Beetroot Cultivars

A comparison of the size, weight, DM, % soluble solids, pH, TA, TPC, sucrose concentration, betacyanin and betaxanthin concentrations, and nitrate concentrations between the three beetroot cultivars from the December planting is presented in Table 5.1. Large 'Pablo' beetroot contained the highest nitrate content in all the collected samples. Hence, large 'Pablo' beetroot grown between 84 and 98 days may be the preferred option for producing a high-nitrate drink (Section 4.16.5).

In the present study, as shown in Table 5.1, 'Monty' beetroot were the largest and heaviest, had the highest DM (14 to 16 %), the highest % soluble solids (12 to 13.8°Brix), as well as the highest betacyanin (903.25 to 1405.56 mg/L) and betaxanthin concentrations (484.77 to 763.30 mg/L) of all three cultivars. However, the nitrate concentration of the extracted juices from 'Monty' were significantly lower than those from 'Pablo' and 'Betty' cultivars over the entire sampling period ($P<0.05$). Due to its high DM content, 'Monty' beetroot may be better used for dry products such as beetroot chips (Ruk Zwaan, 2019). In addition, the 'Monty' cultivar may be a good choice for extracting 'beetroot red', which is a natural red colorant containing betacyanin pigments used in the food industry (Barba-Espin et al., 2018).

Table 5.1 Comparison of size, weight, DM, % soluble solids, pH, TA, TPC, sucrose concentration, betacyanin and betaxanthin concentration, and nitrate concentrations between the three beetroot cultivars from the December planting, harvested on different days after planting (December 2018 planting).

	Pablo	Monty	Betty	Effects of growth time on beetroot composition
Size and weight		largest*	smallest*	increasing with longer growth time
Dry matter (%) (w/w fresh weight)		highest*		constant over entire sampling period
Percentage of soluble solids (ºBrix)		highest*		constant over entire sampling period
pH	-	-	-	decreasing from day 70 onwards
TA (%) (w/v as malic acid)	highest (large 'Pablo')	lowest*		maximum on days 91 to 105
TPC (GAE mg/mL)		highest (medium 'Monty')		maximum on days 70 to 84
Sucrose (mg/L)		highest (large 'Monty')		maximum on days 84 to 91
Betacyanins and betaxanthins (mg/L)		highest (medium 'Monty')		maximum on days 91 to 105
Nitrate (mg/L)	highest (large 'Pablo')	lowest*		maximum on days 84 to 98

Note: *the quality parameter in each row of this cultivar was significantly higher/ lower than that of the other two cultivars on most sampling dates for both medium and large beetroot from the December planting ($P<0.05$).

'Betty' is one of the newest beetroot cultivars available today (Gerhard Smit, 2019). No data on 'Betty' has been published. In the present study, 'Betty' were the smallest beetroot of the three cultivars, however, medium 'Betty' beetroot contained a significantly higher nitrate content (1755 ± 274 mg/L) than that from the medium 'Monty' (1076 ± 29 mg/L) over the entire growth period (70 to 112 days). Besides nitrate content, the DM, % soluble solids, TPC, betacyanin and betaxanthin concentrations of medium 'Betty' were similar to medium 'Pablo' and lower than medium ‘Monty’. According to the information from the beetroot seed company, ‘Betty’ were claimed to have a better sensory freshness taste than the other cultivars, and they are preferred to be used for bunched fresh baby beetroot in supermarket and organic vegetable shops due to their small size (Ruk Zwaan, 2019).

5.14 Conclusions

Large ‘Pablo’ beetroot were found to contain the highest nitrate content of all the samples collected. Hence, large ‘Pablo’ beetroot grown between 84 and 98 days would be a good option for producing high-nitrate juice.

The ‘Monty’ beetroot were larger and heavier, had a higher DM content, higher % soluble solids, and higher betacyanin and betaxanthin concentrations than the other two beetroot cultivars. The recommended harvest time to obtain high betacyanin and betaxanthin beetroot juice from the medium ‘Monty’ cultivar harvested was between 91 and 105 days of growth. Juice from medium ‘Monty’ harvested between 70 and 84 days of growth had the highest TPC.

‘Betty’ beetroot were the smallest beetroot of the three cultivars, medium ‘Betty’ had a higher nitrate content than medium ‘Pablo’ and medium ‘Monty’, although DM, % soluble solids, TPC, betacyanin and betaxanthin concentrations were similar to medium ‘Pablo’. Medium ‘Betty’ may be a good option for bunched fresh beetroot.

Chapter 6 Overall Discussion

6.1 Nitrate in Beetroot

For ‘Pablo’ beetroot, nitrate content in beetroot was constant with growing time from day 70 to day 91. Then after this time, this study found a significant decrease in nitrate content in ‘Pablo’ beetroot from day 91 to day 138 (Section 4.15). The nitrate content of barseem, oats, toriya, maize and Napier bajra was also lower in mature plants compared to the younger plants, and a study suggested that the nitrate decrease in mature plants may due to either a decreasing nitrate absorption rate or the plants assimilating the nitrate faster (Sidhu et al., 2011).

6.1.1 Nitrate Absorption in Beetroot

One of the potential reasons that nitrate decreased as the beetroot mature is that there was a decrease in nitrate content in the soil (Darwinkel, 1975). As mentioned in Section 2.1, the plant nitrate absorption rate decreases with decreasing nitrate content in the soil (Marschner & Marschner, 2012), which depends on if fertilizer was applied to the beetroot and soil during the growing season. If no fertilizer was applied to the field, the nitrate concentration in the soil decreases with time, therefore, less nitrate is available to be absorbed by the plants (Darwinkel, 1975; Pindozzi, 2019).

In the present study, no fertilizer was applied for the two growing seasons, therefore, the nitrate content in the soil probably decreased with longer beetroot growth time. For future studies, soil mineral tests are recommended to confirm whether the nitrate content changes over the growing season.

6.1.2 Nitrate Assimilation in Beetroot

Another possible reason for the observed nitrate content decrease in the ‘Pablo’ beetroot grown for 91 to 138 days may be due to a faster nitrate assimilation in the beetroot. As mentioned in Section 2.1, nitrate can be converted to nitrite and then to amino acids and proteins in roots, however, when the nitrate concentration is high in the roots, the nitrate will be translocated to the shoots and further being assimilated in leaves, which had a higher nitrate reduction capacity (ability to reduce nitrate) than roots (Andrews, 1986; Taiz & Zeiger, 2010). This is because that nitrate assimilation is highly linked with photosynthesis and needs NADPH and other photosynthesis products to support the assimilation (Andrews, 1986; Smirnoff & Stewart, 1985; Taiz

& Zeiger, 2010). When the nitrate concentration in the taproot is high, the taproot can no longer assimilate the nitrate stored in the roots due to the limited energy and light supply, the nitrate may be translocated to the leaves where photosynthesis mainly occurs and provides more energy and photosynthesis products for the nitrate assimilation (Andrews, 1986; Foyer & Quick, 1997; Smirnoff & Stewart, 1985; Taiz & Zeiger, 2010).

For the further studies, to better understand the nitrate assimilation in beetroot plant, the nitrate content and amino acids content in both beetroot taproot and leaves are recommended to be determined.

6.1.3 High-Nitrate Beetroot Juice Production

As mentioned in Section 5.12, based on the results of this study large 'Pablo' grown for between 84 and 98 days are recommended for high-nitrate beetroot juice production. The present study also suggested that the beetroot can be stored in the dark at a low temperature (4°C) without the loss of nitrate during storage prior to processing. Also, the 'Pablo' beetroot grown under fleece from the September planting had a much higher nitrate content (almost two times higher) than those not under fleece, this probably due to the dark growth condition.

As mentioned before, the nitrate reductase (NR) is affected by light intensity, when light intensity increases, the NR activity is increased (Taiz & Zeiger, 2010). Also, when light intensity increased, more NADPH is produced, which donates the electrons for the nitrate reduction process, then more nitrate is reduced and this leads to a lower nitrate content (Taiz & Zeiger, 2010). In contrast, when plants grown in dark, the NR activity is inhibited and lead to a higher nitrate content in plants (Taiz & Zeiger, 2010). Therefore, the dark storage condition is important to maintain the high nitrate content in beetroot prior to juicing.

6.2 Betacyanins and Betaxanthins in Beetroot

6.2.1 Betacyanins and Betaxanthins Accumulation in Beetroot

The study found the concentration of red pigment betacyanins in 'Pablo', 'Monty' and 'Betty' beetroot varied during the growing season with the concentration increasing or remaining constant within the growth period between day 70 and day 112. In contrast, the yellow betaxanthin pigment appeared to gradually increase over the same growth period (Section 5.10 and 5.11).

The present study results agreed with the previous studies (Łukaszewska & Gawęda, 2014). Other publications reported that the betacyanins in beetroot may increase, decrease or fluctuate while the betaxanthin in beetroot continuously increase with beetroot growth (Michalik & Grzebelus, 1995; Shannon, 1970; Waston & Gabelman, 1982).

Scientists are still trying to identify more betacyanin and betaxanthin compounds in the plants and trying to understand the regulation of their levels (Hussain et al., 2018; Kugler, Graneis, Stintzing, & Carle, 2007; Li et al., 2019).

To understand better the betalain biosynthesis, not only the betacyanin and betaxanthin levels in beetroot juice, but also the compounds which produce the betacyanins and betaxanthins in beetroot are essential. In the present study, the betacyanin and betaxanthin concentrations in beetroot juice were quantified by a spectrophotometer. The compounds which produce betacyanins and betaxanthins were not further quantified in the present study.

As mentioned in Section 2.5, betacyanins are produced from betalamic acids and cyclo-DOPA-5-O-glucoside, while betaxanthins are produced from betalamic acids and amino acids (Li et al., 2019; Timoneda et al., 2018). The change of betacyanin and betaxanthin content in beetroot with longer growth time may be affected by the level of change of betalamic acids, cyclo-DOPA-5-O-glucoside and amino acids. For a further study, a HPLC technique is recommended to identify and quantify the compounds which participate in the betalain biosynthesis, for example, betalamic acids and cyclo-DOPA-5-O-glucoside and observe the effect of the growth period on these compounds.

6.2.2 High-Betalain Beetroot Juice Production

As mentioned in Section 5.10 and 5.11, ‘Monty’ beetroot contained the highest betacyanin and betaxanthin content of the three cultivars, and ‘Monty’ beetroot has been reported to be a good option for beetroot juicing due to its higher juice yield (Ruk Zwaan, 2019). As betacyanins and betaxanthins (betalains) degrade easily, maintaining the stability of betalains while juicing can be challenging (Santos et al., 2017; Herbach, Stintzing, & Carle, 2006).

For the production of high-betalain beetroot juice (e.g. from 'Monty' beetroot), the juice will need to be pasteurised, however, thermal treatment (pasteurization) will decrease the betalain concentration in the juice (Kathiravan, Nadanasabapathi, & Kumar, 2014). Kathiravan et al. (2014) found that heating a pre-packed beetroot juice product (200 ml beetroot juice contained in the polypropylene package) at 96°C for 720 seconds can inactive most microorganisms and degrade less betacyanin and betaxanthin content compared to heating products for 540 and 900 seconds. However, betacyanin and betaxanthin content in beetroot juice after heating at 96°C for 720 seconds still decreased by 42 % and 40 %, respectively.

In addition to the heat treatment processes, light and oxygen can also accelerate the degradation of betalains (Carle & Schweiggert, 2016), with the effect of oxygen degradation being the greater of the two (Attoe & Elbe, 1981; Carle & Schweiggert, 2016). Therefore, lowering the oxygen level in the beetroot juice is recommended, for example, removing the oxygen in the product by filling with nitrogen gas (Azeredo, 2009; Carle & Schweiggert, 2016; Huang & Elbe, 1985).

6.3 Other Beetroot Products

The mean nitrate content (1755 mg/L) of medium 'Betty' beetroot was significantly higher than the other two medium cultivars but lower than the large 'Pablo' beetroot (2195 mg/L) (Section 5.12). The mean weight of medium 'Betty' was 92.64 ± 15.82 g while the mean weight of large 'Pablo' was 224.76 ± 67.61 g (between day 70 and day 112). Based on the 29 % juice yield (v/w) in the present study (Appendix C), 3.44 kg beetroot are required to produce 1 L of beetroot juice, which approximately corresponds to 37 medium 'Betty' or 15 large 'Pablo' beetroot. Therefore, large 'Pablo' beetroot is still a better option for producing high-nitrate beetroot juice.

'Betty' are suitable for producing fresh bunched baby beetroot due to their smaller overall size (Ruk Zwaan, 2019). If 'Betty' beetroot are used for bunched baby beetroot, in order to maintain their high nitrate content, dark and cool storage (4°C) during the transport and in-store storage are suggested for retail shops. This is because these conditions lower the activity of nitrate reductase (NR) which converts the nitrate to nitrite (Corleto et al., 2018; Taiz & Zeiger, 2010). Fresh baby beetroot are probably better consumed uncooked if the consumer desires the high nitrate content, as 86 % of nitrate is lost after boiling peeled beetroot at 100°C for 40 minutes due to the high water solubility of the nitrate (Vasconcellos et al., 2016).

If the beetroot are used for canning or vacuumed packing, the peeling process may lower the nitrate content (Bednar et al., 1991). One study found the mean nitrate content of commercial canned beetroot was 250 mg/kg while that from the fresh beetroot was 400 mg/kg (Lee et al., 1971). The nitrate may be lost when the beetroot skin is removed during peeling process in the factory, with the use of running water (Bednar et al., 1991).

Chapter 7 Conclusions and Recommendations

7.1 Conclusions

Size, weight, DM, % soluble solids, pH, TA, TPC, sucrose concentration, betacyanin and betaxanthin concentrations, nitrate and nitrite concentrations were able to be monitored for *Beta vulgaris* 'Pablo' from September and December plantings. The same parameters for quality and composition were compared between *Beta vulgaris* 'Pablo', 'Monty' and 'Betty' from a December planting.

With 'Pablo' beetroot grown between day 70 and day 138, there was a significant nitrate concentration decrease in the 'Pablo' beetroot juice from day 91 to day 138. The nitrite concentration in the 'Pablo' beetroot juice also decreased over the same sampling period. The results suggested that the recommended cultivar and harvest time for producing high-nitrate beetroot juice was large 'Pablo' grown for 84 to 98 days.

After a 21-day storage in the dark at 4 ± 1°C, the % soluble solids, TPC, sucrose concentration, betaxanthin concentration, nitrate and nitrite concentrations in juice extracted from fresh large 'Pablo' did not change significantly after the storage. The results indicated that after being stored in the dark (4 ± 1°C) for 21 days, the beetroot composition was general stable.

The preliminary results from the trial with fleece found that the nitrate content in beetroot grown under fleece was almost two times higher than that in the beetroot not grown under fleece. However, this information needs further investigation.

When comparing composition and quality parameters between 'Pablo', 'Monty' and 'Betty' beetroot grown between day 70 and day 112, 'Monty' were the largest and heaviest, had the highest DM content (14 to 16 %), and contained the highest betalain content (betacyanins and betaxantins). 'Monty' cultivar also had the highest % soluble solids (between 12.0 and 13.8 °Brix).

7.2 Recommendations

The results suggested that the cultivar and harvest time to obtain higher betalain (betacyanins and betaxanthins) beetroot juice was from the medium 'Monty' grown between day 91 and day 105.

'Betty' was the smallest beetroot of the three cultivars, and medium 'Betty' had higher nitrate content (1755 mg/L) than the other two medium cultivars, however, these levels were still lower than the nitrate content of large 'Pablo' beetroot (2195 mg/L). When considering fresh baby beetroot, the 'Betty' cultivar is recommended due to the small size and high nitrate content.

For the 'Pablo' beetroot, to better understand the nitrogen cycle in beetroot, not only nitrate and nitrite content, but also the nitrate and amino acids content of the beetroot taproot and leaves should be measured in future studies. In addition, the mineral content of the soil where the beetroot are being grown should be measured. To better understand the effects of growth time on beetroot pigments, a HPLC technique is recommended to identify and quantify the relevant compound which may participate in the betacyanin and betaxanthin biosynthesis.

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Appendix

Appendix A Preliminary Test on Nitrate and Nitrite Concentration of Juice Extracted from Small, Medium and Large Commercial Beetroot

	No.	Nitrite Concentration mg/L	Nitrate Concentration mg/L
Large	1	589.22	521.90
	2	544.41	1721.03
	3	289.04	2276.06
	4	522.60	560.19
	5	483.30	749.92
	Average	485.71	1165.82
	SD	116.41	789.82
Medium	1	233.25	614.96
	2	263.93	744.43
	3	184.18	1737.21
	4	218.90	836.63
	5	303.32	331.54
	Average	240.71	852.95
	SD	45.24	529.73
Small	1	301.95	361.75
	2	181.39	181.70
	3	378.85	166.90
	4	444.28	212.07
	5	321.97	403.72
	Average	325.69	265.23
	SD	97.83	109.51
Total	Average	283.00	559.09
	SD	84.67	475.39

Appendix B Preliminary Test for Weight, Volume and Size of Commercial Beetroot

Juicing date		Weight (g)	Volume (ml)	Diameter (cm)
23/10/2018	Large	532.13	510.00	10.00
		634.01	610.00	10.25
		618.79	600.00	11.50
		543.54	600.00	10.00
		746.32	750.00	11.00
26/10/2018		454.57	420.00	8.50
		528.99	455.00	10.00
		385.27	300.00	8.10
		440.51	350.00	8.45
		627.58	528.00	11.25
	Average	551.17	512.30	9.91
	SD	86.02	86.20	0.67
23/10/2018	Medium	131.66	120.00	6.50
		125.92	110.00	6.20
		175.68	180.00	6.30
		128.26	100.00	6.30
		81.78	70.00	5.70
	Average	128.66	116.00	6.20
	SD	33.26	40.37	0.30
23/10/2018	Small	32.65	30.00	4.00
		26.94	30.00	3.80
		23.41	20.00	3.65
		41.55	40.00	4.40
		68.11	60.00	5.10
	Average	38.53	36.00	4.19
	SD	17.90	15.17	0.58

Appendix C Juice Yield and Mass Balance Calculation of Fresh Beetroot Juicing

According to the mass balance principle (Varzakas & Tzia, 2015)

total solids in = total solids out

weight of fresh beetroot before juicing

= weight of beetroot pulp + weight of beetroot juice

= weight of DM + beetroot juice lost during juicing + weight of beetroot juice for analyse

Weight of beetroot before juicing = 454.57 g

Weight of beetroot juice after juicing for analyse =140.95g

Volume of beetroot juice after juicing for analyse = 130 ml

Weight of beetroot pulp = 454.57 g -140.95 g = 331.62 g

Juice yield (w/w) = $\frac{140.95\ \text{g}}{454.57\ \text{g}} \times 100\ \% = 31.01\ \%$

Juice yield (v/w) = $\frac{130\ \text{ml}}{454.57\ \text{g}} \times 100\ \% = 28.60\ \%$

The average of DM = 13 %

Weight of DM of beetroot = 454.57 g × 13 % = 59.09 g

Weight of juice lost during juicing = 331.62 g – 59.09 g = 272.53 g

% of juice lost during juicing (w/w) = $\frac{272.53\ \text{g}}{454.57\ \text{g}} \times 100\ \% = 59.95\ \%$

Appendix D Corrected Results of TA, TPC, Sugars, Betacyanins and Betaxanthins, Nitrate and Nitrite Concentrations to 10°Brix Juice

The concentrations of juices of TA, TPC, sugars, betalains and nitrate/nitrite were standardised to 10°Brix after measurement. The calculation use the mass balance, and the juice was considered as a steady system, then the mass of a specific component in the steady system can be expressed in Equation 1(Varzakas & Tzia, 2015):

total solids in = total solids out   Equation 1

For examples: the average concentration of TA of 12°Brix beetroot juice was A g/100 ml which was measured by the study, the unknown concentration of the 10°Brix beetroot juice was B g/100 ml, assuming there was 100 ml of 12 °Brix beetroot juice, the unknown volume of 10°Brix beetroot juice was V, according to the Equation 1:

total solids in = total solids out

12×100 ml = $10 \times V$ ml

V= 120 ml of 10°Brix beetroot juice   Equation 2

Concentration of TA of 10°Brix beetroot juice B

$= \frac{A}{120 \text{ ml}} \times 100 \text{ ml} = \frac{10}{12} A$ g/100 ml

Appendix E Statistic for 'Pablo' Beetroot from the September and December Plantings (Chapter 4)

1. Beetroot Size

One-way ANOVA with Turkey's test: size of medium beetroot for September planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

M	N	Mean	Grouping	
138	18	7.1917	A	
131	8	6.906	A	B
124	15	6.477		B
110	18	6.419		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: size of large beetroot for September planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

L	N	Mean	Grouping		
138	18	9.711	A		
131	9	8.728		B	
124	18	8.592		B	
110	20	7.830			C

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: size of medium beetroot for December planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

M	N	Mean	Grouping	
84	18	6.114	A	
91	18	5.994	A	B
98	18	5.9694	A	B
105	15	5.953	A	B
112	15	5.777	A	B
77	18	5.6639		B
72	10	5.645	A	B
70	10	5.645	A	B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: size of large beetroot for December planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

L	N	Mean	Grouping	
105	17	8.385	A	
112	15	8.0633	A	B
98	16	7.872	A	B
91	15	7.773		B
84	12	7.563		B

Means that do not share a letter are significantly different.
Two-Sample T-Test and CI for medium beetroot harvested on day 110 and day 112 after planting
Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
112 Dec M	15	5.777	0.398	0.10
110 Sep M	18	6.419	0.641	0.15

T-Value	DF	P-Value
-3.52	28	0.001

Two-Sample T-Test and CI for large beetroot harvested on day 110 and day 112 after planting
Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
110 Sep L	19	7.821	0.710	0.16
112 Dec L	15	8.063	0.281	0.073

T-Value	DF	P-Value
-1.36	24	0.187

2. Beetroot Weight

One-way ANOVA with Turkey's test: weight of medium beetroot for September planting

M	N	Mean	Grouping
138	18	195.28	A

131	9	192.0	A	B
124	17	156.74		B
110	18	154.39		B

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: weight of large beetroot for September planting

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

L	N	Mean	Grouping	
138	18	518.7	A	
124	18	380.4		B
131	9	370.7		B
110	6	315.9		B

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: weight of medium beetroot for December planting

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

M	N	Mean	Grouping
84	18	102.34	A
112	16	96.96	A
91	18	96.28	A
105	15	94.42	A
77	18	93.26	A
70	10	93.05	A
98	18	92.81	A
72	9	90.03	A

Means that do not share a letter are significantly different

One-way ANOVA with Turkey's test: weight of large beetroot for December planting

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

L	N	Mean	Grouping	
105	17	269.4	A	
98	16	223.05	A	B
91	15	218.3	A	B
112	15	211.8		B
84	12	180.7		B

Means that do not share a letter are significantly different.

Two-Sample T-Test and CI for medium beetroot harvested on day 110 and day 112 after planting

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
112 Dec M	16	97.0	14.7	3.7
110 Sep M	18	154.4	36.0	8.5

T-Value	DF	P-Value
-6.22	23	0.000

Two-Sample T-Test and CI for large beetroot harvested on day 110 and day 112 after planting

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
110 Sep L	6	315.9	93.6	38
112 Dec L	13	211.6	48.1	13

T-Value	DF	P-Value
2.58	6	0.042

3. Beetroot DM

One-way ANOVA with Turkey's test: DM of medium beetroot for September planting

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

M	N	Mean	Grouping
138	9	12.981	A
124	7	12.567	A
110	9	12.284	A
131	9	11.258	B

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: DM of large beetroot for September planting

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

L	N	Mean	Grouping
110	3	12.083	A
124	6	11.368	A
138	9	11.356	A
131	9	11.130	A

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: DM of medium beetroot for December planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

M	N	Mean	Grouping	
98	6	16.415	A	
105	6	15.223	A	B
84	9	14.982	A	B
112	6	14.649		B
70	4	14.5919	A	B
77	9	14.214		B
72	6	14.017		B
91	8	13.913		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: DM of large December planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

L	N	Mean	Grouping	
112	6	14.608	A	
84	9	13.586	A	B
98	9	13.262	A	B
91	9	12.669		B
105	5	12.441		B

Means that do not share a letter are significantly different.
Two-Sample T-Test and CI for medium and large beetroot harvested on day 110 after planting for September planting
Descriptive Statistics: 110

	N	Mean	StDev	SE Mean
1	9	12.284	0.502	0.17
2	3	12.083	0.245	0.14

T-Value	DF	P-Value
	7	0.390

Two-Sample T-Test and CI for medium and large beetroot harvested on day 124 after planting for September planting
Descriptive Statistics: 124

	N	Mean	StDev	SE Mean
M	7	12.567	0.572	0.22
L	6	11.368	0.649	0.27

T-Value	DF	P-Value
3.51	10	0.006

Two-Sample T-Test and CI for medium and large beetroot harvested on day 131 after planting for September planting
Descriptive Statistics: 131

	N	Mean	StDev	SE Mean
M	9	11.258	0.864	0.29
L	9	11.130	0.821	0.27

T-Value	DF	P-Value
0.32	15	0.752

Two-Sample T-Test and CI for medium and large beetroot harvested on day 138 after planting for September planting
Descriptive Statistics: 138

	N	Mean	StDev	SE Mean
M	9	12.981	0.729	0.24
L	9	11.36	1.24	0.41

T-Value	DF	P-Value
3.39	12	0.005

Two-Sample T-Test and CI for medium and large beetroot harvested on day 84 after planting for December planting
Descriptive Statistics: 84

	N	Mean	StDev	SE Mean
M	9	14.98	1.53	0.51

L	9	13.59	1.28	0.43

T-Value	DF	P-Value
2.10	15	0.053

Two-Sample T-Test and CI for medium and large beetroot harvested on day 91 after planting for December planting
Descriptive Statistics: 91

	N	Mean	StDev	SE Mean
M	8	13.913	0.784	0.28
L	9	12.669	0.913	0.30

T-Value	DF	P-Value
3.03	14	0.009

Two-Sample T-Test and CI for medium and large beetroot harvested on day 98 after planting
Descriptive Statistics: 98

	N	Mean	StDev	SE Mean
M	6	16.415	0.332	0.14
L	8	13.357	0.671	0.24

T-Value	DF	P-Value
11.20	10	0.000

Two-Sample T-Test and CI for medium and large beetroot harvested on day 105 after planting
Descriptive Statistics: 105

	N	Mean	StDev	SE Mean
M	6	15.22	1.12	0.46
L	5	12.441	0.737	0.33

T-Value	DF	P-Value
4.93	8	0.001

Two-Sample T-Test and CI for medium and large beetroot harvested on day 112 after planting
Descriptive Statistics: 112

	N	Mean	StDev	SE Mean
M	6	14.649	0.922	0.38
L	6	14.608	0.351	0.14

T-Value	DF	P-Value
0.10	6	0.922

4. Percentage of Soluble Solids of the Beetroot Juice

One-way ANOVA with Turkey's test: % soluble solids of medium beetroot for September planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

C9	N	Mean	Grouping		
138	9	11.5000	A		
124	9	10.811		B	
110	9	10.2444		B	C
131	9	10.156			C

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: % soluble solids of large beetroot for September planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

C19	N	Mean	Grouping		
124	9	11.4667	A		
138	9	10.656		B	
131	9	9.956			C
110	3	9.9333			C

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: % soluble solids of medium beetroot for December planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

C11	N	Mean	Grouping
112	9	12.222	A
77	9	11.656	A
84	9	11.511	A
70	9	11.4667	A
105	8	11.400	A

98	7	11.214	A
72	7	11.200	A
91	8	11.163	A

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: % soluble solids of large beetroot for December planting

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

C21	N	Mean	Grouping
112	9	10.400	A
84	9	9.911	A
105	7	9.743	A
98	7	9.229	A
91	8	9.175	A

Means that do not share a letter are significantly different.

5. pH of the Beetroot Juice

One-way ANOVA with Turkey's test: pH of medium beetroot for September planting

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

M	N	Mean	Grouping	
124	9	6.4044	A	
138	8	6.3350		B
131	9	6.3167		B
110	9	6.30444		B

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: pH of large beetroot for September planting

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

L	N	Mean	Grouping	
124	9	6.4067	A	
131	9	6.34778	A	B
138	9	6.3044		B
110	3	6.29000		B

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: pH of medium beetroot for December planting

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

M	N	Mean	Grouping			
70	9	6.5567	A			
72	9	6.4578		B		
77	9	6.4256		B	C	
91	9	6.3689			C	
84	9	6.3589			C	
98	9	6.2344				D
112	9	6.21000				D
105	9	6.1856				D

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: pH of large beetroot for December planting

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

L	N	Mean	Grouping	
84	9	6.4111	A	
91	9	6.3811	A	B
112	9	6.33333		B
98	9	6.3300		B
105	9	6.3300		B

Means that do not share a letter are significantly different.

6. TA of the Beetroot Juice

One-way ANOVA with Turkey's test: TA of medium beetroot for September planting

M	N	Mean	Grouping		
138	9	0.10435	A		
131	9	0.09405	A	B	
110	9	0.09150		B	
124	9	0.07688			C

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: TA of large beetroot for September planting

Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

L	N	Mean	Grouping	
110	3	0.10070	A	
138	9	0.09589	A	
131	9	0.08813	A	
124	7	0.07099		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: TA of medium beetroot for December planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

M	N	Mean	Grouping			
112	9	0.09138	A			
105	9	0.09074	A			
98	9	0.08712	A			
91	9	0.08376	A	B		
84	9	0.073849		B	C	
70	9	0.06393			C	D
77	9	0.06118				D
72	9	0.05655				D

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: TA of large beetroot for December planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

L	N	Mean	Grouping	
98	9	0.10581	A	
105	9	0.10073	A	
112	9	0.09626	A	
91	9	0.09586	A	
84	9	0.08027		B

Means that do not share a letter are significantly different.

7. TPC in Beetroot Juice

One-way ANOVA with Turkey's test: TPC of medium beetroot for September planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

M	N	Mean	Grouping	
138	8	0.9145	A	
124	9	0.8955	A	
110	9	0.8856	A	
131	9	0.7178		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: TPC of large beetroot for September planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

L	N	Mean	Grouping		
131	8	0.9893	A		
138	9	0.8696		B	
110	3	0.7985		B	C
124	9	0.6635			C

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: TPC of medium beetroot for December planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

M	N	Mean	Grouping		
70	9	1.7325	A		
72	9	1.6415	A	B	
77	9	1.5860	A	B	
91	9	1.5585	A	B	
84	9	1.3057		B	C
112	9	1.1757			C
98	8	1.148			C
105	8	1.0396			C

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: TPC of large beetroot for December planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

L	N	Mean	Grouping		
84	9	1.6422	A		
112	9	1.4300	A	B	
105	8	1.2229		B	C
91	9	1.2210		B	C
98	9	1.1136			C

Means that do not share a letter are significantly different.
Two-Sample T-Test and CI for medium beetroot harvested on day 110 (September planting) and day 112 (December planting) after planting

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
112 Dec M	9	1.176	0.137	0.046
110 SepM	9	0.8856	0.0686	0.023

T-Value	DF	P-Value
5.68	11	0.000

Two-Sample T-Test and CI for large beetroot harvested on day 110 and day 112 after planting

Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
112 Dec L	9	1.430	0.216	0.072
110 Sep L	3	0.7985	0.0485	0.028

T-Value	DF	P-Value
8.19	9	0.000

8. Sucrose Concentration in Beetroot Juice

One-way ANOVA with Turkey's test: sucrose of medium beetroot for September planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

M	N	Mean	Grouping	
124	9	83.272	A	
131	9	82.81	A	
138	9	73.26		B
110	9	72.574		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: sucrose of large beetroot for September planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

L	N	Mean	Grouping
124	9	73.13	A
110	3	72.127	A
138	9	71.41	A
131	4	71.25	A

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: sucrose of medium beetroot for December planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

M	N	Mean	Grouping	
112	7	90.76	A	
91	9	85.72	A	
70	9	81.66	A	B
84	8	81.42	A	B
105	9	79.61	A	B
72	9	74.86	A	B
77	8	73.44	A	B
98	9	68.18		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: sucrose of large beetroot for December planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

L	N	Mean	Grouping	
112	6	83.98	A	
84	9	80.51	A	
98	9	74.00	A	B
91	9	72.30	A	B
105	9	66.75		B

Means that do not share a letter are significantly different.

9. Betacyanin Concentration in Beetroot Juice

One-way ANOVA with Turkey's test: betacyanin of medium beetroot for September planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

M	N	Mean	Grouping		
110	9	1077.4	A		
131	9	981.6		B	
124	9	945.03		B	
138	9	725.5			C

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: betacyanin of large beetroot for September planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

L	N	Mean	Grouping		
110	3	1006.21	A		
131	9	809.7		B	
124	9	687.8			C
138	9	622.96			C

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: betacyanin of medium beetroot for December planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

M	N	Mean	Grouping	
70	9	1020.8	A	
77	9	1017.6	A	
72	9	1016.0	A	
112	9	995.5	A	
105	9	994.4	A	
98	9	896.0	A	
84	9	885.8	A	B
91	9	725.1		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: betacyanin of large beetroot for December planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

L	N	Mean	Grouping	
105	9	1176.6	A	
84	9	1083.6	A	
98	9	1064.0	A	
91	9	846.3		B
112	9	770.4		B

Means that do not share a letter are significantly different.

10.Betaxanthin Concentration in Beetroot Juice

One-way ANOVA with Turkey's test: betaxanthin of medium beetroot for September planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

M	N	Mean	Grouping			
131	9	584.55	A			
110	9	518.43		B		
124	9	450.99			C	
138	8	365.89				D

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: betaxanthin of large beetroot for September planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

L	N	Mean	Grouping	
110	3	505.32	A	
131	9	456.0	A	
138	9	333.56		B
124	9	332.43		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: betaxanthin of medium beetroot for December planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

M	N	Mean	Grouping		
112	9	650.0	A		
105	9	565.1	A	B	
77	9	515.3		B	C
84	9	496.91		B	C
98	9	480.4		B	C
72	9	480.4		B	C
70	9	459.48			C
91	9	428.9			C

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: betaxanthin of large beetroot for December planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

L	N	Mean	Grouping		
105	9	674.8	A		
84	9	630.3	A	B	
98	9	586.1	A	B	C
112	9	563.5		B	C
91	9	504.7			C

Means that do not share a letter are significantly different.

11.Betacyanin/betaxanthin Ratio in Beetroot Juice

One-way ANOVA with Turkey's test: betacyanin/betaxanthin of medium beetroot for September planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

M	N	Mean	Grouping	
124	9	2.0960	A	
110	9	2.0797	A	
138	8	1.9982	A	
131	8	1.7380		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: betacyanin/betaxanthin of large beetroot for September planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

L	N	Mean	Grouping		
124	9	2.0694	A		
110	3	1.9913	A		
138	8	1.86816		B	
131	9	1.54395			C

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: betacyanin/betaxanthin of medium beetroot for December planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

M	N	Mean	Grouping				
70	9	2.2262	A				
72	9	2.1071	A	B			
77	9	1.9739		B	C		
98	9	1.86531			C	D	
105	9	1.795			C	D	
84	9	1.7842			C	D	
91	9	1.68957				D	E
112	9	1.52967					E

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: betacyanin/betaxanthin of large beetroot for December planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

L	N	Mean	Grouping		
98	9	1.8099	A		
105	9	1.7416	A	B	
84	9	1.7222		B	
91	9	1.6735		B	
112	9	1.5648			C

Means that do not share a letter are significantly different.

Two-Sample T-Test and CI for medium beetroot harvested on day 110 (September planting) and day 112 after planting (December planting)
Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
112 Dec M	9	1.5297	0.0262	0.0087
110 Sep M	9	2.0797	0.0365	0.012

T-Value	DF	P-Value
-36.73	14	0.000

Two-Sample T-Test and CI for large beetroot harvested on day 110 and day 112 after planting
Descriptive Statistics

Sample	N	Mean	StDev	SE Mean
112 Dec L	9	1.5648	0.0503	0.017
110 Sep L	3	1.9913	0.0176	0.010

T-Value	DF	P-Value
-21.76	9	0.000

12.Nitrate Concentration in Beetroot Juice

One-way ANOVA with Turkey's test: nitrate of medium beetroot for September planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

M	N	Mean	Grouping	
110	9	927.0	A	
124	9	867	A	B
131	9	792.8	A	B
138	9	617.63		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: nitrate of large beetroot for September planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

L	N	Mean	Grouping	
110	3	1123.23	A	
138	9	1001.7	A	
124	9	752.8		B
131	9	745.1		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: nitrate of medium beetroot for December planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

M	N	Mean	Grouping	
91	8	1656	A	
84	9	1653.7	A	
77	9	1601.2	A	B
98	9	1560	A	B
72	9	1475.0	A	B
70	8	1410.2	A	B
112	9	1327	A	B
105	9	1200.1		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: nitrate of large beetroot for December planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

L	N	Mean	Grouping	
91	9	2878.1	A	
98	9	2376	A	B
84	9	2214	A	B
105	9	1897		B
112	9	1613		B

Means that do not share a letter are significantly different.

13. Nitrite Concentration in Beetroot Juice

One-way ANOVA with Turkey's test: nitrite of medium beetroot for September planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

M	N	Mean	Grouping		
124	9	66.09	A		
110	9	49.53		B	
131	9	48.26		B	
138	9	22.26			C

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: nitrite of large beetroot for September planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

L	N	Mean	Grouping		
110	3	47.30	A		
124	8	45.63	A		
131	9	33.43		B	
138	9	23.492			C

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: nitrite of medium beetroot for September planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

M	N	Mean	Grouping		
77	9	212.08	A		
70	8	211.2	A		
84	9	206.63	A	B	
98	9	199.09	A	B	
72	9	194.52	A	B	C
91	8	191.1	A	B	C
105	9	174.84		B	C
112	9	164.36			C

Means that do not share a letter are significantly different
One-way ANOVA with Turkey's test: nitrite of large beetroot for December planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

L	N	Mean	Grouping		
91	9	246.48	A		
98	6	238.05	A	B	
84	9	214.07		B	
105	9	176.64			C
112	9	150.8			C

Means that do not share a letter are significantly different.

14. Effects of Growing under Fleece on Beetroot Composition

14.1 Beetroot Size

One-way ANOVA with Turkey's test: size of covered vs not covered medium beetroot on day 124 and day 131 from the September planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
MC131	9	6.928	A
MN131	8	6.906	A
MN124	15	6.477	A
MC124	18	6.344	A

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: size of covered vs not covered large beetroot on day 124 and day 131 from the September planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
LN131	9	8.728	A
LC124	15	8.677	A
LN124	18	8.592	A
LC131	9	8.372	A

Means that do not share a letter are significantly different

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14.2 Beetroot Weight

One-way ANOVA with Turkey's test: weight of covered vs not covered large beetroot on day 124 and day 131 from the September planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
MN 131	9	191.95	A
MC131	9	185.70	A
MC124	16	162.58	A
MN 124	17	156.74	A

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: DM of covered vs not covered large beetroot on day 124 and day 131 from the September planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
LN124	18	380.39	A
LN131	9	370.66	A
LC124	15	358.49	A
LC131	8	324.74	A

Means that do not share a letter are significantly different.

14.3 Beetroot DM

One-way ANOVA with Turkey's test: DM of covered vs not covered medium beetroot on day 124 and day 131 from the September planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping	
MN124	7	12.567	A	
MN131	9	11.258	A	B
MC131	9	11.104		B
MC124	9	11.016		B

Means that do not share a letter are significantly different

One-way ANOVA with Turkey's test: DM of covered vs not covered large beetroot on day 124 and day 131 from the September planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
LN124	6	11.368	A
LN131	9	11.130	A
LC124	9	10.943	A
LC131	9	10.423	A

Means that do not share a letter are significantly different.

14.4 pH of Beetroot Juice

One-way ANOVA with Turkey's test: pH of covered vs not covered medium beetroot on day 124 and day 131 from the September planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping	
MN124	9	6.4044	A	
MN131	9	6.3167		B
MC131	8	6.2850		B
MC124	9	6.2833		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: pH of covered vs not covered large beetroot on day 124 and day 131 from the September planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping	
LN124	9	6.4067	A	
Lc124	9	6.3611	A	B

LN131	9	6.34778	A	B
LC131	9	6.2822		B

Means that do not share a letter are significantly different.

14.5 Percentage of Soluble Solids of Beetroot Juice

One-way ANOVA with Turkey's test: % soluble solids of covered vs not covered medium beetroot on day 124 and day 131 from the September planting

Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping	
MN124	9	10.811	A	
Mn131	9	10.156		B
MC124	9	10.0556		B
MC131	9	9.856		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: % soluble solids of covered vs not covered large beetroot on day 124 and day 131 from the September planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping		
LN124	9	11.4667	A		
LC124	9	10.556		B	
LC131	9	10.044		B	C
LN131	9	9.956			C

Means that do not share a letter are significantly different.

14.6 TA of Beetroot Juice

One-way ANOVA with Turkey's test: TA of covered vs not covered medium beetroot on day 124 and day 131 from the September planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping	
MC131	9	0.10480	A	
MN131	9	0.09405	A	B
Mc124	8	0.07828		B
MN124	9	0.07688		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: TA of covered vs not covered large beetroot on day 124 and day 131 from the September planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping		
Lc131	9	0.09296	A		
LN131	9	0.08813	A	B	
LC124	9	0.07471		B	C
LN124	7	0.07099			C

Means that do not share a letter are significantly different.

14.7 TPC in Beetroot Juice

One-way ANOVA with Turkey's test: TPC of covered vs not covered medium beetroot on day 124 and day 131 from the September planting

Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping		
MC131	8	1.5912	A		
MN124	9	0.8955		B	
MC124	8	0.8662		B	C
MN131	9	0.7178			C

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: TPC of covered vs not covered large beetroot on day 124 and day 131 from the September planting
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping

LC131	9	1.1760	A
LN131	9	0.9893	A
Lc124	9	0.7681	B
LN124	9	0.6635	B

Means that do not share a letter are significantly different.

14.8 Sucrose Concentration in Beetroot Juice

One-way ANOVA with Turkey's test: sucrose of covered vs not covered medium beetroot on day 124 and day 131 from the September planting

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
MN124	9	83.27	A
MN131	9	82.81	A
MC124	9	68.61	B
MC131	9	66.62	B

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: sucrose of covered vs not covered large beetroot on day 124 and day 131 from the September planting

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
LN124	9	73.13	A
LN131	4	71.25	A
LC131	9	68.37	A
LC124	9	58.46	B

Means that do not share a letter are significantly different.

14.9 Betacyanin Concentration in Beetroot Juice

One-way ANOVA with Turkey's test: betacyanin of covered vs not covered large beetroot on day 124 and day 131 from the September planting

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
MN131	9	981.6	A
MN124	9	945.0	A
Mc124	9	835.7	B
MC131	9	703.9	C

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: betacyanin of covered vs not covered large beetroot on day 124 and day 131 from the September planting

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
LN124	9	687.8	A
LC131	9	596.5	A
LN131	9	588.4	A
LC124	9	575.4	A

Means that do not share a letter are significantly different.

14.10 Betaxanthin Concentration in Beetroot Juice

One-way ANOVA with Turkey's test: betaxanthin of covered vs not covered medium beetroot on day 124 and day 131 from the September planting

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping
MN131	9	584.55	A
MC124	9	468.32	B
MN124	9	450.99	B
MC131	9	445.42	B

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: betaxanthin of covered vs not covered large beetroot on day 124 and day 131 from the September planting

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping		
LN131	9	455.98	A		
LC131	9	380.34		B	
LN124	9	332.43			C
LC124	9	323.26			C

Means that do not share a letter are significantly different.

14.11 Nitrate Concentration in Beetroot Juice

One-way ANOVA with Turkey's test: nitrate of covered vs not covered medium beetroot on day 124 and day 131 from the September planting

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping		
MC124	9	1965.9	A		
MC131	9	1597.9		B	
MN124	9	866.9			C
MN131	9	792.8			C

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: nitrate of covered vs not covered large beetroot on day 124 and day 131 from the September planting

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping	
LC124	9	1707.6	A	
LC131	9	1511.4	A	
LN124	9	752.8		B
LN131	9	745.1		B

Means that do not share a letter are significantly different.

14.12 Nitrite Concentration in Beetroot Juice

One-way ANOVA with Turkey's test: nitrite of covered vs not covered medium beetroot on day 124 and day 131 from the September planting

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping		
MN 124	9	66.09	A		
MN131	9	48.26		B	
MC 124	9	37.18			C
MC131	9	29.69			C

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: nitrite of covered vs not covered large beetroot on day 124 and day 131 from the September planting

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Factor	N	Mean	Grouping	
LN124	8	45.63	A	
LN131	9	33.43		B
LC131	9	31.53		B
LC 124	9	29.49		B

Means that do not share a letter are significantly different.

15. Effect of Postharvest Storage on Beetroot Composition

15.1 Beetroot Size

One-way ANOVA with Turkey's test: beetroot size for storage week 0,1,2 and 3

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Day	N	Mean	Grouping	
7	16	8.509	A	
21	15	8.413	A	B
14	17	7.959	A	B
0	15	7.773		B

Means that do not share a letter are significantly different.

15.2 Beetroot Weight

One-way ANOVA with Turkey's test: beetroot weight for storage week 0,1,2 and 3
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

Day	N	Mean	Grouping
7	15	276.3	A
21	15	265.2	A
0	17	212.6	A
14	17	211.5	A

15.3 Beetroot DM

One-way ANOVA with Turkey's test: beetroot DM for storage week 0,1,2 and 3
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

Day	N	Mean	Grouping		
14	6	13.730	A		
7	6	12.956	A	B	
0	9	12.669		B	
21	7	11.604			C

Means that do not share a letter are significantly different.

15.4 Percentage of Soluble Solids of Beetroot Juice

One-way ANOVA with Turkey's test: beetroot % soluble solids for storage week 0,1,2 and 3
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

Day	N	Mean	Grouping		
14	8	10.850	A		
21	9	10.267	A	B	
0	8	9.175		B	C
7	7	8.800			C

Means that do not share a letter are significantly different.

15.5 pH of Beetroot Juice

One-way ANOVA with Turkey's test: beetroot pH for storage week 0,1,2 and 3
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

Day	N	Mean	Grouping		
0	9	6.3811	A		
7	9	6.3011		B	
14	9	6.18111			C
21	9	6.14333			C

Means that do not share a letter are significantly different.

15.6 TA of Beetroot Juice

One-way ANOVA with Turkey's test: beetroot TA for storage week 0,1,2 and 3
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

Day	N	Mean	Grouping
21	9	0.10603	A
7	9	0.10227	A
14	9	0.10138	A
0	9	0.09683	A

Means that do not share a letter are significantly different.

15.7 TPC in Beetroot Juice

One-way ANOVA with Turkey's test: beetroot TPC for storage week 0,1,2 and 3
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

Day	N	Mean	Grouping	
7	9	1.955	A	
21	8	1.564	A	B
14	9	1.2667		B
0	9	1.2222		B

Means that do not share a letter are significantly different.

15.8 Sucrose Concentration in Beetroot Juice

One-way ANOVA with Turkey's test: beetroot sucrose for storage week 0,1,2 and 3
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

Day	N	Mean	Grouping
0	9	70.37	A
7	9	69.85	A
14	8	66.16	A
21	7	63.48	A

Means that do not share a letter are significantly different.

15.9 Betacyanin Concentration in Beetroot Juice

One-way ANOVA with Turkey's test: beetroot betacyanin for storage week 0,1,2 and 3
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

Day	N	Mean	Grouping		
7	9	1266.7	A		
21	9	1071.9	A	B	
14	9	889.7		B	C
0	9	846.3			C

Means that do not share a letter are significantly different.

15.10 Betaxanthin Concentration in Beetroot Juice

One-way ANOVA with Turkey's test: beetroot betaxanthin for storage week 0,1,2 and 3
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

Day	N	Mean	Grouping	
7	9	741.2	A	
21	9	613.8	A	B
14	9	519.8		B
0	9	504.7		B

Means that do not share a letter are significantly different.

15.11 Nitrate Concentration in Beetroot Juice

One-way ANOVA with Turkey's test: beetroot nitrate for storage week 0,1,2 and 3
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

Day	N	Mean	Grouping		
0	9	2878.1	A		
21	9	2697.1	A		
7	9	2302.0		B	
14	8	1673.4			C

Means that do not share a letter are significantly different.

15.12 Nitrite Concentration in the Beetroot Juice

One-way ANOVA with Turkey's test: beetroot nitrite for storage week 0,1,2 and 3
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

Day	N	Mean	Grouping	
0	9	246.48	A	
21	9	220.69	A	
7	9	213.47	A	B
14	9	181.51		B

Appendix F Statistic for 'Pablo', 'Monty' and 'Betty' Beetroot from the December Planting (Chapter 5)

1. Beetroot Size

One-way ANOVA with Turkey's test: size of medium Pablo beetroot
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

PM	N	Mean	Grouping	
84	18	6.114	A	
91	18	5.994	A	B
98	18	5.9694	A	B
105	15	5.953	A	B
112	15	5.777	A	B
77	19	5.6553		B
72	10	5.645	A	B
70	10	5.645	A	B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: size of medium Monty beetroot
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

MM	N	Mean	Grouping	
84	18	5.9694	A	
98	18	5.8833	A	B
91	18	5.811	A	B
105	13	5.746	A	B
77	18	5.703	A	B
72	9	5.594	A	B
70	9	5.456		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: size of medium Betty beetroot
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

BM	N	Mean	Grouping		
105	17	6.015	A		
91	18	5.972	A		
98	18	5.8056	A	B	
84	18	5.7444	A	B	C
72	9	5.428		B	C
70	11	5.400		B	C
77	18	5.3444			C

Means that do not share a letter are significantly different
One-way ANOVA with Turkey's test: size of large Pablo beetroot
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

PL	N	Mean	Grouping	
105	17	8.385	A	
112	15	8.0633	A	B
98	16	7.872	A	B
91	15	7.773		B
84	12	7.563		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: size of large Monty beetroot
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

ML	N	Mean	Grouping	
105	12	8.729	A	
84	13	7.854		B
91	8	7.788		B
98	15	7.687		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: size of large Betty beetroot
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

BL	N	Mean	Grouping

105	11	7.623	A
98	14	7.5286	A
84	13	7.3577	A
91	12	7.2667	A

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: size of medium beetroot between three cultivars at day 70 (Pablo-1, Monty-2, Betty-3)
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

	N	Mean	Grouping
1	10	5.645	A
2	9	5.456	A
3	11	5.400	A

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: size of medium beetroot between three cultivars at day 105
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

	N	Mean	Grouping
3	17	6.015	A
1	15	5.953	A
2	13	5.746	A

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: size of large beetroot between three cultivars at day 84 (Pablo-1, Monty-2, Betty-3)
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

	N	Mean	Grouping	
2	13	7.854	A	
1	12	7.563	A	B
3	13	7.3577		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: size of large beetroot between three cultivars at day 105 (Pablo-1, Monty-2, Betty-3)
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

	N	Mean	Grouping	
2	14	8.671	A	
1	17	8.385	A	
3	11	7.623		B

Means that do not share a letter are significantly different.

2. Beetroot Weight

One-way ANOVA with Turkey's test: weight of medium Pablo beetroot
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

PM	N	Mean	Grouping
84	18	102.34	A
112	16	96.96	A
91	18	96.28	A
105	15	94.42	A
77	18	93.26	A
70	10	93.05	A
98	18	92.81	A
72	9	90.03	A

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: weight of medium Monty beetroot
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

MM	N	Mean	Grouping	
84	18	107.20	A	
98	18	106.75	A	B
91	18	105.89	A	B
105	15	102.88	A	B
77	18	101.54	A	B
72	9	92.57	A	B
70	7	82.08		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: weight of medium Betty beetroot
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

BM	N	Mean	Grouping		
98	18	101.45	A		
91	18	99.29	A	B	
105	15	97.30	A	B	
84	17	91.04	A	B	C
77	17	86.81		B	C
72	7	83.61	A	B	C
70	10	75.58			C

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: weight of large Pablo beetroot

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

PL	N	Mean	Grouping	
105	17	269.4	A	
98	16	223.05	A	B
91	15	218.3	A	B
112	15	211.8		B
84	12	180.7		B

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: weight of large Monty beetroot

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

ML	N	Mean	Grouping	
105	14	331.0	A	
84	13	248.2		B
91	10	235.8		B
98	14	230.7		B

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: weight of large Betty beetroot

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

BL	N	Mean	Grouping	
105	11	211.2	A	
98	15	199.62	A	
91	12	183.7	A	B
84	13	163.71		B

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: weight of medium beetroot between three cultivars at day 70 (Pablo-1, Monty-2, Betty-3)

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

	N	Mean	Grouping
1	10	93.05	A
2	7	82.08	A
3	10	75.58	A

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: weight of medium beetroot between three cultivars at day 105 (Pablo-1, Monty-2, Betty-3)

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

	N	Mean	Grouping
2	15	102.88	A
3	15	97.30	A
1	15	94.42	A

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: weight of large beetroot between three cultivars at day 84 (Pablo-1, Monty-2, Betty-3)

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

	N	Mean	Grouping	
2	12	253.7	A	
1	11	184.7		B
3	13	163.71		B

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: weight of large beetroot between three cultivars at day 91 (Pablo-1, Monty-2, Betty-3)

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

	N	Mean	Grouping
2	10	235.8	A

1	15	218.3	A	B
3	12	183.7		B

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: weight of large beetroot between three cultivars at day 98 (Pablo-1, Monty-2, Betty-3)

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

	N	Mean	Grouping
1	16	223.05	A
2	17	221.5	A
3	12	204.84	A

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: weight of large beetroot between three cultivars at day 105 (Pablo-1, Monty-2, Betty-3)

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

	N	Mean	Grouping	
2	14	331.0	A	
1	17	269.4	A	B
3	11	211.2		B

Means that do not share a letter are significantly different.

3. Beetroot DM

One-way ANOVA with Turkey's test: DM of medium Pablo beetroot

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

PM	N	Mean	Grouping	
98	6	16.415	A	
105	6	15.223	A	B
84	9	14.982	A	B
112	6	14.649		B
70	4	14.5919	A	B
77	9	14.214		B
72	6	14.017		B
91	8	13.913		B

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: DM of medium Monty beetroot

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

MM	N	Mean	Grouping	
98	6	16.415	A	
70	5	16.272	A	B
105	6	15.981	A	B
91	7	15.947	A	B
72	6	15.556	A	B
84	9	15.100	A	B
77	9	15.041		B

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: DM of medium Betty beetroot

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

BM	N	Mean	Grouping
70	6	14.862	A
72	4	14.551	A
98	6	14.393	A
105	6	14.271	A
77	7	13.877	A
84	9	13.602	A
91	9	13.417	A

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: DM of large Pablo beetroot

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

PL	N	Mean	Grouping	
112	6	14.608	A	
84	7	13.315	A	B
98	9	13.262		B
91	9	12.669		B

105	5	12.441		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: DM of large Monty beetroot
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

ML	N	Mean	Grouping	
98	6	15.956	A	
105	6	14.856	A	B
91	9	14.431		B
84	8	14.060		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: DM of large Betty beetroot
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

BL	N	Mean	Grouping	
98	6	13.851	A	
105	5	13.429	A	B
91	8	12.747	A	B
84	8	11.587		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: DM of medium beetroot between three cultivars at day 70 (Pablo-1, Monty-2, Betty-3)

Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

	N	Mean	Grouping	
2	5	16.272	A	
3	6	14.862		B
1	4	14.5919		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: DM of medium beetroot between three cultivars at day 105 (Pablo-1, Monty-2, Betty-3)
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

	N	Mean	Grouping	
2	6	15.981	A	
1	6	15.223	A	B
3	6	14.271		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: DM of large beetroot between three cultivars at day 84 (Pablo-1, Monty-2, Betty-3)
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

	N	Mean	Grouping	
2	8	14.060	A	
1	7	13.315	A	B
3	8	11.587		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: DM of large beetroot between three cultivars at day 105 (Pablo-1, Monty-2, Betty-3)
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

	N	Mean	Grouping		
2	6	14.856	A		
3	5	13.429		B	
1	5	12.441			C

Means that do not share a letter are significantly different.

4. Percentage of Soluble Solids of Beetroot Juice

One-way ANOVA with Turkey's test: % soluble solids in the beetroot juice of medium Pablo beetroot
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

PM	N	Mean	Grouping
112	9	12.222	A
77	9	11.656	A
84	9	11.511	A
70	9	11.4667	A
105	8	11.400	A
98	7	11.214	A
72	7	11.200	A
91	8	11.163	A

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: % soluble solids of medium Monty beetroot
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

MM	N	Mean	Grouping	
98	9	13.789	A	
91	9	13.756	A	
70	9	12.911	A	B
84	9	12.711	A	B
72	9	12.533	A	B
105	9	12.522	A	B
77	9	12.022		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: % soluble solids of medium Betty beetroot
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

BM	N	Mean	Grouping
77	9	11.7556	A
98	9	11.744	A
72	9	11.633	A
70	9	11.611	A
91	9	11.544	A
84	9	11.456	A
105	9	10.989	A

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: % soluble solids of large Pablo beetroot
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

PL	N	Mean	Grouping
112	9	10.400	A
84	9	9.911	A
105	7	9.743	A
98	7	9.229	A
91	8	9.175	A

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: % soluble solids of large Monty beetroot
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

ML	N	Mean	Grouping
98	7	13.500	A
91	9	13.311	A
105	9	12.578	A
84	9	12.200	A

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: % soluble solids of large Betty beetroot
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

BL	N	Mean	Grouping
91	9	11.056	A
105	9	10.922	A
84	9	10.744	A
98	9	10.589	A

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: % soluble solids of medium beetroot between three cultivars at day 70 (Pablo-1, Monty-2, Betty-3)
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

	N	Mean	Grouping	
2	9	12.911	A	
3	9	11.611		B
1	9	11.4667		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: % soluble solids of medium beetroot between three cultivars at day 72 (Pablo-1, Monty-2, Betty-3)
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

	N	Mean	Grouping

2	9	12.533	A
3	9	11.633	B
1	7	11.200	B

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: % soluble solids of medium beetroot between three cultivars at day 77 (Pablo-1, Monty-2, Betty-3)

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

	N	Mean	Grouping
2	9	12.022	A
3	9	11.7556	A
1	9	11.656	A

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: % soluble solids of medium beetroot between three cultivars at day 84 (Pablo-1, Monty-2, Betty-3)

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

	N	Mean	Grouping	
2	9	12.711	A	
1	9	11.511		B
3	9	11.456		B

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: % soluble solids of medium beetroot between three cultivars at day 91 (Pablo-1, Monty-2, Betty-3)

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

	N	Mean	Grouping	
2	9	13.756	A	
3	9	11.544		B
1	8	11.163		B

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: size of medium beetroot between three cultivars at day 98 (Pablo-1, Monty-2, Betty-3)

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

	N	Mean	Grouping	
2	9	13.789	A	
3	9	11.744		B
1	7	11.214		B

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: % soluble solids of medium beetroot between three cultivars at day 105 (Pablo-1, Monty-2, Betty-3)

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

	N	Mean	Grouping	
2	9	12.522	A	
1	8	11.400	A	B
3	9	10.989		B

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: % soluble solids of large beetroot between three cultivars at day 84 (Pablo-1, Monty-2, Betty-3)

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

	N	Mean	Grouping	
2	9	12.200	A	
3	9	10.744		B
1	9	9.911		B

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: % soluble solids of large beetroot between three cultivars at day 91 (Pablo-1, Monty-2, Betty-3)

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

	N	Mean	Grouping		
2	9	13.311	A		
3	9	11.056		B	
1	8	9.175			C

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: % soluble solids of large beetroot between three cultivars at day 98 (Pablo-1, Monty-2, Betty-3)

Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

	N	Mean	Grouping	
2	7	13.500	A	
3	9	10.589		B
1	7	9.229		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: % soluble solids of large beetroot between three cultivars at day 105 (Pablo-1, Monty-2, Betty-3)
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

	N	Mean	Grouping	
2	9	12.578	A	
3	9	10.922		B
1	7	9.743		B

Means that do not share a letter are significantly different.

5. pH of Beetroot Juice

One-way ANOVA with Turkey's test: pH in the beetroot juice of medium Pablo beetroot
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

PM	N	Mean	Grouping			
70	9	6.5567	A			
72	9	6.4578		B		
77	9	6.4256		B	C	
91	9	6.3689			C	
84	9	6.3589			C	
98	9	6.2344				D
112	9	6.21000				D
105	9	6.1856				D

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: pH in the beetroot juice of medium Monty beetroot
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

MM	N	Mean	Grouping				
72	9	6.5944	A				
70	9	6.5178		B			
77	9	6.4256			C		
84	9	6.41333			C		
98	9	6.3822			C	D	
91	9	6.3500				D	
105	9	6.25444					E

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: pH in the beetroot juice of medium Betty beetroot
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

BM	N	Mean	Grouping			
72	9	6.5756	A			
70	9	6.5600	A			
77	9	6.4522		B		
98	9	6.3422			C	
84	9	6.3389			C	
91	9	6.3344			C	
105	9	6.2156				D

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: pH in the beetroot juice of large Pablo beetroot
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

PL	N	Mean	Grouping	
84	9	6.4111	A	
91	9	6.3811	A	B
112	9	6.33333		B
98	9	6.3300		B
105	9	6.3300		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: pH in the beetroot juice of large Monty beetroot

Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

ML	N	Mean	Grouping	
84	8	6.4300	A	
98	9	6.4144	A	
91	9	6.40000	A	
105	9	6.2300		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: pH in the beetroot juice of large Betty beetroot
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

BL	N	Mean	Grouping
98	9	6.3611	A
91	9	6.3544	A
105	9	6.34444	A
84	9	6.3356	A

Means that do not share a letter are significantly different.

6. TA of Beetroot Juice

One-way ANOVA with Turkey's test: TA in the beetroot juice of medium Pablo beetroot
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

PM	N	Mean	Grouping			
112	9	0.09282	A			
105	9	0.09074	A			
98	9	0.08712	A			
91	9	0.08376	A	B		
84	9	0.073849		B	C	
70	9	0.06393			C	D
77	9	0.06118				D
72	9	0.05655				D

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: TA in the beetroot juice of medium Monty beetroot
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

MM	N	Mean	Grouping		
98	9	0.07397	A		
105	9	0.07366	A		
84	9	0.06932	A		
91	9	0.06662	A		
77	9	0.05750		B	
70	9	0.04820			C
72	9	0.04726			C

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: TA in the beetroot juice of medium Betty beetroot
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

BM	N	Mean	Grouping			
105	9	0.090992	A			
98	9	0.07874		B		
84	9	0.07650		B		
91	9	0.07578		B		
77	9	0.07455		B		
70	9	0.06565			C	
72	9	0.05541				D

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: TA in the beetroot juice of large Pablo beetroot
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

PL	N	Mean	Grouping	
98	9	0.10581	A	
105	9	0.10073	A	
112	9	0.09626	A	
91	9	0.09586	A	
84	9	0.08027		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: TA in the beetroot juice of large Monty beetroot
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

ML	N	Mean	Grouping	
105	9	0.070988	A	
84	9	0.07086	A	
98	9	0.06807	A	
91	9	0.06119		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: TA in the beetroot juice of large Betty beetroot
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

BL	N	Mean	Grouping	
105	9	0.09541	A	
98	9	0.08855	A	
84	9	0.07966		B
91	9	0.07585		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: TA of medium beetroot between three cultivars at day 70 (Pablo-1, Monty-2, Betty-3)
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

	N	Mean	Grouping	
3	9	0.06565	A	
1	9	0.06393	A	
2	9	0.04820		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: TA of medium beetroot between three cultivars at day 105 (Pablo-1, Monty-2, Betty-3)
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

	N	Mean	Grouping	
3	9	0.090992	A	
1	9	0.09074	A	
2	9	0.07366		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: TA of large beetroot between three cultivars at day 84 (Pablo-1, Monty-2, Betty-3)
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

	N	Mean	Grouping	
1	9	0.08027	A	
3	9	0.07966	A	
2	9	0.07086		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: TA of large beetroot between three cultivars at day 105 (Pablo-1, Monty-2, Betty-3)
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

	N	Mean	Grouping	
1	9	0.10073	A	
3	9	0.09541	A	
2	9	0.070988		B

Means that do not share a letter are significantly different.

7. TPC in Beetroot Juice

One-way ANOVA with Turkey's test: TPC in the beetroot juice of medium Pablo beetroot
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

PM	N	Mean	Grouping		
70	9	1.7325	A		
72	9	1.6415	A		
77	9	1.5860	A	B	
91	9	1.5585	A	B	
84	9	1.3057		B	C
98	9	1.2290			C
112	9	1.1757			C
105	8	1.0396			C

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: TPC in the beetroot juice of medium Monty

Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

MM	N	Mean	Grouping	
77	9	1.8141	A	
70	9	1.8066	A	
72	9	1.7985	A	
84	8	1.6290	A	
105	9	1.617	A	
91	9	1.1887		B
98	8	1.0445		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: TPC in the beetroot juice of medium Betty
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

BM	N	Mean	Grouping		
72	9	1.6831	A		
84	9	1.5450	A	B	
77	9	1.5110	A	B	
70	9	1.4201	A	B	
105	9	1.3399		B	
91	9	1.0105			C
98	9	0.8703			C

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: TPC in the beetroot juice of large Pablo
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

PL	N	Mean	Grouping		
84	8	1.6659	A		
112	9	1.4300	A	B	
105	8	1.2229		B	C
91	9	1.2210		B	C
98	9	1.1136			C

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: TPC in the beetroot juice of large Monty
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

ML	N	Mean	Grouping	
84	9	1.6257	A	
105	7	1.1949		B
91	9	1.0193		B
98	9	0.942		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: TPC in the beetroot juice of large Betty
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

BL	N	Mean	Grouping	
84	9	1.5845	A	
98	9	1.0651		B
105	9	1.0457		B
91	9	0.9858		B

Means that do not share a letter are significantly different.

8. Sucrose Concentration in Beetroot Juice

One-way ANOVA with Turkey's test: sucrose concentration in the beetroot juice of medium Pablo
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

PM	N	Mean	Grouping	
112	7	90.76	A	
91	9	85.72	A	
70	9	81.66	A	B
84	8	81.42	A	B
105	9	79.61	A	B
72	9	74.86	A	B
77	8	73.44	A	B
98	9	68.18		B

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: sucrose concentration in the beetroot juice of medium Monty
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

MM	N	Mean	Grouping
72	9	81.52	A
70	9	81.39	A
91	9	80.88	A
84	9	79.00	A
77	9	76.32	A
105	9	75.23	A
98	7	69.81	A

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: sucrose concentration in the beetroot juice of medium Betty
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

BM	N	Mean	Grouping		
105	9	86.79	A		
70	9	84.92	A	B	
98	6	71.74		B	C
72	9	71.215			C
77	9	70.81			C
91	9	65.78			C
84	9	62.68			C

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: sucrose concentration in the beetroot juice of large Pablo
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

PL	N	Mean	Grouping	
112	6	83.98	A	
84	9	80.51	A	B
98	9	74.00	A	B
91	9	72.30	A	B
105	9	66.75		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: sucrose concentration in the beetroot juice of large Monty
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

ML	N	Mean	Grouping	
84	9	99.80	A	
91	9	92.73	A	B
98	9	87.31	A	B
105	9	77.80		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: sucrose concentration in the beetroot juice of Large Betty
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

BL	N	Mean	Grouping
105	6	86.95	A
98	8	82.59	A
84	9	76.11	A
91	6	71.91	A

Means that do not share a letter are significantly different.

9. Betacyanin Concentration in Beetroot Juice

One-way ANOVA with Turkey's test: betacyanin concentration in the beetroot juice of medium Pablo
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

PM	N	Mean	Grouping	
70	9	1021.8	A	
77	9	1017.6	A	
72	9	1016.0	A	
112	9	995.5	A	
105	9	994.4	A	
98	9	896.0	A	
84	9	885.8	A	B

91	9	725.1	B

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: betacyanin concentration in the beetroot juice of medium Monty

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

MM	N	Mean	Grouping
105	9	1544.7	A
77	9	1284.9	B
72	9	1258.8	B
98	9	1195.9	B
84	9	1178.3	B
91	9	1155.3	B
70	9	1131.5	B

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: betacyanin concentration in the beetroot juice of medium Betty

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

BM	N	Mean	Grouping
98	9	1052.9	A
77	9	1038.78	A
72	9	1008.18	A
70	9	994.8	A
84	9	994.7	A
91	9	970.3	A
105	9	885.8	A

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: betacyanin concentration in the beetroot juice of large Pablo

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

PL	N	Mean	Grouping
105	9	1176.6	A
84	9	1083.6	A
98	9	1064.0	A
91	9	846.3	B
112	9	770.4	B

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: betacyanin concentration in the beetroot juice of Large Monty

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

ML	N	Mean	Grouping
98	9	1405.6	A
91	9	1138.8	B
84	9	1072.1	B
105	9	903.3	C

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: betacyanin concentration in the beetroot juice of Large Betty

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

BL	N	Mean	Grouping
98	9	1091.4	A
84	9	978.9	A B
91	9	894.4	B
105	9	680.6	C

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: betacyanin of medium beetroot between three cultivars at day 70 (Pablo-1, Monty-2, Betty-3)

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

	N	Mean	Grouping
2	9	1131.5	A
1	9	1020.8	A B
3	9	994.8	B

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: betacyanin of medium beetroot between three cultivars at day 105 (Pablo-1, Monty-2, Betty-3)

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

	N	Mean	Grouping	
2	9	1544.7	A	
1	9	994.4		B
3	9	885.8		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: betacyanin of large beetroot between three cultivars at day 84 (Pablo-1, Monty-2, Betty-3)
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

	N	Mean	Grouping
1	9	1083.6	A
2	9	1072.1	A
3	9	978.9	A

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: betacyanin of medium beetroot between three cultivars at day 91 (Pablo-1, Monty-2, Betty-3)
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

	N	Mean	Grouping	
2	9	1138.8	A	
3	9	894.4		B
1	9	846.3		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: betacyanin of medium beetroot between three cultivars at day 98 (Pablo-1, Monty-2, Betty-3)
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

	N	Mean	Grouping	
2	9	1405.6	A	
3	9	1091.4		B
1	9	1064.0		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: betacyanin of large beetroot between three cultivars at day 105 (Pablo-1, Monty-2, Betty-3)
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

	N	Mean	Grouping	
1	9	1176.6	A	
2	9	757.9		B
3	9	680.6		B

Means that do not share a letter are significantly different.

10. Betaxanthin Concentration in Beetroot Juice

One-way ANOVA with Turkey's test: betaxanthin concentration in the beetroot juice of medium Pablo
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

C2	N	Mean	Grouping		
112	9	650.0	A		
105	9	565.1	A	B	
77	9	515.3		B	C
84	9	496.91		B	C
98	9	480.4		B	C
72	9	480.4		B	C
70	9	459.48			C
91	9	428.9			C

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: betaxanthin concentration in the beetroot juice of medium Monty
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

C8	N	Mean	Grouping				
105	9	763.3	A				
91	9	672.1		B			
84	9	624.89		B	C		
98	9	622.1		B	C		
77	9	589.94			C	D	
72	9	538.83				D	E
70	9	484.8					E

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: betaxanthin concentration in the beetroot juice of medium Betty
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

C14	N	Mean	Grouping		
98.00	9	730.7	A			
91.00	9	636.8		B		
105.00	9	610.1		B		
84.00	9	565.39		B	C	
77.00	9	515.079			C	D
72.00	9	497.54			C	D
70.00	9	431.2				D

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: betaxanthin concentration in the beetroot juice of large Pablo
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

C5	N	Mean	Grouping		
105.00	9	674.8	A		
84.00	9	630.3	A	B	
98.00	9	586.1	A	B	C
112.00	9	563.5		B	C
91.00	9	504.7			C

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: betaxanthin concentration in the beetroot juice of large Monty
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

C11	N	Mean	Grouping		
98.00	9	750.0	A		
91.00	9	655.75		B	
84.00	9	576.9			C
105.00	9	512.20			C

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: betaxanthin concentration in the beetroot juice of large Betty
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

C17	N	Mean	Grouping	
98.00	9	613.9	A	
91.00	9	595.3	A	
84.00	9	576.7	A	
105.00	9	461.7		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: betaxanthin concentration of medium beetroot between three cultivars at day 70 (Pablo-1, Monty-2, Betty-3)
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

C2	N	Mean	Grouping	
2	8	493.1	A	
1	9	459.48	A	B
3	9	431.2		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: betaxanthin concentration of medium beetroot between three cultivars at day 72 (Pablo-1, Monty-2, Betty-3)
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

C4	N	Mean	Grouping
2	10	526.8	A
3	9	497.54	A
1	9	480.4	A

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: betaxanthin concentration of medium beetroot between three cultivars at day 77 (Pablo-1, Monty-2, Betty-3)
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

C6	N	Mean	Grouping	
2	9	589.94	A	
1	9	515.3		B
3	9	515.079		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: betaxanthin concentration of medium beetroot between three cultivars at day 84 (Pablo-1, Monty-2, Betty-3)
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

C8	N	Mean	Grouping		
2	9	624.89	A		
3	9	565.39		B	
1	9	496.91			C

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: betaxanthin concentration of medium beetroot between three cultivars at day 91 (Pablo-1, Monty-2, Betty-3)
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

C10	N	Mean	Grouping	
2	9	672.1	A	
3	9	636.8	A	
1	9	428.9		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: betaxanthin concentration of medium beetroot between three cultivars at day 98 (Pablo-1, Monty-2, Betty-3)
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

C12	N	Mean	Grouping	
3	9	730.7	A	
2	9	622.1	A	
1	9	480.4		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: betaxanthin concentration of medium beetroot between three cultivars at day 105 (Pablo-1, Monty-2, Betty-3)
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

C14	N	Mean	Grouping	
2	9	763.3	A	
3	9	610.1		B
1	9	565.1		B

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: betaxanthin concentration of large beetroot between three cultivars at day 84 (Pablo-1, Monty-2, Betty-3)
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

C16	N	Mean	Grouping
1	9	630.3	A
2	9	576.9	A
3	9	576.7	A

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: betaxanthin concentration of large beetroot between three cultivars at day 91 (Pablo-1, Monty-2, Betty-3)
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

C18	N	Mean	Grouping	
2	9	655.75	A	
3	9	595.3	A	
1	9	504.7		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: betaxanthin concentration of large beetroot between three cultivars at day 98 (Pablo-1, Monty-2, Betty-3)
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

C20	N	Mean	Grouping	
2	9	750.0	A	
3	9	613.9		B
1	9	586.1		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: betaxanthin concentration of large beetroot between three cultivars at day 105 (Pablo-1, Monty-2, Betty-3)
Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

C22	N	Mean	Grouping	
1	9	674.8	A	
2	9	512.20		B
3	9	461.7		B

Means that do not share a letter are significantly different.

11. Betacyanin/betaxanthin Ratio in Beetroot Juice

Two sample T-test for betacyanin/betaxanthin ratio in juice from medium Pablo beetroot harvested at day 70 and day 112.
Descriptive Statistics: PM

PM	N	Mean	StDev	SE Mean
70	9	2.226	0.118	0.039
112	9	1.5297	0.0262	0.0087

T-Value	DF	P-Value
17.25	8	0.000

Two sample T-test for betacyanin/betaxanthin ratio in juice from medium Monty beetroot harvested at day 70 and day 105.
Descriptive Statistics:

MM	N	Mean	StDev	SE Mean
70	9	2.3366	0.0342	0.011
105	6	1.890	0.101	0.041

T-Value	DF	P-Value
10.40	5	0.000

Two sample T-test for betacyanin/betaxanthin ratio in juice from medium Betty beetroot harvested at day 70 and day 105.
Descriptive Statistics:

BM	N	Mean	StDev	SE Mean
70	9	2.3028	0.0890	0.030
105	9	1.4450	0.0514	0.017

T-Value	DF	P-Value
25.05	12	0.000

Two sample T-test for betacyanin/betaxanthin ratio in juice from large Pablo beetroot harvested at day 84 and day 112.
Descriptive Statistics: PL

PL	N	Mean	StDev	SE Mean
84	9	1.7222	0.0479	0.016
112	9	1.5648	0.0503	0.017

T-Value	DF	P-Value
6.80	15	0.000

Two sample T-test for betacyanin/betaxanthin ratio in juice from large Monty beetroot harvested at day 84 and day 105.
Descriptive Statistics:

ML	N	Mean	StDev	SE Mean
84	9	1.8601	0.0189	0.0063
105	9	1.7642	0.0524	0.017

T-Value	DF	P-Value
5.17	10	0.000

Two sample T-test for betacyanin/betaxanthin ratio in juice from large Betty beetroot harvested at day 70 and day 105.
Descriptive Statistics:

BL	N	Mean	StDev	SE Mean
84	9	1.6956	0.0464	0.015
105	9	1.4747	0.0300	0.010

T-Value	DF	P-Value
11.98	13	0.000

12. Nitrate Concentration in Beetroot Juice

One-way ANOVA with Turkey's test: nitrate concentration in the beetroot juice of medium Pablo beetroot
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

PM	N	Mean	Grouping	
91	8	1656	A	
84	9	1653.7	A	
77	9	1601.2	A	B
98	9	1560	A	B
72	9	1475.0	A	B
70	8	1410.2	A	B

112	9	1327	A	B
105	9	1200.1		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: nitrate concentration in the beetroot juice of medium Monty beetroot
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

MM	N	Mean	Grouping		
77	9	1405.5	A		
84	9	1208.8	A	B	
98	9	1110.6		B	
105	9	1097.3		B	
70	9	1030.3		B	
91	9	1026.2		B	
72	9	654.2			C

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: nitrate concentration in the beetroot juice of medium Betty beetroot
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

BM	N	Mean	Grouping			
91	8	2080.8	A			
105	9	1952.3	A	B		
84	9	1951	A	B		
77	9	1817	A	B	C	
72	9	1608		B	C	D
98	9	1454.5			C	D
70	9	1370.7				D

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: nitrate concentration in the beetroot juice of large Pablo beetroot
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

PL	N	Mean	Grouping	
91	9	2878.1	A	
98	9	2376	A	B
84	9	2214	A	B
105	9	1897		B
112	9	1613		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: nitrate concentration in the beetroot juice of large Monty beetroot
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

ML	N	Mean	Grouping
105	9	1279.4	A
84	9	1275.4	A
91	9	1016.0	A
98	9	996	A

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: nitrate concentration in the beetroot juice of large Betty beetroot
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

BL	N	Mean	Grouping	
84	9	1995.9	A	
105	9	1952.3	A	
91	9	1697	A	B
98	9	1520.8		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: size of medium beetroot between three cultivars at day70 (Pablo-1, Monty-2, Betty-3)
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

	N	Mean	Grouping	
1	8	1410.2	A	
3	9	1370.7	A	
2	9	1030.3		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: size of medium beetroot between three cultivars at day 98 (Pablo-1, Monty-2, Betty-3)
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

	N	Mean	Grouping

3	9	234.3	A	
1	9	199.09	A	
2	9	154.0		B

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: size of medium beetroot between three cultivars at day 105 (Pablo-1, Monty-2, Betty-3)

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

	N	Mean	Grouping	
3	9	1952.3	A	
1	9	1200.1		B
2	9	1097.3		B

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: size of large beetroot between three cultivars at day 84 (Pablo-1, Monty-2, Betty-3)

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

	N	Mean	Grouping	
1	9	242.69	A	
3	9	223.9	A	
2	9	163.06		B

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: size of large beetroot between three cultivars at day 105 (Pablo-1, Monty-2, Betty-3)

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

	N	Mean	Grouping	
3	9	1952.3	A	
1	9	1897	A	
2	9	1279.4		B

Means that do not share a letter are significantly different.

13. Nitrite Concentration in Beetroot Juice

One-way ANOVA with Turkey's test: nitrite concentration in the beetroot juice of medium Pablo

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

PM	N	Mean	Grouping		
77	9	212.08	A		
70	8	211.2	A		
84	9	206.63	A	B	
98	9	199.09	A	B	
72	9	194.52	A	B	C
91	8	191.1	A	B	C
105	9	174.84		B	C
112	9	164.4			C

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: nitrite concentration in the beetroot juice of medium Monty

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

MM	N	Mean	Grouping
77	9	166.4	A
98	9	154.0	A
91	9	139.36	A
105	9	138.77	A
70	9	137.4	A
84	9	137.19	A
72	9	135.74	A

Means that do not share a letter are significantly different.

One-way ANOVA with Turkey's test: nitrite concentration in the beetroot juice of medium Betty

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

BM	N	Mean	Grouping		
84	9	255.0	A		
98	9	234.3	A	B	
91	8	220.7	A	B	
105	9	220.38	A	B	
77	9	214.3	A	B	C
72	9	193.33		B	C
70	9	168.48			C

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: nitrite concentration in the beetroot juice of large Pablo
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

PL	N	Mean	Grouping	
91	9	246.48	A	
84	9	242.69	A	
98	6	238.05	A	
105	9	176.64		B
112	9	172.6		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: nitrite concentration in the beetroot juice of large Monty
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

ML	N	Mean	Grouping	
84	9	163.06	A	
98	9	144.95	A	B
105	9	137.89	A	B
91	9	127.3		B

Means that do not share a letter are significantly different.
One-way ANOVA with Turkey's test: nitrite concentration in the beetroot juice of large Betty
Tukey Pairwise Comparisons
Grouping Information Using the Tukey Method and 95% Confidence

BL	N	Mean	Grouping	
98	9	253.42	A	
84	9	223.9	A	B
105	9	220.38	A	B
91	9	218.74		B

Means that do not share a letter are significantly different.