Identifying the optimal harvest time of mountain pepper based on its flavour and aroma profile

Charlton Shen

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Supervisor: Dr. Malcolm Possell

Assoc Supervisor: Dr. Kim-yen Phan-Thien

Other participant: Dr. Andrew Rath

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Abstract

This study aims to focus on identifying the optimal harvest period and post-harvest treatment for mountain pepper (*Tasmannia lanceolata*). As the popularity of mountain pepper has increased significantly over the past years, the industry finds it difficult to maintain its production rate and product quality in order to satisfy the rising demands. Therefore, it is important to identify the ideal harvest period and post-harvest methods for producing high quality berries. The main quality parameters of mountain pepper this study has focused on include: size, volume, mass, colour, polygodial levels, anthocyanin levels, flavonol concentrations and hydroxycinnamic acid concentrations. Each parameter was examined and analysed in mountain peppers harvested from different periods (April to June), under different drying methods (air-drying and freeze-drying). The result shows that between the two post-harvest drying methods, freeze-dried berries contain higher levels of polygodial (15-18 g kg^{-1} dry weight), anthocyanins (25-28 mg kg^{-1} dry weight), flavonols (2-2.3 mg kg^{-1} dry weight) and hydroxycinnamic acids (6-8 mg kg^{-1} dry weight) compare to air-dried berries. This indicates freeze-dried berries are stronger in flavour and richer in antioxidants. Moreover, freeze-dried berries also have brighter colours (L*=20-23, a*=1.5-1.7, b*=1.2-1.5). Throughout the entire harvest period, the samples harvested in June have the highest polygodial concentrations, flavonols concentrations and hydroxycinnamic acid concentrations. Therefore June is identified to be the optimal harvest period for Tasmanian mountain pepper.

1. Introduction

Mountain Pepper (Tasmannia lanceolata), also named as Tasmanian pepper, is one of the indigenous Australian ingredients that has increased its popularity enormously during the past decades. The plant sits in the family *Winteraceae*, which had been originally grown on the primeval supercontinent Gondwana (Sultanbawa and Sultanbawa, 2016). When the supercontinent splits up, mountain pepper was drifted along with the continent, and is now located in South-East Australia and Tasmania, growing under various ecological environments through numerous altitudes (Read and Menary, 2001). Plant development is optimised in wet and cool environments with open canopy, adequate water source and nutritious soil. The fully grown size varies between 2 to 5 meters high, and the berry has a delightful hot flavour and strong aroma (Winnett et al. 2014).

The timing of harvest and the selection of high quality berries are two vital factors for commercialisation (Dragar et al. 1998). Since the popularity of mountain pepper has increased dramatically, the biggest issues for this evolving industry is to maintain its level of production, in order to fulfil the rising demand domestically and internationally, as well as ensuring the consistency of the product flavour and quality (Druce, 2013).

Mountain pepper's production speed and product quality are closely associated with their harvest time and post-harvest management. The plant is normally harvested between March and July, and when the harvest is kept under favoured condition (1-2°C), berry quality can be maintained for numerous weeks (Druce, 2013). Currently, there are two types of commercial post-harvest approaches for mountain pepper, air-drying and freeze-drying (Druce, 2013). Air-drying involves drying berries by hot air at a temperature of 30-45°C

inside an oven. The final product generally develops similar physical features of the dried peppercorn of black pepper (*Piper nigrum*). Freeze drying involves drying berries inside a vacuum chamber at a temperature of -22°C (Oruña-Concha et al. 1998). Both methods aim to remove moisture from the berries to prevent spoilage and contamination by fungus and bacteria. Moreover, dried berries are more suitable for fine grinding and producing pepper mix (Clarke, 2012).

Studies of various samples of mountain pepper have shown that large variations were found in their polygodial concentrations. Polygodial is an important compound that provides the intense heat and spiciness for mountain pepper, a consistent level of polygodial concentration will provide a consistent level of spiciness in the berries (Dragar et al. 1998). However, previous studies have identified inconsistent patterns of polygodial concentration in mountain pepper, polygodial content may either rise or stay constant over time (Dragar et al. 1998). Dragar et al. (1998) detected varying polygodial intensities in pepperberries, ranging from 2% to 30%. In order to produce high quality products, mountain pepper growers must either choose plants with constant high levels of polygodial, or plants with increasing levels of polygodial during the harvest period (Dragar et al. 1998).

Flavonoids are another group of compounds vital for mountain pepper's quality attributes. Anthocyanins are phenolic compounds of flavonoids popular for their unique colouring characteristics. They present blue, red and purple colours in numerous fresh berries and fruits. However, anthocyanins are fairly unstable and tremendously fragile, frequently causing colour deterioration (Jensen et al. 2011). The colour stability of anthocyanins can be significantly disturbed by many factors such as light, pH, temperature, enzyme availability, concentration of anthocyanins as well as the existence of complex compounds including

phenolic acids and other flavonoids. Yet this unstable matter can be improved by co-pigmentation, which may offer brighter and more stable colours. For mountain pepper, co-pigmentation is believed to be critical, as the appearance of mountain pepper will affect consumers' purchase decisions when determining the berry qualities (Jensen et al. 2011).

Furthermore, flavonols and hydroxycinnamic acids are also compounds that not only make tremendous contributions to mountain pepper's flavour properties, but also its antioxidant activities. Current researches have acknowledged extraordinarily huge amount of antioxidant content in Tasmanian pepper. These findings have suggested that Tasmanian pepper leaves contain antioxidant contents four times more than those found in basil leaves and blueberries, particularly when basil leaves and blueberries are considered high in antioxidant levels by numerous studies (Netzel et al. 2007).

This study aims to find out the optimal harvest period that produces the maximum polygodial and anthocyanin concentration, together with its antioxidant properties, as well as identify the optimal post-harvest process that produces higher quality berries in terms of their flavour, aroma, and antioxidant levels. The outcome of this project may assists mountain pepper farmers to understand when to harvest, and which post-harvest drying method to focus on in order to produce top quality berries. This may potentially solve the problems the industry is currently facing: inconsistency in flavour and production rate. The hypotheses for this experiment is that harvest time and post-processing treatment will have a significant effect on the physical and flavour characteristics of Tasmanian Mountain Pepper.

2. Materials & Methods

2.1 Material

All mountain peppers were supplied by Dr. Andrew Rath, Bronzewing Farms, Tasmania. Peppers were harvested once a month, between April and June 2018. Five separate bushes were harvested at each time point (for a total of 15 bushes over the complete harvest period). The harvest from each bush was then separated into three portions. One portion was immediately frozen (hereafter referred to as fresh), the second portion was air-dried at 90°C for one hour then at 40°C for 3 days (hereafter referred to as air-dried), and the third portioned was freeze-dried for 24 hours (hereafter referred to as freeze-dried). Samples were then air-freighted to Sydney.

2.2 Size and Volume

Thirty berries from each batch of air-dried, freeze-dried and fresh samples were randomly selected. Each berry's height and length were measured using calipers. Volume was calculated from the size data with the equation $(4/3\pi a^2 b) \times 2$, where *a* represents ½ of berry height, and *b* represents ¼ of the berry's length. As one half of an individual mountain pepper has the shape of an oblate ellipsoid, the equation for the volume of an oblate ellipsoid was used, then doubled to obtain the full volume of each mountain pepper. All calculations were recorded with the average volume and standard deviation.

2.3 Mass

Thirty berries from each batch of air-dried, freeze-dried and fresh samples were randomly selected and weighed using an Ohaus AX324 analytical balance and repeated with

3 replicates. All masses were recorded, and average mass and standard deviations were calculated.

2.4 Colour

Samples from each batches were placed inside a holder for a Konica Minolta CR-400/410 Chroma meter (Konica Minolta, Sydney, Australia) and measured in the unit of L*a*b*. L* represents lightness; a* represents the green to red components, with red in the positive direction and green in the negative direction; and b* represents blue to yellow components, with yellow in the positive direction and blue in the negative direction. All measurements were recorded.

2.5 Moisture content of fresh berries

Twenty grams of sample from all fresh berry batches were weighed and placed inside the paper bags with corresponding labels. All samples were dehydrated inside a Thermoline Scientific dehydrating oven for 48 hours and the mass of the dry berries was weighed immediately after to determine their dry weight.

2.6 Polygodial extraction

One gram of sample from all batches were blended individually using a Home & Co coffee grinder (Kmart; Sydney, Australia). 500 mg of each blended samples were then weighed into a 15 mL polypropylene tube. Five mL of hexane (Sigma-Aldrich) was added to each polypropylene tube. All polypropylene tubes were sonicated by an Elma sonicator (John Morris Scientific Pty Ltd; Sydney, Australia) for 15 minutes, rested for 2 minutes, and sonicated for another 15 minutes. After being sonicated, all polypropylene tubes were centrifuged at 4000 rpm for 10 minutes. One mL of the supernatant from all polypropylene

tubes were extracted and transferred to GC vials with corresponding labels, and capped. All GC vials were placed into the freezer until analysis by gas chromatography - flame ionization detector (GC-FID).

2.6.1 Polygodial concentration measurements by GC-FID

Polygodial concentrations in the samples were determined using an Agilent 7890 GC equipped with a flame ionisation detector (Agilent Technologies Pty Ltd, Mulgrave, Australia). One µL of pepper berry extract was injected at a 10:1 split into a split/splitless inlet held at 250 °C onto a SolGel-WAX capillary column (30 m x 0.25 mm, 0.25 µm film thickness; SGE Analytical Science Pty Ltd., Melrose Park, NSW, Australia). Ultra-high purity helium was used as carrier gas (flow rate through the column was 2.3 ml min^{-1}. The initial oven temperature of the GC was 40 °C, held for 8 minute, then heated at a rate of 4 °C min^{-1} to 230 °C, held isothermally for 5 minutes. The temperature of the detector was 280 °C. The polygodial concentration was determined from a calibration of polygodial (Sigma-Aldrich, Sydney, Australia) dissolved in hexane (Sigma-Aldrich).

2.7 Anthocyanin, flavonol and hydroxycinnamic acid concentration

500 mg of sample from all batches were blended individually using a blender. 250 mg of each blended sample were then weighed into a 15 mL polypropylene tube. Five mL of methanolic HCL (80% methanol: 1% HCI: 19% H_2O) were added into each polypropylene tube. All polypropylene tubes were sonicated for 10 minute, then centrifuged for 10 minutes at 4000 rpm. Five mL of the supernatants from each polypropylene tubes were extracted and transferred to new polypropylene tubes with corresponding labels. Another 5mL of methanolic HCL was added to the original tubes with blended samples. The steps above were repeated 2 more times until a total of 15 mL of supernatant is obtained. All polypropylene

tubes with 15 mL of supernatant were shaken on a vortex mixer before transferring 1.5 mL into individual GC vials with corresponding labels. All GC vials were capped, and frozen at -20°C until analysis by high-performance liquid chromatography (HPLC).

2.7.1 HPLC quantification of anthocyanin, flavonol and hydroxycinnamic acids

Quantification of phenolic compounds in extracts was carried out using an Agilent 1200 Series High Performance Liquid Chromatography (HPLC) system (Agilent Technologies, Pty Ltd, Mulgrave, Australia that consisted of a quaternary pump (G1311A), a G1322A degasser, a G1329A autosampler, a G1315D diode array detector and a G1316A column oven equipped with a 150 mm x 4.6 mm I.D., 5 μm ZORBAX Eclipse XDB-C18 column (Agilent). The following solvents in water with a flow rate of 1.0 mL/min were used: A, 3.0% phosphoric acid in water and B, 95% acetonitrile and 3% phosphoric acid in water. The elution profile was a linear gradient elution for B of 10% over 6 minutes followed by an increase to 50% over 27 min, and then to 80% over 9 minutes. The column was then washed with 100% solvent B for 5 minutes. Analytical HPLC was run at 25°C and monitored at 326 nm (hydroxycinnamic acids and stilbenes), 370 nm (flavonols) and 520 nm (anthocyanins).

Cinnamic acids were quantified as chlorogenic acid equivalents (CHA Eq.), flavonols and stilbenes were quantified as rutin equivalents (R Eq.) and anthocyanin compounds were quantified as cyanidin 3-glucoside equivalents (C3G Eq.). The results are presented per gram of dry weight (e.g. mg C3G Eq/g DW). All chemicals were purchased from (Sigma-Aldrich, Sydney, Australia). Calibration was performed by dissolving known quantities of the standards in the same solution used in the extraction process.

3. Results

3.1 Mass

Figure 1 shows that fresh berries have the highest mass compare to air-dried and freeze-dried berries. Fresh berries harvested in June were slightly heavier than fresh berries harvested in April and May, while air-dried and freeze-dried berries have similar mass consistently throughout the entire harvest period. Harvest and harvest × treatment interactions both have a significant effect with regard to the berry mass (Table 1).

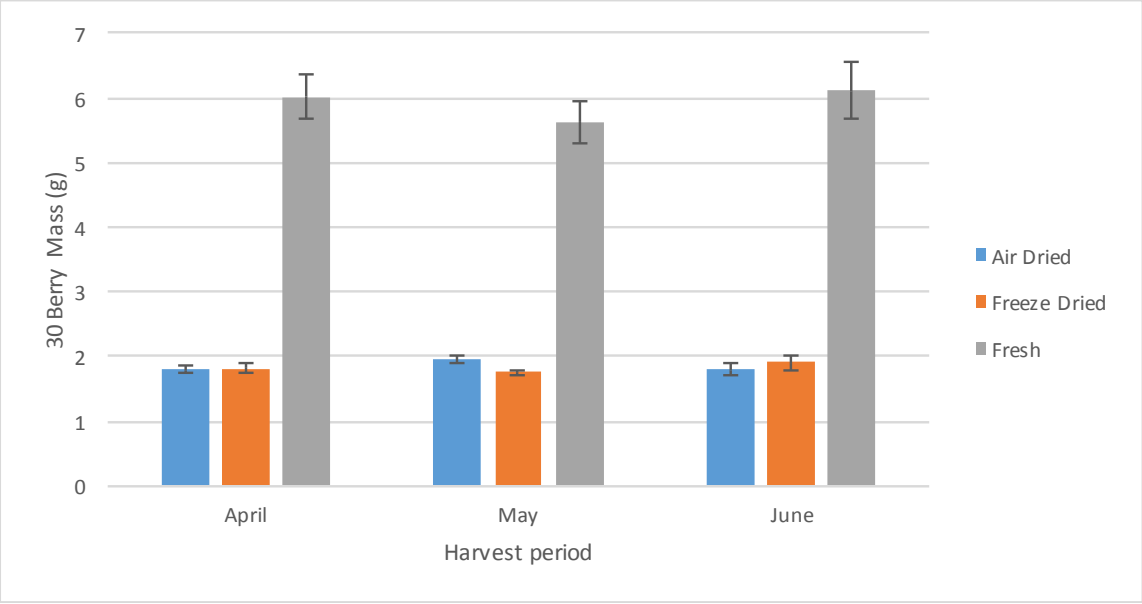

Figure 1: Mass of air-dried, freeze-dried and fresh berry harvested in April, May and June (30 berry mass (g)). Values are reported as means ± standard deviation (n = 3).

3.2 Volume

Figure 2 shows that air-dried berries have the least volume compare to freeze-dried and fresh berry. However, the berry volume was gradually increasing from April to June. Freeze-dried berries have a higher volume than fresh berries in May, but similar volume in April and June. Harvest, treatment and harvest × treatment interactions all have a significant effect on berry volume (Table 1).

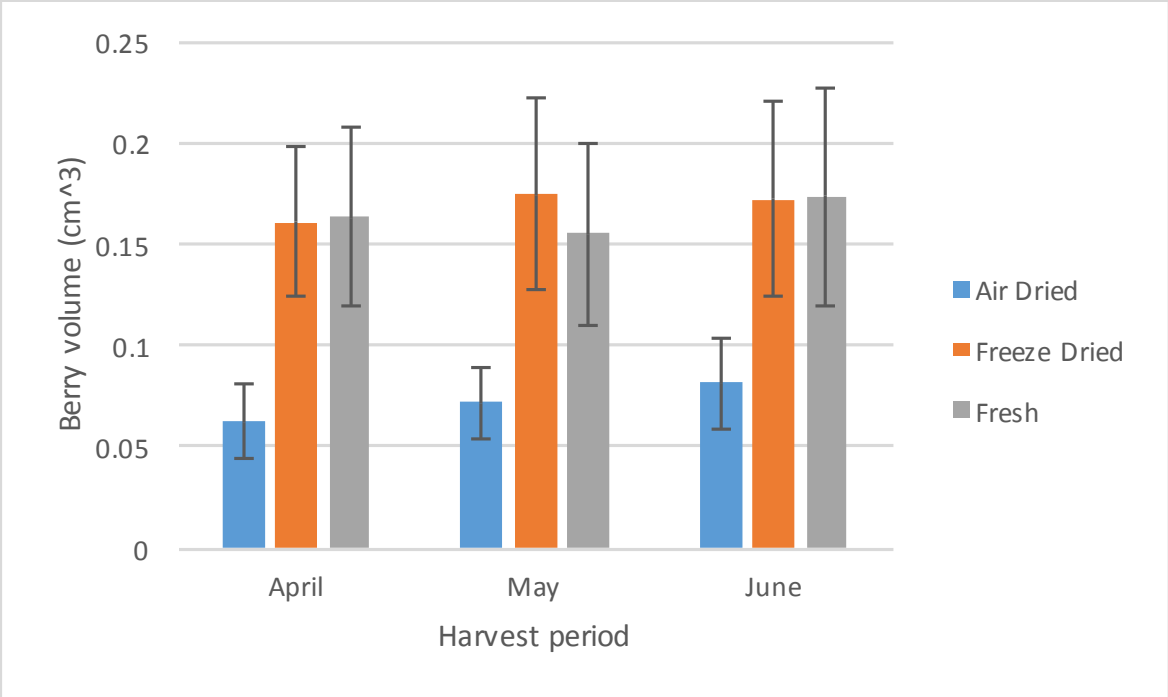

Figure 2: Volume of air-dried, freeze-dried and fresh berry harvested in April, May and June. Values are reported as means ± standard deviation (n = 45).

3.3 Colour

*3.3.1 Colour L**

Figure 3 indicates that freeze-dried berry has the highest colour L* value compare to air-dried and fresh berry. However, L* value of freeze-dried berries gradually decreased from April to June. While the L* value of air-dried berries is consistent throughout the entire harvest period. Fresh berries have the same L* value as air-dried berries in April and June, but slightly higher in May. Harvest, treatment and harvest × treatment interactions all have a significant effect on lightness (Table 1).

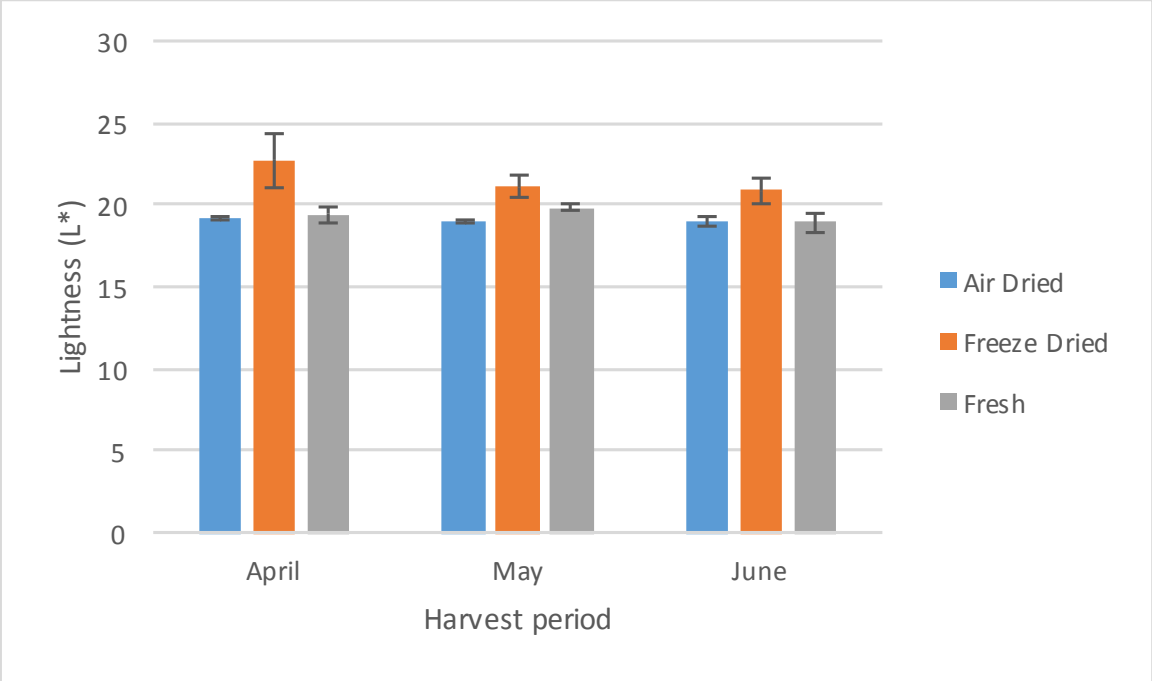

Figure 3: L* value of air-dried, freeze-dried and fresh berry harvested in April, May and June. Values are reported as means ± standard deviation (n = 5).

*3.3.2 Colour a**

Figure 4 shows that air-dried berries have the highest colour a* value compare to freeze-dried and fresh berry. The a* value for air-dried berries is consistent throughout April and May, and increased slightly in June. Freeze dried and fresh berries have the same value in April, but in May, the a* value for fresh berries has reduced slightly while freeze dried berries remained the same. In June, both freeze-dried and fresh berries increased their a* value marginally. Harvest and treatment both have a significant effect on colour a* (Table 1).

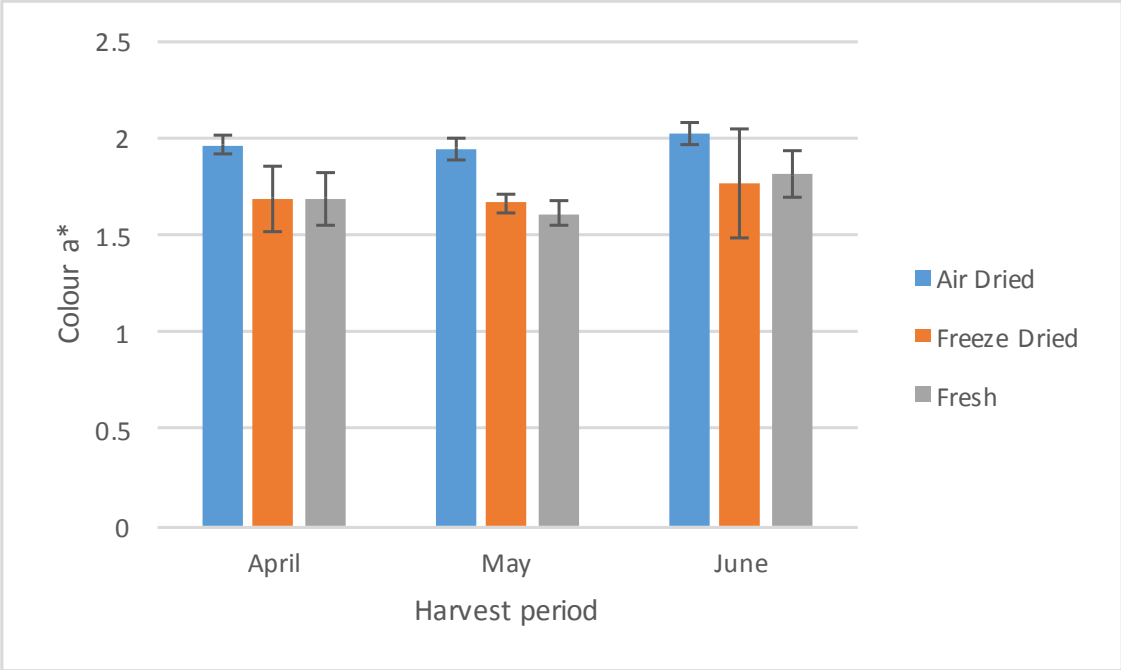

Figure 4: Colour a* of air-dried, freeze-dried and fresh berry harvested in April, May and June. Values are reported as means ± standard deviation (n = 5).

*3.3.3 Colour b**

Figure 5 shows that fresh berries have a negative value in April and May, then positive value in June. While air-dried and freeze-dried berries both received positive values throughout the entire harvest period. The b* value of air-dried berries gradually decreased from April to June, while the freeze-dried berries had their highest b* value in April and lowest in May. Harvest, treatment and harvest × treatment interactions all have a significant effect on colour b* (Table 1).

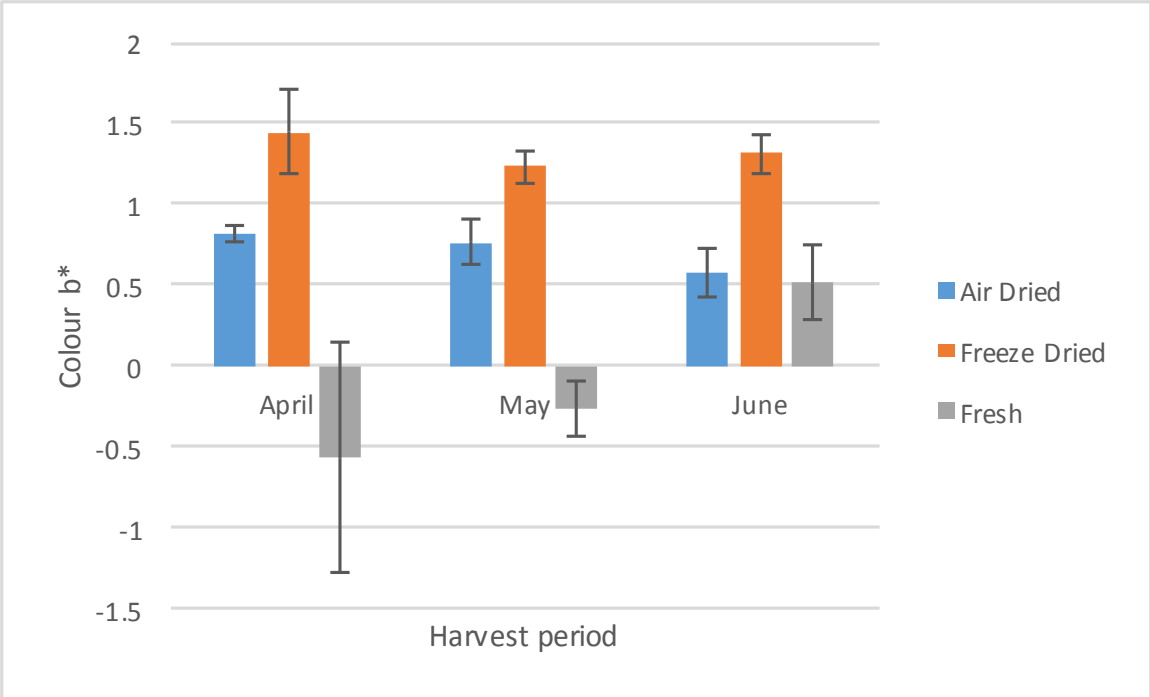

Figure 5: Colour b* of air-dried, freeze-dried and fresh berry harvested in April, May and June. Values are reported as means ± standard deviation (n = 5).

3.4 Polygodial concentration

Polygodial concentration for all samples is shown in Figure 6. Fresh berries have the lowest polygodial concentration, while freeze-dried berries have the highest polygodial concentration, especially the berries harvested in June. Air-dried berries have lower polygodial concentration than freeze-dried berries, but higher concentrations than fresh berries. Treatment and harvest × treatment interactions both have a significant effect on polygodial concentration (Table 2).

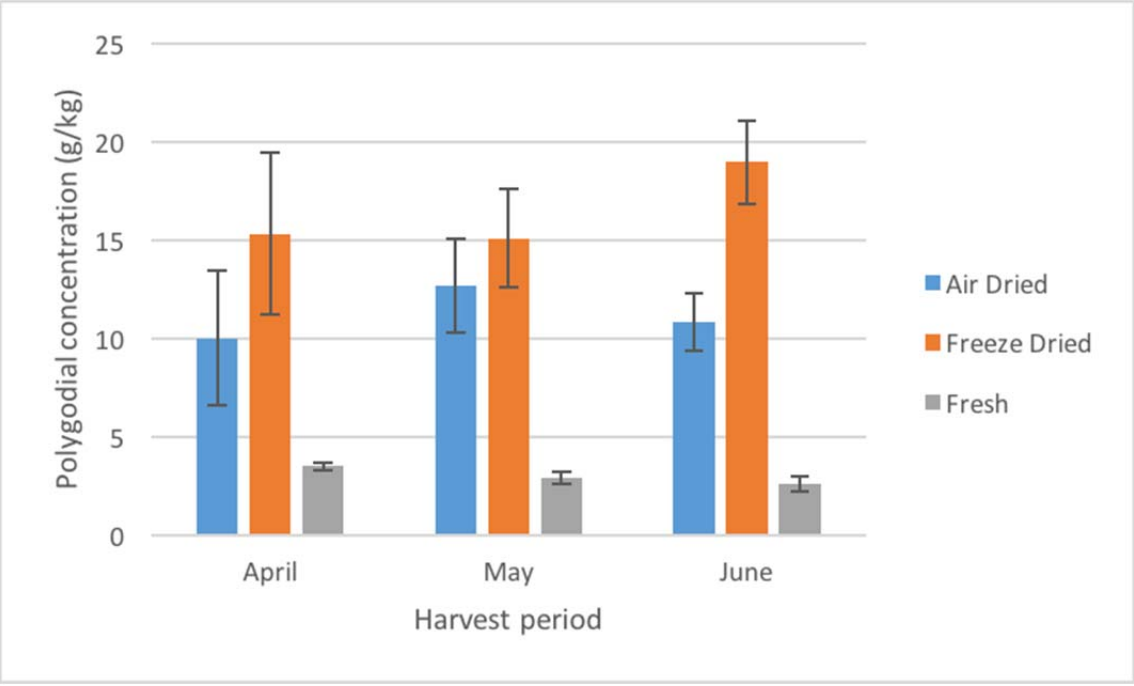

Figure 6: Polygodial concentration of air-dried, freeze-dried and fresh berry harvested in April, May and June. Values are reported as means ± standard deviation (n = 5).

3.5 Anthocyanin concentration

Figure 7 shows that throughout all 3 months, freeze-dried berries have the highest anthocyanin concentrations, especially in April, while air-dried berries have a little amount compare to freeze-dried berries. Fresh berries have none. Harvest, treatment and harvest × treatment all have a significant effect on anthocyanin content (Table 2).

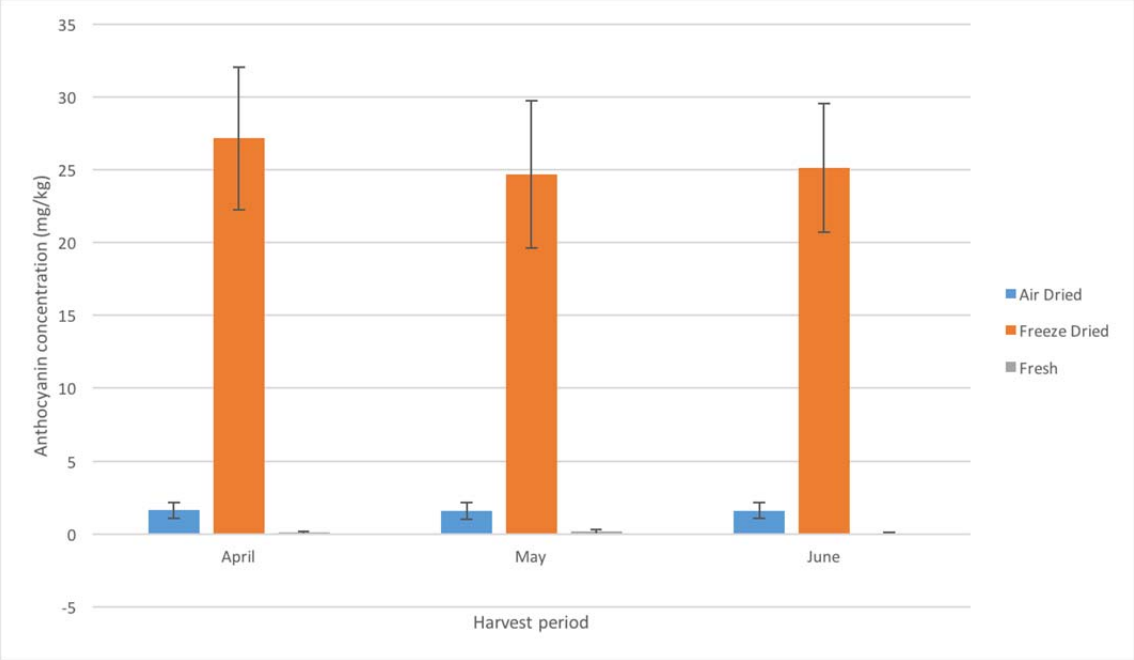

Figure 7: Anthocyanin concentration of air-dried, freeze-dried and fresh berry harvested in April, May and June. Values are reported as means ± standard deviation (n = 5).

3.6 Flavonol concentration

Figure 8 shows that freeze-dried berries have the highest flavonol concentration out of all drying methods, while the berries harvest in June have the highest flavonol level. Air-dried berries have less flavonol concentration than freeze-dried berries, but it gradually increases from April to June. Fresh berries have very little flavonol throughout the entire harvest season. Only treatment has a significant effect for flavonol concentration, no significant differences were found between each harvests (Table 2).

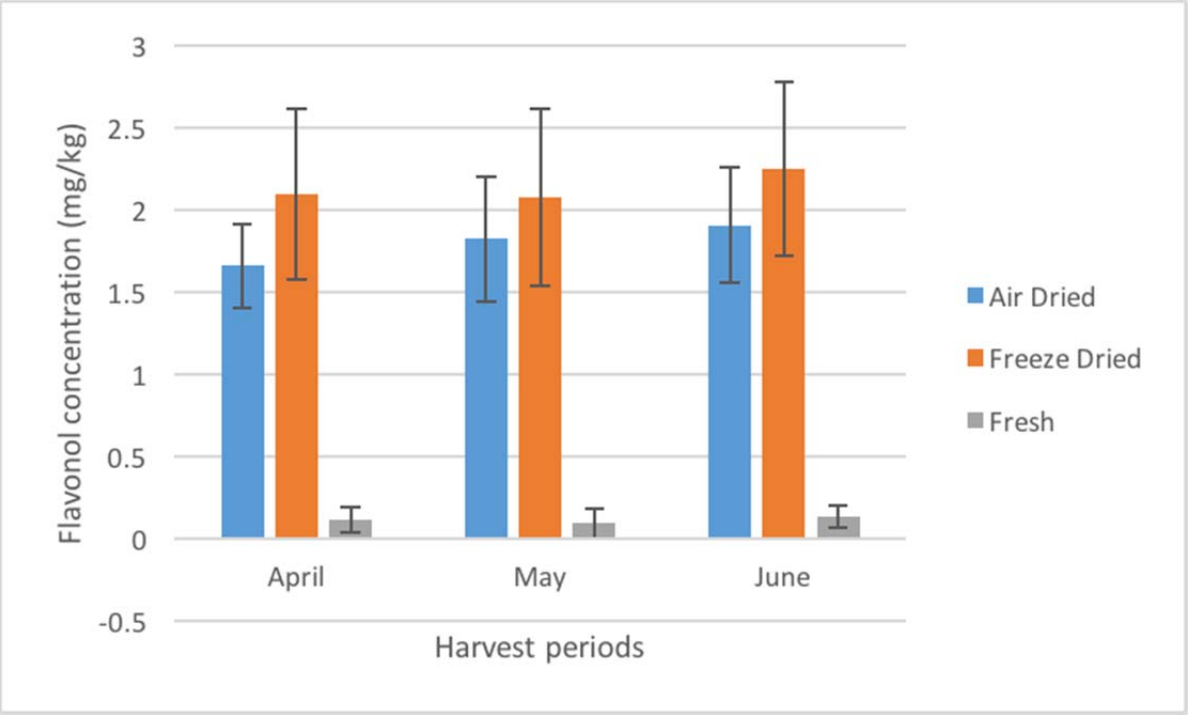

Figure 8: Flavonol concentration of air-dried, freeze-dried and fresh berry harvested in April, May and June. Values are reported as means ± standard deviation (n = 5).

3.7 Hydroxycinnamic acid concentration

Figure 9 shows that freeze-dried berries have the highest hydroxycinnamic acid for all the entire harvest period, while air-dried berries have the second highest, with consistent amount each month. Fresh berries have no hydroxycinnamic acid. Only treatment has a significant effect on hydroxycinnamic acid concentration, no significant differences were found between each harvests (Table 2).

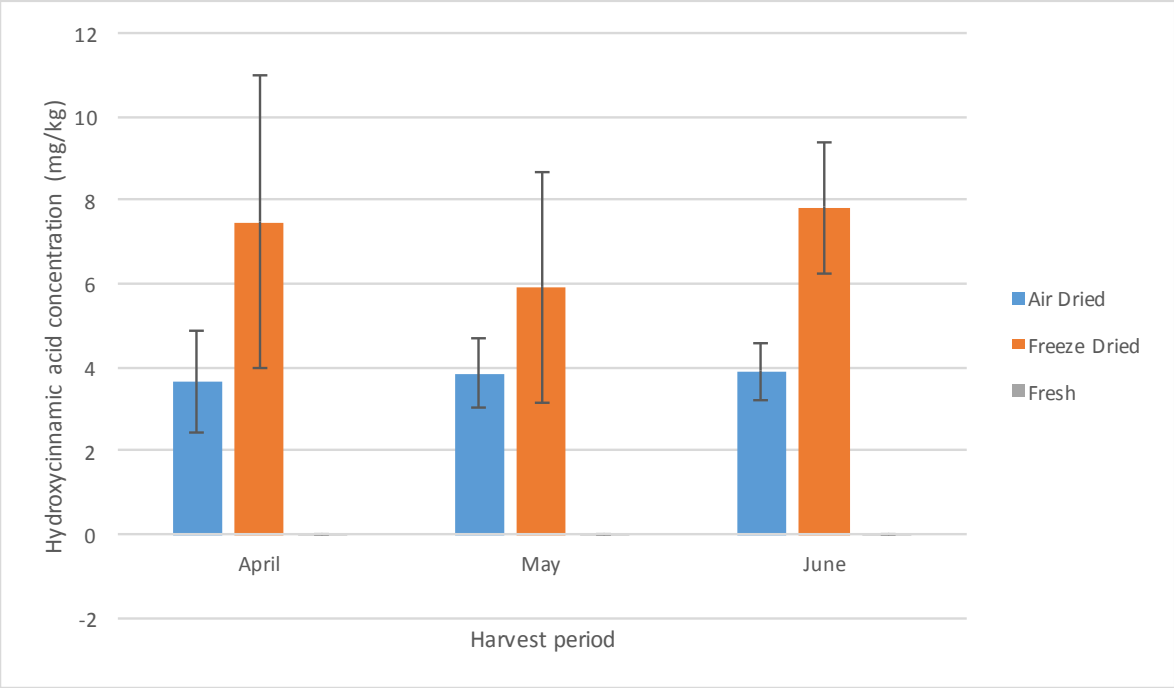

Figure 9: Hydroxycinnamic acid concentration of air-dried, freeze-dried and fresh berry harvested in April, May and June. Values are reported as means ± standard deviation (n = 5).

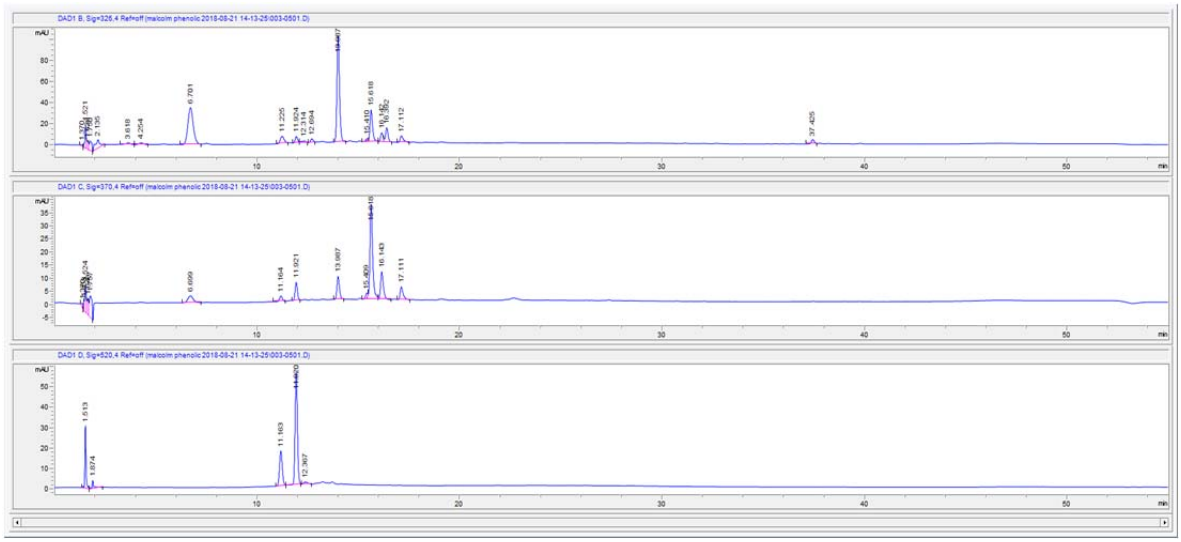

Figure 10: HPLC retention time of air-dried berry (Harvest 1 Block 1). Polygodial, anthocyanin, flavonol and hydroxycinnamic acids were found through HPLC, together with several other compounds (not examined in this study).

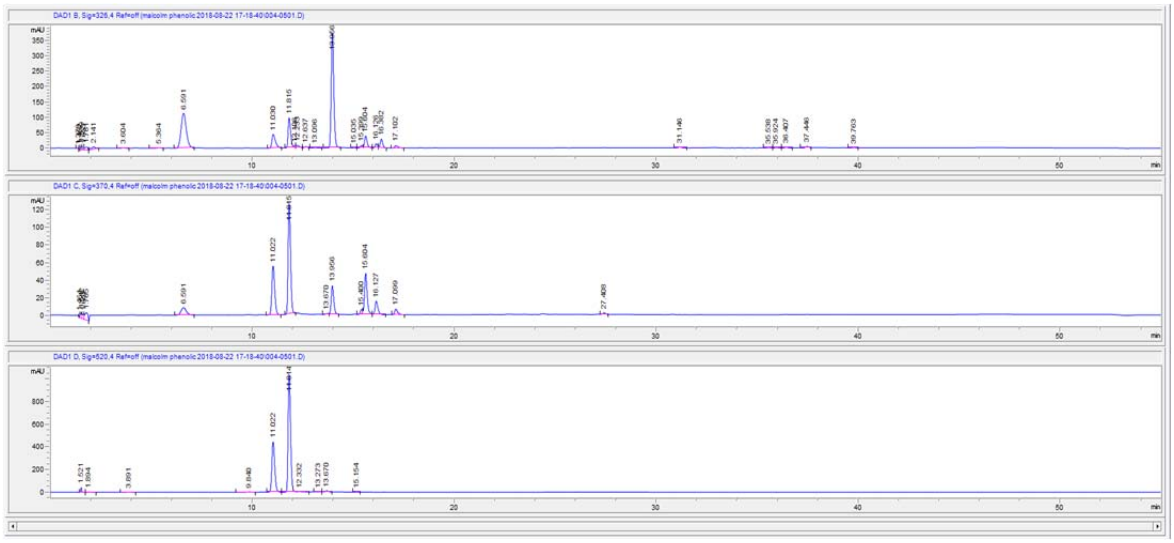

Figure 11: HPLC retention time of freeze-dried berry (Harvest 1 Block 2). Polygodial, anthocyanin, flavonol and hydroxycinnamic acids were found through HPLC, together with several other compounds (not examined in this study

Table 1: F and P values of berry mass, volume and colour L*, colour a* and colour b* for harvest, treatment and harvest × treatment interactions.

	Mass		Volume		Colour					
					L*		a*		b*	
	F	P	F	P	F	P	F	P	F	P
Harvest	1.250	0.290	24.029	0.000	5.627	0.000	3.462	0.042	3.359	0.046
Treatment	2906.166	0.000	1219.748	0.000	61.028	0.000	22.234	0.000	95.234	0.000
Harvest × Treatment	3.788	0.006	9.634	0.000	3.211	0.024	0.304	0.873	8.678	0.000

Table 2: F and P values of polygodial content, anthocyanins content, flavonols content and hydroxycinnamic acids content for harvest, treatment and harvest × treatment interactions.

	Polygodial content		Anthocyanins content		Flavonols content		Hydroxycinnamic – acids content	
	F	P	F	P	F	P	F	P
Harvest	0.099	0.906	8.083	0.001	0.610	0.549	0.497	0.613
Treatment	372.270	0.000	634.039	0.000	133.427	0.000	502.154	0.000
Harvest × Treatment	4.566	0.004	7.604	0.000	0.182	0.946	0.374	0.825

4. Discussion

4.1 Drying Methods and colour

Mountain pepper has its red colour due to their high concentrations of anthocyanins, together with various other pigments. The occurrence of metal cations, oxygen, high temperature, and organic acids can cause a reduction of natural red colour in berry and fruits with high anthocyanin contents (Shewfelt, 1975). Yang and Atallah's (1985) study on blueberry shows a substantial increase in colour L* value after different drying treatments, suggesting a fading of the dark colour in blueberries. Additionally, their results showed that there were no significant differences in L* value between freeze-dried and air-dried berries. Instead of only testing two drying methods, their experiment included vacuum-drying and micro-convection drying. There was a significant increase in L* value found in micro-convection dried berries, demonstrating a reduction in anthocyanin level due to thermal degradation (Yang and Atallah, 1985). Moreover, the positive a* value, which represent redness, was not significantly different among freeze-dried, and vacuum-dried berries in the same study. However, a significant decrease of a* value was found in both air-dried and micro-convection dried berries, which may be due to oxidation of anthocyanin as well as heat degradation during dehydration. Nebesky et al. (1949) suggested that the presence of oxygen and rising temperature were the most effective accelerating factors in the degradation of berry colours. A significant reduction of b* value was also obtained during treatments, which shows a changing of colour from yellow to blue (Yang and Atallah, 1985).

When comparing their results with this study, there was a significant difference of L* value (Table 1) between air-dried and freeze-dried berries, this differs from what Yang and Atallah (1985) found. Also, there was a significantly difference in a* value for air-dried and freeze-dried berries (Table 1), further contrasting with Yang and Atallah (1985). One

explanation to this may be the use of different berries in our experiments, as blueberry may have different properties compare to mountain pepper. Furthermore, their experiment included vacuum-drying and micro-convection drying methods, which this study did not. If these two methods were integrated in this experiment, a more accurate and detailed results may be obtained for mountain pepper.

Yang and Atallah (1985) found that different drying methods may offer different outcomes within samples. Air-drying offers less soluble solids and colour retention period, with low rehydration ratio and berry density. Micro-convection drying allows berry to achieve preferred moisture level within the shortest period of time, but it also gives poor berry quality compare to the other drying methods. Vacuum-dried berries were high in soluble solids and colour retention period, and this method is also low in cost, with a fast processing speed. Freeze-drying provides the highest retention period for soluble solids and colour, it also gives the highest rehydration ratio with the lowest berry density, this could potentially make freeze-dried berries more attractive to consumers when comparing berries processed from different drying methods (Yang and Atallah, 1985). In this study, freeze-dried berry contains the highest polygodial (Figure 6), highest anthocyanin (Figure 7), highest flavonol (Figure 8) and highest hydroxycinnamic acid concentrations (Figure 9) compare to air-dried berry. Although vacuum-drying was not examined in this experiment, a combination of freeze-drying and vacuum-drying on mountain pepper may potentially produce the highest quality in dried berries.

4.2 Polygodial concentration

Mountain pepper is well known for its aromatic characteristics, the plant is one of few rare species around the world known to produce polygodial (Wilson, 2015). Examining the polygodial concentration (Figure 12) is essential for this study as polygodial is the source of the hot flavour in mountain pepper. According to Menary et al. (1999), the Japanese market has discovered that the polygodial concentration is a vital influence in the evaluation of product quality in mountain pepper. Therefore, identifying the polygodial concentration is a crucial part for this experiment.

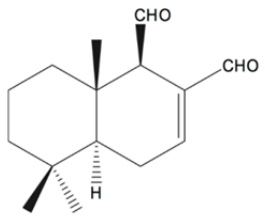

Figure 12: Chemical structure of Polygodial.

Menary et al. (2003) conducted a primary investigation on populations of mountain peppers in Tasmania, and found that polygodial concentration differs from 0% to more than 67% of the extract on a dry matter basis. They also discovered similarity between individuals with respect to other compounds. Out of the 307 mountain pepper samples they tested, the most abundant polygodial concentration was 2-2.5% dry weight, with 70 samples in this category. When compared with the polygodial concentration detected from this experiment (Figure 6), the highest concentration discovered was approximately 18 g kg^{-1} (freeze-dried berry in June), which is 1.8% dry weight. While the lowest level was found to be 15 g kg^{-1}, or 1.5% dry weight. Knowing that polygodial is the main source of the characteristic hot flavour for mountain pepper, it is important to consider this parameter in all future harvests

(Sultanbawa and Sultanbawa, 2016). Similarly, Read and Menary (2001) discovered a polygodial level in mountain pepper between 0.11% to 2.9% dry weight. Their report stated that the polygodial concentration is deeply associated with the environmental factors during growth period. Mountain pepper that is inhabited in disturbed native environments tends to develop a hotter taste than those in favoured environments, this occurs due to self-protection from animal depredation.

Additionally, Muñoz-Concha et al. (2007) examined the level of polygodial in 5 species in *Drimys*, a genus in the same family as mountain pepper, *Winteraceae*. The identification of polygodial was performed by a thin-layer chromatography (TLC), HPLC and GC analysis. As a result, the concentration of polygodial is ranged between 0.01% and 2.76% across all 5 species, this is similar to Figure 6, where the polygodial concentrations for the freeze-dried and air-dried berries were 1.8% and 1.5% respectively. Interestingly, all 5 species examined by Muñoz-Concha et al. (2007) were originated in different locations with different growing altitudes, the average polygodial level was significantly different from each individual samples. This indicates that growing altitudes may influence the polygodial levels in mountain peppers.

Various studies have confirmed that polygodial is the largest component of mountain pepper extracts. Wilson et al. (2016) suggested that the polygodial concentration within mountain pepper can be altered through applying N, P and K fertiliser during growth period. Their report shows that the mountain peppers applied with the highest amount of N have the highest amount of polygodial, approximately 3.5% dry basis, indicating applying N as a fertiliser during growth period may potentially increase the polygodial concentration by 1.5 - 2%. Mountain peppers fertilised with P and K produced significantly low levels of polygodial.

Within the low polygodial samples, the polygodial concentrations remained constant over time, while in high polygodial samples, concentrations changed over time (Menary et al. 1999). As this study aims to focus on improving the quality attributes for mountain pepper, although the use of fertilisers is not considered in this study, it is still relevant as this may impact the polygodial concentrations in mountain peppers.

4.3 Anthocyanin concentration

Anthocyanin concentrations (Figure 13) were tested in this experiment due to their contribution to the overall appearances of mountain peppers, and appearances are important for consumers in determining the product quality. Anthocyanins are the most essential pigments of various vascular plants and these pigments are responsible for the red colour in mountain pepper. These pigments also have substantial features such as their antioxidant activity, which plays a crucial role in inhibition of the development of cancer cells, cardiovascular diseases and diabetes (Castaneda-ovando et al. 2009). However, as mentioned before, anthocyanins are extremely unstable and very vulnerable to degradation. Their stability can be disturbed by various factors such as oxygen level, surrounding pH, light, the occurrence of enzymes and high temperature. Jensen et al. (2011) stated that the degradation speed of anthocyanins rises during post-harvest handling and storage when the surrounding temperature increases.

The stability of anthocyanins can be enhanced through co-pigmentation, where the anthocyanin compound reacts with other natural compounds directly or through weak interactions, forming a brighter and stable colour (Rein, 2005). Anthocyanin co-pigmentation may provides stronger and more consistent colours compared to a single anthocyanin compound.

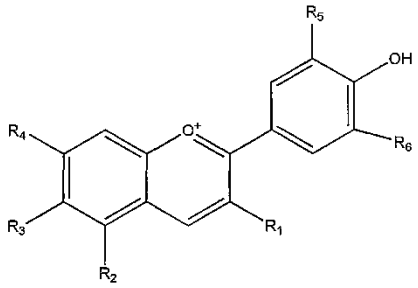

Figure 13: Chemical structure of anthocyanins

In berries, the anthocyanin concentration correlates well with the darkness of the berry colour, the darker the berry, the higher the anthocyanin concentration (Rein, 2005). However, this statement contrasts with this study, as freeze-dried berries have the highest anthocyanin concentrations (Figure 7), they also have the highest L* values, which indicates lightness, or brightness (Figure 3). On the other hand, air-dried berries have significantly low anthocyanin contents compare to freeze-dried berries (Figure 7), and they have lower L* values (Figure 3), suggesting they are darker. This occurred due to different berries were tested between the two experiments, as different berries may have different properties from each other.

The highest anthocyanin concentration found in any berry is the bilberry and black currants, with 300-600 mg per 100g and 80-810 mg per 100g respectively. Netzel et al. (2006) discovered cyanidin 3-rutinoside and cyanidin 3-glucoside as the main anthocyanins in mountain pepper, together making up 73% of the total anthocyanin concentration, approximately 21.13 μmol g^{-1}. Similarly, Konczak et al. (2010) also found cyanidin 3-rutinoside and cyanidin 3-glucoside to be the main anthocyanin compounds in pepperberries, and the concentrations were found at a quite high level (1.25 mg g^{-1} dry weight). When compared to this study, the highest anthocyanin concentration detected was in April, in the freeze-dried berries (Figure 7), at a concentration of 27 mg kg^{-1}, which is a lot smaller than

Konczak et al. (2010). One explanation for this would be that the sample volume used in this study was a lot less compared to existing studies, if more samples were used, more anthocyanin levels would be detected.

Another study by Lohachoompol et al. (2004) suggested that the anthocyanin concentration in freeze-dried blueberries had no significant differences from the fresh blueberries. Furthermore, the concentration of anthocyanins in air-dried blueberries was significantly reduced by a total level of 41% compared to the fresh blueberries. Their results were drastically different compared to this study as freeze-dried mountain pepper had significantly higher anthocyanin concentration compare to fresh mountain pepper. These significantly different results from these experiments may be a result of different methodological approaches. There was a 4-hour preheating step in the Lohachoompol et al. (2004) study, where anthocyanin leakage occurred during preheating, and dewaxing occurred by stirring and soaking during their osmotic pretreatment. Similar results were found by Sapers and Philips (1985), that anthocyanin levels were lost due to leakage from exposed skin to the osmotic solution before and during drying. Another study by Garcia-Viguera et al. (1998) further confirms this as the anthocyanin content in raspberries was lost by 17-40% during heating in their experiment.

4.4 Flavonols concentration

Similar to anthocyanin, flavonols (Figure 14) are polyphenolic phytochemicals within the class of flavonoids that form a large fraction of secondary plant metabolites. These natural chemicals are relevant for this research because of their specialised health-stimulating properties as antioxidant for mountain pepper. The experiment by Hakkinen et al. (1999) on various berries has shown that there are large differences in flavonol content among the berries they tested, ranging from 6-210 mg kg^{-1}. Quercetin, a type of plant flavonol, was

found in all fresh berries with the highest level in cranberry, lingonberry, rowanberry and crowberry. Almost a 20% reduction of flavonols were found in the oven-dried berries compared to freeze-dried berries (Hakkinen et al. 1999). This is comparable to this study as freeze-dried pepperberries contained higher levels of flavonols than air-dried pepperberries. However, the flavonol concentration of mountain pepper is approximately 2-2.3 mg kg^{-1} (Figure 8), which extremely low compare to any other types of berry. The reason for this may be the low volume of samples used in this study, if more volume of the samples were used, more flavonols concentration would be obtained.

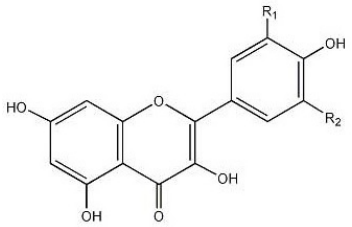

Figure 14: Chemical structure of flavonols

Mattivi et al. (2006) have identified the flavonols concentration in grapes and found out their close relationship with the anthocyanin concentrations. During their experiment, HPLC-DAD was used to analyse flavonol contents in grapes, and the total amount of flavonols, after hydrolysis of the grape extracts ranged from 3.81 to 80.37 mg kg^{-1}, with a mean of 32.46 mg kg^{-1} in the red varieties, and a mean of 10.83 mg kg^{-1} in the white varieties. These levels were significantly higher compare to the flavonol concentration detected in mountain peppers (Figure 8). As mentioned before, this may be due to the low volume of samples used in this experiment. The biosynthesis of flavonols is closely linked with anthocyanins (Jeong et al. 2006). As both compounds are antioxidants, the main difference is that flavonol contains kaempferol, while anthocyanin contains pelargonidin, this leads to the formation of the anthocyanin delphinidin (Mattivi et al. 2006).

Another study of the flavonol concentration of various berries was conducted by Jakobek and Seruga (2009). Their study suggested that berries such as blackberry, strawberry and chokeberry contained relatively high flavonol concentration, approximately 6.5 to 438 mg kg^{-1} fresh weight. Within the flavonols, quercetin was found to be most dominant. This extraction also discovered high levels of anthocyanins in chokeberries, blueberries and elderberries, with approximately 14.7, 13.0 and 11.0 g kg^{-1} fresh weight respectively. Both flavonols and anthocyanins concentrations were significantly higher compare to this study (Figure 7 and 8). This may be due to the differences in samples, as well as the differences in measuring under dry weight and fresh weight. Wang et al. (2014) suggested the average flavonols concentration for vaccinium uliginosum berry was 93 ± 4 mg per 100g fresh weight. This is significantly higher than any other berries tested. Similarly to Jakobek and Seruga (2009), quercetin was the most dominant flavonol compound discovered.

The ripening of berries may also contribute to the total flavonols concentration. Marin et al. (2004) suggested the flavonol level in un-ripened green pepper was commonly 4-5 times greater than the ripened green pepper. A significant reduction of flavonols level was observed during ripening. This decrease was mostly caused by the increase of fruit size. Howard et al. (2000) have stated an increase of total soluble phenolic was observed as peppers reached maturity from different pepper cultivars.

4.5 Hydroxycinnamic acids concentration

Hydroxycinnamic acids (Figure 15) are one of the most vital source of phenolic compounds in berries, the compounds provide an extensive variety of biological effects, including antioxidant, antimicrobial and anti-inflammatory actions (Kahkonen et al. 2001). A study conducted by Maatta-Riihinen et al. (2004) on the quantification of phenolic compounds in raspberries and cloudberries suggested that the content of hydroxycinnamic

acids varied from 9-19 mg kg^{-1} and 52-63 mg kg^{-1} in fresh weight, respectively. This is exceedingly high compare to the results of this experiment (Figure 9) where the highest hydroxycinnamic acid concentration is just under 8 mg kg^{-1} in dry weight. The differences in the result may be caused by the use of different samples.

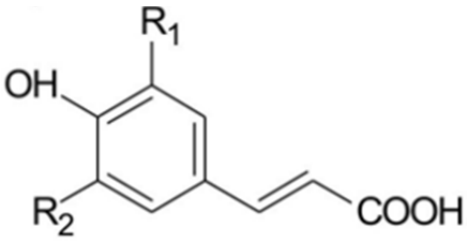

Figure 15: Chemical structure of hydroxycinnamic acid

Ruiz et al. (2013) pointed out that the total hydroxycinnamic acids detected in their study for calafate berries was 1.48 ± 0.8 mg g^{-1} in fresh weight. This is a very high concentration in comparison to not only pepperberries (Figure 9), but also other berries such as bilberries and black currant. Berry ripening may also disturb the hydroxycinnamic acid levels in berries. It has been found that the total concentration of hydroxycinnamic acid decreased almost three times when berries reach to their ripening stage (Ruiz et al. 2013). In contrast, anthocyanin level was found to be increasing during ripening for most berries (Castrejon et al. 2008).

Konczak et al. (2010) suggested that chlorogenic acid (a type of hydroxycinnamic acid), was the main phenolic compound in mountain pepper, quantified at 1.5 ± 0.1 mg g^{-1} dry weight, making up approximately 3% of the sample's dry mass. These results show that chlorogenic acids are one of the main contributors to the high antioxidant levels of mountain peppers. Figure 9 shows that the hydroxycinnamic acid concentration for this study was roughly at 8 mg kg^{-1} dry weight, which is a lot smaller compare to what Konczak et al. (2010) have found.

4.6 Limitations

This study was limited in a few areas. Firstly, only 4 main compounds were focused (polygodial, anthocyanins, flavonols and hydroxycinnamic acids). There were a lot of other compounds detected, but not included in this study (Figure 11). If these compounds were included, the final results may be more accurate and effective, therefore they should be tested for future studies. Similarly, this study mainly focused on two drying methods: air-drying and freeze-drying, there are a few more drying methods that were not considered, such as vacuum-drying and micro-convection drying. These drying-methods were found to be very efficient and suitable for drying other berries such as blueberry, therefore it is possible that they can also be used to dry pepperberries. Another limitation is that the quantity of samples used to measure anthocyanins, flavonols and hydroxycinnamic acids were very low (250 mg for each samples, see *2.7*), as expected the amount of concentrations detected were also very small compared to existing studies, this can be improved by increasing the sample volumes.

4.7 Future directions

For future directions, it is recommended to focus more on the freeze-drying method as this method is able to produce higher quality berries in terms of hot flavour. Freeze-dried berries also contain higher antioxidant levels, indicating they are healthier than air-dried berries. One strategy is to produce three products based on their drying methods: one product with freeze-dried berries only, label it as the hot pepper mix; a second product with air-dried berries only, label it as the mild pepper mix; and a third product mixed with both freeze-dried and air-dried berries. This allows consumers to choose their preferred flavour and give them more options. Moreover, various antioxidant compounds were detected in the extracts of mountain peppers, as antioxidants are healthy, mountain pepper producers may add labels to their product, emphasising the benefits of antioxidants in mountain pepper. This may potentially increase popularity and boost sales, as consumers generally have high awareness of their well-beings. In terms of the optimal harvest period, berries harvested in June have the highest polygodial and antioxidant concentrations, therefore it is recommended to harvest in June. Another recommendation is to try to apply vacuum-drying together with freeze-drying. As mentioned before in *4.1*, vacuum-drying is low in costs with a fast processing speed, the vacuum-dried berries also have high soluble solids and long colour retention period. Although this experiment did not examine the effectiveness of vacuum-drying, various existing studies have confirmed its practicality to dry berries.

5. Conclusion

The results of this experiment showed that harvest time and post-processing treatment have significant effects on the physical and flavour characteristics of Tasmanian Mountain Pepper. The freeze-dried berries have higher polygodial, anthocyanins, flavonols and hydroxycinnamic acid concentrations compared to air-dried berries. Therefore, it is the optimal post-harvest drying method for mountain pepper. Additionally, existing studies showed that freeze-drying method gave the highest retention period for soluble solids and colour, as well as good rehydration ratio and low berry density, further confirming the effectiveness of freeze drying method. Pepperberries harvested in June had the highest polygodial and anthocyanins concentration compared to berries harvested in April and May. Hence, the optimal harvest period for mountain pepper is June. Furthermore, physical quality attributes of air-dried, freeze-dried and fresh mountain pepper were investigated, this included size, volume and colour. As expected, freeze-dried berries have the largest volume and the most desired physical characteristics. The results of this study may allow mountain pepper growers to understand the optimal drying methods to deliver pepperberries with the strongest flavour and spiciness, as well as the optimal period to harvest berries with the highest polygodial, anthocyanins, flavonols and hydroxycinnamic acid concentrations. This may potentially help the mountain pepper industry to improve the consistency in berry flavours, and increase the production rate to supply rising demands.

References

Castaneda-Ovando, A., Pacheco-Hernandez, M.D.L., Paez-Hernandez, M.E., Rodriguez, J.A., Galan-Vidal, C.A., 2009. Chemical studies of anthocyanins: A review. 113, 859-871. https://doi.org/10.1016/j.foodchem.2008.09.001

Castrejon, A.D.R., Eichholz, I., Rohn, S., Kroh, L.W., Huyskens-Keil, S., 2008. Phenolic profile and antioxidant activity of highbush blueberry (Vaccinium corymbosum L.) during fruit maturation and ripening. Food Chem. 109, 564-572. https://doi.org/10.1016/j.foodchem.2008.01.007

Clarke, M., 2012. Australian Native Food Industry Stocktake. RIRDC. 1-75.

Dragar, V.A., Garland, S.M., Menary, R.C., 1998. Investigation of the variation in chemical composition of *Tasmannia lanceolata* solvent extracts. J. Agric. Food Chem. 46, 3210-3213. https://doi.org/10.1021/jf970808b

Druce, H., 2013. Aroma Characterisation of various 'true' and 'false' peppers from around the globe. The University of Sydney. 1-123.

Garcia-Viguera, C., Zafrilla, P., Artes, F., Romero, F., Abellan, P., Tomas-Barberan, F.A., 1998. Colour and Anthocyanin Stability of Red Raspberry Jam. J. Sci. Food Agric. 78, 565-573. https://doi.org/10.1002/(SICI)1097-0010(199812)78:4<565::AID-JSFA154>3.0.CO;2-P

Hakkinen, S.H., Karenlampi, S.O., Heinonen, I.M., Mykkanen, H.M., Torronen, A.R., 1999. Content of the Flavonols Quercetin, Myricetin, and Kaempferol in 25 Edible Berries. J. Agric Food Chem. 47, 2274-2279. https://doi.org/10.1021/jf9811065

Howard, L.R., Talcott, S.T., Brenes, C.H., Villalon, B., 2000. Changes in phytochemical and antioxidant activity of selected pepper cultivars (Capsicum species) as influenced by maturity. J. Agric. Food Chem. 48, 1713-1720. https://doi.org/10.1021/jf990916t

Jakobek, L., Seruga, M., 2012. Influence of anthocyanins, flavonols and phenolic acids on the antiradical activity of berries and small fruits. 15, 122-133. https://doi.org/10.1080/10942911003754684

Jensen, M.B., López-de-Dicastillo Bergamo, C.A., Payet, R.M., Liu, X., Konczak, I., 2011. Influence of copigment derived from tasmannia pepper leaf on davidson's plum anthocyanins. J. Food Sci. 76, 447–453. https://doi.org/10.1111/j.1750-3841.2011.02077.x

Jeong, S.T., Goto-Yamamoto, N., Hashizume, K., Esaka, M., 2006. Expression of the flavonoid 3'-hydroxylase and flavonoid 3', 5'-hydroxylase genes and flavonoid composition in grape (Vitis vinifera). Plant Science. 170, 61-69. https://doi.org/10.1016/j.plantsci.2005.07.025

Kahkonen, M.P., Hopia, A.I., Heinonen, M., 2001. Berry Phenolics and Their Antioxidant Activity. J. Agric. Food Chem. 49, 4076-4082. https://doi.org/10.1021/jf010152t

Konczak, I., Zabaras, D., Dunstan, M., Aguas, P., 2010. Antioxidant capacity and phenolic compounds in commercially grown native Australian herbs and spices. Food Chem. 122, 260–266. https://doi.org/10.1016/j.foodchem.2010.03.004

Lohachoompol, V., Srzednicki, G., Craske, J., 2004. The change of total anthocyanins in blueberries and their antioxidant effect after drying and freezing. J Biomed Biotechnol. 2004, 248-252. https://doi.org/10.1155/S1110724304406123

Maatta-Riihinen, K.R., Kamal-Eldin, A., Torronen, A.R., 2004. Identification and Quantification of Phenolic compounds in berries of fragaria and rubus species (Family Rosaceae). J. Agric. Food Chem. 52, 6178-6187. https://doi.org/10.1021/jf049450r

Mattivi, F., Guzzon, R., Vrhovsek, U., Stefanini, M., Velasco, R., 2006. Metabolite Profiling of Grape: Flavonols and Anthocyanins. J. Agric. Food Chem. 54, 7692-7702. https://doi.org/10.1021/jf061538c

Menary, R.C., Dragar, V.A., Garland, S.M., 1999. Tasmannia lanceolata – Developing a New commercial Flavour Product. RIRDC. 99, 33-47.

Menary, R.C., Dragar, V.A., Thomas, Sonya., Read, C.D., 2003. Mountain pepper extract (Tasmania lanceolata): Quality Stabilisation and Registration. RIRDC. 2, 9-27.

Muñoz-Concha, D., Vogel, H., Yunes, R., Razmilic, I., Bresciani, L., Malheiros, A., 2007. Presence of polygodial and drimenol in Drimys populations from Chile. Biochem. Syst. Ecol. 35, 434–438. https://doi.org/10.1016/j.bse.2006.10.019

Nebesky, E.A., Esselen, W.B., McConnel, J.E.W., Fellers, C.R., 1949. Stability of color in fruit juices. Food Res. 14, 261-274. https://doi.org/10.1111/j.1365-2621.1949.tb16232.x

Netzel, M., Netzel, G., Tian, Q., Schwartz, S., Konczak, I., 2007. Native Australian fruits - a novel source of antioxidants for food. Innov. Food Sci. Emerg. Technol. 8, 339–346. https://doi.org/10.1016/j.ifset.2007.03.007

Netzel, M., Netzel, G., Tian, Q., Schwartz, S., Konczak, I., 2006. Sources of antioxidant activity in Australian native fruits. Identification and quantification of anthocyanins. J. Agric. Food Chem. 54, 9820–9826. https://doi.org/10.1021/jf0622735

Oruña-Concha, M.J., Lopez-Hernandez, J., Simal-Lozano, J.A., Simal-Gandara, J., Gonzalez-Castro, M.J., Garcia, C.D.L.C., 1998. Determination of volatile components in fresh, frozen, and freeze-dried Padron-type peppers by gas chromatography-mass spectrometry using dynamic headspace sampling and microwave desorption. J. Chromatogr. Sci. 36, 583–8. https://doi-org.ezproxy1.library.usyd.edu.au/10.1093/chromsci/36.12.583

Read, C.D., Menary, R.C., 2001. Leaf extract and polygodial yield in Tasmannia lanceolata (Poir.) A.C. Smith. J. Essent. Oil Res. 13, 348-350. https://doi.org/10.1080/10412905.2001.9712229

Rein, M., 2005. Copigmentation reactions and color stability of berry anthocyanins. University of Helsinki. 9-67.

Ruiz, A., Mardones, C., Vergara, C., Hermosin-Gutierrez, I., Baer, D.V., Hinrichsen, P., Rodriguez, R., Arribillaga, D., Dominguez, E., 2013. Analysis of hydroxycinnamic acids derivatives in calafate (Berberis microphylla G. Forst) berries by liquid chromatography with photodiode array and mass spectrometry detection. J. Chromatogr. A. 1281, 38-45.

Sapers, G.M., Philips, John., 1985. Leakage of anthocyanins from skin of raw and cooked highbush blueberries (Vaccinium corymbosum L.). J. Food Sci. 50, 431-439. https://doi.org/10.1111/j.1365-2621.1985.tb13421.x

Shewfelt, R.L., 1986. Flavor and Color of Fruits as Affected by Processing. Springer, Dordrecht. 481-529. https://doi.org/10.1007/978-94-011-7385-8_11

Sultanbawa, Y., Sultanbawa, F., 2016. Australian Native Plants: Cultivation and Uses in the Health and Food Industries. CRC Press. 12, 134-143.

Wang, L.J., Su, S., Wu, J., Du, H., Li, S.S., Huo, J.W., Z, Y., Wang, L.S., 2014. Variation of anthocyanins and flavonols in vaccinium uliginosum berry in Lesser Khingan Mountains and its antioxidant activity. Food Chem. 160, 357-364. https://doi.org/10.1016/j.foodchem.2014.03.081

Wilson, M.D., Hons, B.A.S., 2015. Aspects of developing *Tasmannia lanceolata* for commercial extract production. University of Tasmania. 1-140.

Wilson, M.D., Menary, R.C., Close, D.C., 2016. Effect of N, P and K on polygodial leaf extract of Tasmannia lanceolata (Poir.) A.C. Smith. Journal of Essential Oil Research. 28, 512-517. https://doi.org/10.1080/10412905.2016.1160845

Winnett, V., Boyer, H., Sirdaarta, J., Cock, I.E., 2014. The potential of tasmannia lanceolata as a natural preservative and medicinal agent: antimicrobial activity and toxicity. Pharmacognosy Communications. 4, 42-52. https://doi.org/10.5530/pc.2014.1.7

Yang, C.S.T., Atallah, W.A., 1985. Effect of Four Drying Methods on the Quality of Intermediate Moisture Lowbush Blueberries. J. Food Sci. 50, 1233-1237. https://doi.org/10.1111/j.1365-2621.1985.tb10450.x