EFFECTS OF NITROGEN AND POTASSIUM FERTILIZING AND CLUSTER THINNING ON QUALITY AND ANTHOCYANIN CONTENTS OF CABERNET SAUVIGNON GRAPE AND WINE

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อิทธิพลของการให้ปุ๋ยไนโตรเจน โปแตสเซียม และการตัดแต่งช่ออ่อนต่อคุณภาพ และปริมาณแอนโทไซยานินในผล และไวน์ขององุ่นสายพันธุ์คาร์เบอร์เน ซอวียอง

นายวสันต์ บุญเติม

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
สาขาวิชาเทคโนโลยีชีวภาพ
มหาวิทยาลัยเทคโนโลยีสุรนารี
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Suranaree University of Technology has approved this thesis submitted in fulfillment of the requirements for the Degree of Doctor of Philosophy.

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วสันต์ บุญเติม : อิทธิพลของการให้ปุ๋ยไนโตรเจน โปแตสเซียมและการตัดแต่งช่ออ่อนต่อคุณภาพ และปริมาณแอนโทไซยานินในผลและไวน์ขององุ่นพันธุ์คาร์เบอร์เน ซอวียอง (EFFECTS OF NITROGEN AND POTASSIUM FERTILIZING AND CLUSTER THINNING ON QUALITY AND ANTHOCYANIN CONTENT OF CABERNET SAUVIGNON GRAPE AND WINE) อาจารย์ที่ปรึกษา : ศาสตราจารย์ ดร.นันทกร บุญเกิด, 179 หน้า.

การศึกษาวิจัยได้ดำเนินการในเขตสภาพอากาศกึ่งร้อนชื้นที่เมืองซีชาง มณฑลเสฉวน ทางตอนใต้ของประเทศสาธารณรัฐประชาธิปไตยประชาชนจีน (ละติจูด 27 องศาเหนือ ลองติจูด 102 องศาตะวันออก ความสูงจากระดับน้ำทะเล 1650 เมตร) ระหว่างปี 2005 และ 2006 โดยใช้แปลงองุ่นพันธุ์คาร์เบอร์เน ซอวียอง อายุ 8 ปี ของบริษัทซีชางเจียไต๋ไวน์ ระยะปลูกระหว่างต้น 1.25 เมตร ระหว่างแถว 2 เมตร ในทิศทางเหนือ-ใต้ จัดทรงต้นแบบ 2 แขน กิ่งให้ผลในแนวตั้ง และตัดแต่งแบบกิ่งสั้น แปลงปลูกให้น้ำด้วยระบบน้ำหยด การทดลองให้ปุ๋ยไนโตรเจน และโปแตสเซียม 3 ระดับ คือ ไม่ให้ปุ๋ย 0-0, 100-20 และ 200-60 กรัมต่อต้นและจำนวนช่อต่อต้น 10, 20 และ 30 ช่อต่อต้น โดย 1 ต้นเป็น 1 หน่วยการทดลอง จำนวน 6 ซ้ำ โดยวางแผนการทดลองแบบสปิตพลอตและเพื่อควบคุมทรงต้นจึงจำกัดความยาวกิ่งที่จำนวน 15 ข้อ ผลการทดลอง พบว่าผลผลิตต่อต้นปี 2005 สูงกว่าปี 2006 การเพิ่มปริมาณไนโตรเจนและโปแตสเซียม ไม่ช่วยเพิ่มผลผลิตตรงกันข้ามกับการเพิ่มจำนวนช่อต่อต้นที่ทำให้ผลผลิตเพิ่มอย่างชัดเจน สีและสารฟีนอลในผลองุ่นปี 2006 สูงกว่าปี 2005 ปริมาณไนโตรเจนและโปแตสเซียมที่ 100-20 กรัมต่อต้นมีปริมาณสารฟีนอลในผลต่ำกว่าที่ระดับอื่นๆ ผลองุ่นสายพันธุ์คาร์เบอร์เน ซอวียอง มีปริมาณสารมัลวิดีนมากกว่าสารอื่นๆในกลุ่มสารแอนโทไซยานิน และที่สำคัญพบว่าคุณภาพของผลองุ่นมีผลต่อคุณภาพของไวน์เป็นอย่างมาก ไวน์สด (หลังหมักเสร็จ) และไวน์บ่ม (6 เดือนหลังหมักเสร็จ) ของปี 2006 มีคุณภาพดีกว่าปี 2005 ทั้งนี้เนื่องจากปริมาณผลผลิตต่ำและผลขนาดเล็กกว่า ปริมาณกรดที่สามารถไทเตรทได้ลดลงเมื่อเพิ่มปริมาณไนโตรเจนและโปแตสเซียม แต่การเพิ่มจำนวนช่อต่อต้นทำให้ปริมาณกรดที่สามารถไทเตรทได้เพิ่มขึ้น ปริมาณไนโตรเจนและโปแตสเซียมที่ 100-20 กรัมต่อต้นมีปริมาณสารฟีนอลในไวน์หมักต่ำกว่าที่ระดับอื่นๆ และนอกจากนี้การเพิ่มจำนวนช่อต่อต้นทำให้ดีกรีสารสีแดงและปริมาณสารสีเหลือง/น้ำตาลของไวน์ปรับแต่งเพิ่มขึ้น และทำให้ปริมาณสารสีแดงทั้งหมด สารฟีนอลทั้งหมด ความเข้มสีของไวน์ปรับแต่งลดลง สำหรับไวน์บ่มการเพิ่มจำนวนช่อต่อต้นทำให้ปริมาณสารให้สีที่ทนต่อซัลเฟอร์ไดออกไซด์เพิ่มขึ้น แต่ทำให้ความเข้มสีของไวน์ ดีกรีสารสีแดง ความเข้มสีของไวน์ปรับแต่ง ปริมาณสารสีเหลือง/น้ำตาลของไวน์ปรับแต่ง และดีกรีสารสีแดงของ

ไวน์ปรับแต่งลดลง ปริมาณแอนโทไซยานินในไวน์หมักและไวน์บ่มแตกต่างกันอย่างมีนัยสำคัญยิ่งทั้ง 2 ปี

สาขาวิชาเทคโนโลยีชีวภาพ ลายมือชื่อนักศึกษา_____

ปีการศึกษา 2553 ลายมือชื่ออาจารย์ที่ปรึกษา_____

VASON BOONTERM : EFFECTS OF NITROGEN AND POTASSIUM FERTILIZING AND CLUSTER THINNING ON QUALITY AND ANTHOCYANIN CONTENTS OF CABERNET SAUVIGNON GRAPE AND WINE. THESIS ADVISOR : PROF. NANTAKORN BOONKERD, Ph.D., 179 PP.

ANTHOCYANIN/PHENOLIC COMPOUNDS/MALVIDIN/CABERNET SAUVIGNON

The experiment was carried out in a humid subtropical climate located in the south of China, in Xichang, Sichuan province, (27ºN, 102ºE and 1650 m above mean sea level) during year 2005 and 2006 seasons. Eight years old, irrigated Cabernet Sauvignon vines were used at Xichang Chia Tai Wine & Spirits Co., Ltd. Vine plants were spaced 1.25 m apart and 2.0 m apart between row and oriented approximately N/S. Vines were trained to a vertical shoot positioned training system (VSP) and were bilaterally cordon-trained, spur-pruned, and shoots were vertically positioned upright. Vines were irrigated by drip irrigation. Three different levels of N-K, 0-0, 100-20 and 200-60 g/vine and three different levels of clusters, 10, 20 and 30 clusters per vine were applied as experimental treatments, each replicated 6 times in a split plot design, in which the main plots were number of clusters per vine. Vine shoot lengths were maintained at 15 nodes by shoot trimming. It was found that the yield of grapes in year 2005 was higher than that in 2006. Increasing the rate of N-K application in both years did not increase yields, but increased clusters per vine did in both years. Color and phenolic compounds in the berries were higher in year 2006 than that in 2005. At the 100-20 g/vine level of N-K, phenolic compounds in the grape were lower than that

of the other treatments. It was also found that the Cabernet Sauvignon berry contained more malvidin than other anthocyanins. The grape quality highly affected wine quality. The production of vintage year 2006 pressed and aged wines were better in both quality and color than that in 2005 because of the lower yields and smaller berries. Titratable acidity (TA) was decreased by increasing the rate of N-K application but increasing cluster levels increased it in both years. At the rate of N-K 100-20 g/vine, the phenolic compounds in grape wine were lower than that of the others. Pressed wine of the grape obtaining N-K at the rate of 100-20 g/vine had lower phenolic compounds than that of the others. Increasing cluster levels increased degree of red pigment coloration and modified wine color hue, but decreased total red pigments, total phenolics and modified wine color density. In aged wine, increasing cluster levels increased estimated SO_2 resistant pigment but decreased wine color density, degree of red pigment coloration, modified wine color density, modified wine color hue and modified degree red pigment coloration. Wine anthocyanin content was significantly higher in pressed than that in the aged wine for both years.

School of Biotechnology Student's Signature_____

Academic Year 2010 Advisor's Signature_____

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Vason Boonterm

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LIST OF ABBREVIATIONS

BB	=	budbreak
BL	=	bloom
ºC	=	degree celsius
cm	=	centimeter
DBB - BL	=	days from budbreak 50 % to bloom 50 %
DBB – H	=	days from budbreak 50 % to harvest
DBB - V	=	days from budbreak 50 % to véraison 50 %
DBL – H	=	days from bloom 50 % to harvest
DBL – V	=	days from bloom 50 % to véraison 50 %
DV – H	=	days from véraison 50 % to harvest
ºE	=	degree east
g	=	gram
g/l	=	gram per liter
GDD	=	growing degree days
GDDBB – BL	=	growing degree days from budbreak 50 % to bloom 50 %
GDDBB – H	=	growing degree days from budbreak 50 % to harvest
GDDBB – V	=	growing degree days from budbreak 50 % to véraison 50 %
GDDBL – H	=	growing degree days from bloom 50 % to harvest
GDDBL – V	=	growing degree days from bloom 50 % to véraison 50 %
GDDV – H	=	growing degree days from véraison 50 % to harvest
H	=	harvest

LIST OF ABBREVIATIONS (Continued)

ha	=	hectare
kg	=	kilogram
l	=	liter
l/ha	=	liter per hectare
M	=	molarity
µm	=	micrometer
mg/kg	=	milligram per kilogram
mg/ml	=	milligram per milliliter
mm	=	millimeter
mmol/l	=	millimolar per liter
min	=	minute
MDRPC	=	modified degree of red pigment coloration
MWCD	=	modified wine color density
MWCH	=	modified wine color hue
ºN	=	degree north
nm	=	nanometer
ppm	=	part per million
RMB	=	yuan (chinese's dollar)
rpm	=	round per minutes
TA	=	titatrable acidity
TB	=	started bud break
Temp	=	temperature

LIST OF ABBREVIATIONS (Continued)

TSS	=	total soluble solid
UV	=	ultraviolet light
V	=	véraison
VSP	=	vertical shoot positioned training system
v/v	=	volume by volume

CHAPTER I

INTRODUCTION

Grapevines are commonly grouped in the genus *Vitis* in the family Vitaceae. Grapes are an ancient food; cultivated seeds and evidence of wine making have been found dating back to the Bronze Age in Europe, approximately 3000 BC (Jackson, 2000). Two thousand years ago, wine was traded from one end of the Roman Empire to the other. Wines from different regions were famous for their unique quality and characters and were eagerly sought by connoisseurs. Today we continue using wine to celebrate the important occasions of our lives. A glass of wine is a reason to stop, to relax, to use our senses to explore the wine. We admire the color. We inhale the bouquet. We savor the flavor of the wine in our mouth. A glass of wine can be like a moment of meditation, a short vacation for your spirit, in the middle of a busy day. That is the culture of wine (Wagner, 2006).

1.1 Grape and wine production in the World and China

Worldwide areas under vines should therefore grow slightly between 2007 and 2008, and reached 7.408 million hectares and wine production in 2008 was 27.27 million tons. The China production areas was 0.438 million hectares and wine production in 2008 was 0.800 million tons (FAO, 2010). Modern Chinese winemaking, less than three decades old, has entered an awkward but promising adolescence. Several ambitious wineries are now using Western technology to create

dry wines from vinifera grapes, including Cabernet Sauvignon, Chardonnay and Pinot Noir. But wineries still face with growing pains. Most wineries buy their grapes from local farmers whose viticultural practices are questionable. Also the surface area of China's terroir has only been scratched only a few of the potential growing regions in this vast nation, with dozens of different climates, have been explored. Most of the wineries are located in the East, within a few hundred miles of Beijing. Vines are planted on rocky hillsides and get some breezes from the nearby Yellow Sea. Summer is extremely humid, and moist monsoon winds and occasional typhoons are hazards (Frank, 2005). The Chinese spirits industry is one of the most important in the world. Local spirits are in general strong alcohols made from cereals such as rice or oats. Twenty years ago, the Chinese government turned to the wine industry in view of decreasing the consumption of strong spirits. China became a growing actor in the development of the wine industry. However, contrary to its role as the leading producer of beer, China is still developing its wine production. When China became a member of the World Trade Organization in 2001, this implied that Chinese wines would have to conform to international criteria in relation to wine production, quality and taste. Faced with growing international competition, China's vineyards have not stopped spreading and its winemaking knows how to increase. The Chinese wine industry has undergone rapid changes and positively evolves since 1996. Even if wine production only represents 1% of the alcoholic drinks market with 359,000 hectares of vines and 0.334 million tons in volume, China is the currently the 6th leading operator in terms of surface under vine and wine production. At present, there are 500 Chinese producers (China Wine Information Website, 2006). In 2005, China's total grape wine output reached 434.3 million liters, up to 25.4% over the year

of 2004, profits amounted to RMB 1.256 billion, up to 58.8%, and that of the taxes paid reached RMB 1.207 billion, up by 30.2%. Forecasted by Ministry of Commerce, domestic grape wine output will increase with a growth rate of 15% before 2010, and the figure will reach about 800,000 tons by 2010. Grape wine imports will also increase constantly, with price falling sharply until equal to the price of homemade grape wines. Currently, Great Wall, Changyu, and Dynasty have monopolized China's grape wine market by and large. The market shares of the three giants are as high as 52%, the asset shares reached 38% of the industry, and the sales revenue even amount to 56% of the total. Each of them has their own advantageous markets. In the important grape wine consumption market of South China, the market shares of them exceeded 50% in total (Research in China, 2006).

1.2 Grape and wine quality in China

The typical Chinese wine still tastes like a very poor quality Bordeaux Rouge: sometimes not recognizably vinous, thin from overproduction, tart from under-ripeness, and often tough (Robinson, 2010). The main problems were very high yields and climate. China's continental climate produces hot, usually rainy summers, and very dry and cold winters. It is therefore necessary to supply supplementary irrigation for growing grapes in most regions. Two supplies of irrigation, one before the vines are pruned and the other one after bud break are obligatory to have a normal growth of the vines and to obtain a good yield. Irrigation is not recommended one to two months before harvest, especially for wine grapes. The climatic conditions are very variable in China. For example, it is very hot and humid in summer in the Yangtze valley, but very dry in the region of Xinjiang. Diseases are therefore, found

to be different from one region to another. Several fertilizer applications are usually carried out per year in most vineyards. In general, three or four applications of chemical fertilizers are applied after bud break, at flowering, during rapid growth of young fruits and during the maturation of grape berries. Nitrogenous and phosphorous fertilizers are usually supplied for the first two or three applications while only potash fertilizer is used during the maturation of berries. Moreover, a high quantity of manure (more than 30 t/ha) is often applied after harvest or in late autumn. The last manure application is very important for obtaining a high grape quality because of the rather low content of organic matter in the soil. Only several years ago, grape yields were very high in China, especially in the case of table grapes. Many growers obtained very high yields, as much as 40 to 60 t/ha, and sometimes up to 100 t/ha, but obviously at the expense of grape quality. Consequently, sugar content in the grapes was too low to be used for wine making and table grapes were non-marketable or sold at very low prices. In recent years, growers paid more attention to grape quality than to yield. The productivity is now severely controlled in most vineyards. The grapevines usually enter into bearing in the second or third year after planting and mature vines may produce 20 to 30 tonnes per hectare in irrigated vineyards in order to obtain a higher quality of grapes (Shao-Hua, 2010). Many producers buy their grapes through contracts with farmers. This does not tend to work because the farmers are paid by weight and thus the focus is on quantity, not quality (Demei, 2009).

1.3 Grape and wine quality

The most significant constituent of wine is water (75- 90%). This amount (15%) variation can be explained for by the amount of phenolics, organic acids, mineral salts and pectins which form the wine extract, and which differ from wine to wine. The second largest constituent of wine is ethyl alcohol, which, according to the type of wine, varies from 8% to 13% (v/v) or more. The sugar content of dry wines is generally less than 2 g/L; while in a botrytized sweet wine it can reach almost 200 g/L (Dominé *et al*., 2004). Grape color is an integral and important part of red grape and red wine quality (Gishen *et al*., 2002; Somers and Evans, 1974). It is generally accepted that an increase in grape color coincides with an improvement in phenol structure, an increase in aroma intensity and an increase in wine quality. The color of red or black grapes is caused by the presence of anthocyanins in the grape skins (Marais, 2005). The synthesis of anthocyanins is stimulated by light, both UV and visible, as well as by nutrient stress, especially nitrogen and phosphorus deficiencies and low temperature (Hopkins, 1995). Revilla *et al*., 1997 showed that the content of phenolics (catechin and procyanidins) in grapes is clearly affected by four agroecological factors: the cultivar, the year of production (i.e., the climate condition from year to year), the site of production (the effect of geographic origin of grapes, soil chemistry, and fertilization), and the degree of maturation. Reported levels of anthocyanins in red grapes range from 300 to 7,500 mg/kg, but the levels vary highly according to the cultivar, maturity, production year and environmental conditions (Mazza, 1995). The above characteristics are controlled by cultivation managements; namely weed control, pest control, canopy management, crop loading, irrigation, and fertilizer management. These practices are considered as routine works. Among

these the most immediate effect, which can give rise to the yield and wine quality are crop load and N-P-K in each specific area. In Xichang China the maturity of grape is in rainy season which sunlight is usually low. Thus to obtain high quality, grape cluster management should be conducted. Also soil in this area is high in P and K. Therefore we were interested in finding the effect of rates of N and K on berry quality since P requirement in grape is low. Because the low color of grape and wine that was produced in this areas. Wines needed to add color extract for suitable color. The aim of this research was to investigate the effect of of N-K application and clusters per vine on quality and anthocyanin content in grape and wine.

1.4 Research objectives

Two research objectives were established:

1. To determine the effects of nitrogen and potassium fertilization and cluster thinning on Cabernet Sauvignon grapevine performance and berry quality.

2. To determine the effects of nitrogen, potassium and number of cluster on Cabernet Sauvignon wine color and anthocyanin content.

CHAPTER II

LITERATURE REVIEW

In grapes and young red wine, the red color mainly results from the pigments that are presented, the anthocyanins (Boulton, 2001; Corona *et al*., 2004; Lorenzo *et al*., 2005; Mayen *et al*., 1994; Mazza *et al*., 1999; Ribereau-Gayon, 1982; Schwarz *et al*., 2003; Vian *et al*., 2006). More recently it has been demonstrated that, during fermentation, yeast releases secondary metabolic products into the medium, such as pyruvic acid and acetaldehyde, some of which react with anthocyanins to produce derivatives such as vitisin A, vitisin B, and ethyl-linked anthocyanin-flavanol pigments (Asenstorfer *et al*., 2003; Eglinton *et al*., 2004; Lee *et al*., 2004; Morata *et al*., 2003). Anthocyanin composition is an important quality parameter for red grapes because of the significance of these compounds in determining color of the resulting wines. Anthocyanin profiles of grapes, determined by the relative proportions of the different anthocyanins, are characteristic for each grape variety. Moreover, concentrations of the different compounds can vary significantly within grape cultivars according to environmental conditions, including climate, soil and vineyard management practices (Boulton *et al*., 1996). Vineyard management can alter the proportion of pigments in red grapes; whilst, winemaking techniques and wine aging influence the development of subsequent pigments in the wine (Arnous *et al*., 2002; Bakker *et al*., 1998; Cameira-dos-Santos *et al*., 1996; Gomez-Plaza *et al*., 2002; Tsanova-Savova *et al*., 2002; Zafrilla *et al*., 2003). A broad range of factors

influences wine grape quality and its manipulation has stimulated interest among grape growers and winemakers.

2.1 The importance of grape and wine color

Grape color is an integral and important part of red grape and red wine quality (Gishen *et al*., 2002; Somers and Evans, 1974). It is generally accepted that an increase in grape color coincides with an improvement in phenol structure, an increase in aroma intensity and an increase in wine quality. The color of red or black grapes is caused by the presence of anthocyanins in the grape skins (Marais, 2005). The color can give the judge some indication as to the quality of the wine when it is compared with the wine's age. Ultimately, the color affects the score and the price of the wine (Rovner, 2006). The color of a red wine is primarily due to the type and concentration of anthocyanin, but other factors play a role, including phenolic compounds other than the anthocyanin (Johnston and Morris, 1996). Anthocyanins are considered very good antioxidant agents, their high activity being attributed to their peculiar structure, namely the oxonium ion in the C ring (van Acker *et al*., 1996). The antioxidant functions of anthocyanins have been ascribed to the aglycone moiety, and this was demonstrated for cyanidin and some of its glycosides (Wang *et al*., 1999) but the number of sugar residues at the 3-position (Wang *et al*., 1999) the oxidation state of the C ring (Lapidot *et al*., 1999) the hydroxylation pattern (Espin *et al*., 2000; Rice-Evans *et al*., 1995; Wang *et al*., 1997) as well as the acylation by phenolic acids (Degenhardt *et al*., 2000) are considered crucial factors for the expression of antioxidant effects. The phenolic compounds are important constituents of plant cells and are associated with physiological defense against

infection by bacteria and viruses. Moreover, the phenolic compounds in grape and wines reportedly contribute to the following properties: color, astringency, bitterness, oxidation reactions, interaction with proteins and aging behavior of wine (Carando *et al*., 1999). Recently, the flavonoid compounds have received considerable attention due to their antioxidant, antimutagenic, and anticarcinogenic properties (Karakaya and El, 1999; Soleas *et al*., 2002). Phenolic compounds play an important part in the wine, since they contribute to color, sensory characteristics and health effects (Fischerleitner *et al*., 2003). Considerable evidences accumulate now indicating that moderate prolonged red wine consumption is beneficial for health (Criqui and Ringer, 1994; Renaud and De Lorgeril, 1992; St-Leger *et al*., 1979). This could be explained by significant amount of natural antioxidant phenolics intake present in red wine (Rice-Evans and Miller, 1996). Indeed epidemiological studies show that intake of these compounds is correlated with reduced incidence of coronary heart disease (CHD) (Ghiselli *et al*., 1998; Hertog *et al*., 1993). Furthermore, red wine and its antioxidant phenolic components increase serum antioxidant capacity in vivo (Roig *et al*., 1999; Serafini *et al*., 2000) inhibit low density lipoprotein (LDL) oxidation in vitro (Frankel *et al*., 1995; Ghiselli *et al.,* 1998; Koga *et al*., 1999; Teissedre *et al*., 1995). Dietary consumption, mainly from red fruits, certain vegetables and red wine (Macheix *et al*., 1990) can be as high as 200 mg/day and their consumption in red wine has been proposed as part of the reason for the "French Paradox" (Clifford, 2000) in which a diet rich in saturated fats and moderate alcohol consumption does not lead to elevated levels of heart disease, cancers and strokes found in other countries. Anthocyanins are effective antioxidants (Stinzing and Carle, 2004) but they have also been proposed to have other biological activities independent of their antioxidant capacities that

produce health benefits; examples range from inhibition of cancer cell growth in vitro (Zhang *et al*., 2005) induction of insulin production in isolated pancreatic cells (Jayaprakasam *et al*., 2005) reduction of starch digestion through inhibition of α-glucosidase activity (Matsui *et al*., 2001) suppression of inflammatory responses (Tall *et al*., 2004) to protection against age-related declines in cognitive behaviour and neuronal dysfunction in the central nervous system (Joseph *et al*., 1999).

2.2 Grape and wine phenolic compounds

Grapes contain a variety of phenolic compounds. Phenols represent the third most abundant constituent in grapes and wines after carbohydrates and fruit acids (Singleton, 1980). The phenolic compounds are broadly distributed inside grapes. When extracting a single grape variety, the composition of phenolics depends upon whether the extraction is performed on whole grape pulp, skin, or seeds. The total extractable phenolics in grape are present at only about 10% or less in pulp, 60-70% in the seeds, and 28-35% in the skin (Vernhet *et al*., 1996). Two chemical classes of flavonoids, the flavan-3-ols (catechins and proanthocyanidins) and the anthocyanins, are the natural antioxidants present at the highest concentration in red grape and wine. In the berry, the anthocyanins are localized in the skins, similarly to other highly bioactive phenolics of grape such as the resveratrols and the flavonols, while the flavan-3-ols are contained both in the skins and seeds. During winemaking, only a fraction of the grape flavonoids are selectively extracted into the wine, with a time course and a final yield strongly depending on the grape variety (Mattivi *et al*., 2002). The phenolic compound composition of red and white wines varied greatly. Both the red and white wines contained a complex mixture of phenolic compounds whose

content and composition varied by the brand, indicating that the processing technique would greatly influence the phenolic compounds quality and quantity of the wines rather than the color of the grape (Basha *et al*., 2004). The specific amounts and types of phenolics depend on a number factor, including variety, cultural practices, seasonal conditions, storage, and processing (Mazza, 1995). During véraison, berry skins lose chlorophyll and begin to synthesize and accumulate phenolic compounds that are responsible for development of characteristic colors: yellow-gold (flavonols), pink and red (anthocyanins) colors (Watson, 2003). Crippen and Morrison (1986) found that total soluble phenols increased until véraison, then decreased from véraison to harvest. The percentage of polymerized phenols decreased during early berry growth, and then increased from véraison to harvest. There are four major types of phenolic compounds found naturally in grapes and wines.

- Phenolic acids
- Hydroxycinnamic acids
- Tannin
- Flavonoids

These compounds are non-nutritive secondary plant metabolites concentrates in the skin and the seeds. They are not essential for the plant to live, but they generally help protect the plant from damage.

2.2.1 Phenolic acids

Phenolic acids have one phenol constituent. The four-hydroxybenzoic acids of grapes and wines are p-hydroxybenzoic acid, vanillic acid, syringic acid, and gallic acid (Macheix *et al*., 1990). The major phenolic acid in grapes is gallic acid.

The main sources of gallic acid in wine are grape seeds and oak cooperage. In seeds, gallic acid is present as free gallic acid and as an ester attached to procyanidin polymers. Gallic acid is also present in grape stems and may be increased by whole cluster fermentations. Red wine was found to contain an average of 95 mg/l while white wine had only about 7 mg/l (Frankel *et al.,* 1995).

2.2.2 Hydroxycinnamic acid

These are the quantitatively more important non-flavonoid phenols in wines, and exist in grapes only as tartaric acid esters. Major hydroxycinnamic acid derivates found in grapes are:

- Caffeic acid (3,4 di-OH) is a free cinnamic acid. Caftaric acid is an ester formed from caffeic and tartaric acids. Only caftaric acid is found in grapes. Both the ester and free cinnamate are found in wine. Caftaric acid is found in grape skins, pulp, and stems; it is not present in seeds. Other cinnamate esters in grapes include fertaric and coutaric acids; the relative proportion of the different cinnamic acid esters varies by variety. Caffeic acid is found at concentrations between 1 and 15 mg/l wine or crushed grapes. White wines were at the low end of the distribution.

- Ferulic acid (4-OH, 3-OMe) is less prevalent, with only 0.2-1 mg/l present in wine.

- p-coumaric acid (4-OH) is found at a concentration of 0.3-30 mg/l.

- Chlorogenic acid is a quinic glycoside of caffeic acid (Frankel *et al*., 1995).

Although the free forms of these acids do not exist in grapes, they can be found in wines due to enzyme and acid hydrolysis during the winemaking processes.

The ratio of caffeic to caftaric acid in wines has been used to estimate esterase acivities contained in pectinase preperations (Wightman, 1995). The amounts of hydroxycinnamic acids are high in free run juice as they are the main phenols present in the flesh. In the skins they are present in lower amounts, and the ratio of caftaric acid to other hydroxycinnamic acids is lower. The hydroxycinnamic acids can also be bound to the glucose moiety of anthocyanins in red cultivars (except Pinot noir), and this is important for anthocyanin color stability by intramolecular copigmentation (Dangles *et al*., 1993). Caftaric acid is also the main substrate for enzymatic oxidation in musts, leading to browning and oxidation of anthocyanins (Macheix *et al*., 1991; Sarni *et al*., 1995).

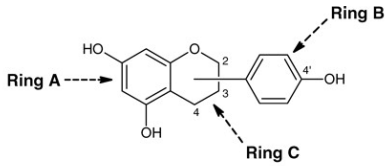
2.2.3 Procyanidins or tannin

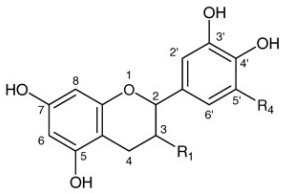
These are usually considered to be the oligomers of two to eight flavan-3-ol units, with larger or more complicated structures referred to as tannins. Often the word ‘tannin’ when used in relation to wine includes the procyanidins. They are also sometimes referred to as proanthocyanidins, and they used to be referred to as leucoanthocyanidins. The monomeric (+)-catechin and (-)-epicatechin units of procyanidins most commonly have C4 to C8 interflavan bonds, but less commonly C4 to C6 bonds will occur. Catechins (5,7, 3′, 4′-tetra OH) are found at levels of approximately 190 mg/l in red wines and 35 mg/l in white wines. Epicatechin is found at 150 mg/l in red wine and 15 mg/l in white wine (Calabrese, 2003). In the mildy acidic medium of wine, these bonds may break and reform readily (Haslam, 1980), leading to a great diversity of sizes and structures. They react with other phenols and anthocyanins, and precipitate with proteins. They are present as

monomers in grapes and wine and are the primary components of polymeric phenols. Both compounds are found in high concentrations in seeds and stems and may be found in the skins of immature grapes. Pinot noir has much higher concentrations of both compounds than Cabernet, Merlot, or Zinfandel.

2.2.4 Flavonoids

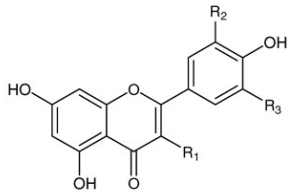
Flavonoids are a class of secondary metabolites widespread in various plants (Tahara, 2007). Flavonoids perform major roles in plants such as UV protection, defense against pathogens and pests, pollen fertility, signaling with microorganisms, auxin transport regulation, and pigmentation (Winkel-Shirley, 2001). Today more than 10,000 varieties of flavonoids have been identified (Dixon and Paiva, 1995; Tahara, 2007). Flavonoids are characterized as molecules possessing two phenolic groups joined by a pyran (oxygen-containing) ring structure (Figure 2.1). All of these phenolic classes have a large number of structures that differ in the number and position of hydroxy (-OH) and methoxy (-OCH3) groups on the basic skeleton. Each structure can also be variously substituted (e.g. glycosylated, acylated, esterified). Most of the phenolic compounds in grapes are flavonoids. Flavonoids are 15-carbon phenolic compounds generally distributed throughout the plant kingdom (Harbone, 1984) and divided into isoflavones, anthocyanins, flavans, flavonols, flavones and flavonones (Peterson and Dwyer, 1998).





Flavanols

	R_1	R_4
Catechin	OH	H
Epicatechin	OH	H
EGC	OH	OH
ECG	Gallate	H
EGCG	Gallate	OH

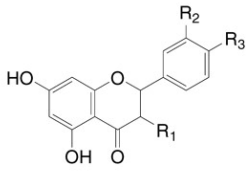


Flavonols

	R_1	R_2	R_3
Kaempferol	OH	H	H
Quercetin	OH	OH	H
Myricetin	OH	OH	OH
Isorhamnetin	OH	OCH_3	H

Flavones

Luteolin	H	OH	H
Apigenin	H	H	H

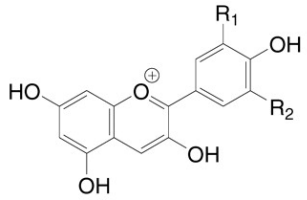


Flavanones

	R_1	R_2	R_3
Naringenin	H	H	OH
Hesperetin	H	OCH_3	H

Flavanonols

Taxifolin	OH	OH	OH
Astilbin	O-rhamnosyl	OH	OH
Engeletin	O-rhamnosyl	H	OH



Anthocyanidins

	R_1	R_2
Pelargonidin	H	H
Cyanidin	OH	H
Delphinidin	OH	OH
Paeonidin	OCH_3	H
Petunidin	OCH_3	OH
Malvidin	OCH_3	OCH_3

Figure 2.1 Basic structure of flavonoid

The main flavonoid species important to the chemical reactions and sensory properties of red wine are the anthocyanins (e.g. malvidin-3-O-glucoside) and flavanols (e.g. catechin and epicatechin). The next most abundant compounds are flavonols, their yellow pigments in the skins of both red and white grapes. Flavonoids

are synthesized via the phenylpropanoid pathway in which the amino acid phenylalanine is used to produce 4-coumaroyl-CoA. Phenylalanine ammonia lyase (PAL) catalyzes the conversion of phenylalanine to cinnamate. PAL also shows activity with converting tyrosine to p-coumarate, albeit to a lower efficiency. The cinnamate 4-hydroxylase (C4H) catalyzes the synthesis of p-hydroxycinnamate from cinnamate and 4-coumarate: CoA ligase (4CL) converts p-coumarate to its coenzyme-A ester, activating it for reaction with malonyl CoA. The flavonoid biosynthetic pathway starts with the condensation of one molecule of 4-coumaroyl-CoA and three molecules of malonyl-CoA, yielding naringenin chalcone. This reaction is carried out by the enzyme chalcone synthase (CHS). Chalcone is isomerised to a flavanone by the enzyme chalcone flavanone isomerase (CHI). From these central intermediates, the pathway diverges into several side branches, each resulting in a different class of flavonoids. Flavanone 3-hydroxylase (F3H) catalyzes the stereospecific 3β-hydroxylation of (2S)-flavanones to dihydroflavonols. For the biosynthesis of anthocyanins, dihydroflavonol reductase (DFR) catalyzes the reduction of dihydroflavonols to flavan-3, 4-diols (leucoanthocyanins), which are converted to anthocyanidins by anthocyanidin synthase (ANS). The formation of glucosides is catalyzed by UDP glucose-flavonoid 3-o-glucosyl transferase (UFGT), which stabilizes the anthocyanidins by 3-o-glycosylation (Bohm, 1998; Harborne, 1994).

2.3 Anthocyanins

Anthocyanins are members of the flavonoid group of polyphenols, meaning they share a basic C15 skeleton structure. Their basic structure comprises the A-ring,

which is a phloroglucinol derivative, linked to a pyrilium ring, which is linked to the B phenolic ring. They are a double bonds carbon 1-2 and 3-4, and a hydroxyl moiet at C3. O-glycosylation usually occurs at carbon 3. Aglycones are called anthocyanidins (Figure 2.1). The aglycones are rarely found in fresh plant material (Clifford, 2000; Prior, 2004) or commercial products such as wine (Waterhouse, 2002), except in trace quantities, because they are quite unstable. This form is principally responsible for the red color of young red wines, and it is also this flavylium chromophore that gives color to the red polymeric wine pigments. The anthocyanidin form has a hydroxy group at position 3, and due to its instability is seldom found in its free form in grapes or wine. The anthocyanins however have an -O-sugar group at position 3 that confers significantly greater stability on them (Furtado *et al*., 1993). It has been proposed that the biosynthesis of anthocyanins in plants involves the formation of the unstable, colorless chalcone form of the 3-deoxyanthocyanidin which then has a glucoside added to form the 3-monoglucoside anthocyanin. This stabilises the molecule by favouring ring closure to give the hemiacetal form that and can rapidly take on other stable equilibrium forms (Brouillard, 1982). Only six anthocyanidins are common in higher plants pelargonidin (Pg), peonidin (Pn), cyanidin (Cy), malvidin (Mv), petunidin (Pt) and delphinidin (Dp). The glycosides of the three non-methylated anthocyanidins (Cy, Dp and Pg) are the most widespread in nature, being present in 80% of pigmented leaves, 69% of fruits and 50% of flowers. The distribution of the six most common anthocyanidins in the edible parts of plants is Cy (50%), Pg (12%), Pn (12%), Dp (12%), Pt (7%), and Mv (7%). The following four classes of anthocyanidin glycosides are common: 3-monosides, 3-biosides, 3,5-diglycosides

and 3,7-diglycosides. 3-glycosides occur about two and half times more frequently than 3,5-diglycosides. Since each anthocyanidin may be glycosylated and acylated by various sugars and acids at different positions, a great number of chemical combinations exist (Delgado-Vargas and Paredes-Lopez, 2003; Harborne, 1998). Based on several reviews, it was estimated that more than 600 anthocyanins had been found in nature. So, the most widespread anthocyanin is cyanidin 3-glucoside (Kong *et al*., 2003). In the grapes and wines of *Vitis vinifera*, the only anthocyanins present are those of malvidin, delphinidin, petunidin, peonidin and cyanidin, with an -O-glucose at position 3. Non vinifera grapes also contain 3, 5 diglucoside forms of these five anthocyanins, and in other fruits and flowers sugars other than glucose can be substituted at positions 3 and 5. The sugar group may be further acylated with an acetic, p-coumaric, or caffeic acid group in grapes such as Cabernet Sauvignon, however Pinot noir has no acylated anthocyanins (Wulf and Nagel, 1978).

Anthocyanins are responsible for the red colour of grapes and wines, which are located in the grape skin of red varieties and predominantly exist in grapes as glucosides, which from through the conjugation of the flavonoid component; called an anthocyanidin, with glucose the sugar component increases the chemical stability and water solubility of the anthocyanidin (Jackson, 2000). Anthocyanin synthesis begins during veraison and anthocyanins are gradually accumulated in the berry skin throughout grape ripening (Bautista-Ortı´n *et al*., 2006; Cacho *et al*., 1992; Canals *et al*., 2005; Cholet and Darne´, 2004; De la Hera Orts *et al*., 2005; Esteban *et al*., 2001; Ferna´ndez Lo´ pez *et al*., 1998; Fournand *et al*., 2006; Gil and Yuste, 2004; Ojeda *et al*., 2002; Pe´ rez-Magarino and Gonza´ lez-San Jose´, 2004; Robinson and Davies, 2000; Roby *et al*., 2004; Ryan and Revilla, 2003), malvidin-3-glucoside being the

most abundant anthocyanin in nearly all grape varieties (Cholet and Darne´, 2004; Fournand *et al*., 2006; Pomar *et al*., 2005; Ryan and Revilla, 2003). Several agroecological factors, such as cultivar (Cacho *et al*., 1992; Pomar *et al.,* 2005; Ryan and Revilla, 2003), climate (Cacho *et al*., 1992; Gil and Yuste, 2004; Yamane *et al*., 2006) soil conditions (Gil and Yuste, 2004; Yokotsuka *et al*., 1999) vine water status (Ojeda *et al.,* 2002; Roby *et al*., 2004) and cultural practices (De la Hera Orts *et al*., 2005; Esteban *et al*., 2001), have been related to the rate of anthocyanin accumulation. However, anthocyanin concentration may decrease slightly just before harvest (Ryan and Revilla, 2003) and/or during over-maturing (Fournand *et al*., 2006). Anthocyanin pigments are of prominent importance in wines because of their dual role; first, they constitute an integral part of the sensory attributes because their levels, various forms and derivatives pertain directly to the coloration of the final product; second, they have been claimed to possess diverse biological properties and therefore are considered as secondary metabolites with potential nutritional value (Kallithraka *et al*., 2005). Anthocyanins are synthesized as a branch of phenyl propanoid pathway. Many of enzymes involved in anthocyanin biosynthesis have been characterized (Figure 2.2), and the corresponding genes have been cloned (Forkmann, 1991). The enzymic steps involved in the synthesis of anthocyanidins from leucoanthocyanidins, however, are not well defined. It is thought that dehydration and an oxidation step are involved (Heller and Formann, 1988). Anthocyanin synthesis in *Vitis vinifera* L. cv. Shiraz grape berries began 10 weeks postflowering and continued throughout berry ripening. Expression of seven genes of the anthocyanin biosynthetic pathway (phenylalanine ammonia lyase [PAL], chalcone synthase [CHS], chalcone isomerase [CHI], flavanone-3-hydroxylase [F3H],

dihydroflavonol 4-reductase [DFR], leucoanthocyanidin dioxygenase [LDOX], and UDP glucose-flavonoid 3-o-glucosyl transferase [UFGT]) was determined. In flowers and grape berry skins, expression of all of the genes, except UFGT, was detected up to 4 weeks postflowering, followed by a reduction in this expression 6 to 8 weeks postflowering. Expression of CHS, CHI, F3H, DFR, LDOX, and UFGT then increased 10 weeks postflowering, coinciding with the onset of anthocyanin synthesis. In grape berry flesh, no PAL or UFGT expression was detected at any stage of development, but CHS, CHI, F3H, DFR, and LDOX were expressed up to 4 weeks postflowering. These results indicate that the onset of anthocyanin synthesis in ripening grape berry skins coincides with a coordinated increase in expression of a number of genes in the anthocyanin biosynthetic pathway, suggesting the involvement of regulatory genes. Boss *et al*. (1996b) suggested that UFGT was the key enzyme in anthocyanin biosynthesis; hence, it is important to investigate UFGT activity in poorly colored berries grown under warm night conditions. It has already been established that in many plants the gene expression of the enzymes involved in anthocyanin biosynthesis is affected by temperature; the expression of the genes PAL, CHS and CHI is induced by low temperature in various plants such as maize (Christie *et al*., 1994), arabidopsis (Leyva *et al*., 1995), petunia (Shvarts *et al*., 1997) and aster (Shaked-Sachray *et al*., 2002). Mori *et al*., 2005 suggested that the temperature at the initial stage of ripening (immediately prior to veraison) has a greater effect on the expression of genes of the anthocyanin biosynthetic pathway and on anthocyanin accumulation. Yamane *et al*., 2006 reported that anthocyanin accumulation in the grape berry skins was significantly higher at 20°C than at 30°C after the temperature treatment and the most sensitive stage for the

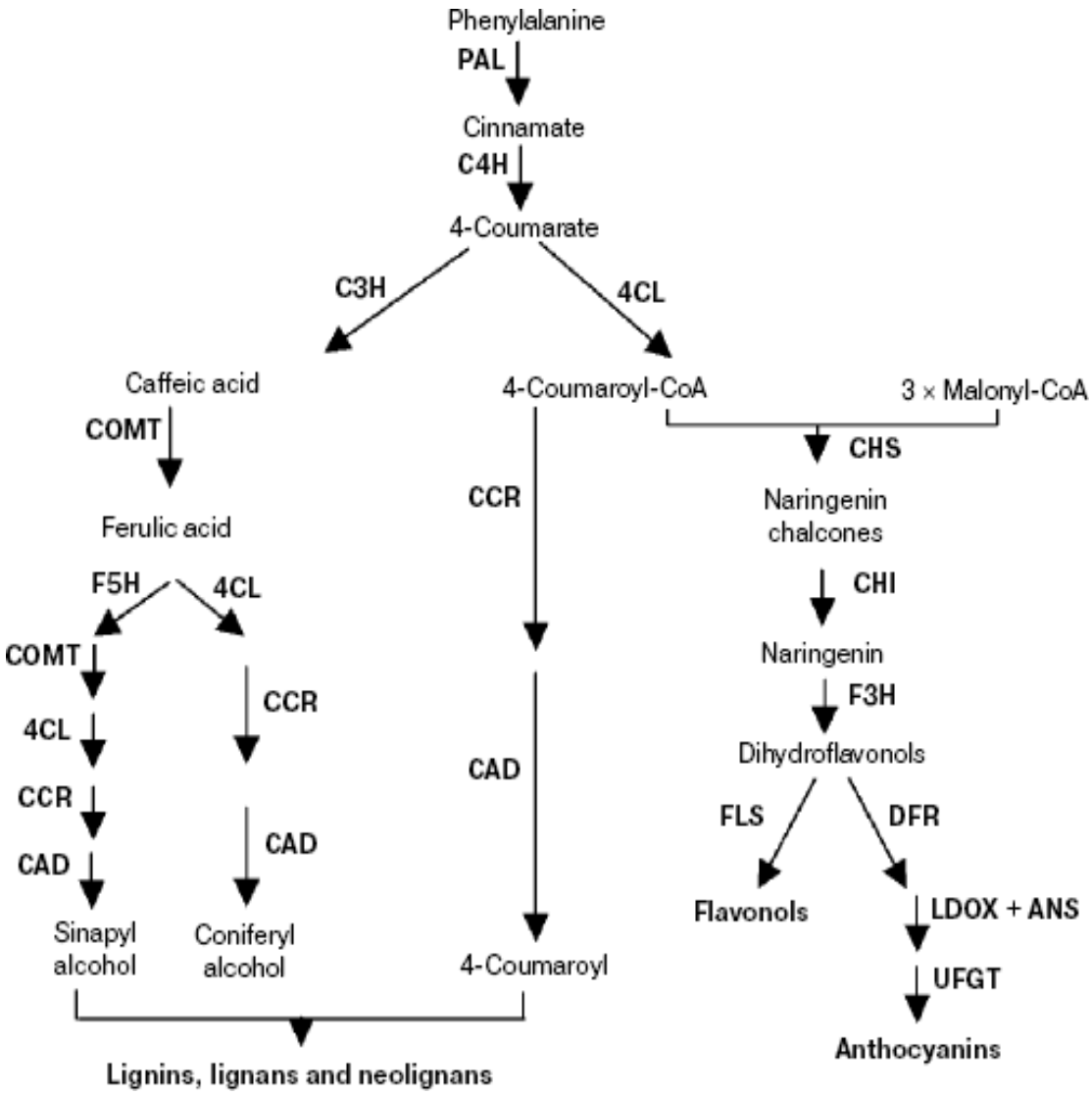


Figure 2.2 Anthocyanin biosynthesis pathway. The genes discussed in this paper are boxed. PAL, phenylalanine ammonia-lyase; C4H, cinnamate 4-hydroxylase; 4CL, 4-coumarate: CoA-ligase; CHS, chalcone synthase; CHI, chalcone isomerase; F3H, flavanone 3-hydroxylase; F30H, flavonoid 30-hydroxylase; F30 50H, flavonoid30 50-hydroxylase; DFR, dihydroflavonol 4-reductase; LDOX, leucoanthocyanindioxygenase; UFGT, UDP-glucose:flavonoid 3-O-glucosyltransferase.

temperature treatment was from one to three weeks after coloring began. Most sensitive stage for the temperature treatment was from one to three weeks after coloring began. Kobayashi *et al*., 2001 found that the expression of the UFGT gene has been shown to be critical for anthocyanin biosynthesis in the grape berry. In 1994, Shiraishi and Watanabe determined the anthocyanin compositions of 59 cultivars of red and black grapes and reported that most red cultivars contain mainly derivatives of the anthocyanidins cyanidin and/or peonidin, while black cultivars contain primarily delphinidin, petunidin, and/or malvidin derivatives. The finding showed that malvidin 3-0-glucoside is the predominant anthocyanin, as demonstrated for a number of other *V. vinifera* species, including Syrah (Roggero *et al*., 1986), Agiorgitiko (Dourtoglou *et al*., 1994), Cabernet Sauvignon and Pinot noir (Wulf and Nagel, 1978), Cabernet Franc, Merlot and Pinot Noir (Mazza *et al*., 1999), Graciano (Nunez *et al*., 2004), Tannat (Neves *et al*., 2004), and Tempranillo (Hebrero *et al*., 1988; Esteban *et al*., 2001). Further, the evolution of different anthocyanins during the ripening of the grape berries has been reported in a number of cultivars including 'Cabernet Sauvignon' (Regules *et al*., 2006; Ryan and Revilla, 2003), 'Merlot' (Regules *et al*., 2006), 'Syrah' (Regules *et al*., 2006; Roggero *et al*., 1986), 'Monastrell' (Fernandez-Lopez *et al*., 1992; Regules *et al*., 2006), 'Tempranillo' (Cacho *et al*., 1992; Ryan and Revilla, 2003), 'Touriga Nacional' and 'Touriga Francesa' (Mateus *et al*., 2002), 'Reliance' (*Vitis vinifera* L. x *V. labrusca* L.) (Gao and Cahoon, 1995), 'Moristel and Garnacha' (Cacho *et al*., 1992) using high performance liquid chromatography (HPLC). Other anthocyanin was distinctive for each cultivar, but the degree of ripeness (Roggero *et al*., 1986) may be critical in this respect, because anthocyanin distribution can be considerably affected during

different maturation stages. Grape varieties, Vapsa, Mandilaria and Mavrodafni were rich in anthocyanin. Moderate values were found for Thrapsa, Sangiovese, Grenache Rouge, Negoska and Cabernet Sauvignon, while Xinomavro, Aidani Mavro, Pardala, Papadiko, Karlachanas, Merlot, Limnio and Araklinos had contents below average (Arnous *et al*., 2002). The red grape, Cabernet Sauvignon, contained substantial quantities of delphinidin, cyanidin, petunidin and malvidin glycosides (Johnston and Morris, 1996; Zhang *et al*., 2003). Boss *et al*., 1996a showed that Cabernet Sauvignon and Shiraz grapes contained anthocyanin monoglucosides as well as acetylated and coumarylated derivatives, predominantly of malvidin, whereas Pinot Noir grapes contained only anthocyanin monoglucosides. Anthocyanins were the main phenolics in red grapes ranging from 69 (Crimson Seedless) to 151 (Flame Seedless) mg/kg fresh weight of grapes, whereas flavan-3-ols were the most abundant phenolics in the white varieties ranging from 52 (Dominga) to 81 (Moscatel Italica) mg/kg fresh weight of grapes. Total phenolics ranged from 115 (Dominga) to 361 (Flame Seedless) mg/kg fresh weight of grapes (Cantos *et al*., 2002). Muscadine grapes, average total phenolics were 2178.8, 374.6, 23.8, and 351.6 mg/g gallic acid equivalent in seed, skin, pulp, and leaves, respectively. Total anthocyanin contents were 2.1 and 132.1 mg/100 g of fresh weight in the skins of bronze and purple grapes, respectively, and 4.3 and 4.6 mg/100 g of fresh weight in seeds and pulps (Pastrana-Bonilla *et al*., 2003).

The synthesis of anthocyanins is stimulated by light, both UV and visible, as well as by nutrient stress (especially nitrogen and phosphorus deficiencies) and low temperature (Hopkins, 1995). Revilla *et al*., 1997 showed that the content of phenolics (catechin and procyanidins) in grapes is clearly affected by four

agroecological factors: the cultivar, the year of production (i.e., the climate condition from year to year), the site of production (the effect of geographic origin of grapes, soil chemistry, and fertilization), and the degree of maturation. Anthocyanins increased linearly as sunlight exposure on the north side of the canopy increased, but declined when cluster exposure on the south exceeded 100 $\mu mol/m^2/sec$. Total phenolics generally followed a similar pattern. The results suggest that the effects of light on fruit composition are heavily dependent upon the extent to which berry temperature is elevated as a result of increased sunlight exposure (Bergqvist *et al*., 2001). The anthocyanin content per berry was significantly higher for vines defoliated from véraison. Sugar levels in berry skins seemed to be associated with anthocyanin concentration. The phenolic content per berry was unaffected by partial defoliation (Hunter *et al*., 1991).

The principal difference between red and white wines is that red wines have very much greater and more diverse phenol content. It is this phenol content that gives color, astringency, mouthfeel and texture to a red wine and it is the extraction and management of wine phenols that are responsible for the differences in vinification between red and white wines. White wine vinification usually seeks to keep phenolic extraction to a minimum as phenols lead to unattractive bitterness and astringency in white wines, and are the main substrate for oxidative browning. By contrast, the most influential factor determining red wine styles is the amount and type of phenols extracted, and their extent of polymerization. Despite variations between cultivars and seasons, red and white grapes have generally comparable qualitative and quantitative phenolic distributions (Slinkard and Singleton, 1984) with the major exception being the presence of high concentrations of anthocyanins in the

outer skin vacuoles of red grapes. Traditional red winemaking practices actually combine three major and very complex processes into essentially a single unit operation. Red wine is a macerated wine, and maceration should represent a managed fractional extraction of the grape (Peynaud, 1984). The degree of extraction influences the amount and stability of color, the astringency and tannin structure of the wine, the potential of the wine to age and the balance between fruitiness and heavier, more complex flavors and aromas (Zoecklein, 1991). The various phenols are located in different regions of the grape and have different solubility and diffusivities with respect to each other, with respect to the aqueous or alcoholic phase, and with respect to the temperature and chemical environment of the must. Thus, only a small proportion of grape anthocyanins that have been extracted from skins during fermentation can be detected in aged red wines, even though the color intensity is largely maintained (Peng *et al*., 2002; Somers and Evans, 1977). About a third of the total available phenols from the grape are ever extracted into the wine, and these can come variously from the pulp, skins, seeds and stems. For this reason berry size (skin to juice ratio), seed number per berry, and the inclusion of 'ripe' (lignified) stems in the maceration are important considerations. The methods and conditions of juice/wine contacting with the pomace (skins, seeds and stems) can have a significant effect on the extraction of phenols into the wine. Non-flavonoid phenols are already present in the juice, and are soluble from the skins in the aqueous phase. Anthocyanins are also water soluble, and the maximum extraction of anthocyanins is often obtained within the first few days of skin contact. Anthocyanins polymerize in red wine, forming stable structures (Singleton *et al*., 1964; Timberlake and Bridle, 1976). Free anthocyanidins have been shown to be destroyed or decolorized by heat

(Flora, 1978; Sims and Morris, 1984), light (van Buren *et al*., 1968), SO_2 (Jurd, 1963; van Buren *et al*., 1968), pH (Ohta *et al*., 1980), and temperature (van Buren *et al*., 1968). The flavan-3-ols and their oligomers, the procyanidins are more soluble in alcohol, and are located largely in the seeds, so the total phenol and 'tannin' content due to these compounds increases rather more slowly as the ferment progresses. Most cultivars also have significant amounts of these flavonoids in the skins, but Pinot noir is the notable exception, lacking both monomeric flavan-3-ols and procyanidins in the skins (Price *et al*., 1995). Grape flavour/aroma compounds are a large and complex group of chemicals that are water soluble, and are found in free volatile or glycosidically bound precursor forms in the mesocarp vacuoles and the pericarp immediately under the skin. Important precursors for some red wine aroma compounds are found in the waxy bloom on the outside of the skin (Brander *et al*., 1980). As well as the above molecules, the extraction of other compounds such as polysaccharides and nitrogenous compounds (amino acids and proteins) is important for vinification and evolution of the wine. The three important control points for influencing phenols and red wine style are in the vineyard, maceration and maturation.

2.4 Factors influence grape and wine quality

2.4.1 Grape nutrition

Soil nutritional status affects all parts of the grape vine, from root growth and distribution through to shoot growth and grape composition. In general, the availability of each nutrient element is dependant on soil pH. Although Seguin (1986) argues that soil pH does not have much influence on the quality of wines, since

quality wines are produced on acidic, neutral and alkaline soils, there are limits to the acidity and alkalinity of the soil tolerable by vines. Vines do not perform well when soil pH lower than 5 due to stunted shoot and root growth (Conradie, 1983a). At these low pH values the increased concentration of exchangeable aluminum is mostly responsible for distorted root growth (Reeve and Sumner, 1970). In soils with pH above 8 availability of nitrogen, calcium, magnesium, iron, manganese, copper and zinc are reduced (Davidson, 1991). Studies of the effect of nutrition on vine performance, in particular vine growth and grape quality, have generally been confined to macro nutrients, in particular nitrogen (Conradie, 1986; Goldspink and Howes, 2001; Retallack, 2002; Robinson, 1992; Treeby *et al*., 1995). The concentration of soil nutrients for wine grape production (mg/kg): N=2-10; K=100-250; P=35-80; Mn=2-4; Zn=1-2; and Cu=0.2-0.4 (Goldspink and Howes, 2001; McMullen, 1995; Robinson, 1992; Robinson *et al*., 1997). Addition of limestone or oyster shells to the native soil significantly influenced the anthocyanin composition in Cabernet Sauvignon berry skins (Yokotsuka *et al*., 1999). The grapevine is a perennial plant that accumulates and stores nutrients in the woody parts such as trunk and roots. The stored nutrients act as a buffer to supply the growing parts of the vines with nutrients as required. If a vine is growing out of balance then these nutrients will be transported to parts of the plant at the wrong time (Wood and Parish, 2003). Grapevine requires 14 elements from the soil. Six macronutrients are needed in large quantities: Nitrogen (N), Phosphorus (P), Potassium (K), Sulfur (S), Calcium (Ca), and Magnesium (Mg). Eight micronutrients are needed in much smaller amounts: Iron (Fe), Manganese (Mn), Boron (B), Molybdenum (Mo), Cupper (Cu), Zinc (Zn), Nickel (Ni), Chlorine (Cl) (Dalton et al., 1988). All must be available as required for

optimal vines and fruit. Nitrogen, potassium, phosphorus, boron, and zinc are the most common deficiencies in vineyards. Nutrient analysis of vineyard's soils and vines can identify nutrients limiting vine health or fruit quality, and strategies for soil and nutrient management.

2.4.1.1 Nitrogen (N)

N is a constituent of many important molecules, including protein, nucleic acids, enzymes, vitamins and certain hormones (e.g., indole-3-acetic acid, cytokinin), and chlorophyll (Beevers, 1976; Hopkins, 1995; Jackson, 2000; Marscher, 1995; Mengel and Kirkby, 1987). Amino acids are the most important form of stored N in grapevines. Of these, arginine accounts for 50 to 90 percent of the soluble N in the roots, trunks, and canes during fall and winter (Christensen *et al*., 1978). N sources can be supplied to vineyards through both inorganic and organic nitrogen fertilizers. There are several commercially available nitrogen sources that supply ammonium, nitrate, or both to the soil solution for plant uptake. When ammonia is combined with nitric acid under heat and pressure, ammonium nitrate fertilizer is formed. Similar reactions with sulfuric and phosphoric acids produce ammonium sulfate and ammonium phosphate, respectively. Urea, a common inorganic nitrogen fertilizer, is formed from the reaction of ammonia and carbon dioxide under heat and pressure. Since industrial nitrogen fertilizers require high temperatures during the formation of both ammonia and ammonium, the cost of fertilizers are dependent on the cost and availability of fuel sources. Therefore, inorganic nitrogen fertilizers that cost the least per unit of nitrogen are preferred. N fertilizer salts such as ammonium nitrate, ammonium phosphate, and calcium nitrate

when applied to the vineyard floor are dissolved into the soil solution and dissociate into their component ions. For example, ammonium nitrate (NH_4NO_3) dissolves into the ammonium cation (NH_4^+) and nitrate anion (NO_3^-). Ammonium cations can absorb onto soil clay particles and the degree of absorption is dependent on the cation exchange capacity and the competition from other cations. Ammonium can be converted to nitrate through the process of nitrification. Nitrate anions, preferentially absorbed by grapevines, are a quick source of nitrogen but they are also subject to leaching. Both ammonium and nitrate make up a small percentage of the total nitrogen in agricultural nitrogen cycles; however, they are the nitrogen forms taken up by grapevines. It is estimated that 70% of all mineral nutrient ions taken up by plant roots are in the form of ammonium or nitrate. Urea is converted to ammonia and then to ammonium through hydrolysis with the urease enzyme. Urea hydrolysis is a biochemical reaction influenced by several factors such as temperature, moisture, and enzyme concentration. Strongly acidic soils and soils with low clay content slow the rate of urea hydrolysis. Urease activity is optimum between a soil pH of 6.5-7.0. The intermediate step in the conversion of urea to ammonium is the formation of ammonia, which can be lost from the system through volatilization. Sandy, alkaline soils, high temperature, wet soils, as well as high and unincorporated urea applications increase ammonia volatilization. N can be lost from the vineyard system through erosion, denitrification, harvesting plant tissues (grapes), and leaching. Erosion leads to the physical removal of organic nitrogen in the upper soil profile. Denitrification is the conversion of nitrate back to atmospheric nitrogen. Grapes and sometimes wood infected with disease removed from the vineyard also removes organic nitrogen from the system. Nitrate leaching is an agricultural concern because

excess leaching leads to soil acidification and potential groundwater pollution. Industrial and organic fertilizers both provide ammonium to the soil where the ammonium is oxidized to nitrate and potentially leached. Efforts should be made to make the most efficient use of nitrogen fertilizers by using the appropriate material, rate, and timing for the individual vineyard goals. Grapevines most critically need N during the period of rapid shoot growth in the spring through bloom and early berry development. The need for N declines from midsummer to senescence (Christensen *et al*., 1978, Winkler *et al*., 1974). Grapevines and deciduous fruit trees depend heavily on redistribution of N previously stored in roots, trunk, and canes or limbs to support spring growth (Alleweldt *et al*., 1984; Conradie, 1980; Conradie, 1983b; Conradie, 1986; Kliewer, 1967; Kliewer and Cook, 1974; Peacock *et al*., 1989; Weinbaum *et al*., 1978; Weinbaum *et al*., 1980; Weinbaum *et al*., 1984). Since the grapevine's need for N is most critical in the spring and highly dependent on storage, it can be inferred that fertilizer should be applied when the vine can best absorb and incorporate it as part of the N reserve while minimizing N loss from the soil (leaching, denitrification). Vegetative imbalance due to high soil nitrogen levels has been shown to adversely affect grape quality by delaying crop maturation (Kliewer *et al*., 1991; Spayd *et al*., 1991), decreasing berry sugar levels (Delas *et al*., 1991; Kliewer, 1977; Smart, 1991; van Huyssteen, 1990) and skin phenolic concentration (Delas *et al.,* 1991; Smart, 1991) and reducing bud hardiness (Ahmedullah and Roberts, 1991). In berry, N accumulation rate is slow before veraison and increases sharply at the onset of ripening (Ollat and Gaudillère, 1996). In the berry and the must, nitrogen can be found under mineral (NH_4^+, NO_3^-, and NO_2^-) and organic (free aminoacids, proteins and other nitrogenated organic compounds such as urea, ethyl

carbamate and nucleic acids) forms. Total nitrogen in the must can vary from 100 to 1200 mg/L, and usually red wines possess higher nitrogen content than white wines. This nitrogen, called fermentable nitrogen, is used by yeast to carry on normal alcoholic fermentation of the must. When fermentable nitrogen is below 150-200 mg/L, ammonium (in the form of phosphate, sulphate or sulphite salts) is added to the must to avoid "stuck" fermentations and formation of hydrogen sulfide and other sulphur odours (Jiranek *et al*., 1995; Kunkee, 1991). Moderate rates of nitrogen fertilization stimulated vine growth and vigor (shoot extension rate) resulting in an increase in canopy density. Prior to flowering, maximum vine vigor was observed upon addition of 100 g N/vine. This effect was no longer evident after flowering (Bell and Robson, 1999). Yield was determined primarily by N availability at bloom, while grape quality was determined predominantly by light conditions during véraison (Keller *et al*., 1998). Increased nitrogen supply appeared to be the most important nutritional factor, significantly increasing grape juice pH and malate concentration in Chadonnay, Cabernet Sauvignon and Riesling varieties, citrate in Cabernet Sauvignon and tartrate and potassium concentrations in Chadonnay (Ruhl *et al*., 1992). The pigment in green chlorophyll and anthocyanins in fruit require N. Excessive levels of N can cause poor fruit set and reduce carbohydrate storage (Christensen *et al*., 1978). Keller and Hrazdina (1998) found that high rates of N supply delayed the accumulation of phenolic compounds, particularly flavonols, in grape skin at véraison and all five anthocyanins started accumulating concurrently at the inception of ripening. However, while peonidin-, malvidin- and cyanidin-3-glucosides were the major pigments at véraison, malvidin- and delphidin-3-glucosides became most abundant towards maturity. The percentage of malvidin-3-glucoside increased with

high rates of N fertilizer. High rates of N increased malic acid and reduced skin phenols, flavonols and anthocynins. Malvidin-3-glucoside was the most abundant anthocyanin in skins and wine (Keller *et al*., 1999). High nitrogen supply decreased anthocyanins in the juice and wine, increased pH and increased the percentage of malvidin-3-glucoside. Repeated shoot topping gave lower wine total phenols and anthocyanins and thus enhanced the nitrogen effect (Keller *et al*., 1999).

2.4.1.2 Phosphorus (P)

In the plant, P is found largely as phosphate ester-including the sugar-phosphates, which play such an important role in photosynthesis and intermediary metabolism (Hopkins, 1995). P is not required in large amounts by grapes. Several factors contribute to this: grapevines have a good ability to extract P from the soil, P is very mobile in the vine, and crop removal of P is relatively small (Hellman, 1997). P does have a place for sod or cover crop maintenance. A soil test is the best way to determine if there is a need to apply this nutrient to the sod cover. In the absence of a soil test, a complete fertilizer (100 kg/ha 10-20-20) could be broadcast and incorporated before seeding a cover crop in a vineyard. Apply P before planting a vineyard, when it can be thoroughly incorporated in the soil if a soil test indicates a need. P soil test values between 12-20 ppm are considered adequate for vineyard establishment and production. P in the soil was not available until we raised the soil pH at which time the red flecking symptoms went away. It seems that most often a P vineyard problem will be associated with low pH or very high applications of Zinc (zinc phosphate is very insoluble). An excess of P has the opposite effect of nitrogen in that it preferentially stimulates growth of roots over shoots (Hopkins

1995). The P status of the vineyards, above 0.2 percent in bloomtime petioles, was good (Christensen *et al*., 1978). Terra *et al*., 2000 found that no effects of increasing dose of P on yield were observed, thus indicating that an annual phosphate application with the lowest level of P_2O_5 is enough, that is, 40 g/plant.

2.4.1.3 Potassium (K)

K is a monovalent cation and has a high rate of uptake by plant tissue (Marschner, 1995; Mengel and Kirkby, 1987). K is an essential element for all living organisms. K is essential for vine growth and yield. K is second in importance only to N in the production of grapes, and K deficiencies have been observed throughout the world (Cahoon, 1985). The important roles of K in plants can be grouped into four physiological-biochemical roles: (1) enzyme activation (Leigh and Wyn-Jones, 1984; Walker *et al*., 1998); (2) cellular membrane transport processes and translocation of assimilates (Patrick *et al*., 2001; Salisbury and Ross, 1992); (3) anion neutralization, which is essential in maintenance of membrane potential (Leigh, 2001; Maathuis and Sanders, 1996) and (4) osmotic potential regulation, which is one of the important mechanisms in the control of plant water relations (Davies and Zhang, 1991), turgor maintenance and growth and K plays an important role in the synthesis and translocation of carbohydrates (Fang *et al*., 2002). Unlike other macronutrients, K does not appear to be structurally bound in the plant. K ion serves to activate a number of enzymes, notably those involved in photosynthesis and respiration (Hopkins, 1995; Jackson, 2000). Grapes require a larger quantity of potassium than fruit trees. Grapevines take up K from soil via the roots by simple diffusion mechanism or energy driven system or a combination of both. Through these two

processes plant roots are able to accumulate potassium to concentrations 10 to1000 times higher than potassium concentration in the surrounding soil solution (Mengel, 1980). Most K is then transported in the transpiration stream to the mature leaves via the water conducting vessels of the plant, the xylem. K is then stored in the vacuoles of a number of specific storage cells located within the mature leaves. The transfer of high amounts of potassium from vine leaves to the ripening fruit has been related to the reduction of photosynthesis activity in the mature leaves (Boulton, 1980 and Iland, 1988). Berry K content generally increases over the season (Boselli *et al*., 1995; Conradie, 1981; Possner and Kliewer, 1985; Rogiers *et al*., 2001) with a sharp increase at the onset of ripening (Ollat and Gaudillere, 1996). Grape berries and clusters contain a large amount of potassium. Because fruit potassium is taken away from the vineyard ecosystem at harvest, K levels tend to decline in a vineyard over time. Some soils have a very large supply of exchangeable potassium, but many vineyard soils do not. K is one of the more important fertilizer elements for grape production. Much of the effect of K deficiency on the fruit is the result of reduced vine growth and premature leaf fall (Peacock and Christensen, 1998). Grape berries are strong sink for K, particularly during ripening. The direction of K transport is often towards the growing plant tissues, and K often redistributed from older to younger plant tissues (Mengel and Kirkby, 1987). K is by far the major cation in ripe berries. The concentrations (ppm) of inorganic cations in De Chaunac berries at harvest were: K=2875, Na=200, Ca=100, Mg=110, Cu=2.2 and Mn=0.8 (Hrazdina *et al*., 1984). Factors such as cultivars, crop load, climate and cultural practices that affect rate of berry growth and/or rate of K accumulation in berry will affect berry K concentration. Excess K levels in grape berries may have a negative impact on wine

quality, mainly because it decreases free tartaric acid resulting in an increase in the pH of grape juice, must and wine (Boulton, 1980 and Gawel *et al*., 2000). When adequate, K favors grape quality by enhancing fruit coloration and sufficient acidity (Jackson, 2000). Higher K fertilization had no effect on fruit yield or sugar content, but increased grape juice pH from 3.95 to 4.08, malate concentration from 0.94 to 1.20 g/ L and K concentration from 46.8 to 56.8 mmol/L (Ruhl, 1989). K is found mostly in the skins and stems, and has an important effect on wine pH. Grape juice with high pH often results in unstable musts and wines that are more susceptible to oxidative and biological spoilage, and often produces a wine with high pH and low acidity with a flat taste (Somers, 1977). The high pH of grape juice and wine also decreases the color quality of red wine. The degree of ionization of anthocyanins, which is the percentage of total anthocyanins present in the colored forms, decreases as pH increases (Somers, 1975). Anthocyanins are located in berry skin (Somers and Pocock, 1986) where K concentration is generally higher than in the pulp (Iland and Coombe, 1988; Walker *et al*., 1998). Therefore, berry K levels are often a more important consideration for red wine than for white wine because during the fermentation of red wine, the skin is left for some period after crushing for extraction of anthocyanins, during which time more K may also be extracted. K deficiency produces an interveinal chlorosis that starts at the margin of leaves in the middle (not the base) of shoots. With increasing severity the number of affected leaves and the severity of infection increase. The leaves below are severely affected. At this level fruit development, fruit color, vine growth and winter hardiness would all be reduced. Excessive levels of K fertilization increased the pH and lowered the acid content of fresh and juice. These increases in pH due to excessive K were probably due to the

direct exchange of K cations for protons derived from the organic acids, and probably due to the activity of adenosine triphosphatase (Morris *et al*., 1982).

2.4.1.4 Calcium (Ca)

Plants take up Ca as a divalent ion, which cannot be taken up and translocated in the phloem cell of the plant. Given this deficiency symptoms occur in the young parts of the plant where it is used in cell division. Generally deficiency symptoms include twisted and deformed tissues at the growing tips (Salisbury and Ross, 1992). Just like other nutrients the key to understanding Ca nutrition is to understand its uptake. Seasonal uptake of Ca has been studied by Conradie, 1981 on Chenin blanc/99R. The study showed that little Ca was accumulated by the vine prior to budburst or in the 22 days after budburst, though Ca reserves decreased in the roots as new growth accumulated Ca in this period. In this same period Ca levels in the bark were noted to have doubled and continued to increase over the subsequent 45 days as Ca accumulation continued to increase in the vine. Between flowering and veraison 2,398 mg of Ca was absorbed by the vine, which accounted for nearly half the vines yearly consumption. Between veraison and harvest bunches accumulated no Ca but the vine does slowly take up Ca for other parts of the vine. At harvest Ca levels were as follows: bunches (7.7%), leaves (46.4%), roots (19.8%), shoots (16.7%) and the trunk (9.4%). Ca accumulation was insignificant after harvest; however in the 44 days proceeding leaf drop Ca uptake increased significantly. During leaf drop Ca levels increased in the leaves and decreased in the shoots, with 54% of total vine Ca being lost through the leaves. During the season the vine accumulated 5, 242 mg Ca, with 442 mg Ca removed by the crop and 370 mg Ca

retained by the permanent vine parts. The active accumulation periods for Ca during the season are three weeks after budburst till veraison and in the six weeks before leaf fall. The concentration and the balance of dibasic cations, Mg and Ca, and the monobasic cation, K, are important to vine health and function. Soil that is low in pH is almost by definition low in calcium and magnesium ions. Adding limestone - calcium or magnesium carbonates, raises soil pH. If agricultural lime low in Mg is used to raise soil pH, an imbalance may be created. Dolomitic limestone, which contains both Mg and Ca, is the preferred material to use. Ca accumulation in the berries occurs when xylem water flow is high and almost stops after veraison. Ca is considered to be phloem immobile and the results, Ca is mainly accumulated during the first growing period, are consistent with Ca supply to the fruits by xylem sap (Ollat and Gaudillère, 1996).

2.4.1.5 Magnesium (Mg)

Mg is a component of chlorophyll, and low Mg causes an interveinal chlorosis of leaf blades. One symptom of low pH nutrient disorders is pale yellow leaves that are deficient in Mg. Mg and K ions compete for the same uptake sites in the grape root. Therefore the balance between the concentration of K and Mg is important. If K is added to a vineyard low in both K and Mg, a magnesium deficiency may be created. Similarly adding Mg may create a K deficiency. If there is any doubt about the K status, then K should be added along with lime to correct a Mg deficiency. Mg deficiency can lead to premature fruit drop at harvest. The uptake of Mg was studied by Conradie (1981), which showed that Mg uptake was not significant in the 22 days prior to budburst or the 27 days following budburst in

Chenin blanc/99R. Mg uptake began to increase in the time period surrounding flowering, which was translocated mostly into new growth. Mg absorption continued to increase from flowering to veraison with Mg reserves increasing in the roots, shoots and leaves. The berries absorbed little Mg during this time. From veraison to harvest, Mg absorption continued but at a slower rate. At harvest Mg levels were as follows: bunches (15.4%), leaves (36.8%), roots (15.1%), shoots (26.2%) and trunk (6.4%). After harvest vines accumulated a significant amount of Mg, which was stored in the roots, shoots and woody components of the trunk. Mg accumulation continued to leaf fall with most being stored in the roots and leaves. No Mg was actively absorbed during leaf drop. A total of 1,568 mg Mg was accumulated per vine during the season, with 234 mg Mg removed by the crop and 195 mg Mg retained by the permanent vine parts. Mg accumulation is relatively steady across the season therefore timing for Mg application could occur from just prior to flowering till leaf drop. Accumulation rate of Mg is slowly before veraison and increases sharply at the onset of ripening (Ollat and Gaudillère, 1996).

2.4.1.6 Boron (B)

B is a micronutrient, the vine only needs a low level (>25 ppm) and at high concentration boron is toxic to grapevines (Christensen, 1978). Boron is taken up as a boric acid, which is translocated slowly with in the plant. Deficiency symptoms can include a failure of root tips to elongate, inhibition of DNA and RNA synthesis and inhibition of cell division in the shoot apex of young leaves. Boron is also known to be critical in the elongation of the pollen tube (Salisbury and Ross, 1992). The uptake of B is affected by irrigation and under drought stress it can

become limited to plants, on the others side high rainfall and intensive irrigation can result in the ion being leached from the profile especially in sandy soils (Pearson and Goheen, 1988). In the areas boron deficiency is associated with very high pH soils. B deficiency has great impact on vineyard productivity and B levels should be monitored carefully (Peacock and Christensen, 1998). B deficiency is perhaps the most common of micronutrient deficiencies. It occurs mainly on alkaline soils (pH greater than 6.5), acid soils (pH 3.5-4.5), dry soils, soils low in organic matter, or on sandy knolls. B deficiency can have an effect on growth and fruiting.

2.4.1.7 Zinc (Zn)

Zn is taken up as a divalent ion; deficiency symptoms result in a reduction in growth of young tissues, which can cause small leaves and internodes. Interveinal chlorosis can also occur in many plants. Zn is involved in the production and functioning of many enzymes as well as many growth hormones (Salisbury and Ross, 1992). Zn may become deficient in sandy soils, high pH soils, soils with high P content and where the topsoil has been removed (Pearson and Goheen, 1988). In *Vitis* spp. deficiency symptoms occur as small leaf blades with small petiolar sinuses and sharp teeth. One half of the leaf blade can become larger than the other and the interveinal areas can turn light green to yellow in a mosaic pattern. In red cultivars it can become red to black. Leaf veins can become clear with narrow boarders of green; more advanced symptoms include chlorotic areas that can become necrotic (Pearson and Goheen, 1988).

2.4.1.8 Manganese (Mn)

Mn exists as three different ionic states (Mn^{2+}, Mn^{3+}, and Mn^{4+}) as well as in a chelated form, yet like other ion it is taken up as a divalent ion (Mn^{2+}). In plants deficiency symptoms are seen as interveinal chlorosis of younger and older leaves. Mn itself is known to be important in the photosynthetic split of water molecules and also as an activator of many enzymes (Salisbury and Ross, 1992). In *Vitis* spp. deficiency symptoms as in other plants is seen as interveinal chlorosis that have a mosaic like arrangement. Generally symptoms are more severe on sun-exposed leaves and advanced conditions can affect the growth of berries and shoots, it may also delay veraison. Deficiency symptoms are more likely to occur on alkaline, sandy soils high in organic matter or on limey soils that are deficient in Mn (Pearson and Goheen, 1988).

2.4.1.9 Iron (Fe)

Fe is an essential component of a number of proteins and enzymes as well as acting as a proton carrier during photosynthesis and respiration. Deficiency symptoms have been called iron chlorosis, lime chlorosis and lime-induced chlorosis. Deficiencies normally occur during times of cool wet weather when iron movement in the soil is very slow. Symptoms are normally found in soils that have relative high lime (Ca) content (Pearson and Goheen, 1988). Symptoms are seen, as pronounced interveinal chlorosis that can even be white in colour (Salisbury and Ross, 1992). Fruit set can also be affected by Fe deficiency (Pearson and Goheen, 1988). Bertamini and Nandunchezhian (2005) studied the response of Pinot noir to iron deficiency. Leaves were classified for the study as Fe deficient and Fe

sufficient based on chlorophyll concentration in the leaf, below 10 nmol chlorophyll / cm^2 (deficient) and above 30 nmol chlorophyll / cm^2 (sufficient). Results showed that Fe deficiency decreases vegetative growth, affected membrane integrity, decreased leaf CO_2 exchange and photosynthetic efficiency, reduced leaf area and dry matter accumulation, as well as resulting in increased fruit abscission or drop.

2.4.1.10 Copper (Cu)

Plants need very small amount of Cu and because of this they are very rarely deficient, but in Australia many soils are Cu deficient (Salisbury and Ross, 1992). Cu can be taken up in both the monovalent and divalent form, the monovalent form is generally only taken up in wet soils were oxygen is limiting. Deficiency symptoms occur as dark green and twisted leaves, the ion is used in several enzymes and proteins involved in oxidation and reduction (Salisbury and Ross, 1992). Generally it is rare to find Cu deficiency in the vineyard. Cu is also known to be involved in the lignification or hardening of canes and shoots.

2.4.1.11 Molybdenum (Mo)

Little is known about Molybdenum as it is only used in small amounts by plants and deficiency symptoms are rare. Molybdenum is available in a molybdate (MoO_4^{2-}) and MoS_2 form (Salisbury and Ross, 1992). Mo is known to be involved in nitrate reductase, which reduces nitrate ion to a nitrite ion. It is also thought to be involved in the reduction of purines like adenine and also as an oxidase that converts abscisic acid aldehyde to the hormone ABA that is involved in the adaptation of plants to stress. Generally it has been thought that vine do not require

Mo but work conducted by Williams *et al.*, 2004 on Merlot vines has shown that the application of Mo can increase yield as a result of increased bunch weight and a reduction in short berry. An increase in functional seeds and percentage of coloured berries was seen as a result of Mo application. Mo was applied in two foliar applications before flowering at a rate of 118 g Mo / ha as sodium molybdate, 410-800 l / ha. Williams *et al*., 2004 stated that level of Mo in the petiole at flowering of 0.05-0.09 mg / kg was associated with deficient vines.

2.4.2 Canopy management

Typically, vigorous grapevines develop excessive shoot growth and dense canopies. High number of large, dark green leaves occur in the canopy, leading to excessive shading (Koblet, 1987; Saayman and van Huyssteen, 1983; Smart, 1982). Shading canopies cause several problems including delayed ripening, reduce yields (Shaulis and Smart, 1974; Smart, 1987; Smart *et al*., 1989), poor fruit quality, low bud fruitfulness and high disease incidence (Rotem and Patti, 1969). Shaded canopies will delay veraison and reduce ripening, resulting in reduced anthocyanin, sugar and phenol levels and increased titratable acidity, malic and tartaric acid levels (Carbonneau and Huglin, 1982; Smart 1982; Smart, 1985a; Smart *et al*., 1985b; Smart *et al*., 1988). Canopy management has received considerable research attention during the past several decades (Carbonneau, 1984; Koblet, 1988; Smart, 1985a; Smart, 1985b; Smart, 1987; Smart and Robinson, 1991). The purpose of canopy management was to develop viticultural practices that provided adequate exposure of the fruit to sunlight while providing adequate, but not excessive, leaf area to ripen the fruit and improve fruit composition at harvest. These practices included trellis

systems and leaf, shoot, or partial shoot removal (hedging). High canopy densities causing shade vine microclimates have also been implicated in higher grape juice potassium level (Smart and Robinson, 1991). Practices that reduce shading such as leaf removal and the use of open training and trellis systems have been recommended to prevent high potassium in the fruit. However, one must be very careful about removing leaves in the fruit zone as this practice lower the photosynthesis capacity of the vine which, in turn, can have a negative affect on vine growth, fruit yield, sugar content and vine carbohydrate reserves (Petrie *et al*., 2002). Reactions to shading cannot be explained simply by taking the amount of light that penetrates the vine into effect. The quality of the light also plays an important role. Within the vine photon receptors occur, namely phytochrome, which can control and regulate a large amount of enzymes and reactions. Phytochrome acts as a biological switch and has an "on" and "off" position which is determined by the molecular orientation in which it occurs. The phytochrome molecules are also interchangeable from the active to the inactive form. The ratio of active to inactive phytochrome is determined by the amount of red and far-red light that penetrates the vine. Vine leaves absorb a lot more red than far-red light, which transforms more phytochrome to the inactive form. The denser the canopy, therefore, the less phytochrome in the active forms. Phytochrome controls various reactions and enzymes. It has been proven that the enzymes PEP-carboxylase (involved in malic acid synthesis), malic acid dehydrogenase (involved in the breakdown of malic acid), phenylalanine ammonia liase (PAL) and nitrate reductase are controlled by phytochrome. PAL is the enzyme responsible for diverting phenylalanine from protein synthesis to be used for phenol and anthocyanin synthesis. Nitrate reductase impels the reaction during which nitrate is converted to

nitrite within the vine. The reaction requires potassium, which is readily taken up by the vine (van Schalkwyk, 2006). Archer and Strauss (1989) found that an increase in shading significantly decreased the skin color of Cabernet Sauvignon, which is in agreement with the findings of wine tasters that the wine quality was reduced in proportion to the degree of shading. The difference between the effects of leaf shading and cluster shading on grape composition was investigated by Morrison and Noble (1990). They found that shaded bunches caused a reduction in the phenol and anthocyanin concentrations, while shading of the leaves caused a delay in berry growth and sugar accumulation. Wines from highly and moderately exposed cluster positions had higher total anthocyanin levels than those from shaded clusters, while wines from highly exposed clusters had 40% greater polymeric anthocyanin levels than those from shaded treatments (Price *et al*., 1995). It appears that the anthocyanin metabolism responds to changes in both light and temperature conditions. Studies by Pirie and Mullins, 1977 suggest that the optimum temperature for the enzymes involved in the anthocyanin biosynthetic pathway is between 17 and 26 ºC. Crop thinning has become an increasingly common practice in vineyards. However, there is little scientific basis to what a vine can carry - usually long experience of the vineyard and an understanding of the vine's capacity is the best guide to an appropriate cropping level. As a rough rule of thumb, approximately 8 to 10 leaves per shoot (average length 1 to 1.2 meters) are required to ripen two bunches per shoot in a warm to hot climate, 12 to 15 leaves per shoot in a cooler climate and more than 15 leaves in a cool climate (Archer, 2002). If it is necessary to remove crop to allow the vine's canopy to cope with and ripen the remaining crop, then those bunches should be cut off prior to veraison. When done at this stage, the vine continues to

push its photosynthates into the leaves and growth of leaf and shoot is encouraged. If the decision is left too late, until after veraison, then the vine continues to develop its shoots in the similar way to when all fruit was present. Shoot thinning is necessary for young vines that tend to over crop, and should be addressed early in the growing season while the shoot are only 5-10 cm long, and too much energy in growing those shoots and potential berries has not been expended (Davidson, 2002). If vegetative vigor is high due to spring weather, delay the thinning as leaving the "extra" clusters will reduce subsequent growth; thin the extra clusters at veraison to insure proper ripening of remaining crop (Fiola, 2009). Controlling the leaf area/crop weight will improve berry coloration and accelerate ripening (Kliewer and Dokoozlian, 2000).

2.4.3 Crop level (yield/vine)

It is one factor affecting wine grape quality. Since the capacity of a vine to ripen fruit depend largely on the rate of photosynthesis and accumulation of carbohydrates, it follows that a quantitative crop level may be related qualitatively to fruit composition. Of all factors affecting fruit ripening, crop level is the most likely one, which growers can manipulate (Winkler *et al*., 1974). Crop level and grapevine light microclimate can be manipulated to influence fruit and wine composition (Chapman *et al*., 2004). Reducing the node number per vine via increased pruning severity and/or reducing the cluster number per vine via cluster thinning have increased soluble solids concentration (Bravdo *et al*., 1985; Edson *et al*., 1993; Howell *et al*., 1987; Reynolds *et al*., 1986) and anthocyanins (Gao, 1993; Kliewer and Weaver, 1971) in grape berries. The effect that crop control has on titratable acidity (TA) is inconclusive. Some studies have shown that TA decreased with reduced crop

level (Reynolds and Wardle, 1989; Wolpert *et al*., 1983), whereas others have shown that it increased (Bravdo *et al*., 1985; Reynolds *et al*., 1986). Increasing fruit exposure to light penetration has been linked to enhanced accumulation of soluble solids (Morrison and Noble, 1990; Reynolds *et al*., 1986; Smart, 1987), reduced pH and potassium ion concentration (Morrison and Noble, 1990; Smart *et al*., 1985b), decreased TA levels (Archer and Strauss, 1989; Reynolds *et al*., 1986), and elevated levels of anthocyanins and other phenolics in colored cultivars (Gao, 1993; Morrison and Noble, 1990). The crop level and light microclimate of the vine can also influence yield. Limiting crop level through post-set cluster thinning has the potential to decrease yields by reducing berry number per vine. Guidoni *et al*. (2002) found that berry skin anthocyanin and flavonoids were more concentrated in berries from cluster-thinned plants and cluster thinning increased the concentrations of cyaniding-3-glucoside, peonidin-3-glucoside, and, to a less extent, petunidin-3-glucoside. Concentrations of malvidin-3-glucoside and of acylated anthocyanins were not affected by cluster thinning. Vine moderately cluster-thinned shortly after fruit set however may compensate for reduced berry numbers by producing berries at greater weight (Looney, 1981). Increasing light exposure generally increases berry weights (Archer and Strauss, 1989; Smart *et al*., 1988). In some instances, however, berry weights and berry sizes have been reported to decrease when clusters are fully exposed, due to increases in cluster temperatures (Reynolds *et al*., 1986). Berries that had developed on bunches receiving high levels of ambient light generally had the highest relative levels of quercetin-3-glucoside and a lower proportion of their malvidin anthocyanins as the coumarate derivative compared to berries that had developed on bunches in shaded canopy condition (Haselgrove *et al*., 2000). During

maturation, as well as temperature, exposure of the cluster to light is of fundamental importance with greater effects on the anthocyanins than on sugar accumulation (Kliewer, 1970; Smart *et al*., 1988)

2.4.4 Grape maturity

Grape maturity can be defined as the physiological age of the berry on the vine. The berry functions to attract animals for dispersal of grape seeds. Dispersal is always critical, but even more so if the vine is under limiting growth conditions. This is the main reason that a certain amount of vine stress is beneficial for development of grape flavorants. Berry ripening is therefore tightly coordinated with seed development. During veraison, water, sugars, and nitrogen compounds are transported to the berry via the phloem. Sucrose is hydrolyzed to glucose and fructose in the berry (Lang and During, 1991). Berry flavor and aroma compounds are synthesized within the berry. Sugar content increases during ripening and is therefore a function of berry age. Sugar is also relatively easy to assess, adding to its value as an index of ripeness. However, the sugar:acidity ratio is quite variable across different varieties and growing conditions, and these kinds of universal rules of thumb may be of little general predictive value for wine quality - especially if indiscriminately applied (Boulton *et al*., 1996). Changes in acidity level, as they reflect berry metabolic activities, may be useful to assess. It is well known that malate is consumed as an energy source in the berry during veraison, so malate levels decrease relative to tartrate (Amerine and Joslyn, 1970; Amerine *et al*., 1980; Boulton *et al*., 1996; Jackson and Lombard, 1993). Tartrate levels generally remain constant during veraison, but may rise slightly during grape dehydration. Malate

levels decrease as the acid is consumed by the fruit, and seem to plateau at a low level, roughly 2 to 3 g/l (Amerine and Joslyn, 1970; Amerine *et al*., 1980). Anthocyanin levels have also been associated with maturity (Gonzalez-San Jose *et al*., 1990) but dramatic effects of environmental and cultural conditions on anthocyanin pigment accumulation have been reported (Keller and Hrazdina, 1998). Malvidin-3-glucoside appeared to be unresponsive to growing conditions, with levels increasing as a function of maturity only (Keller and Hrazdina, 1998). Guidoni *et al*., 2008 showed that 3'-Substituted anthocyanin biosynthesis is likely more strongly influenced by climatic conditions and cultural practices than is 3', 5'-substituted anthocyanin biosynthesis. An optical fiber probe capable of providing a rapid assessment of both total phenolic and anthocyanin content is being developed that might prove useful in the assessment of optimal maturity (Celotti *et al*., 2001).

2.4.5 Wine making processes

Wine making processes on phenolic composition of wine. The color of a red wine is primarily due to the type and concentration of anthocynin from grape skin during maceration process (Auw *et al*., 1996; Rodríguez-Delgado *et al*., 2002; Sims and Bates, 1994; Zimman *et al*., 2002), but other factors play a role, including phenolic compounds other than the anthocyanins, sulphur dioxide, oxygen content, grape cultivars, yeast/fermentation method/winemaking techniques employed, and final wine pH (Etievant *et al*., 1988; Jackson *et al*., 1978; Joslyn and Little, 1967). The anthocyanin extracted into red wines chemically combines with other wine components, forming stable compounds referred to as polymers (Singleton *et al*., 1964; Somers, 1971; Timberlake and Bridle, 1976; Wildenradt and Singleton, 1974).

During vinification, colorless phenolics increase during alcoholic fermentation, reaching maximum values at pressing, and remain stable during malolactic fermentation and subsequent storage. Anthocyanins and color density, on the other hand, increase during the early stages of alcoholic fermentation, reaching maximum values 2-3 days (3% to 6% ethanol) after the start of fermentation, and decrease during storage. Keller *et al*. (1999) found that the increasing of anthocyanin concentration at the beginning of fermentation and before malolactic fermentation, were followed by declines during the later stages of alcoholic and malolactic fermentation. Others measured maximum color extraction within four days of skin fermentation with optimum maturity fruit, but only at day six with later maturity fruit, while the non-colored phenolics increased with increased skin fermentation time (Auw *et. al*., 1996). Vigorous crushing favours the extraction of the astringent and bitter tannins. During fermentation, the cap formed is mixed with the juice on regular intervals to promote contact between the juice and skins and thus enhance extraction. Different cap management techniques were compared to determine any differences on the extraction of phenolics. It was found that mechanical punch down and pump over treatments significantly enhanced the extraction of all phenolic compounds and their polymerization in comparison to the traditional manual punch down treatments. The total polyphenol concentration and wine quality was also higher for the punching-down and rotor treatments, in comparison with the pumping-over treatments. The pump over regime gave all varieties of wines significantly higher quercetin levels. The maceration temperature greatly affects the transfer of polyphenols from skins to must, with a linear increase in color extraction by increasing the temperature from 15 ºC to 33 ºC (Lee *et al*., 1977). A fermentation temperature around 30 ºC has been

determined to be optimal for the extraction of anthocyanins and the promotion of stable polymers for Pinot noir wines (Gao *et al*., 1997), while a fermentation temperature of between 28 ºC and 32 ºC was also found optimal for the production of high quality Pinotage wines (Marais and Malan, 1999). Johnston and Morris (1996) found that increasing skin contact time result in increased phenolic extraction into red wine but the phenolic extracted into Cabernet Sauvignon and Noble wines polymerize differently, resulting in different UV/Vis and CD spectra. Different skin contact times were investigated for the possible benefit of enhanced extraction of anthocyanins and skin tannins to promote the stabilization of color. Assume 5 to 6 days of skin contact until the end of fermentation. Although the polyphenol extraction rates differed between the skin contact treatments, the concentrations in the final wines increased slightly with an increase in skin contact time. Wines made with extended skin contact of 4, 5 and 10 days, still exhibited increased color characteristics after one year of ageing. The extraction of 280 nm absorbing phenols as well as cinnamic acids was found to reach an optimum at 36 days of skin contact while 520 nm absorbing pigments increased very steeply from days 1 to 4 and then decreased gradually (Yokotsuka *et al*., 2000). Red wines made with pomace contact for 4 to 16 days were judged to have higher complexity, acceptable bitterness and astringency and better appearance than wines made with pomace contact for less than 4 or more than 16 days. Sims and Bates (1994) reported that maximum color extraction of red *Vitis vinifera* wines occurred between 3 and 6 days of skin maceration. Another study that determined that pre- and post-fermentation maceration for 15 days (approximately 10 days of extended skin contact) resulted in a wine with lower anthocyanin concentration than the control with 7 days skin contact, suggesting degradation or

precipitation with extended skin contact. Scudamore-Smith *et al*., 1990 determined that although the color densities of extended pomace treatment wines were higher initially than traditional fermented wines, after 14 months of ageing they were similar. The types and concentration of the anthocyanins in red wine depend on the grape variety, ripening, climatic conditions, wine making practices, (the use of enzymes, maceration conditions, and fermentation temperature), and ageing. Among these factors, maceration conditions have the largest impact on anthocyanins and other sensory characteristics of the red wines. Maceration time had no uniform effect on density, ethanol, pH, residual sugar, volatile acidity, and total acidity. Total phenolic compounds and tannin amounts significantly increased with increased skin contact time and reached the maximum level on the 10^{th} day. Similar results for red wines were reported by Auw *et al*., 1996; Ribéreau-Gayon and Glories, 1987; Ricardo-da-Silva *et al*., 1993; Spiora and Granda, 1998. The color of a young red wine is primarily due to the monomeric anthocyanins (Somers and Evans, 1977), however due to the nature of anthocyanin chemistry there is no direct correlation between the anthocyanin concentration in a young wine and its color (Somers and Evans, 1974). Factors that affect the expression of color in a wine by anthocyanins include pH, SO_2, polymerization and copigmentation. Of these factors, copigmentation is also affected by pH, ethanol concentration, temperature and the amount and type of other compounds in the wine that may act as copigments, such as flavonoids and other phenols. The amount and type of other phenols in the wine will also affect the amount of polymeric pigments, and proteins and polysaccharides can also become involved in these reactions (Ribereau-Gayon and Glories, 1987). Polymerization reactions involving anthocyanins are largely influenced by temperature (Somers and

Evans, 1986) and oxygen contact (Ribereau-Gayon *et al*., 1983; Timberlake and Bridle, 1976), and as a wine mature and ages the polymeric pigments become increasingly responsible for red wine color. Malo-lactic fermentation will usually be completed after the wine has been separated from the pomace, and is either conducted in stainless steel or in barrels. After fermentation is complete and the wine is pressed, the maturation phase begins, and it extends up until the wine is bottled. Conservation in the bottle will be called 'ageing'. As a wine age, it has been demonstrated that the initially present grape pigments slowly turn into new more stable red pigments. That phenomenon goes on for weeks, months and years (Brouillard *et al*., 1997). Most red wines spend time 'maturing' in oak barrels, being periodically racked, then fined and stabilized prior to bottling. During this time the most noticeable sensory changes concern the color and texture of the wine, and are mostly brought about by polymerization of the phenols (Somers, 1971). *Vitis vinifera* wines have been shown to contain acylated anthocyanins (Hebrero *et al*., 1988) while no acylated compounds have been found in *Vitis rotundifolia* wines or grapes (Johnston and Morris, 1996). Acetaldehyde has been shown to increase the chemical age and improve the longterm color stability of Muscadine wines (Sims and Morris, 1986), and has been shown to increase polymerization of anthocyanins in Cabernet Sauvignon wines (Timberlake and Bridle, 1977).

2.4.6 Wine ageing

Red wines vary in their ageing characteristics: some wines appear to age quite rapidly reaching a superior quality within the first year, whilst others require several years of storage before reaching their optimum quality (Somers and Evans,

1977). In young red wine, the red colour mainly results from the pigments that are present, the anthocyanins (Bautista-Ortı´n *et al*., 2004; Bautista-Ortín *et al*., 2005; Garcia-Beneyte *et al*., 2002; García-Beneytez *et al*., 2003; Gonzalez-Neves *et al*., 2004a; González-Neves *et al*., 2004b; Hermosin- Gutierrez and Garcia-Romero, 2004; Kammerer *et al*., 2004; Mateus *et al*., 2002; Mazza, 1995; Munoz-Espada *et al*., 2004; Pérez-Magarino and González-San José, 2006; Ribereau-Gayon, 1982; Romero-Cascales *et al*., 2005; Wang *et al*., 2003a). During maturation and ageing of wine, anthocyanins undergo changes, becoming more complex and higher in molecular weight than the anthocyanin monomers from which they were formed (Atanasova *et al*., 2002; Boido *et al*., 2006; Escribano-Bailón *et al*., 2001; Garcia-Puente Rivas *et al*., 2006; Harbertson *et al*., 2003; Haslam, 1980; Monagas *et al*., 2005; Rivas-Gonzalo, 1999; Somers 1971; Wang *et al.,* 2003a). Polymeric pigments are considered to be red-brown or brown in colour (Somers, 1971; Harbertson, *et al*., 2003). In addition, it is also believed that polymerisation reactions give soluble brown compounds that express more colour than the red anthocyanins (Romero and Bakker, 2001). It is believed that SO_2 stable wine colour is a major contributor to aged wine colour (Garcia-Puente Rivas *et al.,* 2006; Somers, 1971). The percentage of SO_2 non-bleachable pigments is a comparison of the wine colour before and after addition of bisulfite solution (Eglinton and Henschke, 2003; Iland *et al*., 2004). The percentage measurement is an indicator of how much colour is provided by pigments that are stable to SO_2 bleaching. The chemical age defined the relationship between the so-called “polymeric pigments” and “wine anthocyanins” (Somers and Evans, 1977). The chemical age assesses the “variation in the ageing characteristics” of a red wine. The proportion of anthocyanins and other pigments will enhance the color of a wine and may affect its sensory perception (Es-Safi *et al*., 2003; Vidal *et al*., 2004).

CHAPTER III

MATERIALS AND MEDTHODS

3.1 Population, samplings and location of research

Vineyard: 8 year old, irrigated Cabernet Sauvignon vines were used in Xichang, Sichuan province, China (27ºN, 102ºE, and 1650 m above mean sea level) during the 2005 and 2006 seasons at Xichang Chia Tai Wine & Spirits Co., Ltd. Vine plants were spaced at 1.25 m apart with 2 vines and rows were 2.0 m apart and oriented approximately north/south. Vines were trained to a vertical shoot positioned training system (VSP), and were bilaterally cordon-trained, spur-pruned, and shoots were vertically positioned upright. Vines were irrigated 30 l/vine/week from pruning until véraison by drip irrigation.

3.2 Research methodology

Fertilizer and clusters per vine treatments were applied to 1 vine plot; each treatment was replicated 6 times in a split plot, main plot was cluster number and they were arranged in randomized complete block design. For a standard canopy area for each treatment the shoots were thinned to 20 shoots per vine prior to bloom. Vine shoot length was maintained at 15 nodes by shoot trimming. Three fertilizer treatments and 3 clusters per vine treatments were applied and repeated for 2 years of the experiment.

3.3 Treatments

3.3.1 Fertilizer treatments

F1 = Control (no fertilizer)

F2 = N-K (100-20 g/vine) split into 3 soil applications of actual (30-5 g/vine N-K at bud break), (40-10 g/vine N-K at bloom) and (30-5 g/vine N-K at 30-days after bloom)

F3 = N-K (200-60 g/vine) split into 3 soil application of actual (60-20 g/vine N-K at bud break), (80-20 g/vine N-K at bloom) and (60-20 g/vine N-K at 30-days after bloom)

Urea and potassium sulfate, were source of N and K, and applied in the row under treatment vines and incorporated into the soil.

3.3.2 Clusters per vine treatments

C1= 10 clusters per vine (1 unifromity cluster per 2 fruiting shoot was selected)

C2= 20 clusters per vine (1 unifromity cluster per 1 fruiting shoot was selected)

C3= 30 clusters per vine (1 or 2 unifromity cluster per 1 fruiting shoot was selected)

Clusters per vine treatments were applied at véraison.

3.4 Instrumentation and data collection

3.4.1 Growing degree days and number of days

Growing degree days and number of days between phenological stages were calculated for 10 periods: pruning to budbreak (BB), bloom (BL), véraison (V) and harvest (H); BB to BL, V and H; BL to V and H; and V to H. Daily mean temperatures were obtained from a thermograph by averaging hourly temperatures. Budbreak was recorded when half of scale the total bud number for each vine reached stage B (Bud swell) of Baillod and Baggiolini (1993). Bloom was defined as the time when 50 % of the corolla (cap) had fallen. Véraison was defined as the time when 50 % of the clusters of each vine started to change from green to red color. Harvest was defined as the date when total soluble solids (TSS) of berries had reached a maximum and stayed constant for three days.

3.4.2 Tissue analysis

Plant tissue samples were collected once each year for each replication at bloom. Samples consisted of 20 petioles per treatment plot. Leaves opposite flower clusters were sampled at bloom. The petioles were separated from blades and placed in paper bags prior to promptly dry at 70ºC for 48 hours. The N and K contents in the petioles were analysed. Subsequently the N and K contents in the grape berries were analysed after harvest.

3.4.3 Yield components and berry composition

Fifty berries form each replication were randomly sampled and percent soluble solids concentrations were determined with a temperature compensating,

hand-held refractometer. A portable pH meter was used to determine pH. Titratable acidity was be determined by titration with 0.1M of NaOH to a pH 8.2 end point and expressed as g/l of tartaric acid. Phenolic compounds were measured by spectrophotometer and HPLC. At harvesting period, fruits from each vine were harvested and weighed. Other yield components including cluster weights and berry weights were collected. Cane pruning weights were collected after each growing season. Crop load (fruit weight per vine/pruning weight per vine) was determined for each treatment.

3.4.4 Determination of red pigments

Determination of red pigments (color) and total phenolics of grape berries were carried out along with the method of Iland *et al*., 2000. (Appendix 1C).

3.4.5 Detection of grape berry anthocyanin by HPLC

Solvent Profile: Linear Equilibration time: 0.00 min. The extracts from 3.4.4 were centrifuged at 14000 rpm for 10 min and filtrated by 13 mm, 0.20 µm syringe filter (GAT Asia Limited). Ten microliters were used for HPLC analysis. The gradient cycle consisted of an initial 5 min isocratic segment (solution A, 100%). Then the linear gradient was changed progressively by increasing solution B (100% CH_3CN) to 10% at 10 min, 15% at 20 min, 20% at 25 min and then increasing solution A to 100% at 30 min. Solution A is consisted 4/4/92 CH_3OH/CH_3CN/87 mmol/L H_3PO_4 in H_2O (v/v/v). Anthocyanin contents were determined by reverse-phase HPLC using a Luna 5U c18 100A column (4.6 µm, 150 mm) particle diameter 5 µm. Wavelength 520 nm was used.

3.4.6 Wine quality

After harvest, grape berries of each treatment were mixed, crushed, adjusted to 22ºBrix and separated to four replications. Diammonium phosphate (DAP) 0.30 g/l were added and each replicate was treated with 50 mg/l of SO_2 for 6 hour then add yeast (*Saccharomyces cerevisiae*) (Enoferm BDX) 0.36 mg/l. Alcoholic fermentations were carried out in four replications using 2-l vessels that were thermostated at 20ºC. Must temperature and ºBrix, were measured every 24 hours. The fermenting must were pressed at about 5 Brix, Lactic acid bacteria (*Leuconostoc oenos*) was added. After malolactic fermentation finished, wines were racked and added with 75 mg/l of SO_2 Wine sample were collected for pH, TA, and phenol composition analyses. Anthocyanins and phenolics were determined by spectrophotomeric and HPLC methods.

3.4.7 Determination of titratable acidity

Determination of titratable acidity was carried out using the method of Iland *et al*., 2000. (Appendix 2C).

3.4.8 Red wine color and phenolic measure

Red wine color and phenolic measure was carried out using the method of Iland *et al*. (2000). (Appendix 3C).

3.4.9 Detection of red wine anthocyanin by HPLC

Detection of red wine anthocyanin was conducted using HPLC. Red wines were centrifuged at 14000 rpm for 10 min and filtrated by 13 mm, 0.20 µm

syringe filter (GAT Asia Limited). Ten microliters were used for HPLC analysis. The gradient cycle was ascribed as 3.4.5.

3.5 Statistical analysis

The Statistical Package for Social Sciences (SPSS) for Windows release 13.0, from SPSS Inc., 2004, was used. As a parametric methodology, variance analysis was used.

CHAPTER IV

RESULTS

The climacteric data of experiment station at Xichang Chia Tai Wine & spirits Co., Ltd., Xichang district, Sichuan province, PRC were shown in Figures 7.1, 7.2 and 7.3. The data showed seven years of temperature; minimum, maximum and average; and humidity. Minimum temperature was -9 ºC in January 2000; Maximum temperature was 38.0ºC in September 2006. Humidity was 49.37-88.05 %. The data showed temperature was increased every year. Average temperature was increased in 7 years in Jan, Feb, Mar, Apr, May, Jun, Jul, Aug, Sep, Oct, Nov, and Dec from 6.3 to 10.3, 8.5 to 13.2, 13.1 to 17.8, 15.8 to 21.9, 21.2 to 22.3, 22.8 to 23.7, 25.2 to 26.3, 24.4 to 26.7, 20.3 to 23.9, 18.3 to 20.8, 13.7 to 19.0, and 10.4 to 12.7 ºC (Figure 7.2) and Maximum rainfall were in June and July, but in 2005 was in August and September. Total rainfall was 763.6, 1045.5, 966.9, 638.1, 745.5, 748.0, and 999.3 mm in 2000-2006 (Figure 7.3). Growing degree days were higher in July and August than other months (Figure 7.4). The growing degree days increased every year from 2000-2006 except in 2004 from 2609, 2726, 2812, 3118, 2850, 3510, and 3623ºC, respectively (Figure 7.5). Monthly min, max and mean temperature in 2005 was higher than 2006 (Figure 7.6). Total rainfall in 2006 was about 999.3 mm which was higher than 2005 about 250 mm and the rainfall in period of production from March to July were 236.3 and 510.9 mm in 2005 and 2006 (Figure 7.7). That caused serious downy mildew disease at véraison period in 2006.

4.1 Grapevine performance

4.1.1 Grapevine vigor

4.1.1.1 Number of days and growing degree days of grapevine between phenological stages.

Grapevines were pruned on 15th January and started bud break (TB) during 7th-17th March, 50 % bud break (BB) during 14th-21st March, bloom (BL) during 22nd April-2nd May, véraison (V) during 30th June-8th July, and harvested (H) on 1st August in 2005. In 2006 grapevines were pruned at the same time of 2005 and TB during 2nd-15 March, BB during 13th-20th March, BL during 18th-22nd April, V during 3rd-13th July and H on 31st July. All of phenological stages were almost in the same time except V period of 2005 was earlier than 2006.

Table 4.1 Influence of fertilizers (N-K) and number of clusters on duration (days) between phenological periods of grapevine.

Treatments	DBB-BL	DBB-V	DBB-H	DBL-V	DBL-V	DV-H
Fertilizers (N-K)						
F1	38.06b	112.22a	136.25a	74.17a	98.19a	24.03a
F2	37.53ab	112.06a	136.22a	74.53a	98.69ab	24.17a
F3	36.75a	111.78a	136.25a	75.03a	99.50b	24.47a
No. of clusters						
C1	36.86a	111.39a	135.53a	74.53a	98.67a	24.14a
C2	37.72b	111.89ab	136.44b	74.16a	98.72a	24.56a
C3	37.75b	112.78b	136.75b	75.03a	99.00a	23.97a
Mean	37.44	112.02	136.24	74.58	98.79	24.23

In a column and each treatment means followed by a common letter are not significantly different at the 5% level by DMRT.

Increasing N-K levels decreased DBB-BL from 38.06 to 36.75 days and increased DBL-V from 98.19 to 99.50 days. Increasing cluster levels increased DBB-V and DBB-H from 111.39 to 112.78 and 135.53 to 136.75 days, respectively (Table 4.1).

Table 4.2 Influence of years, fertilizers (N-K) and number of clusters on duration (days) between phenological stages of grapevine.

Fertilizers	**DBB-BL**		**DBB-V**	
	2005	**2006**	**2005**	**2006**
Fertilizers (N-K)				
F1	40.89b	35.22a	108.33a	116.11a
F2	39.89ab	35.17a	108.22a	115.89a
F3	38.94a	34.56a	107.78a	115.78a
No. of clusters				
C1	39.22a	34.50a	107.00a	115.78a
C2	40.28a	35.17a	108.00ab	115.78a
C3	40.22a	35.28a	109.33b	116.22a
Mean	39.91	34.98	108.11	115.93

In a column and each treatment, means followed by a common letter are not significantly different at the 5% level by DMRT.

DBB-BL and DBB-V in 2006 was not significantly different by effect of N-K levels and cluster levels. Increasing N-K levels decreased DBB-BL from 40.89 to 38.94 days in 2005. Increasing cluster levels increased DBB-V from 107.00 to 109.33 days in 2005 (Table 4.2)

Table 4.3 Influence of years, fertilizers (N-K) and number of clusters onduration (days) between phenological stages of grapevine.

Treatments	DBB-H		DBL-V		DBL-H		DV-H	
	2005	2006	2005	2006	2005	2006	2005	2006
Fertilizers (N-K)								
F1	135.38a	137.22a	67.44a	80.89a	94.39a	102.00a	26.94a	21.11a
F2	135.17a	137.28a	68.33ab	80.72a	95.28ab	102.11a	26.94a	21.39a
F3	135.17a	137.33a	68.83b	81.22a	96.22b	102.78a	27.39a	21.56a
No. of clusters								
C1	134.56a	136.50a	67.78a	81.28a	95.33a	102.00a	27.56a	20.72a
C2	135.11ab	137.78a	67.72a	80.61a	94.83a	102.61a	27.11a	22.00a
C3	135.94b	137.56a	69.11b	80.94a	95.72a	102.28a	26.61a	21.33a
Mean	135.20	137.27	68.20	80.94	95.30	102.30	27.09	21.35

In a column and each treatment, means followed by a common letter are not significantly different at the 5% level by DMRT.

Table 4.4 Influence of fertilizers (N-K) and number of clusters on growing degree days for various phenological periods of grapevine.

Treatments	GDDBB-BL	GDDBB-V	GDDBB-H	GDDBL-V	GDDBL-H	GDDV-H
Fertilizers (N-K)						
F1	416.05b	1450.59a	1836.21a	1052.13a	1437.75a	385.61a
F2	410.07ab	1446.92a	1834.46a	1056.26a	1443.81a	387.55a
F3	400.19a	1442.66a	1836.17a	1060.62a	1454.01b	393.39a
No. of clusters						
C1	405.87a	1434.93a	1831.67a	1050.18a	1446.91a	396.72a
C2	408.67a	1444.29ab	1833.37ab	1052.46a	1441.53a	389.08a
C3	411.78a	1460.93b	1841.79b	1066.37a	1447.13a	380.76a
Mean	408.77	1446.72	1835.61	1056.33	1445.19	388.85

In a column and each treatment means followed by a common letter are not significantly different at the 5% level by DMRT.

Increasing N-K levels increased DBL-V and DBL-H from 67.44 to 68.83 and 94.39 to 96.22 days in 2005, respectively. Increasing cluster levels increased DBB-H and DBL-V from 134.56 to 135.94 and 67.78 to 69.11 days in 2005, respectively. In 2006, all duration (days) between phenological stages of grapevine were not significantly different (Tables 4.2 and 4.3).

Increasing N-K levels decreased DDBB-BL from 416.05 to 400.19 and increased GDDBL-H from 1437.75 to 1454.01. Increasing cluster levels increased GDDBB-V and GDDBB-H from 1434.93 to 1460.93 and 1831.67 to 1841.79, respectively (Table 4.4).

Table 4.5 Influence of years, fertilizers (N-K) and number of clusters on growing degree days for various phenological periods of grapevine.

Fertilizers	**GDDBB-BL**		**GDDBB-V**	
	2005	**2006**	**2005**	**2006**
Fertilizers (N-K)				
F1	459.07b	373.02a	1453.17a	1448.01a
F2	447.98ab	372.16a	1449.15a	1444.69a
F3	436.82a	363.57a	1444.25a	1441.06a
No. of clusters				
C1	442.50a	369.24a	1431.57a	1438.30a
C2	451.58a	365.76a	1448.21ab	1440.39a
C3	449.79a	373.76a	1466.79b	1455.07a
Mean	447.96	369.58	1448.86	1444.59

In a column and each treatment, means followed by a common letter are not significantly different at the 5% level by DMRT.

Increasing N-K levels decreased GDDBB-BL from 459.07 to 436.82 in 2005. Increasing cluster levels increased GDDBB-V from 1431.57 to 1466.79 in 2005 (Table 4.5).

Table 4.6 Influence of years, fertilizers (N-K) and number of clusters on growing degree days for various phenological periods of grapevine.

Treatments	GDDBB-H		GDDBL-V		GDDBL-H		GDDV-H	
	2005	2006	2005	2006	2005	2006	2005	2006
Fertilizers (N-K)								
F1	1866.54a	1805.87a	1029.26a	1074.99a	1442.64a	1432.85a	413.36a	375.86a
F2	1862.25a	1806.67a	1040.00a	1072.52a	1453.09ab	1434.52a	413.10a	362.00a
F3	1865.02a	1807.31a	1043.53a	1077.71a	1464.30b	1443.72a	420.78a	366.01a
No. of clusters								
C1	1855.52a	1807.81a	1031.31a	1069.06a	1455.27a	1438.56a	423.94a	369.49a
C2	1864.36ab	1802.37a	1030.29a	1074.62a	1446.43a	1436.63a	416.15a	362.01a
C3	1873.93b	1809.67a	1051.20b	1081.54a	1458.34a	1435.91a	407.15a	354.37a
Mean	1864.60	1806.62	1037.60	1075.07	1453.34	1437.03	415.75	361.96

In a column and each treatment, means followed by a common letter are not significantly different at the 5% level by DMRT.

Increasing N-K levels increased GDDBL-H from 1442.64 to 1464.30 in 2005. Increasing cluster levels increased GDDBB-H and GDDBL-V from 1855.52 to 1873.93 and 1031.31 to 1051.20 in 2005 (Table 4.6).

Table 4.7 Influence of fertilizers (N-K) and number of clusters on grapevine performances during 2005 and 2006.

Treatments	Cluster before thinning (bunch)	Shoot weight (g)	Crop load
Fertilizers (N-K)			
F1	35.36b	0.73b	2.51a
F2	32.47a	0.61a	2.57a
F3	31.78a	0.56a	3.03b
No. of clusters			
C1	30.28a	0.57a	1.73a
C2	32.83b	0.63a	2.79b
C3	36.50c	0.69b	3.58c
Mean	33.20	0.63	2.70

In a column and each treatment means followed by a common letter are not significantly different at the 5% level by DMRT.

Increasing N-K level decreased cluster before thinning from 35.36 to 31.78 bunches, shoot weight from 0.73 to 0.56 g and increased crop load from 2.51 to 3.03. Increasing cluster levels increased cluster before thinning from 30.28 to 36.50 bunch, shoot weight from 0.57 to 0.69 g and crop load from 1.73 to 3.58 (Table 4.7).

Table 4.8 Influence of years, fertilizers (N-K) and number of clusters on grapevine performances.

Treatments	Cluster before thinning (bunch)		Shoot weight (g)		Crop load	
	2005	**2006**	**2005**	**2006**	**2005**	**2006**
Fertilizers (N-K)						
F1	30.61a	40.11b	0.65b	0.80b	3.62a	1.40a
F2	28.39a	35.17a	0.59b	0.63a	3.73a	1.40a
F3	28.67a	36.28a	0.49a	0.62a	4.59ab	1.47a
No. of clusters						
C1	27.89a	32.67a	0.52a	0.62a	2.49a	0.97a
C2	27.94a	37.72b	0.58a	0.67ab	4.12b	1.47b
C3	31.83b	41.17b	0.63b	0.76b	5.32c	1.83c
Mean	29.22	37.19	0.58	0.68	3.98	1.42

In a column and each treatment, means followed by a common letter are not significantly different at the 5% level by DMRT.

Increasing N-K levels decreased cluster before thinning significantly from 40.11 to 35.17 bunchs in 2006 and trended to decrease in 2005. Shoot weight was decreased significantly in both years from 0.65 to 0.49 and 0.80 to 0.62 kg in 2005 and 2006, respectively and crop load was increased significantly from 3.62 to 4.59 in 2005 and trended to increase in 2006. Increasing cluster levels increased cluster before thinning, shoot weight and crop load significantly from 27.89 to 31.83, 0.52 to 0.63 and 2.49 to 5.32 in 2005 and 32.67 to 41.17, 0.62 to 0.76 and 0.97 to 1.83 in 2006, respectively (Table 4.8).

4.1.1.2 Grapevine petiole, berry and soil analysis

Soil pH increased from 5.25 in 2005 to 5.79 in 2006. N and K were decreased from 9.90 to 9.80, 470.00 to 156.00 ppm, respectively. Ca was almost constant amount for 300.00 ppm. P was increased from 9.80 to 19.40 ppm. That result might be changed of available P in the soil by climateric factors (Table B1).

Table 4.9 Influence of fertilizers (N-K) and number of clusters on N and K in petiole at blooming period and in grape berry.

Treatments	Petiole N (%)	Berry N (%)	Petiole K (%)	Berry K (%)
Fertilizers (N-K)				
F1	1.139a	0.638a	4.169a	1.357a
F2	1.175a	0.680a	3.832a	1.023a
F3	1.174a	0.741a	4.130a	0.897a
No. of clusters				
C1	1.176a	0.665a	3.947a	0.979a
C2	1.127a	0.728a	4.089a	1.351a
C3	1.185a	0.667a	4.096a	0.947a
Mean	1.163	0.686	4.044	1.092

In a column and each treatment means followed by a common letter are not significantly different at the 5% level by DMRT.

There were not significantly different N, K content in petiole at bloom and in berry at harvest, petiole N, K, and berry N, K were 1.163, 4.044, 0.686 and 1.092 %, respectively (Table 4.9).

Table 4.10 Influence of years, fertilizers (N-K) and number of clusters on petiole N and K.

Treatments	Petiole N (%)		Petiole K (%)	
	2005	**2006**	**2005**	**2006**
Fertilizers (N-K)				
F1	1.24a	1.04a	4.17b	4.17a
F2	1.21a	1.14b	3.52a	4.15a
F3	1.24a	1.11ab	3.84ab	4.42b
No. of clusters				
C1	1.23a	1.12a	3.79a	4.11a
C2	1.19a	1.07a	3.82a	4.26ab
C3	1.27a	1.10a	3.92a	4.38b
Mean	1.23	1.10	3.84	4.25

In a column and each treatment, means followed by a common letter are not significantly different at the 5% level by DMRT.

Increasing N-K levels increased petiole N at 100-20 level in 2006 and decreased petiole K in 2005 but increased in 2006. Increasing cluster level increased petiole K from 4.11 to 4.38 % in 2006 (Table 4.10).

4.1.1.3 Grape yields and berry performances

Increasing N-K levels decreased berry weight from 1.07 to 1.01 g, TA from 10.79 to 10.34 g/l, and increased pH from 3.32 to 3.40. Increasing cluster levels increased yields from 0.91 to 2.24 kg/vine and TA from 10.07 to 10.85 g/l (Table 4.11).

Table 4.11 Influence of fertilizers (N-K) and number of clusters on yields (kg/vine), berry/clusters (berry), berry weights (g), TSS (Brix), TA (g/l), and pH of grape berry.

Treatments	Yields (kg/vine)	Berry/cluster (berry)	Berry weight (g)	TSS (^{o}Brix)	TA (g/l)	pH
Fertilizers (N-K)						
F1	1.53a	107.79a	1.07b	18.25a	10.79b	3.32a
F2	1.55a	106.41a	1.03a	18.08a	10.50ab	3.36b
F3	1.70a	101.38a	1.01a	18.39a	10.34a	3.40c
No. of clusters						
C1	0.91a	111.21a	1.05a	18.33a	10.07a	3.37a
C2	1.63b	104.43a	1.03a	18.35a	10.85b	3.34b
C3	2.24c	99.94a	1.04a	18.04a	10.70b	3.36a
Mean	1.59	105.19	1.04	18.24	10.54	3.36

In a column and each treatment means followed by a common letter are not significantly different at the 5% level by DMRT.

Increasing N-K levels decreased berry weight significantly from 1.22 to 1.13 g/berry in 2005 and 0.93 to 0.90 g/berry in 2006. Yield and number of berry per cluster decreased significantly from 1.08 to 0.83 kg/vine and 61.21 to 49.84 berry/cluster in 2006. Increasing cluster levels increased yields both years from 1.24 to 3.20 kg/vine in 2005 and 0.59 to 1.28 kg/vine in 2006 and decreased berry per cluster from 64.93 to 46.22 berries in 2006 (Table 4.12).

Table 4.12 Influence of years, fertilizers (N-K) and number of clusters on yields (kg/vine), berry per cluster (berry) and berry weight (g/berry).

Treatments	Yield (kg/vine)		Berry/cluster (berry)		Berry weight (g/berry)	
	2005	**2006**	**2005**	**2006**	**2005**	**2006**
Fertilizers (N-K)						
F1	2.33a	1.08b	154.37a	61.21b	1.22b	0.93b
F2	2.23a	0.93ab	158.28a	54.55ab	1.18b	0.87a
F3	2.18a	0.83a	152.92a	49.84a	1.13a	0.90ab
No. of clusters						
C1	1.24a	0.59a	157.49a	64.93b	1.19a	0.90a
C2	2.30b	0.97b	154.41a	54.45a	1.17a	0.89a
C3	3.20c	1.28c	153.67a	46.22a	1.16a	0.92a
Mean	2.25	0.95	155.19	55.2	1.17	0.90

In a column and each treatment, means followed by a common letter are not significantly different at the 5% level by DMRT.

Increasing N-K levels increased pH both years from 3.45 to 3.52 in 2005 and 3.19 to 3.27 in 2006; While TA was decreased from 12.86 to 12.13 in 2006. Increased cluster levels increased TA both years from 8.20 to 8.61 in 2005 and 11.94 to 12.83 in2006. TSS amount was highest at 20 clusters with the lowest pH (Table 4.13).

Table 4.13 Influence of years, fertilizers (N-K) and number of clusters on TSS (ºBrix), pH, and TA (g/l) of grape berry.

Treatments	TSS (ºBrix)		pH		TA (g/l)	
	2005	**2006**	**2005**	**2006**	**2005**	**2006**
Fertilizers (N-K)						
F1	18.59a	17.92a	3.45a	3.19a	8.71a	12.86b
F2	18.47a	17.69a	3.48b	3.23b	8.43a	12.57ab
F3	18.67a	18.11a	3.52c	3.27c	8.53a	12.13a
No. of clusters						
C1	18.52a	18.15a	3.50b	3.24b	8.20a	11.94a
C2	18.88b	17.81a	3.47a	3.22a	8.87b	12.79b
C3	18.33a	17.76a	3.49ab	3.24b	8.61b	12.83b
Mean	18.58	17.91	3.48	3.23	8.56	12.52

In a column and each treatment, means followed by a common letter are not significantly different at the 5% level by DMRT.

4.1.2 Grape berry red pigments and phenolic compounds

Increasing N-K levels, grape berry at 100-20 level has the lowest mg color/berry, mg color/g berry, total phenolic/berry, and total phenolic/g berry were 0.749, 0.819, 0.869, and 0.955, respectively while 0-0 level showed relative high concentration of these compositions. Increasing cluster levels decreased color/berry from 0.885 to 0.766 and total phenolic/berry from 1.015 to 0.923 (Table 4.14).

Table 4.14 Influence of fertilizers (N-K) and number of clusters on color and phenolic compounds in grape berry.

Treatments	mg color/berry	mg color/g berry	Total phenolic/berry	Total phenolic/g berry
Fertilizers (N-K)				
F1	0.878b	0.890ab	1.037b	1.060b
F2	0.749a	0.819a	0.869a	0.955a
F3	0.827ab	0.897b	0.984ab	1.076b
No. of clusters				
C1	0.885b	0.907a	1.015b	1.059a
C2	0.803ab	0.863a	0.953ab	1.029a
C3	0.766a	0.836a	0.923a	1.003a
Mean	0.818	0.868	0.963	1.030

In a column and each treatment means followed by a common letter are not significantly different at the 5% level by DMRT.

N-K levels, at 100-20 level caused lower color and total phenolic per berry than others in both years. Increasing N-K levels decreased color per berry from 0.947 to 0.741 mg and total phenolic per berry from 0.850 to 0.669 in 2005 (Table 4.15).

Table 4.15 Influence of years, fertilizers (N-K) and number of clusters on color and phenolic compounds in grape berry.

Treatments	mg color/berry		mg color/g berry		Total phenolic/berry		Total Phenolic/g berry	
	2005	**2006**	**2005**	**2006**	**2005**	**2006**	**2005**	**2006**
Fertilizers (N-K)								
F1	0.925b	0.832a	0.886b	0.894a	0.874b	1.201b	0.828b	1.292a
F2	0.710a	0.788a	0.726a	0.913a	0.676a	1.063a	0.690a	1.221a
F3	0.839b	0.814a	0.889b	0.905a	0.721a	1.247b	0.765ab	1.387b
No. of clusters								
C1	0.947b	0.822a	0.889a	0.924a	0.850b	1.181a	0.798a	1.322a
C2	0.786a	0.821a	0.802a	0.925a	0.752ab	1.154a	0.759a	1.300a
C3	0.741a	0.790a	0.810a	0.863a	0.669a	1.176a	0.726a	1.279a
Mean	0.825	0.811	0.834	0.904	0.757	1.170	0.761	1.300

In a column and each treatment, means followed by a common letter are not significantly different at the 5% level by DMRT.

4.1.3 Grape berry anthocyanin

N-K levels at 100-20; grape berries had lower content of Pt, Cy and Mv than others, 0.230, 0.049, and 0.504 mg/g berry, respectively. At 30 cluster levels were found the highest contents of Pt, Cy and Mv with amount 0.331, 0.065, and 0.599 mg/g berry, respectively. The Mv was the highest anthocyanin contents in grape berry (Table 4.16).

Table 4.16 Influence of fertilizers (N-K) and number of clusters on grape berry anthocyanin contents (mg/g berry).

Fertilizers	Pt	Cy	Mv
Fertilizers (N-K)			
F1	0.291b	0.057a	0.517ab
F2	0.230a	0.049a	0.504a
F3	0.268ab	0.060a	0.572b
No. of clusters			
C1	0.243a	0.055ab	0.517a
C2	0.214a	0.046a	0.476a
C3	0.331b	0.065b	0.599b
Mean	0.263	0.055	0.531

In a column and each treatment means followed by a common letter are not significantly different at the 5% level by DMRT.

Table 4.17 Influence of years, fertilizers (N-K) and number of clusters on grape berry anthocyanin contents (mg/g berry).

Treatments	Pt		Cy		Mv	
	2005	2006	2005	2006	2005	2006
Fertilizers (N-K)						
F1	0.379c	0.202a	0.036b	0.078a	0.518a	0.514ab
F2	0.267a	0.194a	0.026a	0.072a	0.562a	0.445a
F3	0.318b	0.217a	0.032ab	0.089a	0.568a	0.575b
No. of clusters						
C1	0.321ab	0.165a	0.035b	0.074a	0.539a	0.495a
C2	0.282a	0.146a	0.028a	0.065a	0.488a	0.464a
C3	0.360b	0.302b	0.031ab	0.100b	0.622b	0.577a
Mean	0.321	0.204	0.031	0.080	0.549	0.511

In a column and each treatment, means followed by a common letter are not significantly different at the 5% level by DMRT.

Pt content was highest at 0-0 level of N-K in 2005 and at 30 clusters in 2005 and 2006. Cy content was highest at 0-0 level of N-K in 2005 and at 10 clusters in 2005 and 30 clusters in 2006. Mv content was highest at 200-60 level of N-K in 2006 and 30 clusters in 2005. The mean contents of Pt, Cy and Mv were 0.321, 0.031, and 0.549 mg/g berry in 2005 and 0.204, 0.080, and 0.511 mg/g berryin 2006 (Table 4.17).

4.2 Wine performances

4.2.1 Wine quality

Increasing N-K levels increased wine pH from 3.59 to 3.63 but decreased TA from 6.78 to 6.31 g/l. Increased cluster levels decreased wine pH and VA from

Table 4.18 Influence of fertilizers (N-K) and number of clusters on wine characteristic.

Treatments	pH	TA (g/l)	Alc (%)	VA
Fertilizers (N-K)				
F1	3.59a	6.78b	11.19a	0.63a
F2	3.62b	6.61b	11.12a	0.64a
F3	3.63b	6.31a	11.11a	0.63a
No. of clusters				
C1	3.63b	6.46a	10.89a	0.68b
C2	3.62b	6.46a	11.29b	0.61ab
C3	3.59a	6.79b	11.24b	0.60a
Mean	3.61	6.57	11.14	0.63

In a column and each treatment means followed by a common letter are not significantly different at the 5% level by DMRT.

3.63 to 3.59, and 0.68 to 0.60, respectively. Increasing of TA and Alc from 6.46 to 6.79 g/l, and 10.89 to 11.29 %, respectively were also detected (Table 4.18).

Table 4.19 Influence of years, fertilizers (N-K) and nunber of clusters on wine characteristics.

Treatments	pH		TA (g/l)		Alc (%)		VA	
	2005	**2006**	**2005**	**2006**	**2005**	**2006**	**2005**	**2006**
Fertilizers (N-K)								
F1	3.49a	3.71a	6.41b	7.16a	11.51a	10.87a	0.48a	0.78ab
F2	3.51ab	3.72a	6.38ab	6.84a	11.42a	10.82a	0.50a	0.76a
F3	3.53b	3.72a	6.03a	6.58a	11.36a	10.87a	0.43a	0.82b
No. of clusters								
C1	3.52a	3.75b	6.20ab	6.71a	11.43a	10.36a	0.45ab	0.91c
C2	3.51a	3.72b	6.10a	6.81a	11.38a	11.00b	0.43a	0.79b
C3	3.49a	3.69a	6.52b	7.06a	11.48a	11.20b	0.53b	0.64a
Mean	3.51	3.72	6.27	6.86	11.43	10.85	0.47	0.78

In a column and each treatment, means followed by a common letter are not significantly different at the 5% level by DMRT.

Wine pH was increased by increasing N-K levels in 2005 but decreased by decreasing cluster levels in 2006. In 2005 wine pH increased from 3.49 to 3.53 and decreased from 3.75 to 3.69 in 2006. TA was decreased by increasing N-K levels but increased by increasing cluster levels in 2005 but none in 2006. Increasing N-K levels caused decreasing of TA from 6.41 to 6.03 g/l and was increased from 6.20 to 6.52 g/l in 2005 but none in 2006 (Table 4.19).

4.2.2 Wine color and phenolic compounds

Wine color density pressed and aged wines were highest at 200-60 level of N-K. The highest wine color density was 5.45 and 5.38 a.u. in pressed and aged wines. Wine color hue was opposite with wine color density. The wine color hue was decreased when N-K levels increased from 0.71 to 0.67 a.u. in pressed wines. Increasing cluster levels decreased wine color density from 5.41 to 4.17 a.u. in aged wines and wine color hue from 0.71 to 0.68 a.u. in pressed wines. The degree of red pigment coloration was increased from 16.38 to 18.51 % in pressed wines and decreased from 34.72 to 23.02 % in aged wines (Table 4.20).

Table 4.20 Influence of fertilizers (N-K) and number of clusters on wine color characteristics (absorbance unit, a.u.).

Treatments	Wine color density (a.u.)		Wine color hue (a.u.)		Degree of red pigment coloration (%)	
	Press	Age	Press	Age	Press	Age
Fertilizers (N-K)						
F1	4.80a	4.70ab	0.71b	0.76a	17.53a	29.93a
F2	4.81a	4.57a	0.69ab	0.78a	17.14a	26.67a
F3	5.45b	5.38b	0.67a	0.73a	18.92a	35.67a
No. of clusters						
C1	4.83a	5.41b	0.71b	0.74a	16.38a	34.72b
C2	5.09a	5.06ab	0.68a	0.73a	18.69b	34.62ab
C3	5.13a	4.17a	0.68a	0.80a	18.51b	23.02a
Mean	5.02	4.88	0.69	0.76	17.86	30.79

In a column and each treatment means followed by a common letter are not significantly different at the 5% level by DMRT.

Table 4.21 Influence of fertilizers (N-K) and number of clusters on wine color characteristics (absorbance unit, a.u.).

Treatments	Estimate SO_2 resistant pigments (a.u.)		Total red pigments (a.u.)		Total phenolics (a.u.)	
	Press	Age	Press	Age	Press	Age
Fertilizers (N-K)						
F1	0.89ab	1.37ab	16.29a	10.12a	24.62a	20.49a
F2	0.85a	1.23a	16.74ab	10.89a	24.75a	20.64a
F3	0.95b	1.88b	17.49b	10.24a	25.72a	20.59a
No. of clusters						
C1	0.89a	0.94a	17.52b	10.27ab	26.09b	20.48a
C2	0.91a	1.65b	16.53ab	9.67a	25.19ab	19.90a
C3	0.89a	1.89b	16.47a	11.32b	23.81a	21.33a
Mean	0.90	1.49	16.84	10.42	25.03	20.57

In a column and each treatment means followed by a common letter are not significantly different at the 5% level by DMRT.

Estimate SO_2 resistant pigments were increased from pressed to aged wines. At each N-K levels SO_2 resistant pigments were increased from 0.89 to 1.37, 0.85 to 1.23, and 0.95 to 1.88 a.u. at 0-0, 100-20, and 200-60 N-K level, respectively. At each of cluster levels from 0.89 to 0.94, 0.91 to 1.65, and 0.89 to 1.89 a.u. at 10, 20, and 30 clusters, respectivly. Pressed wines had lower estimate SO_2 resistant pigments than aged wines. Total red pigments and total phenolics were decreased after aging from 16.84 to 10.42, and 25.03 to 20.57 a.u., respectively. Increased N-K levels increased total red pigments from 16.29 to 17.49 a.u. in pressed wines but total phenolics were not significantly different in both pressed and aged wines. Increasing cluster levels decreased total red pigments from 17.52 to 16.47, and 10.27 to 9.67 a.u. in pressed and aged wines and total phenolics from 26.09 to 23.81 a.u. in pressed wines (Table 4.21)

Table 4.22 Influence of years, fertilizers (N-K) and number of clusters on wine color characteristics (pressed wine) (absorbance unit, a.u.).

Treatments	Wine color density (a.u.)		Wine color hue (a.u.)		Degree of red pigment coloration (%)	
	2005	**2006**	**2005**	**2006**	**2005**	**2006**
Fertilizers (N-K)						
F1	4.361a	5.242a	0.616b	0.798a	16.678a	18.378a
F2	4.398a	5.241a	0.601ab	0.783a	16.011a	18.267a
F3	4.916b	5.979b	0.587a	0.744a	16.756a	21.078a
No. of clusters						
C1	4.276a	5.392a	0.606a	0.817b	14.600a	18.167a
C2	4.654b	5.440a	0.596a	0.756a	16.844b	20.533a
C3	4.744b	5.603a	0.602a	0.753a	18.000b	19.022a
Mean	4.558	5.478	0.601	0.775	16.482	19.241

In a column and each treatment, means followed by a common letter are not significantly different at the 5% level by DMRT.

In pressed wine, wine color density, wine color hue, degree of red pigment coloration and estimate SO_2 resistant pigments in 2006 were higher than those of year 2005 at each treatment levels. There were 4.558, 0.601, 16.482, and 0.878 a.u. in 2005 and 5.478, 0.775, 19.241, and 0.915 a.u. in 2006. Increasing N-K levels increased wine color density both years, in 2005 from 4.361 to 4.916, and 5.242 to 5.979 a.u. in 2006 and wine color hue decreased from 0.616 to 0.587 a.u. in 2005 and estimate SO_2 resistant pigments were highest at 200-60 level in 2005 and trended to

be in 2006. Increasing cluster levels increased wine color density from 4.276 to 4.744 a.u. in 2005 and trended to be in 2006. Increasing cluster levels decreased wine color hue from 0.817 to 0.753 a.u. in 2006. Increasing cluster levels increased degree of red pigment coloration from 14.6 to 18.0 % in 2005. Total red pigments were increased by increasing N-K levels from 16.356 to 18.644 a.u. and decreased by increasing cluster levels from 18.278 to 16.600 a.u. in 2005. Total phenolic was decreased by increasing cluster levels from 27.933 to 22.544 a.u. in 2005 (Tables 4.22 and 4.23).

Table 4.23 Influence of years, fertilizers (N-K) and number of clusters on wine color characteristics (pressed wine) (absorbance unit, a.u.).

Treatments	Estimate SO_2 resistant (a.u.)		Total red pigments (a.u.)		Total phenolic (a.u.)	
	2005	2006	2005	2006	2005	2006
Fertilizers (N-K)						
F1	0.872ab	0.908a	16.356a	16.222a	25.344a	23.889a
F2	0.831a	0.864a	17.244b	16.244a	25.611a	23.889a
F3	0.931b	0.973a	18.644c	16.333a	29.956a	25.478a
No. of clusters						
C1	0.864a	0.914a	18.278b	16.767a	27.933c	24.244a
C2	0.883a	0.930a	17.367a	15.700a	26.433b	23.944a
C3	0.887a	0.901a	16.600a	16.333a	22.544a	25.067a
Mean	0.878	0.915	17.415	16.266	25.637	24.419

In a column and each treatment, means followed by a common letter are not significantly different at the 5% level by DMRT.

Table 4.24 Influence of years, fertilizers (N-K) and number of clusters on wine color (aged wine) (absorbance unit, a.u.).

Treatments	Wine color dentity (a.u.)		Wine color hue (a.u.)		Degree of red pigment coloration (%)	
	2005	**2006**	**2005**	**2006**	**2005**	**2006**
Fertilizers (N-K)						
F1	5.617a	3.777a	0.638b	0.872a	38.989a	20.867a
F2	5.437a	3.706a	0.643b	0.924a	32.522a	20.822a
F3	6.432b	4.322a	0.584a	0.878a	42.411a	29.111a
No. of clusters						
C1	6.066a	4.756b	0.663b	0.821a	36.400a	33.044b
C2	5.996a	4.127b	0.639b	0.816a	44.189a	25.056ab
C3	5.424a	2.922a	0.563a	1.038a	33.333a	12.700a
Mean	5.829	3.935	0.622	0.891	37.974	23.600

In a column and each treatment, means followed by a common letter are not significantly different at the 5% level by DMRT.

In aged wine, wine color density, degree of red pigment coloration and estimate SO_2 resistant pigments were higher in 2005 than those of the year 2006 from 5.829 to 3.935 a.u., 37.974 to 23.600 %, and 1.880 to 1.102 a.u., respectively. While wine color hue was lower in 2005 than 2006. Increasing N-K levels increased wine color density and estimate SO_2 resistant pigments there were highest at 200-60 levels, 6.432 and 2.376 a.u., respectively. Wine color hue was lowest at the same level. Increasing cluster levels decreased wine color density from 4.756 to 2.922 a.u. in

2006, wine color hue from 0663 to 0.563 a.u. in 2005, degree of red pigment coloration from 33.044 to 12.700 % in 2006 and estimate SO_2 resistant pigments from 2.304 to 1.187 in 2005 and 1.620 to 0.693 a.u. in 2006. Total red pigment and total phenolic were increased by increasing cluster levels from 9.156 to 11.367, and 19.189 to 22.189 a.u. in 2006 (Tables 4.24 and 4.25).

Table 4.25 Influence of years, fertilizers (N-K) and number of clusters on wine color (aged wine) (absorbance unit, a.u.).

Treatments	**Estimate SO_2 resistant (a.u.)**		**Total red pigments (a.u.)**		**Total phenolic (a.u.)**	
	2005	**2006**	**2005**	**2006**	**2005**	**2006**
Fertilizers (N-K)						
F1	1.701ab	1.034a	9.900a	10.344a	20.567a	20.411a
F2	1.564a	0.897a	10.756a	10.211a	21.000a	20.278a
F3	2.376b	1.374a	11.578a	9.733a	20.200a	20.978a
No. of clusters						
C1	2.150b	1.620b	11.389a	91.56a	21.778a	19.189a
C2	2.304b	0.992a	9.567a	9.767a	19.511a	20.289a
C3	1.187a	0.693a	11.278a	11.367b	20.478a	22.189b
Mean	1.880	1.102	10.745	10.096	20.589	20.556

In a column and each treatment, means followed by a common letter are not significantly different at the 5% level by DMRT.

Table 4.26 Influence of fertilizers (N-K) and number of clusters on wine color characteristics (absorbance unit, a.u.).

Treatments	MWCD (a.u.)		MWCH (a.u.)		MDRPC (%)	
	Press	**Age**	**Press**	**Age**	**Press**	**Age**
Fertilizers (N-K)						
F1	5.21a	5.22a	0.63a	0.64a	19.79a	33.85a
F2	5.21a	5.37a	0.64a	0.64a	19.41a	31.83a
F3	5.80a	5.97b	0.64a	0.64a	20.38a	39.46a
No. of clusters						
C1	5.57a	5.92b	0.62a	0.65b	19.82a	38.22b
C2	5.33a	5.40a	0.64ab	0.66b	19.84a	36.79ab
C3	5.32a	5.24a	0.65b	0.61a	19.93a	30.12a
Mean	5.41	5.52	0.64	0.64	19.86	35.05

In a column and each treatment means followed by a common letter are not significantly different at the 5% level by DMRT.

Modified wine color density (MWCD) was increased by increasing N-K levels from 5.21 to 5.80 and 5.22 to 5.97 a.u. in pressed and aged wines and was decreased by increasing cluster levels from 5.92 to 5.24 a.u. in aged wine. Modified wine color hue (MWCH) in pressed wines was increased from 0.62 to 0.65 a.u. but in aged wines was decreased from 0.65 to 0.61 a.u. by increasing cluster levels. Increasing cluster levels decreased modified degree red pigment coloration (WDRPC) in aged wines from 38.22 to 30.12 a.u. (Table 4.26).

Table 4.27 Influence of years, fertilizers (N-K) and number of clusters on wine color (pressed wine) (absorbance unit, a.u.).

Treatments	MWCD (a.u.)		MWCH (a.u.)		MDRPC (%)	
	2005	**2006**	**2005**	**2006**	**2005**	**2006**
Fertilizers (N-K)						
F1	4.977a	5.438a	0.550a	0.707a	19.767a	19.822a
F2	5.077a	5.348a	0.542a	0.732a	19.633a	19.178a
F3	5.594b	6.011b	0.538a	0.748a	19.567a	21.200a
No. of cluster						
C1	5.317b	5.813b	0.521a	0.710a	19.144a	20.489a
C2	5.031a	5.638ab	0.550b	0.731a	18.711a	20.967a
C3	5.300b	5.345a	0.559b	0.746a	21.111a	18.744a
Mean	5.216	5.599	0.543	0.729	19.656	20.067

In a column and each treatment, means followed by a common letter are not significantly different at the 5% level by DMRT.

In pressed wine, increasing N-K levels increased modified wine color density from 4.977 to 5.594, and 5.438 to 6.011 a.u. a.u. in 2005 and 2006, respectively. Increasing cluster levels decreased modified wine color density from 5.813 to 5.345 a.u. in 2006 and increased modified wine color hue from 0.521 to 0.559 a.u. in 2005 (Table 4.27).

Table 4.28 Influence of years, fertilizers (N-K) and number of clusters on wine color (aged wine) (absorbance unit, a.u.).

Treatments	MWCD (a.u.)		MWCH (a.u.)		MDRPC (%)	
	2005	**2006**	**2005**	**2006**	**2005**	**2006**
Fertilizers (N-K)						
F1	5.721a	4.726a	0.638b	0.640a	39.322a	28.378a
F2	5.813a	4.933a	0.601a	0.677b	33.944a	29.722a
F3	6.612b	5.329a	0.582a	0.690b	42.722a	36.189a
No. of clusters						
C1	6.400b	5.443a	0.637b	0.654a	37.733a	38.711b
C2	6.002ab	4.800a	0.633b	0.692b	43.211a	30.378ab
C3	5.744a	4.744a	0.551a	0.660a	35.044a	25.200a
Mean	6.049	4.996	0.607	0.669	38.663	31.430

In a column and each treatment, means followed by a common letter are not significantly different at the 5% level by DMRT.

In aged wines, increasing N-K levels increased modified wine color density from 5.721 to 6.612 a.u. and decreased modified wine color hue from 0.638 to 0.582 a.u. in 2005 but increased modified wine color hue from 0.640 to 0.690 a.u. in 2006. Increasing cluster levels decreased modified wine color density and modified wine color hue from 6.400 to 5.744, 0.637 to 0.551 a.u. in 2005 and degree of red pigment coloration from 38.711 to 25.200 a.u. in 2006 (Table 4.28).

4.2.3 Wine anthocyanin

Anthocyanin contents were not significantly different by N-K levels in pressed and aged wines. Cluster levels at 20 clusters have higher anthocyanin contents than 10 clusters level in pressed wine but were not significantly different in aged wines (Table 4.29).

Table 4.29 Influence of fertilizers (N-K) and number of clusters on wine anthocyanin content (mg/ml).

Fertilizers	**Pressed**	**Aged**
Fertilizers (N-K)		
F1	0.1944a	0.1327a
F2	0.2002a	0.1536a
F3	0.1761a	0.1319a
No. of clusters		
C1	0.1661a	0.1414a
C2	0.2251b	0.1259a
C3	0.1795ab	0.1508a
Mean	0.1902	0.1394

In a column and each treatment means followed by a common letter are not significantly different at the 5% level by DMRT.

Table 4.30 Influence of years, fertilizers (N-K) and number of clusters on wine anthocyanin contents (mg/ml).

Treatments	**Press**		**Age**	
	2005	**2006**	**2005**	**2006**
Fertilizers (N-K)				
F1	0.1694a	0.2194a	0.1095a	0.1559a
F2	0.1475a	0.2529a	0.1512a	0.1559a
F3	0.1536a	0.1987a	0.1102a	0.1536a
No. of clusters				
C1	0.1387a	0.1935a	0.1348a	0.1481a
C2	0.1687a	0.2816b	0.0984a	0.1534a
C3	0.1630a	0.1960a	0.1377a	0.1639a
Mean	0.1568	0.2237	0.1236	0.1551

In a column and each treatment, means followed by a common letter are not significantly different at the 5% level by DMRT.

Cluster levels at 20 clusters had higher anthocyanin contents than 10 clusters level in pressed wine in 2006. 2006 pressed and aged wines have higher anthocyanin contents than 2005 wines and pressedwines have higher anthocyanin contents than aged wines (Table 4.30).

Table 4.31 Influence of fertilizers (N-K) and number of clusters on wine anthocyanin contents (pressed wine) (mg/ml).

Treatments	Dp	Cy	Pt	Pn	Mv
Fertilizers (N-K)					
F1	0.0112a	0.0187a	0.0030a	0.0066a	0.1551a
F2	0.0104a	0.0185a	0.0019a	0.0054a	0.1640a
F3	0.0108a	0.0152a	0.0027a	0.0063a	0.1411a
No. of clusters					
C1	0.0098a	0.0117a	0.0026a	0.0057a	0.1363a
C2	0.0116a	0.0276b	0.0023a	0.0051a	0.1785a
C3	0.0109a	0.0131a	0.0027a	0.0075b	0.1453a
Mean	0.0108	0.0175	0.0025	0.0061	0.1534

In a column and each treatment means followed by a common letter are not significantly different at the 5% level by DMRT.

There were no significantly differences in anthocyanin; Dp, Cy, Pt, Pn, and Mv; contents in pressed wines excepted Cy at 20 cluster level and Pn at 30 cluster level which were the highest contents, 0.0276 and 0.0075 mg/ml, respectively. The average contents were 0.0108, 0.0175, 0.0025, 0.0061, and 0.1534 mg/ml, respectively (Table 4.31). The ratio between Mv and Pn was 25.1.

Table 4.32 Influence of years, fertilizers (N-K) and number of clusters on wine anthocyanin contents (pressed wine) (mg/ml).

Treatments	Dp		Cy		Pt	
	2005	**2006**	**2005**	**2006**	**2005**	**2006**
Fertilizers (N-K)						
F1	0.0201a	0.0023a	0.0025a	0.0348a	0.0035a	0.0024a
F2	0.0181a	0.0026a	0.0023a	0.0348a	0.0030a	0.0009a
F3	0.0198a	0.0018a	0.0026a	0.0278a	0.0027a	0.0028a
No. of clusters						
C1	0.0174a	0.0022a	0.0022a	0.0211a	0.0033a	0.0020a
C2	0.0205a	0.0027a	0.0023a	0.0529b	0.0028a	0.0017a
C3	0.0202a	0.0017a	0.0028a	0.0234a	0.0030a	0.0024a
Mean	0.0193	0.0022	0.0024	0.0325	0.0030	0.0020

In a column and each treatment, means followed by a common letter are not significantly different at the 5% level by DMRT.

Mv content was higher in year 2006 than in 2005 pressed wines. Cy in 2006 pressed wines was the highest at 20 clusters level. Pn in 2006 pressed wines was the highest at 30 clusters level (Tables 4.32 and 4.33).

Table 4.33 Influence of years, fertilizers (N-K) and number of clusters on wine anthocyanin contents (pressed wine) (mg/ml).

Treatments	Pn		Mv	
	2005	**2006**	**2005**	**2006**
Fertilizers (N-K)				
F1	0.0068a	0.0063a	0.1365a	0.1736a
F2	0.0060a	0.0048a	0.1181a	0.2099a
F3	0.0056a	0.0070a	0.1228a	0.1593a
No. of clusters				
C1	0.0066a	0.0047a	0.1091a	0.1635a
C2	0.0056a	0.0047a	0.1375a	0.2196a
C3	0.0062a	0.0087a	0.1308a	0.1598a
Mean	0.0061	0.0060	0.1258	0.1809

In a column and each treatment, means followed by a common letter are not significantly different at the 5% level by DMRT.

Table 4.34 Influence of fertilizers (N-K) and number of clusters on wine anthocyanin contents (aged wine) (mg/ml).

Treatments	Dp	Cy	Pt	Pn	Mv
Fertilizers (N-K)					
F1	0.0152a	0.0026a	0.0028a	0.0059a	0.1062a
F2	0.0176a	0.0032a	0.0027a	0.0053a	0.1247a
F3	0.0168a	0.0028a	0.0026a	0.0061a	0.1035a
No. of clusters					
C1	0.0168a	0.0027a	0.0028a	0.0057a	0.1135a
C2	0.0145a	0.0026a	0.0029a	0.0053a	0.1005a
C3	0.0183a	0.0033a	0.0024a	0.0063a	0.1206a
Mean	0.0165	0.0029	0.0027	0.0058	0.115

In a column and each treatment means followed by a common letter are not significantly different at the 5% level by DMRT.

There were not significantly different in anthocyanin; Dp, Cy, Pt, Pn, and Mv contents in pressed wines. The average contents were 0.0165, 0.0029, 0.0027, 0.0058, and 0.1115 mg/ml, respectively (Table 4.34). The ratio between Mv and Pn was 19.2 and Mv was the highest anthocyanin content in pressed wine (Table 4.34).

Table 4.35 Influence of years, fertilizers (N-K) and number of clusters on wine anthocyanin contents (aged wine) (mg/ml).

Treatments	**Dp**		**Cy**		**Pt**	
	2005	**2006**	**2005**	**2006**	**2005**	**2006**
Fertilizers (N-K)						
F1	0.0103a	0.0200a	0.0027a	0.0025a	0.0022a	0.0034a
F2	0.0181a	0.0172a	0.0041a	0.0023a	0.0023a	0.0031a
F3	0.0138a	0.0198a	0.0030a	0.0026a	0.0024a	0.0027a
No. of clusters						
C1	0.0163a	0.0174a	0.0031a	0.0023a	0.0021ab	0.0034a
C2	0.0104a	0.0187a	0.0029a	0.0023a	0.0031b	0.0028a
C3	0.0155a	0.0210a	0.0038a	0.0028a	0.0018a	0.0030a
Mean	0.0141	0.0190	0.0033	0.0025	0.0023	0.0031

In a column and each treatment, means followed by a common letter are not significantly different at the 5% level by DMRT.

Table 4.36 Influence of years, fertilizers (N-K) and number of clusters on wine anthocyanin contents (aged wine) (mg/ml).

Treatments	**Pn**		**Mv**	
	2005	**2006**	**2005**	**2006**
Fertilizers (N-K)				
F1	0.0053a	0.0065a	0.0890a	0.1234a
F2	0.0044a	0.0062a	0.1224a	0.1270a
F3	0.0067a	0.0057a	0.0843a	0.1228a
No. of clusters				
C1	0.0045a	0.0069b	0.1089a	0.1181a
C2	0.0054a	0.0052a	0.0765a	0.1244a
C3	0.0063a	0.0062ab	0.1103a	0.1308a
Mean	0.0054	0.0061	0.0986	0.1244

In a column and each treatment, means followed by a common letter are not significantly different at the 5% level by DMRT.

2006 aged wines were higher Mv than 2005 aged wines. Aged wines from 2005 showed the highest contents of Pt with the value of 0.0031 mg/ml at the level of 20 clusters and aged wines from 2006 showed the highest contents of Pn with the value of 0.0069 mg/ml at the level of 10 clusters. The average contents of Dp, Cy, Pt, Pn, and Mv were 0.0141, 0.0033, 0.0023, 0.0054, and 0.0986 in 2005 and 0.0190, 0.0025, 0.0031, 0.0061, and 0.1244 mg/ml in 2006, respectively. The highest anthocyanin content in aged wines was Mv (Tables 4.35 and 4.36).

CHAPTER V

DISCUSSIONS

5.1 Grapevine performance

5.1.1 Grapevine vigor

The data showed that DBB-BL was decreased but increased DBL-V by increasing N-K levels. A high level of N applied at bloom to veraison delayed ripening, and resulted in a lower berry anthocyanin concentration (Boonterm, 2002; Reynolds *et al*., 1994). N plays an important role in the vegetative growth. Accumulation of starch in leaves with decreasing N availability is commonly reported in vine literatures (Chen and Cheng, 2003) and may be involved in the feedback regulation of photosynthetic activity (Quereix *et al*., 2001). On the other hand, as the capacity of plants to obtain nitrogen is dependent on carbon substrate for root production, enhanced labile carbon pools towards roots under low N conditions increase their morphological and physiological properties (Grechi *et al*., 2007). Generally, fruits have an increasing demand for K supply while fruits are ripening (Zhenming *et al*., 2008) by that much of the K influxed into fruits at the mature fruit stages. Increasing cluster levels increased DBB-V and DBB-H that due to partitioning of photoassimilates of berry. Photoassimilates were partitioned preferentially to the fruit when the grapevines had bunches but to the fine roots when the grapevines had no bunches. This characteristic was observed at every growth stage of the berries, but it was especially remarkable during veraison

(Morinaga *et al*., 2003). Edson *et al*., 1995 reported that yield and berries per vine were significantly higher in vines with more clusters at harvest. When the fruit load on the grapevine increases, the skin color of the berries changes slowly. This is caused by a low integration of sugar (Jackson, 1986). There was the same result about growing degree days in 2005 and 2006. Calo *et al*., 1996 explained that budbreak; the start of the annual growing cycle of the vine is strictly dependent on temperature, which influences the speed of metabolic reaction with a cumulative action to which the buds respond by advancing irreversibly towards a bursting. The period between flowering and the future veraison is usually indicated as the first of three stages of berry growth and development. The weather conditions during this initial growth stage are of fundamental importance, not only for the final dimension of the grape, but also for the future sugar accumulation. Because most of the sugars present in the grape at maturation derive from the translocation of reserves from the different plant parts. Therefore, temperatures compatible with photosynthetic processes, water availability and light intensity are the climatic variables that affect the growth rates of the grapes and shoots (Baldwin, 1966). Increasing N-K levels decreased GDDBB-BL but increasing the cluster levels increased GDDBB-V and GDDBB-H. Normally, wine grape ripening time based on accumulated growing degree days and average monthly temperature at the GDD-derived ripening month, a factor was influencing potential quality (Gladstones, 1992). The grape ripening requires between 1150-1300 GDD above the threshold of 10^{o}C from April to October inclusive (Amerine and Winkler, 1944).

Year 2005 grape yield was higher than year 2006 because of the better distribution of rain in 2005 but the quality of grape berry in 2006 was better than 2005

because of the smaller berry size and lower yield in 2006. Yield, berry per cluster, berry weight, cluster before thinning and shoot weight in both years were decreased but cropload was increased by increasing N-K level and increasing cluster levels increased all of them excepted berry per cluster and berry weight in both years. Bell and Robson, 1999 showed a reduction in berry size and vegetative growth when an application rate of 740.8 kg N / ha was applied. Increases in shoot growth and yield were noted at lower application rates (92.6 kg N / ha and 185.2 kg / ha). Ahlawat and Yamdagni, 1988a; Conradie, 2001; Keller *et al*., 2001b; Zerihun and Treeby, 2002 and yet Matín *et al*., 2004 had noticed no change in vegetative growth as a result of N application. But many researchers reported that increases in N application resulted in increases in crop yield, berry size and fruit set (Ahalwat and Yamdagni, 1988b; Bell and Robson, 1999; Conradie, 2001 and Keller *et al*., 2001a). Unlike the previous research of Delgado *et al*., 2004; Delgado *et al*., 2006 and Martín *et al.*, 2004 have noted that no change in yield and berry size as a response to N application under the condition of their trial. Martín *et al*., 2004 work included the application of 0, 50, and 200 g/vine of N on 'Tempranillo' / 110-Richter. The application of K resulted in increased vine growth, pruning weights, dry mass, shoots / vine, leaves / vine and trunk girth, as noted by Conradie and Saayman, 1989; Morris and Cawthon, 1982 and Wolf *et al*., 1983. Smolarz and Mercik (1997) reported that the lack of K over a number of years could decrease shoot growth considerable in their long-term nutrition study. A reduction of vine growth was also observed by Wolf *et al*., 1983 but they noted it as a result of excessive application of K causing the element to becoming toxic. Ahalwat and Yamdagni, 1988a also reported a similar response but it was noted that the response might have been caused by the use of potassium chloride. As

well as a reduction in growth, Smolarz and Mercik, 1997 also demonstrated that a lack of K resulted in a decrease in crop yield and fruit weight. Many investigators such as Boidron, 1986; Dhillon, 1999; El-Sese *et al*., 1988; Haeseler *et al*., 1981; Kilani, 1979 and Klein *et al*., 2000 reported that potassium fertilization increasing the yield of vine as a result of increasing fruit set, number of cluster, and cluster's weight. Conradie and Saayman, 1989 showed a significant increase in yield at an application rate of 45 kg K / ha while Poni *et al*., 2003 reported no changes in yield, bunches / vine or berry weight as a result of K application. Ahlawat and Yamdagni, 1988a and Delgado *et al*., 2006 also mentioned that no changes in yield but did state that there was a reduction in bunch weights as concentrations of K increased yet it did not affect vine yield. Ahlawat and Yamdagni, 1988a suggested that the decrease in bunch weight might cause toxicity due to the use of Muriate of Potash (Potassium Chloride), indicating that fertilizer selection is important to vine performance. Maras, 2006 found that grape yield depended on number of clusters than from cluster weight. Iacono *et al*., 1994 found that Cabernet Sauvignon grapevine cluster thinning lowered yield appreciably with no changes in leaf area and caused high juice sugar concentration and significantly negative effect of shading on anthocyanin concentration in berry skin. Keller *et al*., 2005 showed that cluster thinning and its timing had little or no influence on shoot growth, leaf area, pruning weight, berry number, and berry weight.

Increasing N-K levels and cluster levels increased petiole N and K at bloom in 2005 and 2006 but berry N and K were not increased. Delgado *et al*., 2006 found that the highest application rate of N and K increased the levels of these nutrients in the leaf tissue, but the lower rates had no effect. Bell and Robson, 1999

also demonstrated an increase in N concentration in the petiole analysis as a result of N application. The increase in petiole analysis was noticed up to an N application rate of 185.2 kg N / ha. Conradie, 2001; Delgado *et al*., 2004 and Wolf *et al*., 1983 have all reported an increase in N concentration in the petiole as a result of N application, indicating that there is a positive response in petiole N concentration to the application of N. Morris and Cawthon, 1982 explored the effects of K on Concord / own roots and suggested that the application of K increased the K concentration in the petiole analysis. Other authors including Cline and Bradt, 1980; Delgado *et al*., 2004; Garcia *et al*., 1999; Morris *et al*., 1980; Morris *et al*., 1987; Poni *et al*., 2003 and Wolf *et al*., 1983 have all noted an increase in the petiole, blade or grape analysis as a result of K application. Morris *et al*., 1987 reported that excessive K fertilization and cluster thinning tended to increase petiole and fruit K and fruit pH in most cultivars. Ruhl, 1989 demonstrated that the concentration of K in the vines petioles was related to pH of grape juice as high K concentration in the petiole resulted in reduced acidity that can result in reduced wine quality.

Berry TSS (^{o}Brix) was not affected by N-K and cluster levels. Increasing N-K levels increased pH, but decreased TA on the other hand; increasing cluster levels decreased pH but increased TA. Delgado *et al*., 2006 reported that both rates of K (60 and 129 g/vine) caused reduction of total acidity. The mechanism seems that excessive K^{+} migrates to the fruit, enhancing the formation of potassium bitartrate, which precipitates, lowering total acidity. Brancadoro *et al*., 1994; Delgado *et al*., 2004; Dundon *et al*., 1984; Morris and Cawthon, 1982; Morris *et al*., 1980; Morris *et al*., 1982 and Morris *et al*., 1983 had all reported a reduction in acidity as a result of K application. A reduction in soluble solids was also observed by Ahalwat and

Yamdagni, 1988b; Christensen *et al*., 1994; Conradie, 2001; Ruhl *et al*., 1992 and Martín *et al.*, 2004. Ahalwat, 1988b noted that the reduction in soluble solids could be a result of growth dilution caused by excessive growth as a result of N application. Ruhl *et al.*, 1992 assessed the affects of N application on other juice composition parameters of Riesling, Chardonnay and Cabernet Sauvignon grown in different regions (Sunraysia, Coonawarra and Mornington Peninsula). The investigation revealed that the N application in Chardonnay significantly increased juice pH, citrate and malate while lowering chloride concentration. In Riesling an increase in juice pH and malate was observed, while in Cabernet Sauvignon increased pH, potassium concentration, citrate, malate and tartrate significantly. The results suggested that the increasing juice pH would result in a poorer quality end product. Ahalwat and Yamdagni, 1988b reported a decrease in juice pH as a result of N application, which contradicted previous findings. Ahalwat and Yamdagni, 1988a showed that the soluble solids increased with increasing applications of K with the highest level recorded at an application of 300 g K / vine. However, this result differed from Delgado *et al*., 2004 and Dundon *et al*., 1984 findings, which showed no changes in soluble solid concentrations. Egger *et al*., 1996 found that the number of bunches per plant play a significantly negative role in determining the sugar content at the time of harvest. The average bunch weight appears to be negatively correlated with the acidity of the must, larger bunches is less acid. Arfelli *et al*., 1996 found that cluster thinning after veraison strongly improves the accumulation of sugars during ripening whereas the titratable acidity decline is less influenced. Nevertheless, Keller *et al*., 2005 reported that cluster thinning and its timing had little or no influence on fruit composition (soluble solids, titratable acidity, pH, and color)

in both the current and subsequent seasons. Guidoni *et al*., 2002 found that soluble solids, berry skin anthocyanins, and flavonoids were more concentrated in berries from cluster-thinned plants. Gao and Cahoon, 1998 reported that juice soluble solids concentration (SSC) was increased significantly by cluster thinning treatments. Weight of individual berries was heavier with 20 clusters per vine than with 60.

5.1.2 Grape berry color and phenolic compounds

The 0-0 and the 200-60 levels of N-K level had the highest grape color and phenolic compounds but increasing cluster levels decreased them. In 2005, total phenolic compounds were lower than in 2006 due to smaller berries. Delgado *et al*., 2006 reported that an increase in N caused a significant decrease in total polyphenols, which found that excessive N reduces polyphenol synthesis in the berry skins. However, when K was also high, the treatments with maximum N had higher polyphenols. In the fruits with the highest polyphenol levels, the N: K ratio was 3.6-4.3. Gao and Cahoon, 1998 reported that fruit red pigmentation was increased dratically by cluster thinning. Total anthocyanin concentration in berry skin was increased linearly by cluster thinning.

5.1.3 Grape anthocyanin

Increasing N-K level seemed to decrease Pt but increase Mv as well as increasing cluster level increased Cy, Pt, and Mv. Delgado *et al.*, 2004 found that the nitrogen dose of 50 g N per vine increased the levels of anthocyanins in the skin (600 mg/l standard extract) compared with the untreated control vines (532 mg/l), and this significantly increased the colour density of the must and an increase in the

anthocyanin levels at an application rate of 60 and 120 g K/vine with an increase of 23% and 40%, respectively. Delgado *et al*., 2006 found that when there was no K or medium levels of K, increasing rates of N decreased the content of total anthocyanins. But when K was high, the anthocyanin levels in the fruit receiving the highest N rates were not different from those of control. Because the formation of malvidin-glucoside appears to be more tolerant of unfavorable environmental conditions than other anthocyanins, it becomes dominant in grapes grown in poor light or excessive heat, particularly in combination with excess nitrogen (Spayd *et al*., 2002). Guidoni *et al.*, 2002 reported that cluster thinning increased the concentrations of cyanidin-3-glucoside, peonidin-3-glucoside, and, to a lesser extent, petunidin-3-glucoside. Concentrations of malvidin-3-glucoside and of acylated anthocyanins were not affected by cluster thinning. Gao and Cahoon, 1998 reported that total anthocyanin concentration in berry skin was increased linearly by cluster thinning. The concentration of individual anthocyanins, including cyanidin-3-glucoside, peonidin-3-glucoside, and acylated cyanidin derivative, was increased linearly by cluster thinning. However, the concentration of delphinidin-3-glucoside, or petunidin-3-glucoside, or malvidin-3-glucoside, was not significantly affected. Cluster thinning linearly increased the percentage of cyanidin-3-glucoside and decreased the percentage of the acylated cyanidin derivative. The percentages of delphinidin-3-glucoside, petunidin-3-glucoside, peonidin-3-glucoside, and malvidin-3-glucoside were not affected by cluster thinning. This experimental showed that Mv was the major anthocyanin in Cabernet Sauvignon grape (Burns *et al*., 2002; Cholet and Darne, 2004; Gomez-Miguez *et al*., 2006; Mateus *et al*., 2001; Pomar *et al*., 2005; Wang *et al*., 2003b).

5.2 Wine performance

5.2.1 Wine quality

Increasing N-K level increased wine pH, decreased wine TA but increasing cluster level decreased wine pH and VA, increased wine TA and Alc. These results almost the same for both years. Keller *et al*., 1999 reported that high N supply increased pH in the juice and wine. Crop thinning may have detrimental effects on wine quality by disturbing the natural balance of the vine and increasing vegetative growth (McDonnell *et al*., 2008).

Wine color density was decreased from pressed to aged wine. Aged wine, which increased cluster levels were decreased. Wine color density of pressed wine in 2005 was lower than 2006 but 2005 aged wine was higher than 2006 aged wine. In both years, increasing N-K levels and cluster levels seemed to increase wine color density. Somers, 1998 found that for red wines differing in age by 2 years, the wine color density values ranged between 3, and 25 a.u. Almela *et al*., 1999 found that treatment involves the use of more water and fertilizers showed no significant improvement on wine color.

Wine color hue was increased from pressed to aged wine and pressed wine which increased N-K and cluster levels were decreased. An increase in the hue value is expected for a red wine as its age describes a shift from purple red via brick red to brown tones of the wine colour. Alcalde-Eon *et al*., 2006 reported that as wine became older, the percentages of anthocyanins decreased slightly, whereas that of the anthocyanin derived pigments increased and, above all, compounds providing the wine with orange hues (pyranoanthocyanins). Pyranoanthocyanins are generated by reaction between monomeric anthocyanins and wine compounds having a polarised

double bond, as do some secondary yeast metabolites (pyruvic acid), 4-vinylphenols, 8-vinylflavanols, and hydroxycinnamic acids (Fulcrand *et al*., 1998; Hayasaka and Asenstorfer, 2002 and Mateus *et al*., 2003). In young wines, the hue was 0.4 to 0.5 which increased to 0.8 to 0.9 in aged red wines (Somers and Verette, 1988). Delgado *et al*., 2006 reported that low application rates of N caused a major increase in hue and yellow component, but did not modify color intensity. However, the highest rate of N increased color intensity as well as the red and blue hues and K, by per se, reduced red hue and increased the yellow component.

Degree of red pigment coloration was increased from pressed to aged wine. During fermentation and as the wine ages, the anthocyanin combine with each other, other phenolic material and fermentation metabolites, eg pyruvic acid to from new compounds. These include both small molecules and larger polymeric material, which may be colorless, red or yellow/brown. The red pigments fromed are less sentitive to pH and SO_2 adjustment than the free anthocyanins. Increasing cluster levels increased degree of red pigment coloration in pressed wine but decreased in aged wine (Iland *et al*., 2000).

Estimate SO_2 resistant pigments were increased from pressed to aged wine. At the highest level of N-K of pressed and aged wine were highest, increasing cluster level of aged wine was increased but not of pressed wine. It is well known that polymeric pigments are more stable to SO_2 bleaching than monomeric pigments (Versari *et al*., 2008).

Total red pigments were increased from pressed to aged wine. Versari *et al*., 2008 suggested that the polymerization of colored phenolics might occur in a similar way in the age of wine probably being the factor making more difference.

Pressed wine, increasing N-K levels were increased total red pigments but increasing cluster levels were decreased total red pigment. Aged wine, increasing N-K level was no effect but the highest level of cluster was highest of total red pigments.

Increasing cluster levels of pressed wines decreased total phenolics but aged wines were not affected. Cliff *et al*., 2007 reported that younger wines had higher concentrations of copigmented, monomeric, and total anthocyanins than did older.

Modified wine color density of pressed and aged wines were increased by increasing N-K levels but were decreased by increasing cluster levels that were the same both years. The color of red wines depends largely on its phenolic composition, notably the levels of anthocyanins, polymeric pigments, and anthocyanin derived pigments (Cheynier *et al*., 2006 and Fulcrand *et al*. 2006). Thus evaluation of the phenolic composition of the grapes may allow a more direct evaluation of the quality of grapes. Although the phenolic compounds in wine originate from the grapes, the relation between grape and wine phenols is complicated due to several factors. Extraction of the phenols from the grapes into the fermenting must is an incomplete process, which rarely extracts more than 50 % of the phenols from the grapes. In addition the phenols are reactive compounds, and will continuously undergo several chemical changes during the entire winemaking process, including condensation reactions with other phenols. Such reactions impact the wine color. Wine color is also highly affected both by pH and sulfite levels, but also by the presence of non-colored compounds, in particular other phenols, that can enhance the color by molecular associations with the pigments.

Modified wine color hue was not significantly different by N-K levels both years but pressed wines were decreased in the other hand aged wines were increased by increasing cluster levels. The variation in the level of polymeric pigments is affected by a number of factors, including vintage, grape composition, fermentation and storage conditions. In particular, the decrease in polymeric pigments may be due to precipitation and/or the lighter hue of the larger polymers. There is general consensus on the increase in large polymeric pigments content in red wines with age (De Beer *et al*., 2004).

Increasing cluster levels decreased modified degree red pigment coloration of aged wines but none of pressed wine. Bakker *et al*., 1986 have shown that oligomeric pigments are also bleached to some extent by bisulphite.

Increasing N-K levels and cluster levels increased wine color density in year 2005 pressed wines. Increasing N-K levels decreased wine color hue. Increasing cluster levels increased degree of red pigment coloration. Estimate SO_2 resistant pigments seemed to increase by increasing N-K levels. Total red pigments were increased by increasing N-K levels but were decreased by increasing cluster levels. Increasing cluster levels decreased total phenolics. But 2006 pressed wines were not significantly different for all of them. Increasing N-K levels and cluster levels decreased aged wines year 2005, wine color hue. Increasing cluster levels decreased estimate SO_2 resistant pigments. In year 2006, increasing cluster levels decreased wine color density and degree of red pigment coloration. Progressive increases in pigment resistance to bleaching by bisulfite and decreases in color gain on polymer acidification are envisaged, as oligomeric and polymeric pigments of increasing complexity are formed during wine aging. Losses of total pigments and

total free anthocyanins were logarithmic with time during wine aging (Bakker *et al*., 1986).

5.2.2 Wine anthocyanin

Pressed and aged wine were not significantly different of wine anthocyanin content by N-K level but increasing cluster level seemed to increase wine anthocyanin content of pressed wine especially in 2006. The 20 clusters level was the highest wine anthocyanin content. Mv was the most abundance anthocyanin in both pressed and aged wines. 2005 pressed wine was higher for Dp, Pt and Pn but lower for Cy and Mv than 2006. 2005 Aged wine was higher Cy but lower for Dp, Pt, Pn and Mv than 2006. The ratio between Mv and Pn was 25.1 and 19.2 in pressed and aged wine, respectively. There were the same results with many experiments (Monagas *et al*., 2003; Revilla *et al*., 2001.). The ratio between malvidins and peonidins (Mv/Pn), which is related to the flavonoid-3'-hydroxylase (FH) and o-dihydroxyphenyl-O-methyltransferase (MT) enzyme activities in plants (Roggero, Coen and Larice, 1986), was lower for Graciano (4.6 and 5.1, respectively, after 1.5 and 12 months in bottle) than for Tempranillo (57.6 and 77.2) and Cabernet Sauvignon (39.2 and 51.3) (Monagas *et al*., 2003). In young red wine, some researchers believe the enhanced perception of wine color is considered to be influenced by copigmentation and self-association reactions of anthocyanins with other polyphenols (Asen *et al*., 1972). In aged red wine, the concentration of malvidin 3-glucoside and other anthocyanins decrease to almost negligible amounts (Hermosin Gutierrez *et al*., 2005; Schwarz *et al*., 2003; Somer, 2003). At wine pH (pH 3.5), the less colored quinodal based forms and a complex mixture of the neutral and anionic species of malvidin 3-glucoside are the predominant forms (Asenstorfer

et al., 2003). Hermosin Gutierrez *et al.*, 2005 found that the fraction of red colour due to copigmented anthocyanins ranged from 32% to 43% at the end of the alcoholic fermentation, and this decreased to 20–34% after 3 months of ageing, when a decrease in both monomeric anthocyanin and flavonol concentrations was also observed and after 9 months of ageing, the amounts of remaining monomeric anthocyanins were 40% and 38% for Syrah and Cencibel wines, respectively, whereas Cabernet Sauvignon wines only retained 32% of the monomeric anthocyanins initialy found at the end of alcoholic fermentation and Pinheiro *et al*. (2009) reported that at the end of the study, a reduction of 59.83% in the content of anthocyanins, in relation to the initial time of storage, was verified. Gambelli and Santaroni, 2004 indicated that high amounts of malvidin-3-glucoside are stilled present in aged wine. The petunidin was determined only in young wines. Peonidin and cyaniding decreased during ageing. The delphinidin content was lower than peonidin and cyaniding.

CHAPTER VI

CONCLUSION

There were no significantly differences of growing degree days between 2005 and 2006. In 2006 there was much more rain in veraison to harvesting period than 2005. All duration and degree days between phenological stages were significantly different except GDDBB-V, and GDDV-H. In 2005, increasing N-K level increased GDDBL-H and increasing cluster level increased GDDBB-V and GDDBB-H. Increasing N-K level increased DBL-V, crop load, pH but decreased DBB-BL, DDBB-BL, cluster before thinning, shoot weight, and TA. Increasing of cluster number increased DBB-BL, DBB-V, DBB-H, GDDBB-V, GDDBB-H, and cluster before thinning, shoot weight, crop load, yields, TA, but decreased phenolic compounds in grape berry, wine pH, and VA. The result showed that at 100-20 of N-K level causeed lower amount of phenolic compounds in grape and wine than others level. Pressed wine at 100-20 of N-K level has lower phenolic compounds than other but increasing cluster level increased degree of red pigment coloration and modified wine color hue, whilst decreased total red pigments, total phenolics and modified wine color density. Aged wine, increasing cluster level increased estimate SO_2 resistant pigment, but decreased wine color density, degree of red pigment coloration, modified wine color density, modified wine color hue, and modified degree red pigment coloration. 2005 pressed wine was lower in wine color density, wine color hue, degree of red pigment coloraion, estimate SO_2 resistant pigment, but was higher in total red pigment,

total phenolic than 2006. 2005 aged wine had higher wine color density, degree of red pigment coloration, estimate SO_2 resistant pigment than 2006 but showed the same amounts of total red pigment and total phenolic. Modified degree red pigment coloration of aged wine was higher than pressed wine in both years. Wine anthocyanin content was significantly different between pressed and aged wine in both years. In both years, the main wine anthocynin of pressed and aged wine was Mv. 2005 wine had higher anthocyanin content than 2006. 2005 pressed wine was higher in Dp, Pt, and Pn but lower Cy and Mv than 2006. 2005 aged wine had higher amount of Cy but lower in Dp, Pt, Pn, and Mv than in years 2006. All these data suggest that the changes in the each anthocyanins content of wines in relation to the each anthocyanin content of grapes probably took place during winemaking and wine ageing. This fact could be explained by several hypotheses. This could reside in the existing differences: rate of anthocyanin degradation, or polymerization during winemaking, and wine ageing. The answer to all these questions needs further research to understand differences between the each anthocyanins content of grapes and corresponding wines.

Results obtained from these researches could be recommended that the application of fertilizer inthis vine yard should be none or at minimal within the next four years. Since wine quality was also influenced by grape berries quality thus the management of cultural practices to control the grape berries quality should be considered.

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APPENDICES

APPENDIX A

EXPERIMENTAL WEATHER DATA

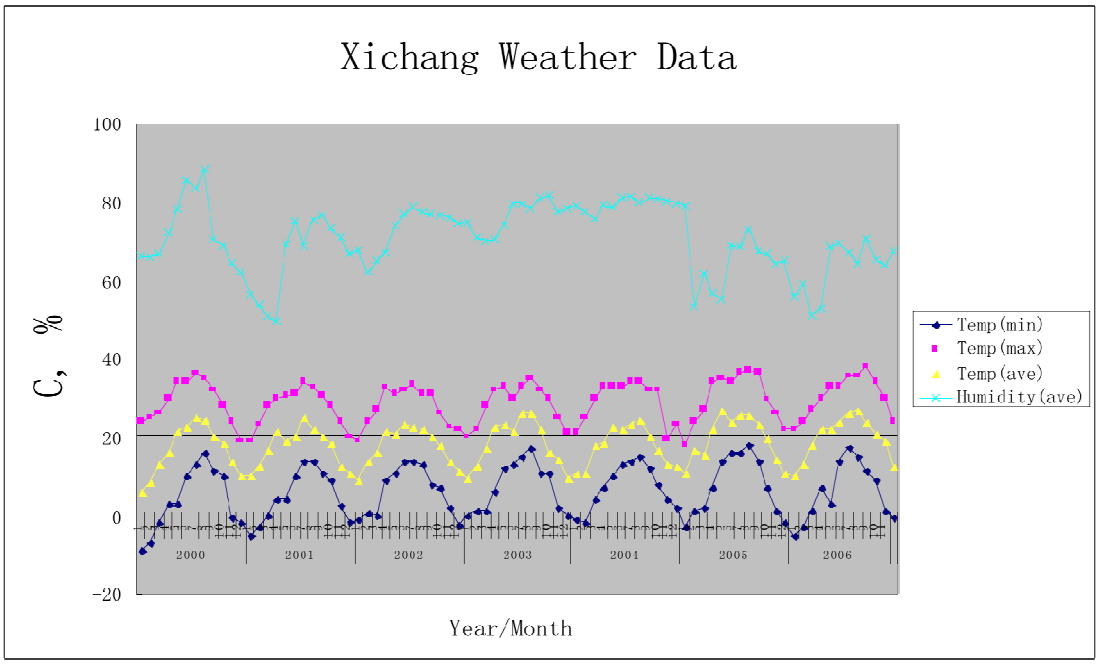

Figure 7.1 Climacterics data at experimental station from 2000-2006.

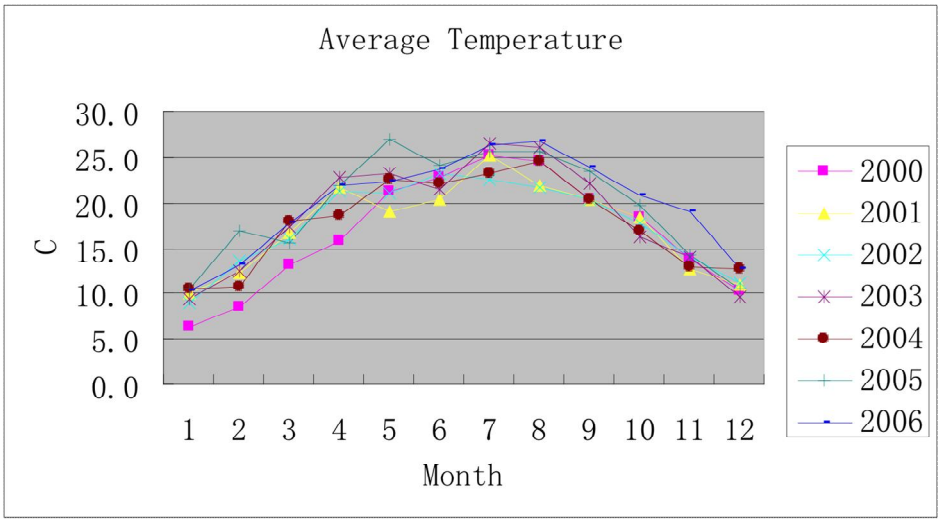

Figure 7.2 Average monthly temperature at experimental station from 2000-2006.

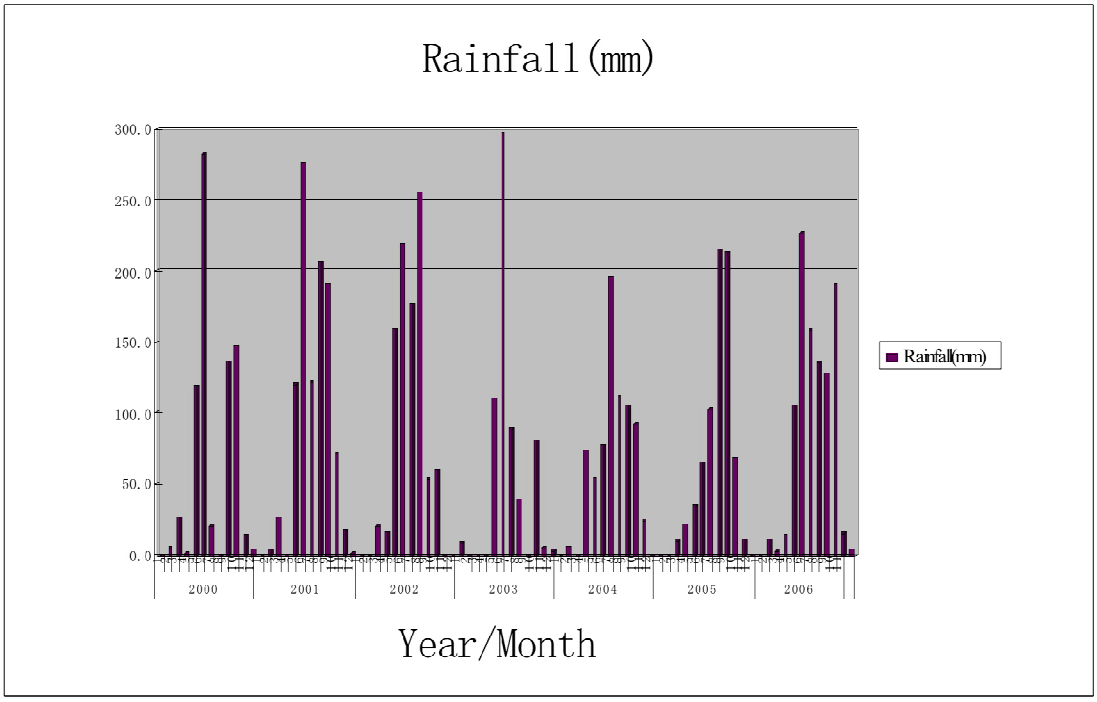

Figure 7.3 Monthly rainfall from 2000-2006 at experimental station.

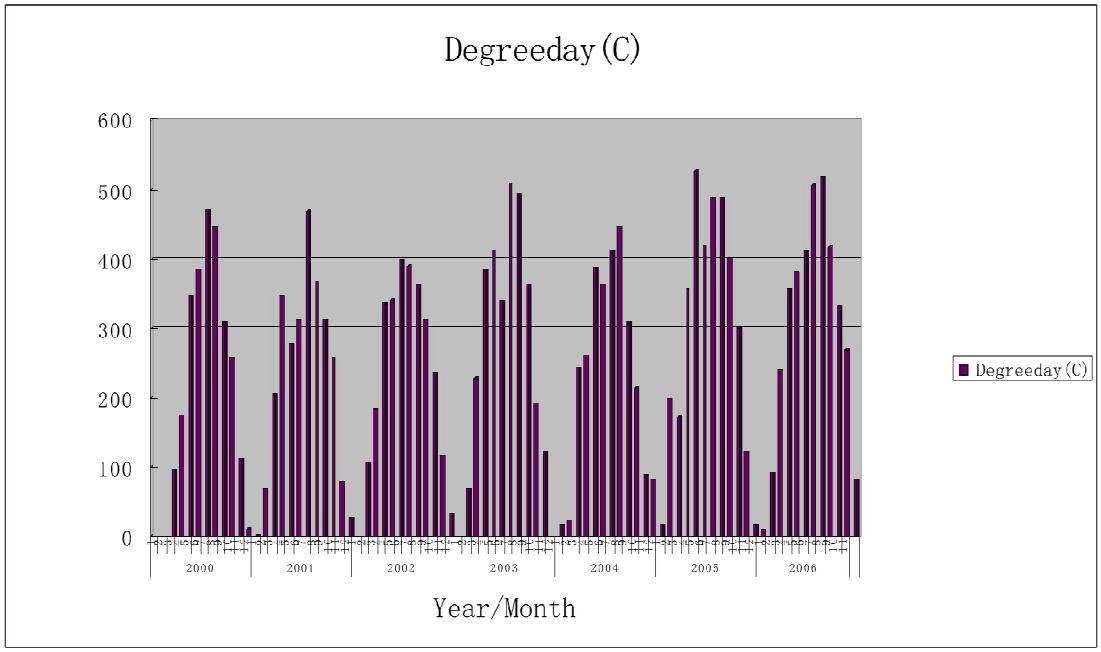

Figure 7.4 Monthly degree days from 2000-2006 at experimental station.

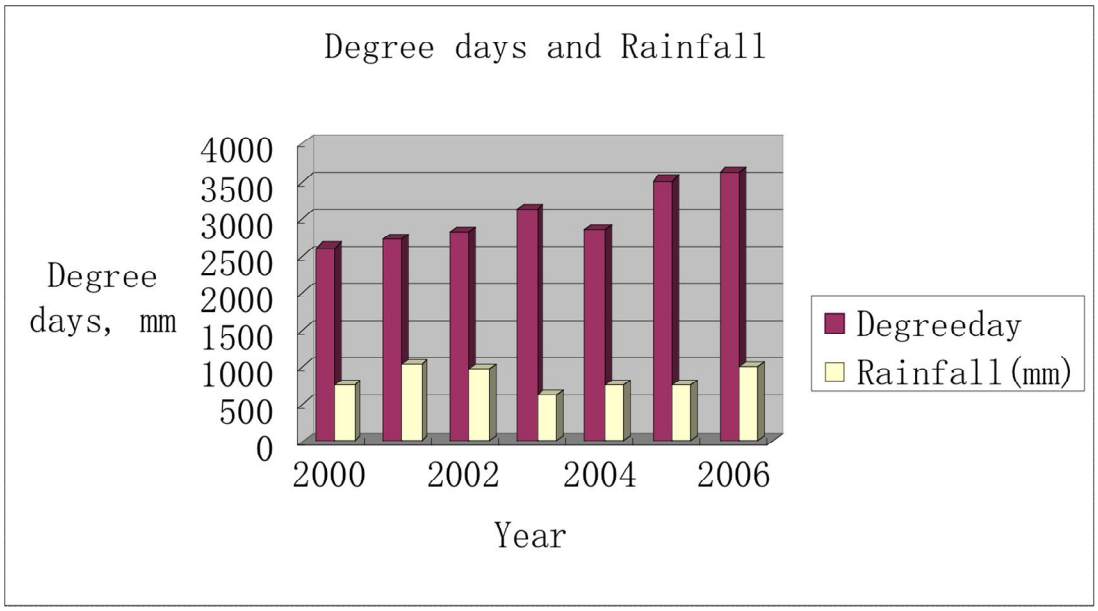

Figure 7.5 Yearly degree days and rainfall from 2000-2006 at experimental station.

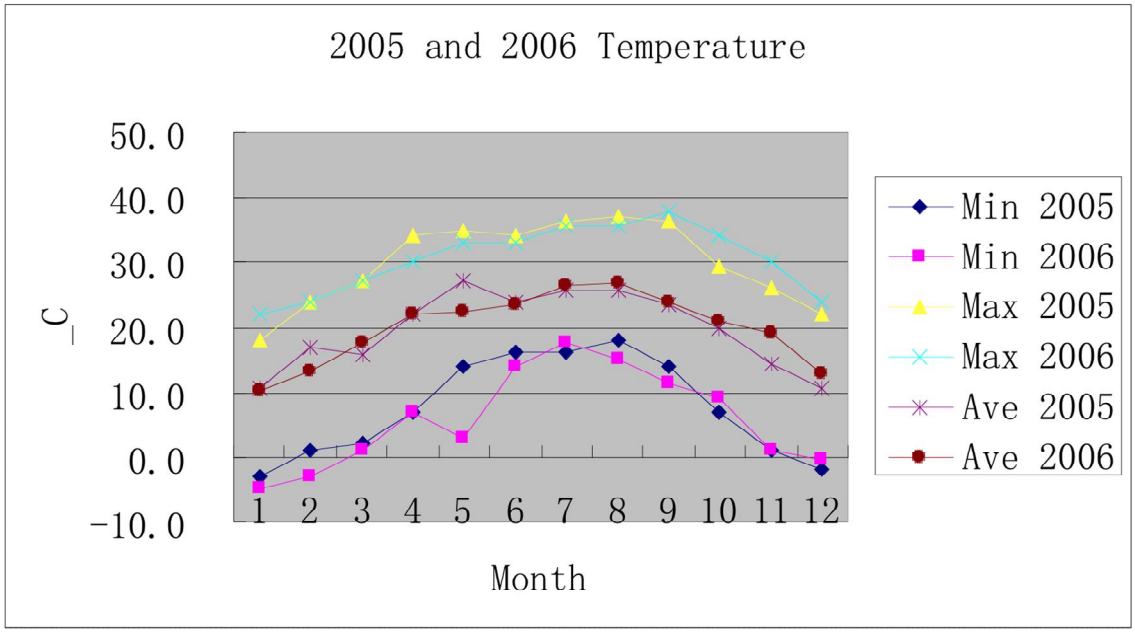

Figure 7.6 Monthly min, max and mean temperatures in 2005 and 2006.

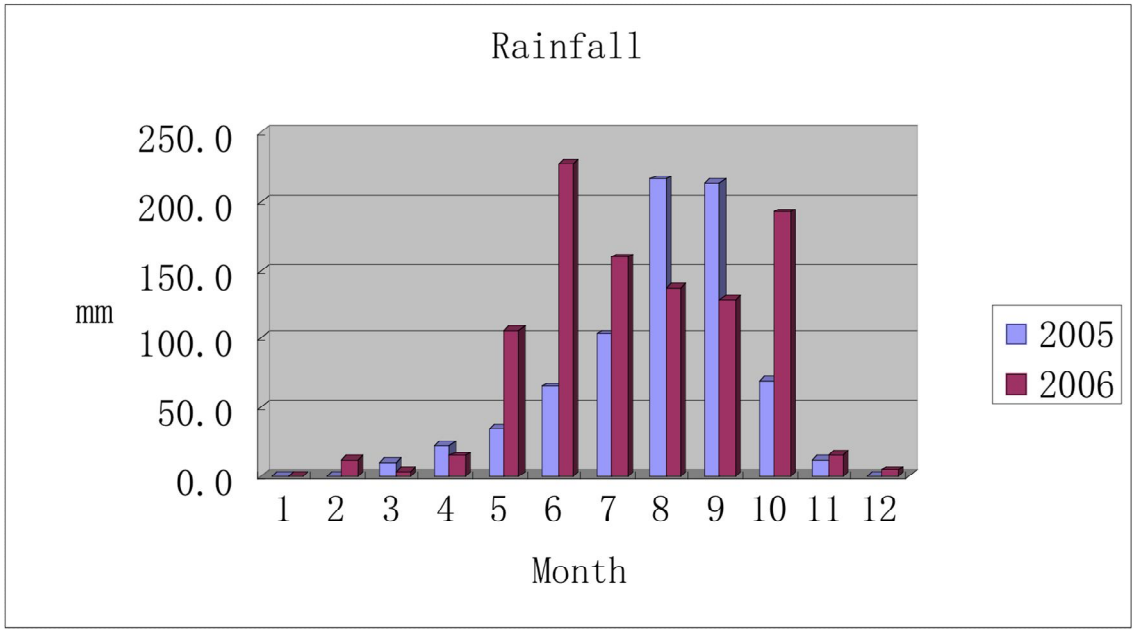

Figure 7.7 Monthly rainfalls in 2005 and 2006.

APPENDIX B

SOIL ANALYSIS DATA AND ANOVA

Table 7.1 Soil analysis data of experimental station.

Title	2005	2006
pH	5.25±0.12	5.79±0.13
OM (%)	0.70±0.24	0.75±0.16
N (ppm)	9.90±0.33	9.80±0.21
P (ppm)	9.80±0.75	19.40±0.13
K (ppm)	470.00±44.60	156.00±50.90
Ca (ppm)	300.00±11.27	229.00±4.12

± Standard error of mean

Table 7.2 Analysis of variance of duration for various phonological periods of grapevine.

SV	df	DBB-BL	DBB-V	DBB-H	DBL-V	DBL-H	DV-H
BLOCK (B)	5	6.69ns	17.26ns	3.28ns	8.99ns	12.48ns	18.56ns
FERT (F)	2	15.53ns	1.82ns	0.01ns	6.73ns	15.62ns	1.86ns
ERROR (a)	10	5.92	7.80	2.63	5.79	6.19	13.25
YEAR (Y)	1	655.15**	1648.95**	116.15**	4382.82**	1323.00**	889.82**
CLUSTERS (C)	2	9.19*	17.82ns	14.57**	6.73ns	1.15ns	3.25ns
FxC	4	1.147ns	3.51ns	8.32*	1.04ns	5.54ns	6.28ns
ERROR (b)	83	2.84	6.62	2.97	5.79	2.99	6.24
TOTAL	107						

** = significant at 1% level; * = significant at 5% level; ns = not significant

Table 7.3 Analysis of variance of growing degree days for various phonological periods of grapevine.

SV	df	DDBB-BL	DDBB-V	DDBB-H	DDBL-V	DDBL-H	DDV-H
BL (B)	5	422.03ns	3675.33ns	1426.49ns	3165.30ns	2385.49ns	9117.02ns
FER (F)	2	2307.30*	567.11ns	35.92ns	649.68ns	2433.44ns	590.89ns
ER (a)	10	548.21	1771.13	707.48	1660.31	1020.28	3942.84
YE (Y)	1	165850.51**	492.42**	90793.78**	37918.89**	7184.88**	78108.99ns
CL (C)	2	314.44ns	6239.67*	1059.91*	2763.34ns	361.63ns	2293.33ns
FxC	4	492.30ns	1095.75ns	96.21ns	1707.96ns	292.78ns	694.57ns
ER (b)	83	538.84	1775.19	324.97	1468.69	400.36	1555.73
TOTAL	107						

** = significant at 1% level; * = significant at 5% level; ns = not significant

Table 7.4 Analysis of variance of Yields (kg), Berry/cluster, Berry weight (g), TSS (Brix), TA (g/l) and pH.

SV	df	Yields (kg)	Berry/cluster	Berry weight (g)	TSS (Brix)	TA (g/l)	pH
BL (B)	5	0.59ns	7.08**	2.32ns	2.83ns	3.72*	1.14ns
FER (F)	2	0.33ns	0.87ns	4.27*	0.91ns	4.70*	10.25**
ER (a)	10	0.27	471.30	0.01	0.93	0.39	0.01
YE (Y)	1	45.71**	459.71**	305.26**	21.90**	680.84**	953.46**
CL (C)	2	15.98**	1.97ns	0.43ns	1.92ns	9.84**	5.03**
FxC	4	0.06ns	0.69ns	0.31ns	3.43*	4.30**	2.02ns
ER (b)	83	0.20	587.17	0.01	0.56	0.62	0.01
TOTAL	107						

** = significant at 1% level; * = significant at 5% level; ns = not significant

Table 7.5 Analysis of variance of Cluster before thinning, Shoot weight (g) and Cropload.

SV	df	Cluster (BF.thin)	Shoot Weight (g)	Cropload
BL (B)	5	68.77ns	2.94ns	1.88ns
FER (F)	2	130.01*	7.06*	3.36*
ER (a)	10	28.77	0.41	0.86
YE (Y)	1	1712.04**	19.10**	276.44**
CL (C)	2	352.15**	8.12**	48.48**
FxC	4	12.51ns	0.78ns	0.31ns
ER (b)	83	28.41	0.02	0.64
TOTAL	107			

** = significant at 1% level; * = significant at 5% level; ns = not significant

Table 7.6 Analysis of variance of color and phenolic compounds in grape berry.

SV	df	mg color/berry	mg color/g berry	Total phenolic/berry	Total phenolic/ g berry
BL (B)	5	0.07ns	0.11**	0.02ns	0.03ns
FER (F)	2	0.15*	0.07ns	0.27*	0.156ns
ER (a)	10	0.03	0.3	0.05	0.05
YE (Y)	1	0.01ns	0.13*	4.62**	7.85**
CL (C)	2	0.13*	0.05ns	0.08ns	0.03ns
FxC	4	0.04ns	0.11**	0.06ns	0.11**
ER (b)	83	0.04	0.2	0.03	0.02
TOTAL	107				

** = significant at 1% level; * = significant at 5% level; ns = not significant

Table 7.7 Analysis of variance of grape berry anthocyanin contents.

SV	df	Pt	Cy	Mv
BL (B)	5	7.46**	0.001ns	0.074**
FER (F)	2	0.03**	0.001ns	0.047*
ER (a)	10	0.002	0.000	0.009
YE (Y)	1	0.37**	0.063**	0.039ns
CL (C)	2	0.13**	0.003**	0.142**
FxC	4	0.02**	0.001ns	0.017ns
ER (b)	83	0.004	0.000	0.016
TOTAL	107			

** = significant at 1% level; * = significant at 5% level; ns = not significant

Table 7.8 Analysis of variance of Nitrogen and Potassium in petiole at blooming period and in grape berry.

SV	df	Petiole N (%)	Berry N (%)	Petiole K (%)	Berry K (%)
BL (B)	5	3.59*	0.70ns	0.34ns	0.64ns
FER (F)	2	0.22ns	1.65ns	1.22ns	1.02ns
ER (a)	10	0.07	0.02	1.77	1.03
CL (C)	2	1.392ns	0.94ns	0.26ns	0.91ns
FxC	4	0.79ns	0.50ns	0.02ns	0.80ns
ER (b)	84	0.03	0.02	0.36	0.86
TOTAL	107				

** = significant at 1% level; * = significant at 5% level; ns = not significant

Table 7.9 Analysis of variance of pressed wine characteristic.

SV	df	pH	TA	Alc	VA
BL (B)	2	0.00ns	0.35ns	0.01ns	0.00ns
FER (F)	2	0.00ns	1.05ns	0.03ns	0.00ns
ER (a)	4	0.01	0.23	0.08	0.01
YE (Y)	1	0.61**	4.62**	4.51**	1.39**
CL (C)	2	0.01**	0.67*	0.83**	0.04ns
FxC	4	0.01**	0.26ns	0.03ns	0.01ns
ER (b)	38	0.01	0.20	0.17	0.01
TOTAL	53				

** = significant at 1% level; * = significant at 5% level; ns = not significant

Table 7.10 Analysis of variance of wine color characteristics (1).

SV	df	Wine color density		Wine color hue		Degree of red pigment coloration (%)	
		Press	Age	Press	Age	Press	Age
BL (B)	2	0.15ns	0.30ns	0.00ns	0.001ns	3.64ns	41.95ns
FER (F)	2	2.48*	3.39*	0.01*	0.013ns	15.72ns	381.70ns
ER (a)	4	0.17	3.39	0.001	0.01	4.91	566.40
YE (Y)	1	11.44**	48.41**	177.85**	0.98**	102.78**	2789.29**
CL (C)	2	0.47ns	7.32**	0.01*	0.27ns	29.62*	815.16*
FxC	4	0.35ns	5.56**	0.00ns	0.008ns	3.62ns	733.67**
ER (b)	38	0.19	1.24	0.002	0.013	8.37	186.51
TOTAL	53						

** = significant at 1% level; * = significant at 5% level; ns = not significant

Table 7.11 Analysis of variance of wine color characteristics (2).

SV	df	Estimate of SO_2 resistant pigments	
		Press	**Age**
BL (B)	2	0.01ns	0.079ns
FER (F)	2	0.05*	2.074*
ER (a)	4	0.02	1.335
YE (Y)	1	0.02ns	8.18**
CL (C)	2	0.00ns	4.35**
FxC	4	0.03ns	2.71**
ER (b)	38	0.01	0.59
TOTAL	53		

** = significant at 1% level; * = significant at 5% level; ns = not significant

Table 7.12 Analysis of variance of wine color characteristics (3) (Modified Wine Color Density (MWCD), Modified Wine Color Hue (MWCH), Modified Degree of Red Pigment Coloration (MDRPC).

SV	df	MWCD		MWCH		MDRPC	
		Press	**Age**	**Press**	**Age**	**Press**	**Age**
BL (B)	2	0.24ns	0.28ns	0.00ns	0.00ns	4.75ns	69.79ns
FER (F)	2	2.11*	2.81**	0.00ns	0.00ns	4.36ns	280.76ns
ER (a)	4	0.13	1.27	0.01	0.00	3.45	411.15
YE (Y)	1	1.98**	14.97**	0.47**	0.05**	2.28ns	706.34*
CL (C)	2	0.34ns	2.26*	0.01*	0.02**	0.06ns	336.50*
FxC	4	0.22ns	2.51**	0.00ns	0.00ns	0.69ns	495.18**
ER (b)	38	0.12	0.59	0.01	0.00	5.79	121.83
TOTAL	53						

** = significant at 1% level; * = significant at 5% level; ns = not significant

Table 7.13 Analysis of variance of wine color characteristics (4).

SV	df	Mod. SO_2 resistance		Total red pigments		Total phenolics	
		Press	Age	Press	Age	Press	Age
BL (B)	2	0.01ns	0.08ns	0.10ns	7.23ns	0.48ns	5.32ns
FER (F)	2	0.05ns	2.07*	6.61*	3.10ns	6.49ns	0.11ns
ER (a)	4	0.02	1.34	0.49	10.89	1.88	5.19
YE (Y)	1	0.02ns	8.18**	17.79**	5.67ns	20.05ns	0.02ns
CL (C)	2	0.00ns	4.35**	6.29ns	12.63*	23.81*	9.35ns
FxC	4	0.03ns	2.71**	0.36ns	16.04**	4.09ns	11.47ns
ER (b)	38	0.01	0.59	2.17	3.77	5.73	4.44
TOTAL	53						

** = significant at 1% level; * = significant at 5% level; ns = not significant

Table 7.14 Analysis of variance of wine anthocyanin contents of pressed wine.

SV	df	Dp	Cy	Pt	Pn	Mv
BL (B)	2	0.000ns	0.000ns	0.000ns	0.000ns	0.000ns
FER (F)	2	0.000ns	0.000ns	0.000ns	0.000ns	0.002ns
ER (a)	4	0.000	0.000	0.000	0.000	0.001
YE (Y)	1	0.004**	0.012**	0.001*	0.001*	0.041**
CL (C)	2	0.000ns	0.001**	0.000ns	0.001*	0.009ns
FxC	4	0.000ns	0.000ns	0.000ns	0.000ns	0.014*
ER (b)	38	0.000	0.000	0.000	0.000	0.004
TOTAL	53					

** = significant at 1% level; * = significant at 5% level; ns = not significant

Table 7.15 Analysis of variance of wine anthocyanin contents of aged wine (6 months after pressing).

SV	df	Dp	Cy	Pl	Pn	Mv
BL (B)	2	0.000ns	0.000ns	0.000ns	0.000ns	0.000ns
FER (F)	2	0.001ns	0.000ns	0.000ns	0.000ns	0.002ns
ER (a)	4	0.000	0.000	0.000	0.000	0.002
YE (Y)	1	0.000*	0.000*	0.000*	0.000ns	0.009*
CL (C)	2	0.001ns	0.000ns	0.001ns	0.000ns	0.002ns
FxC	4	0.001**	0.001ns	0.001ns	0.000ns	0.009**
ER (b)	38	0.001	0.000	0.000	0.000	0.002
TOTAL	53					

** = significant at 1% level; * = significant at 5% level; ns = not significant

Table 7.16 Analysis of variance of wine anthocyanin content of pressed and aged wine.

SV	Df	Pressed wine	Aged wine
BL (B)	2	0.000ns	0.001ns
FER (F)	2	0.003ns	0.003ns
ER (a)	4	0.001	0.002
YE (Y)	1	0.060**	0.013*
CL (C)	2	0.017*	0.003ns
FxC	4	0.017*	0.013**
ER (b)	38	0.005	0.003
TOTAL	53		

** = significant at 1% level; * = significant at 5% level; ns = not significant

APPENDIX C

Appendix 1C. Determination of red pigments (color) and total phenolics of grape berries by the method of Iland *et al*., 2000.

Procedure for preparing the sample

1. Weigh a sample of 50 berries.
2. Transfer the berries to an homogenizing vessel, 125 mL plastic container.
3. Homogenize the berries at high speed, 24000 rpm for 30 seconds. The homogenizer should macerate the flesh, skins and seeds into a homogeneous mixture, so that a representative sub sample can be taken in step 6. There should not be any large pieces of skins or seeds in the mixture.
4. Scrape any homogenate from the shaft of the homogenizer into the homogenizing vessel and repeat the homogenizing step for about 15 seconds.
5. Scrape any homogenate remaining on the shaft of the homogenizer back into the homogenizing vessel.
6. Thoroughly mix the homogenate by stirring it with a small spoon type spatula and immediately take a scoop of approximately 1 gram of homogenate using the spatula.
7. Transfer the scoop of homogenate into a pre-tared centrifuge tube. Record the weight. This is termed ‘the weight of homogenate taken for extraction’.

Procedure for extracting the anthocyanins

1. Pipette 10 mL of 50% v/v aqueous ethanol adjusted to pH 2.0 into the centrifuge tube containing the homogenate. Cap the tube and mix the contents periodically by inverting the tube about every 10 minutes over a period of 1 hour.

2. After 1 hour, centrifuge the tube and contents at 3500 rpm for 5 minutes. The supernatant is termed 'the extract'.

Procedure for determining the red color and total phenolics of the extract

1. Pipette 1.0 mL of 'the extract' into 10 mL 1 M HCl and mix this solution thoroughly. (in this case the dilution factor is 11)

2. Pour the remaining volume of 'the extract' into a tall, thin measuring cylinder and record the volume. Add the value of this recorded volume and the value of the volume of 'the extract' that had previously been taken in step 10 to obtain a value which is termed 'the total extract volume'.

3. Allow the diluted HCl extract solution to stand for about three hours.

4. After 3 hours, using a spectrophotometer, read the absorbance of the diluted HCl extract in a 1 cm cell at 700 nm, 520 nm and 280 nm.

Calculation

Color per berry (anthocyanins mg per berry) = $A_{520}/500$ x dilution factor x final extract volume (mL)/100 x weight of 50 berries (g)/weight of homogenate taken for extraction (g) x 1000/50

Color per gram berry weight = Color per berry/weight of 50 berries (g)

Total phenolics per berry = A_{280} x dilution faction x final extract volume (mL)/100 x weight of 50 berries (g)/weight of homogenate taken for extraction (g) x 1/50

Total phenolics per gram berry weight = Total phenolics per berry/weight of 50 berries (g)

Remark: The measure of absorbance at 700 nm (A_{700}) is a check for sample turbidity; excessive turbidity is an indication of insufficient clarification at step 9. The A700 should typically be < 0.01.

Appendix 2C. Determination of titratable acidity by the method of Iland *et al.*, 2000.

Sample preparation

The determination is normally carried out on an undiluted, clarified sample of juice or wine. A wine sample needs to be degassed.

Procedure for determining the titratable acidity of juice or wine using an indicator to determine the end point.

1. Fill the burette with 0.1 M NaOH.
2. Add approximately 100 mL of distilled water to a conical flask.
3. Add 3 to 5 drops of phenolphthalein indicator to the flask. Mix.

4. Add 0.1 M NaOH from the burette (normally will only be a few drops) until the color of the solution turns a pale pink which persists for 30 seconds. It is not necessary to record this volume of NaOH.

5. Pipette 10.0 mL of juice/degassed wine into the flask (the pink color will change back to clear).

6. Record the initial burette reading.

7. Titrate the solution in the flask with 0.1 M NaOH until the color of the solution again changes to a pale pink which persists for 30 seconds.

8. Record the final burette reading.

9. Calculate the difference between the final and the initial burette readings. This is called the Titre value.

Calculation

This calculation applies to both methods.

Titratable acidity (g/L as H_2T) = 0.75 x Titre value (mL)

Appendix 3C. Red wine color and phenolic measure by the method of Iland *et al*., 2000.

Procedure for determining wine color and phenolic measures at natural wine pH

1. Measure and record the pH of the wine.
2. Set up four test tubes labeled:

i) wine ii) wine + CH_3CHO

iii) wine + SO_2 iv) wine + HCl

3. Add accurately 2 mL of the wine to each of the test tubes i, ii and iii.

4. Add 20 µL of 10 % w/v CH_3CHO solution to the wine in test tube ii. Mix thoroughly. Wait 45 minutes before taking spectral measures.

5. Add 30 µL of 25 % w/v $Na_2S_2O_5$ (sodium metabisulfide) solution to the wine in test tube iii. Mix thoroughly.

6. Add accurately 10 mL of 1 M HCl solution to test tube iv, then add accurately 100 µL of the wine. Mix thoroughly. Wait 3 hours before taking spectral measures.

7. Measure the absorbance of each of the solutions in test tubes i, ii and iii at 520 nm and 420 nm, using a 1 mm cell.

8. Measure the absorbance of the solutions in test tube iv at 520 and 280 nm, in a 10 mm cell.

9. Calculate the various spectral measures by applying the appropriate values to the formulae opposite.

Procedure for determining wine color measures at a common pH (3.5)

1. Adjust a portion (about 100 mL) of the wine to pH 3.5 by adding dropwise, with mixing, either 1 M NaOH or 1 M HCl, depending on the initial pH of the wine.

2. Repeat the procedure for steps 2 to 7 and step 9 using the pH adjusted wine.

Expression of the results

Wine color density = $A_{520} + A_{420}$

Wine color hue = A_{420}/A_{520}

Degree of red pigment coloration = $A_{520}/A^{HCl}{}_{520}$ x 100

Estimate of SO_2 resistant pigments (a.u.) = $A^{SO2}{}_{520}$

Total red pigments (a.u.) = $A^{HCl}{}_{520}$

Total phenolics (a.u.) = $A^{HCl}{}_{280} - 4$

Modified wine color density = $(A^{CH_3CHO}{}_{520} + A^{CH_3CHO}{}_{420})_{pH\ 3.5}$

Modified wine color hue = $(A^{CH_3CHO}{}_{420}/A^{CH_3CHO}{}_{520})_{pH\ 3.5}$

Modified degree of red pigment coloration = $(A^{CH_3CHO}{}_{520}/A^{HCl}{}_{520})_{pH\ 3.5}$ x 100

Modified estimate of SO_2 resistant pigments (a.u.) = $(A^{SO_2}{}_{520})_{pH\ 3.5}$

BIOGRAPHY

Mr. Vason Boonterm was born on June 14, 1961 in Samutsakhon, Thailand. In 1980, he attended College of Agriculture, Kasetsart University Bangkok. He graduated the Bachelor of Sciences in Horticulture with the first class honor in 1984. After that, he started working as an agronomist in Chareonpokphan Group of Company (CP) in field of hybrid corn seed production for 16 years. In 1999 he started to work as a viticulturist for Xichang Chia Tai Wine & Spirits Co., Ltd. in Xichang, Sichuan province, China. In 2000, he graduated the Master of Science degree in Plant Production Technology, Suranaree University. During that he had attended training courses for grape growing and winemaking at University of California, Davis (UC Davis), California USA. His master thesis topic was "Influence of N, K Fertilizers and Fruiting Shoots on Yields and Quality of Vinegrape Variety Cabernet Sauvignon". In 2006, he started his Ph.D. program. His dissertation title was "Effects of Nitrogen and Potassium Fertilizing and Cluster Thinning on Quality and Anthocyanin Contents of Cabernet Sauvignon Grape and Wine". Two papers under the titles of "Effects of Nitrogen, Potassium Fertilizer, and Cluster per Vine on Anthocyanin Content in Cabernet Sauvignon Grape" and "Effects of Nitrogen, Potassium Fertilizer, and Cluster per Vine on Anthocyanin Content in Cabernet Sauvignon Wine" were accepted to be published in Suranaree Journal of Science and Technology.