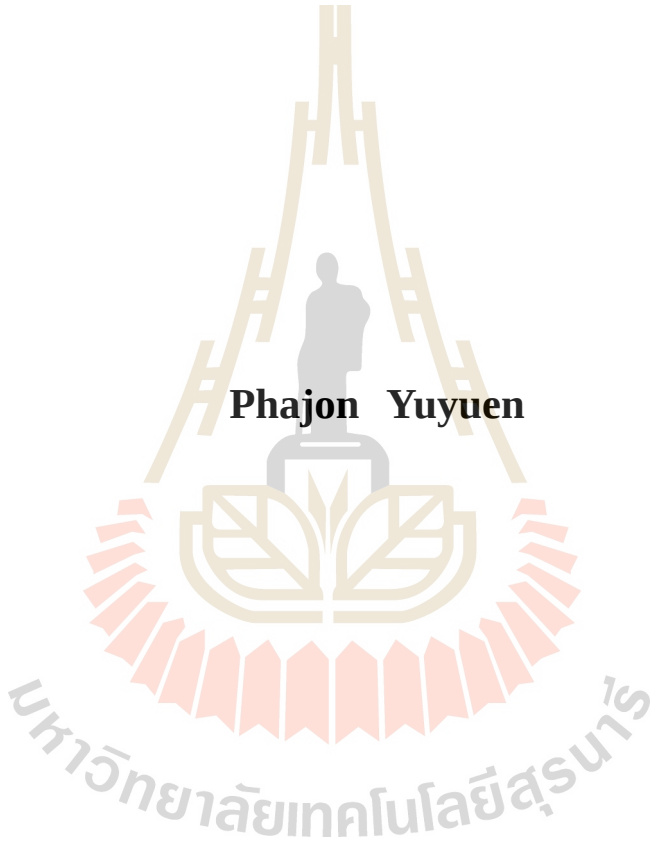
INFLUENCE OF WINE GRAPE BERRY QUALITY AND WINE MAKING CONDITIONS ON WINE QUALITY

Phajon Yuyuen



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อิทธิพลของคุณภาพองุ่นและสภาวะการทำไวน์ที่มีผลต่อคุณภาพไวน์องุ่น

นายผจญ อยู่ยืน

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาเทคโนโลยีชีวภาพ
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INFLUENCE OF WINE GRAPE BERRY QUALITY AND WINE MAKING CONDITIONS ON WINE QUALITY

Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for a Master's Degree.

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ผจญ อยู่ยืน : อิทธิพลของคุณภาพผลองุ่นและสภาพการผลิตไวน์ต่อคุณภาพของไวน์ (INFLUENCE OF WINE GRAPE BERRY QUALITY AND WINE MAKING CONDITIONS ON WINE QUALITY) อาจารย์ที่ปรึกษา : รองศาสตราจารย์ ดร. โชคชัย วนภู, 85 หน้า.

คุณภาพของไวน์มีความสำคัญมากต่อธุรกิจไวน์ คุณภาพของผลองุ่นเป็นปัจจัยหนึ่ง ในการควบคุมคุณภาพไวน์ วัตถุประสงค์ของการศึกษาเรื่องนี้เพื่อหาความสัมพันธ์ระหว่างคุณภาพของผลองุ่นที่มีต่อคุณภาพของไวน์ โดยทำการทดลองในสภาพภูมิอากาศกึ่งร้อนชื้นในบริเวณภาคตะวันตกเฉียงใต้ของประเทศจีน (ตำบลซีซาง จังหวัดเสฉนวน) โดยใช้องุ่นพันธุ์ กาแบร์เน โซวีญงอายุ 15 ปี ปลูกโดยวิธีปักชำในปี 1998 ใช้ระยะปลูก 1.25 x 2.00 เมตร ในแนวเหนือ-ใต้ จากนั้นนำผลองุ่นที่มีระดับความเข้มข้นของน้ำตาล 5 ระดับ (14°Brix, 16°Brix, 18°Brix, 20°Brix และ 22°Brix) มาทำการผลิตไวน์ในปี 2013 จากผลการทดลองพบว่าไวน์ที่ผลิตจากผลองุ่นที่ความเข้มข้นของน้ำตาล 22 °Brix ได้ไวน์ที่มีคุณภาพดีที่สุด และเมื่อมีการปรับเพิ่มปริมาณน้ำตาลในองุ่น 18°Brix และ 20°Brix พบว่า ทำให้ไวน์มีคุณภาพดีเท่ากับไวน์ที่ทำจากองุ่น 22°Brix คือมีปริมาณแอลกอฮอล์ กรด น้ำตาล และ สีของไวน์ ดีขึ้น ยกเว้นปริมาณกรดรวมและค่าความเป็นกรดด่าง ในการควบคุมคุณภาพของผลองุ่นในไร่ในช่วงองุ่นติดผล พบว่าการใช้พลาสติกคลุมร่ององุ่นมีผลต่อคุณภาพของผลองุ่นดีกว่าร่องที่ไม่ได้คลุม

สาขาวิชาเทคโนโลยีชีวภาพ
ปีการศึกษา 2559

ลายมือชื่อนักศึกษา_____
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PHAJON YUYUEN : INFLUENCE OF WINE GRAPE BERRY QUALITY AND WINE MAKING CONDITIONS ON WINE QUALITY. THESIS ADVISOR : ASSOC. PROF. CHOKCHAI WANAPU, Ph.D., 85 PP.

CHAPTALIZATION/ FERMENTATION/ MUST/ VITIS VINIFERA

Wine quality is very important in the wine business. The quality of grape berries is one of the factors in controlling wine quality. The aim of this study was to find the grape berry quality in relation to the production of wine quality. An experiment was carried out in a humid subtropical climate site in the southwest of China (Xichang district, Sichuan province). A fifteen-years-old vine variety of Cabernet Sauvignon (*Vitis vinifera*) was planted by own root cutting in 1998 with a spacing of 1.25 × 2.00 m in a north-south direction. Five different total soluble solid levels of grape berries (14°Brix, 16°Brix, 18°Brix, 20°Brix and 22°Brix) were picked and their wines were producted in 2013. It was found that wine made from grape 22°Brix had the best quality, but the chaptalization process also helped grape 18°Brix and 20°Brix have the same quality in terms of alcohol content, volatile acidity, reducing sugar and wine color with the exception of total acidity and pH. In addition, to protect the quality of grape berries from rain damage, plastic roofing was used. It was found that the quality of grape berries under roofing was better than no roofing.

School of Biotechnology

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Phajon Yuyuen

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LIST OF ABBREVIATIONS

ANOVA	Analysis of varience
°C	Degree Celsius
et al.	et alia (and others)
(m, μ) g	(milli, micro) Gram
h	Hour
(m, μ) l	(milli, micro) Liter
(m, μ) M	(milli, micro) Molarity
min	Minute
(m, μ) mol	(milli, micro) Mole
N	Normality
%	Percentage
rpm	Round per minute
s	Second
SAS	Statistical analysis system
%v/v	Percentage volume by volume
%w/v	Percentage weight by volume
U/g	Unit/g

CHAPTER I

INTRODUCTION

1. Significance of the study

Grape berry maturity is a critical factor in determining wine quality. The importance of picking the fruit at the optimum maturity has led to rational vineyard sampling and improved harvesting of wine grapes. It has always been recognized that wine quality is related to the quality of the fruit. The picking of mold-free or insect-free grapes for wine, that most molds, beyond a small percentage of fruit infection, can cause off-tastes and aromas. Acidity, acetic acid, provides one means, by indicating the activity of yeast and bacteria on the broken grapes (Ough, 1980). Grapes need full sunlight and high temperatures to ripe, so vine planted should be on southern slopes, the south side of windbreaks, or the south sides of buildings. Avoid northern slopes and low ground since these will be cooler throughout the growing season and delaying ripening of the fruit. Choose deep, well-drained soils to avoid standing water in the spring and encourage early growth (Hoover and Hemstad, 2000). Such plants are able to use sunlight to make sugar from carbon dioxide and water through the process of photosynthesis (Vine *et al*., 1999). Berry ripening is therefore tightly coordinated with seed development. During véraison, 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 is increased

during ripening and is therefore a function of berry age. Primarily sugars; sucrose, fructose, and glucose, are measured in the term of percentage of total soluble solids (TSS) which 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 especially in humid subtropical climate 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).

Total acidity (TA) and pH are of great importance for grape juice and wine stability, and both parameters are commonly used as indicators of quality (De La Hera Ort *et al.*, 2005). Tartaric acid and malic acid represent 90% of the acids in wine grapes, which are secondary product related to sugar metabolism and are synthesized primarily in the grapes and also in leaves. Tartaric acid and malic acid range vary with maturity, variety, site climate and vintage. During ripening, the TA decrease and the pH increase. Wine grapes from warmer sites or warmer seasons have lower TA and higher pH than wine grapes from cooler sites or cooler seasons. Wine with low to moderate pH also tends to have crisper, fruity flavors and tend to age better than wine with higher pH level (Watson, 2003).

Therefore, the objective of this study was to investigate the ripeness of the grape berries in relation to make a different wine style and quality in humid sub-tropical climate area.

2. Research hypothesis

1) Different of wine grape berry qualities at harvesting have influence on quality of wine.

2) Extended maceration during fermentation has the effect on quality of wine.

3) Grape's seeds and stem have the effect on quality of wine.

4) Plastic cover roofing has the effect on quality of berries which will be affected on wine grapes.

3. Research objectives

The overall aim of this research will be to find the effect of wine grape quality and fermentation conditions on wine quality, especially to determine the factors affect to phenolic formation. The specific objectives were

1) To find the effect of wine grape quality and fermentation conditions on wine quality, especially to determine the factors effect on phenolic compounds formation.
2) To determine the factors affecting the increasing of phenolic compounds (color intensity).
3) Investigate the phenolic compounds formation after seeds removal during fermentation and maturation.

4. Scope and limitations of the study

Cabernet Sauvignon, Merlot and Zinfandel grapes planted in humid subtropical climate located in the Southwest of People's Republic of China (Moon Valley Vineyard, Xichang district, Sichuan province at 27 ºN, 102 ºE and 1,650 meters above mean sea level) was used throughout at this study.

Red wines fermentation will be done with fresh various quality of grape juice following wine making with and without removing seeds. Wines will be inoculated with a commercial yeast strain then fermentation will be done until the end of alcoholic fermentation. After that malolactic fermentation will be carried out and

followed by cold stabilization and aging for six months. Wine components will be determined from all samples and products. Finally, ten trained panelists and quantitative descriptive analysis method will used for sensory evaluation.

5. Expected results

Knowledge from this research was applied for improving wine quality in humid subtropical climate especially in the Southwest of The People's Republic of China (Xichang District, Sichuan Province).

CHAPTER II

LITERATURE REVIEWS

2.1 Wine grape quality

2.1.1 Wine grape variety

Wine grape varieties represent only a small portion of the more than 600 kinds of grapes and are economically the most important fruit crop worldwide (Fortes and Pais 2016). Each grape variety has its own unique combination of characteristics including color, size, skin thickness, acidity, yield per vine and flavors. Only a few grape varieties are suited to produce fine quality wine. While many grape varieties are used to produce wines, only a few grapes have distinguished themselves as being particular suited for the production of fine wine (Jackson 2014). These noble grape varieties must still be matched with the right microclimate and winemaking techniques in order to live up to their potential (Dhekney 2016). This research was used three wine grape varieties were Cabernet Sauvignon, Merlot and Zinfandel.

2.1.1.1 Cabernet Sauvignon

Cabernet Sauvignon is a variety of red grape mainly used for wine production, one of the most widely planted of the world's noble grape varieties (Reisch *et al*., 1993). This variety is native of the Bordeaux area (ENTAV, 1997). It is one of the coldest hardy and disease resistant *Vitis vinifera* varieties, although late ripening, satisfactory levels of sugar. However, sugar alone does not determine the wine quality, and consistent superior wine quality has only been achieved in the warmer production

areas (Reisch *et al*., 1993). Cabernet Sauvignon makes the most dependable candidate for aging, more often improving into a truly great wine than any other single varietal. With age, its distinctive black currant aroma can develop bouquet nuances of cedar, violets, leather, or cigar box and its typically tannic edge may soften and smooth considerably (Lamar, 2005). As with Sauvignon Blanc, methoxypyrazine (mainly 2-methoxy-3-isobutylpyrazine) plays an important role in the green pepper, grassy and herbaceous nuances of Cabernet Sauvignon. According to the literature, Cabernet Sauvignon grapes generally have higher methoxypyrazine levels than Sauvignon blanc grapes at veraison, and retain these components better as grape ripening proceeds. However, contrary to Sauvignon Blanc, where relatively prominent levels of these pyrazines are acceptable, these aromas are not preferred with Cabernet Sauvignon (Gonzalez-Barreiro, Rial-Otero et al. 2015). They can provide complexity when in balance with other aroma components, but at too high levels they can become over powering. Therefore, canopies are manipulated to introduce more sunlight in order to reduce methoxypyrazine levels and to weaken the intensities of these aromas. Due to the sensitivity of these components to light and temperature, Cabernet Sauvignon wines from warm regions normally have lower intensities of grassiness and herbaceousness than wines from cool regions (Marais, 2005). A “hard” grape, it helps make wines of classic breed, intensity and complexity that often need to bottle-age for at least 5-10 years in order to reach peak flavor condition (Hawkins, 2005).

2.1.1.2 Merlot

Merlot is to the American wine consumer in the 1990s as "burgundy" was in the 70s: the new generic red. A "boom" in wine consumption, combined with the consumer trend to move away from generic wine blends and into varietals, stimulated plantings of "new" as well as "proven" wine grapes in California during the '70s.

Merlot did not appear as a California varietals label until the end of the decade and was not a big seller until the end of the '80s. Less than 2,000 acres existed in California in 1985; there are over 50,000 acres in 2003 (Lamar, 2005). Merlot has been produced as superior wines in New York. However, it has a very long vegetative growth cycle and tends to produce dense, shaded canopies. This leads to bunch rot and reduced winter cold tolerance (Reisch *et al*., 1993). Merlot is quite sensitive to cold injury and crown gall and thus can only be recommended in sites where experience has demonstrated that winter injury is not a serious threat. Merlot fruit is also highly susceptible to bunch rots. Rot development often necessitates early harvest and less than optimal fruit quality. In good sites with relatively dry harvest seasons, Merlot grape and wine quality can be exceptional (Wolf *et al*, 1999). Wines are strong round, alcoholic and colored, but slightly acid. Wine is robust and balanced with supple tannins and keeping qualities benefit from oak aging (ENTAV, 1997).

2.1.1.3 Zinfandel

Zinfandel is only grown under this name in California. As a result, historians have long debated the appearance of this variety in the state. Some believe Zinfandel was first imported from Hungary in 1852; others point to evidence that New England nurseries had cultured it as a table grape and introduced the variety in California during that decade. By the mid-1860s, wines made from Zinfandel grapes were seen as an improvement to those made from the popular Mission grape variety, and plantings of Zinfandel vines increased (Bettiga *et al*, 2003). Essentially, Zinfandel is unique in vinifera varieties in California in that it has a larger cluster and is a fairly vigorous vine. It is a vinifera variety that the berries within the bunch ripen unevenly and, hence you have berries that are slightly underripe, perfectly ripe, and some actually verging on dehydrated or raisiny in the same cluster. This leads to the unique

flavor dynamics of great Zinfandel. It produces bright acidity from the slightly unripe areas, perfect fruit flavors from the ripe berries, and concentration and depth from the slightly withered or overripe berries. It is also the factor that makes Zinfandel so difficult to pick at the right moment because choices of proportion of those various elements in fruit are determined the quality of the wine characteristics during process of production. Aromas and flavors in a wine come primarily from the grapes from which it was made. These aromas and flavors will depend upon where the grapes were grown, the age of the vines, the clone of Zinfandel, the pruning method and subsequent crop level. The elevation of the vineyard, the ripeness of the grapes at harvest and the impact of weather the year were harvested, and complex winemaking decisions regarding type of fermentation and aging protocol. No rigid protocols dictate how Zinfandel grapes are made into wine, however, grape growers and winemakers recognize the importance of using site selections and winemaking techniques to enhance Zinfandel wine characteristics rather than to disguise them (ZAP, 1999).

2.2 Composition of grape berry

2.2.1 Physical Composition

The fruit of the grape is a berry. Berries are attached to the stem. Many berries make up the cluster or bunch of grapes. The essential parts of the berry include the skin, pulp, and seeds were shown in Figure 1. The skin consists of an outer layer covering the berry. It is made up of six to ten layers of thick walled cells. The outer surface of the skin is covered with a wax-like coating called the cuticle, which renders the berry waterproof. The main components in the skin are: coloring matter (red and yellow pigments), tannins, aromatic substances, and potassium and other minerals.

Below the skin layer lies flesh or pulp, which makes up most of the berry volume. Cells in the pulp have large vacuoles containing the cell sap or juice. When the berry is gently crushed, the fragile cells in the pulp are broken and the juice is released. This juice is commonly referred to as the free run. The seeds are localized in the center of the flesh. The berry contains two to four seeds. They are rich in tannin that is extracted during fermentation (in red wines) (Dharmadhikari, 1994).

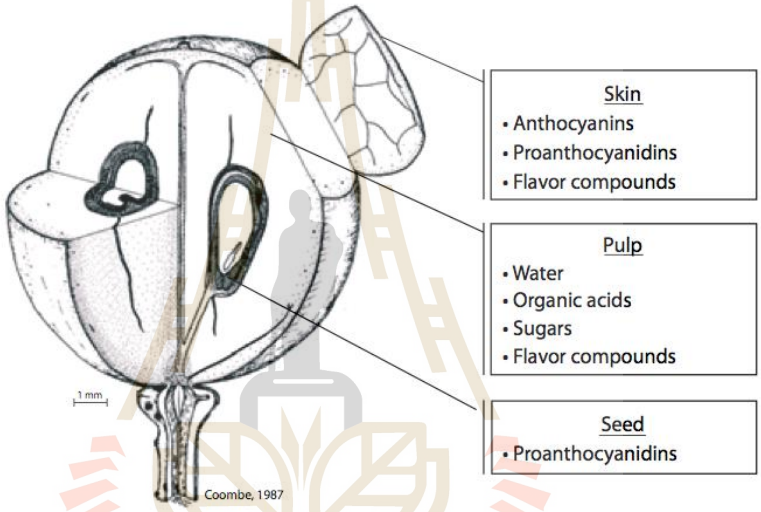


Figure 2.1 Distribution within the grape berry of compounds important to wine quality

Ref: Kennedy, 2008

2.2.2 Chemical Composition

Freshly expressed grape juice consists of 70 to 80% water and many dissolved solids. These soluble solids include numerous organic and inorganic compounds (Dharmadhikari, 1994). The grape berries consist primary of skin, pulp and seeds. The pulp accounts for about 78% of the weight of the berries and its primary constituents are sugar (mainly glucose and fructose), organic acids,

potassium, nitrogenous compounds, pectin and nonflavonoid phenolic compounds (primarily benzoic acid and cinnamic acid derivatives). The phenolic compounds in pulp represent about 10% of the phenolic content in whole berry. The skins typical represent about 15% of the berry weight and are the principle source of aromatic compounds and flavor precursors. They also contain flavonoid compounds (including flavonols, anthocyanins and large polymeric flavonoid compounds known as tannins). Phenolic compounds in the skins represent about 30% of the total phenolic content of the berry. The seeds, which represent about 4% of the berry weight, contain both nonflavonoid and flavonoid compounds including tannins. The seed phenols represent about 60% of the phenolic content of the berries. They also contain significant levels of nitrogenous compounds, minerals and oils (primarily oleic and linoleic acids) (Watson, 2003).

The main organic acids in grapes (Figure 2) are illustrated according to the conventional structure system. Tartaric acid is one of the most prevalent acids in unripe grapes and must. Tartaric acid has a stereo-isomer in which the absolute configuration of the two asymmetrical carbons is L, but whose optical activity in water, measured on a polarimeter, is d (or +). Indeed, at the end of the vegetative growth phase, concentrations in unripe grapes may be as high as 15 g/L. In musts from northerly vineyards, concentrations are often over 6 g/L whereas, in the south, they may be as low as 2–3 g/L since combustion is more effective when the grape bunches are maintained at high temperatures. Tartaric acid is not very widespread in nature, but is specific to grapes. For this reason, it is called *Weinsaure* in German, or “wine acid”. It is a relatively strong acid giving wine a pH on the order of 3.0–3.5 (Ribereau-Gayon *et al*, 1998).

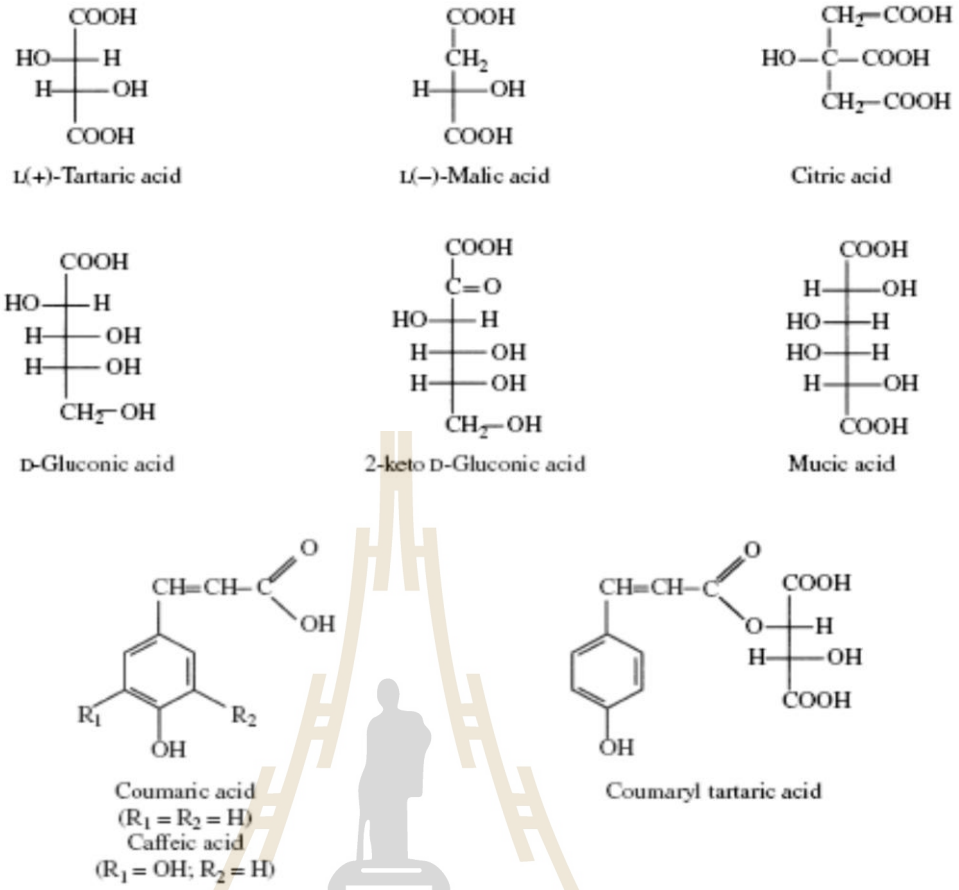


Figure 2.2 The main organic acids in grapes (Ribereau-Gayon *et al*., 1998)

2.3 Factors affecting grape berry quality

Factors affecting grape berry quality do not account for microclimate variations and do not mean a great deal in predicting physiological response of the vines. It does correlate with distinct compositional changes in the fruit used for wine and quality differences in the wines. Growing quality wine grapes has become increasingly important for the wine industry. Over the last few years, there has been an emphasis by the industry to improve quality through reducing yield. However, grape composition and quality is the result of many complex interactions that occur in the field throughout the growing season and yield is only one part of the equation.

Environmental factors and a whole range of viticulture practices all have a place in determining grape quality and composition and these are highlighted (NWGIC, 2012).

2.3.1 Climate, temperature and humidity

Grape growing is limited by certain climatic conditions. A reasonably long growing season (150–180 days) with relatively low humidity (less than 800 mm per year) but sufficient soil moisture is necessary. The temperatures from April to September are crucial for reaching good development of the vine and ripening of the fruits. When temperatures are below 10 °C, vines are dormant. The optimum temperature is between 25 and 30 °C. Temperatures higher than 38 °C will stop growth, Frosts (–1 °C and lower) occurring after vine growth has started in spring could kill off most of the fruitful shoots and reduce the harvest to nil and variations in the microclimate, location and topography of individual vineyards contribute to the diversity of wines and their respective quality (FAO, 2009).

Temperature has an important role throughout the whole grape growing process. Warm temperatures hasten the ripening process, temperatures in excess of 33 °C slow down photosynthesis and subsequently berry ripening. It is not surprising therefore, that in the warm inland grape growing regions of Australia, viticulturists say their vines have “shut down” in very hot conditions during summer. Conversely, very cool temperatures also slow down the ripening process. Without a doubt, humidity can cause many problems in a vineyard. Very humid conditions favor disease development and consequently it is important to consider some of the viticultural practices that can impact on vine growth and development. Excessive irrigation and high fertilizer use will encourage a dense canopy that will not only shade fruit but will create a microclimate within the vineyard that encourages the spread of diseases (NWGIC, 2012).

2.3.2 Rainfall and irrigation

Irrigation is one of the most powerful tools a viticulturist has to ensure quality fruit. Regulated deficit irrigation and partial rootzone drying are explored further in the irrigation section of this guide and winegrape growers should familiarize themselves with these concepts. Successful viticulturists have a good understanding of their soils, monitor soil moisture and understand the importance of irrigation for quality winegrape production. In high rainfall zones, cover crops and inter row swards can be used to manage problems caused by excess moisture (NWGIC, 2012).

2.3.3 Sun exposure

Light and shade play an important part in fruit ripening and fruit quality. Leaves require light for photosynthesis and bunches need light to promote good colour and enhance aroma and flavor development. Generally, speaking wines made from fruit from shaded canopies have reduced sugar, color and phenolic. Fruit that is overexposed and is subject to high temperatures can also be of lower quality because of the negative effect of high temperatures on aroma, color and flavor development. Canopy management techniques should ensure that maximum light interception is achieved without the risk of sunburnt fruit (NWGIC, 2012).

2.3.4 Soils

A good understanding of soils and their water and nutrient holding capacity is important so that you are better able to manage irrigation and fertilizer application. Soils can vary substantially across a vineyard and it is important that your irrigation/fertigation system can accommodate these changes. Heavier soils will retain moisture longer and if irrigation is not managed properly, waterlogging can result. Vines that have waterlogged roots are unable to function at optimum levels, thereby slowing down the ripening process. Naturally, lighter soils will not hold water for as

long as heavier soils and these soils will require shorter, more frequent irrigations (NWGIC, 2012).

2.3.5 Crop level

Crop level studies are related both to the vine's ability to produce the largest crop possible and still survive and to the quality of the wine produced. In the 1950s U.C.researchers proposed relating the crop level of wine grapes to their Brix/acid ratio and, later, relating leaf area to grape quality. Crop level was related also to chemical composition and wine headspace volatiles. Researchers have found that a "normal" crop may vary over a significant range with little change in overall wine quality (Ough, 1980). Crop level (yield/vine) 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 that growers can manipulate (Winkler *et al*, 1974). 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). It is generally believed that heavy crop loads inhibit the development of quality wine grapes and many winemakers are showing a preference for fruit sourced from low yielding vineyards. Studies have shown that increased yields do have a negative effect on grape composition and subsequent wine quality. High crop loads generally delay ripening and if the crop load is too high, grapes may not ripen to a desirable level. However, other research has shown that there was little response to variations in yield. In some cases, an increase in crop load coupled with improvements in canopy microclimate led to improvements in berry composition. During the 1980s in Bordeaux, some of the finest vintages came from vineyards in which yields were

relatively high (NWGIC, 2012).

2.3.6 Nutrients and fertilizers

Applications of fertilizer should be based on the results of soil and petiole tests. High levels of nutrition coupled with adequate temperatures and moisture will result in increased shoot vigor that in turn can result in a shaded canopy with negative effects on fruit quality. Some research has shown that an increase in fungal infection has occurred after high nitrogen applications. High levels of potassium can have a detrimental effect on wine quality. Fertilizers should be used judiciously and only on the basis of objective information provided by a petiole analysis (NWGIC, 2012).

2.3.7 Pests and diseases

Disease is generally minimized if the canopy is open and has good airflow. Growers should aim to create an ecosystem within their vineyard that supports a competitive environment for pests and diseases. One way to achieve this is by establishing a vineyard floor that is botanically diverse. This encourages a wide range of insect species and beneficial predators (NWGIC, 2012).

2.3.8 Grape maturity

Grape maturity can be a critical factor in determining wine quality. The importance of picking the fruit at the optimum maturity has led to rational vineyard sampling and improved harvesting of wine grapes. It has always been recognized that wine quality is related to the quality of the fruit. The picking of mold-free or insect-free grapes for wine, that most molds, beyond a small percentage of fruit infection, could cause off-tastes and aromas. Mold sampling techniques have been worked out for visual detection of moldy and rotten juice. Acetic acid provides one means, by indicating the activity of yeast and bacteria on the broken grapes (Ough, 1980). Grapes need full sunlight and high temperatures to ripen**,** so plant on southern slopes, the

south side of windbreaks, or the south sides of buildings. Avoid northern slopes and low ground since these will be cooler throughout the growing season, delaying ripening of the fruit. Choose deep, well-drained soils to avoid standing water in the spring and encourage early growth (Hoover and Hemstad, 2000). Such plants are able to use sunlight to make sugar from carbon dioxide and water through the process of photosynthesis (Vine *et al*., 1999). 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 is increased 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, Singleton et al., 1998).

2.3.9 Canopy management

Canopy management has received considerable research attention during the past several decades (Smart, 1987; Smart and Robinson, 1991). The purpose of canopy management was to develop viticulture 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 effect on vine growth, fruit yield, sugar content and vine carbohydrate reserves (Petrie *et al*, 2002). 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).

2.4 Effect of wine making condition on wine quality

2.4.1 De-stemming and crushing

The removal of stems, leaves and grapes stalks before crushing has several advantages. The extraction of stem phenols is of potential value only when dealing with red grape varieties low in phenol content (Jackson, 1994). Crushing is employed to cause berry breakage and juice release from the grapes, and nearly 100% of berry will be broken. It is the beginning of the juice, skin, pulp and seed contact that will influence the extent of extraction from these grape components (Boulton *et al*., 1998).

2.4.2 Sugar concentrate of must

Musts with high sugar levels generally need higher yeast numbers to ferment out, and the greater the mass of yeast cells made during the fermentation the greater the nitrogen requirement. Yeast needs oxygen to synthesize sterols such as ergosterol to maintain plasma membrane integrity in the high alcohol levels at the end of the fermentation. The addition of oxygen or air during fermentation increases yeast growth because aerobic metabolism is much more efficient than anaerobic. In

addition, high sugar levels are likely to result in fermentations that tail off leaving some residual sugar, predominantly fructose (Kelly and Paul, 2002).

2.4.3 Juice clarification

The solids were detrimental to wine quality in white wine fermentations. Others have shown that the amount of solids, although changing the style of wines, was not necessarily detrimental. The presence of free sulfur in the grapes is a major cause of off-odor from hydrogen sulfide, and in this case the removal of solids, including the sulfur, is mandatory. The effect of the solids on the yeast during fermentation, musts relatively low in nutrients that grow a poorer crop of yeast will not finish properly, because the yeast settles out. A certain level of solids, whether grape solids or some other inert particulate matter, is essential to keep the yeast circulating and juice fermenting. Wines that do not finish fermenting properly tend to oxidize, lose fruitiness, and, deteriorate in quality. One important factor in increasing wine quality has been the understanding and use of temperature control during fermentation. The effects of fermentation temperature indicated that the concentration of maximum volatile esters depends on the ester and the fermentation temperature as well as on other factors. White wines fermented at lower temperatures (10 to 15 °C) and red wines fermented with skin contact at higher temperatures (25 to 32 °C) were superior (Ough, 1980).

2.4.4 Alcoholic fermentation

The alcoholic fermentation, the conversion of the principle grape sugars glucose and fructose to ethanol and carbondioxide, is conducted by yeast of the genus *Saccharomyces*, generally by *S. cerevisiae* and *S. byanus* (Boulton *et al*., 1998). The type of yeast can be classified by type of alcohol product, resistance to degree of

alcohol production, aroma release, color and fermentation characteristics (Wanapu, et al., 2001).

Yeasts are eukaryotic unicellular microfungi that are widely distribution in the natural environment (Walker, 1999). It is taxonomically classified as follows (Vine *et al*., 1999):

Phylum	Thallophyta
Subphylum	Fungi
Class	*Eumycetes*
Subclass	*Ascomycetes*
Order	*Endomycetales*
Family	*Saccharomycetaceae*
Subfamily	*Saccharomycoidea*
Genera	*Saccharomyces*

Fermentation is an energy releasing from of metabolism in which both substrate and by product are organic compounds (Jackson, 1994). Ethanol gives wine their principle character (Gayon *et al*, 2000). Rate of fermentation depends on fermentation condition, especially fermentation temperature impact to yeast growth rate. Higher temperatures generally lead to increase in fermentation rate. Higher temperatures generally lead to increase in fermentation rate. Charoenchai, Fleet and Henschke (1998) studied the effect of temperature on the fermentation rate for 22 different strains of yeast. The experiment indicated the higher temperatures increased growth rates. However, some different species of yeast have different temperature limitation. *S. cerevisiae* and *S. bayanus* have the trend to be more ethanol tolerant at higher temperature than non-*Saccharomyces* yeast, such as *Klockera apiculata*. Yeast has difficultly remaining viable in levels of alcohol exceeding 14% by volume (Vine *et al*.,

1999). Eglinton *et al* (2004) used *S. bayanus* to modify the chemical and sensory profile of wine, the results indicated that *S. bayanus* produced higher concentration of some higher alcohols, particularly 2-phenylethanol than that of *S. cerevisiae.*

Saccharomyces metabolize glucose and fructose to pyruvate via the glycolytic pathway (Figure 3). One molecule of glucose or fructose yields two molecules each of ethanol and carbon dioxide. The theoretical conversion of 180 g of sugar into 88 g of carbon dioxide and 92 g of ethanol means that yield of ethanol is 51.1% on a weight basis. In model fermentation starting about 22 to 24% sugar, 95% of the sugar is converted into ethanol and carbon dioxide, 1% is converted into cellular material, and the remaining 4% is converted to other end products. This percentage may vary depending upon inoculum size, fermentation temperature and nutrient availability (Boulton *et al*., 1998).

2.4.5 Skin-contact time and cap management

The amount of skin-juice contact is a controllable variable that the winemaker can use in determining wine style. The amount of skin contact can determine how long a red wine will be aged and the amount of color and flavor that will be extracted from the skins. The amounts extracted are related to the fermentation temperature, the amount of alcohol at time of press, and the color and flavor available in the grape. For white wine, the length of desirable skin-juice contact is much shorter than for red wines; again style of wine is the determining factor. Varying the juice-skin contact caused significant changes in wine composition, with mainly increased in pH and total phenols among the variables measured. Differences in quality or “style” were also significant (Ough, 1980). Johnston and Morris (1996) found that increasing skin contact time resulting 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. 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 phenolic. 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 °C to 33 °C (Lee, Hin, Ough, and Berg, 1977).

2.4.6 Pressing

The purpose of pressing is to recover the juice (or wine) associated with the pulp and skin section of the grapes that are not readily release by natural draining

(Boulton *et al*., 1998). Increasing the drainage surface area was another momentous development. Not only did it speed juice release, but it also reduced the flow path for fluid escape. Enlarging the area over which pressure is applied also has been a major design concern. By diminishing the force required for juice extraction, the release of grape solid, tannin, and color is reduced (Jackson, 1994).

2.4.7 Malolactic fermentation

The principle effect of malolactic fermentation is a reduction in acidity (Jackson, 1994). The malolactic fermentation is analogy term with alcoholic fermentation, the metabolizing of L-malic acid (dicarboxylic acid) to L- lactic acid (a monocarboxylic acid) is not a fermentative pathway (Figure 2.3), but decarboxylation. Malic acid decarboxylation is catalized by malolactic enzyme (Van Vuuren and Dicks, 1993). During this conversion, CO_2 is also produced. This reaction will decrease total acidity and enhance wine stability. Malolactic bacteria are capable of direct decarboxylation of malic acid to lactic acid by the enzyme malate carboxylate, which is present in various lactic bacteria, but particularly in three genera: *Lactobacillus*, *Leuconostoc* and *Pediococcus* (Bozoglu and Yurdugul, 1999). High alcohol levels also inhibit the malolactic fermentation, meaning a longer delay before SO_2 addition (Kelly and Paul, 2002).

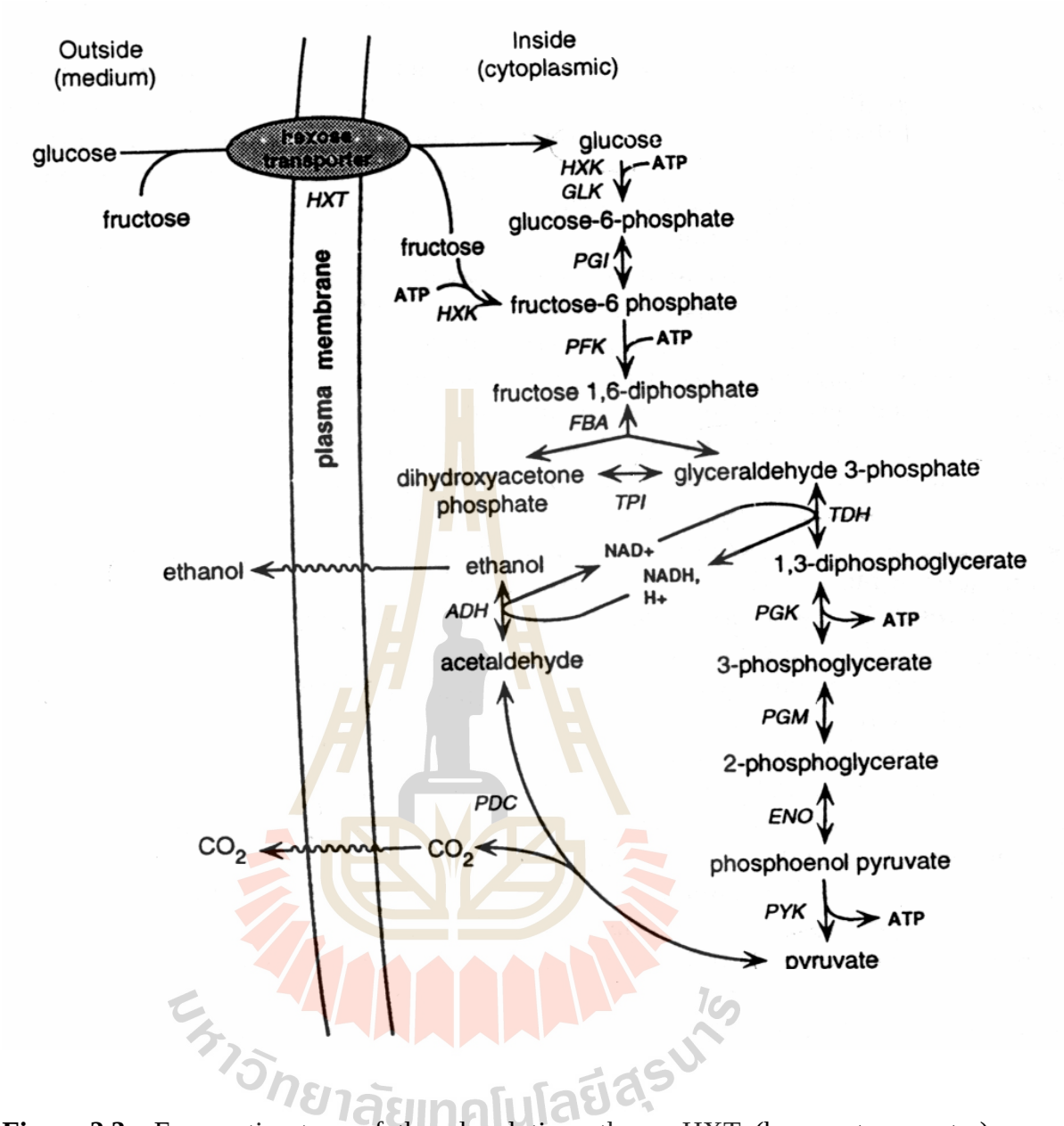


Figure 2.3 Enzymatic steps of the glycolytic pathway HXT (hexose transporter), HXK (hexokinase) GLK (glucokinase), PGI (phosphoglucose isomerase), PFK (phosphofructokinase), FBA (aldolase), TPI (triosephosphate isomerase), PGM (phosphoglycerate mutase), ENO (enolase), PYK (pyruvate kinase), PDC (pyruvate decarboxylase) and ADH (alcohol dehydrogenase) (Boulton *et al*., 1998).

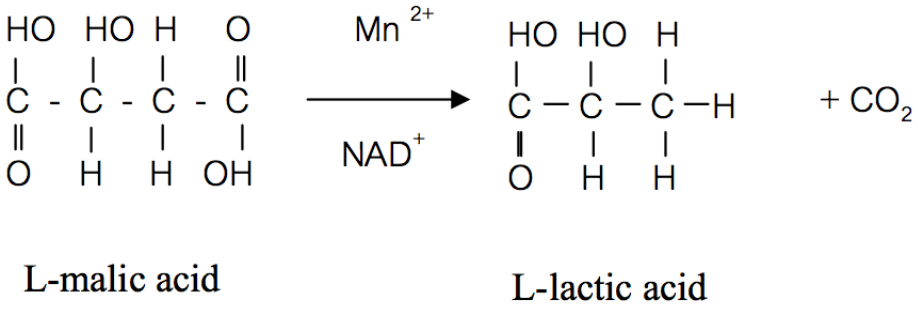


Figure 2.4 Transformation of malic acid to lactic acid (Kelly and Paul, 2002).

2.4.8 Cold stabilization

Tartrate stabilization is one of the facets of wine technology most influencedby consumer precipitation. The presence of even a few tartrate crystals is inordinately feared, or at least misinterpreted, by some wine consumers. Therefore, considerable effort and expense are expended in avoiding the information of crystalline deposit in bottled wine. Stabilization is normally achieved by enhancing crystallization, followed by removal. Less frequently, it may be achieved by delaying or inhibiting crystallization (Jackson, 1994). Organic acids make major contributions to the composition, stability and organoleptic qualities of wines, especially white wines. Their preservative properties also enhance wines microbiological and physicochemical stability. Thus, dry white wines not subjected to malolactic fermentation are more stable in terms of potassium bitartrate (KTH) and calcium tartrate (CaT) precipitation. Young white wines with high acidity generally also have greater aging potential. Red wines are stable at lower acidity, due to the presence of phenols which enhance acidity and help to maintain stability throughout aging. Most organic acids in must and wine have one or more chiral centers. The absolute configuration of the asymmetrical

carbons is deduced from that of the sugars from which they are directly derived. This is especially true of tartaric and malic acids (Rib´ereau-Gayon *et al.*, 1998)

At the pH of wine, and in view of the inevitable presence of K^+ and Ca^{2+} cations, tartaric acid is mainly salified in the following five forms, according to its two dissociation balances; potassium bitartrate, potassium tartrate, calcium tartrate, potassium calcium tartrate and calcium tartromalate. In wine, simple salts are dissociated into TH^- and T^{2-} ions. The last two tartrates share the property of forming and remaining stable at a pH of over 4.5. On the other hand, in terms of solubility, they differ in that potassium calcium tartrate is highly soluble, whereas the tartromalate is relatively insoluble and crystallizes in needles. The properties of this mixed salt may be used to eliminate malic acid, either partially or totally. The solubility, in water at 20°C, of tartaric acid and the salts that cause the most problems in terms of crystalline deposits in wine (Ribereau-Gayon *et al.*, 1998).

2.4.9 Aging

The tendency of wine to improve, or at least change during aging is one of its most fascinating properties. Regrettably, most wines improve only for a few months to years before showing irreversible loss in quality. Quality loss is commonly explained as a dissipation of the fresh, fruity bouquet, along with any aroma donated by the grape variety. Wines noted for continued improvement typically show similar aromatic losses but they gain in aged bouquet. Aging is considered desirable when the development of an aged bouquet, subtle flavor and smooth texture more than compensate for the fading varietals and fruity character of the young wine. Aging is occasionally considered to possess two phases. The first, called maturation refers to changes that occur between alcoholic fermentation and bottling. Although maturation often lasts from 6 to 24 months, it may continue for decades. During maturation, the

wine may undergo malolactic fermentation, be stored in oak cooperage, be racked several times and be treated to one or more clarification techniques. During racking and clarification, wines may absorb about 40 ml O_2/year an amount insufficient to give the wine a noticeably oxidized character. Only in some fortified wines is obvious oxidation an important component of maturation. The second phase of aging commences with bottling. Because this stage occurs essentially in the absence of oxygen, it has been called reductive aging. This contrasts with oxidative aging, an alternative term for maturation that is occasionally used for the aging of some fortified wines (Jackson, 1994).

2.4.10 Bottling

A bottle of wine is the end product of the winery. Wines are generally bottled in dark green or brown bottles to decrease deleterious effects on quality that can be caused by sunlight. Small amounts of sulfite may be added to inhibit oxidation. Sweet wines may be fortified with ethanol (18-20%) or the wines may be pasteurized or sterile filtered to prevent growth of contamination in the bottle (Steinkraus, 1992).

2.5 Phenolic compounds

Phenolic compounds are important components of many fruits, vegetables and beverages; which contribute to their color and sensory properties such as bitterness and astringency (Arnold, Noble, and Singleton, 1980). Although phenolic compounds found in wine can also originate from microbial and oak sources, the majority of the phenolic constituents found in wine are grape-derived in white wine, the most important phenolic compounds are the hydroxycinnamic acids and of minor quantities, the flavan-3-ol monomers. These compounds are important with regard to the visual

quality of white wine (Kennedy, 2008). Phenolic compounds are an important group of substances that contribute to several sensorial characteristics such as color, flavor, astringency and hardness of wine. The types and concentrations of the phenolic compounds in wine depend on grape variety and ripening, atmospheric con-ditions and the techniques employed in producing the must, and on aging (Rodr´ıguez-Delgado *et al*, 2001). Wine contains many phenolic substances, most of which originate in the grape berry. The phenolics have a number of important functions in wine, affecting the tastes of bitterness and astringency, especially in red wine. Second, the color of red wine is caused by phenolics. Third, the phenolics are the key wine preservative and the basis of long aging. Lastly, since phenolics oxidize readily, they are the component that suffers owing to oxidation and the substance that turns brown in wine (and other foods) when exposed to air (Waterhouse, 2002).

The biosynthesis of the phenolic compounds those are important to wine quality share a common pathway (Figure 2.5). While many of the genes in this pathway have been identified, portions of it remain speculative. In particular proanthocyanidin production in plants is still incompletely understood. Despite of this, the recent advances have been made with regard to the factors that are involved in gene regulation. The concentration of grape phenolics increases throughout berry development. Beginning at fruit set and when expressed on a weight basis, tannins and hydroxycinnamic acids increase until véraison. Beginning at véraison, anthocyanins accumulate in the berry and increase during fruit ripening. There is evidence that suggests that anthocyanins can decline in late berry development. It is accepted that phenolic compounds are important to the quality of wine and therefore a considerable amount of research has been directed towards the understanding of how vineyard management practices influence their concentration in grapes (Kenedy, 2008).

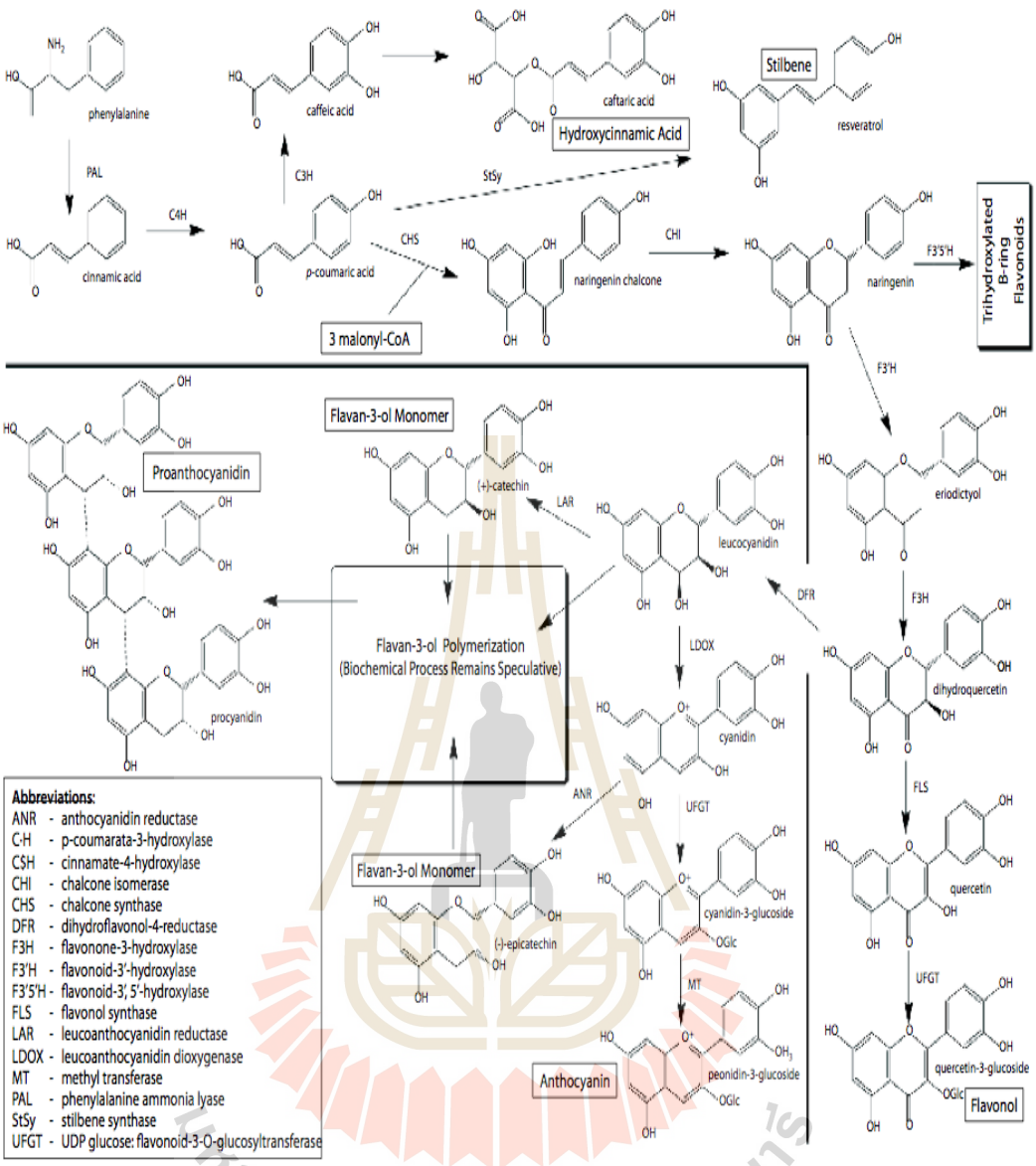


Figure 2.5 Phenylpropanoid biosynthetic pathway for the major phenolic classes found in wine (Kenedy, 2008).

2.5.1 Flavonoids

Over 4,000 flavonoids have been identified, many of which occur in fruits, vegetables and beverages (tea, coffee, beer, wine and fruit drinks) (Rodr´ıguez-Delgado *et al.*, 2001). The major phenolic compounds classes in *V. vinifera* wines are non-flavonoids or phenolic acids imamates (Figure 2.6) and derivatives, low volatility

benzene derivatives, tyrosol, volatile phenols, flavonoids (figure 2.7) (catechins, epicatechins, anthocyanins, flavonols, soluble tannin derivatives and other flavonoid derivatives) and tannins or polymerized phenols. These are located in the skins, seeds, and stems, and are higher in red wine than white wine (Basha, Musingo, and Colova, 2004). The flavonoids may exist free or polymerized. These include monomeric flavan-3-ols, such as catechin, epicatechin, anthocyanins, and oligomeric and polymeric flavan-3-ols such as procyanidins. The monomeric and oligomeric flavonoids are substrates of enzymatic as well as non-enzymatic browning in wines, and they brown more intensely than non-flavonoids (Jaworski and Lee, 1987; Lee and Jaworski, 1989). Procyanidins are polymers from catechin and flavan-3, 4-diols (leucocyanidin), and can be found in dimer, trimer, tetramer or other polymer forms. The procyanidins are found in the skins and parts of the grape cluster. Non-flavonoid phenols are derivatives of hydroxycinnamic and hydroxybenzoic acids, such as coutaric (coumaroyl tartaric acid), caftaric (caffeoyl tartaric acid), gallic acid, and ellagic acid, as well as other lower molecular weight phenolics. These are the predominant phenols in white wines since there is minimal contact with skin, stem and seed during the processing of white wines (Robichaud and Noble, 1990). Zoecklein *et al* (1995) reported that the normal light yellow color and the undesirable brown color of white wines are due to phenolics. In young white wine, the yellow color is derived from the limited extraction and oxidation of flavonoids. In addition, some of the phenolic acids are browning substrates for polyphenol oxidase and can contribute to browning in white wines when oxidized. Therefore, the phenolic acids from the pulp are the primary phenols in white wines (Sims, 1994). There are four main classes of flavonoids: catechins, flavonols, anthocyanins and tannins (Harbertson, 2007).

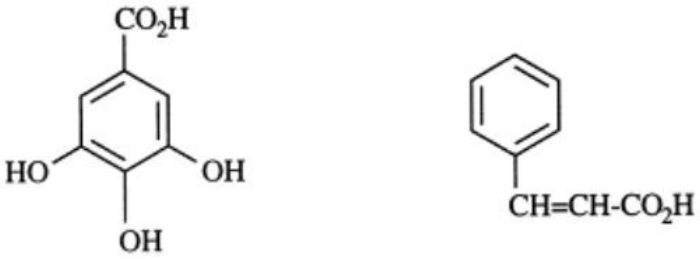


Figure 2.6 Chemical structures of non-flavonoids or phenolic acids imamates (Kerry and Abbey, 1997).

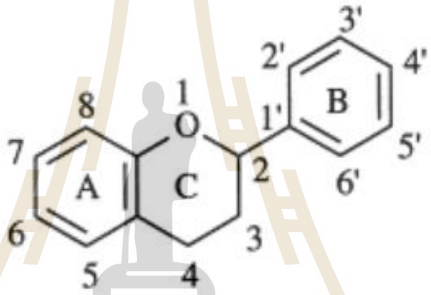


Figure 2.7 Chemical structures of flavonoids (Kerry and Abbey, 1997).

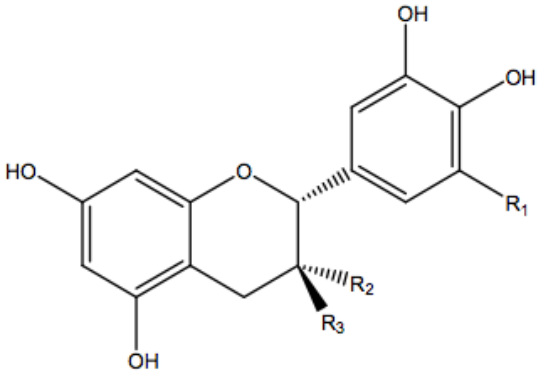
2.5.1.1 Catechins (flavan-3-ols)

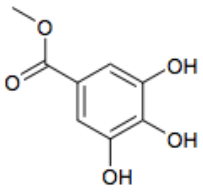
The catechins, or flavan-3-ols are found in the seeds and are known for being bitter. Flavonoid bitterness in wines is primarily due to the flavan-3-ols, catechin and its epimer, epicatechin (Figure 2.8). White wine flavan-3-ol concentration ranges from 10 to 50 mg/L while in red wines they may reach 800 mg/L. Young white and red wines have been estimated on an average to contain 25 mg/L and 75 mg/L, respectively. The threshold bitterness values of flavan-3-ols in water are 20 mg/L. However, ethanol concentration has been demonstrated to influence the perception of

bitterness from catechins. Higher alcohol concentrations enhance bitterness while acidity has no effect on perception of bitterness (Harbertson, 2007).

2.5.1.2 Flavonols

Flavonols (Figure 2.9) are found in the epidermis of grape. The most recognized flavonol is quercetin-3-glucoside. During wine making and aging, quercetin-3-glucoside is de-glycosylated and found as its aglycone form quercetin. The aglycone forms are only sparingly soluble in wine, and the amounts reported are generally in the μg/L levels. Ultra violet light exposure has been shown to increase flavonol accumulation in Merlot and Pinot noir berries. While in site experiments that were able to control temperature and light exposure concluded that while solar radiation increased, flavonol production temperature had little effect on their accumulation. Flavonols are also known as co-factors for the color-enhancing phenomenon known as copigmentation. They are well--known antioxidants and have reputed anti-inflammatory and anti-carcinogenic effects. They have yet to have been attributed a sensory component in wine (Harbertson, 2007).

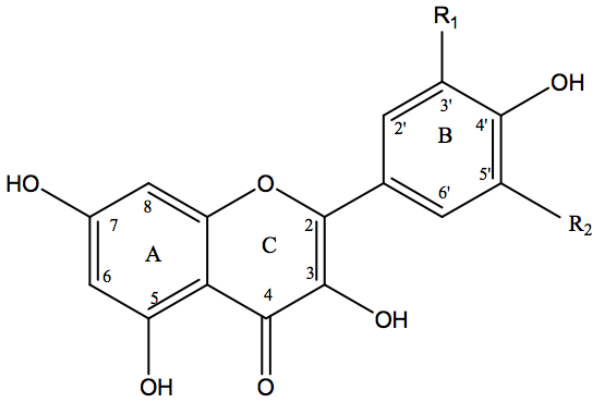




Gallate

Flavan-3-ol	R_1	R_2	R_3
(+)-Catechin (C)	H	H	OH
(+)-Catechin-3-gallate (CG)	H	H	Gallate
(-)-Epicatechin (EC)	H	OH	H
(-)-Epicatechin-3-gallate (ECG)	H	Gallate	H
(-)-Epigallocatechin (EGC)	OH	OH	H
(-)-Epigallocatechin-3-gallate (EGCG)	OH	Gallate	H
(+)-Gallocatechin (GC)	OH	H	OH
(+)-Gallocatechin-3-gallate (GCG)	OH	H	Gallate

Figure 2.8 Structure of flavan-3-ols (catechins and epicatechins) (Bhagwat, Haytowitz and Holden, 2011).



Flavonol	R_1	R_2
Isorhamnetin	OMe	H
Kaempferol	H	H
Myricetin	OH	OH
Quercetin	OH	H

Figure 2.9 Chemical structure of flavonols (quercetin, kaempferol, myricetin, isorhamnetin) (Bhagwat *et al*., 2011).

2.5.1.3 Anthocyanins

Anthocyanins are biosynthesized in the grapes (Figure 2.10), which are extracted during vinification and maceration, are responsible for grape color, and contribute to the color of young red wine. In addition, anthocyanins derived pigments are formed during fermentation by reaction of anthocyanins with yeast fermentation by products and polyphenolic compounds of grape origin. Also, anthocyanins derived pigments continue to be formed as the wine ages provided there are anthocyanins present (Birse *et al*, 2005). The color of a red wine is primarily due to the type and concentration of anthocyanins, but other factors play a role, including phenolic compounds other than the anthocyanins, sulphur dioxide, oxygen content, grape cultivar, yeast/fermentation method/winemaking techniques and final wine pH (Bakker *et al*, 1986). The anthocyanins extracted into red wines chemically combine with other wine components, forming stable compounds referred to as polymers (Somers, 1971).

Anthocyanins are one class of flavonoid compounds, which are widely

distributed plant polyphenols. Flavonols, flavan-3-ols, flavones, flavanones, and flavanonols are additional classes of flavonoids that differ in their oxidation state from the anthocyanins. Solutions of these compounds are colorless or pale yellow (Wrolstad, 2003; Lohachoompol, Srzednicki, and Craske, 2004). During fermentation and as the wine ages, the anthocyanins combine with each other, other phenolic material and fermentation methabolites, e.g. pyruvic acid to form new compounds. These include both small molecules and larger polymeric material that may be colorless, red or yellow/brown. The red pigments formed are less sensitive to pH and SO_2 adjustment than free anthocyanins (Iland, Ewart, Sitters, Markides, and Bruer, 2000). Anthocyanins are highly unstable molecules in food matrix. The color stability of anthocyanins is strongly affected by pH, solvents, temperature, anthocyanins concentration and structure, oxygen, light, enzymes, and other accompanying substance (Rein, 2005). Anthocyanins polymerize with other wine components (and themselves) over time, leading to greater color stability (Johnston and Morris, 1996).

Anthocyanins are also found in the skin of the grape. Anthocyanins are the principle source of pigmentation in red wine and have no flavor or organoleptic property. Of the different anthocyanins found in grape, the most abundant is malvidin-3-glucoside. A phenomenon known as copigmentation occurs in young red wines. Copigmentation is the enhancement of color due to formation of complexes between anthocyanins and colorless cofactors such as flavonols and hydroxycinnamates. Some cofactors can also cause the wine to take on a bluish appearance. This spectral change is known as a bathochromic shift or “blue shift” (Harbertson, 2007).

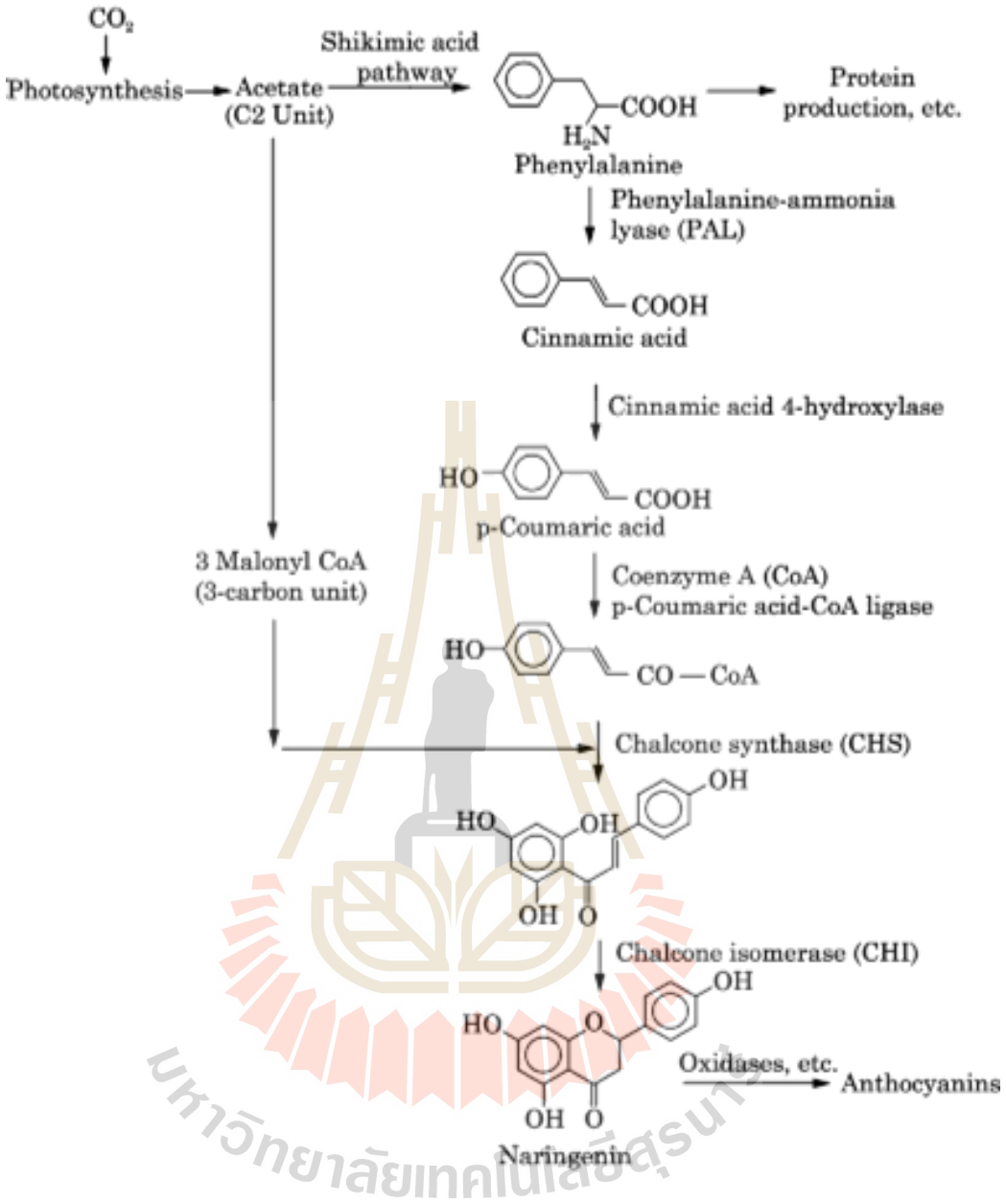


Figure 2.10 Anthocyanins biosynthesis pathways (Pang *et al*, 2007).

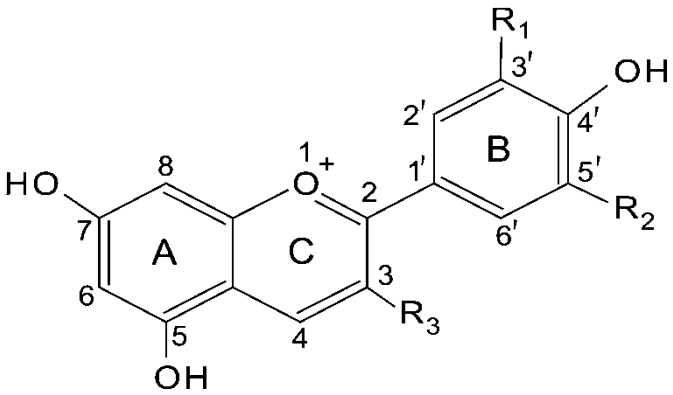
The anthocyanins are conjugated anthocyanidins, which provide the distinctive and vibrant palate of colors found in dark berries. Anthocyanins classification is based primarily on the position of the hydroxyl and methyl group on the B ring of the anthocyanidins molecule. The B ring is made of six carbon atoms each which are

bonded together to form a special structure known as an aromatic ring. The numbers next to each point are called “positions” on this structure. At each position is a carbon atom where specific small groups of atoms called functional groups may attach. The B ring is attached to each other by a “three-carbon bridge”. On this basis, grape anthocyanidins are dividing in to five classes, namely cyanins, delphinidins, malvins, peonins and petunins (Figure 10). They form conjugates with a number of sugars, in particular glucose, sophorose, rutinose, rhamnose, galactose, arabinose and xylose. The proportion and amount of each class vary widely among cultivars and with growing condition. The proportion of anthocyanins markedly influences both hue and color stability. Both properties are directly affected by the hydroxylation pattern of anthocyanidins B ring. Blueness increases with the number of free hydroxyl group, whereas redness intensifies with the degree of methylation (Jackson, 1994).

Wines with longer skin maceration times promoted a greater extraction of phenolic compounds from the skins and had, at the moment of bottling, higher color density (Gómez-Plaza *et al*, 2001). In contrast, sulfur dioxide levels and cold-soak treatments have frequently been shown to have no or little lasting effect or to lead to a decrease in phenolic levels (Karna, Linda and Douglas, 2005). The best color characteristics were obtained, when low-temperature maceration wines were clarified with polyvinyl pyrrolidone. Color quality also improved with lower storage temperature (Gómez-Plaza *et al*, 2000). Wine made by thermo-vinification was much more colored than a traditional wine but it contained less anthocyanins and more polymeric compounds. The carbonic aceration wines were generally less colored than the traditional ones, but they might contain high concentration of proanthocyanidins or total polyphenols (Spranger *et al*, 2004).

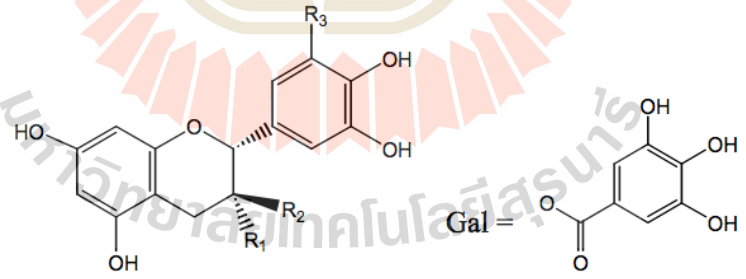
2.5.1.4 Tannins or procyanidins

Tannin is the common name given to several classes of phenolic compounds. Tannins can be divided into two sub categories: condensed and hydrolyzable. Condensed tannins (proanthocyanidins) are the most abundant class of phenolics found in the grape and wine while the hydrolyzable tannins found in wine are the non-flavonoid ellagitannins derived from oak barrels (Harbertson, 2007). Condensed tannins are polymers of flavan-3-ol subunits and are composed of procyanidins and prodelphinidins. The tri-hydroxylated prodelphinidin subunits consist mainly of epigallocatechin but with trace amounts of gallocatechin and epigallocatechin 3-*O*-gallate (figure 11) (McRae and Kennedy, 2011). They are found in the skin and seed of the grape and the mean length of the polymers has been estimated to be 27 and 6 at harvest, respectively (Harbertson, 2007). The natural grape tannins or tannins in wines are extracted from the skins, seeds and stalks. The structures of condensed tannins are made up of a linked series of monomers based on flavan 3-ols or their derivatives. The polymerization of the flavan-3-ols is an essential feature of the structure and properties of grape tannins (Obradovic, 2005). Tannin quality in wines has been a challenge to define and has been a strong research interest. Only a few studies have examined the impact of skin and seed tannins in winemaking (Lee *et al*, 2008). Red wines made by skin fermentation with stem-contact contained much higher polymeric phenols than those made by skin fermentation without stem contact wine, and extending pomace contact time increased both total and polymeric phenol levels of red wines (Spranger *et al*., 2004).



Aglycone	R_1	R_2	R_3
Delphinidin	OH	OH	OH
Cyanidin	OH	H	OH
Petunidin	OCH_3	OH	OH
Peonidin	OCH_3	H	OH
Malvidin	OCH_3	OCH_3	OH
Pelargonidin	H	H	OH

Figure 2.11 Structures of the major anthocyanidins (Kay, 2006).



	Flavan-3-ol Monomer	R_1	R_2	R_3
1	Epigallocatechin	OH	H	OH
2	Gallocatechin	H	OH	OH
3	Epigallocatechin 3-*O*-gallate	O-Gal	H	OH
9	Catechin	H	OH	H
10	Epicatechin	OH	H	H

Figure 2.12 Structures of condensed tannin subunits (flavan-3-ol monomers) (McRae and Kennedy, 2011).

The tannin content of wine has been reported to vary considerably (50 mg/L-1500 mg/L Catechin Equivalents) and wines made from different cultivars also show different average amounts. Tannins are responsible for the astringency of red wines. It has been found in model wine systems that larger polymers have harsher astringency descriptors. As discussed earlier, tannins will combine with anthocyanins to form polymeric pigments. It has been long speculated that these "pigmented tannins" are the source of the mellowing of astringency during wine aging. It has also been speculated that tannins polymerize during wine aging till they become insoluble and the decline of astringency is simply the loss of tannins. Neither of these theories has been proven, and the most recent evidence shows that tannins actually de-polymerize during ageing, however, the definitive work on this subject has yet to be done. Thus, it is clear the precise mechanism for the loss of astringency during wine aging has yet to be proven and further research is still necessary (Harbertson, 2007).

Higher-alcohol solutions tend to extract more tannins from the skins and seeds than do lower-alcohol solutions (Berger, 2005). Samples of wine made after seeds removal were compared with those prepared in the standard way, that is, with seeds present (control). Only minor differences were observed, the regular wine having a slightly greater percentage molar proportion of such seed proanthocyanidins as (-)-epicatechin-3-*O*-gallate extension and terminal subunits, this resulting from a higher degree of seed tannin extraction, while wine produced with seed presence had a higher percentage molar proportion of such proanthocyanidin indicators as (-)-epigallocatechin extension subunits. Wines made after seed removal had higher levels of total anthocyanins, but only minor differences were seen in their color measurement values when these were compared with those of standard wines; control wine was slightly more orange, lighter and more saturated (Lee *et al*., 2008). Total phenols are

extracted from grape seeds during the fermentation of red wines. The amount of total phenols increases with increased seed volume and seed contact time. The skins, although they contain less total phenols, contribute more phenols than the seeds because the skin tannins are more readily extractable. Excessive handling of the skins or prolonged skin-juice contact time can increase tannins in the wine (Meyer and Hernandez, 1970).

Seed tannins are important to obtain a well-balanced wine and help to form stable color. However, the extractable tannin from seeds quantity decreases during maturation. This decrease depends on seasonal and vineyard management practices. As maturity increases, the degree of polymerization of these tannins increases, as does their reactivity towards proteins. It appears that polymerization can be influenced by temperatures during the season (Zoecklein, 2002).

2.5.1.5 Proanthocyanidins (PAs)

PAs (or condensed tannins) are a grape-derived phenolic class of compound that provides wine with bitterness and astringency. Because of this, PAs are considered to be essential components of wine quality. PAs are localized in the seed, skin and stem tissue of the grape berry and the composition of PAs will vary depending on the tissue of origin. Empirical evidence suggests that PAs are seed-derived impart harsh and aggressive tannins to red wine, and minimizing the crushing of fruit prior to fermentation and maceration will minimize the extraction of seed material (Calderon and Karen, 2007).

PAs are oligomers and polymers of polyhydroxyflavan-3-ol monomer units linked most commonly by acid-labile 4 → 8 and in same cases by 4 → 6 bonds. The 4 → 8 bonds are more common than 4 → 6 bonds and some branching may occur in the chain of higher oligomers and polymers (Figure 2.13). The PAs are colourless

compounds which can be release coloured anthocyanidins by cleavage of the interflavan C-C bond on heating in acidic medium. According to their increasing degree of polymerization, PAs are termed as follows: dimers, trimers, oligomers and condensed (polymers). The fundamental structural unit of PAs is the phenolic flavan-3-ol nucleus (Figure 2.14) (Sun and Spranger, 2005).

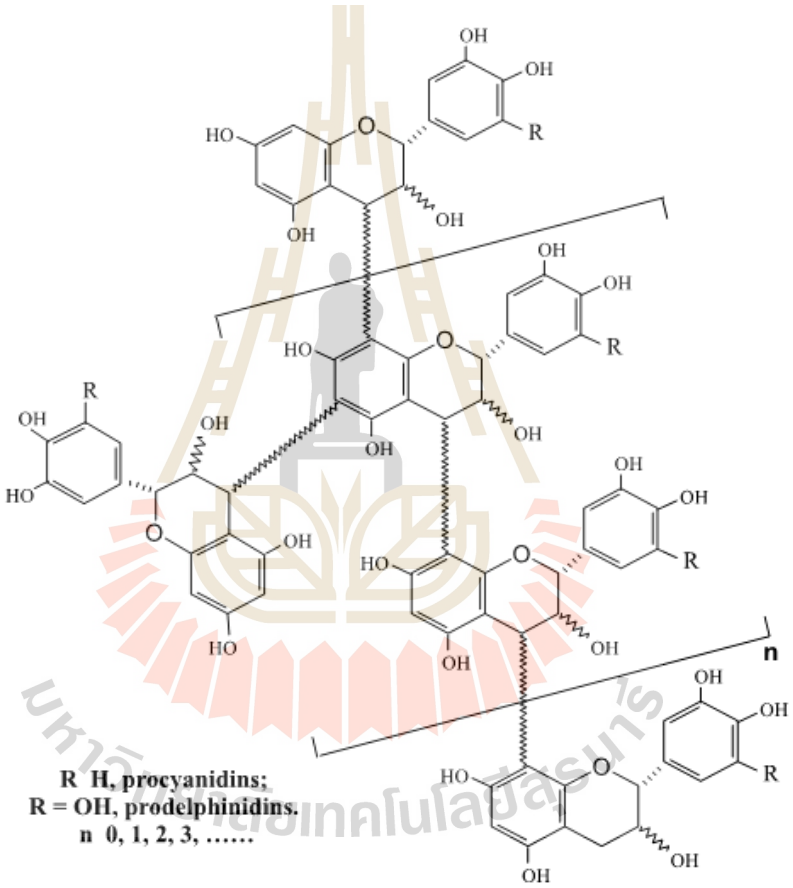


Figure 2.13 General structure of PAs (Sun and Spranger, 2005).

Berries can contain substantial amounts of the flavan-3-ol monomers , (+)-catechin and (-)-epicatechin as well as dimers, trimers and polymeric PAs. The

concentration of the polymers is usually greater than the monomers, dimers and trimers and overall (Amakura *et al*, 2000).

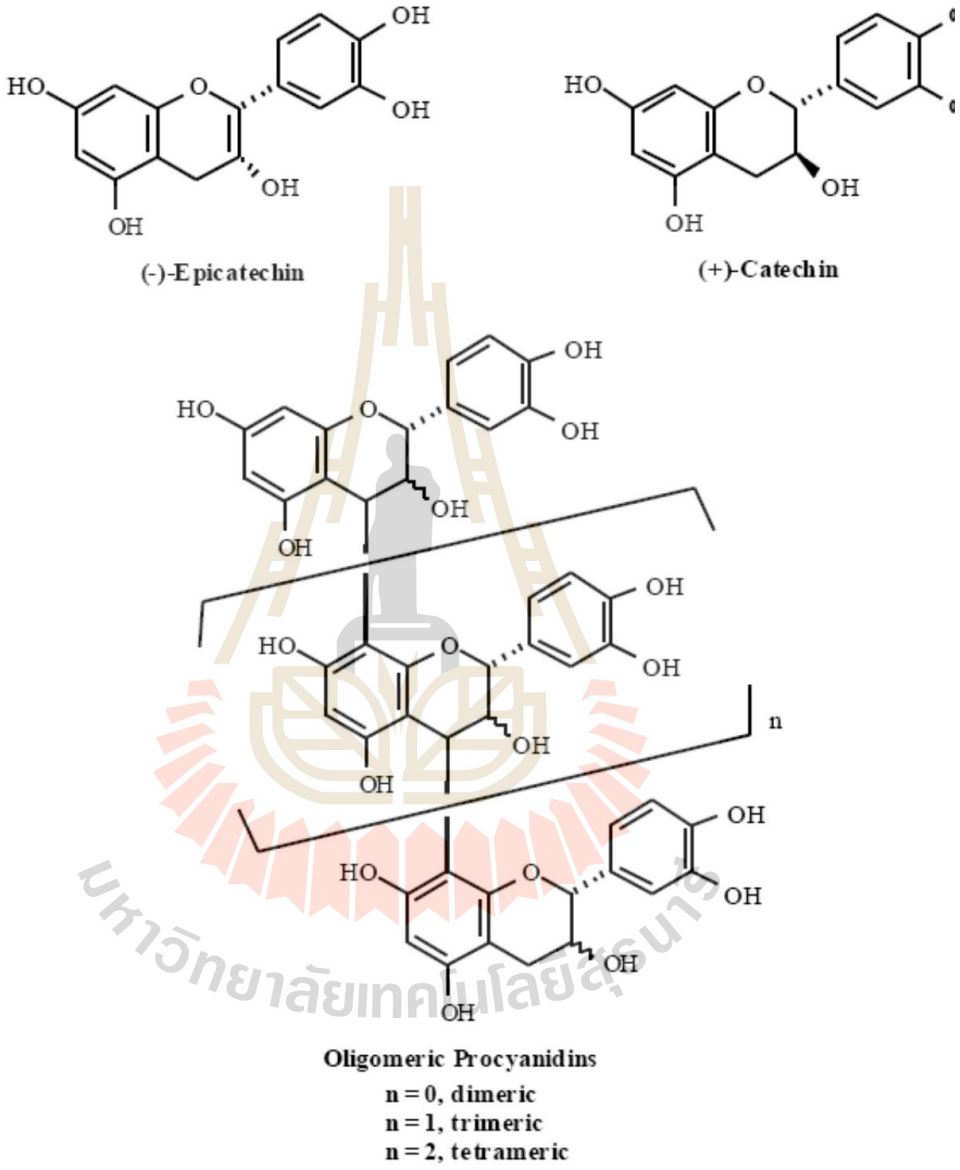


Figure 2.14 Structures of the flavan-3-ol monomers (+)-catechin and (-)-epicatechin and oligomeric PAs (Beattie, Crozier, and Duthie, 2005).

PAs are oligomeric and polymeric end products of the flavonoid biosynthetic pathway (Dixon, Sharma, and Xie, 2005). PAs are derived from the pathway leading to anthocyanins, a class of flavonoids well understood both biochemically and genetically. Three enzymes (leucoanthocyanidin reductase [LAR], anthocyanidin synthase [ANS] and anthocyanidin reductase [ANR]) function at branches between anthocyanin and PA biosynthesis (Figure 2.15). LAR (a member of the reductase-epimerase-dehydrogenase family and closely related to isoflavone reductase from the isoflavonoid pathway and ANS (a member of the 2-oxoglutarate-dependent dioxygenase [2-ODD] family) share the same substrate, flavan-3, 4-diol (leucoanthocyanidin), which is converted to the PAs unit 2,3-trans-flavan-3-ol (catechin) by LAR or to anthocyanidin by ANS. The latter compound can serve as substrate for ANR to produce another major PAs unit, 2,3-*cis*-flavan-3-ol (epicatechin), or be converted to anthocyanins by glycosylation and esterification (Pang *et al*., 2007).

Thus, although catechin and epicatechin only differ by the stereochemical configuration at the C2 and C3 positions (*trans*- or *cis*-, respectively), they are synthesized by two quite distinct pathways. The biosynthesis of PAs oligomers is believed to proceed by addition of an extension unit (derived from leucoanthocyanidin, catechin, or epicatechin) to a starter unit (catechin or epicatechin) with sequential addition of further extension units, although the exact details of the PAs polymerization process are still unclear (Pang *et al*., 2007).

Lee *et al* (2008) found that early seed removal during fermentation contained more PAs, and this could have been due to skin PAs being more readily extracted during fermentation when compared to seed PAs extractability. Seed removed wine

had more intense red color, which was most likely due to higher amount of total anthocyanin present.

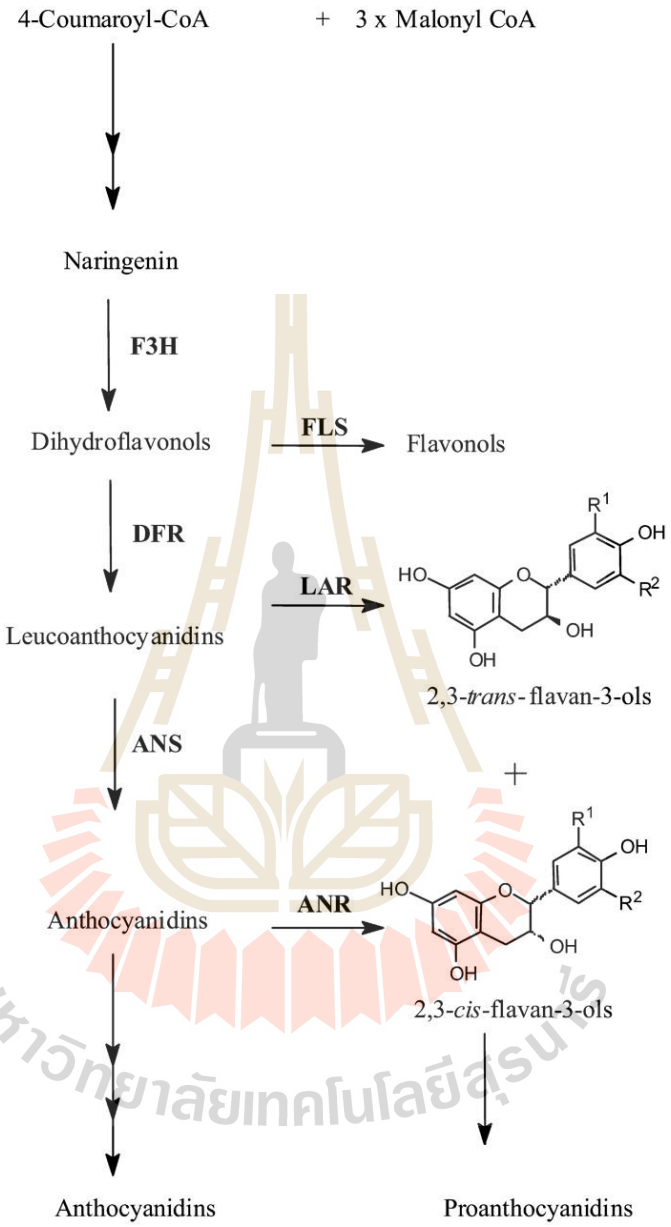


Figure 2.15 Schematic representation of the biosynthetic pathway for anthocyanidins and PAs (ANR, anthocyanidin reductase; ANS, anthocyanidin synthase; DFR, dihydroflavonol 4-reductase; F3H, flavanone 3-hydroxylase; FLS, flavonol synthase; LAR, leucoanthocyanidin reductase) (Pang *et al*., 2007).

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

3.1.1 Microorganisms

The Enoferm BDX (*Saccharomyces cerevisiae*) yeast strain was used in this study. The BDX strain was obtained from the Lallemand Inc., Montreal Canada (LALVIN®). The Viniflora® oenos malolactic bacterial strain (*Oenococcus oeni)* was obtained from Chr. Hansen Holding A/S, Hørsholm Denmark.

3.1.2 Grape berries

The berries were obtained from vines which planted in Moon Valley Vineyard in humid subtropical climate located in the Southwest of People's Republic of China (Xichang district, Sichuan province at 27 ºN, 102 ºE and 1,650 meters high above mean sea level). The berries were harvested in August 2013 at different sugar contents (14°Brix, 16°Brix, 18°Brix, 20°Brix and 22°Brix).

3.2 Methods

3.2.1 Must preparation

To produce must, 5 kilograms of berries were destemmed and crushed by small destemmer and crusher. The must was transferred to PET 5-L vessels and treated with 45 mg/L of sulfur dioxide. Several total soluble solid (°Brix) berries were measured and adjusted up to 22°Brix (Ough and Amarine, 1998).

3.2.2 Starter preparation

The active dry yeast was rehydrated at amount of 200 mg/L in warm water (37ºC) for 30 minutes, according to manufacturer's specifications and then inoculated into must.

3.2.3 Alcoholic fermentation

Must was inoculated with active dry yeast at the amount of 200 mg/L according to specification of manufacturer. Must was fermented under control temperature at 25 ºC and stirred twice a day. Fermentation was finished about 7-9 days or until sugar reduced to 0 ºBrix.

3.2.4 Malolactic fermentation

Malolactic fermentation with *O. oeni* (Viniflora® oenos) in dried powder was employed after alcoholic fermentation finished. The reducing sugar was remained at lower than 4 g/L. The inoculation at initial step was 2 ppm. The pH changes derived from malolactic fermentation progress was checked by paper chromatography to determine malic acid and lactic acid (Iland *et al.*, 2000).

3.2.5 Chemical analysis

Part I

1.1) Extended maceration

Cabernet Sauvignon wine were fermented with seeds and skin and then wine were pressed at 7, 14, 21, 28 days after fermentation. The experimental design were used CRB with three replications.

1.2) Effect of grape seed on wine quality

Grape seeds of Cabernet Sauvignon were added at different amounts (25, 50, 75 and 100% w/w) to must before fermentation and compared with no grape seed was control. The experimental design was used CRD with three replications.

1.3) Effect of cracked grape seeds on wine quality

Grape seeds of Cabernet Sauvignon were cracked by drum pressing method. Then the cracked seeds were added to must at different amount (25, 50, 75 and 100% w/w) before fermentation and compared with no addition as control. The experimental design was used CRD with three replications.

1.4) Effect of stems on wine quality

Stems of Cabernet Sauvignon were added at different amounts (25, 50, 75 and 100% w/w) to must before fermentation using no addition as a control. The experimental design was used CRB with three replications.

1.5) Effect of plastic roofing on vine before berry harvesting on wine quality

Cabernet Sauvignon, Merlot and Zinfandel vines roofing with plastic sheets after véraison period. The experimental design was used RCBD with three with replications. Each replication was treated as followings:

1. A1T1 = Cabernet Sauvignon vines without plastic roofing
2. A1T2 = Cabernet Sauvignon vines with plastic roofing
3. B1T1 = Merlot vines without plastic roofing
4. B1T2 = Merlot vines with plastic roofing
5. C1T1 = Zinfandel vines without plastic roofing
6. C1T2 = Zinfandel vines with plastic roofing

Part II

2.1) Determination of total soluble solid in must and wine

The TSS was measured using hand refractometer (Ough, and Amarine, 1998).

2.2) Determination of pH in must and wine

The pH was determinated on a portion of clarified grape juice or clear wine with pH meter. The temperature of the must or wine was the same temperature as that of the standard buffers used in calibration step (Iland*et al.*, 2000).

2.3) Determination of total acidity in must and wine

Total acidity was analyzed by titration technique with 0.1 M NaOH and phenolphthalein was used as indicator, and using pH meter to observed end point at pH 8.2 (Ough, and Amarine, 1998).

2.4) Determination of volatile acidity in must and wine

The volatile acidity (VA) analysis was carried out on a degassed (by a vacuum system) sample of wine. Distillation method and titration technique were used for analysis acetic acid (Iland*et al.*, 2000).

2.5) Determination of alcohol in wine

The alcohol was recovered by distillation. The specific gravity of the distillate was determined by hydrometer. Hydrometer was calibrated with an appropriate correction. The correct reading of the alcohol strength was expressed as % (v/v) at 20 °C (Iland *et al*., 2000).

2.6) Determination of reducing sugar in wine

Determination of reducing sugar was followed the method of Lane and Eynon (Iland *et al*., 2000).

Fehlings A solutions (69.3 g of $CuSO_4$ $5H_2O$ was dissolved in distilled water to 1000 ml) and Fehlings B (346 g of $KNaC_4H_4O_6$, $4H_2O$ and 100 g of NaOH were dissolved in distilled water to 1000 ml) were pipetted 10.0ml and 20.0ml of the dealcoholised, decolorized (and if necessary, diluted) wine sample into a 250 ml conical flask, wines were contained greater than 4 g/l reducing sugars, require dilution

prior to add to the mixture of Fehlings A and B. The burette was checked that is filled with 0.5% w/v glucose solution. The initial burette reading was recorded. Boiling chips or pumice powder was added some to the flask and titrate with 0.5% w/v glucose. The final burette reading was recorded and calculated the difference between the final and initial burette readings. The reducing sugar using formula:

Calculation:

Reducing sugars (g/l) = (Dilution factor/4) × ［Standard titre (ml) - Sample titre (ml) ］

2.7) Determination of free sulfur dioxide and total sulfur dioxide

Determination of free sulfur dioxide and total sulfur dioxide were done by aspiration method (Iland *et al*., 2000).

A. Standardization

Distilled water was added 10 ml and 3 drops of indicator to the pear flask. The purple color of the solution was adjusted to turquoise green by add small drops of NaOH solution then remember the color. Standard sulfuric acid solution was pipetted 5 ml into the flask and swirled. The burette was filled with 0.01N NaOH to the zero mark and titrated the solution to the same green color and record the titer. The standardization was repeated until 3 titers agree within 0.05 ml. This was also excellent practice in recognizing the endpoints. The base normality was 0.01 x 5/(average ml base used in standardization)

B. Sample Determination

A continuous stream of cold water was passed through the condenser during sample testing. The vacuum adapter was draw air at 200 ml/min. H_2O_2 solution was added 10 ml and 3 drops of indicator to the pear flask, the purple

color of the solution was adjusted to turquoise green by adding small drops of NaOH solution and the flask was attached to the end of the vacuum adapter.

a) Total SO_2: wine and phosphoric acid solution were pipetted 10 ml into the 50 mL pear shaped flask. If the wine was gassy or foamy, add a drop of anti-foam.

b) Free SO_2: wine and H_2PO_4 were pipetted 100 ml and 20 ml into 250 ml round bottom flask. The vacuum adapter will be draw air at 200 ml/min for 15 minutes.

For total SO_2: The vacuum adapter was draw air at 200 mL/min while gently boiling the pear flask with a micro-burner for 15 minutes, carefully removed the pear flask, The tip of the vacuum adapter was rinsed into the flask. The burette was filled to the zero mark with 0.01N NaOH and the solution was titrated to a turquoise green color and recorded the titer. The free SO_2 was determined using formular;

Calculation:

$$\text{Free/total (mg/l)} = \frac{\text{(ml base titer) x 32000 x (normality base)}}{\text{10 (for total) or 100 (for free)}}$$

2.8) Determination of total red pigment and phenolic compound

Total red pigment and phenolic compound were measured by Spectrophotometer at 420 nm and 520 nm (Iland *et al*., 2000).

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

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

Total red pigments (absorbance units) = A^{HCl}_{520}

Total phenolics (absorbance units)= $A^{HCl}_{280} - 4$

A. Sample preparation:

Fifty berries were transferred in to a vessel, 125 mL plastic container. The berries were homogenized at high speed at 3,500 rpm for 30 seconds. The flesh, skins and seeds were macerated. Then a representative sub sample was taken into centrifuge and spin at 3,500 rpm for 5 minutes. The supernatant was termed 'the extract'. There was not any large pieces of skins or seeds in the mixture. Any homogenate from the shaft of the homogenizer was scraped into the homogenizing vessel and repeated the homogenizing step for about 15 seconds. It was scraped back into the homogenizing vessel. Thoroughly, the homogenate was mixed by stirring it with a small spoon type spatula and immediately take a scoop of approximately 1 gram of homogenate using the spatula. The scoop was transferred of homogenate into a pre-tared centrifuge tube. The weight was recorded in termed 'the weight of homogenate' for the extraction'.

B. Anthocyanin extraction :

Aqueous ethanol 50% v/v was pipetted 10 mL adjusted to pH 2.0 into the centrifuge tube containing the homogenate. The tube was caped and mixed the contents periodically by inverting the tube about every 10 minutes over a period of 1 hour. After 1 hour, the tube and content was centrifuged at 3500 rpm for 5 minutes. The supernatant was termed 'the extract'.

One milliliter of the extract was pipetted into 1 M HCl and mixed. The solution was poured the remaining volume into a tall, thin measuring cylinder and record the volume. The value of this record volume and the value of the volume of 'the extract' was termed 'the total extract volume'. The diluted HCl extract solution was stood for about three hours. After 3 hours, the solution was read the absorbance of the diluted HCl extract in a 1 cm cell at 700 nm, 520 nm and 280 nm using spectrophoto meter.

Calculation:

1) Color per berry (anthocyanins mg per berry) =

$$\frac{A_{520}/500 \times \text{dilution factor} \times \text{final extract volume (ml)}/100 \times \text{weight of 50 berries (g)}}{\text{weight of homogenate taken for extraction (g)} \times 1000/50}$$

2) Color per gram berry weight = $\frac{\text{Color per berry}}{\text{weight of 50 berries (g)}}$

3) Total phenolics per berry =

$$\frac{A_{280} \times \text{dilution faction} \times \text{final extract volume (ml)}}{100 \times \text{weight of 50 berries (g)}/\text{weight of homogenate taken for extraction (g)} \times 1/50}$$

4) Total phenolics per gram berry weight = $\frac{\text{Total phenolics per berry}}{\text{weight of 50 berries (g)}}$

3.2.6 Sensory evaluation

Wines were sensory-evaluated by ten experienced panellists at 25°C. The wines were compared by Paired Comparison scoring testing to determine whether there were three repetitions of each treatment in random order (Wanapu *et al.*, 2012). Ranking method was used in order to determine quality difference between tested wines. The panellists were trained to evaluate the individual characteristics of wines and to define characteristic aroma descriptors used in sensory analysis. Each sample of wine was judged for intensity of appearance, aroma, taste, aftertaste, and overall using chart (Modified 20-point cardinal scale) of Purdue University (Vine *et al.*, 1999).

Questionnaires and water for mouth rinsing between each tasting were provided. The panellists were asked to read through the questionnaires, and the meaning of each attribute was explained to the panellists to avoid any misinterpretation.

3.2.7 Data analysis and hypothesis testing

Significant differences among wines and their variability were assessed by analysis of variance (ANOVA) and least significant difference (LSD). The statistical package IBM® SPSS® Statistics for Mac release 20.0.0 from IBM Corp was used. Duncan Multiple Range Test (DMRT) was used to separate the means ($p \leq 0.05$) when the ANOVA test was significant.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Size and weight of grape berry

Results of grape berry size and berry weight are shown in Table 4.1. The berry size and weight was increased from véraison to maturity. The availability of water led to greater increases in berry weight. Berry size and weight were significantly different among the treatments. Kok *et al.* (2013) found that the berry size was broadly accepted as a factor determining wine grape quality. In wine grape growing, there were demand in not only small berry and cluster but also abundant grape must. The increase was very fast during the first days of Stage III (defined as the period of time from véraison to maturity).

It has been widely recognized that berry size is an important factor determining wine grape quality. Berries do not grow by 'pumping' water into a berry vessel of flavor solutes (Matthews and Nuzzo, 2007). It was found in this study that at 18^{o}-22^{o}Brix the berry size and weight were significantly higher than the lower ^{o}Brix (14^{o}-16^{o}).

4.2 Color analysis of grape berry

The color of berry was very important for wine quality. Table 4.2 shows the evolution of anthocyanins and phenolics during ripening. A higher concentration of anthocyanins was found in more ripen grape berry (22°Brix) that had color/berry

0.880 mg of anthocyanins/berry and total phenolics/berry 0.660 a.u. while lower ripen grape berry (14°Brix) had lower in color/berry and total phenolics obviously. Coombe *et al.* (1987) reported that the sugars in ripe berries were present at high concentrations in the flesh, and are not localized in the skin. Gonzáles-San *et al.* (1991) found that the anthocyanins accumulate in the grape berry when beginning with véraison, and correlates with sugar accumulation. Anthocyanin concentrations reach a maximum at full maturity, but are broken down if the grapes become overripe (Ribéreau-Gayon, P. *et al.*, 2000).

Table 4.1 Physical analysis of various grape berries quality.

TSS (°Brix)	Size (mm.)	Weight per berry (g)	Fifty berry weight (g)
14	11.73[a]	1.18[a]	60.49[a]
16	12.00[b]	1.25[b]	60.77[ab]
18	12.33[c]	1.30[c]	61.23[b]
20	12.43[c]	1.32[c]	61.40[bc]
22	12.57[c]	1.33[c]	61.63[c]

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

Table 4.2 Color and phenolic content analysis of various grape berries quality; color / berry (mg of anthocyanins / berry), color/berry weight (mg anthocyanins / g berry), total phenolics / berry (absorbance units / berry) and total phenolics / berry weight (absorbance units per g berry).

TSS (°Brix)	Color / berry	Color / berry weight	Total phenolics / berry	Total phenolics / berry weight
14	0.813[a]	0.667[a]	0.480[a]	0.390[a]
16	0.833a[b]	0.690[ab]	0.513[b]	0.423[b]
18	0.857[b]	0.707[b]	0.577[c]	0.477[c]
20	0.873[bc]	0.713[b]	0.633[d]	0.520[d]
22	0.880[c]	0.713[b]	0.660[e]	0.533[d]

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

4.3 Color analysis of wine

It was shown in Table 4.3 that the higher concentration of sugar of grape berry gave higher color in wine. This study showed that grape berry 14°Brix were gave lower value in total red pigment, total phenolics, wine color density and wine color hue than another treatment. While total red pigment of grape berry 18°Brix was not had a significantly difference of with grape berry 22°Brix. Berry was ripen at maturity and gave wine higher in both total red pigment and phenolic. Higher concentration of sugar in grape berry had higher level in wine color density and wine color hue. Oberholster *et al.* (2010) found that wine color or color density (A420 nm + A520 nm) correlated with the anthocyanin concentration (mg/g berry). This finding indicated that differences in grape composition and color were visible in the

subsequent wines. There was a strong relationship between brix and the general quality of grape extracts and subsequently affected wine characters. Anthocyanin concentration (mg/g berry) and wine hue was also correlated.

Table 4.3 Color and phenolic content analysis of wine from various TSS concentration; total red pigment (a.u.), total phenolics (a.u.), wine density and color hue.

Treatment	Total red pigment (a.u.)	Total phenolics (a.u.)	Wine color density	Wine color hue
14 °Brix	6.070[a]	18.830[a]	2.760[a]	0.660[a]
16 °Brix	8.200[b]	19.700[b]	3.833[b]	0.727[a]
18 °Brix	9.033[c]	21.467[c]	4.250[c]	0.903[b]
20 °Brix	9.133[c]	21.633[c]	4.460[c]	0.927[b]
22 °Brix	9.233[c]	28.200[d]	5.283[d]	0.933[b]

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

4.4 Chemical analysis of wine

Total acidity of wines were a significantly different (Table 4.4). Young berries, 14°Brix, had a higher TA and lower pH. The sugar content in berries at harvest influenced must TSS values, and this in turn is directly related to alcohol content of the wine, principally in the form of ethanol. It was found that alcohol contents were not significantly different because before fermenting all treatments were chaptalized up to 22°Brix. Peynaud (1984) found that sugar additions of up to +2% (2 grams per

100 mL or 20 g/L) are normally allowed, and up to 4% may be used in very difficult vintages. To raise the alcohol level by 1% by volume, about 17 g/L of sugar needs to be added to white juice and about 20 g/L to red must. Greater additions are necessary for red wine to compensate for evaporation of alcohol during warmer red fermentations. VA was acids formed by spoilage bacteria (Vine *et al*., 1999). The VA was founded in wine include mainly acetic acid and to a lesser extend other acids such as butyric, formic and propionic. The concentrate of acetic acid formed during fermentation is usually less than 0.5 g/L (Iland *et al*., 2000). VA had a significantly difference by 14°Brix had higher VA more than other treatments. Reducing sugar is a fermentable sugar and decreases with increasing period of fermentation. Reducing sugar is most important sugar for fermentation as it is easy to metabolize by yeast (Singh *et al*., 2013). The value for the concentration of reducing sugars at the end of fermentation is about 2 g/L or less (Iland *et al*., 2000). Residual reducing sugar had a significantly difference when grape berry at 14°Brix had higher reducing sugar after fermented.

Table 4.4 Chemical analysis of wine from various TSS concentration; total acidity (TA as g/L tartaric acid), pH, alcohol content, volatile acidity (VA as g/L acetic acid) and reducing sugar.

TSS (°Brix)	TA (g/L)	pH	Alcohol content (%v/v)	VA (g/L)	Reducing sugar (g/L)
14	11.30[e]	3.13[a]	12.47[a]	0.55[c]	2.78[c]
16	10.43[d]	3.20[b]	12.47[a]	0.43[b]	2.63[b]
18	9.13[c]	3.35[c]	12.50[a]	0.33[a]	2.48[a]
20	7.47[b]	3.45[d]	12.50[a]	0.31[a]	2.40[a]
22	6.63[a]	3.51[e]	12.53[a]	0.31[a]	2.39[a]

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

4.5 Effect of plastic roofing vine before berry harvesting on wine quality

Xichang is located in the high rainfall area of China where grape is berry harvesting time usually exposed to rain resulted in poor quality of grape berries are obtained. To solve the problem plastic roofing were manipulated during ripening of grape berries (Table 4.5). Wine made from *Cabernet Sauvignon* with plastic roofing, the total red pigments, total phenolic, and wine color were significantly higher than without plastic. The TA was higher under no plastic which pH was higher. Alcohol, VA and RS were similar. Wine made from Merlot, wine color density and wine color hue were significantly higher with plastic roofing which the phenolic and red pigments were not different.

The TA and pH were higher under no roofing which alcohol, VA and RS were not different. Wine made from Zinfandel; under plastic roofing, total red pigments, total phenolic compound and wine color hue were higher than the no roofing but wine color density was higher under no roofing. Overall roofing during harvesting provided a good protection of grape berries for making wine. It was interesting to find that extended maceration, adding, seeds, stems and roofing gave a significant of improving wine quality under tropical rainfall condition.

Table 4.5 Effect of plastic roofing vine before berry harvesting on wine quality.

Wine		TA	pH	Alc	VA	RS	Total red pigments (a.u.)	Total phenolics (a.u.)	Wine color density	Wine color hue
Cabernet Sauvignon	No Plastic roofing vine	7.00^{b}	3.37^{a}	12.53^{a}	0.32^{a}	2.13^{a}	9.03^{a}	20.87^{a}	4.40^{a}	0.62^{a}
	Plastic roofing vine	6.20^{a}	3.45^{b}	12.60^{a}	0.30^{a}	2.17^{a}	9.27^{a}	21.30^{a}	4.64^{a}	0.71^{b}
Merlot	No Plastic roofing vine	5.73^{b}	3.79^{b}	12.77^{b}	0.50^{a}	2.60^{a}	11.93^{a}	29.03^{a}	4.97^{a}	0.78^{a}
	Plastic roofing vine	5.47^{a}	3.65^{a}	12.33^{a}	0.48^{a}	2.63^{a}	13.17^{a}	26.83^{a}	6.58^{b}	0.72^{a}
Zinfandel	No Plastic roofing vine	5.80^{a}	3.79^{b}	12.57^{a}	0.53^{a}	3.90^{a}	9.47^{a}	19.03^{a}	2.96^{a}	0.62^{a}
	Plastic roofing vine	6.60^{b}	3.52^{a}	12.80^{b}	0.49^{a}	3.87^{a}	10.10^{a}	20.47^{a}	4.30^{b}	0.65^{a}

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

4.6 Extended maceration

The maceration of wine from 14-28 days was significantly decreased TA and RS but slightly increased in VA while pH and alcohol were not different. The increasing in maceration up to 28 days were significantly increasing in total red pigments, total phenolic, wine color density and wine color hue. This finding was proved that maceration of wine is important in production wine quality. This was agreed with the finding of other investigators (Olejar, et al. 2015) who reported that using different maceration techniques could increase phenolic and antioxidants in wine (Table 4.6).

Table 4.6 Effect of extended maceration at 7-28 days.

Wine	TA	pH	Alc	VA	RS	Total red pigments (a.u.)	Total phenolics (a.u.)	Wine color density	Wine color hue
7 day	7.93^{b}	3.41^{a}	12.77^{a}	0.32^{a}	2.77^{c}	9.23^{a}	16.53^{a}	5.07^{a}	0.72^{a}
14 day	7.80^{a}	3.41^{a}	12.73^{a}	0.33^{a}	2.60^{b}	9.33^{ab}	18.07^{b}	5.29^{ab}	0.81^{b}
21 day	7.83^{a}	3.41^{a}	12.57^{b}	0.36^{b}	2.48^{ab}	9.47^{b}	22.87^{c}	5.46^{ab}	0.93^{c}
28 day	7.83^{a}	3.41^{a}	12.23^{a}	0.39^{c}	2.40^{a}	9.50^{c}	22.53^{c}	5.59^{b}	0.94^{c}

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

4.7 Effect of grape seeds on wine quality

Addition of seeds at 75-100% had significantly increased TA, RS but decreased pH. Alcohol concentration and VA were not changed (Table 4.7). It has been

recognized that phenolic composition is one of the main determinants of the quality of red wines. Wines attributes such as color, body and astringency are closely related to its composition in anthocyanins and proanthocyanidins (Glories, Y. 1984; Ribereau-Gayon, P. et al 2000; Vida, S. et al. 2003). Proanthocyanidins which are found in grape seeds, also known as condensed tannins are the main determinant of texture sensations such as body and astringency. It was found that addition of seeds from 50-80% in wine could significantly increase total phenolic. Wine color density was also significantly increased when seeds were added at 50-75%. Riberen-Gayon et al (1998) reported that by combining with anthocyanins, proandthocyanidins also contributed to long-term color stability. Therefore, grapes seeds are important in wine quality stabilization.

Table 4.7 Effect of grape seeds addition on wine quality.

Wine	TA	pH	Alc	VA	RS	Total red pigments (a.u.)	Total phenolics (a.u.)	Wine color density	Wine color hue
0%	6.03^{a}	3.42^{c}	12.53^{a}	0.26^{a}	2.17^{a}	10.13^{b}	13.13^{a}	4.29^{a}	0.81^{b}
25%	6.03^{a}	3.44^{c}	12.57^{a}	0.27^{a}	2.20^{a}	7.70^{a}	16.50^{b}	5.43^{b}	0.77^{b}
50%	6.93^{ab}	3.26^{a}	12.60^{a}	0.35^{a}	2.27^{a}	7.87^{a}	20.63^{c}	6.60c	0.57^{a}
75%	7.43^{b}	3.30^{a}	12.57^{a}	0.27^{a}	2.50^{b}	9.73^{b}	21.93^{c}	7.37^{d}	0.62^{a}
100%	8.53^{c}	3.36^{b}	12.47^{a}	0.35^{a}	2.77^{c}	7.37^{a}	24.63^{d}	4.02^{a}	0.81^{b}

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

4.8 Effect of cracked grape seeds on wine quality

Adding cracked seeds during wine fermentation on wine characteristics are shown in Table 4.4. Adding cracked seeds significantly decreased TA but increased pH and RS while the amount of alcohol was not different. Adding seeding cracked seeds significantly increased total phenolic compounds, wine color density and hue which was similar trend as uncracked seeds in Table 4.8. Eliminating and adding seeds considerably affects the color, phenolic compounds composition and astringency (Canals, R., et al. 2008; Poudel, P. R., et al. 2008).

Table 4.8 Effect of cracked grape seeds addition on wine quality.

Wine	TA	pH	Alc	VA	RS	Total red pigments (a.u.)	Total phenolics (a.u.)	Wine color density	Wine color hue
0%	7.50^{b}	3.35^{a}	12.67^{a}	0.41^{a}	2.30^{a}	9.60^{a}	16.47^{a}	4.35^{ab}	0.69^{a}
25%	6.57^{a}	3.41^{ab}	12.53^{a}	0.47^{ab}	2.37^{a}	8.10^{a}	22.40^{b}	4.06^{a}	0.68^{a}
50%	5.97^{a}	3.50^{b}	12.60^{a}	0.60^{b}	2.57^{b}	8.90^{a}	20.33^{ab}	4.26^{ab}	0.85^{ab}
75%	5.90^{a}	3.50^{b}	12.47^{a}	0.46^{ab}	2.90^{c}	9.23^{a}	21.67^{ab}	4.53^{b}	0.82^{ab}
100%	6.20^{a}	3.54^{b}	12.53^{a}	0.46^{ab}	3.10^{d}	8.37^{a}	22.63^{b}	4.03^{a}	0.90^{b}

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

4.9 Effect of stems addition on wine quality

Effect of stems adding during wine fermentation are demonstrated in Table 4.9. It was found that adding stems affected on pH and RS increasing while TA and alcohol did not. Total phenolic compounds and wine color were increased with

increasing stems added. The highest phenolic was obtained when 75% of stems were added. Suriano, S., et al. (2015) found that the higher percentage of stems used during fermentation, the higher the content of total polyphenols was detected. Normally, undergo natural evolution evidenced by a progression decay of total flavonoids, total polyphenols, flavors reactive to vanillin and proanthocyanidins high quality of wine (Solinas, M. et al 2003).

Table 4.9 Effect of stems on wine quality.

Wine	TA	pH	Alc	VA	RS	Total red pigments (a.u.)	Total phenolics (a.u.)	Wine color density	Wine color hue
0%	6.57^{a}	3.48^{a}	12.27^{b}	0.61^{a}	2.23^{a}	7.57^{a}	15.33^{a}	2.98^{b}	0.62^{ab}
25%	6.33^{a}	3.53^{a}	12.33^{b}	0.55^{a}	2.33^{ab}	9.53^{c}	21.47^{b}	3.13^{c}	0.65^{b}
50%	6.03^{a}	3.59^{ab}	12.43^{b}	0.59^{a}	2.37^{b}	7.73^{a}	24.83^{c}	2.89^{a}	0.65^{b}
75%	5.37^{a}	3.68^{b}	11.27^{a}	0.68^{a}	2.67^{c}	8.60^{b}	26.33^{d}	5.51^{d}	0.60^{a}
100%	5.80^{a}	3.68^{b}	12.27^{b}	0.65^{a}	2.80^{d}	7.30^{a}	22.17^{b}	3.20^{c}	0.74^{c}

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

4.10 Total acidity and pH of grape berry

TA and pH values are shown in Figure 4.1. At the time of harvest, TA levels were similar for all the grapes. As TA decreased during ripening, pH values increased. A small changing in pH reflects large changes in TA. This finding agrees with

Esteban *et al.*, (2002) who observed that pH increased linearly with berry ripening while TA decreased exponentially. At the beginning, when TA was high, a decrease in TA did not bring about a substantial changed in pH as ripening advanced, the variation in pH became larger. However, the pH values found in the must at maturity were high. The decrease in TA during ripening is normally attributed to falling concentrations of malic acid because tartaric acid is considered to be unaffected (Calo *et al.*, 1997).

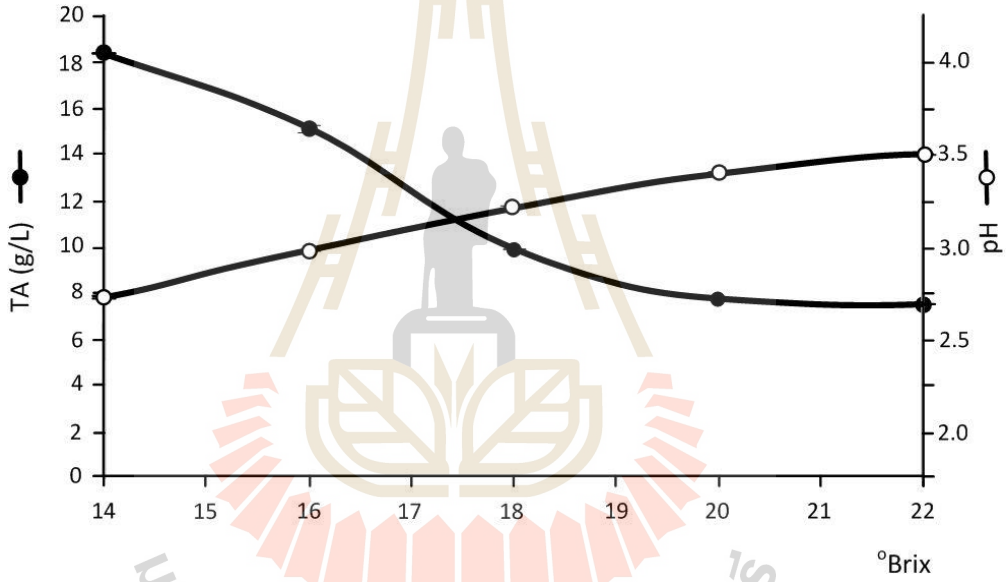


Figure 4.1 Total acidity and pH analyses of various grape berries quality; 14°Brix, 16°Brix, 18°Brix, 20°Brix and 22°Brix

4.11 Sensory analysis

Chen *et al.* (2013) found that the sensory evaluation by tasters were commonly used on evaluating the grape wine sensory quality. During the evaluation, tasters grade several indexes of the grape wines after tasting them. Based on the summation of the indexes, the quality of grape wine was finally evaluated.

The results of 20-point cardinal scale of Paired Comparison scoring test was converted to percentage which showed differences in all parameter between young grape berries (14 ^{o}Brix and 16^{o}Brix) and higher ripeness grape berries (18 ^{o}Brix, 20 ^{o}Brix and 22 ^{o}Brix) taste, color, and aftertaste among the treatment wines (Fig. 4.2). However, The wines made from 18 °Brix, 20 °Brix and 22 °Brix berries had not significantly different in appearance, aroma, taste, aftertaste and overall characters. The experienced wine tasters were more influenced by color (Pangborn *et al.*, 1963). Oberholster*et al.* (2010) found that the wines made during the highest grape color peak did not different significantly from the wines on either side of the maturity period. This indicated that grapes could have been harvested earlier at lower sugar concentrations. However, this does not mean that other sensory criteria did not change significantly.

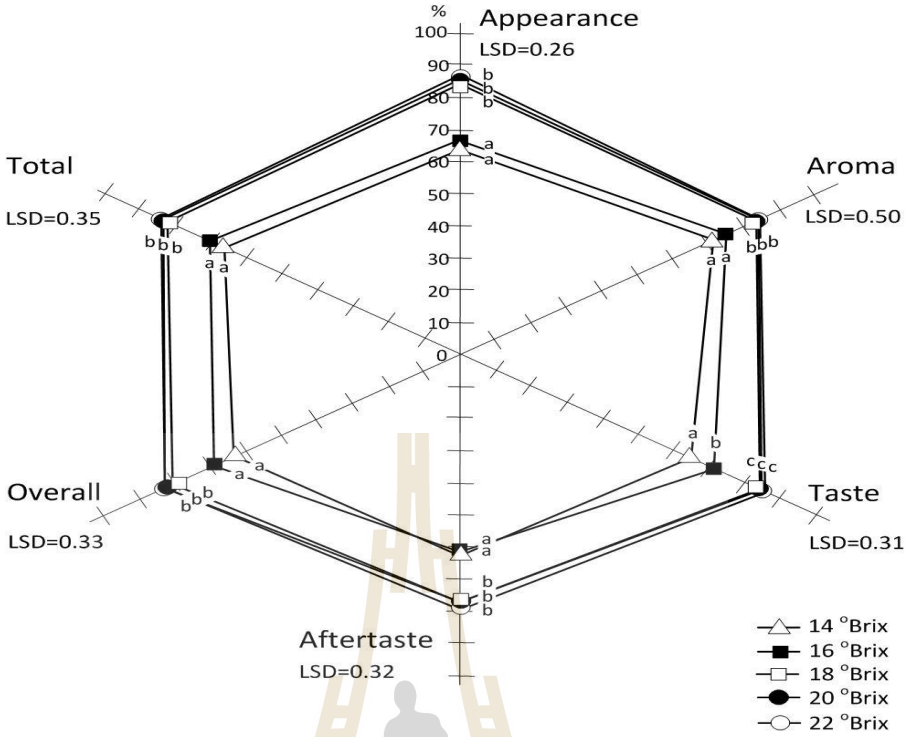


Figure 4.2 Sensory scores of wines from various grape berries quality. The LSD value is the mean least significant difference among wines. The percentage of mean is in each sensory parameter followed by the same letters are not significant different (P>0.05).

CHAPTER V

CONCLUSION

Quality might be defined as those attributes of the grape that make it attractive or pleasant to drink as wine. The absolute concentrations of sugar and acids, as well as their ratio, play important role in flavor of wine. Phenolics determine color quantity in nearly all wines and they are major factor in flavor in red wines. Aroma compounds that are important in wine flavor arise from both the fruit and as product of fermentation. The high ripen maturity of grape berry containing TSS 22°Brix gave wine with high density in color, low TA, low VA and low reducing sugar. The low ripen berry containing TSS14°Brix gave the conjunction result in high TA, VA and reducing sugar that made wine lower quality. However, berries had TSS more than 18°Brix gave acceptable wine quality but must be used the chaptalization method to increase TSS to desirable level sugar of must. Finally, grape berries quality was directly affected on wine quality and it could be used as the index to predict the quality of wine. A range of parameters could effectively differentiate between grapes and wine on the basis of ripeness. The quality of grape berries from rain damage plastic roofing was used and found that the quality of grape berries under roofing was better than none roofing. The parameters that could improve wine quality in humid subtropical climate over seasonal and climatic variance will be investigated further.

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APPENDIX

A. **Sample preparation （decoloristing and dealcoholisting the wine):**

Wine sample will be accurately filled 100ml in to volumetric flask and then transferred it quantitatively to a 250 ml beaker, boiling chips will be added to the wine and boiled to about half its volume to dealcoholise the wine. If the wine has any color, added about 0.5 grams activated (decolorising) charcoal to the dealcoholised wine sample and boiled for 30 seconds. It will be cooled to room temperature and filtered through Whatman No.5 filter paper. The dealcoholisted and decolorised wine were transfered quantitatively to the 100ml volumetric flask and make up accurately to the mark with distilled water at 20 ℃, cap the flask and mix the solution thoroughly.

B. Procedure for standardizing the soxhlet solution:

Fehlings A and 10.0ml of Fehlings B were pipette 10.0 ml of solutions into a 250 ml conical flask (Soxhlet solution is the mixture of Fehlings A and B). It will be added some boiling chips or a small spatula tip of pumice powder to the flask then filled the burette with 0.5%w/v glucose solution and recorded the initial burette reading and bring the Soxhlet solution to the boil and start titrating with the 0.5% w/v glucose solution. The solution will be blue in color. While boiling, keep titrating with further glucose until only a faint blue color remains, then added 5 drops of 1% w/v methylene blue indicator .The blue color will be intensify. Continue titrating, with the solution still boiling until the blue color in the solution is dissipated. A precipitate of cuprous oxide will be formed which imparts a brick red color to the solution, but when this settles out, the ‘solution’ will be cleared and colorless. This end point will be difficult to recognize and it was easier if look through the solution edges against a white

background for the disappearance of the blue color. The titration will be timed of is critical, it will be taken as close to 3 minutes as possible from the commencement of boiling until the end point is reached then recorded the final burette reading. The difference between the final and the initial burette readings were calculated. This will be called the standard titre.

E. Monitoring of malolactic fermentation.

Malic acid disappearing will be monitored by paper chromatographic method. The solvent mixture will be used contain the pH indicator bromocresol green, which undergoes a color change from yellow to blue in the pH range of 3.8-5.4. The presence of an acid will be indicated as a yellow spot on a blue background. Wine acid including of malic acid, tartaric acid and lactic acid (0.3%w/v) were used as standard. The ten microliters of all acids and wines were spotted on Whatman No.1 chromatographic paper, and then put into chromatography developing tank contained with chromatographic solvent. The solvent will be prepared from 100 ml of n-butanol, 100 ml of deionized water, 10.7 ml stock formic acid and 15 ml of 1% bromocresol green indicator solution prepared by deionized water. Solvent mixture will be taken to separatory funnel for discarded organic phase (Iland *et al*., 2000).

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. Phajon Yuyuen was born in Chiangmai, Thailand on November, 6, 1978. He attended Rajamangala Institute of Technology Agricultural at Bangpra, Thailand and received his Bachelor's degree in food science and technology in 2000 with 2nd Honor. In 2001, He teach at Rajamangala Institute of Technology Phitsanulok Campus, Thailand in Food Technology Department and researched many kinds of fruit wine and herbaceous wine production. A year later, he worked at Charoen Pokaphand Seeds Co.,Ltd. as a research employee. In 2003, he worked at Xichang Chia Tai Wine&Spirits Co.,Ltd. Sichuan, People' s Republic of China as a researcher and winemaker. In 2012, he was master's student in School of Biotechnology at Suranaree University of Technology.