TRANS-RESVERATROL AND OTHER PHENOLIC COMPOUNDS OF SOME SUT GRAPES AND WINES AND THEIR CHANGES DURING VINIFICATION PROCESS

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TRANS-RESVERATROL และสารประกอบฟีนอลิกอื่นๆขององุ่นและไวน์ มทส และการเปลี่ยนแปลงระหว่างกระบวนการผลิตไวน์

นางสาวจิรายุส วรรัตน์โภคา

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

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จิรายุส วรรัตน์โภคา : *TRANS*-RESVERATROL และสารประกอบฟีนอลิกอื่นๆขององุ่นและไวน์ มทส และการเปลี่ยนแปลงระหว่างกระบวนการผลิตไวน์ (*TRANS*-RESVERATROL AND OTHER PHENOLIC COMPOUNDS OF SOME SUT GRAPES AND WINES AND THEIR CHANGES DURING VINIFICATION PROCESS) อาจารย์ที่ปรึกษา : รองศาสตราจารย์ ดร.กนกอร อินทราพิเชฐ, 141 หน้า. ISBN 974-533-519-3

ปริมาณและองค์ประกอบของสารประกอบฟีนอลิกขององุ่นและไวน์มีความหลากหลายตามพันธุ์องุ่น ฤดูกาล พื้นที่เพาะปลูก และกระบวนการผลิตไวน์ ดังนั้นวัตถุประสงค์ของงานวิจัยเพื่อประเมินและเปรียบเทียบปริมาณสารประกอบฟีนอลิกเบื้องต้นและคุณสมบัติในการต้านออกซิเดชันขององุ่นและไวน์บางสายพันธุ์ที่ผลิตในประเทศไทย โดยเฉพาะสายพันธุ์ที่ปลูกและผลิตที่ฟาร์มมหาวิทยาลัยเทคโนโลยีสุรนารี (มทส) และ เพื่อศึกษาการเปลี่ยนแปลงสารประกอบฟีนอลิกระหว่างกระบวนการผลิตไวน์จากยีสต์ต่างสายพันธุ์ร่วมกับการเติมสาร *t*-resveratrol

ปริมาณสารประกอบฟีนอลิกทั้งหมดในองุ่นและไวน์วิเคราะห์โดยวิธี Folin-Ciocault วิเคราะห์คุณสมบัติในการต้านออกซิเดชันด้วยวิธี การจับอนุมูลอิสระ DPPH และ ความสามารถของสารต้านออกซิเดชันในการรีดิวซ์เฟอร์ริก (ferric reducing antioxidant power, FRAP) น้ำองุ่นที่ปั่นรวมทั้งเมล็ดมีปริมาณสารประกอบฟีนอลิกทั้งหมดและคุณสมบัติในการจับกับอนุมูลอิสระได้สูงกว่าในน้ำองุ่นแยกเมล็ด นอกจากนั้น น้ำองุ่นแดงแสดงคุณสมบัติในการจับกับอนุมูลอิสระได้สูงกว่าน้ำองุ่นขาว ไวน์แดงมีปริมาณสารประกอบฟีนอลิกและคุณสมบัติในการเป็นสารต้านทานการเกิดออกซิเดชันมากกว่าไวน์ขาวอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) พบ *t*-resveratrol ในไวน์สายพันธุ์ชีราจ ซินฟานเดล และไวน์ผสม ในไวน์ พบสาร catechin มากกว่า epicatechin ไม่พบสารประกอบที่ออกฤทธิ์ทางชีวภาพ ในไวน์สายพันธุ์ Chasselar Dore เช่น *t*-resveratrol, epicatechin, catechin, rutin และ quercetin ส่วนในไวน์แดง พบว่ามีปริมาณ phenolic acid ชนิด gallic acid สูงที่สุด

วัตถุดิบที่ใช้ในการผลิตไวน์เพื่อศึกษาการเปลี่ยนแปลงของสารประกอบฟีนอลิกระหว่างกระบวนการหมักแอลกอฮอล์ การหมัก malolactic และการบ่ม ได้แก่ องุ่น (*Vitis vinifera*) สายพันธุ์ Exotic และ Shiraz ที่ปลูกในฟาร์ม มทส. การหมักแอลกอฮอล์ใช้ยีสต์สายพันธุ์ต่างๆ (K1V1116, CY3079 และ EC1118) ร่วมกับการเติม *t*-resveratrol (ความเข้มข้น 0 5 และ 10 ppm) ส่วนการหมัก malolactic ใช้แบคทีเรียสร้างกรดแลคติก *Oenococus oeni* สายพันธุ์ MBR.B1 จากนั้นบ่มไวน์กับชิ้นไม้โอ๊กและเก็บที่อุณหภูมิ 15 องศาเซลเซียส การหาปริมาณ *t*-resveratrol และสารประกอบฟีนอลิกชนิดอื่นๆ ทำได้โดยใช้วิธี capillary electrophoresis

ระหว่างกระบวนการหมักแอลกอฮอล์ของไวน์ทั้ง 2 ชนิด พบว่า ความเข้มข้นของปริมาณ *t*-resveratrol เพิ่มขึ้นจนถึงวันที่ 3 และลดลงหลังจากวันที่ 5 ของการหมัก การเติม *t*-resveratrol ก่อนกระบวนการหมักทำให้มีปริมาณของ *t*-resveratrol สูงกว่าตัวอย่างที่ไม่ได้เติม เมื่อกระบวนการหมักแอลกอฮอล์สิ้นสุดลง พบว่า ความเข้มข้นของฟีนอลิกที่ได้จากยีสต์ต่างสายพันธุ์ไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ ($p>0.05$) ส่วนการเติม *t*-resveratrol บริสุทธิ์ไม่มีผลต่อความเข้มข้นของปริมาณสารประกอบฟีนอลิกอื่นๆ

หลังจากการหมัก malolactic พบว่า *t*-resveratrol เพิ่มขึ้นในทุกสภาวะ โดยเฉพาะในสภาวะที่มีการเติม *t*-resveratrol นอกจากนี้ยังพบว่า phenolic acid บางชนิด เช่น gentisic *p*-hydroxybenzoic salicylic caffeic และ protocatechuic acid เกิดขึ้นระหว่างกระบวนการหมัก malolactic ด้วย

ระหว่างการบ่มไวน์ พบว่า มีการลดลงของปริมาณสารประกอบฟีนอลิกทั้งหมด *t*-resveratrol monomeric flavonoids รวมถึง phenolic acid บางชนิด อีกทั้งยังพบว่าค่าความเข้มของสี ปริมาณสาร anthocyanin ทั้งหมด และปริมาณสาร anthocyanin ที่ทำให้เกิดสีทั้งหมดลดลงอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) ในขณะที่ค่า chemical age เพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ ($p<0.05$)

สาขาเทคโนโลยีอาหาร ลายมือชื่อนักศึกษา_____

ปีการศึกษา 2548 ลายมือชื่ออาจารย์ที่ปรึกษา_____

JIRAYUS WORARATPHOKA : *TRANS*-RESVERATROL AND OTHER PHENOLIC COMPOUNDS OF SOME SUT GRAPES AND WINES AND THEIR CHANGES DURING VINIFICATION PROCESS. THESIS ADVISOR : ASSOC. PROF. KANOK-ORN INTARAPICHET, Ph.D., 141 P.P. ISBN 974-533-519-3

PHENOLIC COMPOUND/GRAPES/WINES/ANTIOXIDANT/FREE RADICAL SCAVENGING/RESVERATROL/ALCOHOLIC FERMENTATION/ MALOLACTIC FERMENTATION/AGEING

The amount and composition of phenolic compound of grapes and wines vary greatly with the grape variety, seasonal condition, production area and vinification process. Therefore, the objectives of this work were to preliminarily evaluate and compare phenolic contents and antioxidative properties of some grape and wine varieties produced in Thailand, particularly grown and produced on Suranaree University of Technology (SUT) Farm and to investigate changes of phenolic compounds during vinificantion process due to different yeast strains and added *t*-resveratrol.

Total phenolic content (TPC) in grapes and wines were determined by Folin-Ciocault method. Antioxidative properties were evaluated by $DPPH^{\bullet}$ radical scavenging and ferric reducing antioxidant power (FRAP). Grape juices with seeds contained higher ($p<0.05$) TPC and free radical scavenging properties than those of without seeds. Moreover, red grape juices showed higher free radical scavenging activity than white grape juices. In general, red wines had significantly higher ($p<0.05$)

phenolic compounds and antioxidant activity than those of white wines. *t*-Resveratrol was found in Shiraz, Zinfandel and blended wine varieties. Catechin was present in wines higher than epicatechin. The bioactive compounds such as *t*-resveratrol, epicatechin, catechin, rutin and quercetin were not found in wine produced from Chasselar Dore grape variety. Gallic acid was the highest phenolic acid found in red wine.

The *Vitis vinifera* via Exotic and Shiraz grape grown on SUT Farm were used to produce wines to investigate changes of phenolic compounds during alcoholic fermentation, malolactic fermentation (MLF) and ageing process. Alcoholic fermentation was carried out using different fermentative yeast strains (K1V1116, CY3079 and EC1118) with the addition of pure *t*-resveratrol (concentration of 0, 5 and 10 ppm). Lactic acid bacteria, *Oenococus oeni* strain MBR.B1, was used for MLF. Wine samples were aged with oak chips and stored at 15°C. *t*-Resveratrol content and other individual phenolic compounds were determined using capillary electrophoresis technique.

During alcoholic fermentation, the concentration of *t*-resveratrol increased up to the third day of fermentation and decreased after the fifth day for both wine varieties. The additions of *t*-resveratrol to the must prior to fermentation led to higher amount of *t*-resveratrol. After alcoholic fermentation was finished, there were no significant differences ($p>0.05$) in phenolic concentrations due to different yeast strains. The addition of pure *t*-resveratrol did not affect the contents of other phenolic compounds as well as *t*-resveratrol.

After MLF, *t*-resveratrol increased for all treatments, especially for the treatment with added *t*-resveratrol. Furthermore, some of the phenolic acids such as

gentisic, *p*-hydroxybenzoic, salicylic, caffeic and protocatechuic acid were generated during MLF process.

During ageing process, the decreasing of TPC, *t*-resveratrol, monomeric flavonoids as well as some of phenolic acids were observed. Color density, total anthocyanin and total colored anthocyanin significantly decreased ($p<0.05$), whereas chemical age value significantly increased ($p<0.05$) after ageing process.

School of Food Technology Student's Signature J. Wen

Academic Year 2005 Advisor's Signature K. Tiaraprichat

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Jirayus Woraratphoka

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LIST OF ABBREVIATIONS

% V/V	=	percent volume by volume
°C	=	degree Celsius
g	=	gram
mg/L	=	milligram per liter
g/mL	=	gram per liter
HDL	=	high density lipoprotein
LDL	=	low density lipoprotein
µg/kg	=	microgram per kilogram
ng/g	=	nanogram per gram
V/cm	=	volt per centimeter
µM	=	micromolar
CZE	=	capillary zone electrophoresis
SPE	=	solid phase extraction
Nm	=	nanometer
mg GAE/L	=	milligram gallic acid equivalent per liter
Fe^{II}	=	ferrous
Fe^{III}	=	ferric
$FeSO_4$	=	ferrous sulfate
H_3BO_3	=	boric acid
Na_2HPO_4	=	disodium hydrogen phosphate
NaOH	=	sodium hydroxide
mmol/L	=	millimolar per liter

LIST OF ABBREVIATIONS (Continued)

CFU	=	colony forming unit
W/V	=	weight by volume
kV	=	kilo volt
µm	=	micrometer
i.d.	=	internal diameter
cm	=	centimeter
s	=	second
N	=	normality
M	=	morlarlity
Abs	=	absorbance
UV	=	ultra violet
ND	=	not detected
ppm	=	part per million
Resv	=	resveratrol
K1V	=	K1V1116
CY3	=	CY3079
EC1	=	EC1118
min	=	minute
U/mg	=	unit per milligram

CHAPTER I

INTRODUCTION

1.1 Introduction

Wines have been well known that benefit for health. St.Leger, Cochrane and Moore (1979) showed that there was an association between increased wine consumption and reduced the rate of coronary heart disease (CHD) mortality. The 'French Paradox' was born: despite high fat intake in Frence population, the rate of CHD mortality was low due to regular wine consumption. Beside, the alcohol, there are the important components in wine which absent in others alcoholic beverage favorably affect for reduce CHD mortality (Gronbaek et al., 1995). These unique components are polyphenols which some originating from the grapes and some metabolic by products of yeast activity during fermentation (Soleas, Diamandis, and Goldberg, 1997). Polyphenolic compounds of grapes and wines usually include derivatives of hydroxybenzoic (syringic, gentisic, salicylic, gallic and protocatechuic acid) and hydroxycinnamic acid (*p*-coumaric, cinnamic and caffeic acid), trihydroxystilbene (resveratrol and its glucoside (piceid)), flavonoids include flavonols (e.g. quercetin), flavan-3-ols (e.g. catechin, epicatechin) as well as polymer of the latter, defined as procyanidin and anthocyanins. A wide range of studies has shown the antioxidative properties of flavonoids in protection against arteriosclerosis and CHD (Estruch, 2000; Stupans, Kirlich, Tuck, and Hayball, 2002; Sun, Simonyi, and Sun, 2002). Resveratrol was first considered when Siemann and Creasey (1992)

reported its presence in wine. These authors suggested that this compound might be the biologically active component of red wine. Since then, the properties of resveratrol have been extensively investigated.

Resveratrols (3,4′,5-trihydroxystilbene) are grapevine phytoalexin which synthesized by stress factors such as fungal infection (*Botrytis cinerea*), injury, UV radiation. Given that it is present in the skin but not in flesh. It is a parent molecule of a family of polymers named viniferin. Resveratrol exists in *trans (t)*- and *cis (c)*-isomeric form as shown in Figure 1.1. However the *c*-form is a by-product of fermentation and it is rarely found in grape. Both *t* and *c*-resveratrol also occurred as glucosides (bound to glucose molecule) called piceid (Fig 1.1). The major resource of resveratrol and piceid in the human diet are grape, wine and peanuts. It is also found in the root of *Polygonum cuspidatum* that are usually used in the traditional Asian medicine for the treatment of several ailments.

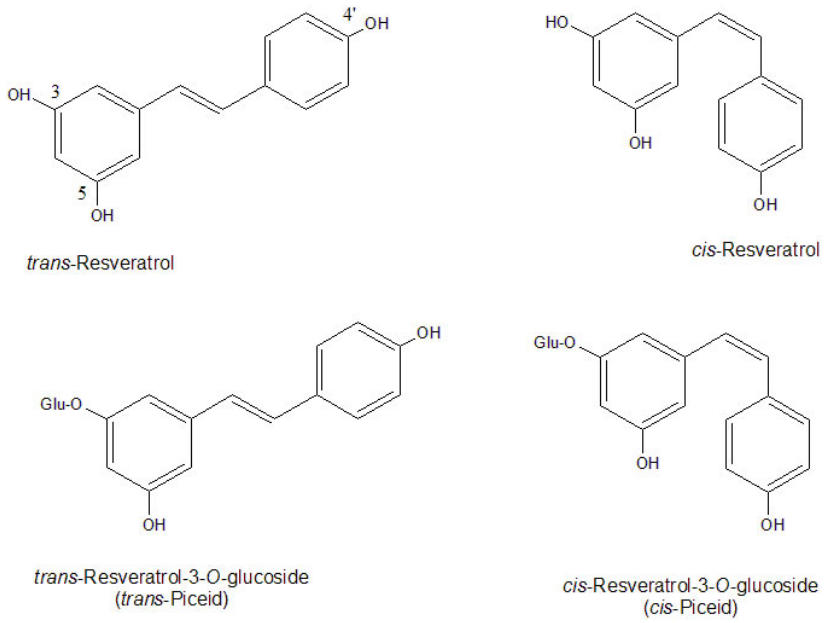

Figure 1.1 Structure of resveratrol and its glucoside

Source: http://lpi.oregonstate.edu/infocenter/phytochemicals/resveratrol/cistrans.html

Numerous studies are interested in *t*-resveratrol on the beneficial for health. *t*-Resveratrol as antioxidant, is more effective than butylhydroxytoluene (BHT), quercetin or tocopherol on lipid peroxidation in liposomes and rat liver (Blond, Denis, and Bezard, 1995). *t*-Resveratrol can inhibit low-density lipoprotein (LDL) oxidation (Frankel, Waterhouse, and Kinsella, 1993). It mainly acts by reducing the copper-catalyzed oxidation but cannot chelate ferrous ions, while flavonoids have better free radical scavenging property. Resveratrol also has the ability to inhibit platelet aggregation (Bertelli et al., 1995; Pace-Asciak, Hahn, Diamandis, Soleas, and Goldberg, 1995). Furthermore, resveratrol had a cancer chemopreventive activity in assays representing the three major stages of carcinogenesis, initiation, promotion and proliferation (Jang et al., 1997). *t*-Resveratrol suppressed the activation of cycloxygenese-2 (COX-2) gene expression by inhibiting the protein kinase C signal transduction pathway and directly inhibited the activity of COX-2 (Subbaramaiah et al., 1998). At high concentration of resveratrol (20 – 160 μmol/L), they were binding to the estrogen receptor then inhibited the cell proliferation (Mgbonyebi, Russo and Russo, 1998).

Polyphenolic compound (flavonoid, phenolic acid and stilbene) in grapes and wines have been found in different concentrations. It was depending on the grape variety, environmental conditions in vineyard, and the wine processing techniques (Frankel, Waterhouse, and Teissedre, 1995). The identification of the active phenolic compounds or the phenol class that is responsible for antioxidant properties of red wine has raised much interest. These antioxidant properties provide a rational for exploring the phenolic content in wines to define those that are particularly abundant and to stimulate the development of enological techniques for their enrichment (Minussi et al., 2003; Soleas, Dam, Carey, and Goldberg, 1997).

To identify phenolic compounds in wine, high performing analytical techniques have been used. Capillary electrophoresis (CE) is a high efficiency and capacity to resolve a complex of natural compounds. The successful application of CE for wines and polyphenols has been demonstrated (Gu, Chu, O'Dwyer, and Zeece, 2000; Minussi et al., 2003).

There are very little knowledges in the composition and antiradical properties of grapes and wines produced in Thailand. Therefore, the investigations were done to obtain a useful data for promoting some Thai wines as the rich source of active compounds and benefit for health.

The traditional red wine vinifications are comprised of alcoholic fermentation, malolactic fermentation, clarification, filtration, and ageing process. The processing techniques are different in some wine-maker. The thermovinification (mush heating up to 60-65°C) (Netzel et al., 2003) and the combination of heating and pectiolytic enzyme (Burn et al., 2001) significant increased the flavonoids and stilbenes (such as *t*-resveratrol and *t*-resveratrol glucoside) levels.

The composition of phenolics in wine can be also modified by yeast as a result of conversion of nonphenolic substances, solubilization, extraction of phenolics by the ethanol produced during fermentation (Singleton and Noble, 1976) and hydrolysis of glucoside molecules by the intrinsic enzymes such as β–glucosidase. Therefore, the effects of yeast strains used for fermentation on the phenolic compounds in grapes and wines were also evaluated.

Oak wood or chips are mainly used to accelerate the ageing process. It influences the sensorial and chemical characteristics of wines, because many constituents can be extracted from woods during ageing (Francis, Sefton, and

Williams, 1992; Fernandez de Simon, Caldahia, Conde, and Garcia-Vallejo, 1996). Then, some chemical characteristics of wine in the presence of oak chips were also investigated.

1.2 Research objectives

(1) To evaluate phenolic compounds contents and antioxidant qualities of some commercial grapes and wines of Suranaree University of Technology (SUT) Farm

(2) To evaluate the effects of yeast strains and the addition of pure *t*-resveratrol on the changes of *t*-resveratrol and other phenolic compound during alcoholic fermentation of grapes from SUT farm

(3) To evaluate the changes of *t*-resveratrol and other phenolic compound during malolactic fermentation

(4) To evaluate the changes of *t*-resveratrol and other phenolic compound during ageing with oak chips

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CHAPTER II

LITERATURE REVIEWS

2.1 The grapes

There are more than 60 species of *Vitis* known. *Vitis* is a deciduous plant, which climbs by grasping supporting objects with outgrowths of very special leaf-fraction organs called tendrils. *Vitis vinifera* are cultivated around the world. The major white varieties are Chardonnay, Sauvignon Blanc, and major red varieties are Cabernet Sauvignon, Merlot, Pinot Noir, Zinfandel and Shiraz. Another species so called 'fox' grape is *V. labrusca*, such as Delaware, Niangara, Concord, Isabella. The 'post-oak' or 'frost' grape is referred to *V. riparia* species (Vine, Harkness, Browning, and Wagner, 1997).

Grape consists of a group of tissues or pericarp surrounding the seeds. The pericarp is divided into the exocarp (the skin), the mesocarp (the pulp) and the endocarp (the tissue that lines the seed receptacles containing the seeds but is not distinguishable from the rest of the pulp) (Ribereau-Gayon, Dubourdieu, Doneche, and Lonvaud, 2000).

2.2 The wines

Wine is the result of fermenting grapes. For all white wines, the juice is pressed out prior to fermentation. Red wines are maceration wines (fermenting with skin and seed). In the traditional method, maceration and alcoholic fermentation are

practical simultaneous following by malolactic fermentation. The fermented juices are precipitated for removing matter and undergo ageing process.

2.2.1 Alcoholic fermentation or maceration

The alcoholic fermentation is the most important reaction involved by yeast. It is usually conducted with yeasts belonging to the *Saccharomyces* genus. It is clear that *Saccharomyces* is well adapted to grow on high sugar-containing media under anaerobic conditions. During fermentation, yeasts utilize sugars and other constituents of grape juice as the substrates for their growth and convert these compounds to ethanol, carbon dioxide and other metabolic end products that contribute to chemical composition and sensory quality of the wine (Fleet and Heard, 1993). Each degree of °Brix will convert to about 0.535% alcohol during fermentation (Vine et al., 1997). Approximately 50% of the total available sugar is usually consumed during the growth phase, the remainder being fermented by stationary phase cells. A typical fermentation profile was presented by Fleet (2003) as shown in Fig. 2.1, displaying sugar concentration, change in specific gravity (°Brix), culture absorbance and ethanol concentration (%v/v). During fermentation, the viable population of yeasts in the grape juice increased from initial values of $10^4 – 10^6$ CFU/mL to $10^8 – 10^9$ CFU/mL.

The time of fermentation is varied according to the style and quality of wine required. Short fermentations are suitable for light wines, while longer times are used for wines destined for long-term keeping.

Nowadays, killer yeasts have been used in most wineries. Killer yeast is the yeast that has killing action due to toxins that are produced and secreted by the killer strain which lethal to sensitive yeasts. Killer yeasts were classified into 10 groups

(K1-K10) based on cross-reactions between the killer strains of various genera and species (Young and Yagiu, 1978). Killer strains of *S.cerevisiae* has suitable properties in wine fermentation due to good fermentation kinetics, production of good quality wine and has killing activity under the extreme environment of wine. β–Glucosidase in yeast is important to the wine industry since it has the ability to hydrolyze the bond between glucose and other compound on an anomeric carbon. This compound may be an aroma compound, or phenolic compound (such as resveratrol). Different strains of *S.cerevisiae* also produce different in β-glucosidase activity (Brocku, 2002).

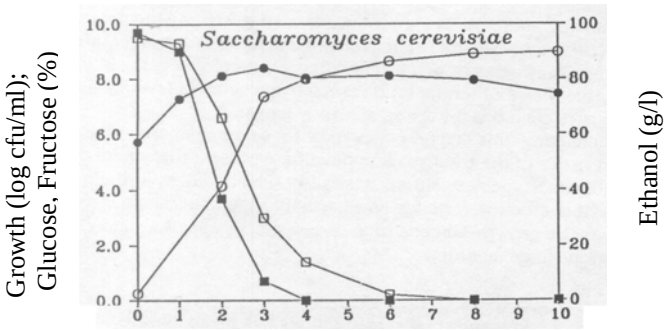


Figure 2.1 Kinetics of growth, sugar utilization and ethanol production by *S.cerevisiae* during fermentation of synthetic grape juice medium. Colony forming units •; ethanol o; glucose ■; fructose □.
Source: modified from Fleet, 2003

During fermentation, there are three major factors affecting on the growth of yeast:

a) Temperature

The rate of yeast growth and alcoholic fermentation increases as the temperature increases, with maximum rates occurring at temperatures between 20 and

25°C (Amerine et al., 1980). Red wines are generally fermented at 20 – 30°C, while white wines are often fermented at 10 – 20°C. Heat is generated during fermentation. Unless cooling is applied, a temperature increase of 5 – 10°C may occur from the beginning to end of fermentation (Sablayrolles and Barre, 1989). Increase temperature above 30°C will ultimately kill yeast cells, particulary at higher ethanol concentration, and lead to stuck fermentation (Van Uden, 1989). The temperature of the fermentation effects on ethanol yields, aroma of wine, rate of yeast growth, time course of ethanol formation and the extraction of phenolic compounds.

b) Sulfur dioxide (SO_2)

Sulfur dioxide has effect on the kinetics of yeast growth by increasing the lag phase and delaying the onset of fermentation, decreasing the growth rate and increasing the time to complete fermentation, and accelerating the decline/death phase (Fleet, 2003).

c) pH

The pH of grape juice varies between 3.0 and 4.0, depending upon the concentrations of tartaric and malic acids. However, the rates of growth and juice fermentation by *S. cerevisiae* decreased as the pH decreased from 3.5 to 3.0 (Ough, 1996).

2.2.2 Malolactic fermentation

Generally, spontaneous malolactic fermentation (MLF) occurs after completion of alcoholic fermentation, but occasionally it can occur at the same time

as alcoholic fermentation. To avoid undesirable interference between yeast and bacterial growth, and best control of MLF, the preferred time for inoculation of malolactic bacteria is just at completion of alcoholic fermentation.

MLF, the enzymatic decarboxylation of L-malic acid to L-lactic acid (Fig 2.2), is an important secondary fermentation carried out by lactic acid bacteria (LAB) such as the genus of *Leuconostoc, Lactobacillus* and *Pediococcus* during the vinification of red wine. The changes of malic acid as showed in Figure 2.3. MLF is justified as an important process in winemaking for three reasons (Henick-Kling, 1994):

(a) For deacidification

The conversion of malic to the lactic and the loss of carbon dioxide decrease the acidity and increase pH of the wine. This function is important in wines, which often have a high acid (tartaric plus malic) content and low pH (Henick-Kling, 1994).

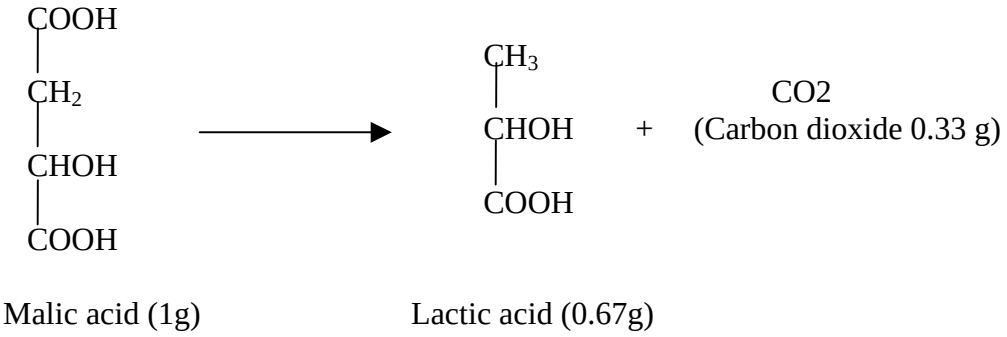


Figure 2.2 The conversion of malic acid to lactic acid
Source: modified from Ribereau-Gayon, Dubourdieu, et al., 2000

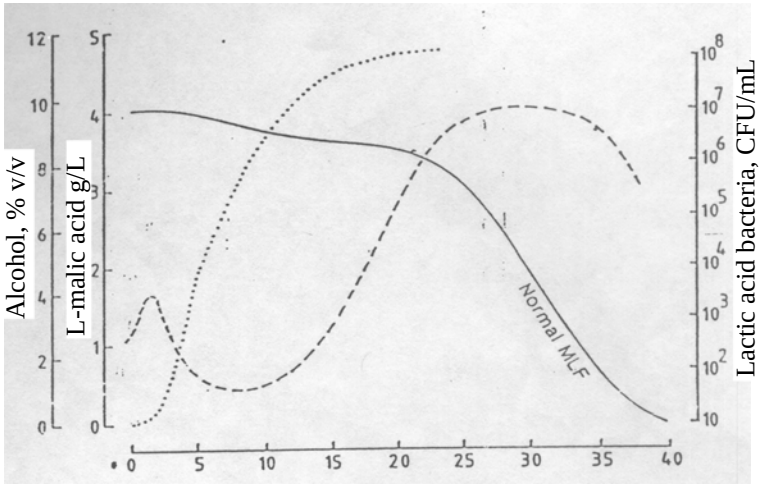


Figure 2.3 Development of native LAB during alcoholic fermentation of must and wine

Source: modified from Jackson, 2000

(b) For flavor modification

MLF not only affects the taste of wine through deacidification but also can contribute to flavor characteristics. These malolactic flavors have been generally described as ‘malolactic’, ‘buttery’, ‘lactic’, ‘nutty’, ‘yeasty’, ‘oaky’ and ‘sweaty’. Furthermore, it is believed that MLF can enhance the fruity and mouthful character of a wine (Henick-Kling, 1994).

(c) For microbial stability

Completed MLF gives the wine some microbial stability by removing malic acid, which can be used as substrate for some microorganisms and some sugars, and by producing antimicrbial agents such as lactic acid and most likely bacteriocins (Henick-Kling, 1994).

Oenococcus oeni (previously known as *Leuconostoc oenos*) is particularly well adapted to the low pH and high ethanol concentration of wine and, therefore, is one of the most frequently associated with MLF. Furthermore, three glycosidase activities of *O. oeni* have been found, namely β-glucosidase, α-glucosidase, and N-acetyl –β-glucosaminidase (Alexandre, Costello, Remize, Guzzo, and Guilloux-Benatier, 2004) that enhance the releasing of volatile compounds in wine.

2.2.3 Ageing process

During the period from the end of the fermentations until bottling, a wine is said to be ageing. Ageing duration is highly variable according to the wine origin, type and quality. It must be long enough to stabilize the wine, as well as preparing great wines for bottle ageing (Ribereau-Gayon, Glories, Maujean, and Dubouedieu, 2000). During the ageing process, wine undergoes important modifications so that their stability increases and their organoleptic characteristics improve.

2.2.3.1 Oak composition

Modern winemaking frequently involves fermentation and / or storage in oak, involving interaction of air, wine and wood. The transference process of different phenolic and volatile compounds from oak wood into wine during ageing depends on potentially extractable compounds initially present in the wood. The chemical composition of this oak is influenced by different factors such as its species, its geographical origin, or its processing in the cooperage, especially the method to obtain the staves, their seasoning and the degree of oak toasting during the ageing system’s manufacture (Zoecklein et al., 1995). Ageing in oak wood allows wine to

extract a series of benzoic and cinnamic compounds (vanillin, vanillinic acid, syringaldehyde, syringic acid, coniferaldehyde, sinapaldehyde), gallic acid, ellagic acid and cummarines (scopoletine, umbelliferone) from the wood. Oak chips are mainly used to accelerate the ageing process. Spillmann (1999) studied factors affecting aroma properties of wine ageing in oak barrels and oak chips, and noticed that the oak chip gave a less satisfactory result than that of barrel.

2.2.3.2 Phenolic composition

Phenolic compounds play a major role in wine quality. They contribute to sensory characteristics of wines, in particular color and astringency. The most rapid changes in color composition occur during the first year of storage (Somers and Evans, 1986). Phenolic compounds undergo a number of transformations that depend on the temperature, sulfur dioxide concentration, degree of oxidation, time and the anthocyanin to tannin ratio.

During maturation in barrels or tanks, where oxygen is present, the redness (A520) of red young wine decreases and the absorbance in the yellow/brown region, near 420 nm increases (Mazza and Miniati, 1993). The reactions considered responsible for the formation of polymeric pigment molecules, acetaldehyde-mediated condensation, copigmentation and self-association. These reactions lead to color enhancement and pigment complexes less sensitive to pH change than the anthocyanins.

a) Acetaldehyde-mediated condensation

Timberlake and Bridle (1976) proposed a reaction mechanism initiated by the generation of an acetaldehyde carbonium ion, which is capable of reacting with

the active positions (6 and 8) of the catechin-type compound, whereby the carbonyl carbon readily undergoes nucleophilic attack by position 8 of catechin. The acetaldehyde-catechin complex forms a reactive carbonium ion which combines with the anthocyanin flavylium ion, most probably at position 8. Formation of quinonoidal form occurs more readily as a result of the substitution pattern which accounts for the subsequent increase in color and the violet shift. Further condensation onto the polymer could occur via the reactive 6 position. By analogy with the behavior of simple flavylium salts, the 6 and 8 positions of the anthocyanin A-ring have been proposed as the reactive positions, with position 8 being the most reactive.

b) Copigmentation

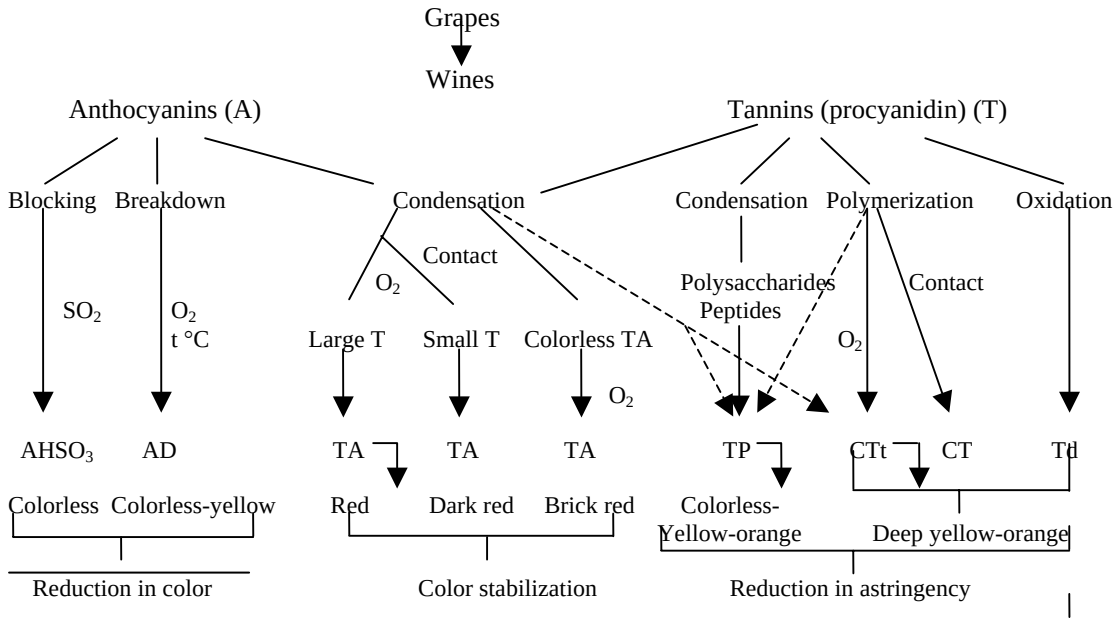
A significant role of the copigmentation reaction of anthocyanins with other polyphenols in the maturation and ageing of wine was recognized by Somers and Evans (1977). It is now known that noncovalent complex formation between the anthocyanin-colored structures and the copigment is responsible for intensification and change in the initial solution color. Molecules capable of acting as copigments include a large variety of structurally unrelated compounds, such as flavonoids, other polyphenols, alkaloids, amino acids and anthocyanins themselves (Mazza and Miniati, 1993). Therefore, the copigmentation reaction is probably a major molecular interaction mechanism involved in variations of color and astringency during production and ageing of wine.

c) Self-association

Another phenomenon associated with maturation and ageing of the

wine is self-association of anthocyanins and of other polyphenols. Hoshino (1991) has proposed a helically stacked conformation for the homo-association of the neutral and ionized quinonoidal bases of anthocyanins. According to this model, the stacked anthocyanin chromophores form a hydrophobic area, the benzopyrylium ring would be protected from water attack and as such retain its color.

Moreover, anthocyanins and tannins extracted from grapes are involved in various reactions that depend to a great extent on external conditions and produce a variety of compounds. These reactions include degradation, color stabilization, polymerization of tannins and condensation with other components. These reactions are summarized by Ribereau-Gayon, Glories et al. (2000) as shown in Figure 2.4.



A = Anthocyanins
T = Tannin
P = Procyanidin
CTt = Very condensed tannins
CT = Condensed tannins
TP = Tannin polysaccharides
= Precipitates
DA = Degraded anthocyanins

Figure 2.4 Reactions of tannins and anthocyanins

Source: Ribereau-Gayon, Glories, et al., 2000

The main consequences of these reactions involving phenols in red wines changing in color intensity, a tendency to develop a yellow-orange hue (generally accompanied by loss of color) and various modifications in the tannins, responsible for their gradual softening (Ribereau-Gayon, Glories, et al., 2000). Tannins appear to stabilize anthocyanins by combining to form larger polymeric pigments. The presence of anthocyanins increases the solubility of phenol-tannin complexes in red wines (Singleton and Trousdale, 1992). These authors hypothesized that the incorporation of the flavylium salt and its attached sugars into the polymer increase the solubility of a given condensed tannin molecules.

2.2.4 Quality parameter of wine

Color is certainly one of the most important attributes in red wine quality. Wine color is strongly influenced by grape composition and enological practices, such as wine-making techniques, storage temperature, length of storage and oxygen exposure (Gomez-Plaza, Gil-munoz, Lopez-Roca, and Martinez, 2000). Human perceive color as a characteristic of the wavelength and intensity of light being reflected off the surface or being transmitted through an object. A measure of color intensity or density can be achieved by summation of absorbance reading at 420 and 520 nm. By comparison, hue or tint is measured as the ratio of absorbance at 420/520 nm (Zoecklein et al., 1995). The spectrum of red wines has a maximum at 520 nm, due to anthocyanins and their flavylium combinations, and a minimum in the region of 420 nm. Color intensity and hue only take into account the contribution of red and yellow to overall color. These two values are suitable for studying wines with some age, but do not always cover the relatively deep colors of young wines. Most of young

wines have a transmittance minimum (absorbance maximum) at 520 nm and a transmittance maximum (absorbance ninimum) at 420 nm. As wine matures, there is a shift in absorption maximum to between 400 and 500 nm, usually near 450 nm. With excessive oxidation of red wines, the spectral characteristics approach those of a sherry and the polymerized red pigments may precipitate (Zoecklein et al., 1995).

Color intensity represents the amount of color. It varies a great deal from one wine and grape variety to another (ranging 0.3 – 1.8) (Ribereau-Gayon, Glories, et al., 2000). The hue indicates the development of a color toward orange. Young wines have a value on the order of 0.5 – 0.7 which increases throughout ageing, reaching an upper limit around 1.2 – 1.3 (Ribereau-Gayon, Glories, et al.). Chemical age (CA) value is used to define the degree to which polymeric pigment forms have replaced monomeric pigment forms (Zoecklein et al., 1995). To calculate this value, the wine is bleached by an excess of SO_2, at the normal pH of wine (A_{520}^{SO2}). An identical measurement is made at pH lower than 1.2, when 95% of the anthocyanins present are colored (A_{520}^{HCl}). CA value calculated by A_{520}^{SO2} / A_{520}^{HCl}. This value represents the combined anthocyanins at optical density of 520 nm. The higher of CA indicate the higher in polymeric compounds.

2.3 Chemical constituents of grapes and wines

Wine contains more than 500 compounds, some originating from the grapes and some metabolic by-products of yeast activity during fermentation (Soleas et al, 1997). Most of them are present in very low concentrations, but a few compounds occur at concentrations above 100 mg/L. The most important alcohol in wine is ethanol, with concentrations ranging from 10 to 14%. Ethanol is crucial to the stability,

aging, and sensory properties of wine. It plays a role in extraction of pigments and tannins during skin and seed fermentation (van de Wiel, van de Golde, and Hart, 2001).

2.3.1 Acids

Malic acid may constitute about haft the total acidity of grape and wine. The concentration in fruit decreases as the grape mature, especially during hot period. Under cool conditions, malic acid levels remain high and give a sour taste of the resultant wine. Thus malic acid content is one of the indicators used in determining harvest dates. Tartaric acid is the other major grape acid. It does not decline during grape ripening, but it metabolized by few microorganisms (Jackson, 2000). This acid commonly presents as a potassium salt in leaves and grapes. During wine aging, tartaric acid forms crystal with potassium salt as potassium bitartrate and tends to precipitate. Normally, wines often are cooled near the end of maturation to enhance early tartrate precipitation and avoid crystal deposition in the bottle. Lactic acid occurs as a major constituent in wine, it comes from the metabolic activity of bacteria during malolactic fermentation. Acetic acids are produced in small amount by yeasts during fermentation. At normal concentration (< 300 mg/L), it can be a desirable flavorant, adding to the complexity of taste and odor. If it is high, it progressively gives the wine a sour taste and taints its fragrance (Jackson, 2000).

2.3.2 Sugars

Glucose and fructose are principle grape sugars. Sucrose is rarely found in grape. The sugar content of grape varies depending on the species, variety, maturity, and health of the fruit. At maturity stage, *V. vinifera* generally reach a sugar content of

20% or more. Grape sugar content is critical to growth and metabolism of yeast. In dry wines, the unfermented sugar so called residual sugar consists primarily of pentose sugar such as arabinose, hamnose, mannose, xylose, and small amount of residual glucose and fructose (1 – 2 g/L) (Jackson, 2000).

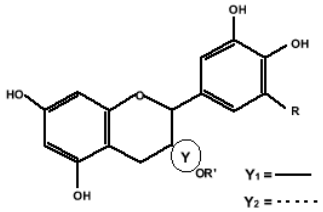
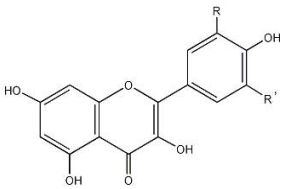
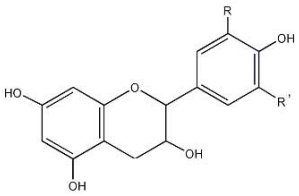
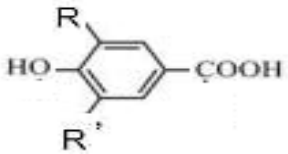
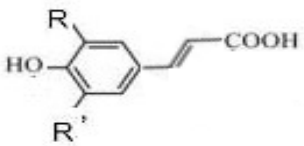
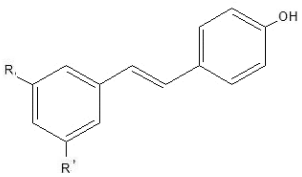
2.4 Phenolic compound and related polyphenols

The term ‘phenolic’ or ‘polyphenol’ can be defied chemically as a substance which possesses an aromatic ring bearing one or more hydroxy substituents, including functional derivatives (esters, methyl esters, glycosides etc.) (Harborne, 1989). Waterhouse and Teissedre (1997) reported the variability in the amounts of total phenolic content (TPC) and individual component in California wine. TPC ranged from 1850 – 2200 mg/L for red and 220 – 250 mg/L for white wines. White wine as usually made from free-run juices, so they have much lower resveratrol and others phenolic compound than the red ones, due to minimal skin contact time. Phenolic compounds in wine are usually subdivided into two groups: flavonoids and non-flavonoids. The total amount of flavonoids present is 1 – 3 g/L in red wines and 0.2 g/L in whites. In addition, wine contains a significant amount of non-flavonoids typically 0.2 – 0.4 g/L (Waterhouse, 1995). The structure and source of phenolic compounds are summarized in Table 2.1.

2.4.1 Flavonoids

The flavonoid has a common core, the flavane nucleus, consisting of two benzene rings (A and B) linked by an oxygen-containing pyrane ring (Fig. 2.5).

Table 2.1 Principal phenolic compounds in grapes and wines[a]

General type	General structure	Examples	Major source[b]
Flavonoids			
Flavan-3-ol		R=H,Y=Y1,R'=H: Epicatechin	G
		R=H,Y=Y2,R'=H: Catechin	G
Flavonols		R=OH,R'=H : Quercetin	G
		R=R'=H : Kaempferol	G
		R=R'=OH : Myricetin	G
Anthocyanins		$R=R'=OCH_3$: Malvidin	G
		R=R'=OH : Delphinidin	G
		$R=OCH_3$,R'=H : Paeonidin	G
		R=OH,R'=H : Cyanidin	G
		$R=OCH_3$,R'=OH : Petunidin	G
Non-flavonoids			
Hydroxybenzoic acid		R=R'=H : *p*-Hydroxybenzoic acid	G, O
		R=OH,R'=H : Protocatechuic acid	G, O
		$R=OCH_3$,R'=H : Vanillic acid	O
		R=R'=OH : Gallic acid	G, O
		$R=R'=OCH_3$: Syringic acid	G
Hydroxycinnamic acid		R=R'=OH : *p*-Coumaric acid	G, O
		R=OH,R'=H : Caffeic acid	G
		$R=OCH_3$,R'=H : Ferulic acid	G, O
		$R=R'=OCH_3$: Sinapic acid	O
Stilbene		R=R'=OH : *trans*-Resveratrol	G
		R=O-Glc,R'=OH : *trans*-Piceid	G

[a] Data adapted from Amarine and Ough (1980)
[b] G = Grape, O = Oak

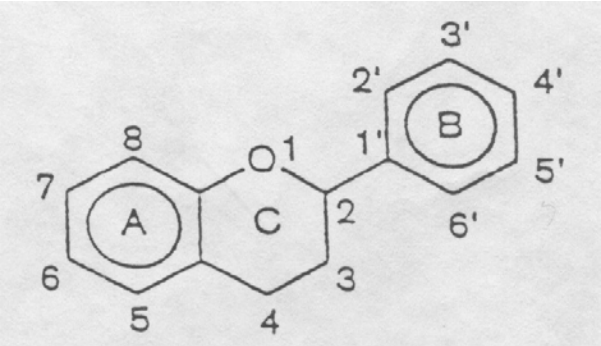


Figure 2.5 Flavonoid structure
Source: van de Wiel et al., 2001

Differences in the degree of oxidation of the heterocycle (C ring) and hydroxylation of the three rings led to a large family of structures with essential differences in biological behavior, bioavailability and efficacy (Soleas et al, 1997). Flavonoids are derived primarily from the skins and seeds of the fruit, and less frequently from the stems (Jackson, 2000). The most common flavonoids in wine are flavan-3-ols (catechin, epicatechin, tannins), flavonols (quercetin, kaempferol, myricetin), and anthocyanins (cyanin). Flavonoids exist either free or polymerized with sugars to form glycosides. The biosynthetic pathway of phenolic compounds, known as the shikimic acid pathway (Fig 2.6), leads to the production of cinnamic acid and coumaric acid. The condensation of three acetyl CoA molecules, derived from Krebs cycle reactions, also leads to the formation of a benzene ring.

The condensation of second ring with the cinnamic acid molecule produces a molecule group known as the flavonoid (Fig 2.7). Anthocyanins were most abundant in red grapes, and flavonols were most abundant in white grapes (Yi, Meyer and Frankel, 1997).

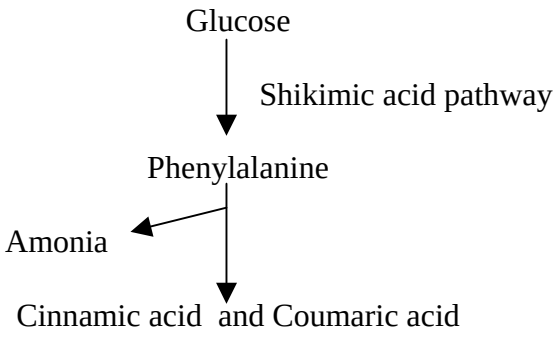


Figure 2.6 Shikimic pathway

Source: Balentine, 1992

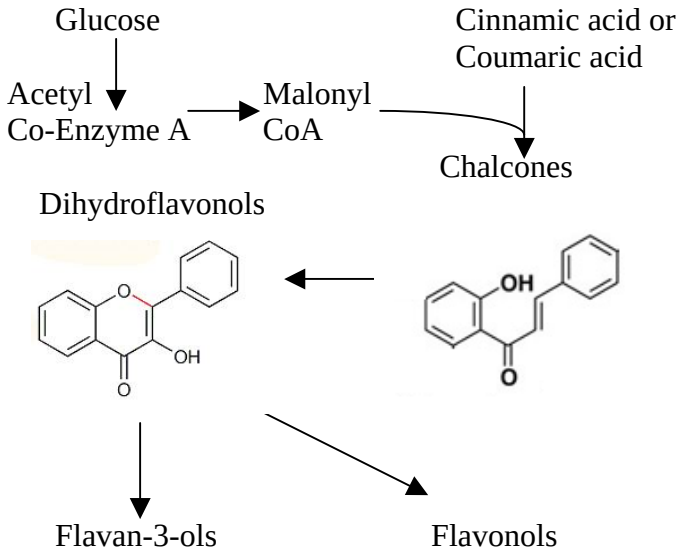


Figure 2.7 Flavan-3-los and flavonols synthesis

Source: Balentine, 1992

2.4.1.1 Flavan-3-ols

Catechin and epicatechin are primarily flavan-3-ol found in grapes and wines. Both compounds are found in high concentration in seeds and stems and may be found in the skins of immature grapes (ETS laboratories, 2006). Their

concentration in white wines ranges from 10 to 50 mg/L and may reach 200 mg/L in reds (Singleton and Esau, 1969). Flavan-3-ols may exist in dimeric and polymeric forms (Zoecklein, Fugelsang, Gump and Nury, 1995). Polymeric forms are referred to as procyanidins or condensed tannins.

2.4.1.2 Flavonols

Flavonols are localized in the grape skin, occurring in glycosidic forms. The most commonly sugar moiety reacted with flavonols is glucose. Glucosides of kaempferol, quercetin, and myricetin are present in 3-glucoside form. Quercetin comprises the majority of the flavonol fraction. Quercetin accumulates in grape skins to protect against damage from UV light (Price, Breen, Valladao, and Watson, 1995). In wines produced with skin contact, quercetin was reported at zero or only traces concentrations (McMurtrey, 1997) as showed in Table 2.2

2.4.1.3 Anthocyanins

The color of red grapes is attributed to the presence of members of a large group of pigments, the anthocyanins. These compounds are present as glycosides. In varieties of *V. vinifera*, glycosidation occur as single sugar residues attached to carbon 3 positions, form the 3-glucoside. Anthocyanins are generally found in red grapes are malvidin-, delphinidin-, peonidin-, cyaniding-, and petunidin-3-D-glucoside. Of these, malvidin is the most common pigment in *V. vinifera*. The color of these pigments and their stability is a function of pH and other factor, which are summarized below (Zoecklein et al., 1995).

(a) pH

Anthocyanins are amphoteric, so their color is depending on pH values (Fig 2.8). At low pH values (pH < 4), the main equilibrium forms are the red-colored flavylium ion and its colorless pseudobase. The pK for equilibrium between these forms (pK = 2.6) favors the colorless form. Therefore, at wine pH values greater than 3.0, less than 50% of the potential red color are visible (Zoecklein et al., 1995).

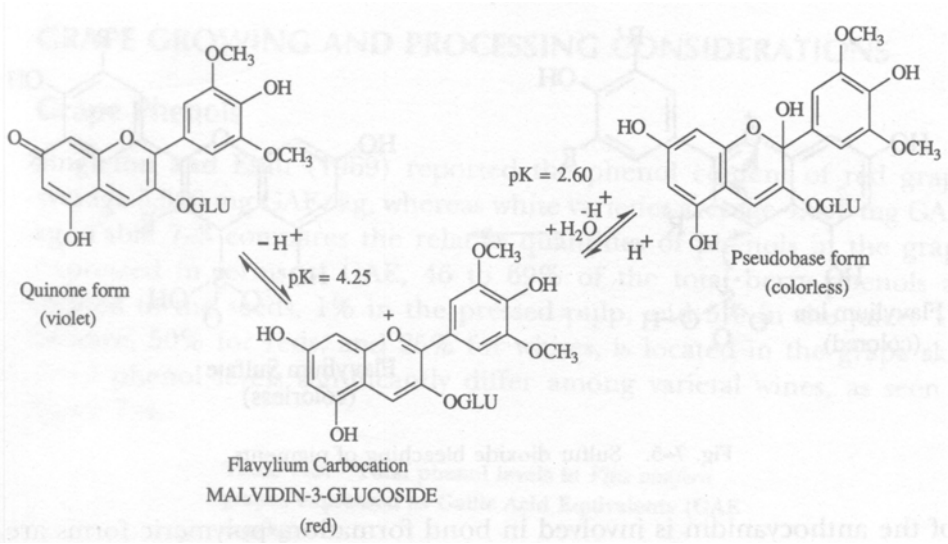


Figure 2.8 Equillium forms of anthocyanin
Source: Zoecklein et al., 1995

(b) Sulfile bleaching

The addition of SO_2 can result in temporary color reduction via a reversible reaction (Fig 2.9). The site of sulfite binding (carbon 4) is also the point at which react with other phenolics, thus polymerized pigments are resistant to decolorization by SO_2 (Zoecklein et al., 1995).

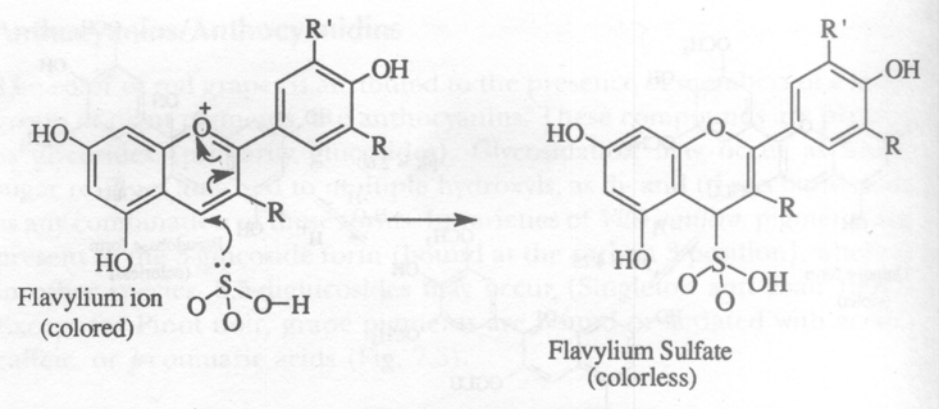


Figure 2.9 A reversible reaction of SO_2 bleching
Source: Zoecklein et al., 1995

(c) Temperature

In general, increasing storage temperature results in an increase in red or brown color. It is presumably due to accelerated rates of polymerization and other reactions occurring at high temperature (Zoecklein et al., 1995).

(d) Polymerization

Copolymerization may occur between C_4 of the anthocyanidin and either C_6 or C_8 of the tannin to yield a red-colored dimer. The presence of SO_2 inhibits polymerization as a result of occupying the C_4 position of flavonoid. Polymeric forms are not reactive toward SO_2 or responsive to changes in pH. By comparison to monomeric anthocyanidins, polymeric pigments show increase in stability (Zoecklein et al., 1995).

2.4.2 Non-flavonoids

The non-flavonoids in wine, phenols with only one aromatic ring, are derivatives of hydroxycinnamic acid and hydroxybenzoic acid. The numerous of these compounds are esterified to sugars, organic acids, or alcohol. Another classes of non-flavonoids in grape products are stilbenes and stilbene glycosides, with *trans*-resveratrol as its most well known representative (Zoecklein et al., 1995).

2.4.2.1 Hydroxybenzoic acids

The hydroxybenzoic derivatives in wine consist of syringic, *p*-hydroxybenzoic, gentisic, salicylic, gallic and protocatechuic acid. The levels of benzoic acids and its derivatives in red wines range from 50 to 100 mg/L and in whites from 1 to 5 mg/L (Singleton, 1985). Salicylic acid is present at levels of more than 10 mg/L (Muller and Fugelsang, 1994), and other derivatives are present in only trace amounts. The main sources of gallic acid in wine are grape seeds and oak cooperage. In seeds, gallic acid is present as free acid and as ester attached to procyanidin polymers (ETS laboratories).

2.4.2.2 Hydroxycinnamic acids

The hydroxycinnamate derivaties in wine consist of cinnamic, *p*-coumaric, caffeic and caftaric acid (an ester from caffeic and tartaric acid). Only caftaric acid is found in grapes. Both the ester and free cinnamate are found in wine (ETS laboratories). During alcoholic fermentation, slow hydrolysis of non-flavonoid esters occurs, resulting in free acid and ester forms. Caftaric acid and similarly bound or acylated phenols are hydrolyzed to varying degrees, yielding the corresponding

free cinnamic acids (Zoecklein et al., 1995). Somers (1987) reported that fermentation caused total hydroxycinnamates to decrease by nearly 20% due to adsorption by yeasts.

2.4.2.3 Stilbenes

Stilbenes are ethylene derivatives substituted by two phenyl rings. Ring A usually carries two hydroxyl groups in *m*-position, while ring B is substituted by hydroxy and methoxy groups in the *o*-,*m*- and *p*-position. The most important substances are *trans*-resveratrol and its glucoside so called picied. They are widely distributed in liverworts and higher plants, in monomeric form and as dimer, trimer and polymeric stilbenes, the so-called viniferin (polydatin) as shown in Figure 2.10 (Gorham, 1980).

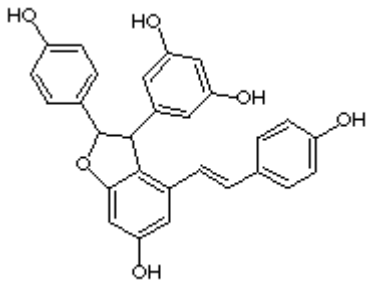


Figure 2.10 The structure of viniferin

Source: http://pages.unibas.ch/mdpi/ecsoc-4/c0002-07/cyphost1.gif

2.5 Resveratrol

Resveratrol (3,5,4'-trihydroxystilbene) is a naturally occurring phytoalexin produced by some higher plants in response to microbial infection or injury or UV light exposure. It has been quantified in grapes, mulberries, peanuts, and in their

by-products (Linus Pauling Institute's Micronutrient Information Center, 2006). In folk medicine, humans also have resveratrol from the use of medicinal plants, for example the roots and rhizomes of *Polygonum cuspidatum* (Ko-Jo-Kun in Japanese) that are usually used in the traditional Asian medicine for treatment of several ailments (Arichi et al., 1982). In grape berries, this compound is primarily located in the skin cells and is absent or low, in the fruit flesh (Creasy and Coffee, 1988). There are two isomeric forms of resveratrol present, *cis (c)* and *trans (t)* form. However, the *c*-resveratol is a by-product of fermentation, and it is rarely found in grapes (Jeandet et al., 1995).

2.5.1 Resveratrol synthesis and gene expression

The condensation of *p*-coumaroyl CoA with three molecules of malonyl CoA is accomplished through the activity of the stilbene synthase (Fig 2.11). The *Vitis* possess another enzyme, chalcone synthase, which catalyzes a reaction involving one molecule of *p*-coumaroyl CoA and three of malonyl CoA as well, the product is naringenin chalcone, which in a further series of reactions, gives rise to the flavonoid family. (Soleas, Diamandis, and Goldberg, 1997). This includes tannins and anthocyanins, which account for the bitterness and the pigmentation, respectively and are present in the skins of mature grapes. Chalcone synthase is expressed constitutively, such that its activity is substrate-driven and its products accumulate in proportion to the generation of precursor sugars during ripening and maturation of the berries. By contrast, resveratrol synthase is normally unexpressed and induced by UV-irradiation, trauma and infection (Hopwood and Sherman, 1990).

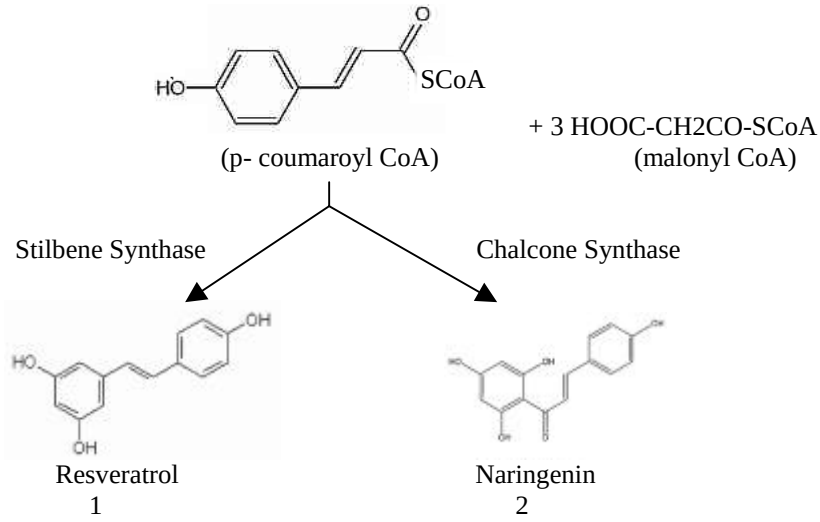


Figure 2.11 Biosynthetic pathway of resveratrol
Source: modified from Soleas, Diamandis, et al., 1997

2.5.2 Concentration of resveratrol in grapes and wines

In grape juices, total resveratrol content in red juices is between 0.69 and 14.47 mg/L (mean 4.73 mg/L) and in white ones is between not detected and 1.44 mg/L (mean 0.49 mg/L) (Romero-Perez, Ibern-Gomez, Lamuela-Raventos and de la Torre-Boronnat, 1999). In juices, the differences in resveratrol content between white and red grape juices have to be distributed to the juice processing technology (i.e. pressing and skin color extraction procedures) since the levels of *t*-resveratrol in grapes skins are similar for red and white grape berries (Okuda and Yokotsuka, 1996). In juices, the glycosides account for 90% or more of the total reseratrol content in juices (Romero-Perez et al., 1999). The concentration of resveratrol was found in different levels as summarized in Table 2.2.

Table 2.2 Resveratrol and free quercetin levels in red wines

Wine (Vintner)	Vintage	Origin*	Resveratrol	Quercetin
Merlot/ Cabernet Sauvignon	1990	France	8.26 ± 0.24	3.57 ± 0.06
"Burgundy"	NV	CA	0.50	3.33 ± 0.37
75% Cab. Sauv./ 25% Mer.	1991	Chile	1.56 ± 0.08	1.46 ± 0.07
Barbera, Pinot noir,				
Zinfandel, Ruby Cabenet	NV	CA	2.74 ± 0.08	-
Barbera (Sebastiani)	1991	CA	9.20 ± 0.40	13.40 ± 0.60
Beaujolais (Jadot)	1992	France	3.55 ± 0.06	0.15 ± 0.02
" (St. Louis)	1992	France	3.27 ± 0.14	0.19 ± 0.02
Cabernet Sauvig. (Gallo)	1986	CA	0.99 ± 0.08	-
" (Gallo)	1992	CA	2.78 ± 0.16	3.06 ± 0.28
" (Avia)	1989	Slovenia	0.95 ± 0.01	3.33 ± 0.37
" (Kendall-Jackson)	1991	CA	4.33 ± 0.54	-
" (Vendange)	1993	CA	0.53 ± 0.02	5.03 ± 0.48
Chianti (Ruffino)	1993	Italia	4.80 ± 0.10	-
" (Placido)	1993	Italia	2.76 ± 0.10	7.34 ± 0.08
Citra (Montapulcitano)	1993	Italia	2.60 ± 0.15	3.37 ± 0.21
Gamay Beaujolais	1994	CA	2.09 ± 0.17	-
Merlot (Marcus James)	1991	Brazil	3.30 ± 0.10	4.00 ± 0.27
" (Walnut Creek)	1992	CA	5.42 ± 0.23	3.04 ± 0.00
" (Rosemount Estate)	1993	Australia	5.78 ± 0.09	9.62 ± 0.13
" (Deer Valley)	1992	CA	3.57 ± 0.33	-
Petit Syrah (Mirassou)	1991	CA	1.76 ± 0.11	-
Pinot noir (Sebastiani)	1990	CA	5.01 ± 0.25	1.82 ± 0.16
" (Mt. View)	1991	CA	7.99 ± 0.09	3.32 ± 0.06
" (Beaulieu)	1991	CA	3.72 ± 0.10	0.90 ± 0.12
" (Mirassou)	1992	CA	8.70 ± 0.10	-
Zinfandel (Sutter Home)	1991	CA	1.38 ± 0.18	0.90 ± 0.10
" (Sebastiani)	1991	CA	4.90 ± 0.50	4.96 ± 0.58

* CA = California

Source: McMurtrey, 1997

2.5.3 Factors affecting wine resveratrol concentration

The concentration of resveratrol is varying in some regions. Differences in grape varieties and winemaking practices also contribute to its concentration in the products involved.

2.5.3.1 Varieties

Lamuela-Raventos and Waterhouse (1999) and Goldberg, Hahn, and Parkes (1995) found that Pinot noir variety wine contained the highest average concentration of resveratrol as similar to McMurtrey (1997) who also observed high level of resveratrol in Pinot noir (Table 2.2). This is 2 ½ times more than Cabernet Sauvignon and Merlot, 4 times more than Syrah, 7 and 18 times more than Zinfandel and Cabernet Franc, respectively. Pinot noir appears to have a high intrinsic ability to synthesize resveratrol due to it has a relatively thin skin that renders it very sensitive to traumatic damage, *Botritis* infection, and UV-light (Soles, Diamandis, and Goldberg, 1997a). Furthermore, the climates affect resveratrol levels. In warm and dry climates where fungal attack is low, lower concentration is produced, whereas in the cooler and damper climates of Bordeaux and Canada, wines with much higher concentrations of resveratrol are produced (Soleas et al., 1997a).

2.5.3.2 Fungal infection

Jeandet, Bessis, Sbaghi, Meunier, and Trollat (1995) suggested that 10% infection by mold could lead to enhance resveratrol production. However, extensive fungal development may destroy induction of resveratrol formation. The degradation of resveratrol by the pathogen *Botrytis cinerea* in highly infected grapes could be due to the presence of laccase activity. Furthermore, it is possible that some yeast strains used for artificial inoculation contain laccase-like enzymes capable of degrading resveratrol.

2.5.3.3 Skin contact

Grape skins contain various important polyphenols, especially *t*-resveratrol, which only present in this part. It is not surprising that most investigators have found an intimate relationship between duration of skin contact during fermentation and the degree of maceration of the skins. Mattivi and Nicolini (1993) reported that fermentation of wine with grape skin in Trentino region of Italy, an increase in the *t*-resveratrol content of wine was up to 4 days of fermentation followed by a plateau up to 14 days and a sharp fall thereafter. Lower values were obtained if the skins were removed at day 8. The presence of high sulfite concentrations protected resveratrol against losses due to increase in pH and temperature. Jeandet et al (1995) confirmed the importance of skin maceration in causing a 10-fold increase in the two isomers (*trans*- and *cis*-) in both red and white wines. Pezet and Cuenat (1996) measured the content of *t*- and *c*-resveratrol of Gammy variety in the fermenting must as well as in skins and seeds up to day 6 of macerative fermentation, at which point the skins and seeds were removed then, malolactic fermentation was allowed to continue until day 46. They pointed out the significance of their finding after removing the skins and seeds, there was a 4-fold increase in both resveratrol isomers that could only be due to their formation from a pool of glucosides or oligomeric forms through the enzymatic activity of malolactic bacteria.

2.5.3.4 Other enological practices

Manipulations that can be applied during wine making to increase stilbenes, as well as other phenolics, include treatment of juice with commercial pectolytic enzymes. This can increase the amount of resveratrol by 50%. The enzymes

breakdowns the hypodermis of the skins, releasing flavor and phenolic compounds (Wightman, Price, Watson, and Wrolstad, 1997). Many wines, after fermentation is complete, are transferred to oak barrels for a period of ageing. Different types of oak impart different flavor characteristics that are enhanced by toasting the barrels. Soleas, Goldberg, Kuramanchiri, Diamandis, and Ng (1995) reported that ageing Ontario red wines in oak for one year led to a mean loss of 68% for *t*-resveratrol and 58% for *c*-resveratrol. When the wines are aged in oak, major losses of resveratrol isomers occurred even when the wines were kept cool and without light. Precipitation, oxidation or absorption by oak during the ageing process seems to be the operative mechanisms. Filtration prior to bottling is another widely used practice in winemaking. Most of the clarifying agents traditionally used (e.g. egg white, diatomaceous earth, bentonite and gelatin) caused little, while PVPP decreased the levels of all resveratrol form up to 90% (Vrhovsek, Wendelin, and Eder, 1997). Commercial filter pads selectively remove significant amounts of *t*-resveratrol, while charcoal treatment causes major losses in both isomers (Soleas et al., 1995). Finally, the fact that resveratrol and other polyphenols are present in high concentrations in grape seeds led to the proposal that grape musts enriched in seeds will extract more resveratrol during fermentation (Kovac, Alonso, and Revilla, 1995).

2.6 Wines and health benefits

Wine is an alcoholic beverage that benefit for health. In the very first study, St Leger, Cochrane, and Moore (1979) showed that there was an association between reduced coronary heart disease (CHD) mortality and increased wine consumption. The term “Franch Paradox” (despite high fat intake, mortality from CHD is lower in

some regions of France than in the other developed countries due to regular wine consumption) was born. In addition, epidemiology studies have shown that wine consumption is correlated with a greater reduction in CHD mortality than other alcoholic beverages (Fig 2.12). These data suggest that wine contains components absent in other alcoholic beverages and it is unique components, which favorably affect CHD mortality. Beside, moderate consumption of alcohol can raise HDL subfractions that have been found to be protective against CHD, the effective phenolic compounds has several physiological activity contribute to health benefit.

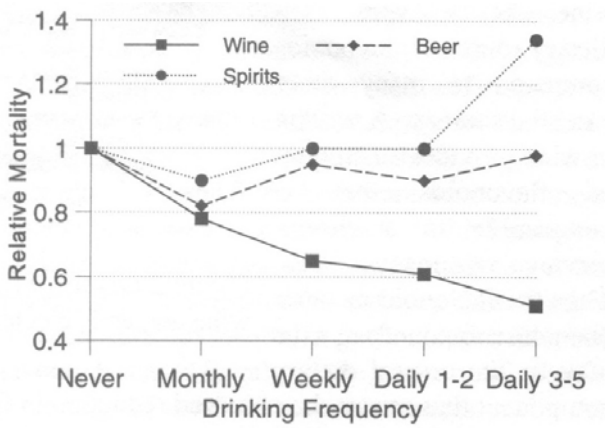


Figure 2.12 Relative mortality rates at varying consumption rates for specific beverages

Source: Gronbaek et al., 1995

2.6.1 Antioxidant

Free radicals are unstable molecules that attack with cell membranes and other intracellular structures causing damage to body tissues and often generating more harmful molecules as well. The damages can eventually lead to diseases such as cancer and atherosclerosis (Beckman and Ames, 1998). Phenolic compounds of wine

have very high antioxidative power to protect several cells against free radical molecules. *t*-Resveratrol has been demonstrated to be more effective antioxidant than butylhydroxytoluene (BHT), quercetin or tocopherol on lipid peroxidation in liposomes and rat liver (Belguendouz, Fremont, and Gozzelino, 1998; Belguendouz, Fremont, and Linard, 1997; Frankel, Waterhouse and Kinsella, 1993; Fremont, Belguendouz, and Delpal, 1997). Furthermore, Goldberg et al. (1995) noted that flavonoids such as quercetin, rutin, catechin, epicatechin were individually and collectively 10 to 20 times more potent than vitamin E in protecting LDL oxidation. Frankel et al. (1993) reported both resveratrol and quercetin to be more powerful antioxidant than vitamin E in protecting human LDL against copper-catalyzed oxidation. Resveratrol can inhibit LDL oxidation (Belguendouz et al., 1997). Resveratrol mainly acted by chelating copper whereas flavonoids (quercetin, epicatechin, catechin) were better scavengers of free radicals. However, resveratrol cannot chelate ferrous ions. This is potentially useful in vivo since LDL is known to have a high affinity for binding copper. The chelating properties of the *cis*-isomer are about half that of the *trans*-isomer. However, both isomers are equally as effective as scavenging free radicals (Belguendouz et al., 1998). The antioxidant activities of resveratrol are believed to be due to its protective effects on the cellular membranes. It has been suggested that the effects of resveratrol are due to its amphiphatic character (when the structure has both hydrophilic and hydrophobic sites), which allows the protection of cellular and subcellular components (Chanvitayapong, Draczynska-Lusiak, and Sun, 1997).

Furthermore, anthocyanins are in large part responsible for the scavenging activity of red wines (Sainst-Cricq de Gaulejac, Glories, and Vivas, 1999). The ability

of anthocyanins to counter oxidants makes them strong–atherosclerosis fighters. Anthocyanins prevent a key step in atherogenesis, oxidation of LDL. Researchers in a USDA-funded study concluded that anthocyanins is a "more potent" antioxidant than vitamin C or BHT, which is used as a preservative (Mazza and Miniati, 1993).

2.6.2 Antiaggregation properties

Arachidonic acid (AA) predominates in mammalian cells, being stored in cell membranes. AA is transformed into the powerful pro-inflammatory and platelet aggregating thromboxanes through the cyclooxygenase (COX) enzymes. AA becomes the powerful inflammatory and white cell stimulating agents known as leukotrienes, hepoxillins and lipoxins through the lipoxygenase (LOX) enzymes. The 50% inhibition concentration or IC50 concentrations for both resveratrol and quercetin in platelet aggregation were approximately 100 micromoles, while others antioxidant, BHT, and vitamin E, and the major wine phenolics, catechin, and epicatechin were ineffective at inhibiting platelet aggregation (Pace-Asciak, Hahn, Diamandis, Soleas, and Glodberg, 1995). Soleas et al. (1997a) noted that resveratrol at micromolar concentrations is able to inhibit thromboxane A2 production by inhibiting cyclooxygenase and hydroperoxidase function, and quercetin could likewise inhibit the formation of hepoxillins. Resveratrol has been shown to counteract the induction of collagen that causes platelets to aggregate (Bertelli et al., 1995). In addition, it is also been noted to decrease the adhesion of blood constituents that promote platelet aggregation to vessel wall (Bertelli, Bertelli, Gozzini, and Giovannini, 1998).

Keli, Hertog, Feskens, and Kromhout (1994) reported that flavonoids markedly inhibit platelet aggregation as well due to a consequence of the production and release of nitric oxide by endothelial cells of blood vessels.

2.6.3 Cancer chemopreventive and chemotherapeutic agent

Resveratrol inhibits cellular events associated with tumor initiation, promotion, and progression. It has antiinitiation activity because of its antioxidant and antimutagenic effects, and it inhibits the induction of phase II drug-metabolizing enzymes. It possesses antipromotion activity because of its anti-inflammatory effects, causing inhibition of production of AA metabolites catalyzed by either COX-1 or COX-2, and chemical carcinogen-induced neoplastic transformation of mouse embryo fibroblast. Resveratrol suppresses the activation of the COX-2 gene expression leading to the inhibiting of the protein kinase C signal transduction pathway (Jang et al., 1997; Jang and Pezzuto, 1999). It is also a major inhibitor of human P450 1A1 (Chun, Kim, and Guengerich, 1999). Antipropression activity is demonstrated by its ability to induce human promyelocytic leukemia (HL-60) cell differentiation (Jang and Pezzuto, 1999). Furthermore, resveratrol has been shown to induce apoptosis in human breast carcinoma and human leukemia cells (Darrie, Gerauer, Wachter, and Zunino, 2001).

2.6.4 Cardioprotective action

Resveratrol has ability to inhibit the oxidation of LDL, antiplatelet aggregation and has possible phytoestrogenic activity that contributes to its possible cardioprotective action (Fig 2.13) (Fremont, Belguendouz, and Delpal, 1999). Moreover, phenolic compounds and alcohol in red wine affected to several risk

factors leading to reduce CHD. The alcohol appears to provide effects at the hemostatic level, whereas the phenolic compounds found in red wine and grape juice are postulated to be involved primarily with LDL (Wollin and Jones, 2001).

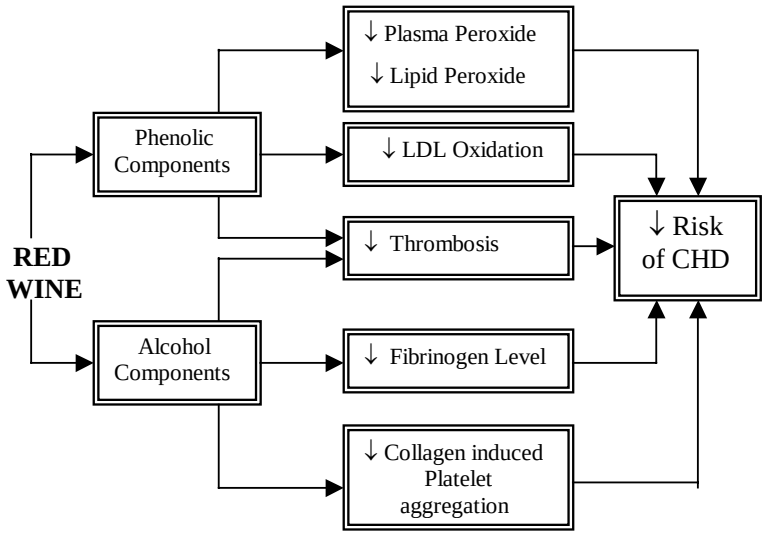


Figure 2.13 Schematic representation of the effects of alcohol and phenolic compound on risk factors for CHD
Source: Wollin and Jones, 2001

2.6.5 Neurodegenerative protection

Resveratrol possesses many of the properties of the drugs used for preventing or treating Alzheimer's disease because it has anti-inflammatory, antioxidant, estrogenic and hypolipemic properties (Tredici et al., 1999). Furthermore, resveratrol lowers the levels of the amyloid-beta peptides which cause the telltale senile plaques of Alzheimer's disease (Bethesda, 2005). Resveratrol acts by stimulating the degradation of amyloid-beta peptides by the proteasome, a barrel-shaped multi-protein complex that can specifically digest proteins into short polypeptides and amino acid.

2.7 Absorption and bioavailability of phenolics

In rat, oral administration, resveratrol (86 µg/kg) was shown to be quickly absorbed and reached its peak concentration in the plasma and kidneys approximately 15 – 60 min after red wine ingestion, while the peak concentration in liver was reached 30 min after administration at the concentration of 20.7 ng/g. The maximum concentration in the heart was reached after 120 min of ingestion (Bertelli et al., 1998). Resvertrol is rapidly absorbed at the intestinal levels and reaches its highest concentration in blood. Absorbed flavonoids, after metabolism in the small intestine, are subjected to various reactions in the liver, including methylation, sulphation, and glucuronidase leading to a variety of conjugated forms (Stahl et al., 2002). The metabolism of flavan-3-ols in term of plasma levels of conjugates and metabolites has been studies in rat (Stahl et al., 2002). Methylated and glucuronidated metabolites have been detected in rat.

However, the absorption and bioavailability of phenolic compounds were not clear, it have been recommended that drinking alcoholic beverage in moderation (fewer than two drinks a day, ≈ 200 mL as suggested by Renaud and de Lorgeril, 1992) decreases risk of heart disease and stoke. If more than 2 drinks a day cannot be recommended because of its possible negative effects. This small amount of alcoholic drink recommendation is sufficient enough to obtain effective phenolic compound for positive effects on health such as decreasing the risk of cancers, and CHD (Wollin and Jones, 2001).

2.8 Measurement of phenolic compounds

Phenolic compound determinations are usually done by using HPLC and GC-MS. Recently, CE application is also used for analysis. The measurement of phenolic compounds is required organic phase extraction followed by one or more high performance chromatography steps.

2.8.1 High performance liquid chromatography (HPLC)

HPLC can be used in routinely analysis of wines because it is rapid and does not require any sample pretreatment. The reversed-phase C_{18} column followed by gradient elution and diode array detection was used (Soleas et al., 1997a).

2.8.2 Gas chromatography-mass-spectrometry (GC-MS)

Most GC methods require a pre-derivatization with bis-[trimethylsilyl]-trifluoroacetamide (BSTFA) prior to column application with detection by flame ionization or mass-spectrometry (MS). Although GC-MS techniques have many advantages in the assay of phenolic compounds in wine, including higher sensitivity and identification by mass spectral characteristics. The disadvantages of GC methods for stilbenes compared to the liquid chromatography methods are (Lamuela-Raventos, Romero-Perez, and de la Torre-Boronat, 2001):

(a) Time required for the extraction or for the derivatization (60min).

(b) *Trans*- to *cis*- isomerizaiton may occur during derivatization.

(c) *Trans*-polydatin (aggregated form of resveratrol) is converted to the free isomers, resulting in an overestimation of the aglycone.

2.8.3 Capillary electrophoresis (CE)

CE is a relatively new separation technique and represents an alternative method for the analysis of a variety of compounds, including antioxidants in food matrices. The principle of separation in CE is based on the differential migration of the analytes in an electric field resulting from intrinsic differences in mass to charge ratios. The high field strength typically employed (300 V/cm) results in high efficiency separation. Electroosmotic flow of water from the anode to cathode gives the high field strength. This flow creates a pumping action originating at the capillary wall that is devoid of a radial pressure gradient (Gu, Chu, O,Dwyer, and Zeece, 2000). CE method has been shown to separate various types of phenolic compounds as complied by Gu et al. (2000) and Sadecka and Polonsky (2000) shown in Table 2.3.

Table 2.3 Summary of CE for separation of flavonoid antioxidants

Analyte	Separation condition	Remarks
Catechin, epicatechin,rutin, quercetin, myricetin	30 mM sodium phosphate buffers of pH 8.85	CZE separation of antioxidant in red wine at pH 8.85
Catechin, epicatechin quercetin, resveratrol gentistic acid, caffeic acid,gallic acid	100 mM sodium borate, pH 9.5	CZE separation of SPE samples
11 phenolic compound	100 mM borate, pH 9.5	CZE separation Liquid-liquid extraction
15 phenolic compound	25 mM phosphate, 10 mM borate pH 8.8	CZE separation Liquid-liquid extraction

Source: Gu at el., 2000; Sadecka and Polonsky, 2000.

Several investigators used the CE to determine the levels of *t*- and *c*-resveratrol in wine samples with good selectivity, speed, and reproducibility. Most of the CE methods for measuring resveratrol in wine were able to reliably detect at 0.2 – 1.0 μM levels (Gu et al., 2000).

2.9 References

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CHAPTER III

PHENOLIC COMPOUNDS AND ANTIOXIDATIVE PROPERTIES OF SOME GRAPES AND WINES OF SURANAREE UNIVERSITY OF TECHNOLOGY (SUT) FARM

3.1 Abstract

The purposes of this work were to evaluate and compare phenolic contents and antioxidative properties of some grape and wine varieties produced in Thailand, using those particularly grown and produced on Suranaree University of Technology (SUT) Farm as a case study. Eight grape and nine wine varieties were used to evaluate for total phenolic content (TPC) by Folin-Ciocault method, free radical scavenging efficacy by DPPH method and reducing power by ferric reducing-antioxidant power (FRAP) method. Grape juices with seeds had higher ($p<0.05$) TPC and free radical scavenging activity than those without seeds. Moreover, red grape juices showed higher free radical scavenging activity than white grape juices. The red wines had significantly higher ($p<0.05$) amounts of total phenols, flavonoids and antioxidant activities (AA) compared to white wines. Capillary electrophoresis (CE) was used as a powerful and high performing tool for analysis of principal phenolic compounds in the wines. *t*-Resveratrol was found in Shiraz, Zinfandel and Blended wine varieties. (+)Catechin was found in all wine varieties, except in Chasselar Dore.

(+)Catechin was present in wines higher that (-)epicatechin. In red wine, gallic acid was the highest phenolic acid found.

Key words: antioxidative, total phenolic, free radical scavenging, ferric reducing-antioxidant power, grape juice, wine, capillary electrophoresis, resveratrol, flavonoids

3.2 Introduction

Lipid oxidation is a free radical chain reaction including initiation, propagation and termination reactions. It represents one of the basic mechanisms in the cell and tissue damage leading to various diseases such as atherosclerosis, inflammation and cancer. Antioxidants can interfere with the oxidation process by reacting with free radicals, chelating metal and acting as oxygen scavengers.

The phenolic antioxidants in red wines have been proposed as an explanation for the lower death rate from coronary heart disease (CHD) in France as referred to "The Franch Paradox" (despite high fat intake, mortality from coronary heart disease is lower in some regions of France than in the other developed countries due to regular wine consumption). Phenolic compounds play roles as antioxidant in both biological and food systems. They have many favorable effects on human health, such as inhibition of the oxidization of low-density lipoproteins (Frankel, Waterhouse, and Kinsella, 1993) thereby decreasing heart disease risks. Phenolic compounds can be classified into two groups: the flavonoids and nonflavonoids. The major C6-C3-C6 flavonoids in wine include conjugates of the flavonols, quercetin, and myricetin; the flavan-3-ols, (+)-catechin and (-)-epicatechin, and other anthocyanins. The

nonflavonoids include the C6-C1 hydroxybenzoic acids, and gallic acids; the C6-C3 hydroxycinnamates, caffeic, caftaric, and *p*-coumaric acids and the C6-C2-C6 stilbenes *trans (t)*-resveratrol, *cis (c)*-resveratrol, and *t*-resveratrol glucoside.

The antioxidant activities of these phenolic compounds in grapes and wines and as well as their individual pure chemicals have been investigated using many procedures such as the stable 2,2-diphenyl-1-picryhydrazyl ($DPPH^{\bullet}$) radical methods (Sanchez-Moreno, Larrauri, and Saura-Calixto, 1999), and ferric reducing-antioxidant power (FRAP) methods (Katalinic, Milos, Modun, Music, and Boban, 2004).

Capillary electrophoresis (CE) is one of the useful techniques to evaluate phenolic constituents in wines. Its high efficiency and capacity to resolve a complex of natural compounds were the reasons for choosing this technique. The successful application of CE for wine and polyphenol determination has been demonstrated by Gu, Chu, O'Dwyer, and Zeece, 2000; Minussi et al., 2003; and Sadecka, and Polonsky, 2000.

The active polyphenols in grapes and wines are depending on grape variety, geology, environment and wine processing technique (Frankel, Waterhouse, and Teissedre, 1995). There is little knowledge of phenolic contents and their antioxidative properties in grapes and wines produced in Thailand. Therefore, the purpose of this study were to preliminarily evaluate the contents and antioxidant qualities of phenolic compounds in some grapes and wines produced from Suranaree University of Technology (SUT) Farm and to obtain a phenolic profiles of such wines.

3.3 Materials and Methods

3.3.1 Materials

2,2-Diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) was obtained from Sigma (St Louise, MO, USA). Tripyridyl-s-triazine was obtained from Acros Organics (New Jersey, USA). Organics Analytical grade methanol, absolute ethanol, formaldehyde, disodium hydrogen phosphate anhydrous, sodium carbonate, and Folin-Ciocalteu's reagent were purchased from Carlo Erba Reagents (Strada Rivoltana, Spain).

3.3.2 Collection of the samples

Eight grape varieties grown on Suranaree University of Technology (SUT) Farm were harvested in year 2003 with sugar content of about 20° Brix, including six red grape varieties; Shiraz, Cabernet Sauvignon, Exotic, Carolina Blackrose, Muscat Hamburg (China) and 8804, and two white grape varieties; Semillon and Niagara. Twenty-five grams wet weight of grape berries (with and without seed) was homogenized for 30 s, filtered and centrifuged at 4°C, 2000 x g for 10 min. The supernatant was used to determine for total phenolic content (TPC) and antioxidant activity.

For wine samples, five red wines; Shiraz, Muscat Hamburg (China), Zinfandel and Barbera (vintage year 2003 and 2004) and Muscat Hamburg (vintage year 2004), three white wines; Italia and Chasselar Dore (year 2003) and Chenin Blanc (year 2004) obtained from SUT farm and a commercial blended wine (red wine; Dong Pa Ya Yen) purchased from local food store were used for this experiment.

3.3.3 Determination of phenolic compounds

3.3.3.1 Total phenolic content

The total phenolic contents (TPC) of the grape and wine samples were determined by Folin-Ciocault method (Matthaus, 2002). Sample solution of 0.1 mL was introduced into a test tube and then 2 mL of 2% of sodium carbonate was added. After incubation for 2 min, 0.1 mL of Folin-Ciocalteu's reagent (Folin:Methanol, 1:1) was added. The absorbance was measured at 750 nm after incubation for 30 min. The TPC content of the sample was calculated using pure gallic acid to establish a standard curve and expressed as mg of gallic acid equivalent (GAE) per liter of sample.

3.3.3.2 Flavonoids

The possible use of formaldehyde to precipitate the flavonoid phenolic compounds has been proposed for wine (Ough and Amerin, 1988). Formaldehyde reacts with 6- or 8-position on the 5,7-dihydroxy flavonoids forming a methylol derivative that will attach to another 6- or 8-position on another flavonoid and so on. These condensed molecules were removed by filtration. The residual nonflavonoid phenolic tannin was analyzed by Folin-Ciocalteu method. The amount of flavonoid was calculated as the differences between total phenols and nonflavonoids in wine. The flavonoid content was expressed as mg of gallic acid equivalent (GAE) per liter of sample obtained from the difference of TPC and nonflavonoid content.

3.3.4 Determination of antioxidant activity

3.3.4.1 Free radical scavenging activity

The $DPPH^{\bullet}$ method (Sanchez-Moreno et al., 1999) was used to

determine free radical scavenging property. For each solution, different concentrations were tested (expressed as mg GAE/L reaction assay). Sample solution of 0.1 mL was added to 3.9 mL of a 2.5 x 10^{-2} g/L methanolic $DPPH^{\bullet}$ solution. The tube was maintained for 45 min in the dark then, the absorbance was measured at 515 nm. The radical scavenging capacity of the sample was expressed as the percentage of $DPPH^{\bullet}$ remaining as following:

$$\% \ DPPH^{\bullet} \text{ remaining} = (A_{(t)} / A_{(0)}) \times 100$$

Where $A_{(0)}$ was the absorbance of control at time = 0 min , and

$A_{(t)}$ was the absorbance of antioxidant at time = 45 min.

Antioxidant activity of the sample was defined as the amount of antioxidant necessary to reduce the initial $DPPH^{\bullet}$ concentration by 50% (Efficient concentration = EC_{50} mg (GAE) sample/ L reaction assay).

3.3.4.2 Ferric reducing-antioxidant power (FRAP)

Measurement of reducing ability of the antioxidative property of grape and wine samples was performed using FRAP method according to Katalinic et al. (2004). FRAP is a simple direct test of antioxidant capacity. This assay measures the formation of a blue colored at 593 nm of Fe^{II}-tripyridyltriazine compound from colorless oxidized Fe^{III} formed by the action of electron donating antioxidants. The working FRAP reagent was prepared by mixing 10 volumes of 1.0 mol/L acetate buffer, pH 3.6 with 1 volume of 10 mmol/L TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mmol/L hydrochloric acid and with 1 volume of 20 mmol/L ferric chloride.

In a reaction test tube, sample solution of 50 μL and 150 μL of deionized water were added into 1.5 mL of the FRAP reagent. Absorbance was measured after 8 min. Standard curve was prepared using different concentrations (100 – 1000 μmol/L) of $FeSO_4.7H_2O$. All solutions were used on the day of preparation. The antioxidant efficiency of the sample solution was calculated with reference to the standard curve given by a Fe^{2+} solution of known concentrations. Ferric reducing power of the sample was expressed in mmol Fe^{2+}/L.

3.3.5 Analysis of phenolic composition

3.3.5.1 Chemicals

The following polyphenol standards commonly found in wines were used; resveratrol, (+)-catechin, (-)-epicatechin, rutin, syringic acid, *p*-coumaric acid, caffeic acid, gallic acid, protocatechuic acid (Sigma Chemical Co., St Louise, MO, USA), cinnamic acid, *p*-hydroxybenzoic acid, quercetin, gentisic acid (Acros Organics, New Jersey, USA) and salicylic acid (Asia Pacific Specialty Chemicals Ltd., Seven Hills, Australia). Boric acid and methanol (HPLC grade) were obtained from Fisher Chemicals (Fisher Scientific UK Ltd, Leics, UK). Absolute ethanol, diethyl ether, sodium hydroxide and disodium hydrogen phosphate were purchased from Carlo Erba Reagents (Strada Rivoltana, Spain)

3.3.5.2 Sample preparation

For the liquid/liquid extraction, 1 mL of wines or juices was extracted twice with 1 mL of diethyl ether. The organic phases were completely dried in the dark under nitrogen gas and resuspended with 1 mL of ethanol (50%). Wine sample

was filtered through 0.45 polyethersulfon membrane (Supor@Acrodisc®, Pall Life Sciences, Gelman Sciences Inc, USA).

3.3.5.3 Capillary electrophoresis procedure

Capillary electrophoresis analyses were performed using an Agilent Technologies model G1600AX (Agilent Technologies Deutschland GmbH, Waldbronn, Germany), equipped with a diode-array detector. An uncoated fused silica capillary tube of 50 μm i.d. (Agilent) with effective and total lengths of 50 and 75 cm, respectively was used. Electrophoretic analyses were performed at an applied voltage of 15 kV at 25°C.

Electrophoretic buffer was a mixture of phosphate 25 mmol/L and borate 10 mmol/L, at pH 8.5. This buffer was obtained by mixing solution of H_3BO_3 (100 mmol/L) and Na_2HPO_4 (100 mmol/L) using 2 N NaOH to adjust the pH to desired pH value.

The column was pre-rinsed with deionized water for 2 min and then, running buffer for 3 min. Sample was hydrodynamically injected for 7 s. Between analyzes, the column was flushed with 0.1 M sodium hydroxide for 1 min, deionized water for 2 min and running buffer for 3 min. All CE analyses were performed in duplicate. Before being used, all solutions were filtered through a 0.45 μm polyethersulfon membrane.

Calibration curve of each phenolic compound was established using pure chemical with concentrations ranging from 2-10 mg/L plotted against peak areas. Each phenolic concentration was calculated by chemstation Rev.A.09.01 (1206) (Agilent Technologies 1990-2001). For each standard compound, four replicate samples were used for analyses.

3.3.6 Statistical analysis

All experiments were performed in duplicate. Each replicate was chemically analyzed in duplicate samples. Statistical analysis was evaluated in completely Randomized design (CRD) with Statistical Analysis (SAS Institute, Inc., 1995) and means comparison by Duncan's Multiple Range Tests (DMRT) were analyzed.

3.4 Results and Discussion

3.4.1 Total phenolic content and antioxidant activity of grape juices

Total phenolic contents (TPC) and antioxidative capacity by 50% reduction of $DPPH^{\bullet}$ (EC50) of grape juices are shown in Table 3.1. The TPC of grape juices were ranging from 631–1628 mgGAE/L for the juices without seed and 1145–3315 mgGAE/L for juices with seed. It was obvious that the TPC content of the juices with seed were higher than those of without seed. During crushing of the whole berries, phenolic compounds of the seed were released into the juice. Moreover, in the juice without seed, TPC of red grape juices (1530.5- 1627.5 mgGAE/L) were significantly higher ($p<0.05$) than white grape juices (910-1100 mgGAE/L), except Shiraz and Cabernet Sauvignon varieties (702.2 and 631.0 mgGAE/L, respectively). In crushed grape with seeds, the highest TPC were found in Exotic and Muscat Hamburg (China) varieties (3115 and 2988 mgGAE/L, respectively), and the lowest amount was found in Semillon variety (1145 mgGAE/L).

The EC50s of red grape juices (5.6 - 11.0 mgGAE/L) were significantly lower ($p<0.05$) than those of white grape juices (23.4 - 24.9 mgGAE/L). The lesser value of the EC50 is indicating the higher efficient antioxidant. There was no significant difference ($p>0.05$) in EC50 values of red grape juices in both with and without seeds.

Table 3.1 Total phenolic content and EC50 of grape juices

Grape Varity	TPC (mgGAE/L)		EC50 (mgGAE/L) (DPPH)	
	without seed	with seed	without seed	with seed
Red grape				
Shiraz	702.2 ± 3.0 e	1241.5 ± 153.4 cd	9.2 ± 0.5 b	6.8 ± 0.8 bc
Cabernet*	631.0 ± 83.5 e	1643.5 ± 75.7 bc	5.6 ± 0.8 b	4.3 ± 1.2 c
Exotic	1530.5 ± 123.7 ab	3115.0 ± 219.2 a	6.6 ± 0.7 b	3.3 ± 0.0 c
Carolina**	1467.0 ± 43.8 b	1819.5 ± 335.9 b	11.0 ± 1.1 b	10.2 ± 0.2 b
Muscat Hamburg	1545.0 ± 76.4 ab	2988.0 ± 282.8 a	9.1 ± 0.2 b	6.6 ± 0.9 bc
8804	1627.5 ± 40.3 a	1946.5 ± 78.5 b	9.2 ± 0.3 b	9.8 ± 1.3 b
White grape				
Semillon	1110.0 ± 0 7.1 c	1145.0 ± 35.4 d	24.9 ± 6.0 a	17.7 ± 3.7 a
Niagara	910.0 ± 14.1 d	1352.5 ± 10.6 cd	23.4 ± 8.2 a	15.6 ± 1.1 a

n = 6
* Cabernet Sauvignon, ** Carolina Blackrose
Numbers with different letters within the same column are statistically different (P≤0.05)

However, the juices with seed of Exotic and Cabernet Sauvignon varieties had fairly low EC50 values (3.3 and 4.3 mgGAE/L, respectively). The results were similar to those of Sanchez-Moreno et al. (1999) who reported that red grape juices had higher TPC and antioxidant activity than white grape juices. It was interesting that red grape juices (Shiraz and Cabernet Sauvignon) gave lower EC50 than did white grape juices (Semillon and Niagara), despite having lower amount of TPC. These results indicated that the phenolic composition in red grape juices had higher free radical scavenging activity than white grape juices. This could be due to anthocyanin present in red grapes which known to be an important antioxidant in the grapes. The results were in

agreement with the data presented by Larson (1988) that red wines had higher antioxidative property and provided lower EC50 values.

The TPC, flavonoid and nonflavonoid contents and antioxidant capacities (EC50 and FRAP value) of selected wines are presented in Table 3.2. The TPC contents of red wine and white wine were ranging from 1498 – 2432 mgGAE/L and 306 – 846 mgGAE/L, respectively. The highest amount of TPC was detected in red wine produced from Shiraz variety in vintage year 2004 (2890.9 mg/L) and the lowest was detected in white wine produced from Chasselar Dore variety in vintage year 2003 (306.6 mg/L). The contents of phenolic compounds were similar to those presented by Katalinic et al. (2004) and Waterhouse and Teissedre (1997) whose reported the variability in the levels of total phenolic content (TPC) ranged from 1850 – 2200 mg/L for red and 220 – 250 mg/L for white wines and mentioned that grape skins and seeds had long contact time during fermentation process for red wines giving high amounts of these compounds. Different vintage years gave differences in phenolic composition. The average TPC of Shiraz and Zinfandel wine produced in vintage year 2004 (2890.9 and 2750.9 mgGAE/L, respectively) had significantly higher ($p<0.05$) than those wine varieties produced in year 2003 (1687.5 and 1856.3, respectively). Moreover, Muscat Hamburg (China) red wine in vintage year 2003 contained TPC twice as much of the one produced in year 2004 (2365.9 and 1458.4 mg/L, respectively). These were in agreement with Waterhouse and Teissedre (1997) that TPC and individual compounds such as catechin, epicatechin, quercetin, gallic acid amounts varied depending on vintage year. The red wines had significantly higher amounts ($p<0.05$) of total phenols and flavonoids compared to white wines, except in Italia white wine variety. The amount of flavonoids was ranging 74.27 – 91.74 % of TPC for red wines and 29.35 – 67.46% for white wines.

Table 3.2 Total phenolic content and antioxidant activity of selected wine

Wine Variety*	TPC (mgGAE/L)	Flavonoid (mgGAE/L)	Non-Flavonoid (mgGAE/L)	DPPH (EC50) (mgGAE/L)	FRAP (mmol Fe^{2+}/L)
Red wine					
Shiraz (03)	1687.5 ± 109.6 bc	1253.3 ± 9.2 cd	434.2 ± 9.2 ab	4.6 ± 0.1 bcd	10.5 ± 2.5 cd
Shiraz_K1V(04)	2843.6 ± 235.9 a	2268.0 ± 105.7 ab	575.6 ± 341.6 a	2.7 ± 0.5 d	19.5 ± 0.2 ab
Shiraz_EC1(04)	2938.2 ± 57.9 a	2647.1 ± 40.7 a	291.1 ± 17.2 bc	4.1 ± 0.8 bcd	19.3 ± 3.0 ab
Muscat (China) (03)	2365.9 ± 19.6 ab	2170.6 ± 3.7 ab	195.3 ± 3.7 bc	3.1 ± 0.0 d	17.1 ± 0.7 abc
Muscat (China) (04)	1458.4 ± 382.1 cd	1190.2 ± 280.7 cd	268.1 ± 101.4 bc	3.6 ± 0.0 bcd	10.6 ± 2.9 cd
Muscat (04)	2184.4 ± 12.9 abc	1901.0 ± 4.0 abc	283.4 ± 8.9 bc	3.8 ± 0.3 bcd	15.6 ± 1.5 abc
Zinfandel (03)	1856.3 ± 133.4 bc	1516.4 ± 41.2 bc	339.9 ± 41.2 bc	3.8 ± 0.1 bcd	12.8 ± 0.1 bcd
Zinfandel (04)	2750.9 ± 623.6 a	2449.3 ± 619.4 a	301.6 ± 4.2 bc	3.3 ± 1.5 cd	20.6 ± 7.2 a
Barbera (03)	2431.5 ± 398.1 ab	2077.4 ± 15.8 ab	354.1 ± 15.8 abc	6.1 ± 0.0 bcd	16.4 ± 3.8 abc
Barbera (04)	2479.7 ± 1029.2 ab	2117.4 ± 999.9 ab	362.3 ± 29.3 abc	4.8 ± 0.3 bcd	15.9 ± 6.2 abc
Blended (02)	1498.1 ± 84.9 cd	1184.0 ± 7.4 cd	314.1 ± 7.4 bc	6.7 ± 0.7 bc	10.8 ± 0.0 cd
White wine					
Italia (03)	845.7 ± 9.5 de	570.5 ± 35.4 de	275.2 ± 35.4 bc	5.7 ± 0.1 bcd	6.4 ± 0.1 de
Chasselar Dore (03)	306.0 ± 22.6 e	89.8 ± 26.4 e	216.2 ± 26.4 bc	13.8 ± 3.0 a	1.9 ± 0.0 e
Chenin Blanc (04)	311.2 ± 9.7 e	151.6 ± 58.7 e	159.6 ± 68.4 c	6.8 ± 3.8 b	2.2 ± 0.6 e

n = 4

* Muscat = Muscat Hamberg, (02), (03) and (04) means vintage year 2002, 2003, and 2004

K1V=K1V1116 yeast strain, EC1=EC1118 yeast strain

Numbers with different letters within the same column are significantly different (P≤0.05)

The amount of nonflavonoid phenols in wines was ranging 195 - 576 mgGAE/L for red wines and 159 – 275 mgGAE/L for white wines. There was no significant difference ($p>0.05$) between nonflavonoids in red and white wines.

The free radical scavenging activity, EC50 determined by DPPH method of red wine and white wine were no significant difference ($p>0.05$) ranging 3.1 – 6.8

mgGAE/L, except in wine produced from Chasselar Dore variety showed the highest ($p<0.05$) amount of 13.8 mgGAE/L. Moreover, red wine also had higher ferric reducing antioxidant power (FRAP) than those of white wines (10.5 – 20.6 mmol Fe^{2+}/L for red wines and 1.9 – 6.4 mmol Fe^{2+}/L for white wines, respectively). Chasselar Dore variety had significantly ($p<0.05$) lowest antioxidant property (highest EC50 and lowest FRAP value) because of its low TPC and flavonoids contents.

3.4.2 Phenolic composition in selected wines

Fourteen pure chemicals of individual phenolic compounds were used as references to determine weather their presentation in selected wines from SUT Farm and those available locally. The calibration and recovery data of 14 phenolic compounds used for capillary electrophoresis (CE) analysis are presented in Table 3.3 and electropherogram is shown in Figure 3.1. The performance of CE for the analysis of *t*-resveratrol, gentisic acid, *p*-hysroxybenzoic acid, and quercetin were range 96.86 - 102.27 %, whereas cinnamic, *p*-coumaric, salicylic, caffeic, gallic, and protocatechuic acid were range 82.62 – 88.74%. The CE recovery of epicatechin and catechin were 67.40 and 76.96%, respectively, the closer in migration time of these two compounds render it sometime overlapped. The extraction recovery of phenolic compound by spiking the mixture of pure 14 compounds at concentration of 10 mg/L into wine sample, were range 76.33 – 113.52 %, except in rutin was only 52.45 %. Rutin is a flavonol glycoside comprised of the flavonol backbone and the disaccharide rutinose. It is more hydrophilic favorably and less soluble in organic phase of diethyl ether (the extraction solvent) then, it showed the lowest of the extraction recovery.

Table 3.3 Calibration and recovery data of capillary electrophoresis

Peak no./compound	Regression equation[a]	Abs	Correlation coefficient	Recovery (%)[b]	Extraction Recovery (%)[c]
1. trans-Resveratrol	y = 5.31018x - 1.26761	206	0.99679	102.27	90.81
2. (-)-Epicatechin	y = 13.75689x - 7.01988	206	0.99236	67.4	76.33
3. (+)-Catechin	y = 18.1251x - 8.90925	206	0.99536	76.96	104.81
4. Rutin	y = 4.2446x - 1.7062	206	0.99677	79.13	52.45
5. Syringic	y = 6.59337x - 3.6545	206	0.98815	74.03	92.49
6. Cinnamic	y = 9.05151x - 0.0192	217	0.99916	85.53	98.46
7. p-Coumaric	y = 7.33965x - 2.17302	206	0.99751	88.74	95.81
8. Gentisic	y = 14.02428x - 3.15387	206	0.99041	99.34	113.52
9. p-Hydroxybenzoic acid	y = 12.68154x + 1.09211	206	0.9984	96.86	93.72
10. Quercetin	y = 8.80158x - 1.35929	206	0.99934	99.41	109.21
11. Salicylic acid	y = 22.40915x - 5.99405	206	0.95843	85.57	83.37
12. Caffeic acid	y = 12.66657x - 4.3738	217	0.998	88.1	102.78
13. Gallic acid	y = 17.80169x - 8.85641	217	0.99568	85.09	96.53
14. Protocatechuic acid	y = 45.78436x - 13.50042	206	0.9982	82.62	91.17

[a] X is the concentration in mg/L and Y is the peak area
[b] Recovery due to the performance of CE instrument, n =7
[c] Recovery due to extraction process, n=7

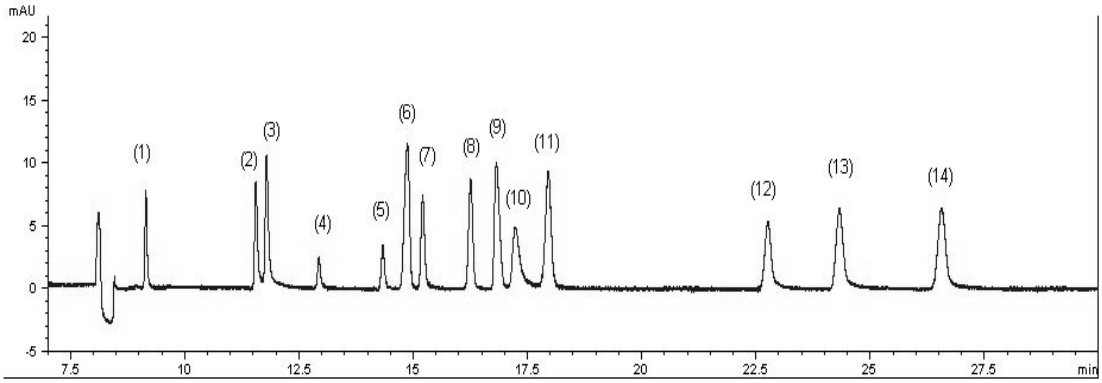

Figure 3.1 Electropherogram of standard 14 phenolic compounds

The bioactive phenolic compositions of selected wines are presented in Table 3.4. *t*-Resveratrol was only found in Shiraz, Zinfandel and blended wine ranging 1.21 – 2.76 mg/L. These results were similar to those of Gu, Creasey, Kester, and Zeece (1999) who reported the low concentration of *t*-resveratrol in Shiraz wine from Australia and Zinfandel wine from California at 6.78 and 3.26 μM, respectively. Shiraz wine produced from vintage year 2003 (2.76 mg/L) had significantly higher ($p<0.05$) amount of resveratrol than year 2004 (average of 1.44 mg/L, data not shown). Moreover, there are no significant difference ($p>0.05$) in resveratrol amounts produced from yeast K1V1116 and CY3079 strain. Resveratrol is a phytoalexin produced by higher plants as environmental stress such as fungal infection, injury or UV light exposure. Therefore it is mostly located in the berry skin. Fremont (2000) reported the large variations in ranges of resveratrol concentrations of red wine originating from various counties. The concentration was depending on grape variety, environmental condition during cultivation, wine processing techniques and alcohol contents. Soleas, Diamandis, and Goldberg (1997) reported the grape variety, which has a thin skin renders its sensitive to traumatic damage, *Botrytis* infection and UV light such as Pinot Noir variety produced the high resveratrol content. Then, the thick skin berries such as Cabernet Sauvignon variety could generate low amount of this compound. It was not detected in this experiment and detected in low amount in California wine ranged 0.4 – 2 mg/L as reported by Chu, Dwyer, and Zeece, 1998, 0.99 – 1.9 μM by Gu et al., 1999, 0.53 – 2.78 ppm by McMurtrey, 1997 and lower than 0.09 mg/L by Lamuela-Raventos and Waterhouse, 1993. Soleas et al. (1997) also reported that the concentration of resveratrol seem to be climate dependant. In warm and dry climates where fungal attack is low, resveratrol production is also low. Therefore, the resveratrol of wines

produced from SUT grapes grown in warm and dry climates, was detected in low concentration. The wine processing also affect the amount of resveratrol, higher resveratrol content are usually present in red wines which there has been prolonged contact between the must and skins, whereas lower contents or no detection are usually present in white wine with absent in must and skins during maceration (Soleas et al., 1997). Finally, Seraini, Maiani, and Ferro-Luzzi (1998) noted that alcohol is a natural stabilizing agent for polyphenolic compound. Resveratrol, is an amphipatic molecule, must take the adequate amount of alcohol to dissolve.

The (-)-epicatechin contents were only found in red wine and there were no significant difference ($p>0.05$). The amount of (-)epicatechin found in the range of not detected to 6.84 mg/L in all red wines which was lower than the (+)catechin (0.72 – 14.46 mg/L). This was in agreement with Minussi et al. (2003) found the higher amount of catechin than epicatechin of Italian wines. The (+) catechin contents were found in both red and white wines ranging 0.72 – 13.17 mg/L, except Chasselar Dore variety. Because there was no bioactive phenolic compound, Chasselar Dore wine variety had the lowest antioxidant activity (Table 3.2). Quercetin is a free form of flavonol group and rutin is a glucoside of this compound. The amounts of quercetin were 0.52 – 3.74 mg/L. The Rutin contents were found in some wine at low amount or no detection. Rutin is a glycoside in nature comprised of quercetin and disaccharide rutinose (rhamnose + glucose) and highly soluble in water than quercetin (PDR health, 2006). Due to its fairly high polar property, extraction and recovery by extraction solvent (diethyl ether) and buffer used for CE analysis were limited (Table 3.3). The blended wine from local food store was the mixture of red grape varieties with Shiraz variety was the main base. Therefore the blended wine and Shiraz wine variety were similar amount and phenolic composition.

Table 3.4 The bioactive phenolic composition (mg/L) of the selected wine

Wine Variety*	Phenolic composition of wine (mg/L)				
	Resveratrol	Epicatechin	Catechin	Rutin	Quercetin
Red wine					
Shiraz (03)	2.76 ± 0.84 a	2.32 ± 0.16 ab	3.56 ± 0.04 cd	5.33 ± 0.27 a	ND
Shiraz_K1V (04)	1.31 ± 0.75 b	4.09 ± 3.18 ab	6.20 ± 4.94 abcd	ND	2.42± 0.62 ab
Shiraz_EC1 (04)	1.53 ± 0.50 b	4.14 ± 1.71 ab	9.29 ± 1.33 abcd	ND	3.74 ± 2.47 a
Muscat (China) (03)	ND	3.93 ± 0.13 ab	13.17 ± 0.09 ab	2.50 ± 0.05 c	ND
Muscat (China) (04)	ND	1.44 ± 0.26 ab	3.59 ± 1.33 cd	ND	1.69 ±0.49 bcd
Muscat (04)	ND	3.11 ± 1.14 ab	9.97 ± 4.79 abc	ND	2.28 ± 0.81 abc
Zinfandel (03)	ND	3.56 ± 0.16 ab	4.15 ± 0.25 bcd	ND	ND
Zinfandel (04)	1.38 ± 1.02 b	6.84 ± 5.50 a	14.46 ± 11.60 a	ND	2.07 ± 0.90 abc
Barbera (03)	ND	ND	0.72 ± 0.10 cd	ND	0.52 ± 0.08 cd
Barbera (04)	ND	3.59 ± 2.79 ab	6.15 ± 4.91 abcd	ND	1.28 ±0.28 bcd
Blended (02)	1.21 ± 0.03 b	2.69 ± 0.27 ab	6.26 ± 0.46 abcd	ND	ND
White wine					
Italia (03)	ND	ND	0.92 ± 0.03 cd	3.46 ± 0.36 b	ND
Chasselar Dore (03)	ND	ND	ND	ND	ND
Chenin Blanc (04)	ND	ND	1.70 ± 0.11 cd	ND	2.12 ± 0.10 abc

n = 4

* Muscat = Muscat Hamberg, (02), (03) and (04) means vintage year 2002, 2003, and 2004
K1V=K1V1116 yeast strain, EC1=EC1118 yeast strain

Numbers with different letters within the same column are statistically different (P≤0.05)

The phenolic acids of selected wine are shown in Table 3.5. Syringic acid contents were found in all wines at moderate concentration (2.52 – 7.84 mg/L). Moreover, *p*-hydroxybenzoic acid and protocatechuic acid contents were 0.51 – 1.99 and 0.57 – 3.13 mg/L, respectively. These compounds have been said to be a principle compound constituted in all plants (Torres et al., 1987). Cinnamic acid and its derivative (*p*-coumaric and caffeic acid) were range 0.49 – 3.71, 0.91 – 25.9 and 0.64 – 9.83 mg/L, respectively.

Table 3.5 Phenolic acids concentration (mg/L) of selected wines

Wine Variety*	Phenolic composition of wine (mg/L)								
	Syringic	Cinnamic	*p*-Coumaric	Gentisic	*p*-Hydroxy benzoic	Salicylic	Caffeic	Gallic	Proto catechuic
Red wine									
Shiraz (03)	7.32 ±0.03 a	3.71 ±0.04 a	18.33 ±0.27 b	0.96 ±0.85 a	1.82 ±0.95 ab	0.62 ±0.13 b	8.45 ±0.37 c	15.38 ±1.31 cd	0.89 ±0.21 cde
Shiraz_K1V (04)	6.58 ±0.69 ab	ND	25.90 ±0.51 a	ND	1.12 ±0.15 abc	ND	9.83 ±1.28 b	14.44 ±0.49 cde	2.04 ±0.22 b
Shiraz_EC1 (04)	7.84 ±4.76 a	ND	14.10 ±5.06 c	ND	1.99 ±1.10 a	1.30 ±0.88 a	13.74 ±0.43 a	20.99 ±2.94 b	3.13 ±1.34 a
Muscat (China) (03)	4.97 ±0.05 abc	2.16 ±0.50 b	1.72 ±0.56 de	0.72 ±0.11 a	1.39 ±0.05 abc	ND	5.73 ±0.32 d	15.11 ±0.28 cd	0.54 ±0.05 de
Muscat (China) (04)	2.52 ±2.09 cd	ND	3.10 ±2.20 de	ND	0.77 ±0.11 cd	ND	13.28 ±0.60 a	10.56 ±1.73 e	1.71 ±0.80 bc
Muscat (04)	ND	ND	ND	ND	0.85 ±0.08 bcd	ND	2.16 ±0.01 f	10.30 ±0.15 e	2.10 ±0.02 b
Zinfandel (03)	5.35 ±1.04 abc	1.36 ±0.29 c	3.19 ±0.28 de	ND	0.84 ±0.01 bcd	0.63 ±0.12 b	8.18 ±0.85 c	13.93 ±1.03 cde	0.58 ±0.08 de
Zinfandel (04)	5.17 ±1.68 abc	ND	4.76 ±1.16 d	ND	1.18 ±0.27 abc	0.85 ±0.09 ab	2.78 ±0.13 f	17.48 ±0.56 bc	1.54 ±0.26 bcd
Barbera (03)	6.05 ±0.06 abc	ND	0.91 ±0.03 de	ND	ND	0.57 ±0.10 bc	1.58 ±0.33 fg	25.69 ±5.01 a	0.81 ±0.16 cde
Barbera (04)	5.50 ±2.10 abc	ND	4.57 ±0.13 d	ND	1.28 ±0.32 abc	0.76 ±0.01 ab	2.45 ±0.01 f	20.92 ±1.19 b	2.17 ±0.11 b
Blended (02)	5.47 ±0.14 abc	0.89 ±0.20 d	3.20 ±1.92 de	0.60 ±0.16 a	0.51 ±0.02 cd	ND	ND	11.91 ±1.46 de	0.57 ±0.04 de
White wine									
Italia (03)	6.09 ±1.32 abc	0.49 ±0.04 e	ND	ND	ND	0.60 ±0.01 b	ND	ND	ND
Chasselar Dore (03)	4.27 ±0.48 abc	1.54 ±0.27 c	1.15 ±0.37 de	ND	0.75 ±0.12 cd	0.50 ±0.89 bc	0.64 ±0.42 gh	ND	0.60 ±0.05de
Chenin Blanc (04)	2.64 ±0.28 bcd	ND	1.33 ±0.40 de	ND	0.73 ±0.04 cd	ND	4.28 ±1.17 e	1.25 ±0.33 f	0.94 ±0.13 cde

n = 4

* Muscat = Muscat Hamburg , (02), (03) and (04) means vintage year 2002, 2003, and 2004, respectively, and K1=K1V1116 yeast strain, EC=EC1118 yeast strain

Numbers with different letters within the same column are significantly different (P≤0.05)

Cinnamic acid plays the key role in the biosynthesis pathway of phenolic compound as it is converted to *p*-coumaric acid, which is a substrate for the formation of flavonoid and some of nonflavonoid family (Soleas et al., 1997). In red wines, gallic acid was the highest of the polyphenols presented, ranging 11.91 – 25.69 mg/L, while it was detected in very low amount (1.25 mg/L) or no detection in white wines. The presence of high amounts of gallic acid in red wines would be expected since this phenolic acid is principally formed by hydrolysis of flavonoid gallate esters, which are largely absent in white wine due to the lack of skin extraction (Frankel et al., 1995). This finding was in agreement with the work of Minussi et al. (2003) who reported that gallic acid was the highest polyphenol in red wine.

Sanchez-Moreno et al. (1999) reported free radical scavenging activity of gallic acid, protocatechuic acid and gentisic acid were the highest, epicatechin, catechin, caffeic acid, syringic acid, rutin and quercetin were intermediate and resveratrol was the lowest. Their report was similar to the findings of this experiment. (data not shown). Cinnamic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid and salicylic acid do not act as antiradical scavenger by DPPH method (data not shown).

Moreover, from many biological evaluations of phenolic compounds, *t*-resveratrol is known to be an active molecule against low-density lipoprotein (LDL) oxidation (Belguendouz, Fremont, and Gozzelino, 1998; Belguendouz, Fremont, and Linard, 1997; Frankel, et al., 1993; Fremont, Belguendouz, and Delpal, 1999). Frankel et al. (1993) suggested that *t*-resveratrol of near 2 mg/L would be required to obtain 80% inhibition of LDL oxidation. In addition, Blond, Denis, and Bezard (1995) found the same level of *t*-resveratrol (2 mg/L) would inhibit 80% of lipid peroxidation in liposomes. Furthermore, Bertelli et al. (1995)

and Kallithraka, Arvanitoyannis, EI-Zajouli, and Kefalas (2001) found that *t*-resveratrol at a concentration of 3.56 μg/L was able to inhibit 50.3% platelet aggregation in vivo of human plasma. In addition, the flavonoid groups such as catechin, epicatechin and quercetin have been also reported to inhibited LDL oxidation, lipid peroxidation and platelet aggregation (van de Wiel, van de Golde, and Hart, 2001). These biochemical properties lead into the anti-cardiovascular disease, anti-cancer, anti-inflammation properties of these phenolic compounds (Kris-Etherton et al., 2002; Soleas et al, 1997; van de Wiel et al., 2001)

The intake of flavonoids has been evaluated by many studies. The reported values differ from 11.5 mg/day to 1000 mg/day (Kuhnau, 1976). Comprehensive studies of Hertog, Hollman, and Katan (1992) suggested the content of 0.025 g/day of an average intake of flavonoids. Renaud, and de Lorgeril (1992) recommended that drinking alcoholic beverage in moderation (fewer than two drinks a day, ≈ 200 mL) decrease risk of heart disease and stoke. Therefore, moderate consumption of 200 ml/day of Shiraz wine variety of both vintage years produced by SUT Farm would give *t*-resveratrol in the amount of 0.262 - 0.552 mg/day and flavonoid content of 250 – 530 mg/day. In addition, a constant moderate consumption of red wine provides a significant amount of other phenolic compounds that have beneficial effects and also act as synergistic effects.

3.5 Conclusion

Crushed grape with seeds contained higher amount of TPC and free radical scavenging activity than the crushed grape without seed. Antioxidant activity of red grape juices and wines were significantly higher ($p<0.05$) than those of the white

ones, except for wine produced from Italia grape. There were no significant difference ($p>0.05$) in antioxidant activity of the red grapes and wines. The health promoting *t*-resveratrol was found only in Shiraz and Zinfandel wine varieties. *t*-Resveratrol was also found in the commercially blended wine in similar amount to those of Shiraz wine produced in 2004 and Zinfandel wine produced in the same year. Gallic acid was the main component in red wines, while it was absent in white wines. These investigations provided the information of health benefits of selected grapes and wines produced from the SUT Farm during the vintage years of 2003 and 2004, that red grape juices and wines had a better health benefit for consumption.

3.6 References

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CHAPTER IV

CHANGES OF PHENOLIC COMPOUNDS DURING RED WINE FERMENTATION BY DIFFERENT YEAST STRAINS

4.1 Abstract

The objectives of this study were to investigate changes of total phenolic, *t*-resveratrol, monomeric flavonoid, low molecular weight phenolic acid and color parameters during alcoholic fermentation, malolactic fermentation (MLF) and ageing process using capillary electrophoresis (CE) procedure. The *Vitis vinifera* via Exotic and Shiraz grape grown on SUT Farm were used to produce wines. Grape musts were fermented using different fermentative yeast strains (K1V1116, CY3079 and EC1118) with the addition of pure *t*-resveratrol (concentration of 0, 5 and 10 ppm). The wine samples were undergone MLF with lactic acid bacteria, *Oenococus oeni* strain MBR.B1 for a month and aged at 15°C for six months. During alcoholic fermentation, an increasing in *t*-resveratrol contents of both Exotic and Shiraz wines were observed up to the third day of fermentation and decreasing after the fifth day of fermentation. Regardless of yeast strains, higher amounts of *t*-resveratrol were observed in Exotic than those of Shiraz wines. The additions of pure t-resveratrol to the must prior to fermentation lead to the higher amounts of *t*-resveratrol in the wines during fermentation. However, similar amounts of *t*-resveratrol were observed at day 13 of

alcoholic fermentation in both wines with and without added pure resveratrol. The monomeric flavonoid, epicatechin, catechin and quercetin, and phenolic acid also increased up to the four to sixth day of fermentation and then, decreased thereafter. At the end of alcoholic fermentation, there were no significant differences ($p>0.05$) of phenolic compounds produced by three yeast strains. After alcoholic fermentation, color density (CD) value, total anthocyanin (TA) and total colored anthocyanin (TCA) of both wine varieties also increased for all treatments. The Exotic wine contained higher amount of these color parameters than those of Shiraz. During MLF, *t*-resveratrol was slightly increased for all treatments. Some of the phenolic acids such as gentisic, *p*-hydroxybenzoic, salicylic, caffeic and protocatechuic acid were generated during MLF process. There were slight increases or no change in chemical age (CA) value during MLF for Exotic and Shiraz.

Ageing process caused decreasing of TPC, *t*-resveratrol, monomeric flavonoid as well as some of low molecular weight phenolic acids. CD, TA and TCA significantly decreased ($p<0.05$), whereas CA value significantly increased ($p<0.05$) during ageing.

Key words: total phenolic, *t*-resveratrol, flavonoid, phenolic acid, color, alcoholic fermentation, malolactic fermentation, ageing, capillary electrophoresis, grape, wine

4.2 Introduction

Wines contain a complex of phenolic components which act as antioxidant. The phenolics can be derived from grapes and wood or metabolite products from yeasts (Soleas, Diamandis, and Goldberg, 1997). Phenolics within the skin, seeds and

flesh of black grapes are extracted into red wines during vinification processes. The processes of viticulture and vinification, which vary between countries, regions and wine-makers, contribute to different in the content and profile of phenolic compounds in wine. Vineyard factors such as grape variety, quality, climate, geographical origin and disease affect the phenolic compounds that accumulate in grapes (Siemann and Creasey, 1992; McDonold et al., 1998). During vinification, the length of skin contact, temperature and presence of seeds, stems and enzymes have all been shown to affect the extraction of phenolics into the fermentating juice or must (Ramey, Bertrand, Ough, Singleton, and Sanders, 1986; Mattivi, Reniero, and Korhammer, 1995; Kovac, Alonso, Bourzeix, and Revilla, 1992).

Beside the alcoholic production into the must by yeast, the intrinsic enzyme such as β-glucosidase has the ability to hydrolyze the bond between glucose and the compound on an anomeric carbon. This compound may be an aroma compound (such as a monoterpenol) or phenolic compounds, which relate to health benefit (such as resveratrol) (Gunata, Bayonove, Tapiero, and Cordonnier, 1990). Various commercial yeast strains, had different amounts of β–glucosidase activity, would produce different levels of phenolic components (Echeverry et al., 2005).

Most of red wine undergoes malolactic fermentation (MLF), after alcoholic fermentation is finished, to improve the quality and stability of this wine. During the MLF process, polyphenolic constituents in wine might as well be altered.

During the ageing process, wine undergoes important modifications so that its stability increase and organoleptic characteristics improve. The transference of characteristic wood compounds-tannins and aromatic substances into the wine cause changes the phenolic profiles of wine. Oak chips are mainly used instead of barrels, to

accelerate the ageing process. Del Alamo-Sanza, Dominguez, Carcel, and Gracia (2004) demonstrated the change of low molecular weight phenolic compounds in red wine aged in oak chip. It was interesting to study changes of the effective health beneficial compounds such as *t*-resveratrol, catechin, epicatechin, quercetin and others low molecular weight phenols during ageing process with oak chips.

Therefore, the objectives of this study were to evaluate the effect of yeast strains on phenolic compounds of wine during fermentation and to investigate changes of health benefit phenolic compounds such as *t*-resveratrol during vinification of red grapes grown at SUT Farm.

4.3 Materials and Methods

4.3.1 Sources of grapes and yeasts

Exotic and Shiraz grapes (*V. vinifera*), obtained from Suranaree University of Technology (SUT) vineyards, Nakhon Ratchasima Province, were harvested with sugar content of about 20°Brix in March and April 2004, respectively. The grapes were washed, destemed and stored at –20°C until use.

Three different yeast strains, the two species of *Saccharomyces cerevisiae*, K1V1116 (Lalvin®, The Lallemand Inc., Montreal, Canada) and CY3079 (CP Co., Ltd., Thailand) and a species of *S. bayanus*, EC1118 (Lalvin®, The Lallemand Inc., Montreal, Canada) were used as starter cultures.

4.3.2 Grape juice and skin extraction

Grape juices and skin extracts were prepared with slight modification from the procedure of Zoecklein, Fugelsang, Gump, and Nury (1995). Grape berries and seed

were removed, weighed approximately 25 g, homogenized (Ystral, scientific promotion, Eastleigh, Hampshire, England) and centrifuged (Universal 16R, Hettich, Germany) at 4500 × g for 10 min. The juice was decanted, kept for determination. The grape skin was extracted with 50% ethanol using 10 mL per gram sample. Both grape juice and skin extract were subjected to analyze for total and individual phenolic compounds by spectrometric and capillary electrophoresis (CE) method. The alcohol was removed from grape skin extract by rotary evaporator before CE determination.

4.3.3 Winemaking

4.3.3.1 Praparation of grape juice

The frozen grapes were thawed and crushed then, the pomaces were removed. Total solid content of grape juice was adjusted to 21 - 22 °Brix by adding sugar. The pH of grape juice was adjusted to 3.6-3.7 by adding tartaric acid. Then, the pomace was added back into grape juice by mixing 1.5 kg of grape skin with 3.5 L of grape juice for each treatment. The must was treated with potassium metabisulfite (100 mg/L free SO_2). After sulphitation, the must was left overnight at room temperature.

4.3.3.2 Alcoholic fermentation

The stock culture of three yeast strains were prepared by adding pure culture into a sterile grape juices with sugar content of about 24 °Brix and shaked for 24 hours. The initial inocular cells were 1.42×10^7 and 5.76×10^6 CFU/mL for Exotic and Shiraz must, respectively. For each yeast strain, three different containers of the must were prepared by adding commercial *t*-resveratrol concentration of 0, 5 and 10

ppm just before fermentation took place. The alcoholic fermentation was carried out for 13 day. The grape skins were removed from fermented juice thereafter.

4.3.3.3 Malolactic fermentation (MLF)

The malolactic fermentation was done with lactic acid bacteria, *Oenococcus oeni* strain MBR.B1 (Microenos®, Laffort Cenologie, St-Simon, France). The culture was rehydrated and added into the wines with initial inocular cells was about 1×10^7 CFU/mL. The malolactic fermentation was carried out for a month.

4.3.3.4. Racking and stabilization

After MLF, the wine was ranked, added with SO_2 of 60 ppm, kept in a cold room (4^oC) for precipitation of potassium bitartrate then, ranked again to separate from the precipitate.

4.3.3.5 Ageing of wine

After precipitation, oak chips (Winetech Supply and Consult Co., Ltd., St-Simon, France) were added (0.5 g/L) to the wine before ageing. The wines were undergone to ageing process at 15°C for 6 months.

4.3.4 Sample collection and analyses

Samples of wines during alcoholic and malolactic fermentation and during ageing were collected for analyses. During the first week of alcoholic fermentation, the fermented juice was collected every day for analyses then, every three days in the second week. For malolactic fermentation the fermented juice was collected just

before adding bacterial culture and a month, thereafter the fermentation was terminated. During aging, collection of wine samples was done at 0, 1, 2, 4, and 6 month. The sample was centrifuged in a refrigerated centrifuge (PK121R, Alc international, Italia) at 4°C, 4500 × g for 15 min to remove particulate matter. The supernatant was used to analyze for total soluble solids, pH, titratable acidity, reducing sugar, alcoholic content and phenolic compounds.

4.3.4.1 Total soluble solids

Total solid content of the sample was measured using a wine refractometer (Atago, WM-7, Nova-Tech International, Inc., Texas, USA). The total solid content was expressed as °Brix.

4.3.4.2 pH

pH of the sample was measured using a pH meter (Mettler Toledo, MP220 , Mettler-Toledo International Inc., New York, USA).

4.3.4.3 Titratable acidity

Titratable acidity of the sample was analyzed by titration method with standard 0.1N NaOH, which was standardized by using potassium hydrogenpthalate prior to used. Phenolphthalein was used as indicator.

Five mL of sample was added into 100 mL of boiled distilled water then titrated with 0.1N NaOH until end-point (slight pink color) held at least 15 seconds (AOAC, 1997). The acid content of the sample was calculated as tartaric acid as following:

$$\text{Calculate as g tartaric/100mL wine} = \frac{(V)(N)(75)(100)}{(1000)(v')}$$

where V = volume of 0.1 N NaOH used for titration (mL)

N = normality of NaOH solution

v' = sample volume (mL)

4.3.4.4 Residual sugar

Residual sugar was analyzed by DNS method. This method was used for observing the sugar consumption and fermentation finishing time. Remained sugar less than 2 g/L defined that fermentation was finished. The main sugar in wine is glucose and fructose, which are reducing sugar. This method tests for the presence of free carbonyl group of reducing sugar.

Dinitrosalicylic reagent was prepared by dissolving 2.5 g of 3,5-dinitrosalicyilc acid and 75 g of sodium potassium tartrate in 250 mL ditilled water, then 4 g of sodium hydroxide was added. The reagent was kept in dark bottle and had shelf life for 15 days in refrigerator (4°C). Sample (wine) 1 mL was reacted with 1 mL dinitrosalicylic reagent, then mixed and boiled at 100°C for 5 min. The reaction was rapidly cooled to room temperature and 10 mL of water was added to each tube. The reaction was measured at absorbance of 540 nm. The solutions of pure glucose of 0, 0.5, 1.0, 1.5, and 2.0 g/L were used for calibration.

4.3.4.5 Alcohol content

The alcohol content was determined by dichromate oxidation method (AOAC., 1997). Kjeldahl apparatus was used for alcoholic distillation. The sample of

1 mL was pipetted into a distillation tube. A 50 mL Erlenmeyer flask containing 25 mL of potassium dichromate solution was placed under the condenser with the tip emerged in the solution to collect the alcohol. After distillation, the potassium dichromate solution was titrated with ferrous ammonium sulfate solution (FAS) and 1, 10–phenanthroline ferrous sulfate was used as an indicator. The percentage of alcohol content was calculated as following:

$$\% \text{ Alcohol by volume} = 25 - (25 \times V/V')$$

where : V = volume of FAS used for wine sample

V' = volume of FAS used for water blank

4.3.5 Spectrophotometric determination

4.3.5.1 Color measurement

Absorbance measurement of the sample was done using UV/VIS 916 spectrophotometer equipped with a Peltier thermocell (GBC, Dandenong, Australia) with 10 mm path length quartz cuvette. The determination was modified from the method of Zoecklein et al. (1995). Sample was diluted with deionized water pH ≈ 3.5 (pH equivalent to wine sample) before measuring the absorbance at 420 (minimum absorbance) and 520 nm (maximum absorbance) against prepared blank (saturated solution of potassium sodium tartrate with 11% ethanol (wine) or 20% glucose (juice). The color density and wine tint were expressed as following:

$$\text{Color density (CD)} = A420 + A520$$

$$\text{Wine tint} = A420/A520$$

To determine total anthocyanins, total colored (ionized) anthocyanins and chemical age (CA) of the sample, the sample aliquot of 100 μL was added with

9 mL of 0.1 N HCl, mixed and left of 4-5 hr then, the absorbance was measured at 520 nm (A_{520}^{HCL}). Another sample aliquot of 1 mL was added with 20 μL of 20 % (w/v) sodium metabisulfite, mixed and left for 1 min then, the absorbance was measured at 520 nm (A_{520}^{SO2}) (Zoecklein et al, 1995). Total anthocyanins, total colored anthocyanins and chemical age were calculated as following:

Total anthocyanins (TA) (mg/L) = $20 [A_{520}^{HCL} - (5/3) A_{520}^{SO2}]$

Total colored (ionized) anthocyanins (TCA) (mg/L) = $20 [A_{520} – A_{520}^{SO2}]$

Chemical age (CA) = $A_{520}^{SO2} \times 100 / A_{520}^{HCL}$

4.3.5.2 Total phenolic content (TPC)

The total phenolic contents of the grapes and wines were determined by Folin-Ciocault method (Matthaus, 2002). Sample solution (0.1mL) was introduced into a test tube and then 2mL of 2% sodium carbonate was added. After incubation for 2 min, 0.1mL of Folin-ciocalteu's reagent (Folin:Methanol, 1:1) was added. The absorbance was measured at 750 nm after incubation for 30 min. The total phenolic content of the sample was expressed as mg of gallic acid equivalent (GAE) per liter of sample.

4.3.6 Capillary electrophoresis (CE) analysis of phenolic compounds

4.3.6.1 Sample preparation

The extraction method was slightly modified from the method of Minussi et al. (2003). For the liquid/liquid extraction, 1 mL of wine sample was twice extracted with 1 mL of diethyl ether. The organic phase was pooled and completely dried in the dark under nitrogen gas and resuspended with 1 mL of 50% ethanol, then

filtered through 0.45 polyethersulfon membrane (Supor@Acrodisc®, Pall Life Sciences, Gelman Sciences Inc, USA).

4.3.6.2 Capillary electropheresis procedure

Capillary electrophoresis analyses were performed using an Agilent Technologies model G1600AX (Agilent Technologies Deutschland GmbH, Waldbronn, Germany), equipped with a diode-array detector. An uncoated fused silica capillary tube of 50 μm i.d. (Agilent) with effective and total lengths of 50 and 75 cm, respectively was used. Electrophoretic analyses were performed at an applied voltage of 15 kV at 25°C.

Electrophoretic buffer was a mixture of phosphate 25 mmol/L and borate 10 mmol/L, at pH 8.5. This buffer was obtained by mixing solution of H_3BO_3 (100 mmol/L) and Na_2HPO_4 (100 mmol/L) using 2 N NaOH to adjust the pH to desired pH value.

The column was pre-rinsed with deionized water for 2 min and then, running buffer for 3 min. Sample was hydrodynamically injected for 7 s. Between analyzes, the column was flushed with 0.1 M sodium hydroxide for 1 min, deionized water for 2 min and running buffer for 3 min. All CE analyses were performed in duplicate. Before being used, all solutions were filtered through a 0.45 μm polyethersulfon membrane.

Calibration curve of each phenolic compound was established using pure chemical with concentrations ranging from 2-10 mg/L plotted against peak areas. Each phenolic concentration was calculated by chemstation Rev.A.09.01 (1206) (Agilent Technologies 1990-2001). For each standard compound, four replicate samples were used for analyses.

4.3.7 Statistical analysis

All experiments were performed in duplicate. Each replicate was chemically analyzed in duplicate samples. Statistical analysis was evaluated in completely Randomized design (CRD) with Statistical Analysis (SAS Institute, Inc., 1995) and means comparison by Duncan's Multiple Range Tests (DMRT) were analyzed.

4.4 Results and Discussion

4.4.1 Phenolic compound of grapes

Total phenolic content (TPC), total anthocyanin and individual phenolic composition of the two grape varieties are shown in Table 4.1. The skin extraction of Exotic and Shiraz grapes (10.31 and 6.82 g/L, respectively) had higher amount of TPC than their juices (1.22 and 0.98 g/L, respectively). The Exotic grapes showed higher TPC than Shiraz grapes in the juices and skin extract. Of both Exotic and Shiraz grapes, anthocyanin was the majority polyphenol found in the juices (23.27 and 17.75 mg/L, respectively) and grape skins (1391.36 and 839.17 mg/L, respectively). The colored anthocyanins were detected in similar amount in the juices (12.94 mg/L for Exotic and 14.13 mg/L for Shiraz wines) and skin extract (125.00 mg/L for Exotic and 110.60 mg/L for Shiraz wines). *t*-Resveratrol was only detected in the skins extract (1.15 mg/L for Exotic and 0.24 mg/L for Shiraz grape), but not detected in the juices. This finding was similar to the result of Jeandet, Bessis, and Gautheron (1991) who reported that *t*-resveratrol was found only in grape skin.

The monomeric form of flavan-3-ols such as epicatechin and catechin of two grape varieties were detected in low amounts (not detected – 2.00 mg/L for epicatechin and 2.11 – 4.70 mg/L for catechin). Shiraz variety showed slightly higher

amount of these compounds, especially for catechin content, it was twice higher than Exotic. The flavan-3-ols may naturally exist in aggregated forms of epicatechin gallate, procyanidin and proanthocyanidin (Zoecklein et al., 1995).

Table 4.1: Phenolic composition of juice and skin in grape berries

Phenolics	Exotic variety		Shiraz variety	
	juice	skin extract	juice	skin extract
Total phenols[a]	1.22± 0.03	10.31 ± 0.56	0.98 ± 0.06	6.82 ± 0.13
Total anthocyanin[b]	23.27 ± 9.77	1391.36 ± 162.21	17.75 ± 6.12	839.17 ± 255.47
Total colored anthocyanin[b]	12.94 ± 3.99	125.00 ± 17.40	14.13 ± 1.23	110.60 ± 24.72
t-Resveratrol[b]	ND	1.15 ± 0.74	ND	0.24 ± 0.05
Epicatechin[b]	ND	1.15 ± 0.30	2.00 ± 0.33	1.69 ± 1.09
Catechin[b]	2.11 ± 0.31	2.74 ± 0.37	3.14 ± 0.66	4.70 ± 3.69
Quercetin[b]	1.39 ± 0.01	1.30 ± 0.00	1.40 ± 0.02	0.95 ± 0.00
Syringic acid[b]	2.15 ± 0.56	2.81 ± 1.06	4.05 ± 1.32	4.67 ± 3.33
Gentisic acid[b]	ND	ND	ND	ND
p-hydroxybenzoic[b]	ND	ND	ND	ND
Salicylic acid[b]	ND	1.64 ± 0.51	ND	ND
Gallic acid[b]	ND	ND	ND	ND
Protocatechuic[b]	ND	ND	ND	ND
Cinnamic acid[b]	0.56 ± 0.08	0.54 ± 0.11	0.47 ± 0.00	ND
p-Coumaric acid[b]	2.00 ± 0.68	0.98 ± 0.20	1.63 ± 0.06	ND
Caffeic acid[b]	ND	ND	ND	ND

n = 4
a = expressed as g/L (for juice) and g/kg grape skin (for skin extract)
b = expressed as mg/L (for juice) and mg/kg grape skin (for skin extract)

Quercetin was detected in both juices and skin extracts ranging 1.30 - 1.39 mg/L for Exotic and 0.95 – 1.40 mg/L for Shiraz grape. This result was in agreement

with Burns et al. (2001) who reported that the free quercetin content of Cabernet sauvignon and Merlot grape were detected in low amount (2.9 and 9.6 nmol/g of grape, respectively). They also reported that most of the quercetin in grape berries exist in conjugated quercetin form (93.76 – 94.04 % of total quercetin).

The hydroxybenzoic acid group; syringic acid was found in both Exotic (2.15 mg/L for juices and 2.81 mg/L for skins extract) and Shiraz grape (4.05 mg/L for juices and 4.67 mg/L for skins extract). Shiraz variety also contained syringic acid content twice as much of the Exotic. Salicylic acid was only found in the skins extract of Exotic grape. In addition, gentisic, *p*-hydroxybenzoic, gallic and protocatechuic acid were not observed in neither the juices nor skins extract of both varieties.

The hydroxycinnamic acid group; cinnamic and *p*-coumaric acid were detected in the juice and skin extract of Exotic grape in the average of 0.55 and 0.99 mg/L, respectively, whereas they were found only in the juice of Shiraz grape (0.47 and 1.63 mg/L, respectively). Caffeic acid was not detected in both juices and skin extracts of both varieties. These were also in agreement with Burns et al. (2001) who reported the free *p*-coumaric and caffeic acid were not detected in grape berries.

4.4.2 Alcoholic fermentation

After alcoholic fermentation, pH, total soluble solid and reducing sugar of both wine varieties decreased, whereas total acidity increased as shown in Table 4.2. There were no differences in these chemical properties among wines produced by the three yeast strains. The total soluble solids decreased from 21.17 to 7.37 °Brix for Exotic and 21.28 to 7.95 °Brix for Shiraz wine. Moreover, reducing sugars decreased from 174.78 to 3.08 g/L for Exotic and 185.99 to 3.57 g/L for Shiraz wine. There

were due to during fermentation, yeast utilized sugars and other constituents of the grape juice as substrates for their growth, converting to ethanol, carbon dioxide and other metabolic end products (Fleet and Heard, 1993). According to the acids or metabolized products such as succinate, malate and lactate produced by yeasts during fermentation of the grape musts (Radler, 1993), the increasing in total acidity (4.00 to 6.11 for Exotic and 5.05 to 6.16 for Shiraz) and decreasing in pH value (3.68 to 3.58 for Exotic and 3.68 to 3.57 for Shiraz) were observed in these experiments.

Table 4.2 Chemical properties of Exotic and Shiraz juice and wine before (day 0) and after (day 13) alcoholic fermentation by different yeast strains

Chemical property	Day 0	Yeast strain		
		Day 13		
		K1V1116	EC1118	CY3079
Exotic variety				
Total soluble solid (°Brix)	21.17 ± 0.08 a	7.43 ± 0.16 b	7.45 ± 0.05 b	7.23 ± 0.05 b
Reducing sugar (g/L)	174.78 ± 25.60 a	3.22 ± 0.22 b	3.08 ± 0.16 b	2.96 ± 0.12 b
pH	3.68 ± 0.04 a	3.58 ± 0.01 b	3.59 ± 0.02 b	3.57 ± 0.01 b
Total acidity (g/100mL)	0.40 ± 0.01 b	0.62 ± 0.02 a	0.61 ± 0.01 a	0.61 ± 0.01 a
Shiraz variety				
Total soluble solid (°Brix)	21.28 ± 0.12 a	7.95 ± 0.22 b	7.98 ± 0.22 b	7.92 ± 0.04 b
Reducing sugar (g/L)	185.99 ± 28.55 a	3.84 ± 0.61 b	3.57 ± 0.35 b	3.29 ± 0.13 b
pH	3.68 ± 0.02 a	3.57 ± 0.01 b	3.58 ± 0.01 b	3.56 ± 0.01 b
Total acidity (g/100mL)	0.50 ± 0.03 b	0.62 ± 0.04 a	0.61 ± 0.03 a	0.63 ± 0.01 a

Numbers with different letters within the same row are significantly different ($p \leq 0.05$)

4.4.2.1 Changes of alcoholic content

During alcoholic fermentation, the alcohol rapidly increased up to the second day of fermentation and remained constant in both of wine varieties (Fig. 4.1). All yeast strains generated the same amount of alcohol. The maximum content was

about 11% at day 4 of fermentation of both wines. The starting points of alcohol content of these two wine varieties were different (average of 2.40 % for Exotic and 7.12 % for Shiraz, data not shown). It was due to the chemical and physical compositions of the juices affect the rate of fermentation. The important variables that are likely to affecting yeast growth are sugar concentration, supply of nitrogenous substrates, presence of adequate vitamins, concentration of dissolved oxygen and concentration of insoluble solids (Fleet and Heard, 1993).

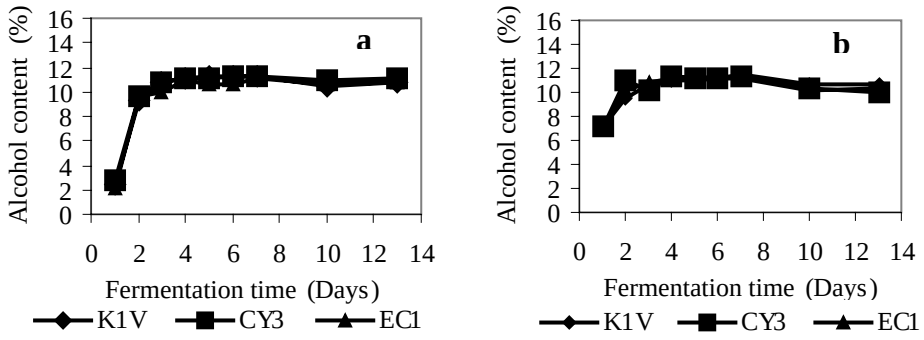

Figure 4.1 Alcoholic content of Exotic (a) and Shiraz (b) wine during alcoholic fermentation

4.4.2.2 Changes of *t*-resveratrol concentration

In vinification without commercial *t*-resveratrol, the amount of *t*-resveratrol showed no significant different ($p>0.05$) in both varieties (Fig 4.2 a and d). In Exotic wine, the maximum amount of resveratrol was observed at the third day of fermentation, ranged 1.26 - 1.84 mg/L for all three yeast strains. However, in Shiraz wine the highest amount was detected in the range of 0.92 - 1.68 mg/L on the fifth and sixth day of fermentation. The amount of *t*-resveratrol of Exotic wine was slightly higher than that of Shiraz wine due to higher resveratrol contents of Exotic grape skins (1.15 mg/L for Exotic as compared with 0.24 mg/L for Shiraz grape) (Table 4.1).

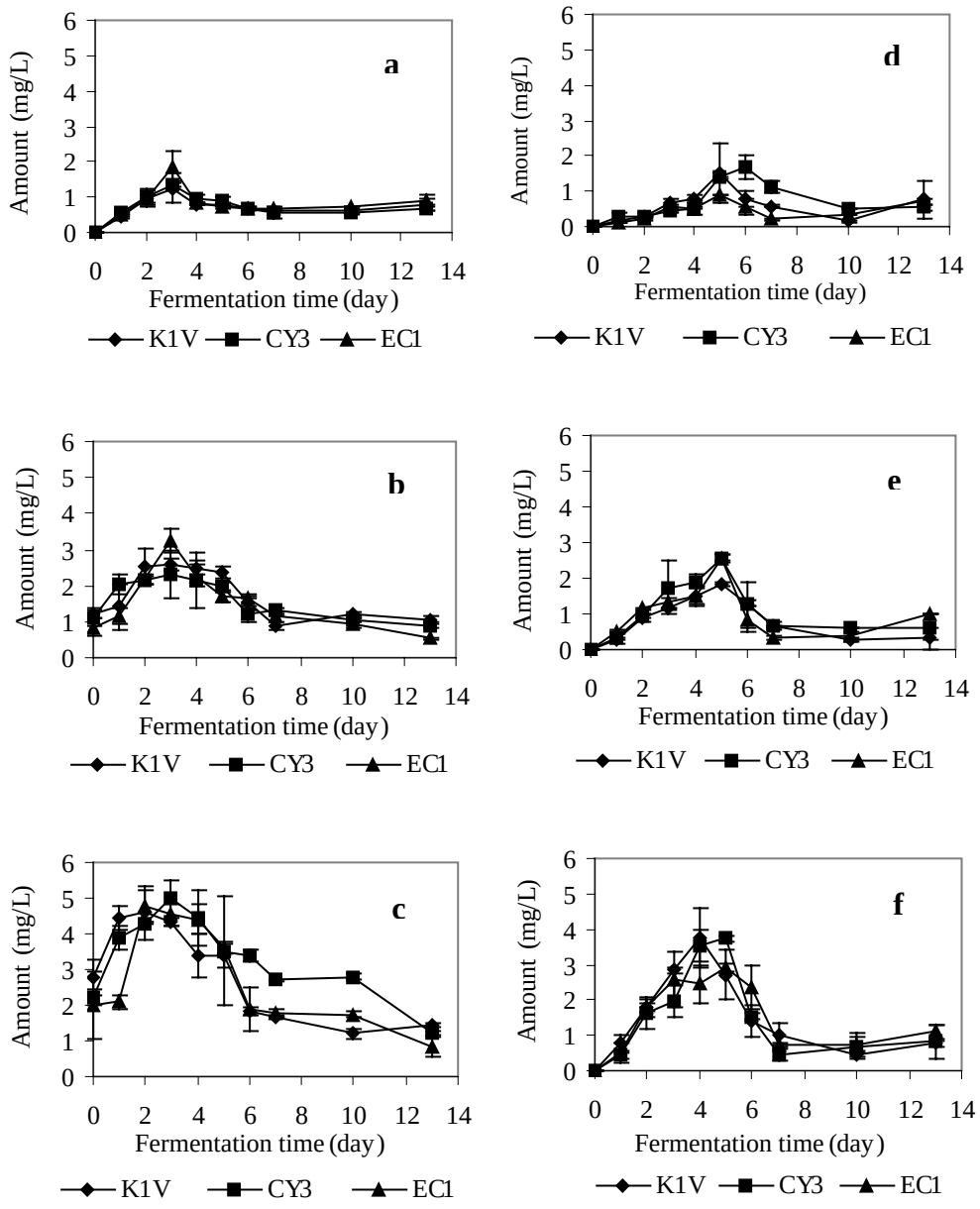

Figure 4.2 Changes of *t*-resveratrol during alcoholic fermentation of Exotic wine: a = Resv 0 ppm, b = Resv 5 ppm and c = Resv 10 ppm and Shiraz wine: d = Resv 0 ppm, e = Resv 5 ppm and f = Resv 10 ppm

Fermentation of Exotic must with addition of commercial *t*-resveratrol, the maximum amounts of *t*-resveratrol were found at the third day of fermentation in concentration of 2.59, 2.31 and 3.26 mg/L for the wine with 5 ppm resveratrol, by

K1V1116, CY3079 and EC1118, respectively and 4.58, 5.00 and 4.78 mg/L for the wine with 10 ppm resveratrol, by K1V1116, CY3079 and EC1118, respectively (Fig 4.2 b, c).

However, in Shiraz wine (Fig 4.2 e, f), the maximum amounts of *t*-resveratrol were detected on the fifth day of fermentation at 1.84, 2.56 and 2.58 mg/L for the wine with 5 ppm resveratrol, by K1V1116, CY3079 and EC1118, respectively and 3.77, 3.73 and 2.92 mg/L for the wine with 10 ppm resveratrol, by K1V1116, CY3079 and EC1118, respectively. There were no significant differences ($p>0.05$) in *t*-resveratrol concentrations due to the three yeast strains. The addition of commercial *t*-resveratrol into grape musts lead to increase in the amount of free resveratrol found in wine, but the observed levels were less than the added levels. This could be due to the aggregation of this compound to form dimeric or polymeric molecules or the adsorption onto the grape skins.

The high concentrations of *t*-resveratrol were found, when alcohol content of the wine reached to 9.38 – 11.26 % during day 2 – 6 of fermentation (Fig 4.1). The increase of *t*-resveratrol was due to either the alcohol produce by yeast, which enhanced the extraction of phenolic compound into wine (Lamuela-Raventos et al., 1997), or the β–glucosidase of yeast, which brokedown the picied molecule (resveratrol glucoside) to aglycon molecule (free resveratrol) (La Torre et al., 2004). After day 6 of fermentation where the activity of yeast reduced, the amount of resveratrol decreased. This result was similar to those of Burn et al. (2001), and Lamuela-Raventos et al. (1997) whose found that the total amount of resveratrol reached its maximum concentration on the sixth and eighth day of fermentation, respectively, and the level decreased thereafter. It would appear that once the maximum amount had been extracted from the skins, these compounds would be

adsorbed by yeast lees and / or further oxidized (Lamuela-Raventos et al., 1997). It was very interesting that maceration of the wine up to day 13 of fermentation (finished alcoholic fermentation), the *t*-resveratrol were closely similar amount in all treatments of three yeast strains used ranging 0.53 – 1.46 mg/L for Exotic and 0.31 – 1.11 mg/L for Shiraz wine.

4.4.2.3 Changes of TPC and others phenolic compound

4.4.2.3.1 Total phenolic content

Total phenolic content (TPC) of Exotic and Shiraz wines increased up to the third day of fermentation and remained constant or slightly decreased for all yeast strains (Fig 4.3). In general, for Exotic wine (Fig 4.3 a), the highest amount of TPC was obtained on 3 day of fermentation at concentration of 3232, 3318 and 3336 mg/L for wine produced by yeast K1V1116, CY3079 and EC1118 strain, respectively. For Shiraz wine (Fig 4.3 d), the maximum TPC content was also observed on 3 day of fermentation at 2553, 2822 and 2569 mg/L for wine produced by yeast K1V1116, CY3079 and EC1118 strain, respectively. TPC values showed high levels at day 3 - 5 because the alcohol produced by fermentation reached the highest amount during these days. The TPC contents of Exotic variety were significantly higher than those of Shiraz variety. This could be due to the Exotic variety contain higher amounts of anthocyanin both in juices and skin extract (1391.36 mg/L for Exotic and 839.17mg/L for Shiraz) (Table 4.1). At the end of alcoholic fermentation, there were no significant differences ($p>0.05$) in TPC of Exotic wines due to yeast strains. In addition, it appeared that addition of commercial *t*-resveratrol in fermentation process did not affect the amounts of TPC of the Exotic

wines. For Shiraz wine without added *t*-resveratrol, there were no significant differences ($p>0.05$) in TPC content due to yeast strains, whereas the wine with 5 ppm resveratrol, the wine fermented by yeast EC1118 strain had a significantly lower ($p<0.05$) than those fermented by K1V1116 and CY3079 strains.

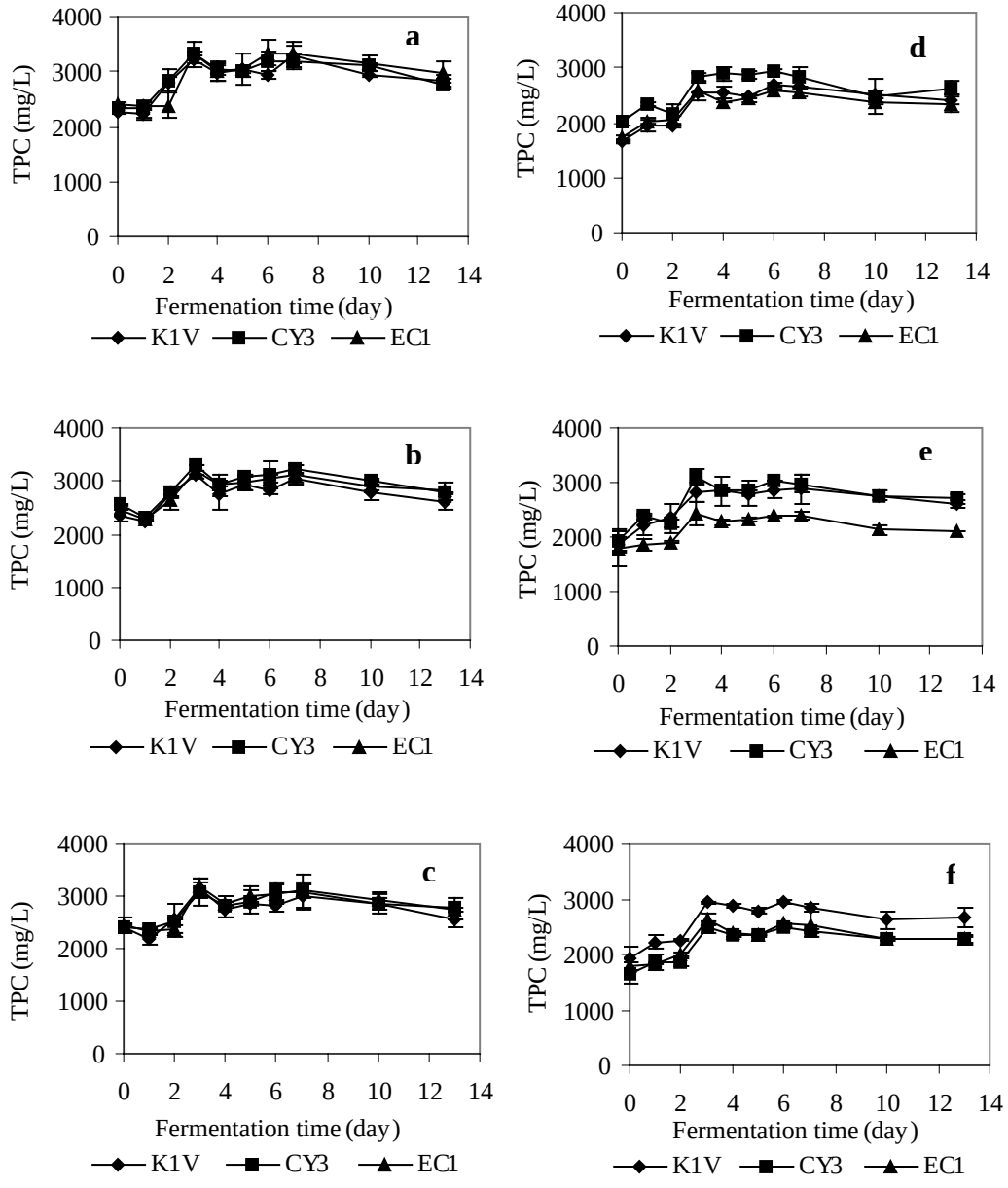

Figure 4.3 Changes of TPC during alcoholic fermentation of Exotic wine : a = Resv 0 ppm, b = Resv 5 ppm and c = Resv 10 ppm and Shiraz wine: d = Resv 0 ppm, e = Resv 5 ppm and f = Resv 10 ppm

4.4.2.3.2 Flavan-3-ol

In wine without added *t*-resveratrol, the amounts of epicatechin increased up to the fifth day of fermentation from the average of 0.78 to 11.27 mg/L for Exotic wine and 5.43 to 29.30 mg/L for Shiraz wine (Fig 4.4 a, d). Catechin content also increased up to 5 days of fermentation from 1.15 to 18.16 mg/L for Exotic and 4.30 to 44.27 mg/L for Shiraz wine (Fig 4.5 a, d). For Exotic wine, the maximum amount of epicatechin and catechin were observed from yeast CY3079 strain at concentration of 13.57 and 20.68 mg/L, respectively on the fifth day of fermentation (Fig 4.4 a and Fig 4.5 a). For Shiraz wine, the high concentrations of these compounds were also observed on day 4 – 5 of fermentation at 32.02 mg/L from CY3079 yeast strain for epicatechin and 50.84 mg/L from K1V1116 yeast strain for catechin (Fig 4.4 d and Fig 4.5 d). It was obvious that the contents of epicatechin and catechin in the wine produced by K1V1116 and CY3079 yeast strain were higher than those produced by EC1118 yeast strain. Sripunya (2005) reported that the former two yeast strains had higher β-D-glucosidase activity (about 90 and 82 U/mg, respectively) than the EC1118 yeast strain (about 58 U/mg). In addition, flavonoid may exist in free form or esterified to nonflavonoid or sugars (Zoecklein et al., 1995). β-Glucosidase enzyme of yeast might breakdown the glycoside molecule to from the free form of epicatechin and catechin. Therefore, due to the higher β-glucosidase activity, the K1V1116 and CY3079 would definitely produce higher amounts of these two compounds.

After day 6 of fermentation, the decreasing of epicatechin and catechin concentration was observed. These results were in agreement with the work of Burn et al. (2001) who observed the flavan-3-ols increased up to day 7 of fermentation and

slightly decreased thereafter. It could be due to the copigmentation of flavan-3-ol derivatives with anthocyanins in wine sediment or due to the adsorption by yeast cell wall (Escribano-Bailon, Dangles, and Brouillard, 1996; Razmkhab et al., 2002).

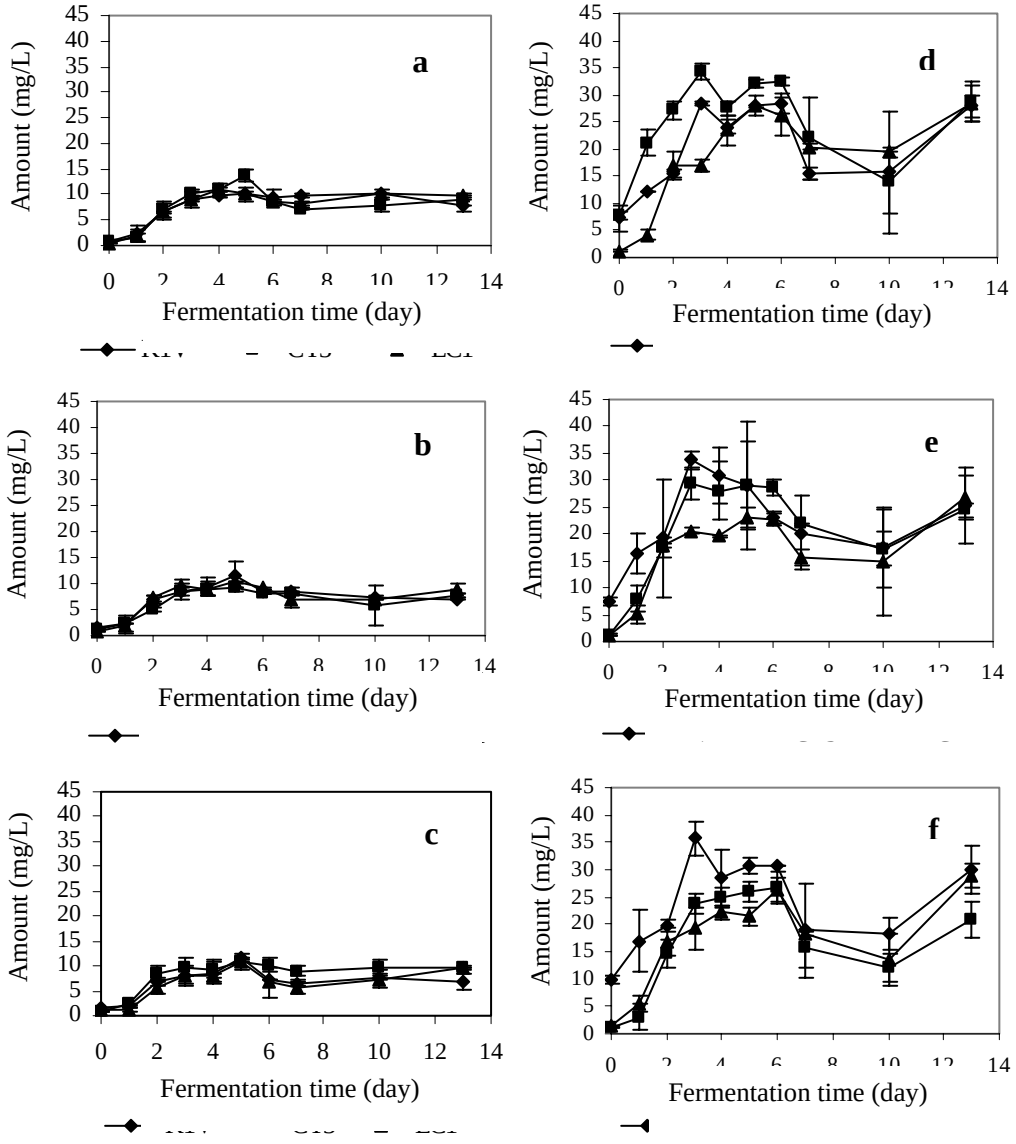

Figure 4.4 Changes of epicatechin during alcoholic fermentation of Exotic wine: a = Resv 0 ppm, b = Resv 5 ppm and c = Resv 10 ppm and Shiraz wine: d = Resv 0 ppm, e = Resv 5 ppm and f = Resv 10 ppm

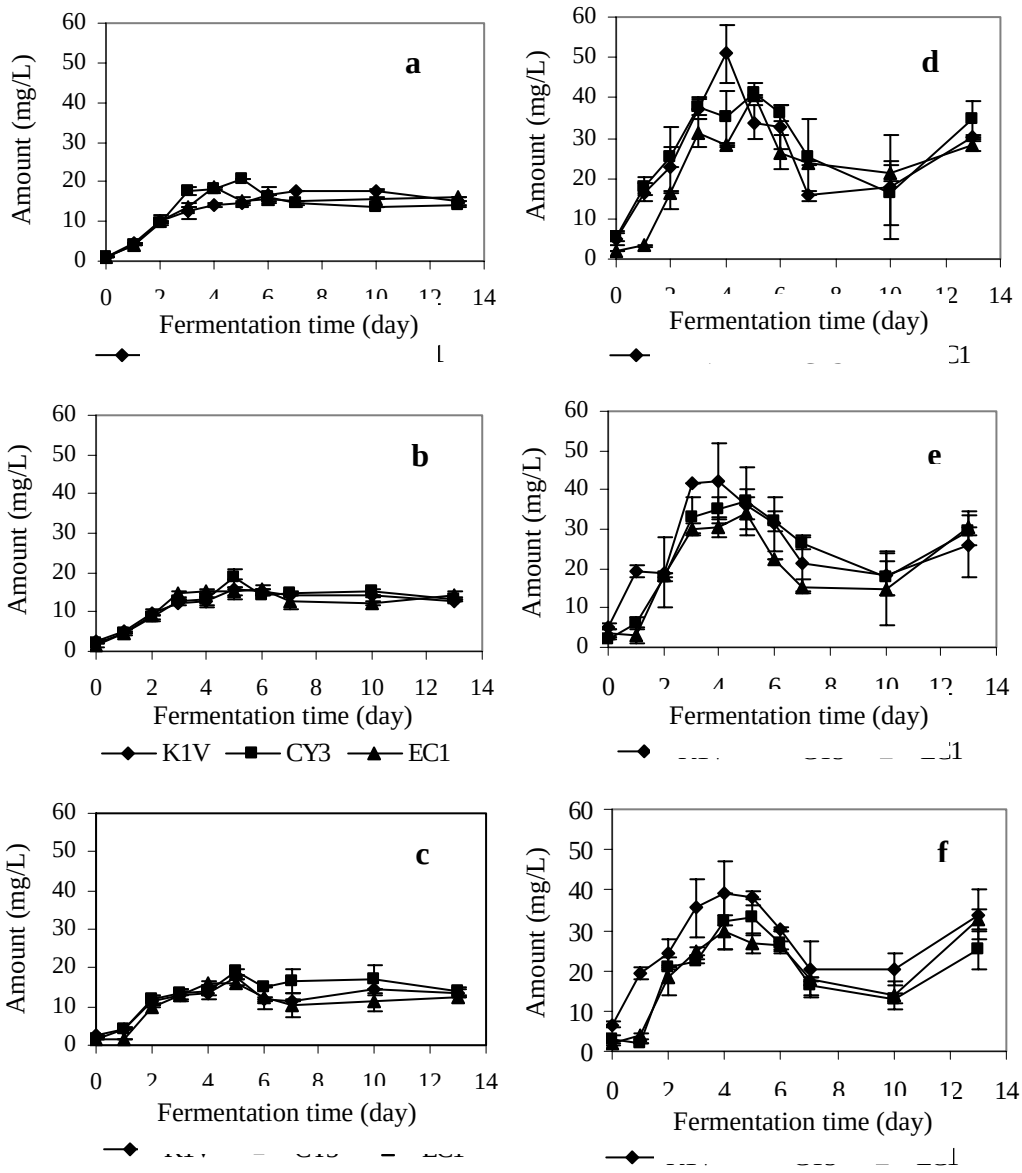

Figure 4.5 Changes of catechin during alcoholic fermentation of Exotic wine: a = Resv 0 ppm, b = Resv 5 ppm and c = Resv 10 ppm and Shiraz wine: d = Resv 0 ppm, e = Resv 5 ppm and f = Resv 10 ppm

At the end of alcoholic fermentation, epicatechin and catechin contents of Exotic wine were significantly lower ($p<0.05$) than those of Shiraz wine. Catechin contents in wines were higher than epicatechin in all treatments that were similar to the results reported by Burn et al. (2002) who observed that catechin in red wine was 2-fold higher than epicatechin. Yeast strains had no effect on epicatechin and catechin

content ($p>0.05$) in Exotic and Shiraz wine. In addition, it appeared that addition of commercial *t*-resveratrol in fermentation process did not affect the amount of epicatechin and catechin of both wines (Fig 4.4 b, c, e, f and Fig 4.5 b, c, e, f).

4.4.2.3.3 Flavonol

For wine without added *t*-resveratrol, quercetin content was undetected in the Exotic and Shiraz musts (day 0 of fermentation) as shown in Fig. 4.6. Quercetin has a hydrophobic favorable, it is normally not found in the grape juices. Therefore, its concentration increased when the alcohol was generated in the solution during fermentation. The maximum content was observed at the fifth day of fermentation both in Exotic and Shiraz wine (average of 3.16 and 2.74 mg/L, respectively) (Fig 4.6 a, d). In Exotic wine, strains of yeasts in fermentation gave no significant differences ($p>0.05$) in the amounts of quercetin, whereas the Shiraz wine produced from CY3079 (2.91 mg/L) was significantly higher than the other two yeasts (2.65 and 2.66 mg/L, for K1V1116 and EC-1118 yeast strain, respectively). The amounts of quercetin reached the maximum amount in day 5 and slightly decreased thereafter. These results were similar to those reported by Burn et al. (2001), the maximum free quercetin was found in day 6 of fermentation and remained relatively steady until day 9 of fermentation. The quercetin content ranged 1.04 – 1.93 mg/L for Exotic and 1.51 – 2.65 mg/L at the end of alcoholic fermentation. In addition, it appeared that addition of commercial *t*-resveratrol in fermentation process did not affect the amount of quercetin of both wines (Fig 4.6 b, c, e, f).

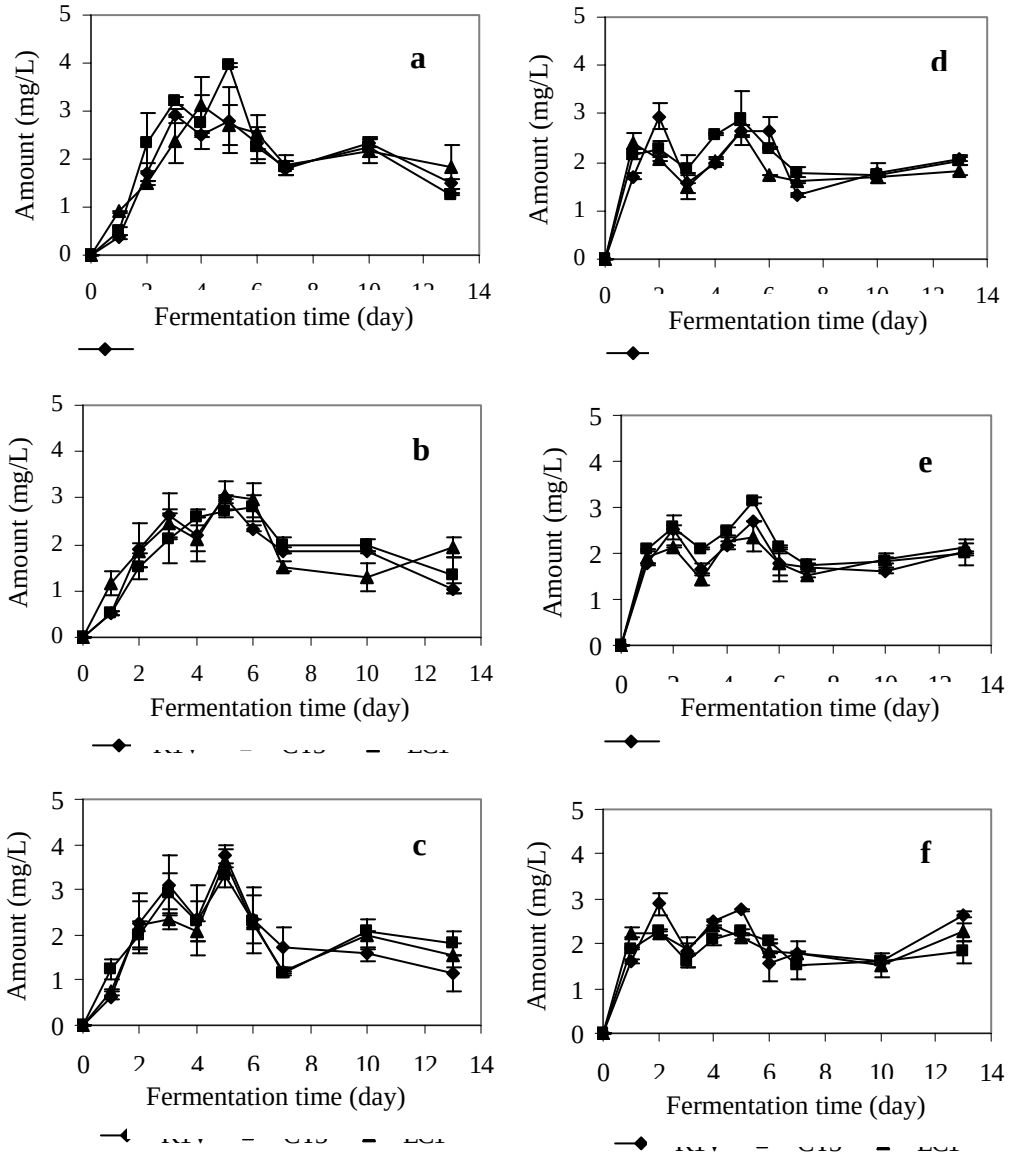

Figure 4.6 Changes of quercetin during alcoholic fermentation of Exotic wine: a = Resv 0 ppm, b = Resv 5 ppm and c = Resv 10 ppm and Shiraz wine: d = Resv 0 ppm, e = Resv 5 ppm and f = Resv 10 ppm

4.4.2.3.4 Hydroxybenzoic acid

For the wine without added *t*-resveratrol, Exotic wine had significantly lower amount ($p<0.05$) of syringic and gallic acid than Shiraz wine (Fig. 4.7 a, d and Fig 4.8 a, d). Yeast strains had no effect on syringic acid concentration in both wines (Fig 4.7 a and d).

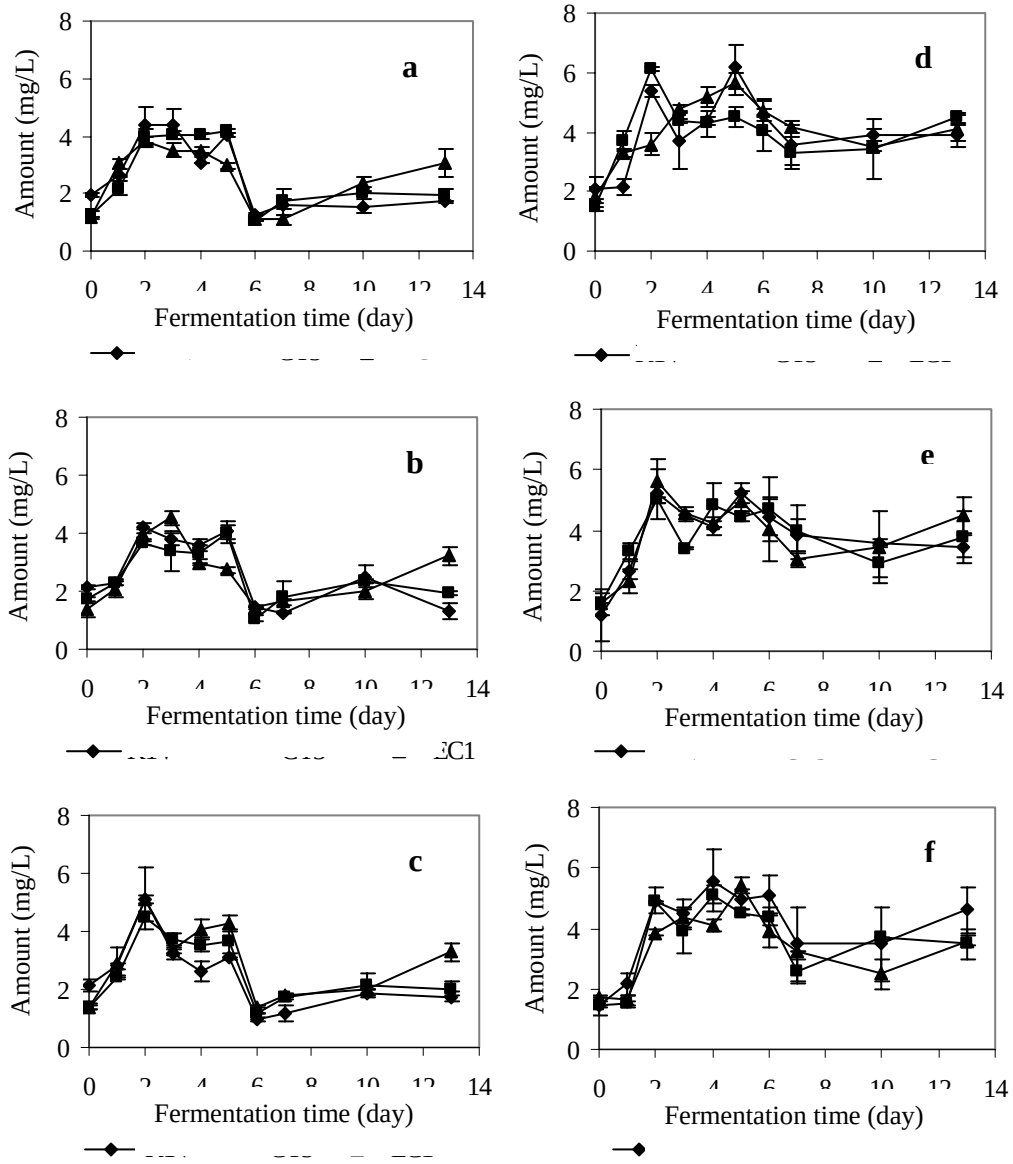

Figure 4.7 Changes of syringic acid during alcoholic fermentation of Exotic wine: a = Resv 0 ppm, b = Resv 5 ppm and c = Resv 10 ppm and Shiraz wine: d = Resv 0 ppm ,e = Resv 5 ppm and f = Resv 10 ppm

For Exotic wine, syringic acid was increased up to day 2 of fermentation from average of 1.61 to 4.29 mg/L. At the end of alcoholic fermentation, syringic acid in Exotic wine produced from yeast EC1118 strain (3.05 mg/L) had higher than those produced by K1V1116 and CY3079 yeast strain (1.71 and 1.96 mg/L, respectively). For Shiraz wine, syringic acid also increased up to day 2 of fermentation from

average of 1.59 to 4.62 mg/L. There were no significant differences in syringic acid among three yeast strains (3.87 – 4.48 mg/L).

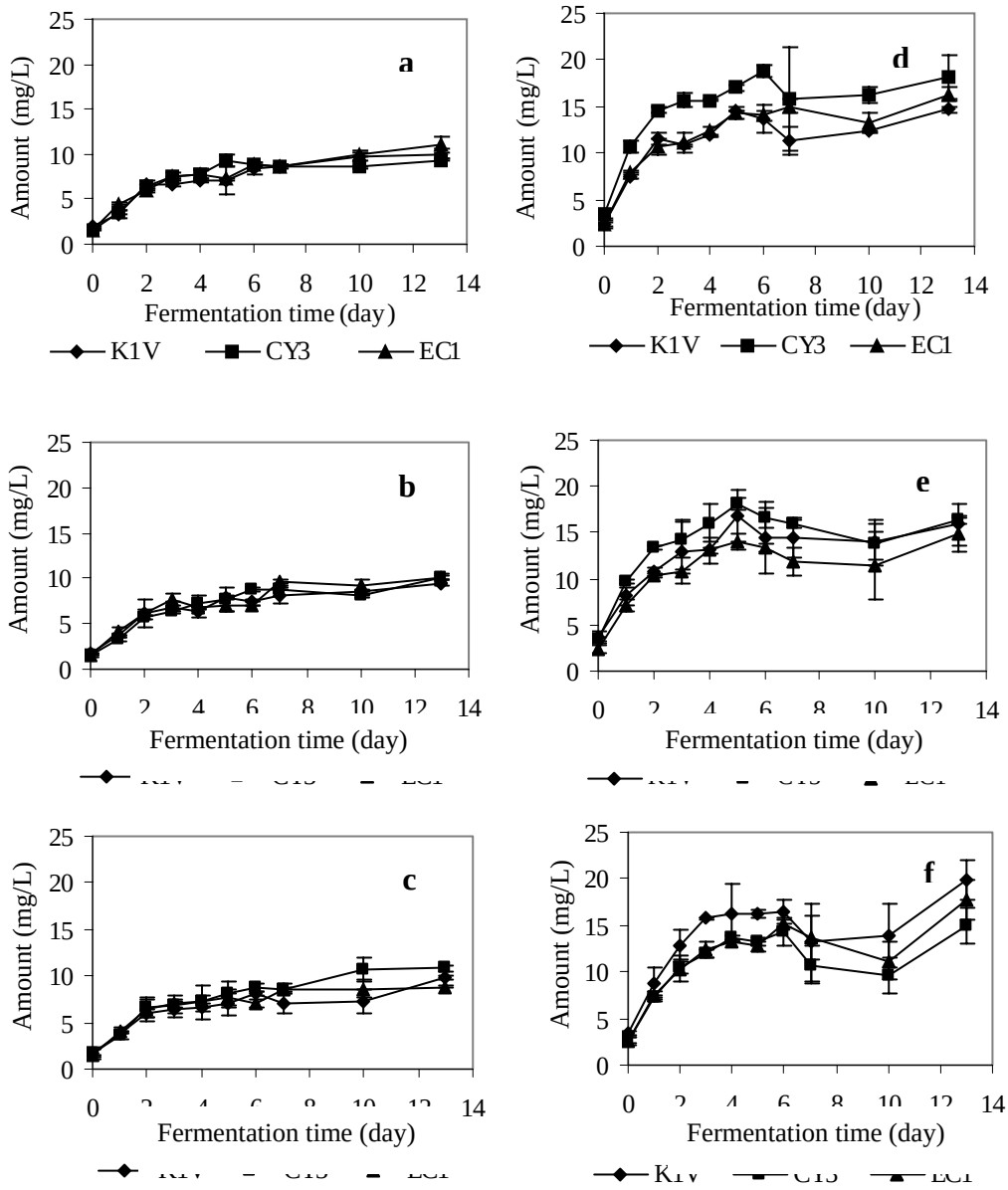

Figure 4.8 Changes of gallic acid during alcoholic fermentation of Exotic wine: a = Resv 0 ppm, b = Resv 5 ppm and c = Resv 10 ppm and Shiraz wine: d = Resv 0 ppm, e = Resv 5 ppm and f = Resv 10 ppm

Gallic acid content drastically increased up to day 2 of fermentation and slightly increased until the end of fermentation from the average of 1.74 to 10.14

mg/L for Exotic wine (Fig 4.8 a) and 2.76 to 16.40 mg/L for Shiraz wine (Fig. 4.8 d). It was obvious that the Exotic wine contained lower amounts of gallic acid than did Shiraz at all time during fermentation. The amount of gallic acid produced from three yeast strains were no significant differences ($p>0.05$) in both wines. The presence of gallic acid would be expected since this phenolic acid is principally formed by hydrolysis of flavonoid gallate esters during maceration of grape skin (Frankel et al., 1995). Moreover, the addition of commercial *t*-resveratrol in fermentation process did not affect the amount of syringic and gallic acid of both wines (Fig 4.7 b, c, e, f and Fig 4.8 b, c, e, f).

4.4.2.3.5 Hydroxycinnamic acid

Cinnamic acid was not detected in the musts (day 0 of fermentation) of both wine verieties (Fig 4.9). In wine without added *t*-resveratrol, it was detected at day 3 and 2 in an average of 2.05 mg/L for Exotic wine and average of 1.95 mg/L for Shiraz wine, respectively (Fig 4.9 a, d). The amounts of cinnamic acid decreased after 6 - 7 days of maceration until the end of alcoholic fermentation. At the end of fermentation, the amount of cinnamic acid in Exotic wine produced from yeast K1V1116 strain (1.44 mg/L) was significantly higher ($p<0.05$) than those from other two yeast strains (0.48 and 0.53 mg/L for CY3079 and EC-1118 yeast strain). For Shiraz wine, there were no significant differences ($p>0.05$) in cinnamic content of wine due to differences in yeast strains.

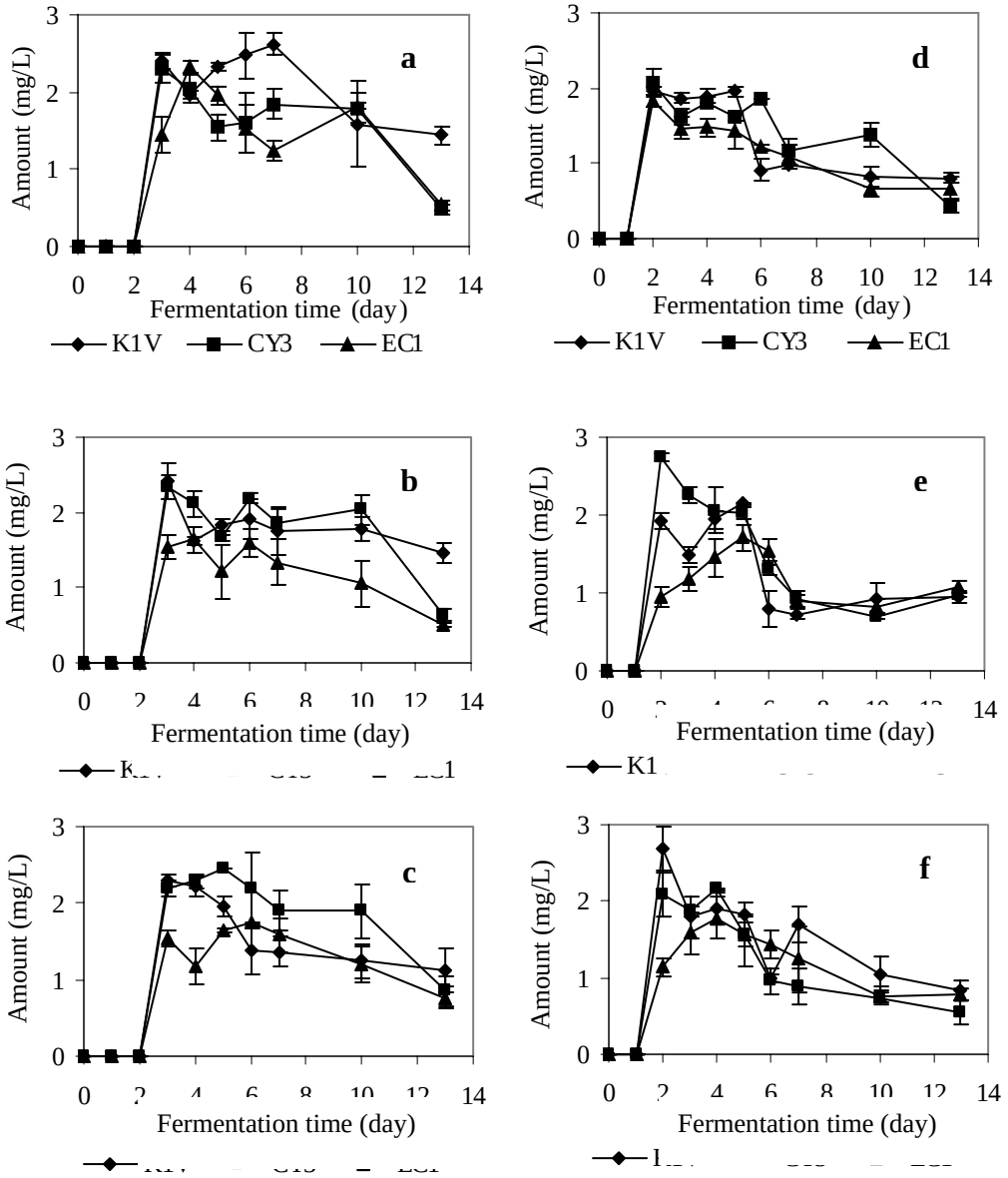

Figure 4.9 Changes of cinnamic acid during alcoholic fermentation of Exotic wine: a = Resv 0 ppm, b = Resv 5 ppm and c = Resv 10 ppm and Shiraz wine: d = Resv 0 ppm, e = Resv 5 ppm and f = Resv 10 ppm

The amount of *p*-coumaric acid of Exotic drastically increased within the first day of fermentation from the average of 1.05 to 5.91 mg/L (Fig 4.10 a). For Shiraz wine, the *p*-coumaric acid increased up to the second day of fermention from the average of 1.10 to 5.77 mg/L (Fig. 4.10 d). Thereafter, the *p*-coumaric acid

content remained steady for Exotic wine and slightly decreased for Shiraz wine until the end of fermentation. At the end of fermentation, the amounts of *p*-coumaric acid

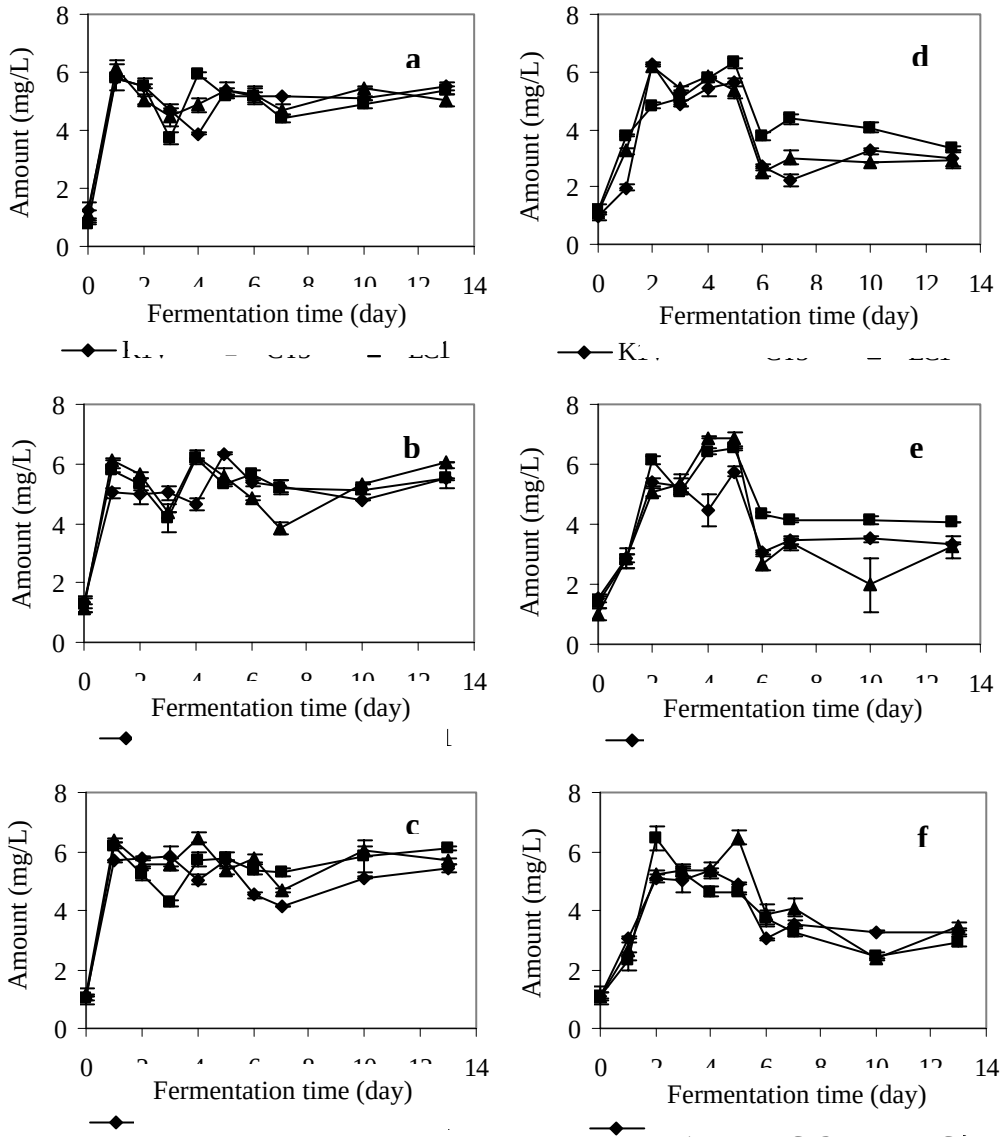

Figure 4.10 Changes of *p*-coumaric acid during alcoholic fermentation of Exotic wine:a = Resv 0 ppm, b = Resv 5 ppm and c = Resv 10 ppm and Shiraz wine: d = Resv 0 ppm, e = Resv 5 ppm and f = Resv 10 ppm

of Exotic wine were not significantly different ($p>0.05$) due to the yeast strains (an average of 5.32 mg/L). However, Shiraz wine produced from yeast CY3079 strain

(3.35 mg/L) contained significantly higher ($p<0.05$) *p*-coumaric acid than those from yeast K1V1116 (2.96 mg/L) and EC1118 strain (2.94 mg/L).

The increasing of both cinnamic and *p*-coumaric acid during alcoholic fermentation was due to the hydroxycinnamic acid derivatives such as caftaric or acylated phenols are hydrolyzed to varying degrees yielding the corresponding free cinnamic acid (Zoecklein et al., 1995). The free hydroxycinnamic acid may be further oxidized to volatile phenols by yeast decarboxylase (Cillier and Singleton, 1989; Chatonnet et al., 1993), or adsorbed by yeast (Somers et al., 1987). These were the reasons of the decreasing of these amounts after day 5 of fermentation. Moreover, Eiro and Heinonen (2002) reported the decreasing of these amounts might be due to they act as copigments of anthocyanins.

4.4.2.4 Changes of color parameters

Chemical density (CD), tint, chemical age (CA), total anthocyanin (TA) and total colored anthocyanin (TCA) of wines without added *t*-resveratrol are shown in Table 4.3. CD and tint indicate the intensity and hue of colored pigment of wines, respectively. The average CD of wines significantly increased in both wine varieties produced by all yeast strains from the average of 0.62 to 4.22 for Exotic and 0.39 to 0.66 for Shiraz wine (data not shown). Wine tint was not changed or slightly decreased from the average of 0.84 to 0.76 for Exotic and from 0.87 to 0.79 for Shiraz wine. The increasing of CD might be due to the releasing of red pigment compounds from the skin into wine.

CA measures the relation between polymeric compounds and monomeric compounds, therefore, the higher of CA, the higher in polymeric compounds. CA of Exotic wines decreased from 10.34 to 9.75 for K1V1116 and from

14.30 to 9.08 for EC1118 yeast strain. But the increasing was found from 6.87 to 10.68 for CY3079 yeast strain. In Shiraz wine, the increasing of CA was found for all yeast strains from the average of 9.00 to 13.73 mg/L. Total anthocyanin (TA) of both wines increased from the average of 117.45 to 175.46 mg/L for Exotic and 90.58 to 97.08 mg/L for Shiraz wine. TA of Exotic wines were higher than those of Shiraz. Total colored anthocyanin (TCA) of both wines also increased 19.57 to 27.94 mg/L for Exotic and 11.50 to 19.68 mg/L for Shiraz wines. It was obvious that the TA and TCA concentration of the wine were related to their amounts presence in grape skin (Table 4.1). All color parameter of both wines added with 5 ppm and 10 ppm resveratrol had similar results as those without added *t*-resveratrol as shown in Table 4.4 and 4.5.

4.4.3 Malolactic fermentation (MLF)

4.4.3.1 Changes of *t*-resveratrol concentration

Resveratrol is found to be one of the major phenolic compounds in grape wines that provide health benefit. Therefore, in order to investigate change of this compound during vinification, pure *t*-resveratrol with the amounts of 5 and 10 ppm were added in the must just prior to fermentation. Table 4.6 shows amount of *t*-resveratrol during fermentation by each yeast strain and after malolactic fermentation (MLF) by lactic acid bacteria, *Oenococus oeni*. The resveratrol concentrations in wines produced by all yeast strains before MLF were not significantly differences ($p>0.05$) with the amounts ranging 0.61 to 1.46 mg/L. After MLF, an increasing of resveratrol content was observed. In vinification without added *t*-resveratrol, the highest increasing of resveratrol was observed in Exotic wine produced from yeast CY3079 strain (from 0.68 to 1.48 mg/L) and Shiraz wine produced from yeast K1V1116 strain (from 0.51 to 0.65 mg/L).

Table 4.3 Color measurements of wine without commercial *t*-resveratrol before and after alcoholic fermentation

	Treatment					
	Exotic (day 0)			Exotic (day 13)		
Parameter	K1V1116	CY3079	EC1118	K1V1116	CY3079	EC1118
CD	0.68 ± 0.16 b	0.66 ± 0.28 b	0.52 ± 0.02 b	4.05 ± 0.01 a	4.24 ± 0.19 a	4.38 ± 0.04 a
Tint	0.88 ± 0.13 a	0.88 ± 0.14 a	0.75 ± 0.04 a	0.71 ± 0.01 a	0.81 ± 0.02 a	0.75 ± 0.01 a
CA	10.34 ± 2.87 ab	6.87 ± 0.66 b	14.30 ± 2.41 a	9.75 ± 0.21 ab	10.68 ± 0.15 ab	9.08 ± 2.93 b
TA (mg/L)	122.33 ± 15.84 ab	147.40 ± 2.54 ab	82.63 ± 18.34 b	171.70 ± 3.11 a	159.06 ± 3.58 ab	195.63 ± 70.57 a
TCA (mg/L)	20.65 ± 3.15 ab	23.24 ± 9.94 ab	14.83 ± 0.47 b	27.32 ± 1.42 a	26.14 ± 0.74 a	30.36 ± 0.40 a
	Shiraz (day 0)			Shiraz (day 13)		
CD	0.42 ± 0.09 b	0.40 ± 0.06 b	0.36 ± 0.01 b	0.68 ± 0.06 a	0.68 ± 0.07 a	0.62 ± 0.01 a
Tint	0.95 ± 0.20 a	0.80 ± 0.07 a	0.86 ± 0.00 a	0.93 ± 0.11 a	0.75 ± 0.02 a	0.70 ± 0.01 a
CA	11.03 ± 1.92 bc	7.81 ± 2.08 d	8.15 ± 0.49 cd	12.92 ± 0.57 ab	13.98 ± 0.35 ab	14.28 ± 0.48 a
TA (mg/L)	84.23 ± 8.82 a	95.87 ± 35.17 a	91.63 ± 6.98 a	100.87 ± 6.22 a	97.00 ± 6.32 a	93.37 ± 4.76 a
TCA (mg/L)	10.09 ± 1.00 c	13.82 ± 1.72 b	10.58 ± 0.08 c	18.52 ± 1.36 a	21.25 ± 2.02 a	19.27 ± 0.24 a

Numbers with different letters within the same row are significantly different ($p \leq 0.05$)

Table 4.4 Color measurements of wine with added 5 ppm commercial *t*-resveratrol before and after alcoholic fermentation

	Treatment					
	Exotic (day 0)			Exotic (day 13)		
Parameter	K1V1116	CY3079	EC1118	K1V1116	CY3079	EC1118
CD	0.53 ± 0.01 c	0.54 ± 0.05 c	0.57 ± 0.03 c	4.18 ± 0.01 b	4.24 ± 0.04 b	4.76 ± 0.20 a
Tint	0.78 ± 0.02 a	0.77 ± 0.03 a	0.78 ± 0.06 a	0.77 ± 0.06 a	0.80 ± 0.02 a	0.85 ± 0.05 a
CA	10.37 ± 0.23 c	10.26 ± 0.26 c	7.64 ± 0.15 d	11.62 ± 0.13 b	11.93 ± 0.15 b	12.44 ± 0.21 a
TA (mg/L)	105.07 ± 8.67 c	112.77 ± 12.30 bc	133.53 ± 12.73 ab	139.92 ± 6.67 a	135.82 ± 6.67 a	142.70 ± 4.00 a
TCA (mg/L)	16.84 ± 0.03 b	16.56 ± 1.16 b	20.12 ± 0.42 b	27.15 ± 1.03 a	26.84 ± 1.72 a	29.20 ± 3.36 a
	Shiraz (day 0)			Shiraz (day 13)		
CD	0.40 ± 0.06 b	0.43 ± 0.01 ab	0.38 ± 0.01 b	0.53 ± 0.06 a	0.53 ± 0.05 a	0.52 ± 0.04 a
Tint	0.79 ± 0.03 b	0.77 ± 0.01 b	0.88 ± 0.02 a	0.68 ± 0.05 c	0.68 ± 0.01 c	0.68 ± 0.01 c
CA	8.93 ± 0.21 b	8.32 ± 0.32 b	9.57 ± 1.05 b	11.90 ± 1.84 ab	13.44 ± 2.74 a	10.71 ± 0.01 ab
TA (mg/L)	93.60 ± 9.24 ab	89.17 ± 4.81 ab	74.03 ± 8.67 b	101.63 ± 15.79 a	89.03 ± 11.27 ab	104.73 ± 6.41 a
TCA (mg/L)	12.26 ± 1.58 bc	16.00 ± 0.06 ab	11.62 ± 0.25 c	16.58 ± 2.97 a	16.12 ± 1.02 ab	17.34 ± 1.87 a

Numbers with different letters within the same row are significantly different ($p \leq 0.05$)

Table 4.5 Color measurements of wine with added 10 ppm commercial *t*-resveratrol before and after alcoholic fermentation

	Treatment					
	Exotic (day 0)			Exotic (day 13)		
Parameter	K1V1116	CY3079	EC1118	K1V1116	CY3079	EC1118
CD	0.52 ± 0.05 b	0.55 ± 0.02 b	0.51 ± 0.02 b	4.26 ± 0.11 a	4.15 ± 0.29 a	4.13 ± 0.20 a
Tint	0.80 ± 0.01 ab	0.77 ± 0.01 ab	0.73 ± 0.02 b	0.84 ± 0.07 a	0.82 ± 0.01 ab	0.75 ± 0.02 ab
CA	8.95 ± 4.03 a	8.42 ± 0.24 a	9.15 ± 1.70 a	11.58 ± 0.39 a	12.54 ± 1.48 a	12.91 ± 0.73 a
TA (mg/L)	132.60 ± 28.47 a	126.83 ± 14.57 a	123.10 ± 17.30 a	144.85 ± 4.03 a	134.37 ± 22.63 a	126.76 ± 3.63 a
TCA (mg/L)	15.71 ± 1.60 b	18.45 ± 0.04 b	16.26 ± 0.06 b	25.58 ± 0.90 a	24.67 ± 2.54 a	26.24 ± 0.97 a
	Shiraz (day 0)			Shiraz (day 13)		
CD	0.47 ± 0.04 a	0.35 ± 0.04 b	0.35 ± 0.04 b	0.49 ± 0.03 a	0.47 ± 0.01 a	0.53 ± 0.02 a
Tint	0.84 ± 0.01 a	0.90 ± 0.05 a	0.84 ± 0.05 a	0.69 ± 0.02 b	0.66 ± 0.01 b	0.69 ± 0.01 b
CA	10.34 ± 0.38 a	8.71 ± 0.84 b	10.06 ± 0.12 a	10.79 ± 0.40 a	10.84 ± 0.46 a	11.27 ± 0.64 a
TA (mg/L)	96.00 ± 1.60 a	77.78 ± 0.07 b	66.58 ± 4.55 b	101.73 ± 8.96 a	98.33 ± 2.07 a	102.40 ± 12.82 a
TCA (mg/L)	13.45 ± 1.32 b	10.62 ± 0.74 c	10.95 ± 0.75 c	15.74 ± 0.99 a	15.35 ± 1.03 ab	17.39 ± 0.01 a

Numbers with different letters within the same row are significantly different ($p \leq 0.05$)

Table 4.6 The concentration of *t*-resveratrol before and after MLF of wine with and without added resveratrol

Resveratrol	Before MLF			After MLF		
added	K1V1116	CY3079	EC1118	K1V1116	CY3079	EC1118
Exotic						
+ 0 ppm	0.83 ± 0.23 bc	0.68 ± 0.07 bc	0.61 ± 0.05 c	0.74 ± 0.11 bc	1.48 ± 0.02a	0.92 ± 0.06 b
+ 5 ppm	1.06 ± 0.07 c	0.91 ± 0.01 cd	0.72 ± 0.05 d	1.74 ± 0.01 a	1.33 ± 0.21 b	1.41 ± 0.04 b
+ 10 ppm	1.46 ± 0.02 cd	1.22 ± 0.03 de	0.76 ± 0.14 e	1.84 ± 0.07 c	3.14 ± 0.39 a	2.33 ± 0.23 b
Shiraz						
+ 0 ppm	0.51 ± 0.19 ab	0.54 ± 0.10ab	0.71± 0.09 a	0.65 ± 0.01ab	0.46 ± 0.04 b	0.60 ± 0.02 a
+ 5 ppm	0.78 ± 0.14 b	0.80 ± 0.05 ab	0.70 ± 0.10 b	0.98 ± 0.01 a	0.72 ± 0.02 b	0.69 ± 0.02 b
+ 10 ppm	1.06 ± 0.07 ab	1.07 ± 0.11 ab	0.91 ± 0.07 b	1.18 ± 0.10 ab	1.49 ± 0.43 a	1.59 ± 0.18 a

Numbers with different letters within the same row are significantly different ($p \leq 0.05$)

The addition of 5 ppm commercial *t*-resveratrol into the must, after MLF, lead to increase the amount of this compound from the average of only 0.90 to 1.49 and 0.76 to 0.80 mg/L for Exotic and Shiraz wine, respectively.

In the wines added 10 ppm resveratrol, the maximum of increasing was observed in Exotic wine produce by yeast CY3079 strain from 1.22 to 3.14 mg/L and Shiraz wine produce by yeast EC1118 strain from 1.07 to 1.49 mg/L. These were in agreement with Pezet and Cuenat (1996) who observed the 4-fold increase in *t*- and *c*-resveratrol on Gamay variety after MLF, which could only be explained by their formation from a pool of glucosides or oligomeric forms through the enzymatic activity of malolactic bacteria. The β-glucosidase activity in *Oenococus oeni* (lactic acid bacteria) was observed by Grimaldi, McLean and Jiranek (2002) and β-glucosidase, α-glucosidase and N-acetyl–β-glucosaminidase were observed by Alexandre, Costello, Remize, Guzzo, and Guilloux-Benatier (2004). These might be related to conversion of glucosides or oligomeric forms to free isomer of resveratrol.

4.4.3.2 Changes of TPC and flavonoid phenols

In vinification without added *t*-resveratrol, for both wines before and after MLF, there were no significant differences ($p>0.05$) in TPC (Table 4.7). However, slight decrease of TPC occurred after MLF. Once the maximum amounts were reached, there were copigmentation and condensation of phenolic compounds causing slight reduction of TPC after MLF. Epicatechin content of Exotic wine were also no significant differences ($p>0.05$). The catechin contents of Exotic wine significantly increased ($p<0.05$) after MLF from the average of 15.08 to 21.66 mg/L. This result was in agreement with the work of Hernandez, Eztrella, Carlavilla, Martin-

Alvarez and Moreno-Arribas (2005) who observed the increasing in both epicatecin and catechin after MLF. On the contrary, epicatechin and catechin contents of Shiraz wine significantly decreased ($p<0.05$). This could be due to high concentrations of both epicatechin and catechin in alcoholic fermentation, then more aggregate and co-pigment could occur. Precipitation of these complexes was expected. Moreover, free quercetin significantly increased ($p<0.05$) after MLF for both Exotic and Shiraz wines for all yeast strains. The presences of β–glucosidase enzyme of *O. oeni* was a factor affecting hydrolysis of quercetin glycoside to free molecule (ETS laboratories). In addition, the addition of commercial *t*-resveratrol in fermentation process showed the similar changes of TPC and flavonoid phenols as in wines without added resveratrol (Table 4.8 and 4.9).

4.4.3.3 Changes of low molecular weight phenolic acids

The changes of phenolic acids of both wine varieties are shown in Table 4.10, 4.11 and 4.12. In vinification without added *t*-resveratrol, syringic acid of Exotic wine significantly increased ($p<0.05$) after MLF for all yeast strain from the average of 2.16 to 4.38 mg/L (Table 4.10). Gentisic, *p*-hydroxybenzoic, salicylic, protocatechuic and caffeic acid were not detected in the wine before MLF. These compounds were generated during MLF. Gentisic and salicyclic acid were only found in Exotic wine at the average concentration of 0.65 and 0.72 mg/L, respectively. *p*-Hydroxybenzoic, protocatechuic acid was generated ranged 0.73 – 1.01 and 0.82 – 1.20 mg/L, respectively. There were no significant differences ($p>0.05$) in generated phenolic acids due to yeast strains after MLF. The amount of caffeic acid was found at concentration of 4.22 mg/L and 6.24 mg/L for wine produced from yeast K1V1116 and CY3079 strain, respectively, but it was not detected in wine produced from yeast EC1118 strain.

Table 4.7 The TPC and monomeric flavonoid content (mg/L) of wine without added *t*-resveratrol before and after MLF

Phenols	Before MLF			After MLF		
	K1V1116	CY3079	EC1118	K1V1116	CY3079	EC1118
Exotic						
TPC	2829.21 ± 112.03 ab	2775.06 ± 89.34 ab	2983.63 ± 199.56 a	2629.42 ± 67.18 b	2754.49 ± 106.94 ab	2743.82 ± 141.22 ab
Epicatechin	7.92 ± 1.00 b	8.52 ± 0.34 ab	9.68 ± 0.15a	7.92 ± 0.17 b	9.18 ± 0.13 ab	8.69 ± 0.74 ab
Catechin	15.14 ± 0.20 b	13.88 ± 0.19 b	16.23 ± 0.01 b	20.31 ± 0.73 a	23.64 ± 0.93 a	21.03 ± 3.05 a
Quercetin	1.54 ± 0.03 e	1.26 ± 0.02 f	2.11 ± 0.06 d	3.99 ± 0.20 b	4.38 ± 0.09 a	3.68 ± 0.16 c
Shiraz						
TPC	2397.08 ± 70.74 cd	2632.74 ± 112.91 bc	2343.21 ± 163.24 d	2549.28 ± 36.36 bcd	2896.38 ± 106.05 a	2701.40 ± 6.06 ab
Epicatechin	28.50 ± 0.82 b	35.67 ± 0.33a	33.19 ± 0.60 a	20.98 ± 0.32 d	24.12 ± 1.35 c	26.65 ± 2.36 bc
Catechin	29.21 ± 0.76 b	38.52 ± 0.78 a	34.75 ± 3.57 a	23.17 ± 0.96 c	29.73 ± 2.61 b	28.12 ± 0.72 b
Quercetin	1.72 ± 0.04 c	1.89 ± 0.18 bc	1.82 ± 0.10 c	2.04 ± 0.07 bc	2.55 ± 0.20 a	2.24 ± 0.18 ab

Numbers with different letters within the same row are significantly different ($p \leq 0.05$)

Table 4.8 The TPC and monomeric flavonoid content (mg/L) of wine with added 5 ppm *t*-resveratrol before and after MLF

Phenols	Before MLF			After MLF		
	K1V1116	CY3079	EC1118	K1V1116	CY3079	EC1118
Exotic						
TPC	2604.59 ± 146.07 abc	2784.08 ± 8.51 ab	2818.18 ± 164.50 a	2430.68 ± 112.43 c	2593.55 ± 46.62 abc	2499.52 ± 154.93 bc
Epicatechin	6.87 ± 0.13 b	6.94 ± 0.39 b	9.33 ± 0.37 a	8.93 ± 0.39 a	6.89 ± 0.52 b	6.64 ± 0.48 b
Catechin	12.55 ± 0.71 c	12.85 ± 0.10 c	14.07 ± 0.94 bc	20.35 ± 0.96 a	15.92 ± 0.63 b	13.41 ± 1.50 c
Quercetin	1.02 ± 0.06 d	1.13 ± 0.09 d	1.93 ± 0.23 c	3.75 ± 0.18 a	3.27 ± 0.01 b	3.38 ± 0.16 b
Shiraz						
TPC	2595.23 ± 68.02 ab	2697.19 ± 92.50 a	2102.73 ± 10.88 c	2753.90 ± 253.01 a	2930.66 ± 181.81 a	2312.52 ± 7.58 bc
Epicatechin	27.91 ± 1.32 ab	31.24 ± 1.14 a	28.87 ± 1.48 ab	27.46 ± 3.04 ab	26.28 ± 1.48 b	24.24 ± 1.72 b
Catechin	28.14 ± 2.06 abc	32.88 ± 2.00 a	32.34 ± 1.50 ab	27.57 ± 3.07 bc	28.51 ± 1.03 abc	25.28 ± 0.05 c
Quercetin	2.06 ± 0.31 ab	1.83 ± 0.32 b	1.73 ± 0.08 b	2.62 ± 0.01 a	2.22 ± 0.14 ab	1.95 ± 0.35 b

Numbers with different letters within the same row are significantly different ($p \leq 0.05$)

Table 4.9 The TPC and monomeric flavonoid content (mg/L) of wine with added 10 ppm *t*-resveratrol before and after MLF

Phenols	Before MLF			After MLF		
	K1V1116	CY3079	EC1118	K1V1116	CY3079	EC1118
Exotic						
TPC	2554.45 ± 148.90 a	2760.02 ± 201.37 a	2740.96 ± 55.31 a	2425.83 ± 122.02 a	2581.92 ± 265.98 a	2572.23 ± 142.59 a
Epicatechin	7.50 ± 0.58 d	9.17 ± 0.18 ab	9.88 ± 0.08 a	7.19 ± 0.15 d	8.34 ± 0.29 c	8.69 ± 0.24 bc
Catechin	13.61 ± 1.43 bc	13.89 ± 0.77 bc	12.87 ± 0.92 c	16.04 ± 0.18 b	20.99 ± 0.15 a	19.70 ± 1.51 a
Quercetin	0.86 ± 0.01 d	1.70 ± 0.17 c	1.79 ± 0.10 c	3.61 ± 0.10 b	4.06 ± 0.15 a	4.33 ± 0.14 a
Shiraz						
TPC	2676.99 ± 180.92 ab	2265.29 ± 82.98 c	2267.22 ± 39.45 c	2827.82 ± 251.50 a	2355.37 ± 92.42 bc	2481.79 ± 34.85 abc
Epicatechin	41.36 ± 2.13 a	28.48 ± 2.57 b	30.08 ± 0.40 b	27.66 ± 0.14 b	26.58 ± 3.22 b	26.15 ± 0.67 b
Catechin	42.33 ± 3.17 a	32.82 ± 1.14 b	33.41 ± 1.21 b	28.16 ± 1.21 c	28.88 ± 0.87 c	27.16 ± 0.62 c
Quercetin	1.72 ± 0.24 c	1.65 ± 0.02 c	1.92 ± 0.03 bc	2.17 ± 0.10 ab	2.32 ± 0.14 a	2.39 ± 0.02 a

Numbers with different letters within the same row are significantly different ($p \leq 0.05$)

Table 4.10 Phenolic acid content (mg/L) of wine without added *t*-resveratrol before and after MLF

Phenolic acid	Before MLF			After MLF		
	K1V1116	CY3079	EC1118	K1V1116	CY3079	EC1118
Exotic						
Syringic	1.71 ± 0.20 c	1.88 ± 0.02 c	2.90 ± 0.29 b	4.11 ± 0.04 a	4.43 ± 0.04 a	4.59 ± 0.45 a
Gentisic	ND	ND	ND	0.67 ± 0.07a	0.70 ± 0.03a	0.57 ± 0.17a
p-Hydroxybenzoic	ND	ND	ND	0.90 ± 0.28 a	0.73 ± 0.05 a	1.01 ± 0.05 a
Salicyclic	ND	ND	ND	0.73 ± 0.04 a	0.71 ± 0.01 a	0.73 ± 0.03 a
Gallic	10.13 ± 0.34 de	9.62 ± 0.27 e	11.37 ± 0.43 bc	10.76 ± 0.15 cd	12.40 ± 0.20 a	11.88 ± 0.56 ab
Protocatechuic	ND	ND	ND	1.20 ± 0.03 a	0.82 ± 0.03 b	1.15 ± 0.07a
Cinnamic	1.50 ± 0.04 a	0.45 ± 0.04 d	0.53 ± 0.07 cd	1.16 ± 0.01 b	1.28 ± 0.11 b	0.69 ± 0.09 c
p-Coumaric	5.54 ± 0.14 a	4.69 ± 0.11 bc	5.04 ± 0.22 b	4.56 ± 0.27 c	4.34 ± 0.16 c	4.44 ± 0.08 c
Caffeic	ND	ND	ND	4.22 ± 0.13 b	6.24 ± 0.10a	ND
Shiraz						
Syringic	2.31 ± 0.06 c	3.34 ± 0.31 b	3.69 ± 0.11 b	4.48 ± 0.01 a	3.73 ± 0.37 b	3.66 ± 0.24 b
Gentisic	ND	ND	ND	ND	ND	ND
p-Hydroxybenzoic	0.67 ± 0.06 a	0.61 ± 0.01 a	0.63 ± 0.04 a	0.74 ± 0.05 a	0.75 ± 0.36 a	0.72 ± 0.01 a
Salicyclic	ND	ND	ND	ND	ND	ND
Gallic	14.37 ± 0.77 d	19.21 ± 0.17 b	18.76 ± 0.53 b	17.09 ± 0.41 c	22.05 ± 0.03 a	19.31 ± 0.22 b
Protocatechuic	ND	ND	ND	1.05 ± 0.08 a	1.07 ± 0.05 a	1.15 ± 0.13 a
Cinnamic	0.63 ± 0.05 c	0.84 ± 0.14 c	0.89 ± 0.10 c	2.24 ± 0.14 ab	2.58 ± 0.22 a	2.20 ± 0.15 b
p-Coumaric	2.43 ± 0.03 b	2.85 ± 0.40 ab	2.45 ± 0.30 b	2.42 ± 0.24 b	3.38 ± 0.15 a	2.26 ± 0.10 b
Caffeic	ND	ND	ND	1.46 ± 0.14 b	1.64 ± 0.02 a	1.56 ± 0.06 ab

Numbers with different letters within the same row are significantly different ($p \leq 0.05$)

Table 4.11 Phenolic acid content (mg/L) of wine with added 5 ppm *t*-resveratrol before and after MLF

Phenolic acid	Before MLF			After MLF		
	K1V1116	CY3079	EC1118	K1V1116	CY3079	EC1118
Exotic						
Syringic	1.54 ± 0.20 b	1.87 ± 0.01 b	4.35 ± 0.03 a	4.53 ± 0.10 a	4.58 ± 0.28 a	4.85 ± 0.69 a
Gentisic	ND	ND	ND	0.72 ± 0.06 a	0.71 ± 0.06 a	0.69 ± 0.03 a
p-hydroxybenzoic	ND	ND	ND	0.66 ± 0.04 a	0.62 ± 0.04 a	0.68 ± 0.02 a
Salicyclic	ND	ND	ND	0.69 ± 0.04 b	0.79 ± 0.07 ab	0.83 ± 0.06 a
Gallic	9.59 ± 0.15 c	10.33 ± 0.15 abc	9.92 ± 0.40 bc	10.89 ± 0.54 ab	9.95 ± 0.19 bc	11.14 ± 0.73 a
Protocatechuic	ND	ND	ND	1.01 ± 0.01 a	1.06 ± 0.09 a	1.07 ± 0.02 a
Cinnamic	1.64 ± 0.10 a	0.84 ± 0.04 b	0.51 ± 0.02 c	0.95 ± 0.05 b	0.98 ± 0.06 b	0.93 ± 0.07 b
p-Coumaric	5.53 ± 0.01 a	5.48 ± 0.01 ab	5.67 ± 0.05 a	5.14 ± 0.01 b	3.84 ± 0.09 c	3.35 ± 0.11 d
Caffeic	ND	ND	ND	4.33 ± 0.17 a	4.26 ± 0.18 a	ND
Shiraz						
Syringic	2.86 ± 0.01 c	3.64 ± 0.04 b	4.22 ± 0.19 a	4.27 ± 0.09 a	3.50 ± 0.02 b	3.68 ± 0.04 b
Gentisic	ND	ND	ND	ND	ND	ND
p-hydroxybenzoic	0.82 ± 0.06 a	0.71 ± 0.15 a	0.64 ± 0.11 a	0.57 ± 0.05 a	0.77 ± 0.11 a	0.60 ± 0.05 a
Salicyclic	ND	ND	ND	ND	ND	ND
Gallic	13.34 ± 0.42 c	17.32 ± 1.63 ab	15.82 ± 0.52 bc	19.78 ± 0.96 a	20.11 ± 1.75 a	18.38 ± 0.08 ab
Protocatechuic	ND	ND	ND	1.06 ± 0.02 a	1.01 ± 0.08 a	1.04 ± 0.15 a
Cinnamic	0.83 ± 0.12 c	1.49 ± 0.10 b	0.61 ± 0.02 c	1.93 ± 0.16 a	1.84 ± 0.13 ab	1.91 ± 0.25 a
p-Coumaric	2.65 ± 0.22 ab	2.84 ± 0.30 a	2.87 ± 0.06 a	2.81 ± 0.15 a	2.10 ± 0.21 c	2.20 ± 0.16 bc
Caffeic	ND	ND	ND	1.50 ± 0.06 a	1.45 ± 0.21 a	1.42 ± 0.21 a

Numbers with different letters within the same row are significantly different ($p \leq 0.05$)

Table 4.12 Phenolic acid content (mg/L) of wine with added 10 ppm *t*-resveratrol before and after MLF

Phenolic acid	Before MLF			After MLF		
	K1V1116	CY3079	EC1118	K1V1116	CY3079	EC1118
Exotic						
Syringic	1.87 ± 0.15 d	2.99 ± 0.10 c	3.10 ± 0.03 c	4.16 ± 0.03 b	4.44 ± 0.02 a	4.63 ± 0.06 a
Gentisic	ND	ND	ND	0.72 ± 0.07 a	0.75 ± 0.03 a	0.68 ± 0.03 a
p-hydroxybenzoic	ND	ND	ND	0.71 ± 0.10 b	0.71 ± 0.04 b	1.23 ± 0.05 a
Salicyclic	ND	ND	ND	0.66 ± 0.02 b	0.70 ± 0.03 b	0.93 ± 0.16 a
Gallic	10.28 ± 0.34 cd	10.67 ± 0.44 bc	8.83 ± 0.08 e	9.42 ± 0.21 de	11.72 ± 0.24 ab	11.98 ± 0.99 a
Protocatechuic	ND	ND	ND	1.02 ± 0.01 b	1.12 ± 0.09 a	1.15 ± 0.01 a
Cinnamic	1.30 ± 0.52 ab	0.71 ± 0.01 bc	0.77 ± 0.14 abc	0.77 ± 0.08 abc	1.32 ± 0.02 a	0.65 ± 0.12 c
p-Coumaric	6.08 ± 0.13 a	6.19 ± 0.17 a	5.66 ± 0.10 b	4.54 ± 0.10 d	4.54 ± 0.07 d	5.26 ± 0.06 c
Caffeic	ND	ND	ND	3.87 ± 0.22 b	4.19 ± 0.19 a	ND
Shiraz						
Syringic	3.95 ± 0.05 b	3.43 ± 0.32 b	3.66 ± 0.26 b	3.89 ± 0.26 b	3.96 ± 0.28 b	4.58 ± 0.12 a
Gentisic	ND	ND	ND	ND	ND	ND
p-hydroxybenzoic	0.59 ± 0.10 ab	0.69 ± 0.07 ab	0.78 ± 0.01 a	0.52 ± 0.02 b	0.78 ± 0.08 a	0.70 ± 0.11 ab
Salicyclic	ND	ND	ND	ND	ND	ND
Gallic	19.49 ± 1.97 a	16.52 ± 0.05 b	16.47 ± 0.73 b	19.56 ± 0.19 a	18.70 ± 1.30 ab	18.35 ± 0.18 ab
Protocatechuic	ND	ND	ND	1.04 ± 0.01 a	1.09 ± 0.08 a	1.04 ± 0.04 a
Cinnamic	1.08 ± 0.18 b	0.77 ± 0.12 b	1.00 ± 0.04 b	1.74 ± 0.04 a	1.90 ± 0.22 a	1.98 ± 0.15 a
p-Coumaric	3.10 ± 0.27 a	2.81 ± 0.13 ab	3.09 ± 0.05 a	2.55 ± 0.05 b	2.50 ± 0.24 b	3.01 ± 0.18 b
Caffeic	ND	ND	ND	1.49 ± 0.16 a	1.38 ± 0.16 a	1.44 ± 0.05 a

Numbers with different letters within the same row are significantly different ($p \leq 0.05$)

Cinnamic acid content remained constant, whereas *p*-coumaric acid content significantly decreased ($p<0.05$) for all yeast strain during MLF (5.54 to 4.56 mg/L for K1V1116, 4.69 to 4.34 mg/L for CY3079 and 5.04 to 4.44 mg/L for EC1118).

For Shiraz wine, there were significant differences ($p<0.05$) in syringic acid contents before and after MLF, only when yeast K1V1116 was used. Changes were not observed in wines fermented by the other two yeast strains. Gentisic and salicyclic acid were not detected in both before and after MLF. The amounts of *p*-hydroxybenzoic acid remained constant ($p>0.05$) during MLF ranging 0.61 – 0.75 mg/L. Protocatechuic and caffeic acid were significantly increased ($p<0.05$) after MLF (not detected before MLF to the average of 1.09 mg/L after MLF and not detected before MLF to the average of 1.55 mg/L after MLF, respectively). Cinnamic acid significantly increased ($p<0.05$) from the average of 0.79 mg/L before MLF to 2.34 mg/L after MLF. *p*-Coumaric acid remained contant ($p>0.05$) ranged 2.58 to 2.69 mg/L. An additional source of *t*-caffeic and *t-p*-coumaric acid may come from the hydrolysis of cinnamoyl-glucoside anthocyanins (Monagas, Bartolome and Gomez-Cordoves, 2005) as well as from other hydroxycinnamic derivatives by lactic acid bacteria enzymatic activity (Hernandez et al., 2005). In addition, it appeared that addition of 5 and 10 ppm commercial *t*-resveratrol in fermentation process did not affect the amount of phenolic compound of both wines during MLF as shown in Table 4.11 and 4.12. Changes of phenolic compounds were in similar patterns as those of wine without added resveratrol in fermentation.

4.4.3.4 Changes of color parameters

The changes of color parameter of both wine varieties are shown in Table 4.13, 4.14 and 4.15. For vinification without added *t*-resveratrol (Table 4.13), CD values significantly increased ($p<0.05$) during MLF for all yeast strains from average of 4.22 to 7.72 for Exotic wine and 0.66 to 3.33 for Shiraz wine. The Exotic wine had significantly higher ($p<0.05$) CD value than Shiraz. After MLF, wine tint also increased for all treatments of both wines. Significant increases ($p<0.05$) were observed for Exotic wines produced form yeast K1V1116 and EC1118 strains and Shiraz wines produced from yeast CY3079 and EC1118 strains. Regarding yeast strains, before and after MLF, there were no significant differences ($p>0.05$) of CA values of both wine varieties. However, Exotic wines had higher CA values after MLF, average of 9.84 before MLF then, increased up to 15.93 after MLF. Shiraz wines had CA average of 13.21 before and after MLF. TA of Exotic wines were not significant differences ($p>0.05$) for all treatments (average of 180.00 mg/L before and after MLF), whereas TCA were significantly increased ($p<0.05$) from the average of 27.94 mg/L before MLF to 43.40 mg/L after MLF. For Shiraz wine, TA were significantly increased ($p<0.05$) from the average of 97.08 to 136.12 mg/L. On the contrary, TCA of Shiraz wines significantly decreased ($p<0.05$) from the average of 19.68 to 10.99 mg/L for all yeast strains. The increasing in wine tint, CA as well as TCA of Exotic wines after MLF indicated that the polymerization could occur on this stage. Moreover, the addition of commercial *t*-resveratrol in fermentation process showed the similar changes of color parameters as in wines without added resveratrol (Table 4.14 and 4.15).

Table 4.13 Color measurements of wine without added *t*-resveratrol before and after MLF

	Treatment					
	Exotic (before MLF)			Exotic (after MLF)		
Parameter	K1V1116	CY3079	EC1118	K1V1116	CY3079	EC1118
CD	4.05 ± 0.01 b	4.24 ± 0.19 b	4.38 ± 0.04 b	7.67 ± 0.24 a	7.54 ± 0.15 a	7.96 ± 0.98 a
Tint	0.71 ± 0.01 b	0.81 ± 0.02 ab	0.75 ± 0.01 b	0.91 ± 0.06 a	0.92 ± 0.03 a	0.91 ± 0.08 a
CA	9.75 ± 0.21 b	10.68 ± 0.15 b	9.08 ± 2.93 b	20.11 ± 7.71 a	14.27 ± 3.28 ab	13.42 ± 0.61 ab
TA (mg/L)	171.70 ± 3.11 a	159.06 ± 3.58 a	195.63 ± 70.57 a	145.47 ± 80.70 a	195.30 ± 40.87 a	212.83 ± 18.15 a
TCA (mg/L)	27.32 ± 1.42 b	26.14 ± 0.74 b	30.36 ± 0.40 b	40.98 ± 0.06 a	42.83 ± 0.69 a	46.40 ± 5.52 a
	Shiraz (before MLF)			Shiraz (after MLF)		
CD	0.68 ± 0.06 c	0.68 ± 0.07 c	0.62 ± 0.01 c	3.12 ± 0.02 b	3.68 ± 0.24 a	3.20 ± 0.11 b
Tint	0.93 ± 0.11 b	0.75 ± 0.02 c	0.70 ± 0.01 c	1.00 ± 0.04 ab	1.07 ± 0.01 a	1.04 ± 0.04 ab
CA	12.92 ± 0.57 a	13.98 ± 0.35 a	14.28 ± 0.48 a	13.00 ± 0.87 a	13.37 ± 1.24 a	11.72 ± 1.96 a
TA (mg/L)	100.87 ± 6.22 b	97.00 ± 6.32 b	93.37 ± 4.76 b	125.40 ± 8.96 ab	136.17 ± 15.70 a	146.80 ± 24.14 a
TCA (mg/L)	18.52 ± 1.36 a	21.25 ± 2.02 a	19.27 ± 0.24 a	10.39 ± 0.21 b	12.25 ± 2.11 b	10.33 ± 0.72 b

Numbers with different letters within the same row are significantly different ($p \leq 0.05$)

Table 4.14 Color measurements of wine with added 5 ppm commercial *t*-resveratrol before and after MLF

	Treatment					
	Exotic (before MLF)			Exotic (after MLF)		
Parameter	K1V1116	CY3079	EC1118	K1V1116	CY3079	EC1118
CD	4.18 ± 0.01 b	4.24 ± 0.04 b	4.76 ± 0.20 b	7.15 ± 0.39 a	7.62 ± 0.58 a	7.83 ± 0.21 a
Tint	0.77 ± 0.06 b	0.80 ± 0.02 ab	0.85 ± 0.05 ab	0.92 ± 0.03 ab	0.93 ± 0.11 ab	0.99 ± 0.12 a
CA	11.62 ± 0.13 b	11.93 ± 0.15 b	12.44 ± 0.21 b	15.35 ± 1.46 a	14.58 ± 0.19 a	14.66 ± 1.44 a
TA (mg/L)	139.92 ± 6.67 b	135.82 ± 6.67 b	142.70 ± 4.00 b	188.50 ± 19.85 a	199.10 ± 8.34 a	179.97 ± 12.12 a
TCA (mg/L)	27.15 ± 1.03 d	26.84 ± 1.72 d	29.20 ± 3.36 cd	35.85 ± 3.63 bc	40.51 ± 0.69 ab	44.03 ± 4.94 a
	Shiraz (before MLF)			Shiraz (after MLF)		
CD	0.53 ± 0.06 c	0.53 ± 0.05 c	0.52 ± 0.04 c	3.41 ± 0.07 a	3.74 ± 0.24 a	2.58 ± 0.22 b
Tint	0.68 ± 0.05 b	0.68 ± 0.01 b	0.68 ± 0.01 b	0.94 ± 0.09 a	1.01 ± 0.01 a	0.99 ± 0.03 a
CA	11.90 ± 1.84 a	13.44 ± 2.74 a	10.71 ± 0.01 a	14.51 ± 0.16 a	12.40 ± 0.37 a	13.80 ± 2.00 a
TA (mg/L)	101.63 ± 15.79 b	89.03 ± 11.27 b	104.73 ± 6.41 b	120.53 ± 7.07 b	151.50 ± 8.91 a	104.27 ± 18.67 b
TCA (mg/L)	16.58 ± 2.97 ab	16.12 ± 1.02 ab	17.34 ± 1.87 a	12.22 ± 0.71 b	13.60 ± 0.03 ab	7.51 ± 2.45 c

Numbers with different letters within the same row are significantly different ($p \leq 0.05$)

Table 4.15 Color measurements of wine with added 10 ppm commercial *t*-resveratrol before and after MLF

	Treatment					
	Exotic (before MLF)			Exotic (after MLF)		
Parameter	K1V1116	CY3079	EC1118	K1V1116	CY3079	EC1118
CD	4.26 ± 0.11 b	4.15 ± 0.29 b	4.13 ± 0.20 b	7.58 ± 0.14 a	7.29 ± 0.65 a	7.83 ± 0.31 a
Tint	0.84 ± 0.07 ab	0.82 ± 0.01 b	0.75 ± 0.02 b	0.86 ± 0.06 ab	0.94 ± 0.04 a	0.94 ± 0.04 a
CA	11.58 ± 0.39 b	12.54 ± 1.48 b	12.91 ± 0.73 b	19.90 ± 2.54 a	16.19 ± 3.44 ab	14.28 ± 0.48 b
TA (mg/L)	144.85 ± 4.03 ab	134.37 ± 22.63 ab	126.76 ± 3.63 b	152.70 ± 0.71 ab	155.63 ± 52.37 ab	192.00 ± 4.43 a
TCA (mg/L)	25.58 ± 0.90 b	24.67 ± 2.54 b	26.24 ± 0.97 b	35.92 ± 4.95 a	41.78 ± 6.82 a	44.55 ± 2.30 a
	Shiraz (before MLF)			Shiraz (after MLF)		
CD	0.49 ± 0.03 c	0.47 ± 0.01 c	0.53 ± 0.02 c	3.88 ± 0.35 a	2.83 ± 0.06 b	3.12 ± 0.14 b
Tint	0.69 ± 0.02 c	0.66 ± 0.01 c	0.69 ± 0.01 c	0.94 ± 0.05 b	1.16 ± 0.06 a	1.14 ± 0.07 a
CA	10.79 ± 0.40 a	10.84 ± 0.46 a	11.27 ± 0.64 a	13.36 ± 0.25 a	12.11 ± 1.55 a	11.08 ± 1.92 a
TA (mg/L)	101.73 ± 8.96 b	98.33 ± 2.07 b	102.40 ± 12.82 b	146.13 ± 5.18 a	127.97 ± 19.94 ab	154.63 ± 32.57 a
TCA (mg/L)	15.74 ± 0.99 a	15.35 ± 1.03 a	17.39 ± 0.01 a	14.93 ± 4.31 a	6.98 ± 1.36 b	8.53 ± 2.16 b

Numbers with different letters within the same row are significantly different ($p \leq 0.05$)

4.4.4 Ageing of wine

4.4.4.1 Changes of TPC, *t*-resveratrol and monomeric flavonoid

After precipitation, oak chips were added into the wine samples. The wines were undergone to ageing process at 15°C for 6 months. For evaluation of changes of phenolic compound during ageing process, wine samples produced by yeast CY3079 strain with added 10 ppm *t*-resveratrol (contained the highest amount of *t*-resveratrol) was selected for this observation. During ageing process, the TPC, resveratrol, epicatechin, catechin and quercetin of two wine varieties diminished with time (Table 4.16). Within six months, TPC slightly decreased ($p>0.05$) from 2425.83 to 2158.41 mg/L for Exotic and significantly decreased ($p<0.05$) from 2666.46 to 1402.11 mg/L for Shiraz wine. The decreasing of *t*-resveratrol was observed ($p<0.05$) from 2.43 – 0.75 mg/L for Exotic and 1.06– 0.41 mg/L for Shiraz wine. These results were in agreement with the work of Soleas, Diamandis, and Goldberg (1997) who observed a mean loss of 68% for *t*-resveratrol during ageing with oak in Ontario red wines.

Epicatechin and catechin content of Exotic wine slightly decreased ($p>0.05$) from 7.12 to 3.08 and 18.44 to 9.78 mg/L, respectively. For Shiraz wine, however, the epicatechin and catechin content significantly decreased ($p<0.05$) in aged wine within a month from 38.82 to 7.14 and 35.70 to 9.20 mg/L, respectively and slightly diminished with time. These results were in agreement with the work of Gomez-Plaza, Gil-Munoz, Lopez-Roca, and Martinez (1999) who observed that catechin and epicatechin levels diminished with time. Flavan-3-ols may suffer oxidation and polymerizations (Singleton and Esau, 1969) and be involved in the formation through condensation of polymeric compounds together with anthocyanins (Timberlake and Bridle, 1977).

Table 4.16 The phenolic compound (mg/L) of wines during ageing

Ageing (month)	Phenolic compound (mg/L)				
	TPC	Resveratrol	Epicatechin	Catechin	Quercetin
Exotic					
0	2425.83 ± 122.02 a	2.43 ± 0.10 a	7.12 ± 1.02 a	18.44 ± 1.92 a	4.02 ± 0.33 a
1	2465.67 ± 141.65 a	2.30 ± 0.16 ab	6.23 ± 1.61 ab	15.90 ± 4.44 ab	3.43 ± 0.26 ab
2	2246.33 ± 149.74 a	1.70 ± 0.51 b	5.54 ± 1.10 ab	15.22 ± 2.96 ab	2.96 ± 0.48 b
4	2148.41 ± 60.91 a	1.01 ± 0.16 c	3.94 ± 0.10 b	12.57 ± 0.93 ab	2.76 ± 0.26 b
6	2158.41 ± 139.15 a	0.75 ± 0.06 c	3.80 ± 0.47 b	9.78 ± 0.97 b	2.67 ± 0.38 b
Shiraz					
0	2666.46 ± 146.18 a	1.06 ± 0.17 a	38.82 ± 6.49 a	35.70 ± 1.92 a	2.29 ± 0.28 a
1	2336.47 ± 139.59 ab	1.10 ± 0.05 a	7.54 ± 0.10 b	9.20 ± 1.24 b	2.53 ± 0.21 a
2	2442.37 ± 124.64 a	0.56 ± 0.12 b	8.28 ± 0.85 b	9.47 ± 0.66 b	1.70 ± 0.13 b
4	2055.97 ± 93.86 b	0.66 ± 0.21 ab	2.59 ± 0.36 b	3.31 ± 0.44 c	1.66 ± 0.22 b
6	1402.11 ± 153.85 c	0.41 ± 0.26 b	1.44 ± 0.14 b	1.56 ± 0.15 c	1.38 ± 0.08 b

Numbers with different letters within the same column are significantly different ($p \leq 0.05$)

4.4.4.2 Changes of low molecular weight phenolic acid

The concentration of phenolic acids varied during 6 months of ageing process (Table 4.17). For the hydroxybenzoic acid group, no changes can be observed for the contents of syringic acid, *p*-hydroxybenzoic, salicylic, gallic acid of both wines. Hydroxybenzoic acid of both wine varieties showed the highest amount ($p<0.05$) at 4 months of ageing at concentration of 1.25 mg/L for Exotic and 1.07 mg/L for Shiraz wine. Protocatechuic acid seems to increase ($p>0.05$) during ageing process. The hydroxycinnamic acid group; cinnamic and *p*-coumaric acid contents of Exotic wine significantly increased ($p<0.05$) after ageing for 4 months from 0.81 to 1.50 mg/L and 4.84 to 6.59 mg/L, respectively whereas caffeic acid contents were not changed.

Table 4.17 The phenolic acid (mg/L) of wine during ageing

Ageing (month)	Phenolic acid composition (mg/L)							
	Syringic	*p*-hydroxy benzoic	Salicylic	Gallic	Proto catechuic	Cinnamic	*p*-Coumaric	Caffeic
Exotic								
0	5.52 ± 0.94 a	0.92 ± 0.06 b	0.69 ± 0.07 b	10.08 ± 0.28 a	1.15 ± 0.02 ab	0.88 ± 0.03 b	5.06 ± 0.31 b	4.04 ± 1.75 a
1	5.12 ± 0.65 a	0.64 ± 0.02 d	0.81 ± 0.12 b	9.78 ± 2.02 a	1.16 ± 0.06 ab	0.83 ± 0.14 b	4.91 ± 0.59 b	4.28 ± 1.68 a
2	4.27 ± 0.36 a	0.84 ± 0.07 bc	1.15 ± 0.19 a	9.92 ± 1.63 a	1.09 ± 0.14 b	0.82 ± 0.11 b	4.90 ± 0.43 b	4.32 ± 1.27 a
4	5.17 ± 0.17 a	1.25 ± 0.10 a	0.86 ± 0.07 b	9.28 ± 0.51 a	1.31 ± 0.15 ab	0.81 ± 0.03 b	4.84 ± 0.11 b	6.95 ± 2.75 a
6	4.63 ± 0.06 a	0.75 ± 0.01 cd	0.74 ± 0.06 b	10.23 ± 1.67 a	1.62 ± 0.37 a	1.50 ± 0.31 a	6.59 ± 0.02 a	8.54 ± 2.04 a
Shiraz								
0	3.29 ± 0.20 bc	0.52 ± 0.02 c	0.64 ± 0.02 ab	22.30 ± 3.03 a	1.11 ± 0.12 b	2.31 ± 0.18 a	2.46 ± 0.34 bc	1.51 ± 0.18 ab
1	3.85 ± 0.39 ab	0.63 ± 0.05 bc	0.58 ± 0.05 b	23.51 ± 1.27 a	1.08 ± 0.06 b	1.75 ± 0.14 b	3.18 ± 0.04 a	1.63 ± 0.03 ab
2	3.01 ± 0.06 c	0.63 ± 0.03 bc	0.61 ± 0.01 ab	16.58 ± 1.46 b	1.06 ± 0.02 b	1.92 ± 0.11 ab	3.06 ± 0.06 ab	1.40 ± 0.03 b
4	4.36 ± 0.26 a	1.07 ± 0.26 a	0.70 ± 0.04 a	20.36 ± 0.67 ab	1.28 ± 0.21 ab	1.82 ± 0.17 b	2.66 ± 0.15 bc	1.64 ± 0.03 a
6	3.63 ± 0.40 abc	0.87 ± 0.02 ab	0.69 ± 0.02 a	19.82 ± 0.90 ab	1.48 ± 0.12 a	1.73 ± 0.18 b	2.57 ± 0.16 bc	1.40 ± 0.01 b

Numbers with different letters within the same column are significantly different ($p \leq 0.05$)

For Shiraz wine, there were no significant differences ($p>0.05$) for all hydroxycinnamic acid content during ageing. Cinnamic acid seemed to decrease with times, while there were no changes on the *p*-coumaric and caffeic acid contents. These observations did not agree with the results of del Alamo Sanza, Dominguez, Carcel, and Gracia (2004) work who observed the increasing in syringic, gallic, protocatechuic, *p*-coumaric and caffeic acid content of wine aged with oak staves, chips and barrles. These phenolic acids come from the hydroalcoholysis of oak wood (del Alamo Sanza et al., 2004). Phenolic acids also involved in co-pigmentation processes with anthocyanin (Brouillard and Dangle, 1994). These two reactions might be simultaneously take place during ageing. As compared to this study, additional amount of oak chips of 0.5 g/L was about five times lower than del Alamo Sanza et al. (2004) work (2.67 g/L). The addition of 0.5 g/L oak chips to wine samples related to the lesser amount of released phenolic acid into wine.

4.4.4.3 Changes of color parameters

Chemical density (CD) value of both wines slightly decreased after ageing process from 7.52 to 5.68 for Exotic ($p>0.05$) and 4.00 to 2.20 for Shiraz wine ($p<0.05$) as shown in Table 4.18. This result was similar to those reported by Gomez-Plaza et al. (1999) who observed that CD decreased with time. The formations of anthocyanin-acetaldehyde-catechin polymer lead to the formation of highly colored intermediates with an increase in the maximum of absorbance of 520 nm. Higher tint and chemical age (CA) values, that is, the wines are more polymerized (Gomez-Plaza et al., 1999). Wine tint of Exotic slightly decreased ($p>0.05$) from 1.04 to 0.89 during ageing process, while this value significantly increased ($p<0.05$) from 0.81 to 1.02 for

Shiraz wine. CA values of both wines increased from 15.14 to 27.62 for Exotic ($p<0.05$) and 13.83 to 24.93 for Shiraz ($p>0.05$). As a result of the increasing of CA value, it was postulated that the polymerization process occurred in ageing process.

Table 4.18 Color measurement of Exotic and Shiraz wine during ageing

Ageing (month)	CD	Tint	CA	TA	TCA
Exotic					
0	7.52 ± 1.05 a	1.04 ± 0.19 a	15.14 ± 2.88 b	169.27 ± 43.56 a	40.11 ± 3.30 a
6	5.68 ± 0.54 a	0.89 ± 0.10 a	27.62 ± 0.16 a	62.33 ± 1.60 b	28.05 ± 1.17 b
Shiraz					
0	4.00 ± 0.20 a	0.81 ± 0.04 b	13.83 ± 0.48 a	116.57 ± 2.59 a	23.20 ± 1.87 a
6	2.20 ± 0.19 b	1.02 ± 0.04 a	24.93 ± 10.44 a	34.70 ± 7.97 b	6.58 ± 4.95 b

Numbers with different letters within the same column are significantly different ($p\leq0.05$)

Total anthocyanin (TA) and total colored anthocyanin (TCA) of Exotic and Shiraz wine significantly decreased ($p<0.05$) after ageing for 6 months. The three different mechanisms of anthocyanins decreasing were the formation of stable polymer by co-pigmentation with flavan-3-ols (Mazza, 1995) or flavonols (Francia-Aricha, Guerra, Rivas-Gonzalo, and Santos-Buelga, 1997), acetadehyde-mediated condensations (Timberlake and Bridle, 1977) and/or the formation of copolymers with the quinone of the caftaric acid and anthocyanins (Cheynier, Souquet, Kontex, and Moutounet, 1994). These complexes might further precipitate (Mazza and Miniati, 1993).

4.5 Conclusions

The phenolic contents of wine samples appeared to vary with grape varieties and vinification process. Exotic grape variety contained higher amount of TPC, TA and TCA than those of Shiraz both in their juices and skin extract. *t*-Resveratrol was only detected in the skin extract, but not detected in juices. The vinification process; alcoholic fermentation, malolactic fermentation (MLF) and ageing process had influences on the phenolic compositions of wine.

In alcoholic fermentation with different of yeast strains, the three yeast strains produced similar changes of phenolic profiles and concentrations ($p>0.05$) at the end of fermentation. The concentration of *t*-resveratrol increased up to the third day of fermentation and decreased after day 6. The addition of pure *t*-resveratrol to the must prior to fementation lead to the higher amount of *t*-resveratrol concentration in the wine during fermentation. It was surprised that the wine fermented with and without added *t*-resveratrol showed similar concentration ($p>0.05$) of resveratrol at the end of alcoholic fermentation. TPC of both wine varieties increased up to day 3 of fermentation and remained constant or slightly decreased until the end of alcoholic fermentatin. The flavonoid and low molecular weight phenolic acids also increased up to 2 - 6 day of fermentation and decreased thereafter. The addition of pure *t*-resveratrol in the fermentation process did not affect ($p>0.05$) the amount of these flavonoid as well as phenolic acids. After alcoholic fermentation, CD, TA and TCA values of both wine varieties also increased for all treatments. The Exotic wine contained higher amount of these color parameters than those of Shiraz.

The amount of *t*-resveratrol increased after Malolactic fermentation (MLF). MLF lead to releasing of *t*-resveratrol into the wine sample by an enzymatic activity

of lactic acid bacteria. The highest increasing of resveratrol content was found in wine with added 10 ppm pure resveratrol to the must prior to fermentation. Three of yeast strains produced the significant differences ($p<0.05$) in resveratrol concentration during MLF. TPC of both wines slightly decreased during MLF. Some of phenolic compounds such as gentisic, *p*-hydroxybenzoic, salicylic, protocatechuic and caffeic acid were generated in this stage. CD value of two wine varieties significantly increased ($p<0.05$) during MLF for all yeast strains. The Exotic wine showed significantly higher ($p<0.05$) in CD value than Shiraz. There were not or slight color polymerization during MLF. The addition of commercial *t*-resveratrol in fermentation process also did not affect on flavonoid and phenolic acid contents.

In ageing process with oak chips at 15°C, TPC including the health benefit compound such as *t*-resveratrol, epicatechin, catechin and quercetin diminished with times. No changes can be observed ($p>0.05$) for syringic, hydroxybenzoic, salicyclic and gallic acid of both wine varieties. Protocatechuic acid content of both wines increased ($p<0.05$) after 4 months of ageing. Cinnamic acid seemed to decrease with times, while there were no changes of the *p*-coumaric and caffeic acid contents. CD, TA and TCA values of both wine varieties significantly decreased ($p<0.05$) during ageing for 6 months, whereas CA values increased.

4.6 Refferences

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CHAPTER V

CONCLUSION

Grapes and wines contain several of phenolic compounds. Crushed grape with seeds contained higher amount of TPC and free radical scavenging property than the without seed. Red grape juices and wines had significantly higher ($p<0.05$) antioxidative activities than those of the white wines. Wine produced from the different vintage years had different phenolic compounds.

In vinification of red wine, the phenolic content of wine samples appeared to vary with grape varieties and vinification process. Exotic grape variety contained higher amount of TPC, total anthocyanin than those of Shiraz grape both in their juices and skin extract. *t*-Resveratrol was only detected in the skin extract, not in juices. The vinification process; alcoholic fermentation, malolactic fermentation and ageing process influnced on the phenolic compositions of wine. Differences of phenolic compounds were found between wines produced from Exotic and Shiraz varieties. The three different yeast strains produced similar changes of phenolic profiles and concentrations ($p>0.05$) at the end of fermentation. The addition of pure *t*-resveratrol had an effect only on the concentration of *t*-resveratrol, but not on other phenolic compounds. MLF lead to releasing more of *t*-resveratrol in wine sample. Some of phenolic acids such as gentisic, *p*-hydroxybenzoic, salicylic, protocatechuic and caffeic acid were generated on MLF. The ageing process caused the decreasing of all health beneficial compounds. The color of wine increased during alcoholic and

malolactic fermentation. A polymerization reaction of colored pigment occurred during ageing process.

These investigations provided the information of health benefits of selected grapes and wines produced from the SUT Farm that red grape and wines have a better health benefit for consumption. Furthermore, information of changes of phenolic compounds during vinification revealed that longer ageing time would reduce more health beneficial phenolic compounds in the wines.

BIOGRAPHY

Jirayus Woraratphoka was born in March 22, 1980 in Maung, Nakhon Ratchasima, Thailand. She attended in Suranaree Wittaya II School and graduated in 1997. She received a Bachelors Degree in Food Technology from Khonkean University, Khonkean in 2001. She continued her Master Degree in School of Food Technology at Institute of Agricultural Technology, Suranaree University of Technology. While she was studying in Master Degree, she had experiences by working as the teaching assistant in school of food technology, SUT, in Food Chemistry and Food Analysis.

PRESENTATIONS

Woraratphoka, J., and Intarapiphet, K. (2004). Phenolic contents and antioxidative properties of some grapes and wines of SUT Farm. 10th World Congress on Clinical Nutrition, November 30 - December 3, 2004, Pearl Village, Phuket, Thailand.