Chapter 2

Review of Literature

2.1 Phenolic compounds

Phenolic compounds are a group of chemical compounds that are widely distributed throughout nature. They are simple compounds present in most fresh fruits, vegetables and herbs, or complex compounds present in bark, root and leaves of plants. A group of polyphenols, responsible for the color of many fruits, vegetables, spices, herbs and flowers are known as anthocyanins. There are several important classes, listed in Table 2.1, of phenolic compounds. According to the basic skeleton, the structure of natural polyphenols varies from simple molecules, such as simple phenols (volatile phenols), to highly polymerized compounds, such as condensed tannins (Harborne, 1980).

Volatile phenols

Simple phenols such as Phenol, o-cresol, 4-ethyphenol, guaiacol, 4-vinylguaiacol and eugenol have been found in volatiles of fruits and vegetables (Buttery *et al.*, 1971; 1976; Tatum *et al.*, 1975). The 4-ethylguaiacol, eugenol and 4-ethylphenol, a group of odorants with high flavor dilution (FD) factors, are a key difference between young and aged red wines (Margarita *et al.*, 2001).

Phenolic acids

Hydroxylbenzoic and hydroxycinnamic acid are the predominant phenolic acids found in plants. Differences between their derivatives consist of the different patterns of hydroxylations and methoxylations of their aromatic rings (Macheix *et al.*, 1990). The structures of some of these compounds are shown in Figures 2.1 (a) and (b).

Hydroxybenzoic acid

Hydroxybenzoic acid has a general structure of C6-C1 (Figure 2.1 (a)) Hydroxybenzoic acid is commonly present in bound form. They are the components of a complex structure such as hydrolysable tannins and lignins. Hydroxybenzoic acids are also found in the form of sugar derivatives (Schuster and Herrmann, 1985). The hydroxybenzoic acids content in foods of plant origin are generally low (Mosel and Hermann, 1974). Gallic acid is one of the most common hydroxybenzoic acids. Its dimeric condensation product and related dilactone, ellagic acid are commonly found in plants. There is a particular interest in ellagic acid in fruits because of the increasing evidence of its anti-carcinogenic and antioxidant effects (Maas and Galleta, 1991; Meyer *et al.*, 1998).

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Table 2.1 The most important classes of phenolic compounds in plants.

Basic skeleton	Class	Examples
C_6	Simple phenols	Phenol, guaiacol
	Benzoquinones	2, 6 -Dimethoxybenzoquinone
C_6-C_1	Hydroxybenzoic acids	Gallic, p-hydroxybenzoic, salicylic
C_6-C_2	Acetophenones	3-Acetyl-6-ethoxybenzaldehyde
	Phenylacetic acids	p-Hydroxyphenylacetic
C_6-C_3	Hydroxycinnamic acids	Caffeic, ferulic, p-coumaric
	Phenylpropenes	Myristicin
	Coumarins	Aesculetin
	Isocoumarins	Bergenon
	Chromones	Eugenin
C_6-C_4	Naphthoquinones	Juglone
C_6-C_1- C_6	Xanthones	Mangiferin
C_6-C_2- C_6	Stilbenes	Resveratrol
	Anthraquinones	Emodin
C_6-C_3- C_6	Flavonoids	Quercetin, catechin
	Isoflavonoids	Genistein
$(C_6$- $C_3)_2$	Lignans	Pinoresinol
	Neolignans	Eusiderin
$(C_6$- C_3- $C_6)_2$	Biflavonoids	Amentoflavone
$(C_6$- $C_3)_n$	Lignins	
$(C_6)_n$	Catechol melanins	
$(C_6$- C_3- $C_6)_n$	Condensed Tannins	

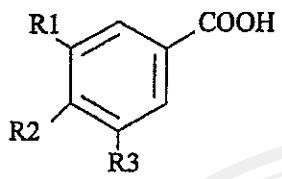
Source: Harborne, 1980.

Hydroxycinnamic acids

Hydroxycinnamic acids (Figure 2.1 (b)) are also found commonly in foods of plant origin. p-Coumaric, caffeic, ferulic and sinapic acids are the major hydroxycinnamic acids found in fruit (Macheix *et al.*, 1990). Among these, caffeic acid is the predominant hydroxycinnamic acid in many fruits. Over 75% of the total hydroxycinnamic acids are caffeic acids. Caffeic acid has been found in plums, apples, apricots, blueberries and tomatoes (Macheix *et al.*, 1990).

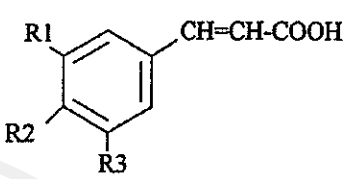
Hydroxycinnamic acids are mainly present in bound form and are rarely found in the free form. Hydroxycinnamic acids usually occur in various conjugated forms. The conjugated forms are ester of hydroxyacids such as quinic, shikimic and tartaric acid and their sugar derivatives. The free hydroxycinnamic acids can be released from chemical or enzymatic hydrolysis during tissue extraction (Schuster and Herrmann 1985; Macheix *et al.*, 1990).

(a)



R1	R2	R3	Compound
H	OH	H	p-Hydroxybenzoic acid
OH	OH	OH	Gallic Acid
H	OH	OH	Protocatechuic acid

(b)



R1	R2	R3	Compound
H	OH	OH	Caffeic Acid
CH_3O	OH	H	Ferulic acid
H	OH	H	p-Coumaric acid

Figure 2.1 (a) Benzoic derivatives (b) Cinnamic derivatives (Shahidi and Naczk, 1995).

Flavonoids

Flavonoids represent the most common and widely distributed group of plant phenolics. Their common structure (C6-C3-C6) consists of two aromatic rings (A ring and B ring) linked through a three-carbons-bridge that is usually an oxygenated heterocycle (C ring) (Harborne, 1980). Figure 2.2 (a) shows the basic structure and the system used for carbon numbering of the flavonoids nucleus. The major flavonoid classes include anthocyanidins, chalcones, flavanols, flavonones, flavones, flavonols and isoflavones. The variability of the flavonoids is based on the hydroxylation of the pyrone ring, absence or presence of a double bond, the number of hydroxyls in A ring and B ring, and/or a double bonded oxygen atom attached to position 4 of the C ring. Flavonoids may be monomeric, dimeric or oligomeric. Polymeric flavonoids, known as tannins, are divided into two groups, condensed and hydrolysable. Condensed tannins are polymers of flavonoids while hydrolysable tannins contain gallic acid.

Flavanols and flavonols

Flavanols known as flavan-3-ols (Figure 2.2(b)), the subunits of proanthocyanidins, have a hydroxyl group attached to the 3 position of the C ring. They have no positive charge on the oxygen atom and no double bond in the C ring. The structure of flavonols (Figure 2.2 (c)) are very similar to those of flavonols, except that there is a double-bonded oxygen atom attached to position 4 of C ring and a double bond in the C ring.

Flavanones and flavones

Flavanones (Figure 2.2 (d)) and flavones (Figure 2.2 (e)) have structures similar to those of flavanols and flavonols, respectively. For each case though, there is no longer a hydroxyl group attached to the 3 position of the C ring.

Anthocyanins

Anthocyanins are widely distributed among fruits, vegetables, spices and herbs. They are one of the main classes of flavonoids. They contribute significantly to the antioxidant activities of the flavonoids (Lapodot *et al.*, 1999). Anthocyanins are water soluble pigments responsible for red, blue and violet colors. Anthocyanins (Figure 2.2 (g)) are glycosylated anthocyanidins, an aglycone with sugars generally attached to the 3-hydroxyl position of the anthocyanidin (Figure 2.2 (f)). In some cases, the sugar residues are acylated by *p*-hydroxybenzoic, *p*-coumaric, caffeic, feruic, sinapic, acetic acid, oxalic acid, malic acid or succinic acid. It means that there

is no sugar group or other functional group attached to the flavone nucleus. Also, the oxygen atom in the C ring has a positive charge on it, and there are two double bonds in the C ring. In addition to hydroxylated anthocyanidins, such as delphinidin, cyanidin and prlargonidin, there are also methylated anthocynidins (malvidin, peonidin and petunidin).

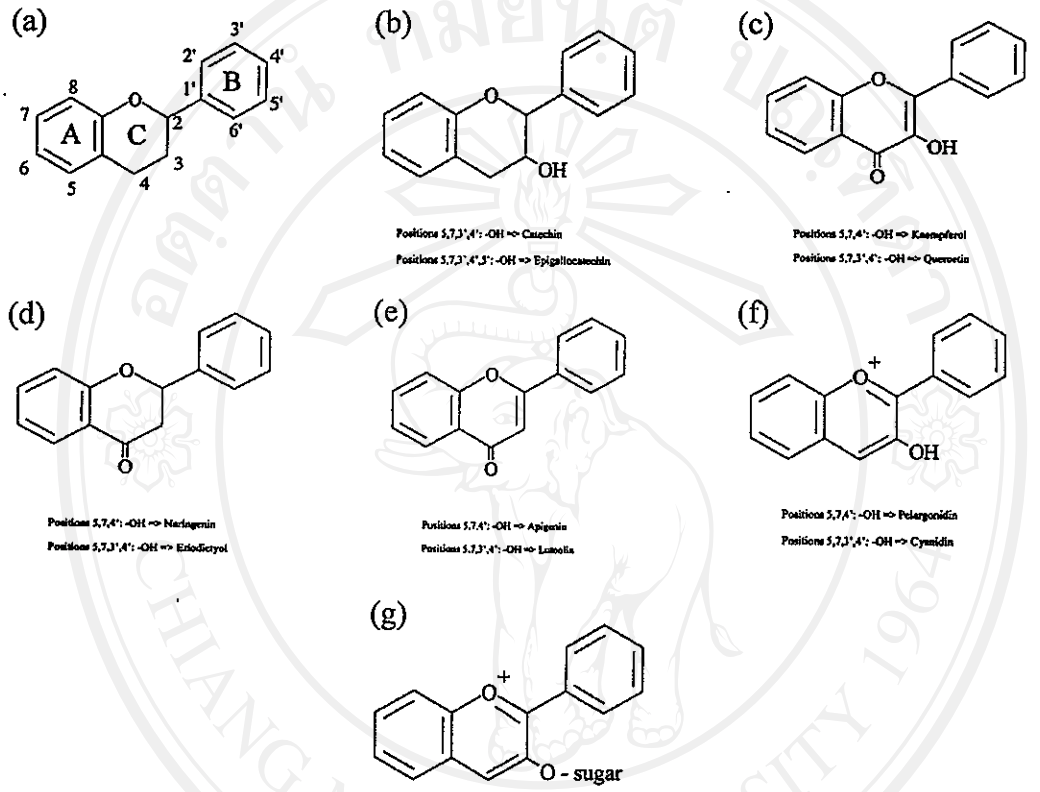


Figure 2.2 (a) Basic flavonoid skeleton (b) Basic flavan-3-ol skeleton (c) Basic flavonol skeleton (d) Basic flavanone skeleton (e) Basic flavone skeleton (f) Basic anthocyanidin skeleton (g) Basic anthocyanin skeleton (Pietta, 2000).

2.2 Lipid oxidation and antioxidant activity

In the biological system, lipids can undergoing oxidation is one of the major causes of food deterioration. Oxidation of lipids produces toxic compounds and initiates other changes in biological systems. In foods, these reactions can lead to the loss of nutritional value, quality changes such as color, texture and flavor and also the formation of organic free radicals. The formation of reaction organic free radicals is mediated by a number of factors such as high oxygen tension, light, heat, metal ions, enzymes and radiation.

The mechanism of lipid oxidation (Figure 2.3) consists of three phases: (1) initiation (the formation of free radicals); (2) propagation (the free-radical chain reaction); and (3) termination (the formation of non-radical products). In figure 2.4 and 2.5, RH is an unsaturated fatty acid. R·, RO·, or ROO· are free radicals formed by

removing a hydrogen from a carbon atom adjacent to a double bond. ROOH is a hydrogen peroxide, which is one of the major initial oxidation products that can decompose to form compounds (such as pentanal, hexanal and malonaldehyde) responsible for off-flavors and odors (Shahidi and Naczk, 1995).

Initiation :
RH --> R• + •H
RH + O_2 --> R• + •OH
Propagation :
R• + O_2 --> ROO•
ROO• + RH --> R• + ROOH
ROOH --> RO• + HO•
Termination :
R• + R• --> RR
R• + ROO• --> ROOR
ROO• + ROO• --> ROOR + O_2
Figure 2.3 Three phases of lipid oxidation (Shahidi and Naczk, 1995).

ROO• + PA-H --> ROOH + PA•
RO• + PA-H --> ROH + PA•
ROO• + PA• --> ROO-PA
RO• + PA• --> RO-PA
Figure 2.4 Antioxidant activities of phenolic antioxidants (Shahidi and Naczk, 1995).

ROOH + Fe^{2+} ---> RO• + Fe^{3+}
RO• + Fe^{2+} + H+ ---> ROH + Fe^{3+}
Fe^{3+} + SSCH- ---> Fe $(SCN)_S^{2-}$
Figure 2.5 Formation of ferric thiocyanate complex (Shahidi and Naczk, 1995).

Antioxidants can interrupt the free-radical chain of oxidative reactions (Figure 2.5) by contributing hydrogen to lipid radicals. The resulting phenoxy radical itself should be a stable free radical. The resulting phenoxy radical itself should be a stable free radical which does not initiate or propagate further oxidation of lipids (Shahidi and Naczk, 1995). Antioxidants also can interrupt the lipid oxidation by acting as an oxygen scavenger, and chelating catalytic metals.

2.3 Oxidative stress and Atherosclerosis

Reactive oxygen and nitrogen species, ROS/RNS are essential to the energy supply, detoxification, chemical signaling and immune system function. They are continuously produced in the human body and controlled by endogenous enzymes (superoxide dismutase, glutathione peroxidase and catalase). When there is an over-production of these species, and exposure to external oxidant substances or a failure in the defense mechanisms, damage to valuable biomolecules (DNA, lipid and proteins) may occur (Aruoma, 1998). This damage has been associated with an increased risk of cardiovascular disease, cancer and other chronic diseases.

This sequence of events is of particular relevance to the development of atherosclerosis. A characteristic feature of atherosclerosis is the presence of unable to

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accumulate significant amounts of native low-density lipoprotein (LDL) regulated by accumulated intracellular cholesterols. In contrast, the uptake of oxidized LDL is rapid and not subject to down-regulation. Oxidized LDL is also chemotactic for macrophages and cytotoxic to the vascular endothelium. Although oxidative mechanisms are clearly not the only pathophysiological events relevant to atherogenesis it is clear that oxidation of lipoproteins represents a significant biological threat and should ideally be prevented (Figure 2.6).(Maxwell, 1997).

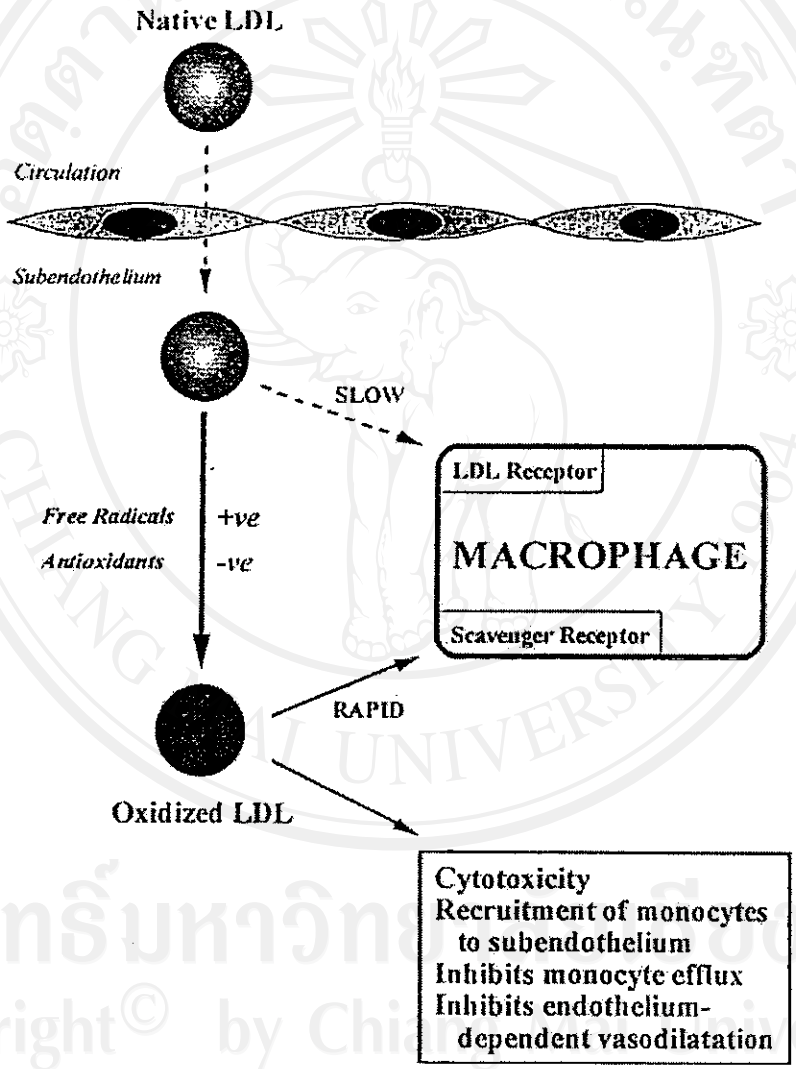

Figure2.6 The oxidative-modification theory of atherosclerosis (Maxwell, 1997).

A number of natural antioxidant mechanisms exists mainly to protect against the ever present threat of oxidation LDL. These include proteins such as transferring, caeruloplasmin and albumin that limit the availability of metal ions. Another very important protective influence is the presence of scavenging antioxidant molecules that are readily sacrificed (oxidized) in preference to more important targets such as LDL. Most of the important sacrificial antioxidants are derived from the diet and

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include vitamins such as ascorbate (vitamin C) and alph-tocopherol (vitamin E) and polyphenolic flavonoids derived from sources such as wine, tea and vegetables. The potent antioxidant properties of dietary flavonoids have stimulated interest in whether flavonoid-rich diets might offer any protection against diseases such as atherosclerosis and cancer, where oxidation is thought to play an important role. Preliminary evidence from epidemiological studies suggests that high dietary antioxidant intake may indeed protect against the development of vascular disease (Maxwell, 1997).

Table 2.2 Top sources of antioxidant plant phenols

Sources	Antioxidants
Fruits	
Berries	Flavanols hydroxycinammic acids, hydroxybenzoic acids, anthocyanins
Cherries	Hydroxycinnamicacids, anthocyanins
Blackgrapes	Anthocyanins, flavonols
Citrus fruits	Flavanones, flavonols, phenolic acids
Plums, prunes, apples, pears, kiwi	Hydroxycinnamic acids, catechins
Vegetables	
Aubergin	Anthocyanins, , hydroxycinnamic acids
Chicory, artichoke	Hydroxycinnamic acids
Parsley	Flavones
Rhubarb	Anthocyanins
Sweet potato leaves	Flavonols, flavones,
Yellow onion, curly, Kale, leek	Flavonols
Parsley	Flavones
Beans	Flavanols
Spinach	Flavonoids, *p*-coumaric acid
Flours	
Oats, wheat, rice	Caffeic and ferulic acids
Teas	
Black, green	Flava-3-ols, flavonols
Alcoholic drinks	
Red wine	Flavan-3-ols, flavonols, anthocyanins
Cider	Hydroxycinnamic acids
Other drinks	
Orange juice	Flavanols
Coffee	Hydroxycinnamic acids
Chocolate	Flavanols
Herbs and spices	
Rosemary	Carnosic acid, carnosol, Rosmarinic acid, rosmanol
Sage	Carnosol, Carnosic acid, lateolin, rosmanul, rosmarinic acid, Rosmarinic acid
Oregano	Rosmarinic acid , phenolic acids, flavonoids
Thyme	Thymol, carvacrol, flavonoids, lubeolin
Summer savory	Rosmarinic, carnosol, carvacrol, flavonoids
Ginger	Gingerd and related companids

Source: Dimitrios (2006).

The antioxidant hypothesis says that 'as antioxidants can prevent oxidative damages, increased intakes from the diet will also reduce the risks of chronic

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diseases' (Stanner *et al.*, 2004). This explains the huge volume of research work and efforts of many researchers to investigate natural antioxidants and degenerative diseases.

Top antioxidant sources are fruits and vegetables. The most important sources and the classes of phenols they contain, are briefly presented in Table 1.

2.4 Methods for assessing total antioxidant activity and total free radical scavenging activity.

Several methods have been utilized to assess the total antioxidant activity of fruits, vegetables, spices, herbs and their products, such as the ferric thiocyanate (FTC) method (Larrauri *et al.*, 1997), the oxygen radical absorbance capacity (ORAC) assay (Ehlenfeldt and Prior, 2001; Kalt *et al.*, 2001), the Trolox-equivalent antioxidant capacity (TEAC) (Sellappan *et al.*, 2002) and heat-induced oxidation of an aqueous emulsion system of β–carotene and linoleic acid or β–carotene bleaching assay (Fukumoto and Mazza, 2000; Yan *et al.*, 2002; Chanwitheesuk *et al.*, 2005). The most widely used total free radical scavenging activity is the DPPH method (Fukumoto and Mazza, 2000).

2.4.1 Heat-induced oxidation of an aqueous emulsion systems of β-carotene and linoleic acid or β–carotene bleaching assay.

For measuring antioxidant activity, β-carotene bleaching and reacting compounds with free radicals are quick and simple methods for measuring potential antioxidant activity. In this method, antioxidant activity is measured by the ability of samples (plant extracts or phenolic compounds) to minimize the loss of β- carotene, aqueous emulsion system of β-carotene and linoleic acid (Marco, 1968). The oxidation of linoleic acid is usually induced by heat. This method can be used in measuring pure compounds with different structures or samples containing different compounds. The reason is that this method is basically based on the inhibition of linoleic acid oxidation reaction by samples. In this method, an aqueous emulsion system of β-carotene and linoleic acid is very important. A successful assay must have a good emulsion system. Usually, Tween 20 is used as an emulsifier. Marco (1968) used this method to rank compounds for their antioxidant activity. Until now, many researchers used this method to measure the antioxidant activities of phenolic compounds (Chanwitheesuk *et al.*, 2005; Burda and Oleszek, 2001; Vilioglu *et al.*, 1998; Jialal *et al.*, 1991) and in-vitro antioxidant and ex-vivo protective activities of green and roasted coffee (Daglia *et al.*, 2000).

2.5 Impact of red wine on antioxidant status

Phenolic compounds are responsible for some of the major organoleptic properties of wines, in particular color and astringency. Wines phenolic composition depends on the grapes used to make the wine and on the vinification conditions (Cheynier *et al.*, 1997). Polyphenolic components of wine fall into one of two major classes. Non-flavonoids comprise hydroxybenzoates and hydroxycinnamates. Flavonoids include flavonols (e.g. quercetin, myricetin), flavan-3-ols (e.g. catechin and epicatechin), as well as polymers of the latter, defined as procyanidins, and anthocyanins that are the pigments responsible for the color of red wines; collectively

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they are 20-fold higher in red than in white wine and other beverages (Figure 2.7) (Soleas and Goldberg, 1999; Maxwell, 1997).

The flavonoid content of red wine has been suggested as an explanation of the "French paradox", i.e. the fact that French people have low incidence of coronary heart disease, despite having a diet high in fat and being heavy smokers (Aruoma, 1996). The mechanism of this protective action of the flavonoids is the subject of considerable debate. As polyphenolic compounds, flavonoids have the ability to act as antioxidants by a free radical-scavenging mechanism (with the formation of less reactive flavonoid phenoxyl radicals) and the metal ion chelation as well (Arora *et al.*,1998; Lodovici *et al.*, 2001). A wide range of studies have shown the antioxidative properties of these compounds in protection against artherosclerosis and coronary heart disease (Estruch, 2000; Santos-Buelga and Scalbert, 2000; Stupans *et al.*,2002; Sun *et al.*, 2002; Visioli *et al.*,2000). Other effects include modulation of eicosanoid synthesis toward a more antiatherogenic pattern and inhibition of tumor growth in vitro and in human cancer patients (Soleas *et al.*, 1997). Thus, the inhibition of human LDL was demonstrated by the addition of the mixture of polyphenols from wine (Frankel *et al.*,1993). Red wine diluted 1000-fold inhibited the in vitro oxidation of human LDL significantly more than α-tocopherol. Other effects of red wine include inhibition of chronic inflammation and thrombotic tendencies (Kinsella *et al.*,1993).

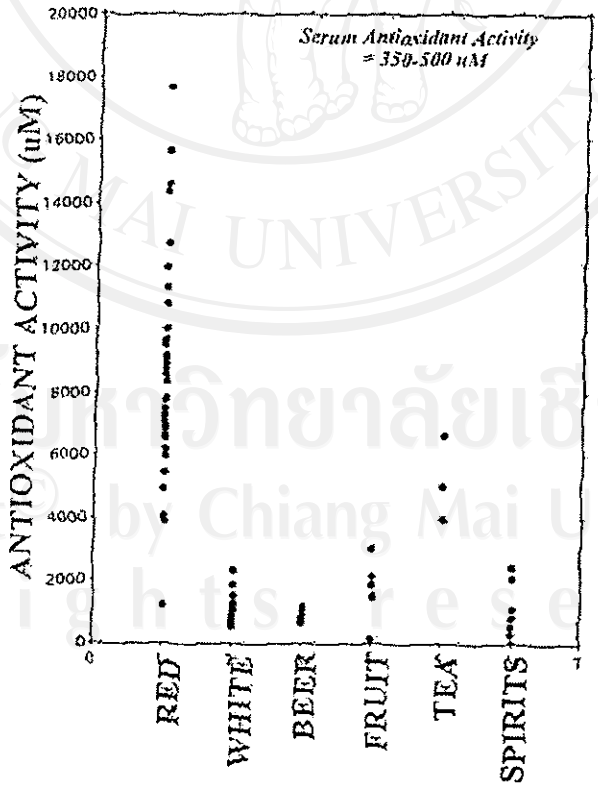

Figure 2.7 Comparison of the antioxidant activity (mol/l) measured by enhanced chemiluminescence of a variety of popular beverages (Maxwell, 1997).

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The flavonoids are the most lipophilic of the natural antioxidants, but less so than α- tocopherol. The α- tocopherol seems to be located in the lipid membrane within the phospholipids bilayer while the flavonoids are mainly located at the polar surface of the bilayer. The aqueous, i.e. transported in the plasma, free radicals would therefore be captured more easily by the flavonoids than by the less accessible α-tocopherol. Thus, the flavonoids could be concentrated near the membranous surface of the low-density lipoprotein (LDL) particles, ready to capture the oxygenated aqueous free radicals. They would in this way prevent the consumption of lipophilic α- tocopherol and thus delay oxidation of the lipids contained in the LDL. Moreover, the initiation phase and the propagation phase of lipid peroxidation takes place, respectively, at the surface and the interior of the membranes, then the flavonoids could well hinder the correct course of the reaction by limiting the initiation phase.

2.6 The overview of Krachai-Dam.

'Krachai-Dam' (*Kaempferia parviflora* Wall. ex Baker) is a herbaceous plant of Zingiberaceae family. Its rhizomes have been used traditionally as a health promoting, stimulating and vitalizing agent (Yenjai *et al.*, 2004; Sennil and Trichalee, 2002). Moreover, because of a belief in the bioreactivities (health promoting, health activating, aphrodisiac activities and etc.) of "Krachai-Dam' rhizomes and its products, "Krachai-Dam" has become very popular in groups of consumers and simultaneously provided good incomes for farmers: and it has been cultivated widely across the country (Thailand), especially the high land provinces with altitudes of 400 to 1400 above average sea level, such as Loei, Phitsnulok, Phetchabun, Tak, Nan, Chiang-rai and etc.

Consumers would purchase the black-internal skin Krachai-Dam rhizomes. According to their attitudes, the darkness of internal skin color was positively correlated with the vitalizing and stimulating agents' contents in the rhizomes. As the main criteria for the selection of the rhizomes for growing in the next season of the farmers was the dark purple to dark internal skin color of the rhizomes, and they used this internal skin color to determine the price and to grade the rhizome (Pojanagaroon and Kaewrak, 2003b). According to the botanical, chemical, pharmaceutical, genetically and agricultural aspects, Krachai-Dam can be divided into 2 main groups of cultivar: Green leaves (pale internal color of rhizomes) and Red leaves (dark internal color of rhizomes) groups (Pojanagaroon and Kaewrak, 2004a, b; 2005; Pojanagaroon *et al.*, 2006a, b). Department of Agriculture (DOA) had pronounced the two registered-Krachai-Dam cultivars, which were 'Phurua-10 (Rom-Klao)' and 'Phurua-12 (Kheg-Noi#2).' Their specific botanical characters were as follows:

'Phurua-10 (Rom-Klao)' is rhizomatous herbs, leafy shoots, 27-41 cm tall. **Rhizome** dark purple, aromatic. **Leaves** simple, alternate: petiole channeled, 2.27x7.24 cm; ligule 0.68x0.45 cm; lamina elliotic, lanceolate or ovate, 11.38x22.26 cm, base cordate apex acute, margin entire and undulate, green except slightly red or purple on the lower surface, midrib channeled on the upper surface and ribbed on the lower surface, lateral veins 8-10, glabrous on both surfaces; sheath glabrous, 0.45x3.36 cm, slightly red or purple on the abaxial surface. **Inflorescence** terminal on the leafy shoot, 0.35x11.2 cm; involucral bracts green, 1.51x3.68 cm. **Flowers** zygomorphic; calyx tubed, 1.5-2 cm long; dorsal petal 1, white and partly pellucid, cuneate, 3x11.88 mm; lateral petals 2, white and partly pellucid, cuneate, 2x11.37

mm; lateral stamenode 2, light yellow on the upper part, cuneate, 4x9.12 mm; labellum (lip) 1, purple, lugulate, 11.28x14.37 mm; fertile stamen sessile; ovary inferior; stigma hairy. **Fruit** globose.

'Phurua-12 (Kheg-Noi#2)' is rhizomatous herbs, leafy shoots, 29-45 cm tall. **Rhizome** light purple, aromatic. **Leaves** simple, alternate: petiole channeled, 2.85x7.59 cm; ligule 0.65x0.41 cm; lamina elliotic, lanceolate or ovate, 12.55x24x67 cm, base cordate apex acute, margin entire and undulate, pale green on the lower surface, midrib channeled on the upper surface and ribbed on the lower surface, lateral veins 8-10, glabrous on both surfaces; sheath glabrous, light green, 0.51x3.97 cm. **Inflorescence** terminal on the leafy shoot, 0.35x14.35 cm; involucral bracts green, 1.68x3.70 cm. **Flowers** zygomorphic; calyx tubed, 1.5-2 cm long; dorsal petal 1, white and partly pellucid, cuneate, 3.13x12.05 mm; lateral petals 2, white and partly pellucid, cuneate, 2.3x11.72 mm; lateral stamenode 2, light yellow on the upper part, cuneate, 3.09x13.67 mm; labellum (lip) 1, purple, lugulate, 10.98x12.98 mm; fertile stamen sessile; ovary inferior; stigma hairy; **Fruit** globose (Pojanagaroon *et al.*, 2006).

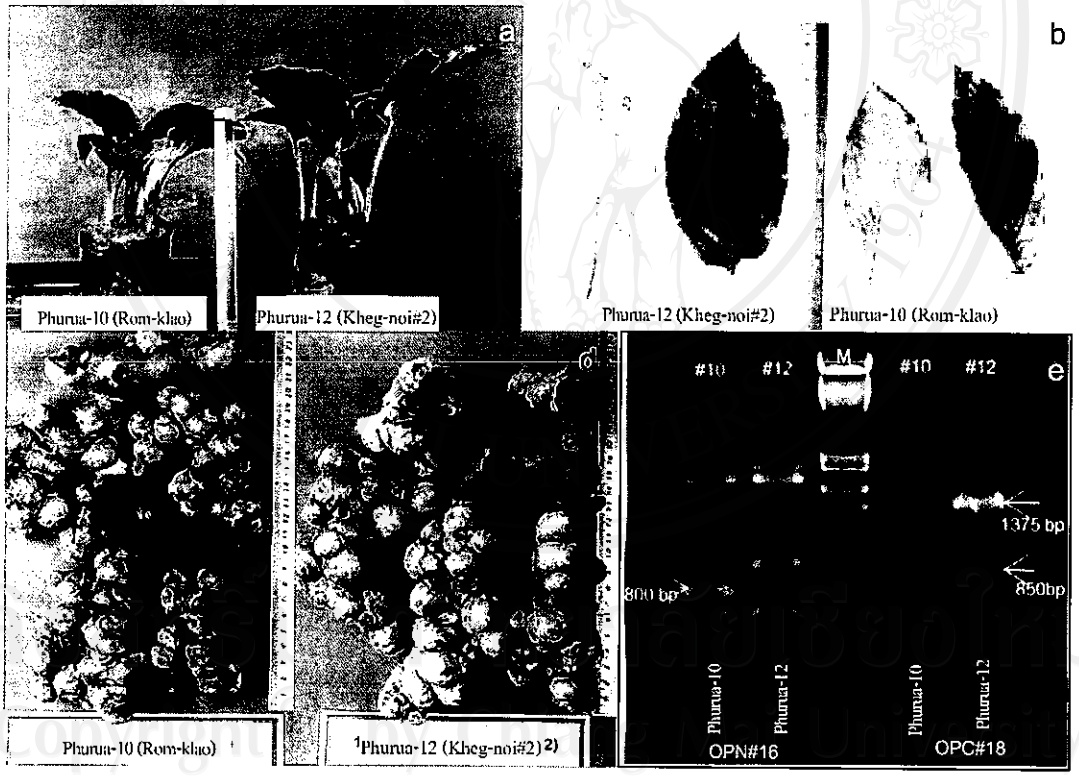

Figure 2.8 Botanical characters of *Kaempferia parviflora* Wall. ex Baker (Krachai-Dam) (Pojanagaroon *et al.*, 2006). (a) Plants of 'Phurua-10 (Rom-Klao)' (left) and 'Phurua-12 (Kheg-Noi#2)' (right); (b) Leaves of 'Phurua-12 (Kheg-Noi#2)' (left) and 'Phurua-10 (Rom-Klao)' (right); (c) Rhizomes of 'Phurua-10 (Rom-Klao)'; (d) Rhizomes of 'Phurua-12 (Kheg-Noi#2); and (e) RAPD patterns using Operon kit N (OPN-16) at 800 bp was the specific band for identification of 'Phurua-10 (Rom-Klao)' (left) and using Operon kit C (OPC-18) at 850 and 1,375 bp were the specific bands for identification of 'Phurua-12 (Kheg-Noi#2) (right).

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Currently, 'Krachai-Dam' has become an economically valuable plant. Its popularity has increased consumer's demand for 'Krachai-Dam' products, and there are various types of products on markets, which the Thai government tries to promote and develop into an export product. The taste, marketing image/fame, products, quality, vitalizing ability and stimulating properties which resemble Korean ginseng were the most important factors related to consumers' purchasing decision of 'Krachai-Dam' products. The most popular products were Krachai-Dam wine, Krachai-Dam honey wine and Krachai-Dam dry gin (Pojanagaroon and Kaewrak, 2003a).

According to the government policy and OTOP program, 'Krachai-Dam' has become a trendy export product. However, the lack of scientific studies for quality control would be a boundary to export 'Krachai-Dam' products. So, the studies focusing on quality control by standardizing the Krachai-Dam rhizomes, finding out the suitable plantation area, planting times, harvesting times, numbers of crops cycles, methods and duration for Krachai-Dam storage. In so doing, the Good Agricultural Practices (GAP) of Krachai-Dam can be drafted for 'Krachai-Dam honey wine' processing purposes which will meet the government policy and possibly make 'Krachai-Dam honey wine' a leading export products in the future.

2.7 Summary of the results of the extraction and isolation and biological activity testing in "Krachai-Dam" rhizomes

Based on the focuses of each previous study, there are two types of studies. Some studies have focused on the extraction and isolation, while others have focused on the biological activities of crude extract and isolated chemical constituents.

2.7.1 Extraction and isolation of "Krachai-Dam" rhizomes

To isolate the chemical constituents of 'Krachai-Dam' rhizomes, various types of solvents have been used during the extraction and isolation, such as Hexane, Dichloromethane, Chloroform and Methanol, giving different chemical compounds as the result. There are several types of compounds isolated from 'Krachai-Dam' rhizomes: flavonoid and chalcone derivatives. There are 21 compounds that have been already found in 'Krachai-Dam' rhizomes; 14 of 21 compounds are flavonoid derivatives, 6 of 21 compounds are chalcone derivatives and the last compound is kawain (Table 2.3). Most of flavonoid derivatives can be extracted by using Hexane and Dichloromethane, while the chalcone derivatives mostly can be extracted by using Chloroform and Methanol as solvents for extraction.

However, the conditions of the extraction also have much influence on quantity and the quality of isolated compounds, their details are as follow:

Jaipetch *et al.* (1983) showed that the 'Krachai-Dam' rhizomes were extracted by using Hexane as a solvent, and then crude extract was obtained as the result. The crude extract was subjected for further isolation, given 11 compounds as the result and then these isolated compounds were elucidated structures (Figure 2.9)

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Table 2.3 The isolated compounds of 'Krachai-Dam' rhizomes

	Flavonoids compounds	Hexane	Dichloromethane	Chloroform	Methanol
1	5-hydroxy-7-methoxyflavone	/	/		
2	5,7- dimethoxyflavone	/	/		
3	5- hydroxy-7-methoxyflavone	/		/	/
4	5- hydroxy-7,4'-dimethoxyflavone	/	/		
5	5,7,4'-trimethoxyflavone	/	/		
6	5,7,3',4'-tetramethoxyflavone	/	/		
7	5- hydroxy-3,7-dimethoxyflavone	/	/		
8	3,5,7- trimethoxyflavone	/	/		
9	5- hydroxy –3,7,3',4'-tetramethoxyflavone	/	/	/	
10	5- hydroxy –3,7,4'-trimethoxyflavone	/	/		
11	3,5,7,4'-tetramethoxyflavone		/	/	
12	5- hydroxy-7,4'-dimethoxyflavanone			/	
13	2'-hydroxy-4',6'-dimethoxychalcone (2',6'-dihydro-4'-methoxychalcone)			/	
14	2'-hydroxy-4,4',6'-dimethoxychalcone			/	
15	5,4'-dihydroxy-7-methoxyflavanone(Sakuranetin)			/	
16	5,7-dihydroxyflavanone(pinocembrin)			/	/
17	Dihydro-5,7-dehydrokawain			/	
18	- (-) hydroxypanduratin A. or 4-hydroxypanduratin A			/	/
19	– (-) panduratin A. or panduratin A			/	/
20	2',4',6'-trihydroxycyclone(pinocembrin chalcone)				/
21	2',4'-dihydroxyflavone-6'-methoxychalcone (cardamin)				/

Source: Jaipetch *et al.* (1983); Herunsulee *et al.* (1987); Tuchinda *et al.* (2002)

Herunsalee *et al.* (1987) studied 'Krachai-Dam' extraction by using Chlorofrom as the solvent. In the process, the milled 'Krachai-Dam' rhizomes were extracted with Chloroform in Soxhlet apparatus, giving heat to the extraction system. Crude extract was obtained and isolated to get pure compounds. The result of extraction and isolation was that 5 compounds were obtained and they were elucidated structures. There are 3,5,7,3' ,4'-pentamethoxyflavone (1), 5,3,7,4'-tetramethoxyflavone (2), 5-hydroxy-7,4'-dimethoxyflavanone (3), 2'-hydroxy-4',6,-dimethoxychalcone (4), 2'-hydroxy-4,4',6-trimethoxychalcone (5) respectively (Figure 2.10).

Tuchida *et al.* (2002) performed "Krachai-Dam" rhizomes extraction in a Soxhlet apparatus in the hot system by using separately and orderly boiling Hexane, Chloroform and Methanol as solvents, the crude extract of each solvent was obtained. The Chloroform crude extract was further isolated and purified, giving 6 compounds as the result. The obtained 6 compounds then elucidated the chemical structures (Figure 2.11).

Sutthanut *et al.* (2007) revealed the diversity of 11 methoxyflavonoid content in ethanolic crude extract of 12-different-origin Krachai-Dam rhizomes, three of them were Phurua-10, 5 and 12 which were used as raw materials for honey wine processing. Each methoxyflavonoid and total flavonoid contents were shown in Table 2.4.

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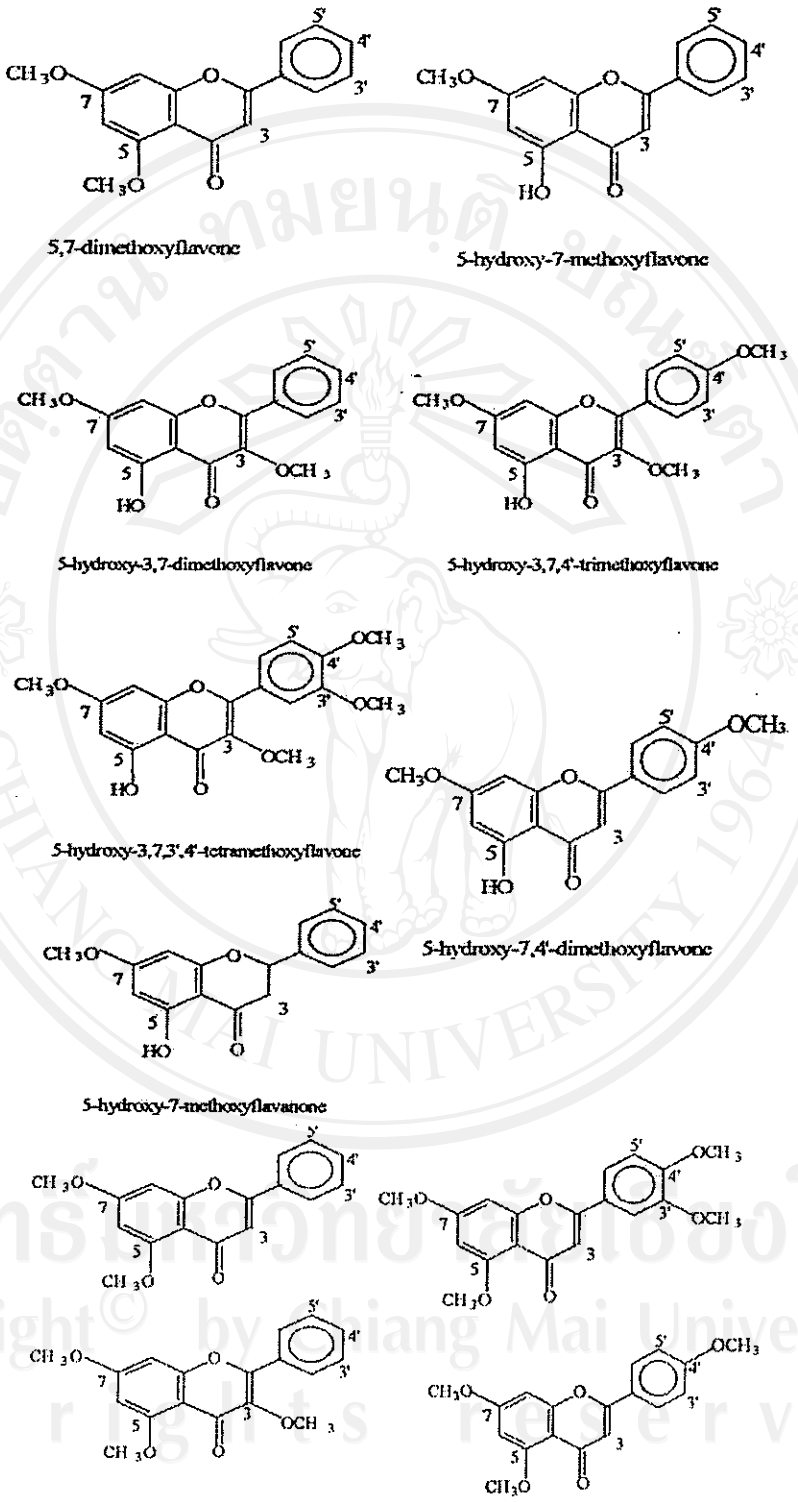

Figure 2.9 The chemical structure of 11 compounds in Krachai-Dam hexane extract (Jaipetch *et al.*, 1983)

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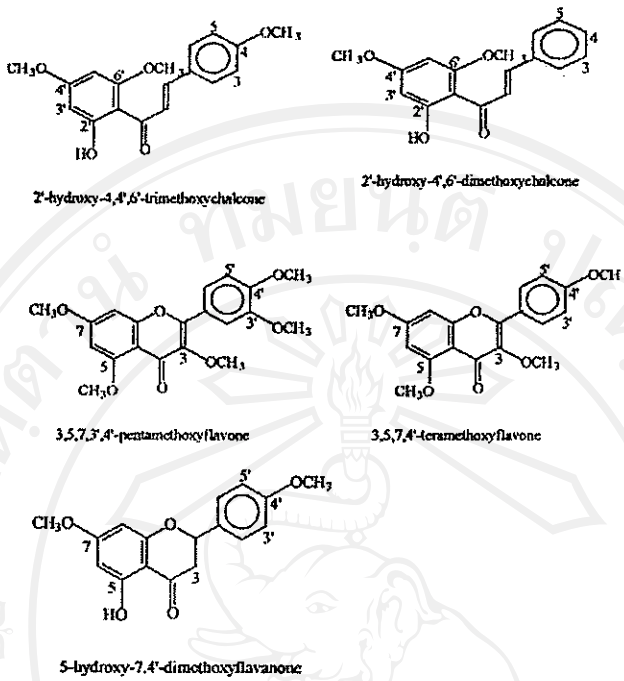

Figure 2.10 The chemical structure of 5 compounds in Krachai-Dam chloroform extracts (Herunsalee *et al.*, 1987).

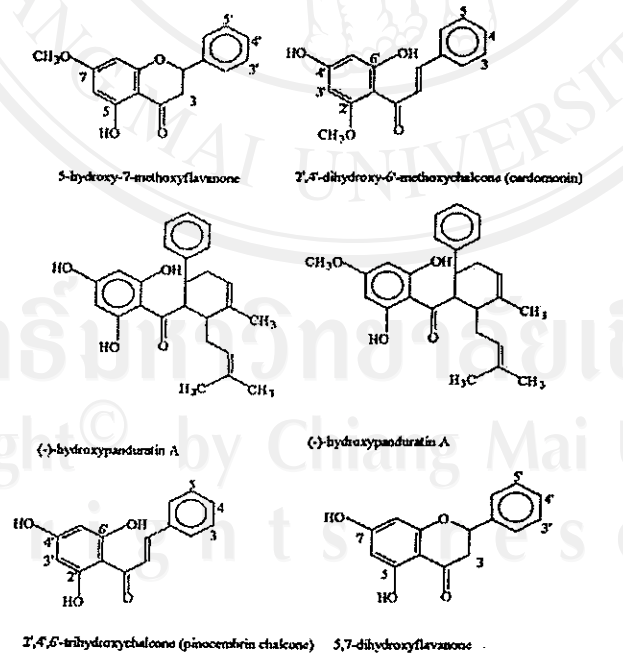

Figure 2.11 The chemical structure of 6 compounds obtained from hot system of boiling hexane, chloroform and methanol as solvents (Tuchinda *et al.*, 2002)

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Table 2.4 Yield and flavonoid content of 3 Krachai-Dam cultivars used as raw materials for honey wine production in this study.

Flavonoid content*	Krachai-Dam Cultivar		
	Phurua-5(Namjuang)	Phurua-10(Romklao)	Phurua-12(Khegnoi-2)
1. 5-hydroxy-7-methoxyflavone	1.946 [5.34]	2.172 [5.38]	0.922 [2.09]
2. 5-hydroxy-3,7-dimethoxyflavone	1.742 [4.78]	2.045 [5.07]	9.986 [22.69]
3. 5,7-dimethoxyflavone	9.166 [25.15]	8.789 [21.77]	20.143 [45.78]
4. 3,5,7-trimethoxyflavone	2.134 [5.86]	1.562 [3.87]	5.109 [11.61]
5. 5-hydroxy-3,7,4'-trimethoxyflavone	1.820 [4.99]	1.627 [4.03]	1.537 [3.49]
6. 5-hydroxy-7,4'-dimethoxyflavone	2.242 [6.15]	1.789 [4.43]	0.767 [1.74]
7. 5-hydroxy-3,7,3',4'-tetramethoxyflavone	0.459 [1.26]	0.362 [0.90]	0.000 [0.00]
8. 5,7,4'-trimethoxyflavone	4.129 [11.33]	5.491 [13.60]	2.825 [6.42]
9. 3,5,7,4'-tetramethoxyflavone	4.045 [11.10]	3.733 [9.24]	2.049 [4.66]
10. 5,7,3',4'-tetramethoxyflavone	1.638 [4.49]	2.941 [7.29]	0.367 [0.83]
11. 3,5,7,3',4'-pentamethoxyflavone	7.119 [19.54]	9.858 [24.42]	0.298 [0.68]
Total flavonoids (mg)	36.44	40.37	44.00
% Yield (ethanolic extract)	5.75	4.82	4.13
Major compound contents ratio (MCR)	2.220	1.601	7.130

*Content values of each compound were expressed as mg/g dried powder or [%] of total flavonoids; MCR : major compound contents ratio (compound no.3 / compound no.8).

Source: Sutthanut *et al.*, 2007.

2.7.2 Biological activity testing

After the extraction and isolation, some chemical constituents underwent the processes of biological activities testing. There are many activities that already have been tested in some chemical constituents, such as cytotoxic, anti-inflammutory, anti-mutagenic, anti-malarial, anti-fungal, anti-bacterial, anti-mycobacterial activities and anti-oxidation (Yenjai, *et al.*, 2002 and 2004; Tuchinda *et al.*, 2002; Trakoontivakorn, 2001; Patanasethanont et al., 2006). All flavonoid derivatives seem to have anti-oxidation activity, but unequal efficacy of each compound, due to their chemical structures (Ivonne M.C.M. R. *et al.*, 2002).

Table 2.5 The summary of biological activity testing in "Krachai-Dam" isolated compounds

Biological activity testing	Chemical constituents	Researcher/year
Anti-inflammation using "TPA-induced ear Edema in rats"	A, B and R	(Tuchinda et.al, 2002)
Anti-mutagenicity using HPLC technique	A,B,C,D,E and F	(Trakoontivakorn et. al, 2001)
Anti-oxidation using TBA method	F, M, N and Q	(Yenjai *et al.*, 2002)
Anti-viral activity	G,H,I,J,K,L,M,N and O	(Yenjai et.al, 2004)
Anti-fungal activity using *Candida albican*	G,H,I,J,K,L,M,N and O	(Yenjai et.al, 2004)
Anti-tuberculosis	G,H,I,J,K,L,M,N and O	(Yenjai et.al, 2004)
Anti-malarial activity using *Plasmidium falciparrum*	G,H,I,J,K,L,M,N and O	(Yenjai et. al, 2004)
Anti-mycobacteria	I and O	(Yenjai et.al, 2004)
Anti-bacterial	B and E	(Yenjai et.al, 2004)
Cytotoxic Activity (Inactive)	A, B, C, D, E, F, I, J and O	(Yenjai et. al, 2004)

A. – (-) hydroxypanduratin A. or 4- hydroxypanduratin A.
B. – (-) panduratin A. or panduratin A.
C. 2',4',6'-trihydroxycyclone (pinocembrin chalcone)
D. 2',4'-dihydroxyflavone-6'-methoxychalcone (cardamonin)
E. 5,7-dihydroxyflavanone (pinocembrin)
F. 5-hydroxy-7-methoxyflavanone
G. 5- hydroxy-7-methoxyflavone
H. 5- hydroxy-7,4'-dimethoxyflavone
I. 5,7,4'-trimethoxyflavone
J. 5,7,3',4'-tetramethoxyflavone
K. 5- hydroxy-3,7-dimethoxyflavone
L. 3,5,7- trimethoxyflavone
M. 5- hydroxy –3,7,3',4'-tetramethoxyflavone
N. 5- hydroxy –3,7,4'-trimethoxyflavone
O. 3,5,7,4'-tetramethoxyflavone
P. 7,4'-dimethoxy-5-hydroxyflavone
Q 3,7-dimethoxy-5-hydroxyflavone
R 5,7-dimethoxyflavone

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Based on the results of previous research, there are at least seven activities that have been tested in some chemical constituents, isolated from "Krachai-Dam" rhizomes (Table 2.5), and six activities have already been found in Krachai-Dam crude extract which are anti-gastric ulcer (Rujjanawate *et al.*, 2005), adaptogenic (Pojanagaroon and Rujjanawate, 2006), vasolidation and aphrodisiac(Thammaaree *et al.*, 2006, a, b; Sudwan and Saenphet, 2007) activities. Confidentially, there are still some more activities, which haven't yet been found and are waiting for further discoveries.

2.7.3 Anti-oxidative activity of Krachai-Dam

Yenjai *et al.* (2002) exhibited that crude dichloromethane and methanol Krachai-Dam extract showed antilipid peroxidation activity by using TBA method with IC_{50} of 0.007% and 0.037% w/v respectively. Chromatographic separation of dichloromethane extract by silica gel column chromatography, preparative layer chromatography and crystallization yielded flavonoids 1-5, 3,7-dimethoxy-5-hydroxyflavone (1), 5-hydroxy-7-methoxyflavone (2), 5-hydroxy-3,7,4′-trimethoxyflavone (3), 7,4′-dimethoxy-5-hydroxyflavone (4) and 5-hydroxy-3,7,3′,4′-tetramethoxyflavone (5). Compound 1-5 exhibited antilipid peroxidation with IC_{50} of 0.39, 0.18, 0.17 and 0.3% w/v respectively. Flavone 5 possessed antilipid peroxidation by 40% inhibitory effect at a maximum concentration of 0.5% w/v. The chemical structure of these compounds were elucidated by the analysis of their spectral data, mainly by 1D and 2D techniques.

Pojanagaroon and Rujjanawate (2004 a) collected rhizomes of *Kaemferia parviflora* with four different intensities of internal color as grouped by the L*a*b* system according to the unweighted pair group method cluster analysis (UPGMA) from production areas in Loei (cv. Bohmuang-Noi #2), Phitsanulok (cvs. Rom-Klao and Nam-Juang) and Phetchabun (cv. Kheg-Noi#2) provinces. The ethanolic extracts of four cultivars of *K. parviflora*, vitamin E and other Zingiberaceous plants were evaluated for their anti-oxidant activity in the form of percent inhibition of lipid peroidation as determined by the Thiobarbituric acid (TBA) method. It was found that all *K. parviflora* extracts at all concentrations used (0.98, 1.95, 3.91 and 7.81 mg/ml) inhibited lipid peroxidation in polyunsaturated fatty acids obtained from Sprague-Dawley rats' brains. The extract of cv. Rom-Klao, which had the darkest internal color, was the highest in percent inhibition of lipid peroxidation. However, the values for the extracts of *K. parviflora* were significantly lower ($p \leq 0.05$) than those for vitamin E and other Zingiberaceous plants. The extracts of *Zingiber officinalis* had the highest percentages of lipid peroxidation (88.85 and 85.06%). All cultivars of *K. parviflora* exhibited an anti-oxidant activity. Those with darker internal color tended to have a higher anti-oxidant activity than those with lighter internal color.

2.7.4 Anti-oxidative activity of Krachai-Dam herbal wine

Pojanagaroon and Rujjanawate (2005) studied the effects of Internal skin color of Krachai-Dam rhizomes as raw materials for wine processing on antioxidative activity of Krachai-Dam herbal wines. Rhizomes with three different intensities of internal color:dark purple (cv. 'Rom-Klao'), purple (cv. 'Boh Muang-Noi # 2') and pale purple (cv.'Kheg-Noi # 2') and herbal wines produced from them were evaluated for their antioxidative activity in the form of percent inhibition of lipid peroxidation as determined by the Thiobarbituric acid (TBA) method. The quantities of phenolic acid and total flavonoids of the extracts were determined by the Folin-Ciocal Teau

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and Al (III) –flavonoids complexation methods. It was found that all Krachai-Dam herbal wine extracts at all concentrations used (0.98, 1.95, 3.91, 7.81, 15.62, 62.50, and 125.00 mg/ml) inhibited lipid peroxidation in polyunsaturated fatty acids from Sprague-Dawley rats' brains. The extract of wine produced from cv. 'Rom-Klao', which had the darkest internal color, was highest in percent inhibition of lipid peroxidation and also gave the highest phenolic acid contents.Where as, the extract of wine produced from cv. 'Kheg-Noi # 2', which had pale internal color, gave the highest total flavonoids content. The higher concentration extracts tended to have a higher antioxidative activity. The average percent ratio of inhibition of lipid peroxidation of herbal wines and their raw materials extracts was 56.21%, according to the average ratio of phenolic compounds (72.62%) Therefore, the internal color of Krachai-Dam rhizomes as raw materials for wine processing had influenced the anti-oxidative activity and phenolic compound content in Krachai-Dam herbal wines.

2.8 Standardization of Krachai-Dam rhizomes

Wongsinkongman *et al.* (2003) had studied on the comparison of the 25 authentic samples (*Kaempferia parviflora* Wall. ex Baker, Family Zingiberaceae), which were fresh rhizomes that purchased from different sources in central part, eastern part and northeastern part of Thailand during December 2001 to May 2002. From the identification from the technique of thin–layer chromatography (TLC), it was found that the chromatograms of the twenty-five dried crude drugs were similar to those of the authentic sample. The results from the quality evaluation, the crude drugs had the following properties: the total ash was not more than 6% w/w, the acid-insoluble ash was not more than 2% w/w, the moisture content was not more than 10% v/w, the water-soluble extractive was not less than 17% w/w, and the ethanol-soluble extractive was not less than 8% w/w. To obtain the 'Krachai-dam' volatile oil, eighteen fresh rhizomes and the authentic samples were distilled with water. The results from the physicochemical evaluation, the volatile oil had the following properties: the refractive index varied from 1.471 to 1.476 at 20°C and the relative density varied from 0.980 to 0.983 at 20°C.

2.9 Winemaking

Winemaking or vinification is the process of wine production, from the selection of grapes to the bottling of finished wine (Robinson, 2003). Wine production can be generally classified into two categories: Still wine production and sparkling wine production. After the harvest, grapes are crushed and allowed to ferment. Red wine is made from the must (pulp) of red or black grapes that undergo fermentation together with the grape skins, while white wine is usually made by fermenting juice pressed from white grapes, but can also be made from must extracted from red grapes with minimal contact with the grapes' skins. Rosé wine is made from red grapes where the juice is allowed to stay in contact with the dark skins long enough to pick up a pinkish color, but little of the tannins contained in the skins. During this primary fermentation, which often takes between one and two weeks, yeast converts most of the sugars in the grape juice into ethanol (alcohol). After the primary fermentation, the liquid is transferred to vessels for the secondary fermentation. Here, the remaining sugars are slowly converted into alcohol and the wine becomes clear. Some wine is

then allowed to age in oak barrels before bottling, which add extra aromas to the wine, while others are bottled directly.

The time from harvest to drinking can vary from a few months for Beaujolais nouveau wines to over twenty years for top wines. However, only about 10% of all red and 5% of white wine will taste better after 5 years, compared to after one year. Depending on the quality of grapes and the target wine style, some of these steps may be combined or omitted to achieve the particular goals of the winemaker. Many wines of comparable quality are produced using similar but distinctly different approaches to their production; quality is dictated by the attributes of the starting material and not necessarily the steps taken during vinification (Robinson, 2003).

Variations on the above procedure exist. With sparkling wines such as Champagne, an additional fermentation takes place inside the bottle, trapping carbon dioxide and creating the characteristic bubbles. Sweet wines are made by ensuring that some residual sugar remains after fermentation is completed. This can be done by harvesting late (late harvest wine), freezing the grapes to concentrate the sugar (ice wine), or adding a substance to kill the remaining yeast before fermentation is completed; for example, high proof brandy is added when making port wine (Robinson, 2003).

Honey wines are primitive types of wine that are cloudy and effervescent containing residues of substrates and fermenting yeasts and other micro-organisms (Steinkraus, 1983). Popular among the indigenous Ethiopian fermented beverages are *Tej*, tella, borde and shamita (Ashenafi, 2002). *Tej* is a home processed, but commercially available honey wine. A mixture of honey and sugar may be used as major fermentation substrate. In cases where sugar is also used as a substrate, coloring is added so that the beverage attains a yellow color similar to that made from honey (Fite *et al.*, 1991). Some *tej* makers also add different concoctions such as barks or roots of some plants or herbal ingredients to improve flavor of potency. Due to concoction, adulteration practices and possibly some other reasons, producers usually are not willing to tell about additives used and their compositions (Bahiru *et al.*, 2006).

One part of honey mixed with 2-5 (v/v) parts of water is placed in the pot, covered with a cloth for 2-3 days. To ferment after which wax and top scum is removed. Some portion of the must is boiled with washed and peeled *Rhamnus prenoides* and put back to the fermenting must. The pot is covered and fermented continuously for another 5 days, in warmer weathers, or for 15-20 days, in colder cases. The mixture is stirred daily and finally filtered through cloth to remove sediment and *R. prenoides* (Vogel and Gobezie, 1983). *Saccharomyces cerevisiae* (of several strains) represents the dominant flora in winery. Although numerically less prevalent, other winery yeasts, including *Brettanomyces*, *Dekkera*, *Zygosaccharomyces*, and *Schizosaccharomyces* may develop as well if conditions permit. In case of successful native yeast fermentations, *Saccharomyces cerevisiae,* of potentially several strains (Mortimer, 1995) eventually takes over in a couple of days, depending on temperature. A natural process method is described for making a beverage from honey with 12 - 14% alcohol. Additives reduce the fermentation time of a 20 - 24% honey solution to about two weeks. Yeast strain 618 (Rahn collection) is used at an incubation temperature of 15 - 18 deg C.E. E. Crane. (Morse, 1980).

Good quality *tej* is yellow, sweet, effervescent and cloudy due to the content of yeasts. The flavor of *tej* depends upon the part of the country where the bees have collected the nectar and the climate (Vogel and Gobezie, 1983). *Tej* is a household commercial product, and each production unit generally sells its product for consumption at point of production.

However, Krachai-Dam honey wine processing in this study have been adapted from those natural process mentioned before and standard herbal wine making process that was used to extend farmers and producers in Loei province.

2.10 Overview of Sensory evaluation

Sensory evaluation is a "scientific discipline used to evoke measure, analyze, and interpret reactions to the characteristics of foods and materials as they are perceived by the senses of sight, smell, taste, touch, and hearing" (Institute of Food Technologists 1981). Without the proper sensory evaluation techniques, it is difficult to interpret sensory responses and make logical and sound decisions. The wine industry has historically relied on "experts" for determination of sensory characteristics of wine. Evaluation of wine by such "experts," highly trained to evaluate the slightest nuances in flavors and aromas may be valuable to a winery, providing detailed descriptions of differences in wines. However, problems can occur if too much reliance is placed on a few individuals making sensory judgments that influence production or marketing decisions. "Experts'" opinions may not be reflective of true sensory characteristics or those important to the wine consumer. External factors such as mental or physical fatigue or distractions can interfere with the "expert's" analysis of the product. If a few "experts" are the common means of sensory evaluation within a winery, limitations of these evaluations are significant. A progressive winery will understand the importance of basing decisions on sensory data that can be statistically evaluated and interpreted (Zoecklein *et al.*, 1995).

In a competitive environment, it is important to base decisions about wine, new products, and wine improvements on the best information possible. Sensory evaluation controls the external variables as much as possible so only the variable of interest is being measured. Sensory results can be interpreted statistically, providing a basis on which decisions can be made. Effects of changes in processing conditions on sensory attributes can be measured and the impact of the processing decision weighed. Sensory evaluation of wines can also assist in relating sensory impressions to others in a meaningful way by establishing a standardized sensory vocabulary. Correlation of sensory analysis with chemical measurements of selected wine compounds can assist in interpreting chemical data relative to wine characteristics. Properly conducted sensory evaluations can lead to improved decision-making with less risk involved, a means of targeting and achieving goals, and a way of categorizing attributes (Zoecklein *et al.*, 1995).

The selection of a sensory evaluation method is determined based on the type of information that is needed. A new grape variety, variations in pressure during pressing, a change in yeast strain or supplier can lead to changes that may impact on the sensory characteristics of the wine. It may be important to know if there are perceptible changes, what those changes are, and the impact of those changes on the consumer's perception of quality or acceptance (Zoecklein *et al.*, 1995).

Wine tasting is the sensory evaluation of wine. The color, aroma, flavor and feel of the wine in the mouth are all assessed (Robinson, 2003). The main aims of wine tasting are to:

- assess the wine's quality.
- determine the wine's maturity and suitability for aging or drinking.
- detect the aromas and flavors of the wine.
- discover the many facets of wine, so as to better appreciate it.
- detect any faults the wine may have

To assess a wine's quality, one must gauge its complexity of aroma and flavor, determine the intensity of the aroma and flavor, check that the flavors and structural elements- such as acid, tannin and alcoholic strength- are well balanced, and finally see how long the wine persists in the mouth after tasting (Robinson, 2003).

Practiced wine tasters will gauge the wine's quality in other ways too. These include, whether the wine is of high quality with respect to other wines of its price, region or vintage; if it is typical of the region it is made in or diverges in style; if it uses certain wine making techniques, such as barrel fermentation or malolactic fermentation; or if it has any wine faults. Many professional wine tasters, such as sommeliers or buyers for retailers, look for characteristics in the wine which are desirable to wine drinkers or which indicate that the wine is likely to sell or mature well (Margalit, 1996).

2.11 Methods of sensory evaluation

Sensory evaluation methods can be divided into two broad classes: affective and analytical methods (Institute of Food Technologists 1981). Affective methods use consumer panels or untrained panelists to answer questions such as Which product do you prefer? Which product do you like? How well do you like this product? and How often would you buy/use this product? Affective methods require a much larger panel size than do analytical methods in order to have greater confidence about the interpretation of the results. The most common analytical methods of sensory evaluation used in the wine industry are discrimination and descriptive methods. Discrimination tests can be used to determine if products are different, if a given wine characteristic is different among samples, or if one product has more of a selected characteristic than another. Experienced panelists can complete discrimination tests. Descriptive methods are used to provide more comprehensive profiles of a product by asking panelists to identify the different characteristics within the product and quantify characteristics. Trained panelists must be used for descriptive methods (Zoecklein *et al.*, 1995). Quantitative Descriptive Analysis (QDA) used in this study is Wine Appreciation chart which is modified from Davis Score (Margalit, 1996).

2.12. Factors influence on Herbal wine qualities

Factors that have the influence on honey wine qualities are as follows:

1. Types of honey. Honeys have distinct odor and other physical properties depend on floral nectar that used to make honeys. The natural honeys have highly sweetened season, fragrance, pale yellow to brown in color. The ratio of glucose and fructose varies from types of honey that influence on honey wine qualities after fermentation process (Charnritthisaen, 2002).

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2. Yeast strains. The variation of yeast strains has influence on fermentation potential and taste of honey wine products (Bahiru *et al.*, 2001). Yeasts of the genus *Saccharomyces* were reported to be responsible generally for the conversion of sugars to ethanol in *tej* or honey wine fermentation is a natural fermentation, variability in lactic acid and yeast flora may result in variability in acidic, flavor and an alcohol content of the product (Bahiru *et al.*, 2006). *Saccharomyces cerevisiae* was reported to be the most popular for wine fermentation. Yeast strains have high influence on taste, odor and fragrance when wine was ready for consumption (Ribereau-Gayon *et al.*, 2000).

3. Temperatures. Controlling the temperature during fermentation in one of the most important factors in good winemaking. The range of temperatures in which the yeast in active and ferments is between 10°C-35°C, (50°F-95°F). At the high temperature range, the fermentation starts faster, but as the alcohol concentration increase, it slows down. At 35°C, sometimes it may even stop, leaving some residual sugar unfermented. At low to moderately temperatures, the fermentation starts slowly, proceeds more moderately, and generally it will go on to dryness. The time lapse between inoculation and the first signs of fermentation can take about on, two, four and seven days for temperatures of 35°C, 25°C, 15°C and 10°C, respectively (Margalit, 1996). The alcohol is an inhibitor for the yeast growth, and its inhibition effect is greater at high temperature. It also evaporates more through the CO_2 bubbles, at higher temperatures, which makes the alcohol yield higher at low temperature fermentation. The variation in alcohol content may be as much as 1% absolute alcohol difference if the fermentation is carried on at temperatures of 20°C and 10°C. So it is expected that the wine will be fuller bodies if fermented at lower temperatures. Also, at lower temperature, the fruitiness of the grapes is better preserved, by reducing evaporation of volatile aroma components from the must. Also the volatile acidity level at low temperature fermentation has been found to be lower than at high temperatures (Ribereau-Gayon *et al.*, 2000).

For all the above reasons and for practical considerations, it has been accepted that the preferred fermentation temperature for white wines is between 8°C-14°C (46°F-57°C). This is also true for rosé wines, and white wines made from red grapes ("blush wine"), as all of them are considered white wines. For red wines, the fermentation temperature should be higher, between 22°C-28°C (72°F-82°C), for two reasons: better color and tannin extraction, and because less fruitiness is desired in red wines (Margalit, 1996).

4. pH of must. Honey has low acidity. Low pH must should be used for protect other microbial. The popular pH used for dry wine is 4.0-4.5 whereas is 3.0-4.0 for sweet wine (Charnritthisaen, 2002).

5. Wine clarification. Bentonite is a good precipitating agent that has been popularly used (Charnritthisaen, 2002). A small amount of bentonite is added to the tirage solution to help with the flocculation of the yeast and honey. 0.5 g/liter bentonite has been suggested for wine clarification (Balik, 2003). There are some works about this as follows: The concentration losses of total anthocyanins in red and rosé wines after their clarification with two commercial types of bentonites were determined; also, the differences in color intensity of clarified and non-clarified wines were evaluated using both analytical methods and sensory evaluation. The loss of anthocyanins increased with increasing doses of bentonite; however, statistically

significant differences were observed only in 0.5 and 1.5 g/liter doses. The dose, and not the type, of bentonite had an important effect on the extent of changes in color intensity of the wine. The sensory evaluation revealed that 0.5 and 1.5 g/liter bentonite in red and rose wines, respectively, significantly reduce the wine color intensity, as compared to non-clarified wines (Balik, 2003). In another work, two batches of sparkling wines were industrially produced, one with addition of bentonite to the tirage solution and the other without bentonite addition. Samples were taken at 20, 40, 90, 180, 270 and 365 days of aging with yeast. Total, free amino-, protein- and peptide-nitrogen concentrations were determined and foam properties and sensory quality were evaluated. It was observed that the bentonite retained part of the peptides and proteins and had an adverse effect on the sensory quality of the wine (Martinez-Rodiguez and Polo, 2003).

2.13. Factors influence on raw materials used for wine production.

Of all factors affecting the quality of a wine, the quality of the grapes more than any other factor determines the quality of the wine (Robinson, 2003). Their quality is not only affected by their variety, but also by the weather during the growing season, the soil, the time of harvest, and the way they are pruned. The combination of these effects is often referred to as their terroir, which means that wines from that terroir are unique, incapable of being reproduced outside that area, even if the variety and winemaking techniques are painstakingly duplicated. The most common genus of wine grape is *Vitis vinifera*, which includes nearly all varieties of European origin.

Factors influence on raw materials used for wine production is as follows:

1. Maturity date or harvest date. The grapes are usually harvested from the vineyard in the fall, in the northern hemisphere from the middle of October until the beginning of November, or the middle of February until the beginning of March in the southern hemisphere (Ribereau-Gayon *et al.*, 2000). The decision to harvest grapes is typically made by the winemaker and informed by the level of sugar (called °Brix), acid (TA or Titratable Acidity as expressed by tartaric acid equivalents) and pH of the grapes, as well as berry flavor, tannin development and overall disposition of the grapevine and weather forecasts. The level of sugar in the grapes is important not only because it will determine the final alcohol content of the wine, but also because it is an indirect index of grape maturity.

Optimal enological maturity depends on grape variety, environmental conditions and wine type (Ribereau-Gayon *et al.*, 2000). The grape crop should be harvested under favorable climatic conditions. The suitable maturity date is a very important factor that influence on wine quality. There were some reports about this as follows: Berries of the cultivars Stover and Conquistador (bunch, *Vitis* spp., subgroup *Euvitis*) and Wander and Noble (muscadine, *V. rotundifolia*) were harvested at 3- or 4-day intervals over 3-6 weeks. Maturity date correlated well with compositional data, except for pH. As ripening progressed, Brix and pH increased while titratable acidity decreased. This trend was less uniform in uneven ripening Conquistador. Despite the compositional differences that exist within a cultivar at different stages of maturity. The maturity-related extremes in grape acidity (and to a lesser extent pH) were attenuated in the wines by cold stabilization (both species) and fermentation (muscadine only). Consequently, mid- to late and early to mid-season harvesting is

recommended for bunch and muscadine grapes, respectively. In all cases, crush pH should be restricted to between 3.0 and 3.5 (Bates, *et al.*, 1987). Another literature was the study of harvesting time for grapes in the Aorta Valley in Italy varies from mid-September to late October. Visual assessments of grape ripeness are unreliable, and a test program was established in 1999, using cultivars Chardonnay and Muller Thurgau (from SE facing vineyards at 750-800 m altitude) and cultivars Petitie Arvine and Moscato Bianco (from S facing vineyards at 600 m altitude). Data were collected for 3 years on the chemical composition of musts and wines. Results should provide a guideline for optimal harvesting time. (Rigazio, 2002). The other study was to study the influence of cultivar, year and harvesting time on the sensory characteristics of wines were studied. Grapes were harvested 3 times at 7- to 10-day intervals. Seven wines (3 white - Chardonnay, Rhine Riesling and Cortese; 4 red - Dolcetto, Barbera, Nebbiolo and Cabernet Sauvignon) from the Instituto Sperimentale per l'Enologia, Italy, from the 1986, 1987 and 1988 vintages were examined. For harvesting time, significant differences between the wines in the intensity of the descriptors sugar, alcohol, acidity, tannins, aromatic finesse and overall judgement were observed. (Ubigli *et al.*, 1997)

2. Temperature. As for grapes, temperature affected photosynthetic activity, metabolism and migration intensity in the vine (Calo *et al.*, 1992). Grape growth and development were directly affected by temperature. High temperatures were unfavorable to cellular multiplication. During the herbaceous growth phase, the optimum temperature was between 20 and 25°C. During maturation, temperature affected migration intensity and thus, indirectly, cell growth. Vine temperature requirements during this period were around 20°C. Temperature also had an influence on the composition of grape phenolic compounds. Intensely colored wines were known to be difficult to obtain in extreme temperature conditions (too low or high) though the phenomenon involved could at first appear paradoxical. High temperatures stimulated metabolic reaction, whereas low temperatures curbed migration. However, this corresponds with poor grape sugar alimentation and thus increased competition between primary metabolism (growth) and secondary metabolism (accumulation). The concentration of phenolic compounds was also affected by thermo-period. Raising the night-time temperature from 15 to 30°C while maintaining a daily temperature of 25°C resulted in a decrease in grape coloration. The anthocyanins were therefore not a blocked metabolic product but, on the contrary, were reversible. Thus, temperature and sun exposure determined phenolic compound accumulation (Ribereau-Gayon *et al.*, 2000).

By comparing a cool viticultural zone with a warmer zone in South Australia, Ewart (1993) showed that the total volatile terpene quantity increased more slowly in the cool zone but was higher at maturity. In a cool climate, and especially with shaded grapes, methoxypyrazine concentrations can attain unfavorable organoleptic thresholds. Conversely, warm climates can lead to high concentrations of certain phenolic compounds in white cultivars such as Riesling. These compounds confer an excessively astringent character to the wine and lead to the development of a diesel-like odor during ageing (Ribereau-Gayon *et al.*, 2000).

3. Water availability.A satisfactory water supply is necessary as much for vine development as for growth and grape maturation (Ribereau-Gayon *et al.*, 2000). Most quality wines are produced in zones where the annual rainfall does not exceed 700 to 800 mm. Evidence suggests that elevated rainfall or excessive irrigation

lowers grape quality. Matthews and Anderson (1989) showed that hydric stress causes an increase in juice and skin phenolic compounds, a higher praline concentration and a lower malic acid concentration. An insufficient water supply leads to high terpenic compound concentrations (Matthews and Anderson, 1990). Conversely, an elevated water supply results in an increase in berry volume but a decrease in phenolic compound concentrations. In viticultural zones where irrigation is used, it should be limited in the weeks preceding the harvest (Ribereau-Gayon *et al.*, 2000).

Situations of extremely low or very high soil water availability result in poor quality grapes. Thus, a moderate stress is the best condition to obtain high quality grapes (Ribereau-Gayon *et al.*, 2000). The relationship between water stress and polyphenolic compounds, and to assess the level of water supply which optimizes polyphenol quality in red grapes using 6-year-old cultivar Merlot plants, grafted on SO4 rootstock and cultivated in a gravelly loam of the Friuli plane (North-Eastern Italy) was studied. Two different levels of water supply (control and stress, 80% and 20% of available water, respectively) we are established from veraison to harvest. Grape samples were collected weekly from veraison to harvest and analyzed for sugars, titratable acidity, pH, total polyphenols and anthocyanins. Wine was made with grapes, and the polyphenolic compounds in wine were analyzed as well. Water stress did not affect sugar concentration in grapes; titratable acidity was slightly reduced and pH increased in water stressed plants at harvest. Total polyphenols and anthocyanins were reduced by water stress during the maturation but not significantly at harvest. Total phenols and anthocyanin concentration was higher in water-stressed wines, but without any significant difference. In conclusion, water stress confirmed the known effect in decreasing polyphenolic compounds in grapes. Berry size reduction is interesting because it can enhance the skin/pulp ratio, thus providing a more abundant polyphenolic source. The dependence of sensory characteristics of wine on the water status of the source vines was determined following vineyard irrigation treatments which included early and late season water deficits and continually irrigated vines (Peterlunger *et al.,* 2002).

The other work showed that the concentrations of anthocyanins and total soluble phenolics were greater in wines from water-deficit-treatment vines than from continually-irrigated vines. For sensory evaluation, a novel protocol for paired comparisons was developed to test separately for differences in wine appearance, flavor, taste and aroma; differences were detected for each wine comparison. In both seasons, wine from continually-irrigated vines differed from wines from early- and late-season water-deficit vines, and wine from early-season water-deficit vines differed from that from late-season water-deficit vines, in their appearance, flavor, taste and aroma. The sensory differences were not attributable to differences in vine yield or fruit maturity. A majority of professional wine tasters using similar tests was able to detect visual but not flavor differences between a selected pair of wines. It is concluded that, where vine water status can be altered, irrigation offers a means of manipulating wine sensory characteristics in the vineyard. (Matthews *et al.*, 1990).

In another work, irrigation increased yield in both seasons due mainly to an increase in berry weight. The concentration of juice soluble solids (Brix) and titratable acidity (TA) was unaffected by the irrigation treatment and a similar pattern was observed for the alcoholic content and TA of the elaborated wines. However, the concentration of anthocyanins, total phenolics and color intensity of both red and rose wines decreased with increasing water application in an inverse pattern to that of

berry size (dilution effect). Yield, berry weight, anthocyanins, total phenolics and color intensity of red wines were closely correlated with the water stress integral (which expresses the intensity and duration of stress) calculated from early morning stem water potential determinations. Sensory evaluation by experienced tasters ranked the red wines in decreasing order of water application with preference for the unirrigated one. The main defects of the irrigated wines were attributed to visual characteristics and poorer aroma quality. (Salon *et al.*, 2004).

4. Meteorological conditions of the year. The three principal climatic parameters (light, heat, humidity) vary considerably from year to year. Their respective influence on maturation processes is consequently of varying importance and leads to a given grape composition at maturity (Ribereau-Gayon *et al.*, 2000).

Variations in meteorological conditions do not have the same influence in all climates. The principal European viticultural regions have been classified into different zones. Examining only the sugar concentration in the northern continental zone (Alsacian, Champagne and Burgundian vineyards in France, and Swiss and German vineyards for the most part), the length of sun exposure seems to be the principal limiting factor during grape development (Calo *et al.*, 1992).

This factor is also important during maturation in the North Atlantic zone (Loire and south-western France vineyards), but is less important in the southern zone (Mediterranean vineyards in Spain, France and Italy). In the latter zone, the hydric factor interferes with the relative consistency of temperature and sun exposure.

Thus the climate/quality relationship can only be represented approximately. The sum of the temperatures, rainfall or length of light exposure does not have the most influence on grape quality; rather, it is their distribution in the course of the vine growth cycle (Ribereau-Gayon *et al.*, 2000).

5. Altitude and latitude of plantation area. Faustino *et al.* (2003) identified and compared Merlot red wines from very different climatic regions of the world, which were Canada, Chile and the United States. Canadian grapes were grown in a cooler climate with American conditions the most temperate. Chilean wines tended to have higher flavonoid content and most of antioxidants examined with Canadian wines intermediate and American wines having the lowest absolute values. It provided a preliminary indication for a mid-range temperature preference to enhance phenolic content in Merlot wines.

Grape cultivars Listan Negro (LN) and Ruby Cabernet (RC) grown in Tenerife, Canary Islands, at 280 and 520 m above sea level were evaluated for fruit yield and quality. RC fruits ripened earlier and recorded higher soluble solid, titratable acid, malic acid, anthocyanin and phenol contents, and a lower (i.e. better) seed phenolic ripening value than LN. A higher pH value, however, was observed in LN. Grapes grown at the higher altitude had higher pH values (except near the harvesting period when the pH value did not significantly vary with altitude); titratable acidity; malic acid, anthocyanin, and total phenol contents; and a lower seed phenolic value than those of grapes grown at the lower altitude. On the other hand, tartaric acid content was higher at the lower altitude. Fruit yield did not significantly vary with cultivar and altitude. The high temperature associated with the lower altitude appeared to have undesirable effects on fruit ripening and quality. RC was more sensitive to climatic variations than LN. (Miguel-Tabares *et al.*, 2002).

6. Varieties of raw materials. Choosing the variety to suit the climate is a deciding factor for obtaining a good maturation and quality wines (Ribereau-Gayon *et*

al., 2000). In general, early-ripening varieties are cultivated in cold climates (Chasselas, Gewürztramine, Pinot) and relatively late-ripening varieties in warm zones (Aramon, Carignan, Grenache). In both cases, maturity should occur just before the average monthly temperature drops below 10°C. The maturation process should not take place too rapidly or abruptly in excessively favorable conditions. Quality cultivars such as Cabernet Sauvignon and Pinot Noir lose much of their aromatic substance and phenolic compound finesse in warm climates. The phonological behavior diversity of these two varieties in the different viticultural regions of the world.

A study carried out on the principal French red grape varieties cultivated in the Mediterranean or Atlantic climate showed that concentration variations according to the climate are significantly less than according to the variety (Bisson and Ribéreau-Gayon, 1978). Similarly, fluctuations in grape phenolic content from one vintage to another and for a given variety are less than the variations between varieties. The genotypic effect of the variety is thus preponderant on grape phenolic compound richness. Anthocyanidic and procyanidic profiles vary greatly with respect to the variety and can therefore be use in varietal discrimination (Calo *et al.*, 1994).

The variability of grape aromatic content is even greater. Some varieties possess characteristic aromas. At present, not all of the molecules responsible for these aromas have been identified. In certain varieties, such as the Concord, descendant of native American vines *(Vitis labrusca, Vitis rotundifolia)*, the grapes always exhibit a foxy odor due to methy1 anthranilate (Bailey, 1988).

In another work, orange wines were made from the varieties Hamlin, Italian, Kozan and Valencia. Sufficient water, $CaCO_3$ and sugar were added to adjust alcohol, total acidity and sugar levels of the wine to 12% (v/v), 7 g/litre and 30 g/liter, respectively. The wines were analyzed by chemical and sensory evaluations. Wines from the variety Kozan were ranked highest, followed by those from Italian. (Canbas and Unal, 1994).

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