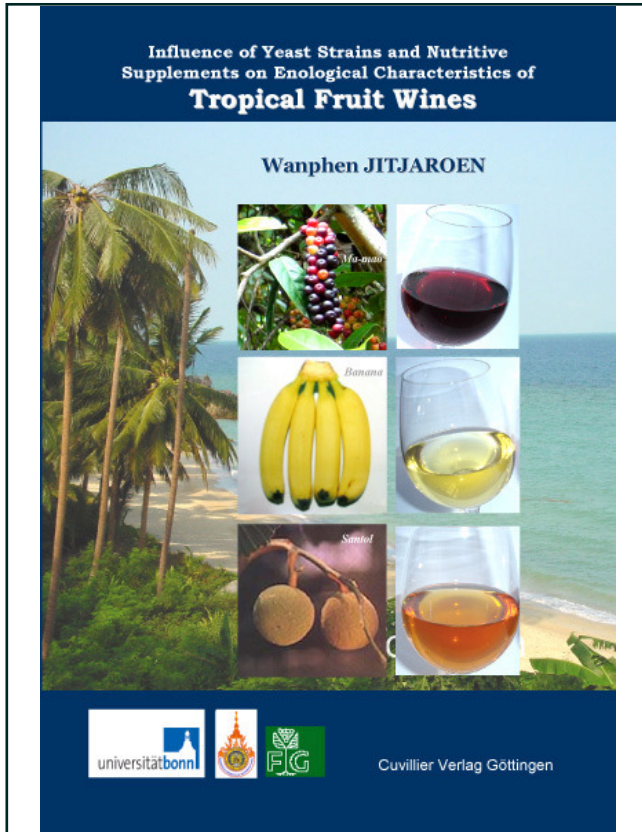




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## **Influence of yeast strains and nutritive supplements on enological characteristics of tropical fruit wines**



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## Chapter 1

### Introduction

#### 1.1 Regional fruit wines in Thailand

The alcoholic beverage production from grape and other fruits using traditional methods is an important post harvest activity in family farms. It has increased economic returns for the households and added value to the fresh fruit (Thompson, 1995). There was a time when Thailand was absent from the world's wine map. As a tropical country, it did not fit into perceptions of where grapevines could be grown. The latitudes of the Southernmost vineyards on our side of the equator are about 35 degrees North. Thailand is 14.3 degrees North, so it is not surprising that it was generally ruled out as a wine country. Since the liberalization of the alcoholic beverage production in 2000 it developed from the monopoly concession to the new market-driven scheme, which allows small and medium-sized industries to enter into this business countrywide. Nowadays, local fruit wines including pineapple, mango, tamarind, strawberry, mulberry, and litchi are produced by approximately 1,600 legal enterprises (Thai Commerce Bank, 2003). Different fruit wines are produced by small farms and a number of them are acceptable to local consumers such as mangosteen wine, rosella wine, longan wine, litchi wine, pineapple wine and a number of herbal wines. Many people become interested in local wines because they are cheaper than imported ones. Publicity about wine has also sparked renewed interest in the fruit wines. Recently the volume of imported wine has obviously decreased by 22% for French wines, 45% for American wines and 64% for Portuguese wines (Weekly Matichon, 2005). That indicates the acceptability of Thai fruit wines by the consumers and is encouraging for the local wine products. Due to the fact that most fruit wines are produced by communities without well-educated and experienced people in winemaking, the products are of low quality. Therefore, the consumption of local fruit wines has decreased in the last years.

The varieties of indigenous fruits are of interest to wineries including ma-mao, santol and the well-known fruit of banana. Very few Thais recognized the two indigenous fruits of ma-mao and santol until it emerged that they could be used to produce fine wines. The former are dark-red, sour, berry-sized fruits growing on branches of trees. The

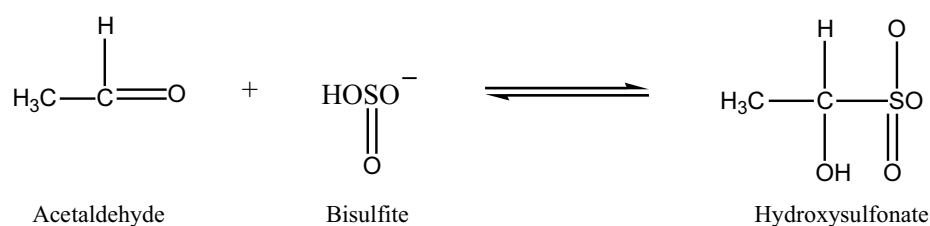
latter are round yellow-orange, sour-sweet apple-sized fruits and are consumed in fresh or pickled form. Ma-mao is ignored and on the verge of extinction and mainly food for wild animals. On the other hand many banana varieties are grown and have only a low market value.

Giving Thailand a local wine supply, the winemaking projects aimed to generate more income to the local economies and give a commercial value to the endangered fruits. This is an effective way of preserving the trees. In addition, villagers can earn extra income and the country saves money from being spent on imported wine. Scientists seriously attempt to manipulate fruit wine research developing new technologies for industries to gain qualitative good wines and more market share.

## **1.2 The role of sulphur binders and the sulphur binding capacity**

Sulphur dioxide is added as a preservative or antioxidant to wine or fruit wine. A part of it combines more or less rapidly with various carbonyl compounds enhancing the natural fruit flavour forming  $\alpha$ -hydroxysulphonates. A part remains free in the inorganic state, mainly as hydrogensulphite ions. It is only the undissociated sulphur dioxide ( $\text{SO}_2$ ) that has any antimicrobial activity. It is important to minimize the sulphite binding capacity of ciders (wines) as far as possible in order to have an adequate amount of free sulphur dioxide in the packaged product (Beech and Jarvis, 1989) and to remain in the legal limits. The use of damaged fruit in cider (wine) making is highly undesirable because of its adverse effect on the microflora for fermentation. Furthermore, this microflora is more difficult to control by sulphiting because of the high sulphur dioxide binding power of the juice (Burroughs and Sparks, 1964). Peynaud and Sapis (1972) showed that wine made from damaged and diseased grapes increased concentrations of sulphur dioxide binding compounds.

In general, the three major  $\text{SO}_2$  binders are those resulting from normal yeast metabolism during fermentation. These are acetaldehyde, pyruvate and  $\alpha$ -ketoglutarate, which are referred to metabolic carbonyls (Whiting and Coggins, 1960). All the sulphur dioxide binding substances so far identified in white wines and ciders contain either one or two carbonyl groups and can react reversibly with sulphur dioxide to form carbonyl bisulphate compounds (hydroxysulphonic acids) (Fig. 1.1). This reaction can be generalized for other carbonyls.



**Figure 1.1** Formation of hydroxysulphonate (Zoecklein et al., 1995).

The simplest and best known of these is acetaldehyde bisulphate, which has long been recognized as chiefly responsible for the bound sulphur dioxide in most wines. At free sulphur dioxide level of  $6.4 \text{ mg L}^{-1}$ , 98% of the acetaldehyde present in solution was bound. This was followed by malvidin 3, 5-diglucoside (63%), pyruvic acid (39%) and  $\alpha$ -ketoglutaric acid (15%) (Burrough, 1975). Acetaldehyde, a pungent fruity smell is found in small amounts in all wines and in high amounts in oxidized wines. The production of too much acetaldehyde in a wine can cause bottle sickness (Adrienne, 2000). It is formed by the decarboxylation of pyruvate through the glycolytic pathway. Utilizing the enzyme alcohol dehydrogenase (ADH), acetaldehyde is reduced to ethanol by accepting hydrogen from the reduced cofactor NADH (Lau et al., 1999). Würdig (1989) reported the binding of acetaldehyde by sulphite as it is formed, and the consequent blockage of the conversion pathway to ethanol, induces accumulation, rather than metabolism of acetaldehyde.

Delfini and Formica (2001) have shown that the formation of acetaldehyde by yeast is influenced by many factors such as pH, degree of aerobiosis, clarifiers and the size of the yeast inoculum. Acetaldehyde also participates in colour development of red wines (James et al., 2002). In fact, acetaldehyde, whose formation at the beginning of fermentation occurs sooner than the formation of the two keto acids. Furthermore, it is the compound that binds most tenaciously to sulphur dioxide. Although pyruvic and 2-ketoglutaric acids are spared, they are available for binding to the excess sulphur dioxide added after fermentation is completed.

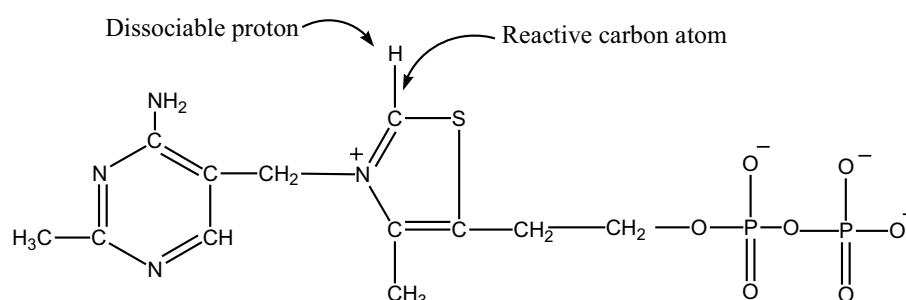
Pyruvic and  $\alpha$ -ketoglutaric acids are secondary products of alcoholic fermentation, considering their low  $K$  value, they can play an important role in the sulphur dioxide combination rate. The equilibrium constant ( $K$ ) gives a measure of the intensity of sulphite binding, a smaller value indicating tighter binding and vice versa. Burroughs and

Sparks (1962) indicated pyruvic acid was shown to be very active in binding sulphur dioxide, and in some ciders its concentration can be enough to account for up to 100 ppm of bound sulphur dioxide. In normal ciders, however, the amounts of pyruvic acid are much smaller and its importance is correspondingly less. The use of ascorbic acid in the cider that would have blocked the fermentation process with the accumulation of excessive quantities of pyruvate and acetaldehyde (Hammond and Carr, 1976; Würdig, 1989).

Jarvis and Lea (2000) confirmed that in minimizing the production of binding compounds, the selection of a yeast strain is of considerable importance. The yeast fermentation capacity, which reached attenuation quickly tended to produce ciders with greater sulphite binding power, for instance: strain NCYC 3627 produced significantly more pyruvate and acetaldehyde at 15°C and more  $\alpha$ -ketoglutarate at 25°C. The sulphite binding capacity of NCYC 3627 cider was greater when fermented at 15°C than at the other temperatures. Similarly, strain NCYC 5169 produced more  $\alpha$ -ketoglutarate and pyruvate at 20°C and more acetaldehyde at 15°C than temperatures. For this yeast strain, the sulphur binding power was higher at 15°C and 25°C than at 20°C. The yeast strains with the highest and lowest sulphite binding capacity were also the highest and lowest producers of sulphite during fermentation. Ribéreau-Gayon et al. (2000a) summarized that an elevated pH and fermentation temperature, anaerobic conditions, and a deficiency in thiamine and pantothenic acid increase the production of ketonic acids.

### **1.3 The role of thiamine in wine fermentation**

Thiamine (Vitamin B1) or thiamine pyrophosphate (TPP) is the cofactor for the enzymatic decarboxylation of pyruvic acid producing ethanal, which is reduced to form ethanol during alcoholic fermentation. TPP and pyruvate form an intermediary compound. More precisely, the carbon atom located between the nitrogen and the sulphur of the TPP thiazole cycle is ionized. It forms a carbonion, which readily combines with the pyruvate carbonyl group (Fig. 1.2) (Ribéreau-Gayon et al., 2000a).



**Figure 1.2** The structure of thiamine pyrophosphate (Ribéreau-Gayon et al., 2000a).

Thiamine effectively decreases significant ketonic acid concentrations (pyruvic acid and  $\alpha$ -ketoglutarate) by decarboxylation (Ribéreau-Gayon et al., 2000a). Thiamine deficiency would seem to be the main factor causing excretion of much pyruvate in anaerobic fermentations of cider and grape juice fermentations. Adding thiamine to thiamine deficient fermentations progressively diminishes pyruvate accumulation. The decrease of 80% in pyruvate on adding thiamine to a synthetic medium was observed. In wine fermentations adding 0.2-0.5 mg of thiamine to 1 L of grape juice at any stage of fermentation decreased the amount of pyruvate. A series of cider fermentations with *Saccharomyces uvarum* showed that adding thiamine decreased pyruvate excretion in juice containing low or high nitrogen by 85% and 2-oxoglutarate excretion, respectively by 40% and 50% in the two juices (Whiting, 1976). The addition of thiamine is mainly justified by its ability to reduce the quantity of sulphite-reacting substances and that of pantothenate to prevent the formation of hydrogen sulphide and volatile mercaptans (Cantarelli, 1989). Whiting (1976), Würdig (1989) and Beech (1993) supported the addition of thiamine and/or pantothenate is known to lower the accumulation of pyruvate and acetaldehyde in susceptible yeast strains. Thiamine supplementing does influence the velocity of fermentation, but does not contribute to the reduction of the latency phase elicited by sulphur dioxide (Delfini and Formica, 2001). In addition to limit carbonyl synthesis, thiamine also reduces the concentration and relative proportions of higher alcohols produced during fermentation (Jackson, 1994).

#### 1.4 The role of nitrogen compounds and the fermentation rate

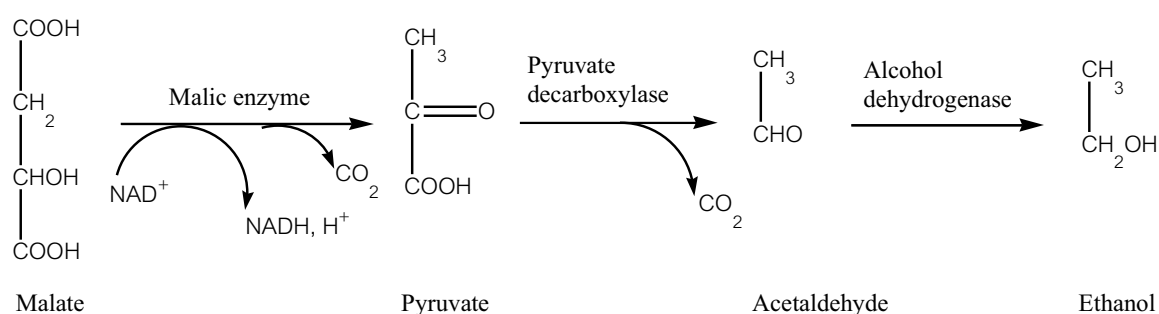
Yeast growth is usually described in terms of the kinetic parameters associated with the different stages of growth: lag, exponential, linear, stationary and decline phases. Those affected by the nitrogen supply are specific growth rate (linear phase), the time when specific growth rate begins to decline, and maximum yield of yeast (biomass) is produced. The maximum fermentation rate was about  $21 \text{ g CO}_2 \text{ L}^{-1} \text{ d}^{-1}$ , which occurred when assimilable nitrogen was maximal ( $300 \text{ mg L}^{-1}$ ). It indicated that nitrogen affects yeast growth and fermentation by different mechanisms. Complex nitrogen supplements may stimulate yeast growth to a greater extent than single sources and may be stimulating growth at later stages of fermentation by satisfying transport systems, which are less inhibited by ethanol. Exponential functions are relating the concentration of assimilate nitrogen with biomass yield or fermentation rate, but the functions were quantitatively different. That is, below about  $140 \text{ mg N L}^{-1}$ , growth and fermentation rates become inadequate for practical purposes while  $400 \text{ mg L}^{-1}$  assimilable nitrogen strongly stimulates the fermentation rate (and reduce the total time) over and above that of simply stimulating biomass yield or growth rate. Resultant increases in growth rate and biomass yield were accompanied by higher rates of fermentation (Henschke and Jiranek, 1992).

Boulton (1980) accounted for the influence of nitrogen on fermentation kinetics. Low initial nitrogen concentration, caused nitrogen exhaustion and the growth rate declined rapidly. Higher initial concentrations of nitrogen delayed or prevented the period before the growth rate declined to a lower rate. The concentration of alcohol was related to the initial nitrogen concentration. It was suggested that ethanol tolerance was, therefore, a function of nitrogen concentration. Maximal carbon dioxide evolution rate was directly proportional to initial nitrogen concentration in the medium. A high initial nitrogen concentration stimulates and sustains the maximum carbon dioxide production rate. Intermediate concentrations have a neutral effect while a low initial concentration reduces carbon dioxide production rate and shorten the phase at maximum rate (Henschke and Jiranek, 1992).

## 1.5 The role of changes in organic acids concentration and distribution during fermentation

### Malic acid

Malic acid is the decomposition of L-malic acid by yeast (Fig. 1.3). The reduction in malate content ranged from 28.8 to 41.5% (Kluba and Beelman, 1975), *Saccharomyces* yeasts utilized L-malic acid from 3 to 45% (Rankine, 1966). In addition, the transformation of malic acid is always accompanied by the fermentation of sugars which although on a modest scale has the result of increasing aroma components such as acetaldehyde, acetic acid, acetoin, diacetyl, 2, 3-butanediol, ethyl lactate and higher alcohols. As a whole, the wine gains in mellowness, roundness and fullness and becomes more pleasant to the palate (Colagrande et al., 1994). The reduction in titratable acidity during normal fermentation is attributed to the loss in malic and tartaric acids (Beelman and Gallander, 1979).



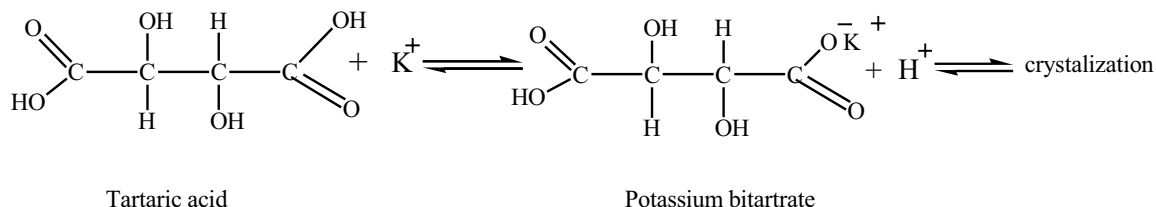
**Figure 1.3** Decomposition of malic acid by yeasts during alcoholic fermentation (Ribéreau-Gayon et al., 2000a).

### Tartaric acid

Tartaric acid ranges from 2 to 10 g L<sup>-1</sup> in grape must. In grapes and wines, tartaric acid is found in ionized form: bitartrate and tartrate. Generally, precipitation will be maximal at pH 3.7 (Zoecklein et al., 1995). Tartrate decreased more than malate during vinification, apparently due to the precipitation of potassium bitartrate. The crystallization of tartrate salts, mainly potassium hydrogen tartrate (KHT), is the major instability in wines (Fig.



1.4; Vernhet et al., 1999). As the alcohol content increases during fermentation, the solubility of potassium bitartrate decreases, and a portion is precipitated in the wine (Kluba and Beelman, 1975). Tartrate salt crystallization occurs spontaneously during both alcoholic fermentation and wine storage.



**Figure 1.4** Potassium bitartrate exists in a dynamic equilibrium between various ionized and salt states (Jackson, 1994).

### Lactic acid

Lactic acid is derived from pyruvic acid. Yeasts form 200-300 mg of D (-) lactic acid per litre and only about a dozen milligrams of L (+) lactic acid. The latter is formed essentially at the start of fermentation (Ribereau-Gayon et al., 2000a). It is absent in fresh musts prepared from healthy grapes but in wines both isomers can be produced by yeasts and lactic acid bacteria (*Oenococcus* and *Lactobacillus*) from glucose, fructose and pentoses. Only during the degradation of malic acid is the L (+)-lactic acid isomer formed exclusively. During alcoholic fermentation the wine yeasts can produce from 0.1-0.6 g L<sup>-1</sup> of both optical isomers of lactic acid. It contributes to the acidity of wine without increasing the aggressive sensation of acidity (Delfini and Formica, 2001)

### Acetic acid

Acetic acid is the principle volatile acid of wine. It is produced in particular during bacterial spoilage but is always formed by yeasts during fermentation. In healthy grape must with a moderate sugar concentration (less 220 g L<sup>-1</sup>), *Saccharomyces cerevisiae* produces relatively small quantities, varying according to the strain. The hydrolysis of acetyl CoA can produce acetic acid. The higher the sugar concentration, the more acetic acid the yeast produces during fermentation. Partly for this reason, sweet wines made

from musts with high sugar concentrations systematically have elevated volatile acidities. Furthermore, the other factors favor the production of acetic acid by *S. cerevisiae* such as anaerobiosis, very low pH (<3.1) or very high pH (>4), certain amino acid and vitamin deficiencies in must, and too high temperature (25-30°C) during the yeast multiplication phase.

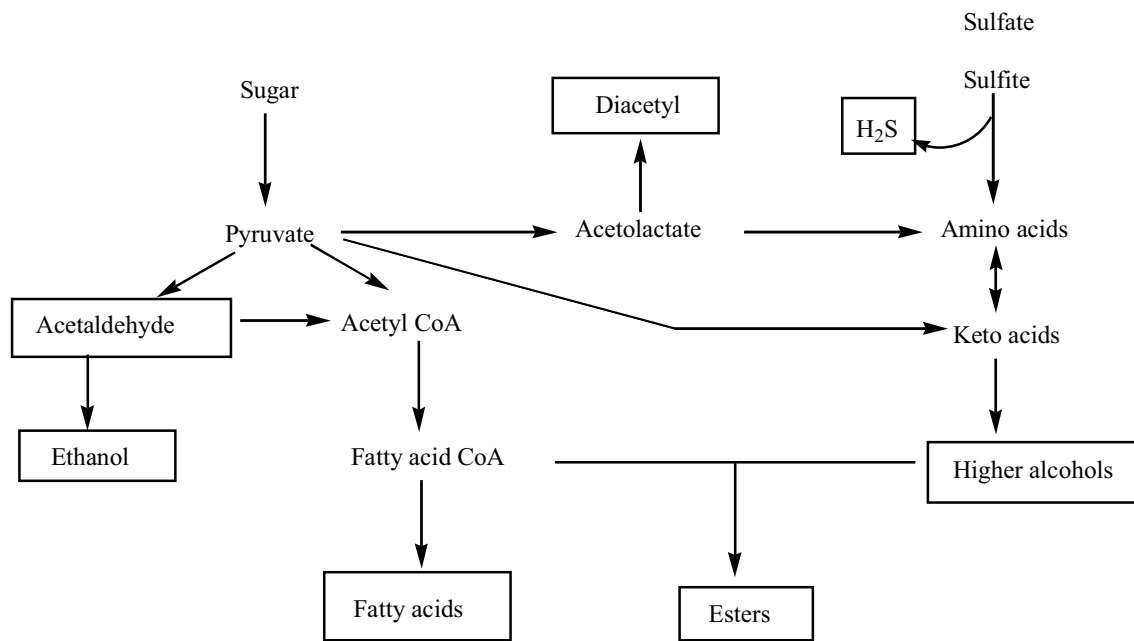
In dry white and rose winemaking, excessive must clarification can also lead to the excessive production of volatile acidity by yeast. Solids sedimentation (must lees) furnishes long chain unsaturated fatty acids (C18:1, C18:2). Yeast lipidic alimentation greatly influences acetic acid production during white and rose winemaking. The volatile acidity of wine made from freshly crushed grape rarely exceeds 0.3 g L<sup>-1</sup> (Ribéreau-Gayon et al., 2000a).

### **Citric acid**

Citric acid involves malolactic acid fermentation first, which is converted by citrate lyase to acetic acid and oxaloacetic acids. The latter was successively transformed by decarboxylation to pyruvic acid, which was subsequently converted to acetoin, diacetyl and acetic acid. Diacetyl is an important aroma component giving a butter-like flavour and as an off-flavour when over 3 mg diacetyl per litre is present, especially in red wines (Shimazu et al., 1985).

## **1.6 The role of aroma compounds**

Wine aroma is determined by a complex balance of several volatiles. More than 800 volatile compounds have been identified in wine (Bayonove, 1998; Ferreira et al., 1998; Noble, 1994). Fermentation increases the chemical and flavour complexity of wine by assisting the extraction of compounds from solids and present in grape must, modifying some grape-derived compounds and producing yeast metabolites (Fig. 1.5). The yeast derived flavour compounds can be placed into three groups: the major volatile products of fermentation, the trace “fermentation bouquet” compounds, and the undesirable or “negative” aroma compounds (Henschke and Jiranek, 1992).



**Figure 1.5** Derivation of flavour compounds from sugar, amino acids and sulphur metabolism by yeasts. (Henschke and Jiranek, 1992).

### Esters

Acetic acid esters are initially produced enzymatically. They are slowly hydrolyzed during storage until equilibrium is reached with the corresponding acids and alcohols, thereby increasing the concentration of acetic acid. A decrease in acetate concentration could be responsible for a loss of fruitiness while an increase in acetic acid concentration could be detected if other aging processes such as oxidation have raised the acetic acid level to above threshold concentrations. Ethyl esters hydrolyze more slowly than acetates, so the apple aroma from ethyl butyrate and ethyl 2-methylbutyrate would not be lost that fast (Ramey and Ough, 1980). The fruity esters ( $C_{4-6}$  acetates) are produced at high concentrations at temperatures less than  $20^{\circ}\text{C}$ , whereas the esters boiling at higher temperatures (ethyl octanoate, ethyl decanoate and 2-phenyl acetate) are generally found in greater amounts at higher fermentation temperatures (Daudt and Ough, 1973; Killian and Ough, 1979).

The increased fruit esters, such as isoamyl, isobutyl and hexyl acetates, are synthesized and retained to a greater degree at cool temperatures. Ethyl octanoate and ethyl decanoate, are produced optimally at  $15^{\circ}\text{C}$ , whereas 2-phenethyl acetate achieves its

highest concentration at 20°C (Ribéreau-Gayon et al., 2000a). Cabaroglu et al. (2002) found that ethyl butanoate, ethyl hexanoate, ethyl octanoate, and 3-methylbutyl acetate are distributed in both Okuzgozu and Bogazkere grape varieties, contributing to apple and fruity aroma, strawberry, green apple and fruity odour, a sweet, ripe fruit and soap odour, and a banana and fruity aroma, respectively (Acree and Am, 1999; Baumes et al., 1986; Girard et al., 1997; Miranda-Lopez, 1992b). The high concentration of 3-methylbutyl acetate is the impact flavour compound mainly responsible for the typical fermentation bouquet of Pinotage wines (Van et al., 1979).

### Higher alcohols

They are produced anabolically from glucose and catabolically from the corresponding amino acids present in the medium (Boulton et al., 1998). The principle higher alcohols produced by yeast are the aliphatic alcohols n-propanol, isobutanol (2-methyl-1-propanol), active amyl alcohol (2-methyl-1-butanol), isoamyl alcohol (3-methyl-1-butanol), and the aromatic alcohol 2-phenylethanol (Fleet et al., 1992). The higher alcohols contribute to the desirable aroma complexity of wine, but when their concentrations exceed 400 mg L<sup>-1</sup>, these compounds are regarded as a negative quality factor. Total concentration of higher alcohols can be affected by numerous factors such as climate conditions, must composition, juice turbidity, temperature and fermentation procedure (Rapp and Versini, 1991). Also, the yeast strains influence it very strongly (Rankine, 1967). Reduced concentrations of higher alcohols both in white or rose wines have been associated with limited amounts of insoluble solids in the must (Ough and Groat, 1978). Progressive increases in the assimilable nitrogen content of the medium reduce the concentration of total higher alcohols. Increasing must nitrogen content over the range 287-766 mg N L<sup>-1</sup> decreased the concentration of total higher alcohols, isobutyl, isoamyl and active amyl alcohols (Ough and Bell, 1980).

Higher alcohols generally surpass esters in concentrations throughout fermentation and finished wines, with isoamyl alcohol found in the highest concentration (Miller et al., 1987). 2-phenylethanol is the most important phenol derived from higher alcohol (Jackson, 1994), which bouquet resembles roses and is also believed to play a sensory role in the perception of wine aroma. Cabaroglu et al. (2002) reported that