CHAPTER 1

Screening of yeast strains and mango fruit cultivars for mango wine fermentation
2.1 INTRODUCTION

2.1.1 Wine and wine-making: Wine is an alcoholic beverage derived from grape juice (grape must), highly popular throughout the world. With a history of more than 8000 years, wine production is one of the world’s oldest biotechnological practices. It is a biological process and is the result of a series of biochemical transformations brought about by the action of several enzymes from different microorganisms, especially yeasts, which are responsible for the principal part of the process, alcoholic fermentation. Lactic acid bacteria are responsible for a secondary process, known as malolactic fermentation (Moreno-Arribas and Polo, 2005). During wine-making, the grape juice is also exposed to numerous enzymes originating from it and other than the yeasts that produce the alcoholic fermentation or the bacteria that produce malolactic fermentation. The endogenous enzymes of grapes, yeast, or bacteria present in juice and wine are often neither efficient nor sufficient under wine-making conditions, and hence commercial enzyme preparations were also widely used.

The initial environment that affects the microbial makeup of a wine fermentation is that of the vineyard. Although a drastically different environment than juice or wine, the types of microbes present on grapes will have an impact on the ensuing ecology in the wine fermentation, particularly in the early stages. Microorganisms appear to colonize around the grape stomata where small amounts of exudate are secreted (Ribereau-Gayon et al. 2000a). The most important transformation that takes place in the grape juice during vinification is the fermentation of the hexose sugars, resulting in ethanol and carbon dioxide production and the generation of a large number of by-products. In the initial phases of fermentation, different species of indigenous yeasts, called wild yeasts, present in the grape make an important contribution (Fugelsang, 1997). The predominant species belongs to the genera Brettanomyces, Candida, Cryptococcus, Debaromyces, Dekkera, Hanseniaspora, Hansenula, Kloechera, Kluyveromyces, Pichia, Rhodotorula, Torulaspora, Schizosaccharomyces, and Zygosaccharomyces. As fermentation progresses, these so-called ‘non-Saccharomyces’ species die off, leaving the more ethanol-tolerant S.
to predominate and complete the fermentation. This species has long been known as “the wine yeast.”

2.1.2 Wine-making process: Wine-making begins with the collection and crushing of grapes. A general schematic of common steps in red and white wine-making is presented in Figure 2.1. For white wines the grape juice is separated away from the skins and clarified via cold settling, filtration or centrifugation. The juice is then moved to a barrel or fermentation tank and the alcoholic fermentation is carried out by yeasts indigenous to the juice, or via inoculation of a selected *S. cerevisiae* starter culture. White wine fermentations are typically carried out for roughly one to two weeks at temperatures around 10 to 18°C. Upon consumption of available glucose and fructose, the main sugars in grape juice, the wine is considered “dry” and separated from the yeast and grape lees (sediment).

Red wines are produced slightly differently than white wines. After crushing the skins are left in the fermentation to allow for color extraction. Like white wines, the alcoholic fermentation commences either through the action of indigenous yeasts or via direct inoculation of a starter culture. During the fermentation the grape material tends to float to the top of the vat forming a “cap”. To better enable extraction of red pigments and to influence wine flavor, wine-makers typically punch down the cap or pump juice from the bottom over the cap. After a suitable period of time, the wine is separated from the grape skins and the fermentation is completed in another vessel. As described for white wines, the red wine is now “dry” and devoid of the main juice sugars.

After the alcoholic fermentation, wines often are spontaneously, or purposely, taken through a malolactic fermentation in which the high level of malate in the juice is converted to lactate, mostly by indigenous or inoculated LAB. Unlike the alcoholic fermentation, the malolactic fermentation is a stylistic consideration by the wine-maker, who, through use of antimicrobial additions (primarily sulfur dioxide) or filtration may choose to prevent this fermentation from initiating.
Fig. 2.1. General schematic for production of white and red wines
(Source: Mills et al., 2008)
Once the wine has been taken through the alcoholic and, if desired, the Malolactic fermentation, the wine is often stored in tanks or barrels to allow flavor development. The residence time for storage is primarily determined by the style of wine and winemaker choice. Often white wines are not stored for long periods of time while reds are frequently stored in oak barrels for several years. While the average wine contains approximately 13% ethanol, the alcohol by itself does not preclude future spoilage. Consequently wine-makers must take great care to prevent exposure of the wine to oxygen, which can encourage microbial growth, as well as judiciously use antimicrobials (again, primarily sulfur dioxide) to prevent microbial spoilage.

2.1.3 Factors affecting wine fermentation: Temperature is one of the most important parameters for the development of alcoholic fermentation since it can affect both the kinetics of the process in terms of duration and rate of fermentation and the final quality of the wine, i.e., production of secondary metabolites (Fleet and Heard, 1993). Other vinification factors, such as the pH, and the sugar concentration of the juice, may affect the growth and metabolic ability of yeast (Dubois et al., 1996; Charoenchai et al., 1998; Calderon et al., 2001). Numerous studies have reported on the influence of enological practices on fermentation rates and on changes in the composition of the grape juice (Alexandre et al., 1992; Angulo et al., 1993, Santamaria et al., 1995). In recent years, by means of several genetic studies, wine-making practices, such as clarification, have been shown to influence selection of the wild yeast strain of *S. cerevisiae* (Epifanio et al., 1999). Also a great number of studies have focused on the multiple factors that can lead to a slower fermentation rate and the possibility of synergistic effects (Bisson, 1999; Bauer and Pretorious, 2000). It is well known that yeast resistance to wine environmental conditions varies not only with the species, but is also strain associated.

The best characterized of the conditions leading to stuck and sluggish fermentations are nutrient deficiency. This nutrient deficiency is mainly due to limitations of assimilable nitrogen and, to a lesser extent, phosphate, lack of vitamins, essentially thiamine and minerals, and a near absence of oxygen. Other known stress factors also occurring during some wine fermentations include, for example, the presence of high levels of SO₂ and CO₂, the presence of competing microorganisms,
i.e., lactic acid bacteria identified as *Lactobacillus kunkeei* spp. Nov (Edwards *et al.*, 1998) as well as of some killer toxins produced by killer yeasts (Hidalgo and Flores, 1994). Otherwise, the supply of nutrients or fermentation activators has been suggested to be adequate for the majority of wine yeast strains.

2.1.4 Fermentation metabolites: From a physico-chemical point of view, wine can be defined as a non-ideal, multicomponent liquid solution containing water, ethanol, glycerol and organic acids as major constituents, as well as other minor components, such as flavours, aromas and phenolic compounds. These are either originally present in the grapes or synthesized during wine-making or formed during ageing of wine (Figure 2.2).

Fig. 2.2. A schematic representation of derivation and synthesis of flavour-active compounds from sugar, amino acids and sulfur metabolism by wine yeast. (Source: Swiegers *et al.*, 2005)
The aroma of wine is caused by hundreds of different compounds (Rapp and Mandery, 1986), and is defined as the fragrant perception that is derived from aromatic grape constituents (Margalit, 1997). This perception is a result of the odor-active volatiles traveling into the nose and binding with odor binding proteins that are found in the mucous membrane of the olfactory epithelium (Snyder et al., 1988). Once the aroma compound is bound, it activates adenylate cyclase, which in turn opens ion channels causing a depolarization and the firing of that receptor (Beauchamp and Bartoshuk, 1997). This impulse is then carried from the basal cells of the receptor to the olfactory bulb and higher areas of the brain for processing.

Most aroma compounds activate multiple receptors (Malnic et al., 1999), all of which have varying levels of sensitivity to each group of aromatic compounds. Volatile compounds also have many different chemical natures, covering a wide range of polarity, solubility, volatility, and pH (Ortega-Heras et al., 2002). Generally, volatile compounds can be detected at very low levels, in the order of $10^{-4} - 10^{-12}$ g/L (Rapp and Mandery, 1986). An important number of aroma-active volatiles, many of which are unstable, are found at low concentrations (µg/mL). They may be easily oxidized and degraded by heat or pH, creating new compounds as well as artifacts (Ortega-Heras et al., 2002).

Flavor, as an attribute of foods and beverages, has been defined (Amerine et al., 1965) as the sum of perceptions resulting from stimulation of receptors that are grouped together at the entrance of the alimentary and respiratory tracts. For purposes of practical sensory analysis, flavor is the interaction of chemical constituents with the sense of taste as well as smell. Flavor is, therefore, composed of volatile compounds, responsible for the aroma, as well as nonvolatile compounds, responsible for taste sensations (Meilgaard et al., 1999; Rapp and Mandery, 1986).

Aroma and flavor constituents of wines have been studied extensively over the last 20 years, and reviews of work on wine aroma and flavor list hundreds of volatile substances detected in different wines. The specific importance of fermentation-derived volatiles responsible for aroma, and their effects on wine quality, has also been
extensively investigated (Simpson and Miller, 1984; Rapp and Mandery, 1986). The presence or absence of these volatile substances is crucial to the identity and complexity of wine. Based upon this, evaluation of wine-making techniques that would function to improve the profile of desirable volatile compounds would be beneficial in the creation of high quality wines.

The aroma is a very important component of the organoleptic quality of wine. The total aromatic content of wine is 0.8-1.2 g/L. Most of these compounds are produced during fermentation and are especially important in the aroma of young wines (Figure 2.2). Acetic acid, acetaldehyde, ethyl acetate, propanol, isobutanol, 2- and 3-methylbutanol account for more than half of these volatiles, the other half being distributed among 600-800 minor volatile compounds present in very low amounts (acetals, organic acids, alcohols, phenolic and heterocyclic compounds, esters, lactones, terpenes and sulfur-containing compounds). The analysis of the contribution to the aroma of these minor compounds is complicated because of their low concentrations and their interactions. The quantity and quality of the aromas and flavours originated in must fermentation depend on environmental conditions, vinification process and the participating yeasts (Thorhgate, 1998; Swiegers et al., 2005). It would therefore be worthwhile to select wine yeast strains that produce the most appreciated aromas and flavours (Romano et al., 2003).

Acetaldehyde, precursor of the acetates and ethanol, is formed from pyruvate by the glycolytic pathway enzyme pyruvate decarboxylase. Freshly made wines usually have acetaldehyde concentrations below 75 mg/L (Zoecklein et al., 1995), although acetaldehyde content was variable from wine sample to sample, these initial values usually decline over time since acetaldehyde is a very reactive compound that combines with polyphenols and other compounds in the wine (Romano and Suzzi, 1993). It has been found that the production of acetaldehyde during must fermentation depends on technological factors (must composition, pH, fermentation temperature, aeration and SO₂ concentration) and on the yeast strain involved (Swiegers and Pretorius, 2005; Romano et al., 1994). With some exceptions, such as fino (pale) sherries, acetaldehyde
is considered undesirable at high concentrations. Hence, it is interesting to perform must fermentation with yeast strains that produce low amounts of acetaldehyde.

Esters are flavor compounds that are generally characterized by their fruity-flowery aromas in wine. At extremely high concentrations they contribute to off-flavours thus making the beverage unpalatable. Many different types of esters have been identified in fermented beverages and were grouped into two categories as acetate and ethyl group of esters. The acetates group consists of compounds such as ethyl acetate (fruity, solvent), isoamyl acetates (banana) and phenyl ethyl acetate (roses, honey, apple) while ethyl or medium chained fatty acid contains compounds such as ethyl caproate and ethyl caprylate (apple like aromas). The ethyl esters are the most dominant presumably because ethanol is the most readily available substrate. It is accepted that Esters are synthesized via two biochemical routes. Firstly, by the decomposition of higher alcohols and acetyl/acyl-CoA in the presence of the enzyme Alcohol acyl transferase (AaTase) (Nordstrom, 1964). Secondly, by esterases working in a reverse direction (Soumalainen, 1981). The rate of ester formation depends mainly on the availability of higher alcohols and acetyl/acyl-CoA substrates and the activity of the AaTase Enzymes. Careful consideration must be taken in selecting yeast strain for controlling the ester formation, since the average ester production and the proportion of the individual esters differs with various strains of yeast (Dufour et al., 2003).

Ethyl acetate is produced by the enzymatic esterification of acetic acid and ethanol. When the must fermentation is protected from aeration, the usual concentration of this compound is 30-50 mg/L, which increases to 60-110 mg/L with aeration. Ethyl acetate is the second most important component (after acetic acid) of wines volatile acidity. Concentrations below 70 mg/L are considered positive for the wine aroma, but higher 150-200 mg/L they are considered negative (Rapp, 1993). Among the wine yeasts, S. cerevisiae is one of the lowest ethyl acetate producers, while oxidative or weakly fermentative yeasts (Candida sp., Debaryomyces sp., Pichia sp. and Hansenula sp.) are the highest producers (Salvadores et al., 1993).
Higher alcohols or fusel alcohols or fusel oils are the main volatile compounds in wines which make up the largest group from a quantitative point of view, arising from either alcoholic or malolactic fermentation (Rapp and Mandery, 1986). There are more than 40 higher alcohols and among these are n-propanol, iso-butanol and isoamyl alcohols (2-methyl and 3-methyl butanol) which have organoleptic importance because they occur at concentrations above flavour threshold. Higher alcohols contribute to wine by intensifying the alcoholic taste, aroma and imparting a warmth mouth feel. In addition, they also serve as a precursor as for ester synthesis. The production of minor fermentation products, such as acetic acid, acetaldehyde, ethyl acetate and higher alcohols, is influenced by numerous factors related to the species and strain of the yeast (Castellari et al., 1994; Giudici and Zambonelli, 1992; Riponi et al., 1997). It was evident that different S. cerevisiae strains can produce significantly different flavor profiles even when fermenting the same must. This is a consequence of the differential ability of wine yeast strains to release varietal volatile compounds from grape precursors, and/or a different capacity to synthesize yeast-derived volatile compounds (Papathanasiou et al., 2006; Ugliano et al., 2006; Vilanova and Sieiro, 2006).

Isobutanol (2-methyl-1-propanol), active amyl alcohol (2-methyl-1-butanol), isoamyl alcohol (3-methyl-1-butanol), n-propanol, tyrosol, hexanol and 2-phenylethanol are the major fusel alcohols produced during must fermentation. In part, they are formed by transamination or disamination of the corresponding amino acid according to the Ehrlich pathway. The resulting acids are decarboxylated to aldehydes, which are finally reduced fusel alcohols. Some fusel alcohols have no possible precursor amongst the amino acids, and are formed from cetonic acids (Henschek and Jiranek, 1993). High amounts of these compounds are considered undesirable in table wines, and concentrations below 350 mg/L may be considered as positive for wine aroma (Rapp and Mandery 1986). The production of fusel alcohols by yeasts depends on the latters' ability to produce amino acids, and it varies according to genus, species and strain (Giudici et al., 1990). Usually, S. cerevisiae produces high amounts, whereas Candida, Kloekera and Brettanomyces produce low amounts. Other factors such as ethanol
production, fermentation temperature, pH, aeration and amount of solids in the must also influence fusel alcohol production by yeasts (Llaurado et al., 2005).

2.1.5 Mechanism of formation of higher alcohols: The formation of higher alcohols takes place via two metabolic routes, namely: the anabolic and catabolic routes. The anabolic route is achieved by synthesis from carbohydrates via pyruvare, while the catabolic route is synthesised as byproducts of amino acid assimilation. In both cases, the immediate precursors are 2-oxo (α-keto) acids. In the anabolic route the 2-oxo acids, arising from carbohydrate metabolism, are decarboxylated to form aldehydes, which are reduced to the corresponding alcohols. Simultaneously, 2-oxo acids also derive from amino acid utilization, which is termed the catabolic (Ehrlich) route to higher alcohol formation (Oshita et al., 1995). The synthesis pathway is governed by amino nitrogen concentration while lower levels of amino acid concentrations favour the anabolic route. Initially, the catabolic route is predominant due to high amino acid concentrations and overlaps with the anabolic route due to reduced amino acid concentrations.

The mechanism involved in the formation of higher alcohols requires a decarboxylation process of the corresponding α-keto acid, followed by a reduction which produces the final alcohol (Figure 2.3) (Rapp and Versini, 1991). When amino acids are catabolized through Ehrlich’s pathway, α-keto acids are mainly produced; for this to occur, glucose is necessary as a source of the α-keto-glutarate which behaves as an acceptor of the amino groups of the amino acids. The alcohols, n-propyl, isobutyl, active amyl and isoamyl, are formed from α-keto-butyrate, α-keto-valerate, α-keto-isocaproat and α-keto-β-methylvalerate which are derived from threonine, valine, leucine and isoleucine, respectively (Ouchi et al., 1980). However, in the absence of appropriate amino acids, such as at the beginning of fermentation, α-keto acids and the corresponding HA formed from these are obtained from glucose through a pyruvate pathway (Henschke and Jiranek, 1993). Using radioactive isotope markers, it has been found that 35% of higher alcohols come from the sugars while the remaining 65% have their origin in amino acids (Zoecklein et al., 1990a).
The relative amount formed by each pathway for every HA depends on various factors, such as yeast strain, yeast growth, fermentation temperature, juice pH, degree of aeration and solids level (Ancin et al., 1996). In addition, grape variety and ripeness affect the concentration of higher alcohols (Cabrera et al., 1988), probably because of the existence of qualitative and quantitative differences in must amino acid composition (Rapp and Versini, 1991). Also, the presence of large amounts of insoluble solids in musts during fermentation produces wines with high levels of higher alcohols and esters when compared to wines made with clarified musts (Groat and Ough, 1978). The size of the solid particles also affects HA concentration; it has been found that musts with an average particle size greater than 53 μm results in wines with isobutyl and isoamyl alcohol concentrations which are significantly higher than for musts where solid particles with average size under 38 μm predominate (Klingshirn et al., 1987).

Some of the volatile components generated by the yeasts that contribute to the aroma of the wines are affected by the type of yeast and the concentration of nitrogen (Bell and Henschke, 2005). Yeasts requiring large quantities of nitrogen produce high concentrations of esters during the alcoholic fermentation, and those need reduced amounts yield large concentrations of higher alcohols (Perez-Coello et al., 1999; Torrea et al., 2003). It was also found that low concentrations of higher alcohols are obtained, through anabolic pathways, in simple media containing ammonium as the only nitrogenous source (Henschke and Jiranek, 1993). The addition of ammonium salts to the must is permitted as the clarification process of juice eliminates part of the Yeast artificial nitrogen (YAN) and that, in some areas, grapes contain low levels of YAN. This increases the development of fruity aromas and reduces the level of higher alcohols as well as that of branched fatty acids in wines (Vilanova et al., 2007). Ough and Bell (1980) showed that increasing must nitrogen content over the range 287-766 mg N/Litre decreased the total concentration of the isobutyl and active amyl alcohols.
Fig. 2.3. Pathway for the formation of higher alcohols from glucose (Source: Boulton et al., 1996).
Also, Hernandez-Orte et al., (2005) found that the addition of YAN to the must reduces the levels of amylic alcohols, β-phenilethanol, and methionol and increases the concentration of propanoic acid. If ammonium salts are used, the levels of ethyl lactate and cis-3-hexanol also increase. When the medium is supplemented with amino acids or when it is complex, a substantial increment in HA concentration is produced; an exception is n-propyl alcohol (Herraiz et al., 1989). Furthermore, the addition of amino acids increases the levels of both γ-butyrolactone and isobutanol. In addition, when the levels of nitrogen are low and ammonium salts are added to the must, the speed of fermentation increases during the exponential phase, resulting in a significant increase in biomass (Hernandez-Orte et al., 2006). Both diammonium hydrogen phosphate and ammonium sulphate are authorised by the EU for increasing YAN levels in musts. Moreover, each of them presents other effects. Thus, diammonium hydrogen phosphate may favour the development of yeasts because it is also a source of phosphate. Ammonium sulphate may reduce must pH, avoiding the development of undesirable microorganisms (Flanzy, 2003).

2.1.6 Wine yeast and alcoholic fermentation: Yeast is the most simple of the eukaryotes and is a group of fungi in which unicellular form is predominant and reproduces predominantly asexually by budding (only Schyzosaccharimyces genera reproduce by binary fission). Yeasts from a complex and heterogeneous group found in three classes of fungi, characterized by their reproduction mode: Ascomycetes, Basidomycetes and the imperfect fungi or Deuteromycetes. The yeast found on the surface of grapes and in the wine belongs to Ascomycetes and Deuteromycetes. Like other soporiferous yeast belonging to the class Ascomycetes, S. cerevisiae can multiply either asexually by vegetative multiplication or sexually by forming ascospores. Under optimal nutritional and cultural condition S. cerevisiae doubles its mass every 90 min. Yeasts are in cosmopolitan distribution and have been isolated from natural substrates like leaves, flowers, sweet fruits, grains, fleshy fungi, exudates of trees, insect, dung and soil (Spencer and Spencer, 1997). Fruits are important microhabitats for a variety of yeast species in nature due to the high concentration of simple sugars, low pH and intense visitation by insect vectors (Lachance and Starmer, 1998).
Natural wine fermentation is a succession of yeast species; fermentation starts with apiculate yeasts (*Hanseniaspora/Kloeckera*), which are replaced by *Saccharomyces* yeasts. During the different stages of fermentation, several other yeast genera are occasionally present, including *Candida*, *Zygosaccharomyces*, *Torulaspora*, *Metschnikowia* and *Pichia* (Domizio et al., 2007). In addition, in spontaneous wine fermentations, a large diversity of microorganisms participates, including oxidative and fermentative yeasts, homo and hetero-fermentative lactic acid bacteria, and acetic bacteria (Heard and Fleet, 1986; Cabranes et al., 1990). However the main argument in favour of these fermentations is that they lend a greater typicality and organoleptic complexity to the fermented products (Romano et al., 1997; Egli et al., 1998; Ciani and Maccarelli, 1998; Henick-Kling et al., 1998). The main drawbacks reported are variability in the product quality and the risk of anomalous fermentations (Splittstoesser, 1982).

The use of pure cultures of yeasts provides a useful tool for standardizing the product (Fleet and Heard, 1993). Indigenous yeasts selected in particular region is a solution towards ensuring adequate control of the alcoholic fermentation and preserving the positive contribution of the indigenous yeasts (Riberau-Gayon, 1985; Martini and Martini, 1990). It is common practice to rely on the indigenous micro flora in the production of wine for both the alcoholic and the malolactic fermentations even in large scale vinifications. While Vilanova and Sieiro, (2006) have shown that wines obtained by spontaneous fermentation are more aromatic than those obtained by inoculating a selected yeast strain. However it was reported that the use of locally-selected yeast strains can positively affect the final quality of the wine (Vilanova and Massneuf-Pomarede, 2005).

Although there have been demonstrated differences in the production of volatiles between yeast strains, these differences have not been unambiguously shown to be reproducible. After exhaustively reviewing the subject, Thorhgate (1998) concluded that, since extrinsic factors can greatly affect a wine's volatile profile, it is too easy to reach erroneous conclusions regarding yeast strain effects. It therefore seems
necessary to conduct comprehensive studies of strain variability, so that wine-makers may know what possible flavour effects to expect for a specific yeast strain.

As wine consumers look for desirable sensory experiences and expect to feel pleasure through drinking an exclusive and complex wine (Bisson et al., 2002). Consequently, the major challenge of today’s wine makers is to fulfill the consumers’ demand and introduce wines with acceptable quality and price (Swiegers et al., 2005). Wine makers are interested in incorporating new enological practices to manage wine flavor (Fleet, 2008) and to distinguish themselves in the marketplace.

Single strains of commercial active dry wine yeasts have been used for many years to control alcoholic fermentation; however, this has resulted in the production of wines with a similar character throughout the world. Nevertheless, wine-makers have the ability to influence the nature and complexity of their wine by utilizing new indigenous yeast strains (Swiegers and Pretorius, 2005) or using mixtures of yeast strains to develop complexity in their wines. Howell et al., (2006) investigated the effects of mixed known *S. cerevisiae* strains on the chemical profile and aromatic properties of Chardonnay wines. They determined that the chemical profiles of the wines fermented with individual and mixed *S. cerevisiae* strains were different and that it was not possible to blend wines produced by the single strains to create the same chemical profile as a wine fermented by the mixed yeast cultures.

The selection of suitable yeast for each kind of fermentation is important to ensure a complete fermentation and to improve the final characteristics of the wine. Although it is clear that the quality of wine is associated with the variety and quality of the grape, yeasts can produce compounds that provide a distinctive touch to the final product. It is also interesting to study the use of selected yeast strains in mango wine production as information on this subject is very limited now-a-days. The aim of this study is to evaluate the behavior of 9 *Saccharomyces* strains which are well known for wine production.
2.2.0 REVIEW OF LITERATURE

In countries where grapes are not extensively produced, other available fruits are utilized for wine making. Mango being a luscious and nutritious fruit is ideal for wine making in India, as many varieties of it is cultivated in all the states (Reddy and Reddy, 2005). The concentration of wine aroma compounds can be influenced by various factors; among these environment (climate, soil and irrigation), grape variety, and degree of ripeness wine production and aging are very important (Rapp, 1998). Several authors have studied the influence on wine quality of yeast added to an alcoholic fermentation (Kunkee and Vilas 1994), since higher alcohols and ester contents in the wine depend on yeast and fermentation conditions (Aragon et al., 1998). According to Chatonnet et al., (1993) the enzyme activities of different yeast strains act differently on the same substrate of a fruit juice. The production levels of byproducts are variable and are yeast strain specific. The yeast strain used during fermentation can have a great influence on the ultimate quality of the final product. So, the selection of the yeast strain is the crucial step for an expected and assured good quality wine and its distillates.

2.2.1 Screening of mangoes for wine-making: Various fruits has been screened for wine production from the past two decades such as caja, banana, pupunha, mango, acerola and cocoa (Duarte et al., 2009) where grapes are not much available or not cost-effective. There are a variety of fruits suited for making a good quality wine. The fruits commonly used for making wine are: apple, pear, peach, plum, cherry, strawberry, blackberry, raspberry and blueberry.

Screening of mango varieties for wine making has been reported previously, for the first time Czyhrinciwk, (1966) reported the technology involved in mango wine production. Later Onkarayya and Singh (1984) screened twenty varieties of mangoes that are available from India for wine production. Obisanya et al. (1987) studied the fermentation of mango juice into wine using locally isolated S. cerevisiae and Schizosaccharomyces species of palm wine. From the physicochemical characteristics of the mango wine produced, it was observed that aromatic components were comparable in concentration with those of grape wine (Reddy and Reddy 2005). According to these reports, the composition of wine is changed with mango variety used
in the fermentation (Kulkarni et al. 1980; Onkarayya and Singh 1984; Reddy and Reddy 2005). In view of the differences noticed in some mango wines with different varieties necessitates the screening and selection of good quality of mango fruit, which is an important step to get good quality product. Reddy and Reddy (2005, 2009) screened six varieties of mangoes and studied the effect of enzymatic maceration on synthesis of higher alcohols during mango wine fermentation and found that more amounts of volatiles were observed in wine produced from Totapuri cultivar than wine from the cultivar Banginapalli. Kumar et al., (2009) had optimized condition for mango wine-making using statistical software, response surface methodology (RSM) by using Saccharomyces bayanus and the volatile aroma composition of mango wine from the two cultivars Banginapalli and Alphonso were reported by Reddy et al., (2010a) by gas chromatography coupled with mass spectrometry (GC-MS). Later, Reddy and Reddy, (2011) reported the effect of fermentation conditions (temperature, pH, SO2 and aeration) on wine fermentation and evaluated yeast growth, duration, fermentation rate and volatile composition and concluded that the temperature (25°C), pH (5), SO2 (100 ppm) for low alcoholic fermentation temperatures in wine making. Li et al. (2011) compared the chemical and volatile composition of mango wine fermented with 3 yeast stains and measured various volatile compounds. Pino and Queris, (2011) reported the volatile compounds of mango wine by using GC-FID and GC-MS and concluded that the compounds potentially most important to mango wine were ethyl butanoate and decanal. Li et al., (2012a) reported the volatile composition of mango wine fermented with two Williopsis yeast strains and concluded that unlike mango wine fermented with Saccharomyces cerevisiae; most terpenoids derived from mango juice were retained in the resultant mango wine fermented with the two Williopsis yeast strains, suggesting the mango wine could retain the aromatic hints of fresh mango. Again from the same research group, based from Singapore Li et al., (2012b) reported the behavior and fermentation performance of mixed yeasts using Saccharomyces cerevisiae and Williopsis saturnus into juices of three mango varieties and concluded that mango wine from cultivar Nam Doc Mai possessed the highest aroma intensity with winey, yeasty, fruity and floral notes that attributed to higher amounts of alcohols, acetate esters and ethyl esters.
2.3.0 MATERIALS AND METHODS

2.3.1 Sample source and processing: Seven cultivars of ripe mangoes were selected that were grown in Andhra Pradesh, South India, viz., Alphonso, Banginapalli, Neelam, Raspuri, Rumani, Sindhura and Totapuri were procured from the local market, all fruits were of ideal in ripeness for consumption and were uniform in size. Fresh edible puree of the fruits was used for the study. Each mango cultivar, the annual fruit available during summer, was procured from three different vendors, and processed.

2.3.2 Preparation of mango juice: Ripe mango (Mangifera indica L.) fruits var. Rumani were procured from the local fruit market in Tirupati, Andhra Pradesh, South India and were processed and homogenized according to (Reddy and Reddy 2005). Juice was recovered manually and up to 50 mg/L SO₂ was added and was treated with previously optimized enzyme concentrations 0.4% of BioTropicase, Pectinolytic enzyme procured from Biocon India Pvt Ltd, Bangalore, India. Juice extraction was made by pressing the treated puree in bi-layered cheese cloth. The juice obtained in this manner was sterilized by autoclaving at 115°C for 10 min and then subjected to analysis of total soluble solids, sugars (total and reducing), total acidity and pH. The final concentration of sugar was adjusted to ~20% (w/v) and pH to 3.8 using L-malic acid.

2.3.3 Yeast strain and inoculum preparation: Saccharomyces cerevisiae CFTRI-101, was procured from Central Food Technological Research Institute, Mysore, India; and S. cerevisiae NCIM-3215, S. cerevisiae NCIM-3090, S. cerevisiae NCIM-3189 and S. cerevisiae NCIM-3045 were procured from National Collection for Industrial Microorganisms, Pune, India. Wine yeasts, S. bayanus (S.B) and S. cerevisiae (S.C) were generous gift from Prof. Roberto Ambrosoli, University of Turin, Italy S. cerevisiae UCD 522 and S. bayanus UCD 595 were a kind gift from Dr. Narayana, Indian Institute of Horticulture Research (IIHR), Bangalore, India were used in the experiments. The culture was maintained on MPYD agar slants containing Maltose 3, Peptone 5, Yeast extract 3, Dextrose 10 and agar 20 (g/L) (Himedia, India), stored at 4°C and subcultured regularly at two months intervals. The inoculum was prepared by inoculating a loop full of culture into 50 mL of the sterile MPYD broth medium taken in
150 mL flask and growing it on a rotary shaker (100 rpm) for 48 h. The culture was harvested by centrifugation at 2000×g for 5 min and washed twice with phosphate buffered saline.

2.3.4 Viability determination: For viability measurements, 100 μL of appropriate dilutions of the cultures were plated (in triplicate) on MPYD plates. Plates were incubated at 30°C until the appearance of colonies (1-3 days), and the number of colony-forming units per ml of cell culture was determined. Microscopic cell viability was measured using methylene blue.

2.3.5 Determination of pH, total acidity, sugars, TSS and SO₂: The pH of the pulp/wine was measured with a hand digital pH meter (Eutech, Japan), pre-calibrated with buffers of pH 4.0 and pH 7.0. Total acidity in puree/wine were determined by titrating with 0.1N NaOH previously standardized using standard oxalic acid and the values were expressed as tartaric acid equivalents; and volatile acidity in the distillate samples are expressed as acetic acid mg/100mL according to Zoeklein et al., (1990b). The total soluble solids (TSS) was determined using a hand refractometer (0-30) (Erma, Japan) in terms of °Brix (°Bx). Free and total SO₂ was determined iodometrically by ripper method and the total reducing sugars were determined spectrophotometrically using dinitrosalicylic acid method (Miller, 1959) while glycerol was determined enzymatically by glycerol kinase method (Wieland 1959) on diluted samples employing the commercial kit from Megazyme International (Ireland).

2.3.6 Estimation of total phenolics: The total phenolic contents of Mango wine was determined colorimetrically using the Folin-phenol method (Singleton and Rossi, 1965). Possible interference of sulfur dioxide in the determination of total phenols was investigated before final determinations. Wines were analyzed before and after addition of acetaldehyde (1 g/L) to bind sulfur dioxide 5 min before total phenol analysis (De Beer et al., 2003). In Brief, 10 mL of wine was centrifuged at 2000 rpm at 20°C for 20 min and the supernatant was diluted with 10 mL ethanol and filtered through 0.45μm Millipore filter and sample aliquot of 100 μL was added to 900 μL of water, 1 mL of Folin-Ciocalteu reagent, and 2 mL of 10% sodium carbonate solution, mixed in a cyclo
mixer, and incubated for 1 h at room temperature. The absorbance was measured at 765 nm with a UV-visible spectrophotometer. The standard curve was drawn using 10-100 μg of gallic acid. The total phenolic content was expressed as gallic acid equivalent (GAE) in milligrams per 100mL of sample.

2.3.7 Determination of sulphydryl groups: The content of sulphydryl (SH) groups was determined using Ellmann's reagent in accordance with slight modifications to method reported by Rice-Evans et al. (1991). Briefly, 2.4 ml of phosphate buffer (5 mM, pH 8) were added to 0.6 ml of wine and the solution mixed. The background absorbance at 412 nm was then measured. Then, 0.3 ml of a solution (1 mM in 5 mM phosphate buffer, pH 8) of the thiol reagent, 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) was added, and the solution mixed and incubated for 1 h at 37°C. For each wine sample, the absorbance at 412 nm was read on a UV-visible spectrophotometer against a blank prepared under the same conditions, containing phosphate buffer instead of DTNB.

2.3.8 Fining of wine: The following experiment was set up: T1 – control, T2 – bentonite (0.5), T3 – bentonite (0.9), T4 – bentonite (1.1), T5 – PVPP (0.3), T6 – PVPP (0.7), T7 – PVPP (1.0), T8 – bentonite+PVPP (0.5+0.7 g/L), T9 – positive control stored at 8°C and T10 – positive control stored at RT. For each variant, 200 mL of wine were used and after treatments (two months) were completed, the wines were separated by centrifugation and analysed.

2.3.8.1 Fining using bentonite: The bentonite suspension solution was prepared by mixing 2.5 g bentonite, dried at 105°C for 4 h previously, with 50ml distilled water (60-80°C) and allowed to stand for a period of 12 h. The mixture was transferred into 100 ml volumetric flask for a constant volume after stirring. The final suspension concentration was 2.5 g/L. The suspension at various concentrations (0.5, 0.9 and 1.1 g/L) was then added with good agitation. For all the trials, slurries were prepared at least 24 h prior to use, allowing it for proper hydration.

2.3.8.2 Fining using PVPP: To evaluate the action of polyvinyl polypyrrolidone (PVPP) on the mango wine, various concentrations (0.3, 0.7 and 1 gm/L) of PVPP was
added to mango wine and were analyzed initially and after two months of storage at room temperature.

2.3.9 **Browning index measurement:** Browning index of the stored juice (100 mL) was measured as light absorbance at 420 nm using the Shimadzu UV-VIS spectrophotometer (Thermo Scientific, USA). Distilled water was used as reference.

2.3.10 **Evaluation of colour:** Colour measurements were made with a Hunter colorimeter (LabScan XE, Hunter Associate Laboratories, Inc., VA). The sample was placed in a 1 cm path length optical glass cell in the total transmission mode, using illuminant C and 2° observer angle. Five colour parameters were recorded: Hunter CIE lightness (L*), a* value, b* value, chroma (saturation, C*), and hue angle (h°).

2.3.11 **Determination of volatiles by Gas Chromatography:** Cell-free samples were obtained by centrifugation at 5000xg for 10 min after the completion of the fermentation and analyzed for alcohols. Ethanol and other major volatiles were determined by Gas Chromatography according to Antony, (1984). An Agilent systems GC-FID Model 6890 plus instrument was used for experiments and the conditions were as follows: Carbopack-B 80/120 mesh glass column (6 ft/2m with 2 mm i.d.; 1/4 mm), nitrogen gas was used as a carrier gas with a flow of 20 mL/min. Eluted compounds were detected by flame ionization detector (FID). Hydrogen with a flow rate of 40 mL/min was used as the fuel gas and the air was used as an oxidant (with a flow rate of 400 mL/min). 4-Methyl-2-pentanol was used as internal standard for all the samples.

2.3.12 **Sensory evaluation:** The sensory characteristics of the final wines were evaluated according to Dias et al. (2007) with a 20-membered panel. The preferences for taste, acidity, mouth feel, aroma, flavour, colour and overall acceptability were determined by 9-point hedonic scale (1, dislike extremely; 2, dislike very much; 3, dislike moderately; 4, dislike slightly; 5, neither like nor dislike; 6, like slightly; 7, like moderately; 8, like very much; 9, like extremely). Randomised refrigerated (10°C) samples, of 50 mL, were served in clear tulip shaped glasses coded with a random 3-digit code. Potable water was provided for rinsing of the palate during the testing.
Evaluations took place in the mornings between 9:00 and 10:00 A.M. and were conducted at room temperature (22-24°C) under white light. Taste was measured in terms of sweetness, where as flavor and aroma were to mango flavor. The mouth feel was assessed in terms of smoothness of the wine. Acidity was assessed in terms of sourness of the wine in the mouth. Colour of the wine was evaluated in terms of its intensity. Overall acceptance was the general preference expressed by the assessor after evaluating the sensory attributes. The mean intensity scores of all the attributes were calculated and plotted.

2.3.13 Statistical analysis: All the experiments were carried out in triplicate and the mean value and standard deviation were presented. Student’s t-test has been used to compare the mean values. The data were analyzed by one-way analysis of variance (ANOVA) using SPSS, version 12.0, post-hoc mean separations were performed by Duncan’s multiple-range test for analysis of variance.

2.4 RESULTS AND DISCUSSION

2.4.1 General composition of mango juice: Mango (Mangifera indica L.) is the most popular and the highly produced fruit of India. The chemical composition of mango pulp varies with the location of cultivation, variety and stage of maturity. The major constituents of the pulp are water, carbohydrates, organic acids, fats, minerals, pigments, tannins, vitamins and flavor compounds. The soluble sugars of the fruit pulp consisted mainly of glucose, fructose and sucrose. The total sugar content of mangoes varies between 11.5 and 25%. An increase in sugar content, decrease in acidity and loss of texture is associated with ripening of mango fruits (Medlicott et al. 1986). It is evident that with fruit ripening, the fruit matrix softens and become juicy; further treatment of pulp with the enzyme pectinase reduces the viscosity of the juice and the pictures of mangoes used in the study were presented in figure 2.4. The juice yield from different mango cultivars in this study ranges from 381-570 mL/Kg. Of all the cultivars studied, Neelam, Totapuri, Raspuri and Sindhura (381, 423, 480 and 495 mL/Kg respectively) were the low juice yield types. However Alphonso, Banginapalli and Rumani were of high juice yield (570, 562 and 560 mL/Kg respectively). The juice
yield is cultivar specific and might also depend on the degree of maturity. In the present study, the juice yield for Raspuri and Neelam cultivars were 480 and 381mL/Kg, however it was 600 and 480 mL/Kg, respectively as reported by Reddy and Reddy (2005).

The nine wine yeast strains collected from various sources were used for production of mango wine individually and their microscopic images were given in Figure 2.5. The physico-chemical composition of pulp/wine was summarized in Table 2.1. The total sugar concentration present in the different mango cultivars were varied and ranged from 14.6-19.2% (w/v). In this study, the highest sugars were in Alphonso (19.2%) and the lowest in Raspuri and Neelam (14.6 and 16.4%, respectively). Even though the cultivar Raspuri has less pectin content than Neelam it has the lowest amount of sugars and this could be attributed to the stage of ripening. Total soluble solids (TSS) also increase with ripening of fruits and in this study they are in the range of 22-24 °Bx. Titratable (Total) acidity in mango juice ranged from 4.1-5.2 (%).

The pH range of the mango juices was between 3.9 and 4.5, the lowest pH of 3.8 was found in the Neelam cultivar. There was a slight decrease in the pH from puree to wine. Amongst organic acids, malic acid was the predominant one whose concentration is ranged from 0.8 to 2.2 mg/L. The titratable acidity of mango wine samples ranged from 5.0 to 6.7 (g/L) and the volatile acidity was between 0.29 and 0.58 (g/L). The total acidity, volatile acidity, pH, residual sugars, higher alcohols and esters values were in comparable amounts in all the wines. The pH value of the wines ranging from 3.6-3.7 for all wines falls within range of the recommended optimal pH level of 3.5-4.0 for wine fermentation (Amerine et al., 1980). Residual sugars are the natural sugars left in a wine after fermentation. Generally, wines <1 mg/L are considered as dry wines however in this study residual sugars were ranging from 2.78-3.21 mg/L.
Fig. 2.4. Images of seven different mango cultivars used for mango wine production.
Table 2.1. Physico-chemical characteristics of mango pulp/wine produced from different varieties.

<table>
<thead>
<tr>
<th></th>
<th>Alphonso</th>
<th>Banginapalli</th>
<th>Neelam</th>
<th>Ruspuri</th>
<th>Rumani</th>
<th>Sindhura</th>
<th>Totapuri</th>
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<tbody>
<tr>
<td><strong>Juice composition:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Juice yield (mL/kg)</td>
<td>570±4.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>562±2.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>381±1.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>480±2.48&lt;sup&gt;d&lt;/sup&gt;</td>
<td>560±2.45&lt;sup&gt;e&lt;/sup&gt;</td>
<td>495±2.7&lt;sup&gt;f&lt;/sup&gt;</td>
<td>423±2.6&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total soluble solids (°Bx)</td>
<td>22.0</td>
<td>23.5</td>
<td>23.0</td>
<td>22.0</td>
<td>23.5</td>
<td>24.0</td>
<td>23.0</td>
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<tr>
<td>Reducing sugars (% w/v)</td>
<td>19.2±0.23&lt;sup&gt;d&lt;/sup&gt;</td>
<td>18.8±0.236&lt;sup&gt;d&lt;/sup&gt;</td>
<td>16.4±0.36&lt;sup&gt;e&lt;/sup&gt;</td>
<td>14.6±0.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.8±0.34&lt;sup&gt;c&lt;/sup&gt;</td>
<td>19.1±0.31&lt;sup&gt;d&lt;/sup&gt;</td>
<td>18.9±0.4&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Titratable acidity (%)</td>
<td>4.8</td>
<td>4.1</td>
<td>4.7</td>
<td>5.0</td>
<td>5.0</td>
<td>5.2</td>
<td>4.9</td>
</tr>
<tr>
<td>pH</td>
<td>4.2</td>
<td>4.4</td>
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<td>4.5</td>
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<tr>
<td>pH</td>
<td>4.0</td>
<td>3.9</td>
<td>3.6</td>
<td>3.9</td>
<td>4.1</td>
<td>4.0</td>
<td>3.8</td>
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<tr>
<td>Titratable acidity (g/L)</td>
<td>6.5</td>
<td>5.3</td>
<td>5.0</td>
<td>6.7</td>
<td>5.3</td>
<td>6.1</td>
<td>5.4</td>
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<tr>
<td>Volatile acidity (g/L)</td>
<td>0.29</td>
<td>0.51</td>
<td>0.58</td>
<td>0.49</td>
<td>0.31</td>
<td>0.48</td>
<td>0.4</td>
</tr>
<tr>
<td>Malic acid (mg/L)</td>
<td>1.76±0.29&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.54±0.32&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.2±0.23&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.39±0.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.45±0.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.81±0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.97±0.23&lt;sup&gt;f&lt;/sup&gt;</td>
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<tr>
<td>Residual sugars (mg/L)</td>
<td>2.78±0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.96±0.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.12±0.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.21±0.41&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.93±0.2&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>3.0±0.42&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>3.11±0.51&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Total polyphenolic content (mg/L)</td>
<td>537±4.8&lt;sup&gt;e&lt;/sup&gt;</td>
<td>456.4±2.3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>322.7±3.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>490.0±2.6&lt;sup&gt;e&lt;/sup&gt;</td>
<td>324.5±4.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>213.4±5.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>202.7±1.5&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>SH groups (mg/L)</td>
<td>3.04±0.68&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.68±0.21&lt;sup&gt;f&lt;/sup&gt;</td>
<td>5.02±0.46&lt;sup&gt;e&lt;/sup&gt;</td>
<td>4.24±0.54&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.89±0.61&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.95±0.37&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.81±0.47&lt;sup&gt;g&lt;/sup&gt;</td>
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<tr>
<td>Free SO&lt;sub&gt;2&lt;/sub&gt; (mg/L)</td>
<td>12</td>
<td>11</td>
<td>10</td>
<td>10</td>
<td>15</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>Total SO&lt;sub&gt;2&lt;/sub&gt; (mg/L)</td>
<td>40</td>
<td>52</td>
<td>46</td>
<td>51</td>
<td>54</td>
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<td>Ethanol (%)</td>
<td>12.3</td>
<td>12.2</td>
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<td>11.4</td>
<td>11.3</td>
<td>12.8</td>
<td>11.9</td>
</tr>
<tr>
<td>Glycerol (g/L)</td>
<td>7.5±0.17&lt;sup&gt;e&lt;/sup&gt;</td>
<td>7.2±0.11&lt;sup&gt;f&lt;/sup&gt;</td>
<td>4.5±0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.1±0.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.7±0.24&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.2±0.17&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.7±0.27&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values not sharing a common superscript letter differ significantly at p≤ 0.01 Duncan’s Multiple Range Test (DMRT).
Fig. 2.5. Microscopic ($40 \times$) images of nine different yeast strains used for mango wine production.
2.4.2 Screening of wine yeast strains for mango wine production: The budding yeast *Saccharomyces cerevisiae* has a long history in the fermentation industry. Owing to its efficiency in producing alcohol, *S. cerevisiae*, counted as “Generally Regarded As Safe” (GRAS) (Pretorius *et al.*, 2003) and is the mankind’s oldest domesticated organism used for brewing beer and other alcoholic beverages. Although the use of yeast was extended in modern molecular genetics and molecular biotechnology now-a-days, ethanol production by yeast fermentation still remains in the prime place. Ethanol is an important industrial chemical with emerging potential as a biofuel to replace vanishing fossil fuels (Alfenore *et al.*, 2002). So, the demand of yeast strains with a good fermentative efficiency and increased alcohol tolerance remains topical.

The selection of a good yeast strain having desirable properties is a prerequisite for the quality wine production (Degree *et al.* 1993). Enological traits of *S. cerevisiae* have been divided into two groups, i.e. technological and qualitative, and both groups have to be considered in the selection of wine yeasts. The technological ones influence the fermentation efficiency, and the qualitative ones determine the chemical composition and sensorial characteristics of wines. Yeast should have a corresponding metabolic profile, i.e. it should be chosen according to aroma and flavour that is typical for each wine (Reed and Nagodavithana, 1991; Romano *et al.*, 1998).

The quality and quantity of volatile compounds found in mango wine depend upon the *S. cerevisiae* strain used in this study. Therefore, it is important to know the potential differences in volatile production by various yeast strains in order to select the best one to produce desirable wine. The results show that the yeast strain *S. bayanus* showed better performance than the other yeast strains. Hence further optimization studies were carried out with *S. bayanus* for the production of mango wine for better quality.

The yeast strain used during fermentation can have a great influence on the ultimate quality of the final product. So, the selection of the yeast strain is the crucial step for an expected and assured good quality wine. Based on the fermentation kinetics and of the six yeast strains *S. cerevisiae* (UCD 522), *S. bayanus* (UCD 595), *S. bayanus*
(S.B), *S. cerevisiae* (S.C) and *S. cerevisiae* CFTRI 101 gave promising results at all conditions and selected for further use in mango wine-making.

2.4.3 Ethanol: Ethanol in wine is mainly produced by the alcoholic fermentation of sugar in must. Although small quantities are produced at various fruit ripening stages, the primary source of ethanol in wine is yeast fermentation. Ethanol is the principal organic by-product of fermentation. Under standard fermentation conditions, ethanol can accumulate at up to about 15-16%. In the present study, the percent alcohol in wines produced from seven cultivars of mangoes (Table 2.1) is in the range of 11.2-12.8%. The high ethanol production was found in wine produced from the *S. cerevisiae* (UCD 522), *S. bayanus* (UCD 595) *S. bayanus* (S.B) (12.8, 11.8, 11.5 % v/v) and lowest alcohol (8.8%) in wine from *S. cerevisiae* (3045). Also, there exists variation in ethanol (%) concentration amongst different cultivars of mangoes. The cultivars viz., *Alphonso, Banginapalli, Sindura and Rumani* which showed more juice yield more ethanol concentration (Figure 2.6), which could be because of the presence of more soluble sugars where as in remaining cultivars these have more pectins and insoluble fibers. The ethanol concentration of the wines, particularly from warm climates where grape sugar content is high, would reach above 15% (v/v) (de Barros Lopes et al. 2003). Higher levels can also be reached by the sequential addition of sugar during fermentation. Generally, it was accepted that the ethanol concentrations in the final product was proportional to the initial sugar content of the fruit before fermentation. In various non-grape fruits like apples, papaya, palm sap, bananas and berries were fermented for wine production but the final ethanol concentrations did not exceeded 10% however, ethanol concentrations in wine above 15% are the result of fortification. However the prime factors controlling ethanol production are sugar content, fermentation temperature, and yeast strain (Mauricio et al. 1997).
Fig. 2.6. Concentration of ethanol (%) in finished mango wine produced by 9 different yeast strains from 7 different mango cultivars.

Also, it was reported that immobilization affected the ethanol concentration as more concentrations were observed in the free cells than the wine produced by immobilized yeast (Oliveira et al., 2011). It was reported by Munoz et al. (2007) that ethanol concentration gradually decreased during wine ageing, irrespective of the yeasts used. Hence, it is inadvisable to prolong aging beyond the time where desired concentration is reached. Ancin et al., (1996) showed that the concentrations of ethanol did not vary with pre-fermentation treatments in the same variety of grapes.

2.4.4 Methanol: Methanol is not a major constituent in wines. The usual range found in wine is 100-200 g/L and has no direct sensory effect. The amount of methanol found in wine is primarily generated from the enzymatic (pectin methyl esterase) breakdown of pectins. After degradation, methyl groups associated with pectin are released as methanol. In the present study, the Methanol concentrations in wine produced from seven cultivars of mangoes are in the range of 121-168.5 mg/L (Figure 2.7). However, Reddy and Reddy (2005) reported the methanol content between 300 and 500 mg/L in wine from six cultivars of mangoes treated with pectinase but reported only 134 mg/L.
in untreated samples indicating that methanol content in the mango wine was ascribed to the use of pectinolytic enzymes, which are commonly used in mango wine production. Further Reddy and Reddy, (2009) reported that the methanol in pectinase enzyme treated wines were 120 and 143 mg/L in two mango cultivars Banginapalli and Totapuri respectively, however, it was 80 and 93 mg/L in control wine made from Banginapalli and Totapuri, respectively.

They also found that a lower amount of methanol was found in mango wine made from Banginapalli than Totapuri cultivars. There were no significant differences in methanol concentrations since its presence was due solely to pre-fermentation operations and it was independent of microbiological metabolism. However higher methanol concentration was observed in the cultivars Neelam and Totapuri when compared to others. Methanol in wine is toxic to humans if present in high concentration and the human oral lethal dose is 340 mg/kg body weight. Higher levels of methanol 485-768 mg/L were also reported in kiwi-fruit wine (Soufleros et al., 2001). Pectolytic enzymes, added to juice or wine to aid clarification, can inadvertently increase the methanol
content. Unlike most fruits, grapes are low in pectin. As a result, grape wine generally has the lowest methanol content of any fermented beverage. The methanol produced in the mango wine was significantly higher when compared to the grape wine, which contains less than 100 mg/L. Oliveira et al., (2011) showed that the fruit wine from cagaita showed methanol values up to 350 mg/L and found that more amount of methanol was present in the wine produced with immobilised yeast.

2.4.5 Acetaldehyde: Acetaldehyde was another important substance both from an organoleptic point of view and for its reactivity as a precursor of other odorous substances such as acetals. Most wines with high acetaldehyde concentration were poorly appreciated by the wine tasters, which is consistent with the perception threshold of this volatile compound from 100 to 125 mg/L according to Zoecklein et al., (1995). Yeast strains differ in their ability to produce acetaldehyde depending on the enzymatic activity (alcoholic dehydrogenase) related with the formation of the latter (Perez-Coello et al., 1999). In the present study, acetaldehyde did not show significant differences related to the yeast strains or mango cultivar used. In the present study, the concentration of acetaldehyde is ranging from 65.6-87.8 mg/L (Figure 2.8), which were within the range (50-120 mg/L) as cited by Fleet and Heard, (1994) in wines fermented by S. cerevisiae. However Reddy and Reddy (2005) reported the concentrations of acetaldehyde in six different cultivars of mango wine were in the range of 15-23mg/L, which were less when compared to that of concentrations in the present study. Further Reddy and Reddy (2011) observed that the acetaldehyde concentrations decreased with increase in temperature from 15-25°C however no significant differences were observed at 30 and 35°C. However in support to the present study, similar result was reported by Eglinton et al., (2000) in which they observed the higher concentration of acetaldehyde with S. bayanus mediated fermentations. It has been found that the production of acetaldehyde during must fermentation depends on technological factors such as must composition, pH, fermentation temperature, aeration and SO2 concentration (Romano et al., 1994) and on the yeast strain involved (Mateos et al 2006). Oliveira et al., 2011 found that the concentration of acetaldehyde was decreased when immobilized cells were used when compared to the free cell fermentation.
Fig. 2.8. Concentration of acetaldehyde (mg/L) in finished mango wine produced by 9 different yeast strains from 7 different mango cultivars.

2.4.6 Esters: Among of secondary products of fermentation, ester plays an important role in wine odors, which impart pleasant smell. Ester is formed when an alcohol functional group reacts with an acid function and water molecule is eliminated. Esters in wine have two distinct origins; enzymatic esterification during the fermentation process and chemical esterification during long term aging (Ribereau-Gayon et al., 2000b). At initial stages, trace amounts of ethyl acetate were observed in mango juice; it was synthesized de novo and increased significantly during fermentation (Reddy and Reddy 2009) and reported that in mango wine ester concentration varied among the two cultivars Rumani (17.3 mg/L) has a higher amount of ethyl acetate compared to Totapuri (9 mg/L). Recently, Reddy and Reddy (2011) found that temperature affects not only the fermentation kinetics (rate and length of fermentation) but also the yeast metabolism, which determine the chemical composition and in turn the quality of produced mango wine and reported that the highest total ester concentration were in the samples from low temperature (40 mg/L at 15°C). However this ester concentration was drastically decreased as temperature increased (18 mg/L at 35°C). In the present study, the ethyl acetate concentrations were varying with respect to the different cultivars of
mango and with the yeast strains used. Ethyl acetate in the samples were in the range from 12.4 to 22.4 mg/L (Figure 2.9).

These variations observed in the present study could be because of the fermentation temperature, mango cultivar used and also to the yeast strain applied. The production of esters also depends on various factors like fruit variety, yeast strain and fermentation conditions. In the present study the ester concentration is very much different between the wines produced with different yeast strains. Reddy and Reddy, (2005) have also observed similar results. It clearly shows that the ester concentration is influenced by yeast variety and other environmental conditions. Ethyl acetate is responsible for fruity flavor in wine at very low concentrations. The threshold value for its presence is between 10-12.5 mg/L and higher concentrations >75 mg/L produces a vinegar smell which negatively affects the wine aroma (Francis and Newton, 2005). In the present study, the amount of ester production varied with the nine yeasts that are used for the mango wine fermentation. The formation of volatile compounds during alcoholic fermentation depends not only on the particular species but also on the particular strain of the species (Romano et al., 2003; Lambrechts and Pretorius, 2000). Selected strains
of *S. cerevisiae* are now used in wine production in many countries and the results have been found to be excellent (Estevez et al., Gill, 2004). The composition and quality of wine are closely related to the yeast strain used (Estevez et al., 2004).

2.4.7 Higher (fusel) alcohols: Higher alcohols commonly account for about 50% of the aromatic constituents of wine, excluding ethanol (Ron, 2000). The principal 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) (Ribereau-Gayon et al., 2000b). The concentrations of higher alcohols in wine represents important variables for yeast strain differentiation, due to their strict relation with yeast metabolism (Romano et al., 2003). Also the concentration of these components in wine is effected by many factors like variety of fruit, clarification and fermentation conditions. Rapp and Mandery (1986) found that total higher alcohols in wine were found to be in the range of 80-540 mg/L and concentrations up to 300 mg/L contributed to pleasant flavor, but above this concentration provoked unpleasant flavor and harsh taste. These compounds could be synthesized by yeast through either the anabolic pathway from glucose, or the catabolic pathway from their corresponding amino acids (valine, leucine, isoleucine and phenylalanine). Higher alcohols (n-propanol, isobutanol, iso and act-amyl alcohols) analysed in this study were in good agreement with what has been previously published (Muratore et al., 1997; Reddy and Reddy 2009). The analyzed higher alcohols have their origin in fruit, except ethyl acetate, iso-amyl alcohol and 2-phenyl ethanol that were metabolized predominantly during fermentation as a result of yeast activity.

In the present study, isobutanol in the mango wine from different cultivars were in the range of 56.2-84.1 mg/L (Figure 2.10), more isobutanol was present in wine produced from cultivar Raspuri, which contains a lower protein level, which is in agreement with the earlier reports of Beech and Carr 1977, the lower isobutanol was present in the cv. Alphonso. However Reddy and Reddy (2005) showed that isobutanol was in the concentration of 29.3-94 mg/L but Reddy and Reddy (2009) observed that the kinetics of higher alcohol formation and the level of isobutanol increased gradually during fermentation 58 and 78 mg/L in the cultivars Banginapalli and Totapuri.
respectively. Also, they observed that isobutanol was present in wine produced from cultivar Totapuri.

![Fig 2.10. Concentration of Isobutanol (mg/L) in finished mango wine produced by 9 different yeast strains from 7 different mango cultivars.](image-url)

Recently Reddy and Reddy, (2011) reported that the isobutanol concentration varied with respect to temperature. From low temperature with increase to room temperature, they found that a gradual increase (73-112 mg/L) till 30°C was evident. However at temperature of 35°C there was a drop in the isobutanol concentrations (90 mg/L). These differences observed by them also in the present study could be because of the variation in temperature, cultivar and yeast stain used. Brown and Ough, (1981) reported that the increased isobutanol concentration has a positive effect on the quality of red wines. Pectinase treatment could increase the isobutanol synthesis by the oxygen diffusion in mash. Valine is the main precursor of isobutyl alcohol through Ehrlich’s pathway (Henschke and Jiranek, 1993). Ancin et al., (1996) showed that the initial concentrations of valine in clarified garnacha musts, and its consumption during fermentation, increased substantially with respect to the control. However, the more clarified musts resulted in a lower production of isobutyl alcohol. Mauricio et al. (1997) found an increase of isobutanol during must fermentation under semi-aerobic conditions.
in contrast with anaerobic conditions. Synthesis of isoamyl alcohol depends primarily on fermentation temperature and on juice total nitrogen content. Isoamyl alcohol below its threshold level contributes a positive effect on wine flavor (Ohkubo and Ough, 1987).

n-Propanol was present in fresh fruit but fermentation provoked further increase of n-Propanol and started to accumulate at the very beginning of fermentation (Reddy and Reddy 2009), however, Pino et al. 2005 reported that n-Propanol is not present in all mango juices. In the present study, n-propanol in the mango wine were in the range of 24.6-45.3mg/L, wine made from Alphonso contained higher amount of n-Propanol when compared with Neelam cultivar. Reddy and Reddy (2005) found that the n-Propanol was in the range from 22.25 to 56 mg/L among six cultivars and found that wine from the cultivar Alphonso showed the highest. However Reddy and Reddy (2009) found that wine produced from the cultivar Totapuri (43 mg/L) had high amount of n-propanol compared to wine from the cultivar Banginapalli (30 mg/L). They also observed that during fermentation kinetics, pectinase enzyme treatment caused an increase in n-propanol concentration, probably because of abundant O₂ availability.

Fig 2.11. Concentration of n-propanol (mg/L) in finished mango wine produced by 9 different yeast strains from 7 different mango cultivars.
Giudici and Kunkee (1994) found that the n-propanol production strongly depends on yeast strain; *S. cerevisiae* strain 6527 always produced lower amounts of n-propanol irrespective of the total nitrogen in synthetic medium, while at the same time, strain 6392 responded strongly to the amount of nitrogen in the medium. Similarly in the present study, more amount of n-propanol was found in the mango wine fermented with *Saccharomyces cerevisiae* (S.C), CFTRI-101, NCIM-3215 and NCIM-3090 strains with the cultivar *Alphonso* (Figure 2.1). It was also found by Reddy and Reddy (2011) that the n-Propanol varied with respect to concentration of SO$_2$ used and also with temperature. With increase in fermentation temperature from 15-30°C, n-Propanol increased from 40±0.83 to 50±1.53 and later observed drop in its concentration at 35°C (42.5±3.6). The main precursors for n-propanol are threonine and γ-aminobutyric acid (Ancin *et al.*, 1996). However studies on wine fermentation supplemented with nitrogen or amino acids showed that values of n-propanol concentration found in all wines were independent of the concentration and consumption of these amino acids, and of the clarification level of initial musts which could be due to that the solids of the juice affect higher alcohol production through its effect on amino acid uptake. However Giudici and Kunkee (1994) found that the n-propanol production is strongly yeast strain dependent.

In the present study, iso and act-amyl alcohols were in the range of 100.4-119.6 mg/L, the highest amount were observed from *Neelam* when compared to the wine from *Sindhura* and *Rumani* (100.4 mg/L) cultivars (Figure 2.12). In earlier report by Reddy and Reddy (2005), amyl alcohols were in the range from 60-148 mg/L; the highest amounts were found in the cultivars *Beneshan* followed by *Alphonso* and the lowest was found in *Neelam*. It was found that pectinase treatment enhanced the synthesis of iso and act-amyl alcohols, because of higher aeration during enzyme treatment (Reddy and Reddy 2009). Bosso (1993) found an increase in iso-amyl alcohol in white wine obtained from macerated grapes. Ohkubo and Ough (1987) reported that active amyl and isoamyl alcohols were formed most abundantly at 21-27°C and are dependent on juice total nitrogen content.
Fig 2.12. Concentration of amyl alcohols (mg/L) in finished mango wine produced by 9 different yeast strains from 7 different mango cultivars.

Reddy and Reddy (2011) found that the iso and act-amyl alcohols were increased with increase in temperature from 95.5±8.3 to 200±5.68 till 30°C and observed a drop in their concentration to 152±7.49 at 35°C. Iso-amyl alcohol below its threshold level contributes a positive effect on wine flavor. Other higher alcohols in high concentration, particularly isoamyl alcohol, contribute to unpleasant flavor (Kourkoutas et al., 2003). Kunkee and Vilas (1994) reported that the synthesis of acetic acid, iso-butanol and iso-amyl alcohol during fermentation depended primarily on yeast strain.

2.4.8 Glycerol: It is the most abundant by-product of wine fermentation after ethanol and carbon dioxide. This does not directly contribute to wine aroma due to its nonvolatile nature, but it contributes to sweetness, fullness and smoothness. Glycerol is a major product of yeast fermentation and is reported to range up to 9.9 g/L in Australian white table wines (Rankine and Bidson, 1971), and 9.36 g/L in South African dry white wines (Nieuwoudt et al., 2002). In its pure form glycerol is a viscous liquid at room temperature and recently Nurgel and Pickering, (2005) reported
enhanced perceived viscosity of a model wine upon increasing its glycerol concentration from 10 to 25 g/L. The amount of glycerol formed during fermentation is influenced by several factors, such as grape variety, degree of ripeness, fermentation temperature, SO₂ concentration, pH, nitrogen composition, aeration, yeast strain and inoculation level. The threshold taste level of glycerol is observed at 5.2 g/L in wine. Typical glycerol levels in mango wine are as 1-15 g/L, with average values approximately 7 g/L (Kumar et al. 2009). In this study the concentration of glycerol was ranged from 4.5-7.5 g/L (Table 2.1).

2.4.9 Total phenolic content: These compounds are among the most important components of wines and are directly related not only to its colour, astringency, bitterness and oxidative level, but also to well-known health beneficial effects as antioxidants. Berardini et al., 2005 reported that the polyphenolic compounds in mango peel were in the concentration of 129.4 and 71.0 mg/g (d.m) according to two different recovery processes. However they reported that the profiles of the polyphenols were almost identical in both processes and found that significant loss in flavonol glycosides depending on heat exposure in the cultivar Tommy Atkins. Garcia-Solis (2008) reported the polyphenolic compounds in the juice of Ataulfo mango as 51.0±3.2 µg/mL. Schieber et al., (2000) characterized Polyphenols from mango puree concentrate using HPLC-MS-DAD and five quercetin glycosides and one kaempferol glycoside were identified, also gallic acid was predominant among the phenolic acids. Ribeiro et al., (2007) reported that phenolic compounds content ranged between 48.40 and 208.70 mg/100 g in mango pulp of Brazilian varieties. In Indian cultivar Alphonso, the total polyphenol content is reported to have 44 mg/g in pulp (Ravindra and Shivashankar, 2004). In the present study, the total polyphenolic compounds present in the mango wine were in the range of 537.4-202.7 mg/L. The highest were in wine from Alphonso (537 mg/L) and the lowest was in the wine from the cultivar Totapuri (202.7 mg/L) (Table 2.1). These results were in agreement with Kumar et al. (2012) who reported the total polyphenolic compounds in wine from south Indian mango cultivars, who reported that the highest content of polyphenolics were found in wine from Alphonso (537.3±4.8), Raspuri (490.0±2.6) and Banginapalli (456.4±2.3). The lowest values were found in Sindhura
and Totapuri (202.7±1.5 mg/L). However these results were also in agreement with Gorinstein et al. (1993) who reported 420 and 390 mg/L in two Israeli white grape wine cultivars, Sauvignon Blanc and French Colombard respectively.

2.4.10 Hunter colour parameters: The determination of the coordinates L*, a*, b* characterizes wine color. At this scale, L* measures luminosity that varies from zero (black) to 100 (pure white); a* and b* values represent the levels of tonality and saturation, with +a (indicating red), -a (indicating green), +b (indicating yellow) and -b (indicating blue). Lightness (L*), hue angle (h°) and chroma (C) are the attributes defining the colour. Lightness is the brightness of a colour. Hue angle is defined as the colour of the material black and chroma indicates the strength of the chromatic response. Hue angle and chroma are the parameters associated with a* and b* values. According to Patras et al. (2009), hunter L, a* and b* or some combinations of a* and b* are the physical characteristics used to indicate the visual colour.

Among the wines, the wine from Banginapalli cultivar was the darkest (L* value of 28.1), while the wine from Totapuri was the lightest (L* value of 16.72) in colour (Table 2.2). However, all the wines were lighter when compared to that of their respective puree; this could be due to filtration of puree to remove insoluble pectic substances. Chen et al. (1995) reported that the heating process has significant decrease in the lightness, hue angle and chroma leading the pineapple juice to appear darker because of the degradation of carotenoid pigment which led to the loss of yellowness in juice. Also this decrease may related to the partial precipitation by unstable, suspended particles in juice (Genovese et al., 1997) and the non-enzymatic browning (Maillard) reaction taking place between amino acids and reducing sugar present in the juice. Though all the wines are in yellow visually in terms of colour, there were little differences among the wines in b* parameter (yellowness). Positive values of a* and b*, as observed in this work, attributed to the carotenoids present in the wine. C* (Chroma) is a parameter that indicates the contribution of of a* (redness) and b* (yellowness) and Chroma of the wines were ranging from 10.33 to 16.05 (Table 2.2). Wine from Alphonso was with larger chroma (16.05) than wine from Totapuri (10.33). However a significant reduction of Chroma was observed by Cortes et al. (2008), who found that
the chroma of thermally pasteurised orange juice decreased during refrigerated storage. This indicates that the juices' colour became significantly less saturated with increasing of storage time. Hue angle (colour, $h^\circ$) was ranging from 77.6 to 82.69° and Overall, wines from *Alphonso, Sindhura, Banginapalli* and *Rumani* (mean hue angle value = 81.35°) were slightly more orange than the wines from other cultivars and were in comparison with the white wines, reported by Lee and Rennaker, (2007). However Rivas *et al.*, (2006) reported that the hue angle of thermally pasteurised mixed orange and carrot juice diminished significantly during storage and the juice became redder and less yellow when the hue decreases (Esteve and Frigola, 2007).

Colour degradation in juice may due to non-enzymatic Maillard browning, which is the reaction between sugars, amino acids and organic acids, which has a severe impact on colour and thus reduces the consumer appeal (Klim and Nagy, 1988). In addition, the colour of juice may change due to heating, air and light, which cause carotenoids to undergo oxidation, cis/trans changes and alterations in epoxide rings as a function of storage (Esteve and Frigola, 2007).

Table 2.2. Hunter color values of mango wine.

<table>
<thead>
<tr>
<th>Variety</th>
<th>$L^*$</th>
<th>$a^*$ value</th>
<th>$b^*$ value</th>
<th>Chroma (C)</th>
<th>Hue angle ($h^\circ$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Alphonso</em></td>
<td>25.54</td>
<td>2.04</td>
<td>15.92</td>
<td>16.05</td>
<td>82.69</td>
</tr>
<tr>
<td><em>Banginapalli</em></td>
<td>28.11</td>
<td>2.09</td>
<td>12.71</td>
<td>12.88</td>
<td>80.65</td>
</tr>
<tr>
<td><em>Neelum</em></td>
<td>22.79</td>
<td>1.88</td>
<td>10.44</td>
<td>10.6</td>
<td>79.78</td>
</tr>
<tr>
<td><em>Sindhura</em></td>
<td>22.05</td>
<td>1.75</td>
<td>12.00</td>
<td>12.3</td>
<td>81.7</td>
</tr>
<tr>
<td><em>Rasapuri</em></td>
<td>18.37</td>
<td>2.34</td>
<td>10.54</td>
<td>10.79</td>
<td>77.47</td>
</tr>
<tr>
<td><em>Rumani</em></td>
<td>20.5</td>
<td>1.93</td>
<td>11.36</td>
<td>11.52</td>
<td>80.36</td>
</tr>
<tr>
<td><em>Totapuri</em></td>
<td>16.72</td>
<td>2.22</td>
<td>10.09</td>
<td>10.33</td>
<td>77.6</td>
</tr>
</tbody>
</table>

Values presented are mean of duplicate analysis.

Chroma (C) = $[(a^*)^2 + (b^*)^2]^{1/2}$ and Hue angle ($h^\circ$) = $\arctan (b^*/a^*)$

2.4.11 Glutathione (SH groups): Glutathione (GSH) was the main non-volatile sulphur compound (tri-peptide) found both in the must and in the finished wine. Primarily glutathione steadily increased towards the end of fermentation and the final wine
concentration of GSH were in the range of 0.1-5.1 mg/L in white grape musts (Park et al., 2000a). However Cimino et al., (2007) reported GSH content in the range of 3.04-10.0 mg/L in red wines. In the present study, final GSH concentration in wines ranging from 3.0 to 5.81 mg/L, and is in agreement with the above reports (Table 2.1). Park et al. (2000b) had found that Palomino grape juice contained 1.28 mg/L GSH but not in Sauvignon blanc grape juice. However GSH increased during fermentation in both samples. Following fermentation, 5.1 mg/L GSH was found in the Palomino wine and 2.1 mg/L for the Sauvignon blanc. According to Lavigne et al. (2007), the amount of glutathione present in a wine at the end of the alcoholic fermentation depends on the yeast strain, and decreases as the lees are removed and also during the aging in barrels. In yeast, GSH accounts for about 1% of dry weight of S. cerevisiae and represents more than 95% of the low molecular thiol pool (Elskens et al. 1991); thereby some of this glutathione could possibly be released into the wine.

Sulfur dioxide is typically used as an antioxidant and antiseptic in wine-making. As a result, this substance occurs in two different forms in wine as free (aqueous SO₂) and bound SO₂ (to Aldehydes). The total and free SO₂ contents of wine are key analytical parameters for must and wine quality control. In the present study, the free SO₂ are in the range of 10-15 mg/L whereas bound SO₂ are in the range of 40-57 mg/L. These results are in agreement with that of different white and red wines as reported by Cmelik et al. (2005) and are in comply with existing legal limits. In the EU, the maximum level of total SO₂ permitted is 160 mg/L for red wines and 210 mg/L for white and rose wines (European Union Council 1999).

2.4.12 Fining trials: The object of fining is to aid to create a product, which is near perfect in terms of taste, colour, bouquet and clarity. The fining method should not take away from any of these characteristics and should allow the clarified conditions to be maintained for as long as necessary before the wine is consumed. Any fining treatment preferably should have little or no effect on the essential aromatic and flavor compounds of wine.
Table 2.3. Effect of fining treatments on the polyphenolic compounds and Hunter colour measurements in wine from two cultivars Banginapalli and Rumani.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Treatments (T)</th>
<th>Total phenolics</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>Abs. at 420</th>
<th>Total phenolics</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>Abs. at 420</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>345.6</td>
<td>4.08</td>
<td>1.05</td>
<td>1.64</td>
<td>0.42</td>
<td>462.3</td>
<td>5.24</td>
<td>1.11</td>
<td>1.85</td>
<td>0.48</td>
</tr>
<tr>
<td>2</td>
<td>Bn 0.5g/L</td>
<td>335.5</td>
<td>3.52</td>
<td>-1.17</td>
<td>1.58</td>
<td>0.33</td>
<td>456.1</td>
<td>4.40</td>
<td>-1.32</td>
<td>1.66</td>
<td>0.27</td>
</tr>
<tr>
<td>3</td>
<td>Bn 0.9g/L</td>
<td>324.7</td>
<td>3.52</td>
<td>-1.45</td>
<td>1.76</td>
<td>0.31</td>
<td>442.3</td>
<td>3.96</td>
<td>-1.90</td>
<td>1.66</td>
<td>0.27</td>
</tr>
<tr>
<td>4</td>
<td>Bn 1.1g/L</td>
<td>311.6</td>
<td>3.59</td>
<td>-1.43</td>
<td>1.56</td>
<td>0.31</td>
<td>431.6</td>
<td>3.85</td>
<td>-1.56</td>
<td>1.69</td>
<td>0.25</td>
</tr>
<tr>
<td>5</td>
<td>PVPP 0.3g/L</td>
<td>328.6</td>
<td>3.86</td>
<td>-1.28</td>
<td>1.84</td>
<td>0.32</td>
<td>452.8</td>
<td>4.04</td>
<td>-1.71</td>
<td>1.68</td>
<td>0.29</td>
</tr>
<tr>
<td>6</td>
<td>PVPP 0.7g/L</td>
<td>324.9</td>
<td>4.00</td>
<td>-1.28</td>
<td>1.81</td>
<td>0.29</td>
<td>443.5</td>
<td>4.14</td>
<td>-1.44</td>
<td>2.14</td>
<td>0.30</td>
</tr>
<tr>
<td>7</td>
<td>PVPP 1.0 g/L</td>
<td>319.4</td>
<td>3.99</td>
<td>-1.21</td>
<td>1.53</td>
<td>0.29</td>
<td>429.7</td>
<td>4.30</td>
<td>-1.69</td>
<td>1.48</td>
<td>0.28</td>
</tr>
<tr>
<td>8</td>
<td>Bn 0.5g/L+PVPP 0.7g/L</td>
<td>327.1</td>
<td>3.95</td>
<td>-1.37</td>
<td>1.59</td>
<td>0.28</td>
<td>441.4</td>
<td>3.76</td>
<td>-1.47</td>
<td>1.24</td>
<td>0.25</td>
</tr>
<tr>
<td>9</td>
<td>Positive Control (8°C)</td>
<td>340.4</td>
<td>3.98</td>
<td>-1.21</td>
<td>1.50</td>
<td>0.22</td>
<td>458.4</td>
<td>6.03</td>
<td>-1.37</td>
<td>1.80</td>
<td>0.22</td>
</tr>
<tr>
<td>10</td>
<td>Positive Control (RT)</td>
<td>338.8</td>
<td>3.82</td>
<td>-1.13</td>
<td>1.59</td>
<td>0.35</td>
<td>451.2</td>
<td>4.81</td>
<td>-1.68</td>
<td>1.96</td>
<td>0.35</td>
</tr>
</tbody>
</table>

Bn = Bentonite; PVPP = Poly vinyl poly pyrrolidine; Abs = Absorbance at 420nm.
In this work, the fining selected agents were: Bentonite and polyvinylpolypyrrolidin (PVPP). In this way only Spectrophotometric determinations were carried out in order to check the effect of these agents on the evolution of colour density during storage in two cultivars (Banginapalli and Rumani) of mango wine.

Treatment with bentonite suspension under different concentrations (0.5 g/L+0.9 g/L, 1.1 g/L) in water together with PVPP or individually (0.3 g/L, 0.7 g/L, 1.0 g/L) depressed the amount of polyphenols up to 17-32% irrespective of the mango cultivar (Table 2.3). This data show similar amounts of polyphenols in white wines. Similarly, apple juice has been reported to show a decrease in turbidity when treated with combination of bentonite and gelatin (Tajchakavit et al., 2001). These authors also reported an increase in turbidity during storage. Fining helps remove active precursors and thus, decrease the potential for haze formation during storage, while providing a more limpid juice (Hsu et al., 1990).

Stankovic et al., 2004 also reported that the bentonite treatments resulted in considerable decrease in polymers and anthocyanins, which is correlated with higher decrease in colour intensity.

The parameter $a^*$ ranged between 1.05 to -1.37 in rumani cultivar and 1.11 to -1.90 in Banginalli, and the $b^*$ was positive in the wines before fining and after fining, $b^*$ was negative. The luminance ($L^*$) increased after clarification. The variations of color were consistent with the level of oxidation. Accumulation of oxidized products led to gain in orange-yellow saturation. Ultrafiltration has been reported to preserve the initial color of the wine in comparison to different fining treatment. For instance, the absorbance of the depectinized ultrafiltered must was higher than the wines. This indicated that the clarification method was indeed a point on which the producers could act to modulate the organoleptic qualities of wines.

The browning index (Absorbance at 420 nm) increased with storage temperature irrespective of the mango cultivar. The wine treated with fining agents had lower browning index than control wine. There was an increase in browning index at higher storage temperature, also an increase in browning index during three months storage at 37°C. The browning index increased in clarified wine during storage due to rapid onset
of browning. The interaction between fining treatment and storage time on the browning index of the juice showed an increase in browning index of control and fining treated wine with increase in storage time regardless of storage temperature. Maillard type reactions are responsible for browning and both enzymatic and non-enzymatic browning has been implicated in the darkening that occurs in fruit juices and wines during processing and storage.

The results obtained from the different treatments used for processing suggest that post bottling haze formation could be reduced by fining treatment. Both bentonite PVPP treatments produced better juice quality than control. These treatments were effective in reducing turbidity, total polyphenol and browning and clarified wine maintained good organoleptic quality after fining treatment. Both haze formation and browning were enhanced by higher storage temperature. Therefore, storage temperature recommended to enhance stability of product, which prevent haze formation was 4-8°C.

2.5 CONCLUSIONS

This study concludes that the mango cultivars Alphonso, Banginapalli and Sindhura and Rumani are suitable for wine-making from mango. The yeasts used in the study S. cerevisiae UCD 522, S. bayanus UCD 595, S. cerevisiae (S.C) and S. bayanus (S.B) from University of Turin were found to have more fermentation power and could ferment mango wine to dry (residual sugars <2 mg/L). Since the concentrations of all the wine compounds reported here proved to be acceptable in terms of wine quality, the yeasts selected were potentially suitable for vinification. The sensory evaluation has indicated that the wine possesses novel characteristics in aroma and taste and good acceptability. Wine from the cultivars Banginapalli, Alphonso Sindhura and Rumani has better sensorial notes and overall acceptability. The glycerol concentration and Hunters colour measurements were good even after fining. For fining trials, the combination of bentonite+PVPP at a concentration of Bn 0.5g/L+PVPP 0.7g/L was finalised for mango wine-making.