WIDE HYBRIDIZATION TO DEVELOP NEW MALE STERILE LINES AND RESTORERS WITH FLORAL TRAITS IN RICE (*Oryza sativa* (L.))

Thesis submitted in part fulfilment of the requirements for the award of

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to the Tamil Nadu Agricultural University,Coimbatore-3

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CERTIFICATE

This is to certify that the thesis entitled " **WIDE HYBRIDIZATION TO DEVELOP NEW MALE STERILE LINES AND RESTORERS WITH FLORAL TRAITS IN RICE (*Oryza sativa* (L.))"** submitted in part fulfilment of the requirements for the award of the degree of **DOCTOR OF PHILOSOPHY (Agriculture)** in **PLANT BREEDING AND GENETICS** to the Tamil Nadu Agricultural University, Coimbatore is a record of *bonafide* research work carried out by **Mrs. P. JAYAMANI** under my supervision and guidance and that no part of this thesis has been submitted for the award of any other degree, diploma, fellowship or other similar titles or prizes and that the work has not been published in part or full in any scientific or popular journal or magazine.

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ABSTRACT

STUDIES ON THE SELECTION OF SUITABLE YEAST CULTURES AND STANDARDIZATION OF FERMENTATION CONDITIONS FOR WINE MAKING FROM BANANA, PAPAYA AND GRAPES

By

JEYARAM, K.

Degree : **Doctor of Philosophy in Biotechnology**

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One hundred and twenty two fermentative yeast strains were isolated from samples collected from different parts of Tamil Nadu. They were analyzed for their oenological, enzymatic characters, stress tolerance, higher alcohol, esters aldehyde, organic acids and glycerol production. By preliminary screening in comparison with three standard commercial yeasts (*S. ellipsoideus* CFTRI 101, MTCC 180 and the baker's yeast *S. cerevisiae*), 24 efficient isolates were selected based on their oenological characters. All the 24 isolates were identified based on morphological and physiological characters. Their genetic diversity was analyzed by RAPD-PCR and from the phylogenic tree, it was found that *Saccharomyces* isolates had 72% genetic similarity.

Fermentation kinetic studies revealed that *S. cerevisiae* KJSK-57, 87, 96, *S. bayanus* KJSK-100, *H. anomala* KJSK-69, *C. maynolice* KJSK-58 and the yeast isolate KJSK-114 were found to be highly efficient fermenting yeasts. The maximum level of sugar tolerance upto 70% w/v was noticed in *S. cerevisiae* KJSK-96. Low temperature was found to slow down the fermentation processes and at higher temperature, drastic reduction of both ethanol production and fermentation rate was noticed. pH range of 5.0 to 3.0 did not affect the fermentation process. Ethanol tolerance upto 8% v/v was noticed in most of the selected yeast isolates. Only two yeast

isolates (*H. anomala* KJSK-69 and *C. intermedia* KJSK-90) were able to tolerate upto a maximum of 300 ppm SO_2. Non-*Saccharomyces* yeasts were found to be the major source of hydrolytic enzymes like polygalacturanse, amylase, cellulase, protease and β - glucosidase.

Broad host range killer toxin was produced in *S. cerevisiae* KJSK-57 and *H. anomala* KJSK-69 with killer range of 92% and 68% respectively against the yeast isolates tested. The origin of killer toxin was located as dsRNA in *S. cerevisiae* KJSK-57. Among the higher alcohols, isoamyl alcohol contains major volatile compound produced by the yeast isolates. From the principal component analysis, it was noticed that higher amount of n-propanol and glycerol was produced by *Candida* sp. than *Saccharomyces* sp. In fermented must, acetic acid and succinic acid were found to vary with yeast isolates.

Based on the above characters, exclusion tests and cluster analysis were carried out and five efficient wine yeast isolates *viz. S. cerevisiae* KJSK-57, *H. anomala* KJSK-69, *S. cerevisiae* KJSK-96 *S. bayanus* KJSK-100 and *S. ellipsoideus* KJSK-106 were selected for wine production from fruits. From the locally available commercial varieties screened, Robusta for banana wine, CO 2 for papaya wine and Muscat for grape wine were found to be the suitable varieties for fruit wine production. A sugar level of 24°B and pH of 3.5 were standardized as optimum conditions for banana and papaya fermentation. Acidification process using citric acid and lime juice yielded quality banana wine but in papaya lime juice acidification was found to give negative effects.

By adjusting pH to 3.5 and TSS to 24°B, a new concept called "Biophysical separation" of fruit juice extraction and clarification during fermentation was standardized for banana wine production. Wine recovery was found to be the maximum at pH 3.5 and TSS 24°B due to their interaction effect. In low temperature (20°C) fermentation, the fruit flavour was found to be enhanced. Three major anthocyanin pigments *viz.*, malvidin glucoside complexes, peonidin and malvidin were separated and found that malvidin glucoside complexes were responsible for wine colour. It was observed that this compound disappeared during aging, which led to the poor colour stability in Muscat grape wine. To improve the colour, treatments like increased skin contact time and must heating time were tried. More than 78% colour increase was observed in

heating (70°C) for 10 min but increased skin contact time led to more tannin extraction and bitter taste.

To prevent the risk of unwanted indigenous yeast growth during fermentation, three killer toxin producing yeasts *viz.*, *S. cerevisiae* KJSK-57, *S. ellipsoideus* KJSK-106 and *H. anomala* KJSK-69 were co-inoculated in both banana and grape must fermentation and their population dynamics during fermentation was studied by a PCR based yeast colony identification protocol (PYCIP). From the result, it was found that the yeast population build-up was lower in banana pulp than grapes must during fermentation. Low indigenous yeast population load was observed in banana wine fermentation. *S. cerevisiae* KJSK-57 was found to be a superior killer yeast dominating in all fermentation conditions studied.

From the HPLC study, it was observed that phenolic acids (P-coumaric acid and caffeic acid) were predominant in banana wine, stilbenes (*trans*-resveratrol) in grape wine and flavonoids (catechin, epicatechin, quercetin) in papaya, which were reported to have medicinal values as anti oxidants, platelets aggregation inhibitors, antimutagenic, anticancer and decreased risk of cardio - vascular diseases. The level of *trans*-resveratrol, one of the key anticancer compound, was increased from 0.67 mgL^{-1} to 3.8 mgL^{-1} during fermentation with Muscat grape cluster stem, which was totally destroyed during colour extraction by heating.

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(K.JEYARAM)

CHAPTER I
INTRODUCTION

In the present context of the development of Indian Agricultural Economy, fruit and vegetable processing industry has a very significant role to play. A large variety of fruits are grown in India, among which mango, banana, citrus, pine apple, papaya, guava, sapota, grapes, apple, pear, plum and peach are important. India accounts for 10% of the total world production of fruits. It leads the world in the production of mango, banana, sapota and acid lime besides recording highest productivity in grapes. Fruit production is with increasing trend, since independence, from 55 lakh tonnes in 1952-53 to 455 lakh tonnes in 1999 – 2000 (Reddy, 2002). Among the most important fruit crops, banana ranks third in area with 4.64 lakh ha and first in total production of fruits with 150.73 lakh tonnes. Papaya ranks sixth in area (0.68 lakh ha) with a production of 15.82 lakh tonnes. Grape occupies 0.43 lakh ha and the production is 10.83 lakh tonnes (Chadha, 2002). There has been a considerable increase in the consumption of fruits in the world during the last few years and there are possibilities of its further increase. The export value of fruits and nuts amount to Rs. 247.14 crores, Banana (Rs. 12.67 crores from 7017 tonnes) and grape (Rs. 64.52 crores from 23679 tonnes) occupying second and fourth position respectively.

The perishable nature of fruits leading to annual loss of Rs. 23, 000 crores approximately, can be further effectively utilized by producing value added products like fruit juice, fruit wine, squash, cordial, punches, sherbets, pulps and other semi liquids, culinary products etc., (Shukla *et al.,* 1987, Rouhangiz and Dhawan, 1992, Charanjit and Khurdiya, 1993, Chakraborty *et al.,* 1993, Neelima *et al.,* 1999 and Khader, 2001). Even though India ranks first in the world fruit production, we have not been able to use more than 0.3%, of fruits for processing. Lack of technology for proper utilization of the horticultural crops results in considerable postharvest losses which have been estimated to be more than 30-35% (Joshi *et al.,* 1990 and Eipeson and Bhowmik, 1992). Fermentation is a potential tool in the development of

new products from fruits with modified physico-chemical and sensory qualities especially flavour and nutritional components (Dubley, 2002). The increased production of fruits can be consumed profitably, if fruit based alcoholic wines are produced. This will help to avoidpostharvest losses, fetch remunerative prices to the orchardists, reduce wastages of fruits and provide healthful drinks for the internal market as well as a tool for earning a good amount of foreign exchange due to export of fruit wines.

The word 'wine' signifies the fermented product from grapes only and for wine from other fruits, the name of fruit is prefixed. Fruit wines were used extensively in the Vedic times, in the 'Durbar' of 'Indira' and is referred to in the scriptures as 'Soma'. Rigveda has also mentioned the medicinal powers of fruit wine (Vyas and Chakravorty, 1972 and Joshi and Attri 1990). Many phenolic compounds present in fruit and vegetables (Crozier *et al.,* 1997) demonstrate potent and desirable antioxidant activity i.e. their ability to trap free radicals which are toxic elements produced in human body during stress. Some polyphenols are having anti-inflammatory and anti-cancer activity. Intake of two glasses of red wine per day will provide approximately 40% of the total antioxidant polyphenol requirement in a healthy diet (Goldberg, 1995). Alcohol derived from distillation and that derived from fermentation has totally different effects on the human body. Fruit wines are produced and consumed in large quantities in all advanced countries of the world. Although a large quantity of hard liquors are produced and consumed in India, fruit wine production is negligible in spite of tremendous increase in the fruit production (Rao, 1989 and Joshi *et al.,* 1999).

According to industrial estimates, about 63 million regular drinkers are there in India.

Epidemiological evidence from many studies, involving hundreds of thousands of human subjects of both sexes, overwhelmingly supports the notion that light to moderate alcohol consumption is associated with a reduction in overall mortality, primarily due to a reduced risk of coronary heart disease (Doll *et al.,* 1994). If sufficient information is provided, the Indian consumers will accept wine more readily than other Asian communities (Malhotra 2001). So the production of fruit wine offers a new avenue in India for the fruit processing industry, which can profitably be explored in changing the socio-economic fabric of the country. Setting up of the fruit based wine industry will generate employment and help in the economic upliftment of the fruit producing belts of our country (Sandhu and Joshi, 1995). Survey of literature showed that in

India appropriate technologies have been developed for preparation of wine from various fruits like grapes, apple, apricot, plum, pear, *etc.* But only very few research works have been carried out on wine production with banana and papaya fruits (Yang and Wiegant, 1949, Patwardham, 1970, Palmer, 1979, Muttamana and Nirmala, 1982, Onkarayya, 1985, Shukla *et al.*, 1987, Kalra *et al.*, 1991, Teotia *et al.*, 1991, Adsule *et al.*, 1992 and Patrick –Sundar, 1993).

Yeast and fermentation conditions *viz*., pH, temperature, sugar level, etc. are claimed to be the most important factors that influence the flavour of fruit wine. Other factors include the fruit variety used, its geographical origin, the ripeness of the fruit, the characteristics of the soil, the technology used for wine making methods etc., (Fundria *et al*., 2002). Spontaneous fermentation of the grape must is carried out by sequential action of different yeast genera and species. The composition of yeast flora can vary according to the climatic condition, the grape variety and the vinification technology. In early stages of fermentation, significant growth of indigenous species of non-*Saccharomyces* predominantly, *Kloeckera, Cryptococcus, Hanseniaspora, Candida, Pichia* and *Hansenula* participate in the fermentation process. When the ethanol concentration increases *Saccharomyces* species with higher ethanol tolerance complete the fermentation. Continuous growth of these indigenous species may in some circumstances enhance the chemical complexity and sensory quality of wines. There are other circumstances in which their contribution may result in spoilage (Egli *et al*., 1998). Several authors have shown the performance of indigenous yeasts in spontaneous fermentation and emphasized the importance of tapping the hidden wealth of indigenous yeast species and their impact on the sensory character of wines. No extensive geographic survey of the diversity of wine yeast strains, which is a key player in wine fermentation and their wine making potential, has been studied (Versavaud *et al.*, 1995). Because of increasing demand for potential wine yeast and feasible fruit wine production technology, the present investigation on fruit wine making with banana and papaya along with grapes was undertaken with the following objectives.

i. To isolate fermentative yeast from samples collected from different places in the Western zone of Tamil Nadu.
ii. To select wine yeast with the best oenological and enzymatic characters.
iii. To characterize the selected wine yeast isolates based on morphological, biochemical and molecular characteristics.

iv. To study the phylogenic relationship of wine yeasts isolated from the various samples collected from different parts of Tamil Nadu.

v. To study the suitability of commercially grown banana, papaya and grapes for fruit wine making.

vi. To study the population dynamics of yeast isolates with killer toxin production during co-inoculation.

vii. To standardize fermentation conditions for banana, papaya and grapes for fruit wine making.

viii. To standardize the methodology of wine making from banana, papaya and grapes.

ix. To analyse the various phenolic compounds of medicinal importance in the fruit wines of banana, papaya and grapes.

CHAPTER II

REVIEW OF LITERATURE

The history of wine making parallels that of civilization: Historians believe that wine was made in the Caucassus and Mesopotamia as early as 6000 BC. Cultivation of the grape vine spread into India during 100 BC (Robinson, 1994). A look at the early days of wine making makes it obvious that, while different techniques produced varied forms of wine, the basic principles changed very little. In 1863, Louis Pasteur revealed for the first time the hidden world of microbial activity during wine fermentation. He proved conclusively that yeast is the primary catalyst in wine fermentation. This fundamental innovation in wine making practices revolutionized the wine industry (Pretorius, 2000).

Two main components of wine making are the grape cultivars and wine yeasts. The diversity of yeast species associated with wine making and tailoring of wine yeast for fermentation makes new approaches to wine making in the twenty first century. Fruit wines have been in use and were part of the diet of ancient people. These are nutritious unlike distilled liquor. Their consumption in moderate or lesser amount is good for health. Wine can be produced from various fruits like grape, apple, apricot, plum, pear etc. (Joshi *et al.,* 1990).

2.1. Wine fermentation

Wine fermentation is a complex microbial process involving the transformation of must into wine by the action of different species of yeasts and lactic acid bacteria originally present in the grapes and winery equipment. Natural grape juice fermentation is carried out by a succession of different yeast populations. The oenological survey on naturally fermenting grape musts revealed a sequential occupation of the substrate. Initially apiculate and lemon shaped cells (*Kloeckera apiculata)* take over and after 3-4 days they are replaced by elliptical and oval shaped cells (*Saccharomyces cerevisiae*)(Fleet *et al.*,1984 and Heard and Fleet,1985). Yeasts of the genera *Candida, Hanseniaspora, Pichia, Torulaspora* and sometimes *Hansenula* grow during early stages of fermentation and with the increasing ethanol concentration, *Saccharomyces cerevisiae* becomes the dominant yeast species (Lema *et al.*,1996). Recent quantitative studies on wine-

making ecology showed that non-*Saccharomyces* species survive during fermentation at significant levels for longer periods than previously thought (Morais *et al.,* 1997). Therefore, non-*Saccharomyces* yeasts could contribute to the analytical composition of wine. Furthermore, there may be positive and interesting features of non-*Saccharomyces* yeasts that may be used in wine making and industrial fermentation process (Pretorius *et al.,*1999).

2.2. Characterization of wine yeasts

Morphological and physiological characteristics can be performed by traditional methods or by identification kits. Comparison of unknown strains with descriptions of recognized species can be achieved by identification keys (Kreger –Van Rij, 1984). In recent years chemotaxonomic and genomic studies have led to great advancements in yeast taxonomy. Commercial systems for the identification of food borne yeasts include the simplified identification method, the API 20C method series (API Analab products, Division of Sher wood Medicals, New York), the microtitre tray procedure (Nunclon Delta S1, Denmark) and the enzyme based identification system (Welthagen and Viljoen, 1997). Gomes *et al.* (2000) used differential staining for industrial yeasts characterization. Perez *et al.* (2000) used cycloheximide resistance as marker for monitoring inoculated yeast in wine fermentation. Kopke *et al.* (2000) suggested that epiflourescence staining, as a faster technique can be used as an indication of microbiological wine quality. Velazquez *et al.* (2001) developed a new kit for yeast identification called ***Yeast Ident – Food / Prole food***. This comprises a set of 24 physiological and biochemical tests with a computer software found to be very efficient in yeast characterization.

Modern methods based on molecular biological techniques have been developed in order to simplify identification. Among such technique electrophoretic karyotyping, PCR-RAPD assay and mitochondrial–DNA (mt-DNA) are valuable (Welthagan and Viljoen, 1997). Pulsed field gel electrophoresis is used for karyotyping at generic level (Mesa *et al.,* 1999, Perez-Coello *et al.,* 1999 and Gomes *et al.,* 2000). PCR-RAPD using random primers (Mitrakul *et al.,* 1999 and Gomes *et al.,* 2000), ribosomal region specific primers (Hayford and Jakobsen, 1999 and Pramateftaki *et al.,* 2000) and microsatellite primers (Caruso *et al.,* 2002) are available.

Mitochondrial DNA restriction digestion pattern was used to identify wine yeast upto strain level (Pramateflaki *et al.,* 2000 and Esteve –Zarzoso *et al.,* 2000).

2.3. Yeast genetic diversity and yeast population dynamics in wine fermentation

A number of different strategies, based on the detection of DNA polymorphism, such as electrophoresis (PFGE), polymerase chain reaction (PCR) and mitochondrial DNA restriction fragment length polymorphism (mt DNA-RFLP) analysis have been used to differentiate intergeneric and intraspecific diversity of yeast biota for wine fermentation (Nadal *et al.,* 1999 and Mortimer, 2000). Gupta *et al.* (1994) studied yeast biodiversity in flowers, fruits etc. in Nainital using traditional biochemical and physiological methods and reported *Candida* spp. were dominant followed by species of *Trichosporon* and *Rhodotorula.*

Versavaud *et al.* (1995) differentiated 35 predominant *S. cerevisiae* strains responsible for wine producing area of Charentes, France using electrophoretic karyotyping, mt DNA-RFLP and RAPD and observed correlation between geographic location and genetic affinity. Sabate *et al*.(1998) used *Rsa I* restriction enzyme for mt- DNA restriction pattern to differentiate all *S. cerevisiae* strains. Couto *et al.* (1999) used microsatellite oligonucleotide primers $(GAC)_5$ and $(GTG)_5$ for 15 strains of *S. cerevisiae.* Using PFGE and PCR analysis. Pataro *et al.* (2000) found a very high molecular variability of *S. cerevisiae* strains at different fermentation ages in Brazilian distilleries.

Trivedi and Ethiraj (1979) characterized a total of eleven different species belonging to *Kloeckera, Candida, Rhodotorula, Schizosaccharomyces* and *Saccharomyces* population during wine fermentation. The predominance of *Lactobacillus, Saccharomyces* and other microflora populations during dough fermentation was also studied (Viljoen and Lues, 1993). Un inoculated wines were characterized by large population of non *Saccharomyces* whereas the wines inoculated with *S. bayanus* had the smallest population of non-*Saccharomyces.* This result was obtained by genetic assay using δ primer based PCR (Eghi *et al.,* 1998). The initial yeast flora of non-*Saccharomyces* species was replaced by wine yeast, predominantly *S. cerevisiae* (Pina and Hogg, 1999, Gutierrez *et al.,* 1999, Povhejemect *et al.,* 2001 and Zarzoso *et al.,* 2001).

Granchi *et al.* (1999) used PCR- RFLP analysis of the rDNA – ITS (Internal Transcribed Spacer) for monitoring the development of the yeast community throughout wine fermentation. Heyford and Jesperson (1999) differentiated *S. cerevisiae* karyotypes based on chromosome length polymorphism and found population dynamics of major *S. cerevisiae* strains were quite similar in all five spontaneous fermentations studied.

2.4. Selection of wine yeasts

The microbial process of must fermentation has been the subject of numerous studies, in which variability and diversity of wine yeast populations have been well established. These studies demonstrated that the main agents responsible for the alcoholic fermentation are *S. cerevisiae* strains. These strains are well adapted to must conditions and can grow under these conditions to complete the alcoholic fermentation (Martini and Martini, 1990). In the last few years, there has been an increasing use of native yeasts for controlled must fermentation in countries such as Spain with a wine making tradition. Though there are commercial yeast to accomplish must fermentation, the use of native yeasts is believed to be much more effective (Ciani *et al.*, 1991).

Native yeasts are presumed to be more competitive because they are better acclimatized to the environmental conditions. Therefore they would be able to dominate the fermentation and become the most important biological agent responsible for the vinification. Selection of the appropriate local yeasts assures the maintenance of the typical sensory properties of the wine produced in any given region (Regodon *et al.*, 1997).

2.4.1. Oenological characteristics

To our knowledge, there has been no simple and effective procedure for selecting wine yeasts for industrial use. Selected yeasts should have certain technological characteristics that make them suitable for industrial wine production. The importance of each of these characteristics varies not only according to the kind of wine but also according to the different opinions of the experts consulted.

Thorton (1991) included following practical attributes during selection of wine yeast in Australia. The wine yeast must exhibit, rapid initiation of fermentation, uniform rate of

fermentation, complete utilization of fermentable sugar, fermentation at low temperature (10-15°C), ethanol tolerance, sulfur dioxide tolerance, low foaming, low H_2S or mercaptan formation, low higher - alcohol production, low acetic acid and acetaldehyde production, low sulfite production, production of desirable fermentation bouquet and strong flocculation at the end of fermentation in a medium (grape juice) where fermentable sugar may exceed 20 per cent w/v and at a pH in the range of 3.0 to 4.0. Later killer phenotype, good glycerol production, autolytic capacity, adherence to glass and hydrolytic enzyme production are also included for selection (Regodon *et al.,* 1997 and Martinez – Rodriguez *et al.,* 2001)

Unfortunately measuring all these properties to select yeast would be time consuming and require sophisticated equipment and specially skilled personnel. Regodon *et al.* (1997) designed a simple and effective procedure for industrial yeast selection by using only some easy - to - measure technological characteristics of the yeasts (resistance to sulfur dioxide, presence of killer K_2 phenotype, growth at 42°C, low foam production, volatile acidity, ethanol production and residual sugars). Out of 86 local *Saccharomyces cerevisiae* strains, nine yeast strains were selected in this way from Spanish region. Six of the nine local strains (66.6%) produced wines of superior quality by means of spontaneous fermentation. Three of these yeasts (33.3%) produced more appreciated wines. Iranzo *et al.* (1998) concluded that only 10 of the 74 *Saccharomyces* strains screened, possessed the most suitable oenological properties (non SH_2 production, tolerance to SO_2 and ethanol, and synthesis/resistance to killer toxin). Principal compounds analysis showed little capacity to hydrolyse esters but found to secrete proteinases and β-glucuronidase. It is difficult to find a strain having both oenological and enzymatic properties that are of practical oenological interest.

2.4.1.1. Fermentative capacity

Ethanol and CO_2 are the major end products of fermentation. The rate and efficiency of ethanol production are important factors in wine making fermentations (Ciani and Rosini, 1987). The production of ethanol was influenced by yeast strain, temperature and growth medium. Under comparable conditions, different yeast strain produced significantly different amount of ethanol. Ciani and Maccarelli (1998) characterized non- *Saccharomyces* yeast based on fermentation rate (g CO_2 produced per day), fermentation vigour (% v/v ethanol production) and fermentation purity

(volatile acidity in relation to total ethanol yield). Nearly ninety strains of *Torulaspora delbrueckii* have 7 to10 per cent fermentation vigour with highest classes of fermentation purity. *Candida stellata* and apiculate strains (*Kloekera apiculata* and *Hanseniaspora uvarum)* appear to have very low fermentation vigour and purity.

The first criterion to discriminate among yeast strains was the total fermentation time (Esteve –Zarzoso *et al.*, 2001). Regodon *et al.*, (1997) selected wine yeasts with following criteria: Volatile acidity less than 0.8 g l^{-1} (which is the maximum value for a wine to be granted appellation control, according to European community legislation), ethanol concentration higher than 8 per cent and residual sugars lower than 4 g l^{-1}.

Iranzo *et al.* (1998) judged yeasts with fermentation rate of over 0.25g CO_2 evolved per litre per hour was an adequate rate for wine making. Ciani and Picciotti (1995) studied growth kinetics of non-*Saccharomyces* yeasts and found that *S. cerevisiae* test strain was a fast grower than non-*Saccharomyces* (*H. uvarum, T. delbrueckii* and *K. apiculata*). *Candida stellata* was found to be the slowest grower. *C. stellata* and some apiculate yeast strains exhibited very low ethanol yield and productivity. But the specific rate of ethanol production was similar to *S. cerevisiae.* Bell *et al.* (2001) studied the fermentation kinetics of yeasts related to other carbon sources (*eg*. maltose). For secondary fermentation of the sparkling wines, Martinez –Rodriguez *et al.* (2001) selected yeast that produced at least 1% (v/v) of ethanol in base wine added with 25 g saccharose L^{-1}. Any residual sugar remaining in wine is a risk. Only 20.6 per cent out of 34 initial strains of yeasts, which satisfied this requirement.

2.4.1.2. H_2S Production

Selection of low H_2S forming yeast strains is essential to minimize the off-flavour production during wine making. However, the low H_2S producing property is an apparently variable character. Methionine and cystine are the major source of sulfur in grape juices. Methionine inhibits sulfate metabolism and thus H_2S formation. When nitrogen supply is limiting, low levels of methinoine and cystine do not inhibit the sulfate reduction pathway and consequently H_2S accumlates. H_2S production is decreased by Diammonium Phosphate (DAP) addition. Iranzo *et al.* (1998) screened 250 *Saccharomyces* strains for H_2S production. In this 61 per cent produced H_2S. Perez-Coello *et al.* (1999) eliminated 54 of the 143 strains possessing a

suitable kinetic value based on H_2S formation during selection of yeast using exclusion tests. During secondary fermentation, Martinez-Rodriguez *et al.* (2001) noticed none of the seven strains produced H_2S. During selection of wine yeast, Esteve-Zarzoso *et al.* (2001) used H_2S and foam production as an unfavourable criteria with second priority followed by fermentation time.

2.4.1.3. Flocculation

Aggregation of microorganisms (i.e. the process leading to the formation of a group of microbial cells in intimate contact) is of particular importance in many industrial processes such as brewing, wine making, waste water treatment and bioconversions (Straver *et al.*, 1993). In the brewing industry, the ability of yeast cells to flocculate is very important. Yeast cells flocculate spontaneously at the end of fermentation and as a result, the majority of cells are separated from the culture medium. Iranzo *et al.* (1998) reported 42 per cent of *Saccharomyces* strains studied were flocculent type to varying extents.

2.4.1.4. Autolytic capacity

During aging of the wine, the autolysis of the yeast takes place. In this process, the yeast released extracellularly different constituents that may importantly modify the organoleptic and foaming properties of the wine. It has been observed that there is a relationship between the yeast strain used and the compounds released into the surrounding medium during autolysis. Martinez-Rodriguez *et al.*(2001) observed the amino acids, proteins and polypeptides were released during induced autolysis. *Saccharomyces bayanus* EC-1118 and *S. cerevisiae* P-29 released greatest quantities of proteins and amino acids in the first 24 h of autolysis (35 mg BSA (Bovine Serum Albumin) l^{-1} and 130 mg leucine l^{-1} respectively). But *S. cerevisiae* IFI-461 released very small quantity of proteins and amino acids (0.21 mg BSA l^{-1} and 15 mg leucine l^{-1} respectively).

2.4.1.5. Killer toxin production

The killer activity was first reported in *Saccharomyces cerevisiae* (Bevean and Makower, 1963). Since then the killer character was detected among many yeast genera and species in culture collection, industrial strains and in ascomycetous, deuteromycetous and basidiomycetous yeasts colonising different substrates. Killer yeast strains (K) produce an extra cellular protein or

glycoprotein (Killer factor) that kills other sensitive yeasts (S). Neutral type yeasts (N) are resistant to killer factor but do not produce it. Killer sensitive strains (KS) have also been discovered, which are immune to their own toxins but may be sensitive to other killer strain toxins.

Hidalgo and Flores (1994) obtained a high frequency of killer strains in *S. cerevisiae* during different phases of fermentation in the Spanish viticultural areas. The prevalence of this character in native yeasts could influence the process of fermentation by modifying the percentage of sensitive phenotype strains. In this situation, the sensitive strains would be eliminated by the killer strains. So that killer character is an important oenological characteristic in the selection of wine yeasts. It is a factor to be considered in the control and composition of microbial populations that intervene wine making. Izgu *et al.* (1997) tested the killer toxin activity of the *Candida tropicalis* isolate at various pH, covering the range 2.9 –6.1 in intervals of 0.2 pH units and at 18-35°C in 1.0°C increments. Killer activity was found to be maximum at pH 3.9 – 4.1 and at 22-25°C.

Two different killer phenotypes, differeing in their degree of killer activity, were detected by Musmanno *et al.* (1999) from spontaneous wine fermentation using a plate bioassay. They were designated as SK^+ (Strong Killer) and WK^+ (Weak Killer). Growth in must negatively affected expression of the killer activity of both phenotypes. Sangorrin *et al.* (2001) studied the occurrence of killer wine yeasts in Comahue region (Argentina). Out of 135 samples, 37 per cent were sensitive to K_1 –K_{10} killer toxins and did not show killer activity. Neutral phenotypes constituted 21 per cent and 42 per cent demonstrated killer activity. Irrespective of grape must type, the neutral and sensitive yeasts were over predominant at initial stages of fermentation.

2.4.2. Enzymatic activities of wine yeasts

Enzymes play a definite role in the production of wine, which could be seen as the product of enzymatic transformation of the grape juice. The enzyme activities originate not only from the grape itself, but also from yeast and other microorganisms. The wine maker now reinforces and extends the action of these endogenous enzymes by the use of exogenous industrial enzyme preparations. There is little information on the production of extra cellular enzymes by non-*Saccharomyces* wine yeast, although some strains of *Kloeckera apiculata* show

extra cellular enzymes and some other strains show extra cellular protease activity. Various authors have reported glycosidase production by *S. cerevisiae* and the potential of these enzymes to enhance wine flavour. Glycosidase activity has been reported in strains of *Candida*, *Pichia* and *Hanseniaspora* (Zoecklin, *et al*., 1997 and Pretorius, 2000).

Another desirable enzyme in a wine yeast is the esterases which hydrolyse the esters. The instability of the proteins in the must could be counteracted by enzymatic hydrolysis which would yield nitrogen compounds of small molecular weight that stimulate metabolism of yeast. This hydrolysis might be due to the action of acid proteinase secreted during the fermentation by these microorganisms. There are very few wild *S. cerevisiae* strains with β-glucosidase activity. Some researchers have modified the genome, introducing the genes that code for expression and synthesis of this enzyme (Bundock and Hookaass, 1996, Raugai *et al.,* 1996, Volchenk *et al.,* 1997, Hama and Kumagai, 1999 and Ostergaard *et al.,* 2000).

Iranzo *et al.* (1998) found 10 *Saccharomyces* strains as most suitable for wine making which have little capacity to hydrolyse esters but secrete proteinases and β-glucuronidase. Study conducted by Straus *et al.* (2001) revealed the potential of indigenous wine yeasts to produce a wide range of extra cellular enzymes. They found that the strains with the most enzymatic activity are the ones that have the biggest effect on wine aroma (Hagedorn and Kaphammer, 1994, Lilly *et al.,* 1999 and Lilly *et al.,* 2000). The terpenes, another secondary metabolite also linked with glucose, does not contribute the wine aroma. Endoglucanolytic wine yeast secreates β (1-4) endoglucanase which release terpenes and increase the fruit aroma (Zoecklein *et al.,* 1997, Santos *et al.,* 1999, Ma *et al.,* 2000 and Pretorius, 2000).

2.4.3. Stress tolerance

2.4.3.1. Osmotic tolerance

Osmotolerance is an important property for fermentation of the musts in hot regions, which have high sugar contents. In Mediterranean zones, must from dried grapes (40°B) is used for wine making. Caridi *et al.*(1999) have shown that all *S. cerevisiae* strains produced abnormal quantities of volatile acidity and SO_2 in must with very high sugar concentration. Osmotic stress induces a defense mechanism in the yeasts, activating the metabolic processes and causes high volatile acidity.

On the other hand, osmotic stress has a clear detrimental effect on ethanol production (Mager and Varela, 1993, Gough *et al.,* 1996 and Chaudhari and Chincholkar, 1999). Perez-Coello *et al.* (1999) selected 13 strains of *S. cerevisiae* out of 174 screened with 30° B sugar tolerance for wine making.

2.4.3.2. Temperature tolerance

Manufacturers know that wines produced at low temperature were of good quality with a distinctive flavour profile and a pleasant taste. Therefore, important products such as the Greek semi sweet vinosanto and the semi-sparkling champagne are produced by a secondary fermentation during the winter. Wine making at temperatures lower than 15°C is not usual on an industrial scale since the productivity is very low. For low temperature fermentation in sparkling wine psychrotolerant yeast have been selected.

Bakoyianis *et al.* (1998) proved that continuous wine making by immobilized cells produce wines with lower alcohols and higher concentrations of ethyl acetate as compared with free cells. Kourkontas *et al.* (2002) immobilized alcohol-resistant psychrophilic *Saccharomyces cerevisiae* AXAZ-1 on apple cuts which was found to be suitable for continuous wine fermentation at temperature between 5 and 15°C.

Efficient, thermotolerant strains of *Kluyveromyces marxianus* capable of growth at 52°C and of fermentation at 50°C were isolated from the distillery environment in India (Banat *et al.,*1992). Normally these thermotolerant (Mesophilic, 28-38°C) yeasts were used for ethanol production in fermentation reactors (Tchango *et al.,* 1997 and Ueno *et al.,* 2001). Caridi *et al.* (1999) screened 15 thermotolerant *S. cerevisiae* strains for wine making at high osmotic strength to assess the preference of thermotolerant strains for wine making from must with elevated sugar concentration.

2.4.3.2. SO_2 tolerance

The physical and chemical reactions of SO_2 (in the form of potassium meta bi sulphite) added during wine making includes the killing and growth inhibition of unwanted bacteria and yeasts, the inhibition of phenol oxidase activity, the interaction with wine phenol in the competitive oxidation, the reaction of sulfite with peroxide, the binding of acetaldehyde, pyruvate, keto-glularate and the anthocyanin pigments and the delay of brown pigment development (Eschenbruch, 1974 and Boulton *et al.,* 1997). Iranzo *et al.*(1998) and Regodon *et*

al. (1997) used 300 mg of SO_2 l^{-1} and 250 mg of SO_2 l^{-1} respectively for the selection of yeast for wine making.

2.4.3.3. Tolerance to CO_2 pressure

Sparkling wine undergoes two fermentations. The first one, in hermetic tanks to avoid CO_2 loss by autochthonous flora. Recently very high density inoculum was used to eliminate the need to add SO_2 which helps in preventing the spoilage microorganisms. The second fermentation, which takes place in the bottles once the wine has been filtered and bottled, is carried out by inoculating selected wine yeasts whose properties are very different from those of the yeasts which carried out the first fermentation, that is tolerance to low temperature, ability to flocculate and above all resistance to CO_2 (Benitez *et al.*, 1996).

2.4.3.4. Tolerance to Ethanol

Saccharomyces species are the most ethanol tolerant of eukaryotic organisms, with yeasts in general being more ethanol tolerant than brewer's yeast. Ethanol tolerant hybrids of *Saccharomyces* wine yeasts had wine making qualities comparable to those of the parents but with increased sugar conversion efficiency (from 84 to 93 per cent) (Thornton, 1991).

The presence of ethanol enhances the lethal effects. The ability of a yeast strain to resist the inhibitory effect of ethanol decreases as temperature increases. Ethanol tolerance is strongly influenced by environmental and nutritional conditions (Lloyd *et al.*, 1993). However, different strains differ in their ability of ethanol tolerance. This ethanol tolerance is a reproducible characteristic implying that it is genetically controlled (Benitez *et al.*, 1996).

2.4.3.5. Acid tolerance

Saccharomyces cerevisiae is frequently inhibited by acidic environment. Many yeast species can be recovered at pH 3.7 –3.8 during isolation. *Bettanomyces* and *Dekkera* secreted high levels of weak acids which could spoil the wine fermentations (Piper *et al.*, 2001). The acidity of a juice, in particular the pH, plays an important role in many aspects of wine making and wine stability. The growth of most bacteria, solubility of tartarate and effectiveness of SO_2 are influenced by juice pH (Piper *et al.*, 2001).

2.4.3.6. Molecular basis for stress tolerance

Ivorra *et al.* (1999) studied the molecular mechanism involved in stress response in *Saccharomyces cerevisiae* strains. The better understanding of these mechanisms in wine yeast could open the possibility to improve the fermentation process. A correlation between resistance to stress and the ability to complete the fermentation was found. Osmotic stress affects the yeast cells immediately when they are added to the must and several genes are expressed in order to allow cells to resist this adverse situation. One of these is the *GPD 1* gene (Glycerol –3 phosphate dehydrogenase) which catalyzes the production of glycerol. They only require to keep glycerol levels appropriate to protect them from the external osmolarity. Heat shock protein, *Hsp 104,* plays a major role in the acquisition of tolerance to a variety of stresses such as heat, ethanol and sodium arsenite and acts as an excellent stress indicator (Brosnan *et al.*, 2000).

The stationary phase of wine fermentation is an ideal stage to express enzymes that improve the floral and fruity aroma of wine (Fuge *et al.,* 1994). This stage is usually reached by nitrogen source limitation (Goni and Azpilicutea, 1999), high ethanol concentration or a combination of both. Cells reach the stationary phase very quickly, when sugar concentration is still around 100-150 g l^{-1}. Therefore, at least two-thirds of the fermentation time is undergone after arresting the cells growth. Thus genes induced under stress conditions are putative candidates to be highly transcribed at the stationary phase (Riou *et al.,* 1997).

2.4.4. Glycerol Production

Glycerol is a non-volatile compound which has no aromatic properties, but significantly contributes to wine quality by providing sweetness and fullness. The amount of glycerol usually formed by *Saccharomyces cerevisiae* in wine varies between 2 and 11 g l^{-1} although normal concentrations are in the range 4-9 g l^{-1}. Red wines have been described as containing more glycerol than white wines. Upto 30 g l^{-1} is found in wines formed from *Botritys cinerea* infected grapes. (Yunome *et al.,* 1981, Grazia *et al.,* 1995 and Ciani and Ferraso, 1996)).

Lubbers *et al.* (2001) showed that glycerol did not change the relative volatility of aroma compound in the range of 5-20 g l^{-1} in water and model wine (Noble and Bursick, 1984). Significantly more glycerol was formed at 25°C than at 15°C. Yeast strains responded differently in terms of glycerol production to the addition of 100 ppm SO_2 and to a pH increase from 3.3 to

3.8 (Thornton, 1991). For baking, added glycerol had no apparent effect on final bread quality in terms of flavour, colour or texture but slightly better oven –spring especially as yeast was aged (at 4°C) (Myers *et al.,* 1998).

Increasing the level of glycerol even more has been attempted by the selective hybridization of wine yeast strains, leading to the construction of yeast producing 10-11 g of glycerol per liter. More recently, genetic engineering approaches have been successful in redirecting the carbon flux towards glycerol. GPDH, a limiting enzyme for glycerol formation, is encoded by *GPD1* and *GPD* 2 (Remize *et al.,* 1999). Over expression of *GPD* 1 in a laboratory strain and in a haploid strain (V5) derived from a wine strain resulted in marked increases in glycerol production at the expense of ethanol (Remize *et al.*, 2000). Up to 28 g l^{-1} of glycerol was formed by an engineered *S. cerevisiae.*

2.4.5. Higher alcohols, organic acids, esters and aldehydes production

The higher alcohols, or fusel oil are important secondary fermentation end products in wine. Different yeast strains produced different proportions of the three alcohols. Ciani and Picciotti (1995) showed a high glycerol production by *C. stellata* with lower production of higher alcohols *viz.*, propanol, isobutanol and amyl alcohol. Ciani and Maccarelli (1998) found that the amounts of 2,3-butanediol produced are clearly comparable among different non-*Saccharomyces* species. Only iso-amyl alcohol was produced in amounts that could alter the sensory properties of wines.

Some yeast strains that can produce significantly higher concentrations of the individual esters aldehydes and acids, which may be undesirable at very high levels. Generally total ester aldehydes and acids production is similar for strains of *Saccharomyces* but the spectrum of these byproducts vary dramatically in non – *Saccharomyces* wild yeasts. Ethyl acetate is always present in wines with concentrations well below the thresh hold taste level of 150 mg l^{-1} (Jackson, 1994). This limit was largely surpassed by all the apiculate species, which consistently formed higher amounts of ethyl acetate.
T. delbrueckii, characterized by a high fermentation purity (low acetic acid, acetaldehyde and ethyl acetate), is an interesting yeast for the production off base wine in sparkling wine production. (Ciani and Picciotti 1995 and Boulton *et al.,* 1997). Ciani and Maccarelli (1998)

reported *T. delbrueckii* and *C. stellata* were constant but low acetic acid producers independent of ethanol production. Succinic acid and acetic acids are formed during fermentation and the levels are influenced by the malic and amino acid concentrations and the yeast strain involved. Succinic acid and acetic acid were found at levels in the range of 0.5 –1.5 g/L (Romano *et al.,* 1992, Boulton *et al.,* 1997, Montanari *et al.,* 1999, Castineir *et al.,* 2000, Guzel –seydin *et al.,* 2000).

2.5. Fruit wine making

2.5.1. Enzymatic fruit juice extraction

All types of fruit and berries of nutritional significance contain larger or smaller amounts of the starch–like substance, pectin (polymethyl galacturonic acid). In the unripe fruit, pectin is present in an insoluble form called proto pectin, imparting hardness to the unripe fruit. Ripening of the fruit results in a partial breakdown of proto pectin to more soluble form and thus the flesh of the fruit becomes soft. Because of its partial solubility at this stage, some of the pectin passes into the juice of the fruit which becomes viscous and difficult to separate from the flesh causing cloudiness of the fruit extract. The result is a relatively low yield of juice and soluble fruit components, such as colour and flavour compounds. These difficulties can be overcome by subjecting the fruit pulp to pectin –splitting enzymes. In such cases, the pectin will be broken down to highly soluble substances, resulting in higher yields of juice, easier filtration, better colour and quantity of the extracted juice (Jain and Lacey, 1991, Boopathy, 1994).

In the manufacture of wine or fruit juice, pectinase is added after pulping but before it is pressed. The enzymes degrade the compounds that hold the cells together and thus release entrapped juice, resulting in both higher free–run and pressed juice yield (Kulkarni *et al.,* 1980 and Gothe, 1998). Koetecha *et al.* (1994 and 1995) used PEC (Pectin Enzyme Extract) @ 0.2 percent at 28 ± 2°C for 4 hours for obtaining juice from banana and apple for wine production. Enzyme increases the presence of aromas, flavours, anthocyanins, tannins, sugar and a range of minerals which contribute to the body and aroma of the wine. Gallifuoco *et al.* (1998) used chitosan immobilized β-glucosidase for industrial wine making with improved qualities.

2.5.2. Standardization of fruit wine making

2.5.2.1. Grape wine

"Great wines are made in the vineyard". Suitable grapes are the first indispensable key to success in wine making and especially distinctive, high quality table wine. Suresh and Nagi (1975) have tested 30 grape varieties for wine production. White grape variety "Chemin Blanc" and red grape variety "Black Cornichon" produced good quality dry table wines containing 10 per cent alcohol. Kundu *et al.* (1980) evaluated seven exotic grape cultivars *viz.,* Pearl of "Csaba", "Champion", "Early Muscat", "Mandeline Angevine", "Bianshi Rai", "Jaosbeh" and "Riesling" grown in Haryana for white table wine making.

Suresh *et al.* (1985) conducted four years study to evaluate new grape cultivars for preparation of wine and found four cultivars (Arka Vati, Arka Kanchan, Arka Shyam and Akra Hans) which produced good quality dry wines. Red dry wine from 'Arka Shayam' had good colour and less foxy flavour. White dry wine prepared from 'Arka Kanchan' was characterized by strong Muscat flavour. Dry wines from 'Arka Hans' had light straw yellow colour with natural flavour.

Venkataramu *et al.* (1977) have studied wine yeast and their fermentation products. They evaluated yeast strains namely *S. cerevisiae* var. *ellipsoideus* No. 101, 374, 379 and 801 and reported strain No. 101 was found to be highly suitable for wine making with production of 0.0102 per cent volatile acidity as acetic acid, 293.2 ppm of esters as ethyl acetate, 110 ppm of aldehyde as acetaldehyde, 240 ppm of higher alcohols and 7.82 per cent of ethyl alcohol. Martini and Martini (1990) have used selected yeasts namely *S. cerevisiae* A, *S. cerevisiae* B, *S. chevalieri, S. capensis* and *Torulospora delbrueckii.* Velazquez *et al.* (1991) used mixed cultures of *Saccharomyces* wild strains and reported increased level of ethanol production with very low level of residual sugar.

S. cerevisiae strains from the same ecological area exhibited significant differences in the production of volatile compounds during fermentation (Lurton *et al.,* 1995). Ubeda and Briones

(2000) studied the concentration of volatiles in wines produced on a laboratory scale using different yeasts strains, which were found to be similar in all the samples. GC (Gas Chromatography) studies have shown that the differences in volatile composition were not sufficiently large to suggest clear differences in aroma quality.

Rupela and Tauro (1979) studied the factors affecting H_2S level in Indian wines. Major contributors to the final H_2S content of wines are the variety and the KMS (Potassium meta bisulphite) used. However the major agency bringing about this transformation is the yeast, since the amount of H_2S is negligible in the unfermented juice. Therefore yeast selection with less H_2S production during fermentation is desirable. Suresh *et al.* (1983) blended 'Gulabi' variety with deep coloured varieties such as 'Baily Alicante' and Rubi red' in 2:1 and 3:1 proportion, which yielded wines with desirable colour. White varieties 'Thompson Seedless' and 'Anab –e Shahi' were blended with 'Rubi red' in different proportions to produce red table wine.

Grappa and Grapa – spirit are alcoholic beverages made from direct steam marc distillation. Grape –spirit is a fermented fruit distillate. Porto (1998) suggested a modified method for distillation technology for alcoholic beverages. Morales *et al.* (2001) characterized the acetification process of sherry wine vinegar using submerged fermentation. Several studies have been performed in order to classify wines according to their geographical origin or vintage year based on chemical analysis of volatile aroma components.Etievant *et al.* (1989) utilized pigments and flavonoids, amino acids, aromatic alcohols and major acids to classify French red wines according to their geographical origin.

Etievant (1991) included amino acids in addition to volatile compounds to classify wines of the same variety according to vintage. Srivertsen *et al.* (1991) classified 22 red wines from four main regions in France, based on both sensory and chemical analyses. The chemical data included major acids, alcohols, esters, pH, total phenols and colour. A better choice of sensory attributes and a well trained panel can improve the correlation between sensory descriptions and chemical analyses.

2.5.2.2. Banana wine

Banana fruits are perishable. The increased production and over ripening results in the significant reduction of their market value. Degree of ripening is an important property for juice extraction. Ramane and Jayaraman (1994) described a simple analytical method by analysing the total soluble solids and total solids. The difference between these values, ranged from 15.22 to 1.01% during ripening. The value 1.01% indicated 100% ripening. Kotecha *et al.* (1994) extracted juice from over ripened banana from local market of Maharastra and fermented with *S. ceverisiae* var. *ellipsoideus* for banana wine production. Sensory evaluation studies showed that wine from overripe fruits was comparable to wine from normal ripe fruits (Kundu *et al.*, 1976).

Juice yield is another criteria. Ahmed (1996) studied the enzymatic juice extraction of four varieties of banana *viz.*, Singapuri, Kathali, Chep and Martaman. Among these varieties Singapuri gave maximum juice yield. Most growers and retailers are left with no option other than to discard their overripe banana and plantain or crossbred species (cooking banana). When demand is less than supply, the overripe *Musa* species could be fermented into wines. Onwuka and Awam (2001) investigated the possibility of producing wine from *Musa* species fruits from Nigeria markets using baker's yeast (*S. cerevisiae*) and proved the feasibility of fruits wine. The wine produced compared favorably with an imported wine and showed better attributes of taste, colour, alcohol content and general acceptability.

2.5.2.3. Papaya wine

Among the various papaya cultivars, CO2, CO6 and Sunrise solo are commonly cultivated in Tamil Nadu. CO2 and CO6 are extensively used for papain extraction. Papaya is rich in vitamin C (75 mg/100g) and carotenoids (1152.50 mg/100g) and other nutrients (Singh and Singh, 1998 and Aruna *et al.*,1997). Papaya fruit is highly perishable and after papain extraction in the field such lanced fruits, having stripes on the surface are unattractive to the purchasers and subsequently undergo microbial spoilage. The culled fruits could be economically utilized for papaya wine production. Narasimman (1994) tried culled papaya fruits for wine fermentation.

2.5.2.4. Other fruit wines

2.5.2.4.1. Apple wine

Apple (*Malus sylvestris)* is an important fruit crop of temperate zone of Northern India. Cider preparation is a common process of utilising culled apples. It is a low alcoholic drink produced by fermentation of apple juice. Nagi and Manjrekar (1975) have prepared cider from five juicy and high sugar containing varieties of apple namely Ambri Kashmiri, Red Delicious, Golden Delicious, Granny smith and Yellow Newton.

Karni *et al.* (1977) have reported the cider preparation from two Kashmiri apple varieties, Red Delicious and Maharaj using there *S. cerevisiae* strains. A sugar nitrogen balance of 16:0.1 was reported to be the best in the fermentable juice for maximum alcohol yield of 7.4 per cent. Patel *et al.* (1977) have prepared cider and brandy from apple and found that the yield of cider obtained from different varieties varied from 54.6 to 74 percent. Karni and Shant (1982) have screened the commercial varieties of Kashmir apple namely Ambri and Red Delicious for making quality cider. The juices of these varieties were fortified with sugar to the level of 24 per cent for obtaining hard cider. Mir *et al.* (1988) fermented culled apples with four strains of *Saccharomyces cerevisiae.* All strains tested exhibited more alcohol production and greater fermentation efficiency at pH 3.5 than at 4.5 or 5.0.

Supplementing juice with sugar (16%) and diammonium phosphate (0.06%) enhanced the fermentation efficiency of test cultures. Grading of wine was highest for all characteristics found in Golden Delicious and 'Rus Pippin' cultivars (Barwal, 1990 and 1991). Vyas and Kochhar (1993) have studied the preparation of cider and wine from culled apple in Himachal Pradesh. Red Delicious produced cider with maximum alcohol content of 6.02 and wine obtained from a combination of Golden Delicious and Red Delicious was found to be in superior quality. Sparkling apple wines are produced by carbonation in tank or bottle by secondary fermentation of base wine. Blending of honey with fruit juice greatly accelerated the fermentation rates and out of various fruits tried, apple produced the wine with desirable physico–chemical characteristics and best sensory

qualities (Sandhu and Joshi, 1995). Quality Control and Product Standardization Laboratory, Department of Horticulture, Himachal Pradesh studied apple brandy preparation from culled apple fruit and found the volatile acids and higher-alcohol levels within the limits specified by ISI for fruit brandies with pH ranging from 4.5 to 4.9 (Kochhar and Vyas, 1996).

2.5.2.4.2. Mango wine

There are nearly 1000 mango varieties in India but only 20 are cultivated on commercial scale. The characteristics of each variety vary widely, and the ultimate quality of the mango products largely depends on the selection of suitable variety. Czyhrincius (1966) has studied the fermentation of two varieties of mango, namely Hilcha and Bolado and recommended Hilcha for wine making. The wine contained 13.2 per cent alcohol and was organoleptically excellent. Kulkarni *et al.* (1980) screened ten varieties of mangoes namely 'Bombay Green', 'Chausa', 'Deshehari', 'Faxri', 'Langra', 'Mallika', Lucknow' and 'Mylepelian' for wine making. Sweet wine made from 'Dashehari' was judged as having good fruit flavour. 'Faxri', Zangra' and 'Chausa' were good for wine making.

Mango dessert wines were added with ascorbic acid (0.1 w/v) which helped in aging rapidly upon modernization at 50°C for 7 days. For this 'Totapuri' mango variety was more acceptable (On Karayya, 1986). Baduyala and Awasthi (1989) evaluated twelve cultivars of mango from Kangra valley for their physio –chemical characters, suitability to produce Ready – to serve beverages (Beerch *et al.,* 1989) like Mango Yoghurt (Curd mixed with mango pulp) (Sahni and Khurdiya, 1993) and canned mango juice (Gowda and Ramanjaneya, 1995).

Thippesha *et al.* (1997) evaluated mango varieties for wine making using *S. cerevisiae* var. *ellipsoideus* strain Montrachet (No. 522). The wines made from Totapuri, Nekkare and Mallika mango varieties were reported to be excellent in quality. Homogenized ripe mango pulp of cv. Deshehari was inoculated with *S. cerevisiae* and *Acetobacter aceti* for preparation of mango vinegar. This mango vinegar was further flavoured with ginger, garlic, mint and artificial mango flavours (Garg *et al.*, 1999).

2.5.2.4.3. Plum wine

Like other fruits plum can also be made into wine and methods for its preparation have also been investigated and reported. But a product of consistently high quality could not be produced due to high acid content, improper balance of tannins, astringency and lack of fruity flavour. Vyas and Joshi (1982) fermented plum juice with water in the ratio of 1:1 with 24 per cent sugar and 0.1 per cent diammonium hydrogen phosphate and inoculated with *S. cerevisiae*, to produce wine of acceptable quality with 8.65% alcohol. Water blanching of plums followed by osmotic treatment were found to be quite effective in increasing the total soluble solids and decreasing the acidity of the fruits. Wines of acceptable acidity and sensory quality attributes were obtained from the plum fruits with pre treatment (Vyas and Sharma, 1989).

2.5.2.4.4. Guava wine

In order to minimize the post-harvest losses and to avoid market glut, guava fruits can be effectively utilized for making wine. Jawahar *et al.* (2001) optimized a good quality guava wine production using Lucknow–49 cultivar fruits. The strain *S. cerevisiae* 3287 at 22 per cent of sugar pH 4.0 and 0.05 per cent of DAHP were optimum conditions for the preparation. Guava juice was also used for preparation of ready to serve beverages (Ronteke *et al,*. 1990) and mixed fruit beverages with ber and pomegranate (Vaidya *et al.*, 1998).

2.5.2.4.5. Sapota wine

Sapota *(Manilkara achras* Forsberg) is one of the important fruit crops in India. India is the largest producer of sapota in the world. Considering the seasonal glut in the market and limited shelf life of the sapota fruit, it is necessary to develop suitable technology for its processing. Conventional processing has not yielded any acceptable product which is economically viable. To utilize this fruit which is high in sugar in a profitable manner, fermented product like sapota wine is one approach. Honde and Adsule (1998) studied the effect of different levels of SO_2 and pH on the sapota wine from 'Kalipatti' cultivar and standardized the use of 100 ppm SO_2 and adjustment of pH of the must to 4.0 are the optimum conditions for good quality sapota wine preparation.

2.5.2.4.6. Orange wine

Mature eight orange cultivars grown in Garhwal hills, Lucknow were evaluated for wine preparation. The balling –acid ratio were found to be low (7.87 to 17.86) due to low sugar content. Hence all the must were ameliorated to 23° Brix before fermentation. The orange wines secured 59 to 69 percent marks in organoleptic evaluation. 'Nagpur Santara' was found to be highly suitable for wine making. Bitterness was not encountered in any of the wines prepared (Shukla and Revis, 1985).

2.5.2.4.7. Pineapple wine

Alain *et al.* (1987) have reported high ethanol production from pineapple juice with pre selected yeast strains. Pine apple blended with grape juice (1:1) with carbonation received the higher sensory quality scores (Saxena *et al.*, 1996).

2.5.3. Fining and clarification of fruit wines

Fining is the addition of a reactive or adsorptive substance to remove or reduce the concentration of one or more undesirable constituents. Turbidity in a juice and wine may be due to grape tissue, yeast and bacteria, colloids derived from the grape, or due to the changes occurring during aging or storage. These particles may be in the form of proteins, pectins, gums, metallocolloids and degradation products of polyphenols. Boutlon *et al.*, 1997 Zoecklein *et al.*, 1997 and Isabelle *et al.*, 2000). The clarifying substances commonly used in the wine are bentonite, charcoal, gelatin, polyvinyl poly pyrrolidone, potassium caseinate and colloidal silicon dioxide, enzymes etc. The oenological gelatin are mainly used for clarification and stabilization in order to reduce the turbidity or to decrease the astringency of musts and wines. Use of oenological gelatin of 0.45 g -0.9g/100 litre decreased the total polyphenol from 803 to 770 mg litre^{-1}, while the colour intensity average increased from 2.74 to 2.83 (Versari *et al.*, 1998). Girard and Fukumoto (1999) reported PVDF (polyvinyldine fluoride) and PS (polysulfone) membranes gave higher clarification than PES (polyether sulfone) and PCE (cellulose). Soleas and Goldberg (2000) found addition of bentonite or kieselsol to the must or to the finished wine would reduce the final

content of 15 pesticides added prior to fermentation. Soleas and Goldberg (2000) found addition of bentonite or kieselsol to the must or to the finished wine would reduce the final content of 15 pesticides added prior to fermentation.

2.6. Wine quality

2.6.1. Wine colour

The extraction of sufficient colour from many red grape varieties, except in a few varieties, have always been a problem due to the presence of anthocyanin pigments in the epidermal cells which are surrounded by semi-permeable membranes. In order to extract maximum colour, the semi-permeable membrane of skin has to be damaged or destroyed. In traditional method, CO_2 and alcohol produced by intermittent mixing releases the pigments. Ethiraj and Suresh (1978) heated musts of eight grape varieties upto 70°C for 30 min for more colour extraction. Organoleptic analysis of the wines showed no variation in quality but reported higher tannin content. The amounts of anthocyanins present in red grapes vary markedly with the variety, maturity, seasonal conditions and the amount of crop. The concentrations of wine colour depends on the fermentation conditions, aging, temperature duration, sulfur dioxide and alcohol concentration (Forteza *et al.,* 1995, Mazza, 1995, Huerta *et al.,* 1998, Pokomy *et al.,* 1998).

Bakker *et al*. (1998) observed the effect of total SO_2 and extraction of anthocyanin composition and colour by using HPLC and found the wines made without SO_2 had a higher colour percentage due to polymers than the wines made with SO_2. Colour changes are more intense during oxidative aging (in wood) than during reducing aging (in bottle).

Almela *et al.* (1996) classified young red wines based on its colour. The variables allowed the highest discrimination (95%) of six different varietal wines, when colour parameters (colour intensity, anthocyanin content, colour thin, per cent of yellow pigments etc.) were included. Romero and

Bakker (2000) reported that the anthocyanin composition of 4 port wines prepared from 4 different grape varieties was mainly based on malvidin and demonstrated the possibility of adding pyruvic acid to all red wines to improve their quality, yielding high amount of vitisin A, which is a very stable monomeric anthocyanin.

2.6.2. Wine aroma

The three major flavour compounds produced by yeasts during fermentation are carbon dioxide, ethanol and glycerol. Carbon dioxide gives the characteristic tingle and the level can its also affect the perception of other flavours. Ethanol produces a warming effect, contributes to perceived sweetness, smoothness, body and is itself a flavour enhancer. Glycerol contributes to fullness, sweetness and mouthfeel (Russell and Stewart, 1992). The other major flavour compounds produced by yeast during fermentation are higher alcohols, vicinal diketones, aldehydes, sulfur compounds, esters and fatty acids. Substances showing aroma values of 1-20 are ethanol, acetates of fusel alcohols, ethyl acetate and ethyl esters of C_6-C_{10} fatty acids, 2 and 3 methyl butanols and acetaldehyde. Substance characteristic of the odour of particular wine showing aroma values of 1 to 10 are ethyl cinnamate, β-ionone, linalool, geranicol and nerol (Muscat), β-damascenone (Riesling, Chardonnay), 4-vinylguaniacol (Traminer), Sotolon, 1,1-diethoxyethane (flor sherries), 2-methoxy 3-isopropylpyrazone and 2-methoxy-3 (2-methyl-propyl) pyrazine (Sauvignons), Oak lactones (wines aged in new barrels) and vitripirane (matured white wines) (Etievant, 1991).

Forcen *et al.*(1993) studied the volatile aroma composition of two major red varietal wines viz., Manto Negro and Callet and the corresponding grape must belonging to two different regions. The greatest differences observed in musts by stepwise discriminate analysis (SDA) were due to grape variety. Manto Negro contained higher concentration of cis-3-hexen-1-ol, White callet was richer in trans-2-hexen-1-ol and benzyl alcohol. Frasse *et al.*(1993) identified more than 43 volatile compounds, which includes 20 alcohols, seven esters, six lactones, six aldehydes, three alkenes and one sulphur compound during *S. cerevisiae* fermentation. Labbers *et al.* (1994) investigated the influence of mannoproteins released from yeast cell wall during alcoholic fermentation on the volatility of aroma substances and found that the proportion of proteins is an important factor to the interactions with the volatile compounds. *Hansenula* yeasts

are potent producers of esters (ethyl acetate and isoamyl acetate). Isoamyl acetate is one of the fruit flavour esters of wine (Inoue *et al.,* 1994). Lurton *et al*.(1995) found under standard conditions *S. cerevisiae* strains from the same ecological area exhibited significant differences in the production of volatile compounds. Yeast species (*S. cerevisiae* 141 or *S. exiguus* M14) associated with lactic acid bacteria were fundamental in determining different volatile profiles (Gobbetti *et al.,* 1995, Petka *et al.,* 2001). Dominance of apiculate yeasts during initial stage of fermentation with mixed cultures improved the volatile compounds in wine (Gill *et al.,* 1996).

The volatile composition of wine is strongly affected by origin and grape variety. Origin affects mainly the amount and proportion of compounds derived from unsaturated lipids in the grape and volatiles derived from yeast amino acid (Ferreira *et al.,* 1996, Gonzalez and Bravo, 1989, Ferreira *et al.,* 1998, Reganon *et al.,* 1998 and Rogerson *et al.,* 2001). Schafer *et al.* (1999) extracted aroma concentrates from organoleptically similar wines and found coupling effect of esters with alcohol. With higher concentration of ethanol in must, the aroma effect of esters are highly enriched. Lopez *et al.* (1999) stated that the aroma of the young red wine is formed by short-chain ethyl esters, fusel alcohols and by non-isoprenoid derivatives.

Vzochukwn *et al.* (1999) analysed the palm wine aroma by Gas-chromatography – Mass spectrometry and sensory evaluation and found that palm wine volatiles and aroma were reproduced in *Saccharomyces* fermented wines, while bacteria fermented ones retain the odour of the unfermented sap. Several studies have been performed in order to classify wines according to their geographical origin. Sivertsen *et al.* (1999) classified 22 red wines based on both sensory and chemical analyses. Burgundy wines were associated with ethyl hexanoate, methyl furfural, 1-butanol, 1-propanol, 2,3-butandiol and lactic acid ethyl ester. The esters *viz.,* diethyl succinate, ethyl acetate and ethyl octanoate were identified to be discriminating factors for the Beaujolais wines. Arozarena *et al.*(2000) differentiated Spanish red wine based on its region and variety using 20 analytical parameters including alcohols, total polyphenols and anthocyanins, colour and several normal oenological variables such as pH, total acidity and dry extract. Ferreira *et al*. (2000) determined 47 odorants of 52 young monovarietal red wines of different varieties and revealed that the five most important odorants are ethyl octanoate, β-damascenone, ethyl

hexanoate, isoamyl acetate and isovaleric acid. The difference between the varieties are due to isoamylacelate, isovaleric acid and isobutyric acid and their esters.

Yeast isolates used were also important odorants in the wines (Kotseridis and Baumes, 2000). Ubeda *et al.*(2000) studied the volatile composition of wines produced on laboratory scale using different strains of *Saccharomyces*. Thirty volatile components analysed by GC exhibited differences in certain variables such as acetaldehyde, butyric acid, isobutyric acid, capric acid and 9-deconeic acid. Fraile *et al*. (2000) found that the evolution and final concentration of the majority of wine volatiles depends on the yeast strain which predominates during the fermentation. In general, the esters are formed at the end of fermentation process. White wines, fermented in the presence of oak (*Quercus alba)* chips, exhibit both higher fermentation yields and a higher production of volatile compounds during fermentation. Oak lactones, euganol and vanillin are increasing the complexity of wine aroma (Perez-Coello *et al.*, 2000).

Morales *et al.* (2002) studied the changes in the aroma profile of five sherry wine vinegar aging with wood for 24 months and found significant change in the concentration of volatiles such as methyl acetate, methanol, diacetyl and γ-butyrolactone. Volatile esters contribute important floral and fruity sensory properties to wine. Vianna and Ebeler (2001) monitered fatty acid ethyl esters and acetate esters formation in grape juice fermentation using solid phase micro extraction coupled with GC-MS. Begale *et al.* (2002) and Favretto *et al.*, 1998 used Headspace solid-phase micro extraction GC/MS for the analysis of aroma constituents in wine and identified 76 abundant aroma components in Cannonau of Jerzi wine.

2.6.3. Wine phenolics

Wine contains many phenolic substances most of which originate in the grape berry. The phenolics in wine have a number of important functions, affecting the taste of bitterness and astringency, especially in red wine. Second, the colour of red wine is carried by phenolics. Third, the phenolics are the key wine preservative and the basis of long aging. Phenolics undergo oxidation readily, they are the components that suffer owing to oxidation and turn the colour of the wine to brown when exposed to air. Wine phenolics include the non-flavonoids; hydroxycinnamates, hydroxybenzoates and the stilbenes; plus the flavonoids: flavan-3 –ols, the

flavonols and the anthocyanins while polymeric condensed tannins and pigmented tannnins constitute the majority of wine phenolics. The total amount of phenols found in a glass of red wine is in the order of 200 mg versus about 40 mg in a glass of white wine (Water house, 2002).

Mazza *et al.* (1999) studied the changes in phenolics (anthocyanins, flavonols, tartaric esters and total phenolics) during ripening of grapes and colour during vinification and aging of cabernet Franc, Merlot and Pinot Noir wines. For each variety colourless phenolics, anthocyanins and colour density in red wines were influenced by cluster stem exposure and season, but yeast used for fermentation has minimal effect. Using LC-DAD-MS, Pascual – Teresa *et al.* (2000) identified 14 flavanols in wine four monomers (catechin, espicatechin, gallocatechin and epigallocatechin), eight dimers and two trimers.

First confirmation of direct anthocyanin- tannin complex formation was reported by Remy *et al.* (2000), LC/MS analysis of different wine fractions before and after thiolysis proved the formation of covalent structures between tannins and native pigments. In dicarbonyl compounds, α -diketones are found in abundant level in wine. Revel *et al.* (2000) found a new dicarbonyl compound, phenylglyoxal in wine. Flavan –3- monomers and diamers play a prominant role in the oxidative browning of white wines (Baron *et al.,* 2000).

2.6.4. Sensory evaluation

In sensory research, people use their senses to evaluate certain properties of wines. Systemic methods for assessing sensory reactions for food and beverages are being used to maintain the sensory aspects of product quality and to reduce risks in the consumer acceptance of innovative food products. Sensory evaluation depends upon several critical factors, including the level of professional training given to sensory scientists and the level of understanding of the test principles by clients and managers.
If a logical approach to testing is followed and methods are not abused by using them for unintended or illogical purposes, then the stature of the field should continue to improve (Lawless and Claassen, 1993).

Aroma components of wine have been isolated and identified using various laboratory methods such as High Pressure Liquid Chromatography (HPLC), Gas Chromatography (GS), Atomic

Adsorption Spectroscopy (AAS) and more recently Capillary Gas Chromatography (CGS) and Mass Spectrometry (MS) (Arrvanitoyannis *et al.,* 1999). The judgments of the assessing members of a sensory panel as well as the chemical results of analyses can take different forms and they represent specific properties. Multivariate statistical analyses were used to assess the results of sensory and chemical studies (Seeber *et al.,* 1991 and Sagrado *et al.,* 2002) . Members of a well –trained laboratory panel are supposed to give small variation in their analytical evaluations.

Descriptive analysis has been used to characterize wines such as Zinfandel, Chardonnay, Semillon and Sauvignon, Seyval Blank, Pinot Noir, Riesling and Gewurztraminer and Spanish white wines. Carlucci and Monteleone (2001) defined a mythological procedure in the statistical validation of sensory data in order to describe the sensory characteristics of wines. Sivertsen *et al.* (2001) evaluated bottled wines made from cabernet sauvignon grapes grown in Chile in terms of both sensory and chemical changes. Descriptive sensory patterns of above two wines stored at different temperature gave different profiles, but it was not possible in this study to predict the shelf life of wines stored at optimum temperature.

Pinheiro *et al.* (2001) investigated the possibility of monitoring the evaluation of complex aroma profile during must fermentation with an electronic aroma – sensing technique, the so called 'electronic nose', and demonstrated without pretreatment, the electronic nose used can only perceive the evaluation of ethanol. After selective enrichment, the electronic nose detected the aroma compounds, even in the presence of ethanol.

2.7. Health benefits of wine

2.7.1. Nutritional aspects

Spontaneous wine consumption depends on multiple cultural, social, economic, gender and age–related factors. This statistically isolated behaviour patterns are highly correlated with increased longevity. Major nutritional components in g/100 ml are water (80-90), alcohol(8.0-15.0), glycerol (0.03-1.4), higher alcohol (0.03-0.19), organic acids (0.3-1.10) and phenolic compounds. Joshi and Chopra (1989) reported that fruit wines produced from apple, pear, plum and apricot could become good contributor of minerals. Caballero –Cordoba and Sgarbieri (2000) reported that the essential amino acids profile of yeast autolysate is well balanced than other foods.

Ethyl alcohol in wine has an energy contribution of 7 Kcal per g. Other non-fermentable form of carbohydrates and proteins will also contribute to the calorie count (Bamforth, 2002). Although ethanol is rich in energy, chronic consumption does not produce the expected gain in body weight. In those individuals consuming more than 30 per cent of total calories as alcohol, significant decreases in protein and fat intake too occur and the consumption of vitamins A, C and thiamin may descend below the recommended dietary allowances. Calcium, iron and fibre intake were also lowered (Lieber *et al.,* 2000).
So moderate or light alcoholic consumption is good for health.

2.7.2. Antioxidant activity

Many studies have been carried out on wine's antioxidants. Wines are rich source of polyphenol and flavonoid antioxidants. These compounds show multifunctional properties as they can act as reducing agents (free radical terminators), metal chelators and singlet oxygen quenchers. Examples of antioxidant phenols and flavanoids present in fruits are kampherol, quercetin, myriccetin and catechin have been shown antioxidant, anti –inflammatory, antiallergic, anticancer and antihemorrhagic properties (Tiwari, 2001).

In France, the diet is high in saturated fat, but the incidence of coronary artery disease is low. This French Paradox has been attributed by relatively high wine consumption.To assess this, Hurtado *et al.* (1997) studied *in vitro* oxidation of LDL (Low Density Lipoprotein) by opper ions in the presence of polyphenolic extracts of wines. Lipoprotein peroxidation was monitored as the formation of conjugated dienes and found that polyphenols in wines delayed lipid peroxidation (Robbins and Seeley, 1977, Fantozzi *et al.,* 1998). Consumption of red wine polyphenols reduces the susceptibility of LDL to oxidation (Nigdikar, *et al.*,1998).

Pellegrini *et al.* (2000) evaluated eight commercial Italian wines for their antioxdant activity. The average flavanol content was found to be 424.7 ± 121.3 mg/L catechin equalents and the total antioxidant activity of 16.8± 3.8 m mol/L Trolox equivalents. Rousselot *et al.* (2001) showed that ethanol itself acts as an antioxidant toward *in vitro* LDL peroxidation initiated by RO_2 / O_2 which would result in a decreased oxidative stress during moderate wine drinking. Burns *et al.* (2001) quantified individual phenolic compounds by HPLC and their

antioxidant activity by electron spin resonance spectroscopy. Over all antioxidant activity of red wines was contributed by larger polyphenolic complexes and condensation products that appear during aging.

Pignatelli *et al.* (2000) indicated that flavonoids inhibit platelet function by blunting hydrogen peroxide production and in turn phospholipase C activation. This may be the reason for decreased cardiovascular disease during moderate consumption of wine.

Traditionally the antioxidant power of wines have been measured using *in vitro* tests primarily based on the inhibition of human LDL oxidation and also using the ORAC (Oxygen Radical Absorbance Capacity) with different reactive species. Mannino *et al.* (1998) described a new method based on FIA (Flow Injection Analysis) system with electrochemical detection, based on the chemical structure of polyphenols. Recently, Alonso *et al.* (2002) developed a new electrochemical method for antioxidant power.

2.7.3. Resveratrol

Resveratrol (3, 5, 4' – tri hydroxystilbene) belongs to a family of stylbenes, observed in limited number of spermatophyta, among which are grapes, peanuts and pines. Their role in plant physiology seems to be the inhibition of fungal infection (i.e. phytoalexins). Resveratrol exists in two isomeric forms *trans* and *cis*. The *trans* –isomer is easily transformed to the *cis* form under intensive UV light.

cis–Resveratrol and its glucoside have been detected in almost all wines analysed so far, regardless of the origin and the technology applied. It is likely therefore that cis –resveratrol is derived from its trans isomer during vinification and there is no evidence about factors that could facilitate such conversion during wine making. Gehm *et al.* (1997) found that the resveratrol is a phytoestrogen and exhibits variable degree of estrogen receptor agonism. The estrogenic action of resveratrol broaden the spectrum of its biological actions and may be relevant to the reported cardio vascular benefits of drinking wine. Resveratrol was found to act as an antioxidant and antimutagen and to induce phase II drug –metabolizing enzymes (anti- initiation activity). It mediated anti-inflammatory effects and inhibited cyclooxygenase and hydroperoxidase functions (anti promotion activity) and also induced human promyelocytic leukemia cell differentiation (anti progression activity) (Cui *et al.,* 1997).

Resveratrol causes a complete and reversible cell cycle arrest at the 5 phase check point in promyelocitic cell line HL-60. By using this arrest in cell cycle, anticancer activity was explained by Ragionne *et al.* (1998). Resveratrol administration to rats inoculated with a fast growing tumor caused a very significant decrease (25%) in the tumour cell content. Carbo *et al.*(1998) suggested that resveratrol causes apportions in the tumour cell population resulting in a decreased cell number.

Gomoh and Nakashima (1999) extracted *trans*-resveratrol using solid-phase extraction and quantified 0.2 –1 mg/L using liquid chromatography Mass spectrometer. Dourtoglou *et al.* (1999) developed direct injection – HPLC method for estimation of *trans* –resveratrol. Using this method, they found that red wines from French and Greek cultivars were having relatively higher amounts (1.2 to 1.5 mg/L) of resveratrol than native cultivars. Bavaresco *et al.* (2000) reported that the dropping of stem process (0.9 g/200ml) in the fermenting must could increase the *trans*- resveratrol (about 1,960 μg/L). Damianaki *et al.* (2000) suggested that low concentration of polyphenols (particularly *trans*-resveratrol) and consecutive consumption of wine or other polyphenol –rich foods and beverages could have a beneficial antiproliferative effect on breast cancer cell growth.

Dobiasova *et al.* (2002) standardized a rapid and sensitive capillary zone electrophoresis for estimation of resveratrol in wine. They identified and quantified both isoforms of resveratrol (trans-resveratrol 1.85 -3μg/ml and *cis* -resveratrol 0.90 –2.5 μ/ml) in wine samples from France. *In vitro* resveratrol inhibited growth of 4T1 breast cancer cells in a dose and time dependent manner. It is a potent inhibitor of 4T1 breast cancer cell *in vitro*, non toxic to mice at 1-5 mg/kg and has no growth-inhibitory effect on 4T1 breast cancer *in vivo* (Bove *et al.*, 2002).

2.7.4. Others

Greenwalt *et al.* (1998) reported that the antimicrobial activity of the traditional fermented beverages is due to its acetic acid content. Polyphenols in food and beverages, also having tumor arresting effects, have been demonstrated in different *in vitro* and *in vivo* systems. Damianaki *et al.* (2000) reported that the decreased cell proliferation of breast cancer cells is due to the inhibitory action of red wine polyphenols.Cacetta *et al.* (2000) concluded that red wine and dealcoholized red wine consumption acutely increase plasma phenolic acid and serum uric acid concentrations, but the increase is insufficient to influence *ex vivo* lipoprotein oxidation. Anthocyanin products are prescribed as medicines in many countries for treating various

diseases. Cao *et al.* (2001) detected the wine anthocyanins as glycosides in plasma and urine and plasma anthocyanin complex is appeared to have a half –life of 132.6 min.

Moderate intake of red wine was also reported to be advisable for diabetes patients (Tessari *et al.*, 2002). Epidemiological evidence from various populations around the world has consistently identified wine consumption with increased longevity and reduced atherosclesotic mortality (German and Walzem, 2000). Cardio protection effect of red wine is related to their biologically active antioxidant compounds, ethanol and phenolics, which are able to prevent oxidation of low density cholesterol (LDL-C) (Gorinstein *et al.*, 2002). Cardio protective effect of white wine is mediated by antioxidants which include resveratrol, catechin and caffeic acid (Chi *et al.*, 2002).

2.8. Health hazards of wine

Alcohol is known to have a major impact on public health in most western countries. Most of the elucidated impact is that higher alcohol intake leads to cancer, cirrhosis, suicide, traffic accidents, abuse and series of socio- economic conditions. However, a large number of prospective population studies indicated that there is a beneficial effect due to moderate alcohol intake. Wine drinkers are at decreased risk of mortality from cardiovascular disease and incidence of isocheim stroke than non wine drinkers, which suggest that substances present in wine, but not in beer and spirits, may be responsible for a beneficial effect on the outcome in addition to a light intake of ethanol (Gronback, 2001). The partial or total dealcoholization of wines to obtain low alcoholic drinks seems to be a good choice for market expansion (Gome –Plaze *et al.* 1999). Sulfite additives have been implicated as a major cause of wine induced asthma. Vally and Thompson (2001) found that only a small number of wine sensitive asthmatic patients responded to a single dose challenge with sulfited wine under laboratory conditions. This may suggest that the role of sulfites and / or wine in triggering asthmatic responses has been overestimated.

CHAPTER III

MATERIALS AND METHODS

3.1. ISOLATION AND MAINTENANCE OF YEAST CULTURES

3.1.1. Source

The yeast cultures used in this study were isolated from different samples collected from all over Tamil Nadu, India (Table 1). Samples were collected in sterile polybags under sterile condition and stored at 4°C till use.

3.1.2. Isolation of fermentative yeasts (Kreger -van rij, 1984; Gupta *et al.*, 1994)

Samples were inoculated into 5 ml sterile Yeast Extract Malt Extract (YM) broth (Appendix I) in test tubes. In order to prevent fungal contaminants and to establish the fermentative ability, surface of the broth in the tubes was sealed with 1 cm layer of sterile paraffin. The tubes were incubated at 28±1°C for 48h.

After incubation yeast cultures were isolated from the YM broth by dilution plate technique (Pelczar and Reid, 1958). Diluted broth (10^{-6}) was spread over Yeast Extract Peptone Dextrose (YEPD, pH 3.5) (Appendix II) agar plates and incubated at 28±1°C for 48h. After incubation, yeast colonies were streaked on YEPD agar plates for purification. Purification was repeated twice and a single clone selected was then grown on YEPD agar slants and stored at 4°C for further study. Purified yeast isolates were stored in -20°C as glycerol stock for long term storage and a yeast germplasm collection was preserved for future research.

3.2. OENOLOGICAL CHARACTERIZATION OF THE YEAST ISOLATES

Oenological characters of the yeast isolates *viz.*, initiation of fermentation, fermentation rate, SH_2 production, flocculant capacity, fermentation vigour, residual sugar, final pH, titrable acidity, volatile acidity, fermentation purity, desirable bouquet, foam production and autolytic

capacity were studied for a total of 125 cultures (122 local + 3 standards) by following the standard protocols.

3.2.1. Fermentation conditions (Ciani and Maccarelli, 1998)

Grape must (var Bangalore blue, June 2000) brought to a sugar concentration of 20°B by addition of glucose, enriched with 0.1% of yeast extract and steam sterilized at 90°C for 15 min. was used to study the oenological properties. All micro- fermentations were carried out in 250 ml Erlenmeyer flasks containing 180 ml of grape must and inoculated with 5 ml of a 48h old culture (≅ 2 x10^6 cells /ml) at 28 ±1°C. The anaerobic condition was fully maintained by using a cork and water sealed special valve on the mouth of the flasks. The CO_2 produced inside escaped the system.

3.2.2. Initiation of fermentation (Thornton, 1991)

The initiation of fermentation by the cultures was observed at every 30 min. interval after inoculation up to 15h. This was seen from the bubble formation by the release of CO_2. Cultures showing initiation of fermentation within 6h were considered as desirable, while after 6h as undesirable.

3.2.3. Fermentation rate (Iranzo *et al.,* 1998)

The weight loss due to CO_2 production during fermentation was followed up to 72h. The flasks were weighed both at the time of inoculation and at every sampling time. The difference in weight indicates the CO_2 production. Fermentation rate was calculated from the amount of CO_2 produced and expressed as g CO_2 produced day^{-1}).

3.2.4. SH_2 production (Iranzo *et al.,* 1998)

Whatman No.1 filter paper strip impregnated with 0.1% lead acetate (PbAcO) was introduced into the flasks through the side of the cotton plug. The strips was placed in such a way that it won't touch the broth. This set up was kept up to 72h. Intensity of blackening indicates the quantity of H_2S production.

3.2.5. Flocculation capacity and adherence to glass (Bellal *et al.*, 1995)

Flocculation capacity (aggregation of yeast cells which settles down leaving clear supernatant) and adherence to glass was determined visually. Flocculation only after complete fermentation was taken as a positive oenological character.

3.2.6. Fermentation vigour (Zoecklein *et al.*, 1997)

Fermentation vigour is the concentration of ethyl alcohol (as % v/v) production by the yeast isolates under excessive amount of sugar (20°B). The ethanol content was estimated by Gas Chromatography.

GC make	- SHIMADZU 14 B
Column	- Poropack Q
Detector	- FID
Carrier gas pressure	- N_2 (300 KPa)
Carrier gas mass flow	- 130 KPa
H_2	- 60 KPa
Air	- 120 KPa

3.2.6.1. Oven condition

Step	Temperature (°C)	Rate (°C/Min)	Hold (Min.)	Total (Min.)
Initial	200	-	1.00	1.00
1	225	12	2.00	5.00

Injector temperature -225°C

Detector temperature - 225°C

3.2.6.2. Internal standard (0.2% v/v Iso-propanol)

Two ml of reagent grade 2 –propanol was diluted to 1000 ml with deionized water.

3.2.6.3. External standard (0.2% v/v ethyl alcohol)

Diluted 10 ml of absolute ethanol to 100 ml using deionized water. One part of ethanol standard was diluted with 99 parts of 2-propanol internal standard solution.From this, 1 µl of aliquot injected to GC and the peak area and retention time for both internal standard and ethanol standard were recorded.

Samples were also diluted (1+99) with 2-propanol internal standard and 1µl was injected thrice into the GC. The response was compared with external standard and the ethanol content was estimated as follows

$$\% \text{ Ethyl alcohol (v/v)} = \frac{RR \times \% \text{ alcohol in standard}}{RR'}$$

RR – Response ratio of the sample alcohol peak area to internal standard.

RR' – Response ratio of the standard ethanol peak area to internal standard.

3.2.7. Residual sugar (Zoecklein *et al.,* 1997)

Residual sugar (sugar level after 72 h after fermentation) was measured as Brix (B°) using hand refractometer (scale 0-32 B°).

3.2.8. Final pH

The pH of the inoculated must was monitored both at the time of inoculation and 72h after using a pH meter (CYBERSCAN 500)

3.2.9. Titrable acidity

Titrable acidity was estimated based on A.O.A.C. (1991) procedure. One ml of phenolphthalein indicator was added to 200 ml of hot boiled deionized water. Five ml of degassed fermented must sample was transferred volumetrically to this and titrated against 0.05N standard NaOH. The end point was the appearance of pink color which coincided with pH 8.2. The titrable acidity was calculated as follows and expressed in g l^{-1} of tartaric acid.

$$\text{Titrable acidity } (gl^{-1} \text{ of tartaric acid}) = \frac{(\text{Volume of base})\,(\text{N base})\,(0.075)\,(1000)}{\text{Sample volume (ml)}}$$

where,

Volume of base - volume of NaOH used in ml

N base - Normality of NaOH

3.2.10. Volatile acidity (Martinez – Rodriguez *et al.,* 2001)

Ten ml of 72h fermented must was added into 200 ml of deionized water in the boiling chamber of the distillation apparatus. One hundred ml of the distillate was collected and 2-3 drops of phenolpthalein indicator was added to the distillate and titrated using standard sodium hydroxide (0.1N NaOH) to a pink end point. The volume of NaOH used in titration was recorded and the volatile acidity was calculated and expresed in g l^{-1} acetic acid.

$$\text{Volatile acidity } (g\,l^{-1} \text{ acetic acid}) = \frac{(\text{Volume of base})\,(\text{N base})\,(0.060)\,(1000)}{\text{Sample volume (ml)}}$$

Where,

Volume of base - volume of NaOH used in ml

N base - Normality of NaOH

3.2.11. Fermentation purity (Ciani and Maccarelli, 1998)

Fermentation purity was calculated as the amount of volatile acidity formed in relation to ethanol produced.

$$\text{Fermentation purity} = \frac{(\text{g volatile acidity } l^{-1})}{(\text{ethanol \% v/v})}$$

3.2.12. Foam production (Martinez – Rodriguez *et al.* 2001)

Yeast cultures were inoculated in test tubes (16 mm x 160 mm) with 10 ml sterile must (Var. Bangalore Blue June, 2000) and incubated at 28±1°C. Foam height less than 4 mm was considered as positive oenological character.

3.2.13. Autolytic capacity (Martinez – Rodriguez *et al.* 2001)

One hundred ml of 48h old yeast broth cultures were centrifuged at 5000 rpm for 10 min. at room temperature. The pellet was washed thrice with 0.9% NaCl and suspended in a 10 ml model wine (Appendix III) and incubated at 30±1°C for 24 h with shaking at 100 rpm. The autolytic capacity was estimated by the quantity of protein released during autolysis, which was measured by optical density change of more than 0.05 OD in Bradford reaction(Bradford,1976) and was taken as a positive oenological character.

3.2.14. Production of a desirable bouquet (Esteve – Zarzoso *et al.*, 2001)

The bouquet of the fermented must was evaluated through smelling by persons belonging to heterogeneous group.

3.3. IDENTIFICATION OF THE SELECTED YEAST ISOLATES

Out of 122 local isolates, 24 isolates were selected for further study based on ethanol production and fermentation rate and identified based on following morphological and physiological characters (Kreger –Van Rij, 1984; Mesa *et al.,* 1999). Standard culture of *Saccharomyces ellipsoideus* CFTRI 101 was used for comparison.

3.3.1. Morphological characterization of the selected yeast isolates

3.3.1.1. Cell morphology

Twenty four hours old yeast cultures were inoculated at 5 percent level in 100 ml of sterile Glucose yeast extract peptone broth in 250 ml conical flask and incubated at 28±1°C for 48h and examined for the morphological characteristics *viz.*, shape of the vegetative cells and budding pattern under microscope after staining with lacto phenol cotton blue.

3.3.1.2. Ascospore formation

Ascospore formation was studied using restricted growth medium (Appendix IV) (Herman, 1971). The selected yeast isolates were first brought to a state of active growth by using pre-sprorulation medium (YM agar for 48h at 28±1°C). The sporulation medium was inoculated with actively growing cultures of the selected yeast isolates and incubated at 28±1°C for 72h and examined under microscope for sporulation. If not sporulated, the culture was incubated for further period up to 4 weeks and sporulation was studied. Ascospore formation was verified by spore staining (Kreger –Van Rij, 1984). The mature ascospores were stained blue-green whereas the vegetative cells appeared red.

3.3.1.3. Pseudomycelium formation

The selected yeast isolates were grown on Corn Meal Agar (Appendix V). Slide culture technique was used to study the formation of pseudomycelium and true mycelium. Petri dish containing a U shaped glass rod supporting 2 glass slides was sterilized by dry heat at 180°C for 2 hr. A drop of melted corn meal agar was directly placed onto the center of the slides. After solidification of the agar, the yeast culture was inoculated very lightly and a sterile cover slip was placed over it. A little quantity of sterile water was poured into the Petri dish to prevent the agar from drying out. The culture was then incubated at 28±1°C for 120h. After the incubation period, the growth around the cover slip was examined microscopically.

3.3.2. Physiological and biochemical characterization of the selected yeast isolates

3.3.2.1. Fermentation of carbohydrates

The ability of the yeast isolates to ferment sugars (glucose, galactose, sucrose, maltose, lactose and raffinose) was examined based on visible gas production. The concentration of the sugar solutions except raffinose was 2% (w/v) while 4% (w/v) concentration was used for raffinose. Five ml of the Defined medium without carbon source (Appendix VI) were dispensed into sterile tubes (150 x 12 mm) carrying insert tubes (Durham tubes) (50 x 6 mm) and sterilized by autoclaving at 15 lbs pressure for 15 min. After sterilization, sugar solutions were filter sterilized and 1 ml quantity was added aseptically into each tube. The fermentation medium was inoculated with 0.1 ml of 48h old yeast cell suspension. The tubes were incubated at 28±1°C for 14 days with regular shaking. Based on the time required for the formation of visible amount of gas in Durhams tube, the rating was given as below.

Amount of gas produced	Rating
Strong gas filling in the insert tubes with in 24-72h	+
Insert tubes partially filled (Weak)	+ w
Very weak (VW) bubble formation in the insert tube	+ vw
Slow (S) gas filling in the insert tube	s
No growth	-

3.3.2.2. Assimilation of carbon compounds

The assimilation of 18 carbon compounds by the yeast isolates (galactose, sucrose, maltose, cellobiose, trehalose, lactose, raffinose, soluble starch, D-xylose, L-arabinose, D-ribose, L-rhamnose, erythritol, ribitol, D-mannitol, succinic acid, citric acid and inositol) was studied using Yeast nitrogen broth (Appendix VI). The concentration of all sugar solutions used was 5 per cent except raffinose whose concentration was 10 per cent (w/v). All the sugar solutions were filter sterilized and added at 0.5 ml rate to 5 ml of broth in test tubes and inoculated with 0.5 ml of 48h old yeast cultures. Broth with inoculation but without carbon source served as control. The tubes were incubated at 28±1°C for 72h and the growth was observed based on the visible turbidity and rated as positive and negative growth.

3.3.2.3. Assimilation of NO_3 in liquid medium

The yeast cultures were inoculated in Nitrate assimilation broth (Appendix VI) as detailed in previous section and rated for the presence (+) or absence (-) of NO_3 assimilation.

3.3.2.4. Growth at 37°C

The yeast cultures were inoculated in glucose assimilation medium (Appendix VI) as detailed in previous section and incubated at 37°C for 21 days. Growth is scored as low (+) moderate (++) and good (+++) based on visual observation.

3.3.2.5. Growth at vitamin free medium

The yeast cultures were inoculated in glucose assimilation medium without vitamins (Appendix VI) as described in previous section and incubated at 28±1°C for 7 days. The growth was measured as before.

3.4. GROWTH KINETICS OF THE SELECTED YEAST ISOLATES (Bell *et al.,*2001)

Defined medium (Appendix VI) with 20% (w/v) sucrose was used for this study. The fermentations were carried out in 500 ml Erlenmeyer flask with 250 ml sterile broth. The broth was inoculated with 48h old yeast culture at 5 per cent level. The population of initial inoculum was adjusted to a cell concentration of 2 $x10^6$ cells ml^{-1} by standard dilution plate technique. The flasks were incubated at 28±1°C with gentle agitation of 100 rpm till the completion of fermentation as indicated by the constant ethanol concentration. The final yeast biomass was assessed on dry weight basis.

Ethanol production and uniform rate of fermentation as g of CO_2 production (*i.e* weight loss during fermentation) were studied after 24 h of inoculation as per the standard protocol described in section 3.2. The reducing sugar was estimated by following the method of Lane–Eyron (1984) and expressed as g l^{-1}. Kinetic parameters were evaluated according to Ciani and Picciotti (1995) and Thronton (1991).

Kinetic parameters studied

1. Ethanol yield (Yeth) $= \frac{\text{ml of ethanol produced}}{\text{One g of sugar}}$ (ml /g)

2. Specific growth rate $= \frac{\text{Biomass production}}{\text{Fermentation time}}$ hr^{-1}

3. Ethanol productivity $= \frac{\text{ml of ethanol produced}}{\text{L of the medium}}$ ml /L. hr^{-1}

4. Fermentation rate $= \frac{\text{Amount of } CO_2 \text{ produced (weight loss)}}{\text{Fermentation time}}$ (g of CO_2/hr)

3. 5. TOLERANCE TO HIGH OSMOTIC PRESSURE (Caridi *et al.*, 1999)

With a view to assess the osmotic tolerance, the selected yeast cultures were grown in test tubes with 10 ml defined medium supplemented with 30-70% sugar concentration. Based on the growth, the cultures were designated as

+++	-	Very strong	- 1/3 rd of the Durham insert was filled with CO_2 gas on first day after inoculation.
++	-	Strong	- 1/3rd of the Durham insert was filled with CO_2 gas 2 days after inoculation
+	-	Weak	- 1/3rd of the Durham insert was filled with CO_2 gas 3 days after inoculation.
-	-	Nil	- No gas production upto 3 days.

For detailed osmotolerance study, three very strong osmotolerant yeast isolates at 60% sugar level namely *S. cerevisiae* KJSK-37, *K. marxianus* KJSK-49 and *S. cerevisiae* KJSK-96 and one susceptible *S. bayanus* KJSK-105 (showed poor growth at 40% sugar level) cultures were tested in comparison with the standard *S. ellipsoideus* CFTRI 101.

The above cultures were grown in must samples for 48h and 10 ml of inoculum was inoculated to 150 ml must sample in 250 ml flask, already adjusted to 20B°, 30B°, 40B°, 60B°

and 70B° with sucrose solution and pasteurized at 100°C for 20 min. The flasks were incubated at 28±1°C. The experiment was proceeded till the completion of fermentation. The ethanol yield and fermentation rate were estimated as described earlier in section 3.2. Residual sugar was estimated after hydrolysing in 10% 4N HCl for 10 min. at 75°C and the reducing sugars in the neutralised samples were determined using dinitrosalicylic acid reagent (Miller,1959).

3.6. TOLERANCE TO TEMPERATURE (Thermotolerant and Psychrotolerant) (Argiriou *et al.*, 1996)

Based on the initial screening in defined media incubated with 10-40°C, following 2 psychrotolerant cultures namely *H. anomale* KJSK-69 and *S.bayanus* KJSK-100 and 2 thermotolerant cultures namely *S. cerevisiae* KJSK-87 and *K. thermotolerans* KJSK-111 were selected and tested in comparison with the standard *S. ellipsoideus* CFTRI.-101 for temperature tolerance study. The cultures were inoculated in must samples with 20B° and incubated at 10, 15, 20, 25, 30, 35 and 40°C for a period of 30 days in BOD incubator. CO_2 production and alcohol content were estimated as per the method described in section 3.2 and the residual sugar was estimated using hand refractometer for a period of 30 days at daily intervals.

3.7. TOLERANCE TO ACIDIC pH

Based on the growth in defined medium adjusted with low pH range from 2.0 –5.0, the selected yeast isolates were designated as very strong, strong, weak and no growth as described in section 3.5. For low pH tolerance studies, 2 strong acidic pH tolerant cultures (pH 2.0) namely *C. oleophila* KJSK-29 and *C. intermedia* KJSK-90 and 2 weak acidic pH tolerant cultures (pH 2.5) namely *H. anomala* KJSK-69 and *S. ellipsoideus* KJSK-106 were tested in comparison with the standard *S. ellipsoideus* CFTRI-101. The cultures were inoculated in must samples adjusted to a pH of 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 using citric acid and sodium hydroxide with 20°B sugar level and incubated at 28±1°C for a period of 30 days. CO_2 production, alcohol content and residual sugar were estimated as per the method described in section 3.2.

3.8. TOLERANCE TO SO_2 AND ALCOHOL

Modified methodology of Parish and Carroll (1987) was used to test the tolerance of the yeast isolates to high concentration of SO_2 and ethanol. To test the tolerance, the selected yeast isolates were subjected to different SO_2 concentration (50, 100, 150, 200, 250, 300 ppm SO_2) by addition of potassium metabisulphite (KMS) and to different ethanol concentration (6, 8, 10, 12, 14, 16 % v/v) by addition of ethanol in Defined medium (Appendix VI). Fermentation was performed separately in test tubes with Durham's tube. The CO_2 produced was collected under Durham insert. The tests were considered positive if, within a maximum of a 3 days incubation period at 28±1°C, the inserts were filled with CO_2 gas up to one third of their capacity.

3.9. KILLER TOXIN PRODUCTION

Twenty four yeast isolates along with standard culture were tested for their killer toxin production and interaction by the method of Hidalgo and Flores (1994). A lawn was formed by inoculating each yeast isolate having a population of 10^5 cells/ml in YEPD medium (pH 3.5) with 0.003 per cent methylene blue. Each lawn was cross streaked with other yeast isolate and the plates were then incubated at 28±1°C for 96 h. Killer activity was scored positive when the killer strain was surrounded by a region of bluish stained cells or by a clear zone of growth inhibition surrounded by green stained cells. Genetic basis of this killer toxin production was also studied based on ds RNA recovery during total genomic DNA isolation (Yap *et al.* 2000).

3.10. ENZYMATIC CHARACTERIZATION OF THE SELECTED YEAST ISOLATES (Iranzo *et al.*, 1998 ; Strauss *et al.*, 2001)

3.10.1. Polygalactouranase

The methodology proposed by Kay (1988) was modified and used. The defined medium (Appendix VI) with polygalacturonic acid as carbon source at 1% level was used. The selected yeast isolates were streaked over agar plates and incubated at 28±1°C for 98h. The enzyme activity was detected by staining with ruthenium red (0.1%) and the colonies showing a purple halo were identified as positive enzyme production.

3.10.2. Cellulase

The Defined medium (Appendix VI) with 1% cellulose as carbon source was used for this study. Selected yeast isolates were streaked on agar plates. After seven days incubation at 28±1°C, visible powdery growth on the agar plates indicated the production of cellulase enzyme.

3.10.3. Amylase

The Defined medium (Appendix VI) with 1% starch as carbon source was used for this study. A loop full of 48h old yeast isolates were streaked on agar plates and incubated at 28±1°C for 7 days. At the end of incubation, agar plates with yeast growth was stained with 0.1% iodine solution. Clearing zones surrounded by yeast growth on blue background indicates production of amylase.

3.10.4. β-Glucosidase

A screening method described by Iranzo *et al.* (1998) was carried out on agar plates with arbutin (1%) as substrate. Selected yeast isolates which hydrolyze the substrate and produce dark brown colour in the agar are considered to be positive for β-glucosidase activity.

3.10.5. Proteinase production

The method proposed by Bilinski *et al.* (1987) was followed. The strains were inoculated in radial streaks on skin milk agar plates and the appearance of clearing zone confirmed the proteinase enzyme production by the selected yeast isolates.

3.10.6. Esterase activity

The method proposed by Iranzo *et al.* (1998) was followed. Yeast cells harvested from 5 ml liquid culture (Appendix VII), washed twice with cold saline (NaCl $9gL^{-1}$) and the pellet was suspended in 10 ml of citrate phosphate buffer (0.03 M, pH 7.5). Esterase activity was assayed by measuring the amount of p-nitrophenol (PNP) substrate. Enzyme solution (cell suspension) was mixed with 2μ mol pNPA (p-nitrophenol acetate) in 0.03 M phosphate buffer. Visible yellow color formation due to PNP formation was considered as positive enzyme production.

3.11. ANALYSIS OF FUSEL OILS (HIGHER ALCOHOLS, ALDEHYDES AND ESTERS) PRODUCED BY THE SELECTED YEAST ISOLATES (Esteve - Zarzoso *et al.,* 2001)

The fusel oils (methanol, propanol, isopropanol, butanol, isobutanol, isoamyl alcohol, acetaldehyde and ethyl acetate) produced by the selected yeast isolate were determined by gas chromatography.

GC make	-	VARIAN CP –3800 with auto injector and Star WS software programmed
Column	-	Stainless steel column packed with 5% carbowax 20M on 60-80% mesh.
Detector	-	FID (Flame ionization detector)
Carried gas	-	N_2 (5.8 $kgcm^{-2}$)
H_2	-	2.6 kg/cm^{-2}
Air	-	4.5 kg/cm^{-2}
Injector temperature		250°C
Detector temperature		250°C
Range (Attenuation)		11

3.11.1. Oven conditions

Step	Temperature	Rate (°C/Min)	Hold (Min.)	Total (Min.)
Initial	60°C	-	2.00	2.00
1	120°C	4.0	0.50	17.50
2.	160°C	4.0	3.50	31.00

3.11.2. Carrier gas flow conditions

Step	Flow (ml / min)	Rate (ml/Min)	Hold (Min.)	Total (Min.)
Initial	5.0	-	10.00	10.00
1	10.0	0.5	0.50	20.50
2.	5.0	0.5	0.50	31.00

The conditions listed above were set for operating the GC.

3.11.3. Working standard preparation

The working standard contains 2 ml each of ethyl acetate, acetaldehyde and methanol and 1 ml each of iso-propanol, n-propanol, isobutanol, isoamyl alcohol, ethyl acetate and volume made up to 100 ml with 1:1 ethanol and water. One µl of the working standard was injected into GC. The retention time and peak area were recorded and used for estimation of the amounts of individual fusel oils in wine distillate.

One µl of wine distillate (100 ml of 72h old selected fermented must) was transferred into Kjeldahl distillation flask. Ten ml of distillate was collected by using Klevencher apparatus cooled by Hoofer Scientific Company gel apparatus water circulation cooler (cooled at temperature 5°C) and was injected into GC. Fusel oils were identified based on retention times with those of standards and the amounts of individual fusel oils present was estimated by using the standards. The internal standard of pentanol at 0.5% v/v concentration was used to avoid injection error.

3.12. ANALYSIS OF ORGANIC ACIDS AND GLYCEROL PRODUCTION BY THE SELECTED YEAST ISOLATES (Zoecklein *et al.*, 1997)

Organic acids produced by the selected yeast isolates were separated and quantified by HPLC.

3.12.1. Sample preparation

Mixed 2 ml of 72 h old selected yeast fermented must and 0.2 ml of concentrated ammonium hydroxide in a capped vial and passed on through a preparative exchange column and allowed to drain thorough and washed the sugars by deinonized water. Carefully added 2 ml of sulfuric acid and demineralized water (1:4) to calculate 10 ml of the elutent containing the organic acids and glycerol. Samples filtered through 0.45 µm syringe filter were used for injection.

HPLC make : VARIAN *prostar*
Column : C-18 Reverse phase
Detector : UV / VIS detector

Mobile phase : 0.1 M Ammonium dihydrogen phosphate + Phosphoric acid (pH 2.5)

Flow rate : 1 ml/min

Detector water length : 210 nm

Injector volume :1 µl

3.12.2. Standard preparation

Standard solution of the various organic acids expected in the sample (acetic, citric, fumaric, lactic, malic, succinic, tartaric, ascorbic acids and glycerol) were prepared at 1 mg /ml concentration and their retention times and peak area were recorded.

From chromatography standard solution of various organic acids and glycerol at known concentration, peak area ratios were compared to those from wine samples and the un known concentrations were calculated.

$$\text{Organic acid concentration } (g\ L^{-1}) = \frac{R}{R'} \times \text{Organic acid concentration in standard}(g\ L^{-1})$$

R - Peak area of the organic acid in the sample

R' - Peak area of the standard organic acid.

3.13. EXCLUSION TESTS TO SELECT FRUIT WINE YEASTS (Perez-Coello *et al.,* 1999)

A modified method of Perez-Coello *et al.* (1999) was used for the best fruit wine yeasts selection. In this selection, oenological properties *viz.* fermentation rate, SH_2 production, flocculation, sugar exhaustion, tolerance to 200 ppm SO_2, 12% ethanol, 30° Brix sugar, growth at 15°C, 3.0-4.0 pH, killer toxin production, hydrolytic enzyme production and other characters were studied using the protocols described in earlier sections. On priority basis, best five yeast isolates with the best oenological properties were selected and used for further standardization of fruit wine making studies.

3.14. CLUSTER ANALYSIS (Iranzo *et al.,* 1998)

Cluster analysis of the yeast isolates based on principal oenological characters *viz.* fermentation rate (g of CO_2 produced /day), SH_2 production, fermentation vigour (alcohol content % v/v), flocculent capacity, 12% alcohol tolerance, osmotic tolerance and SO_2 tolerance were performed using Euclidean Co-efficient statistical packages SYSTAT and NTSys. Further clustering was also done for other oenological characters *viz.,* hydrolytic enzyme production, glycerol production, uniform rate of fermentation, fermentation purity, initiation of fermentation, fermentation time, bouquet, sugar exhaustion, higher alcohols, esters and aldehydes production, organic acids production, higher and lower temperature tolerance, specific growth rate, ethanol productivity, ethanol yield, foam production, adherence to glass and autolytic capacity.

3.15. TOTAL GENOMIC DNA ISOLATION

The selected 24 yeast isolates along with 2 type strains of *S. ellipsoideus* CFTRI 101 and MTCC 180 were grown on YEPD agar plates and after 48h of incubation the cells from single plate streak growth were swapped out using sterile spatula and pasted inside of sterile pestle and morter for each yeast isolates and kept for 2 hrs at –70°C in a cryoscientific freezer. After freezing the cells were ground well and suspended in 5 ml of TE buffer (pH 7.4) (Appendix VIII). To this 0.5ml of 10% SDS solution was added, mixed well and transferred into 50 ml centrifuge tube and incubated at 65°C for 30 min. Then 0.5 ml of 5 M sodium acetate was added immediately and the centrifuge tubes were placed at –70°C for 1 h in a freezer. Then tubes were centrifuged at 10,000 rpm speed for 10 min. The supernatant was transferred to a fresh tube and the DNA was precipitated by adding 2 volumes of absolute ice cold ethanol. After incubation for 30 min in a freezer, the tubes were centrifuged at 10,000 rpm for 5 min. The pellet was resuspended in 3 ml of TE (pH 7.4) and centrifuged at 10,000 rpm for 15 min. The supernatant was transferred into a fresh tube and added with 2 µl of RNase (10 mg ml^{-1}) and incubated at 37°C for 30 min. DNA was again precipitated by adding one volume of isopropanol. DNA thread was spooled out using sterile 1 ml tips, washed with 70% ethanol and dispensed in sterile

200 µl milli Q water. The DNA concentration was estimated by traditional visual observation in ethidium bromide stained 0.8% agarose gel.

3.15.1. Study on plasmid profile

Total genomic DNA without RNase treatment and with RNase treatment was used to study 2 µ circular plasmid and ds RNA plasmid (Killer toxin producer). Ten µl total genomic DNA of both samples for each culture were run in agarose (0.8%) gel electrophoresis with ethidium bromide staining. λ DNA double digest (ECORI and Bam H III) were used as a molecular weight marker.

3.16. POLYMERASE CHAIN REACTION (PCR) AMPLIFICATION

PCR reactions were carried out in an *eppendorf* thermal cycler. A total of 13 primers were used for studying the genetic diversity of the selected 26 yeast isolates (including type strains).

Primers used

i. EI 1 (5'- CTGGCTTGGTGTATGT –3') complementary to intron consensus splicing sites (Pataro *et al.* 2000).

ii. M 13 (5' –GAGGGTGGCGGTTCT –3') (Guerza *et al.* 2001)

iii. RF_2 (5' –CGGCCCCTGT-3') (Suzzi *et al.*2000)

iv. 10 random primers (10 mers from Operon technologies,USA).

OPA1	-	5' CAGGCCCTTC 3'
OPA2	-	5' TGCCGAGCTG 3'
OPA4	-	5' AATCGGGCTG 3'
OPA14	-	5' TCTGTGCTGG 3'
OPA18	-	5' AGGTGACCGT 3'
OPA20	-	5' GTTGCGATCC 3'
OPD 2	-	5' GGACCAACC 3'
OPD 5	-	5' TGAGCGGACA 3'
OPD 16	-	5' AGGGCGTAAG 3'
OPD 18	-	5' GAGAGCCAAC 3'

Reaction mixture

Sterile milli Q water	13.8 µl
10x Taq buffer	2.0 µl
d NTPs (10 nM)	1.0 µl
Template DNA (10ng/µl)	2.0 µl
Primer (25 ρmol)	1.0 µl
Taq polymerase enzyme (2U)	0 2 µl
	20 µl

Reaction were carried out in the *eppendorf* thermal cycler. PCR conditions (except EI 1 primer) were

5 min at	95°C	
30 sec at	95°C	35 cycles
1 min at	36°C	
1 min and 30 sec at	72°C	
5 min at	72°C	
Hold	4°C	

for EI 1 primer

5 min at	95°C	
30 sec at	95°C	2 cycles
2 min at	30°C	
30 sec at	72°C	
30 sec at	95°C	32 cycles
2 min at	40°C	
30 sec at	72°C	
5 min at	72°C	
Hold 4°C		

PCR products were analysed in 2% agarose gel stained with ethidium bromide. For EI 1 primer amplified products 5% polyacrylamide gel stained with silver staining was used.

3.17. STANDARDIZATION OF FRUIT WINE MAKING

3.17.1. Screening banana varieties for wine making

Nine varieties, *viz.,* Poovan, Robusta, Karpooravalli, Peyan and Padathi were procured locally (Coimbatore) while Red Banana, Nendran, Rasthali and Matti were procured from Kanyakumari district. Two kg each of sound, healthy and fully ripe fruits were selected peeled and pulped along with 100 ppm of sulphur dioxide (Potassium meta bisulphite) and ameliorated to obtain 20° Brix by adding cane sugar (Kulkarni *et al.,* 1980) in a blender. About 250 ml of pulp of each variety was kept in 500 ml sterile conical flasks (three replications were maintained) and plugged with cotton. After 8 hrs of sulphiting, the pulp was inoculated with *Saccharomyces ellipsoideus* CFTRI 101 (collected from CFTRI, Mysore) at 5 per cent level starter culture (48 h fermented sterile banana pulp heat activated at 42°C for 1 h 20 min). When vigorous fermentation had set in (48 hrs after inoculation), 0.5 per cent pectic enzyme prepared from *Aspergillus niger* (Trizyme, Mysore) was added for clarification and water sealed with special valve (Shukla and Revis, 1985). The fermentation was carried out at room temperature (28-32°C) for 10-12 days. After completion of fermentation, clear liquid was filtered through a clean muslin cloth and stored in sterile glass bottles for a month in BOD incubator when the suspended matter settled leaving clear wine on the top. The wine was further stored in bottles with 100 ppm SO_2 by adding potassium meta bisulphite.

3.17.2. Screening papaya varieties for wine making

Seven varieties *viz.,* CO1, CO2, CO3, CO4, CO5, CO6 and CO7 were harvested from the papaya field at TNAU Orchard, Coimbatore. Two kg each of sound, healthy and fully ripe fruits were selected peeled, pulped and fermented as described in the previous section.

3.17.3. Screening grape varieties for wine making

Five varieties *viz.,* Bangalore Blue, Thompson Seedless, Alquish and Shared were procured from local market (Coimbatore) and Muscat (Panneer) is obtained from Grapes Orchard, Thondamuthure Coimbatore. Two kg each of healthy and fully ripe clusters were selected, destemed and crushed gently with hands along with 100 ppm of SO_2 and the Brix adjusted to 20° with cane sugar. The other procedures employed for the preparation of wines from must were as described earlier.

3.17.4. Standardization of fruit wine making conditions

Under semi solid state fermentation (banana and papaya pulp) and submerged fermentation (Grape must), fermentation conditions (TSS, pH, acidifier, temperature) were standardized for wine making from banana (Robusta), papaya (CO2), and grape (Muscat) using selected yeast isolates.

Yeast cultures used

Y_1 *H. anomala* KJSK –69
Y_2 *S. cerevisiae* KJSK-57
Y_3 *S. cerevisiae* KJSK-96
Y_4 *S. bayanus* KJSK-100
Y_5 *S. ellipsoideus* KJSK-106
Y_6 *S. ellipsiodeus* CFTRI –101
Y_7 *S. ellipsoideus* MTCC –180

3.17.4.1. Fruit pulp / must preparation

Banana pulp

Robusta banana fruits, obtained from TNAU Orchard, Coimbatore were made to over ripe before pulping. Then peeled and homogenized in a blender for about 5-10 minutes to obtain pulp. Potassium metabisulphite (100 ppm SO_2) was added before homogenization to prevent browning as well as for pasturerization.

Papaya pulp

CO2 papaya fruits, obtained from TNAU Orchard, Coimbatore, were made to over ripe before pulping. Then peeled with knife, removed the seeds and homogenized in a blender with 100 ppm SO_2 for 5-10 min to get pulp.

Grape must

Muscat (Panneer) grape fruits, obtained from Grape growers association, Thondamuthore, Coimbatore were destemmed before crushing and manually crushed by hands with 100 ppm SO_2.

3.17.4.2. Experimental design

Four investigations were carried out to determine the effect of TSS (Total soluble solids), pH, acidifier and temperature on wine making. The following sugar levels were used for studying the effect of TSS on wine making.

S_0 - no sugar level adjustment

S_1 - 18B°

S_2 - 20B°

S_3 - 22B°

S_4 - 24B°

S_5 - 26B°

TSS level was adjusted by adding cane sugar in fruit pulp / must and mixed well. The TSS level was estimated by hand refractometer. No adjustment in pH of the pulp/ must was done and incubated at 28 ± 2°C.

The second investigation was to determine the effect of pH on wine making. (In grape wine making, pH was not adjusted, because pH 3.46 is reported to give high quality wine). The treatments were as follows.

P_0 - Control (No pH adjustment) (Original pH of banana and papaya is 4.84 and 5.20 respectively)

P_1 - pH 4.5

P_2 - pH 4.0

P_3 - pH 3.5

P_4 - pH 3.0

Acidification was done by using food grade citric acid mixed well with pulp and pH was adjusted using Cyberscan pH meter. TSS of the pulp was maintained as 24B° and incubated at 28± 2°C.

Third investigation was carried out to determine the effect of acidifier on wine making with following treatments.

A_0 - Control (No acidification)

A_1 - Acidification by citric acid

A_2 - Acidification by lime juice

A_3 - Acidification by tamarind pulp

TSS of the fruit pulp was 24B° and pH was adjusted up to pH 3.5 for acidification and incubated at 28 ± 2°C.

In fourth investigation to determine the effect of temperature on wine making was determined by carrying out the fermentation at various temperatures as described below.

T_0 - Room temperature (28±2°C)

T_1 - 20°C

T_2 - 15°C

Experimental set up was kept inside the BOD incubator to reach 20°C and 15°C. TSS of the fruit pulp / juice was 24B° (26B° for grapes) and pH 3.5 acidified with citric acid.

3.17.4.4. Fermentation

Pulp /must was enriched by adding 100 mg of ammonium sulphate. Above treated pulp / must was inoculated with activated (heating at 42°C for 1 hr 20 min) yeast cultures @ 5% inoculum level. Fermentation was carried out in 500 ml conical flask with 300 ml pulp / must. Two days after aerobic fermentation, the flasks were water sealed. For grape wine, the skin was removed through filtration under sterile conditions after 2 days and kept under desirable temperature as indicated in the experimental design upto 15 days. Upon completion of alcoholic fermentation, the fermented pulp / mark filtered through musline cloth racked thrice for 7 days at 10°C, removed the yeast sediment, clarified using gelatin (0.165%) bottled pasteurized and kept for aging.

3.17.4.5. Wine analysis

Alcohol content, titrable acidity, volatile acidity, pH, Brix and reducing sugar content were estimated as described earlier (Section 3.2.6). Tannin content was estimated based on the method suggested by Riber ean-Gayon and Glories (1986).

3.17.4.6. Sensory analysis

A panel of 10 judges of heterogenous group was formed and using a 20 points score card (Appendix IX) the sensory properties of the fruit wines were evaluated.

3.17.5. Studies on colour extraction and colour stability in Muscat grape wine

Due to heavy loss of colour in Muscat grape wine during aging process, we designed following 2 experiments to increase the colour extraction and stability.

3.17.5.1. Effect of skin contact time on wine colour

Muscat grapes were crushed manually, adjusted the sugar level upto 24 Brix and potassium meta bisulfite (100 ppm SO_2) was added. Following treatment was designed based on the skin contact time.

SC_0 - No skin contact time

SC_2 - 2 days skin contact time

SC_4 - 4 days skin contact time

SC_6 - 6 days skin contact time

SC_8 - 8 days skin contact time

Cultures used

Y_1 - *S. cerevisiae* KJSK-57

Y_2 - *H. anomata* KJSK –69

Y_3 - *S. cerevisiae* KJSK-96

Y_4 - *S. bayanus* KJSK –100

Y_5 - *S. ellipsoideus* KJSK-106

Y_6 - *S. ellipsoideus* CFTRI –101

Y_7 - *S. ellipsoideus* MTCC –180

For each treatment and culture, replicated trails were produced.

Fermentation was carried out at 28 ± 2°C temperature in 300 ml of must in 500 ml conical flask. Based on contact time, the skin was removed through filtration under sterile condition.

3.17.5.2. Effect of heating (Thermovinification) on wine colour

Like above experiment, crushed must was heated upto 70°C for different time durations.

H_0 - No heating

H_{10} - 70°C for 10 minutes

H_{20} - 70°C for 20 minutes

H_{30} - 70°C for 30 minutes

H_{40} - 70°C for 40 minutes

H_{50} - 70° C for 50 minutes

For each treatment and cultures (all the seven cultures selected as listed in previous section) replicated trials were conducted.

Before fermentation, the sulphited must adjusted to 24B° was heated upto 70°C in water bath based on treatment time. Here the skin was removed on the 2nd day of aerobic fermentation and water sealed as described in previous sections.

3.17.5.3. Extraction of grape anthocyanin (Yokotsuka and Nishino, 1990)

A quantity of 0.23 kg of skin was obtained from 1 kg of Muscat grapes after pressing. The wet skins were soaked in 1 litre of 50% ethanol and kept at room temperature (flushed with N_2 and sealed) under dark for a week. The skins were removed by kada cloth filtration. The filtrate was further filtered through Whatman No. 1 filter paper and concentrated to a small amount by rotary evaporator under reduced pressure at 40°C. The concentrate was made upto equal volume (5ml) and used for spotting in thin layer chromatography (TLC). One dimensional TLC was done at room temperature by using the upper phase of n-butanol, acetic acid and water (BAW) (4:1:5 v/v). The Rf values of the pigments separated by chromatography were calculated.

3.17.5.4. Extraction of wine anthocyanin

Samples (0.5 litre each) of freshly prepared 3 months aged wine, heat treated and those with increased skin contact time were lyophilized, made upto equal quantity (5 ml) and used for TLC spotting. Pigments were eluted from the TLC by using 1 ml of 0.01 N HCl methanol. Their absorbance maxima at UV and visible range was studied by using Beckman spectrophotometer DU-64 by dilution (10 times). After elution of the pigments by TLC, they where identified and quantified using Varian HPLC with Novapack C-18 column using upper phase of BAW and the absorbance of the elute was measured at 520 nm by using UV – VIS detector. Anthocyanin pigments were identified based on its Rf value and RT value in both TLC and HPLC and compared with literature data (Yokotsuka and Nishina, 1990, Bakker, 1998 and Mazza *et al.* 1999) and changes of pigments during aging, colour extraction and different treatments (heat and increased skin contact) were studied.

3.17.5.5. Analysis of extracted colours, tannins and colour stability

Wine samples were diluted (1:10) and the absorbance (A) at 420 nm (A_{420}) and 520 nm (A_{520}) were measured. Colour parameters, brightness and hue were calculated in following ways

$$\text{Brightness} = A_{420} + A_{520}$$

$$\text{Hue} = \frac{A_{420}}{A_{520}}$$

Tannin content and phenolics composition were analysed based on method described in sections 3.17.4.5 and 3.17.6 respectively. Changes in brightness, hue and individual pigments were analysed during aging process (initial, 3 months and 6 months).

3.17.6. Analysis of fruit wine phenolic compounds

HPLC analysis is based on modified method published by Fischer *et al.* (2000). Fruit wines were analysed for the presence of various phenolic compounds by reversed phased chromatography using a Varian HPLC system equipped with a UV/VIS detector and *Prostar* software. Wine samples were passed through 0.45 μm filter prior to injection. Separation of phenolics was carried out using following conditions.

Column used – C-18 RP (Varian)

Mobile phase – Solution A: water with 1.5% phosphoric acid.

Solution B: Acetonitrile with 1.5% phosphoric acid

Gradient

0-18 min	A 95%,	B 5%
18-45 min	B 88%,	B 12%
45-60 min	A 80%,	B 20%
60-65 min	A 70%,	B 30%
65-70 min	A 45%,	B 55%
70-75 min	A 10%,	B 90%
75-90 min	A 95%,	B 5%

Flow rate - 0.5 ml/min

Detection wave length - 280 nm

Column was re-equilibriated for 25 min between each run. To maintain constant retention time and stable base line, a blank run was carried out at start of each day. The peaks were identified by comparison to published spectra and retention times (Brenna *et al.*1998 and Revel *et al.,* 2000) and also with the standards (gallic acid, catechin, epicatechin, malvidin –3-glucoside, trans-resveratrol, myricitin, quercitin and kampferol by Sigma–Aldrich). Concentration of phenolic compounds were calculated by comparing the standards. For others, peak area of gallic acid at 280 nm (2 mg/ml) was used for comparative quantification.

3.17.7. Estimation of *trans*–resveratrol concentration in fruit wines

Fruit wine samples, (grape wines, banana wines and papaya wines) were examined for their trans- resveratrol content by using a direct injection HPLC method (Dourtoglu *et al.,* 1999).

HPLC make	-	Varian *Prostar*
Detector	-	UV –VIS detector Model 320
Columns used	-	(18 Reverse Phase 5 μm, 4mm x 250 mm)
Mobile phase		
Solvent A	-	Water: Acetonitrile (7:3)
Solvent B	-	Water methanol (5:5)
Flow rate	-	0.6 ml/min
Column temperature	-	40°C
Elution gradient	-	100% solvent A for 0-18 min. 100% A to 100% B in 1 min. 100% B to 100% A in 6 min
Injection volume	-	20 μl (0.45 – membranes filtered wine)
Detection wavelength	-	308 nm
Standard solution	-	*trans* – resveratrol (Sigma Aldrich) was prepared using ethyl acetate and kept at 18°C .

Identification and quantification of resveratrol was based on retention time and peak area of standard and sample data obtained by analysing triplicate samples.

3.17.8. Studies on yeast population dynamics in fruit wine fermentation using RAPD-PCR during co-inoculation

With an idea to assess the native yeasts domination, the survival and succession of the yeast inoculated individually and as co-inoculation, following experiment was designed. Two fruit wine making processes (Grape wine from Musket and banana wine from Robusta) were studied for yeast population dynamics during fermentation. Three different yeast cultures *(S. cerevisiae* KJSK-57, *S. ellipsoideus* KJSK-106 and *Hansenula anamala* KJSK-69) were co-inoculated in the grape must and banana pulp.

Yeast selection criteria

(i) *S. cerevisiae* KJ-SK-57 – Killer toxin (88.6% Killer activity producer).

(ii) *S. ellipsoideus* KJ-SK-106 – Killer toxin sensitive.

(iii) *H. anomala* KJSK-69 - Killer toxin resistant

Primer selection for RAPD –PCR based yeast enumeration

Primer used – M 13 (5'– GAGGGTGGCGGTTCT –3') (Guerza *et al.*, 2001) Proved to be highly polymorphic and highly differentiated in above three yeast isolates.

Experimental design

(i) Uninoculated must / pulp (Pasteurized with KMS –100 ppm SO_2)

(ii) Inoculated with *S. cerevisiae* KJSK-57

(iii) Inoculated with *S. ellipsoideus* KJSK-106

(iv) Inoculated with *H. anomala* KJSK-69

(v) Inoculation of both *S. cerevisiae* KJSK-57 and (Killer toxin producer) *S. ellipsoideus* KJSK-106(Killer toxin sensitive)

(vi) Inoculation of both *S. cerevisiae* KJSK-57 and *H. anomala* KJSK-69 (Killer toxin producer)

(vii) Inoculation of all three *(S. cerevisiae* KJSK-57 + *S. ellipsoideus* KJSK-106 + *H. anomala* KJSK –69.

Must / Pulp was pasteurized with 100 ppm SO_2 and inoculated with above yeast isolates at 5% inoculation level with an inoculation load of *S. cerevisiae* KJSK-57 (1.86×10^6 cells/ml), *S. ellipsoideus* KJSK-69 (2.12×10^6 cells /ml) and *H. anomala* KJSK –69 (1.71×10^6 cells /ml). The wine making process were carried out under the same conditions (28 ± 1°C, 20°B and pH 3.5).

Samples were taken aseptically at 4 different stages of the fermentation, the beginning (day 3), the middle (day 5) and the end of fermentation (day 8) and at the end of the alcoholic fermentation (day 15). Several dilutions were made at every fermentation stage (10^{-4}, 10^{-5}, 10^{-6}) and their aliquots were spread onto agar plates of YEPD supplemented with chloramphenicol (100 mg/L). The plates were incubated at 28± 2°C. After 24 hr of incubation, 20 colonies on plates were randomly chosen from the highest dilution. Each colony was picked up by sterile tooth pick and suspended in 100 µl of sterile milli Q water in 0.2 ml sterile PCR tubes. The cell suspension were heated at 95°C for 10 min in thermal cycler. After centrifugation, 10 µl of above cell lysate was transferred into another 0.2 ml PCR tube and PCR reaction was performed in a final volume of 20 µl using the following amplification mixture.

Yeast cell lysate	- 10 µl
Sterile milli Q water	- 5.8 µl
10 X Taq buffer	- 2.0 µl
d NTPs (10mM)	- 1.0 µl
M 13 Primer (25 ρmol)	- 1.0 µl
Taq polymerase (2U)	- 0.2 µl
	- 20 µl

The temperature programme for PCR was as described in section 3.16. for M-13 primer.

All reactions were repeated twice duly including both positive and negative controls. After amplification, PCR tubes were centrifuged and 10 µl of the supernatant was loaded in 2%

(w/v) agarose gel by horizontal electrophoresis with TEB buffer. PCR –RAPD banding patterns were compared with the positive control of the 3 yeasts and the population dynamics was established.

3.18. Statistical analysis

Using the statistical packages AgRes, NTSys, SYSTAT and windows based MS-Excel statistical analysis was performed for variance of one factor and a multivariate analysis, cluster and principal component analysis.

CHAPTER IV
RESULTS

4.1. Isolation of the fermentative yeasts from various natural sources of the western zone of Tamil Nadu

One hundred and twenty two yeast strains were isolated from various samples collected from different places in the western zone of Tamil Nadu (Table 1 and Fig. 1). Only one predominant yeast colony based on its colony morphology in higher dilution (10^{-6}) from each fermented sample was selected and the isolates were named as KJSK (K.Jeyaram and S.Kannaiyan) with serial number based on the order of isolation. Purification was repeated thrice using traditional streaking on YEPD agar to get pure culture. Purified yeast isolates were maintained in agar slants at 4°C for further study and in glycerol stock for germplasm collection.

4.2. Screening of yeast isolates for desirable fermentation characteristics

The above 122 yeast isolates were screened for their oenological characters, enzymatic characters, stress tolerance, higher alcohol, esters, aldehydes, organic acids and glycerol production to select suitable yeast cultures with desirable characteristics for wine fermentation.

4.2.1. Oenological characterization of the native yeast isolates

All the above 122 yeast isolates were screened for their oenological character *viz.* initiation of fermentation, fermentation rate, fermentation vigour, titrable acidity, volatile acidity, final pH, residual sugar, fermentation purity, flocculent capacity, H_2S production, foam production, adherence to glass, autolytic capacity and bouquet in comparison with 3 commercial yeasts (*S. ellipsoideus* CFTRI101 and MTCC180 and *S. cerevisiae* (baker's yeast)) (Table 2).

4.2.1.1. Time taken for initiation of fermentation by yeast isolates

It was found that the yeast isolate KJSK-100 was able to initiate fermentation with in 2.30 h after inoculation. Fifty six percentage of the yeast isolates were able to initiate with in 15 h after inoculation. The results also showed that 11.2% of the isolates initiated fermentation within 3-4 h, 6% within 4-5 h, 7.2% within 6-7 h., 1.6% within 7-8 h., 3.2% within 8-9 h., 4% within 9-10 h., 4% within 10-11%, 1.6% within 11-12 h., 0.8% within 12-13 h., 2.4% within 13-14 h. and 2.4% within 14-15 h. Fermentation initiation after 15 h. (44%) was not accounted.

4.2.1.2. pH change due to fermentation

Final pH was found to reduce from the initial level of 3.56. Yeast isolates KJSK-32 and 49 reduced the pH to less than 3.0. The pH reduction by other isolates (55.2%) was in the range of 3.4 to 3.5.

4.2.1.3. Utilization of sugars

The ability of the yeast isolates to utilize the sugars in the fermentation medium was studied by estimating the residual sugar. The residual sugar level was found to be negatively correlated with the fermentation vigour and fermentation rate. Higher residual sugar resulted in

lesser alcohol production. Yeast isolates KJSK-26 and 118 were not able to utilize higher level of sugars (less than 2.2 Brix sugar only utilized). However, some of the yeast isolates fully utilized all the available sugar leaving almost nil or very little residual sugar (KJSK-58 and KJSK-70 (0.0°B), KJSK-69, 106 and 112 (0.2 °B), KJSK- 90 and 100 (0.4 °B) and KJSK- 57, 110 and 114 (0.6 °B)).

4.2.1.4. Fermentation rate

Fermentation rate was directly correlated with ethanol yield. Among the isolates tested, 24.8% produced more than 3g CO_2day^{-1}, 4.8% produced 2-3g CO_2day^{-1}, 38.4% produced 1-2g CO_2day^{-1} and 32% produced less than 1g CO_2day^{-1} Yeast isolate KJSK-100 was found to posses maximum fermentation rate of 4.4g CO_2day^{-1} followed by KJSK-87 (3.9g CO_2day^{-1}) and KJSK-58 (3.87g CO_2day^{-1}).

4.2.1.5. Fermentation vigour

Out of the 125 isolates tested, 71.2% (89 out of 125) were able to produce less than 6% v/v alcohol (Fig.2). Only 8.8% were able to produce more than 10% v/v alcohol, whereas 8.8% of the isolates recorded 8-10% v/v and the remaining 11.2% registered 6-8% alcohol. The maximum fermentation vigour was found in KJSK –90 (11.83%) followed by KJSK-112 (11.80%), KJSK-106 (11.78%) and KJSK-70 (11.07%).

4.2.1.6. Titrable acidity

No correlation was found between titrable acidity and volatile acidity of the yeast isolates. Titrable acidity was found to be high in KJSK-93 (7.06 gL^{-1} of tartaric acid) followed by KJSK-71 (7.39 gL^{-1}), KJSK-83 (7.38 gL^{-1}), KJSK-46 and 65 (7.06 gL^{-1}) and KJSK-119 (7.05 gL^{-1}). Titrable acidity was found to be very low in KJSK-38 (3.55 gL^{-1}) followed by KJSK-68 (3.61 gL^{-1}). In 91.2% of the isolates, titrable acidity was in the range of 4-6.5 gL^{-1} of tartaric acid.

4.2.1.7. Volatile acidity and fermentation purity

Volatile acidity was found to be very high in KJSK-54 (1.2 gL^{-1}) and KJSK-22 (1.12 gL^{-1} acetic acid). Other isolates produced only less than 1 gL^{-1} of acetic acid. It was very low (<0.1 gL^{-1}) in yeast isolates KJSK-59 (0.04 gL^{-1}), KJSK-53 (0.05 gL^{-1}), KJSK-58

(0.08 gL^{-1}) and KJSK-36 (0.1 gL^{-1}). Fermentation purity of most isolates (53.6%) was below 0.1. It was found to be very low in KJSK-53 (0.010) and KJSK-100 (0.019).

4.2.1.8. Flocculation capacity

Ninety thee yeast isolates out of the total 125 isolates (74.4%) were found to have flocculation capacity. Premature flocculation was noticed in most of the yeast isolates including commercial strains.

4.2.1.9. H_2S production

Production of H_2S was scored in 51.2% of total isolates. Among then, 14.4% darkened the filter paper strips heavily due to more H_2S production (Plate.1). Even industrial strains also produced more H_2S.

4.2.1.10. Foam production and adherence to glass

Out of 125 strains, 84% were found to produce less than 4 mm height foaming. High foaming is positively correlated with high fermentation rate (Plate. 2). Adherence to glass was visible in 23.2% of (29 out of 125) yeast isolates predominantly top yeasts.

4.2.1.11. Autolytic capacity

Most of the yeast isolates (93.6%) were autolyzed in synthetic wine with 10% v/v alcohol. Few isolates (KJSK-11, 70, 90, 96, 99, 110, 112, 114 and 121) were found to be resistant to autolysis.

4.2.1.12. Bouquet

Desirable bouquet was sensed only in 12% of the yeast fermented must (yeast isolates KJSK-11, 37, 57, 58, 69, 87, 96, 97, 99, 104, 106, 110, 112, 121 and *S. ellipsoideus* CFTRI 101). Desirable bouquet was found to have a direct relationship with higher alcohol production.

4.2.1.13. Grouping of yeast isolates based on oenological properties

The fermentation vigour and fermentation rate was found to vary widely among the yeast isolates. Fermentation vigour was positively correlated with fermentation rate. All the 125 yeast

isolates were grouped into 4 groups based on these two parameters (Fig. 3). Group A (49 isolates) was found to produce 0.4% v/v ethanol with weight loss of 1.5 - 4 g CO_2, Group B (43 isolates) 3-7% v/v ethanol with weight of 4-6 g CO_2. Group C (17 isolates) 6-9% v/v ethanol with weight loss of 8-10 g CO_2 and Group D (16 isolates) 9-12% v/v alcohol with weight loss of 10-12 g CO_2. Among the yeast isolates from the four different groups, twenty four isolates (belonging to the group C and D) with best oenological properties were selected for morphological characterization and further screening along with *S. ellipsoideus* CFTRI 101 and MTCC 180.

4.2.2. Identification of the selected yeast isolates

Twenty four yeast isolates, selected by the preliminary screening as detailed above were identified based on morphological and physiological characters using the identification keys of Kergar-Van Rij (1984). Colony morphology cell morphology, reproduction, pseudomycelium formation and sporulation pattern were studied (Table 3) (Plate. 3, 4, 5,6,7,8,9 and 10). Standard culture of *S. ellipsoideus* CFTRI-101 was used for comparison. From Table 4, it is obvious that all the 24 isolates and the standard strain *S. ellipsoideus* CFTRI-101 were able to ferment glucose and sucrose. But, none was able to ferment lactose. Most of them (except thee) were able to ferment raffinose and more than 50% (13) were able to ferment maltose. Galactose was ferementd by only 11 out of 25 yeast isolates studied. In carbon assimilation test (Table 5), none of the isolates was able to assimilate L-rhamnose. Most of them were found to assimilate sucrose, maltose, galactose, raffinose and mannitol. Assimilation of cellulose, lactose, starch, erythitol, ribitol, inositol and citric acid was found to be rare characters. Yeast isolates KJSK-11, 69 and 95 were able to utilize only nitrate form of nitrogen. Growth at 37°C was observed in KJSK-11, 49, 58, 69, 70, 87, 95, 101 and *S. ellipsoideus* CFTRI. 101. Based on the above morphological and physiological characters, using identification keys, selected twenty five were compared with yeast isolates with already well characterized yeast species, out of which 22 yeast isolates were identified (Table 6). However, two yeast isolates (KJSK 95 and 114) could not be compared with any one of the already given standard descriptions and hence designated as unknown.

4.2.3. Genetic diversity of wine yeasts

A new protocol for yeast total genomic DNA isolation, independent of cell wall composition with high intact DNA recovery was standardized. Using this protocol, 2 μ circular plasmid and dsRNA (responsible for killer toxin production) were also recovered. (Plate 11 & 12). Totally 26 yeast isolates (24 selected wine yeasts +2 type strains) were studied for their polymorphism at molecular level. The electrophoretic profile of yeast isolates generated by RAPD-PCR by using primer ITS, M13 and RF2 is shown in (Plates 13, 14 and 15). The primers M13 and RF2 established differences at general level. But species level difference was noticed in ITS primer. From the total of 10 random primer studied, two primers (OPA 4 and OPA 14) were not amplified well. Six primers (OPA1, OPA2, OPA18, OPA20, OPD2, OPD16) did not show any polymorphism within *Saccharomyces* and non-*Saccharomyces* yeast isolates. OPD 18 was found to establish strain level differences in *Saccharomyces* sp. OPD 5 was found to be highly polymorphic to non-*Saccharomyces* sp (Plate 16 and 17).

From the electrophoretic banding pattern, cluster analysis was carried out using Jaccard similarity co-efficient. From the result (Fig. 4) it was found that all *Saccharomyces* sp. except *S. cerevisiae* KJSK-99 and *S. bayanus* KJSK-105 were clustered together with more than 72% similarity. *S. cerevisiae* KJSK-87 and *S. cerevisiae* KJSK-96 were found to be similar by 100%. Two *Hansenula* sp. along with yeast isolate KJSK-95 were clustered together showing 75% similarity. *Saccharomyces* and *Hansenula* were found to have only 32% similarity. Five *Candida* sp. were clustered with only 27% similarity. *C. stellata* KJSK-70 and 112 were grouped together with 94% similarity. Other non-*Saccharomyces* yeast isolates were found to be dissimilar (less than 10% similarity).

4.2.4. Fermentation kinetics of the selected yeast isolates

Selected yeast isolates (24) along with *S. ellipsoideus* CFTRI 101, *S. ellipsoideus* MTCC 180 and *S. cerevisiae* (baker's yeast) were studied for their fermentation kinetics. From Table 7, it is evident that fermentation time was found to be very low (48 h) for *S. cerevisiae* KJSK-96 and *S. bayanus* KJSK-100, 49 h for *S. ellipsoideus* KJSK-106. But *Hansenula* sp KJSK-11 and *S. capensis* KJSK-97 required 102 h for fermentation. *S. ellipsoideus* CFTRI 101 recorded the maximum of 113 h.

When the residual sugar was found to be negatively correlated with ethanol production, the sugar utilized was positively correlated with ethanol production. *S. cerevisiae* KJSK-96 and *C.maynoliae* KJSK-58 were able to utilize 188.1 g and 187.7 g of sugar respectively in the medium with a sugar concentration of 200 g/L.

Ethanol production was found to be maximum in *H. anomala* KJSK-69 followed by *S. capensis* KJSK-110. Thirteen out of 27 isolates (48.15%) were able to produce more than 10% v/v ethanol. Ethanol yield (mlg^{-1} of sugar) was found to be maximum in *K. marxianus* KJSK-49 (0.659 mlg^{-1}) followed by *C. intermedia* KJSK –90 (0.645 mlg^{-1}), *S. ellipsoideus* KJSK-106 (0.639 mlg^{-1}) and *H. anomala* KJSK-69 (0.607 mlg^{-1}).

Uniform rate of fermentation was not recorded in most of the yeasts during fermentation. Very less variation in fermentation rate was noticed in *H. anomala* KJSK-69 (0.112 –0.142 g CO_2h^{-1}) and *S. bayanus* KJSK-100 (0.100 –0.137 g CO_2h^{-1}). In some cases, sudden stop in fermentation was also noticed (*C. intermedia* KJSK-90, Yeast isolate KJSK-95 and *T. delbruechii* KJSK-121). As observed earlier, fermentation rate was positively correlated with ethanol production and *S. bayanus* was found to produce more CO_2/day (4.4g CO_2day^{-1}).

No correlation between biomass and ethanol production was observed. *C. stellata* KJSK-70 was able to produce 10.65% v/v ethanol from 1.668 gL^{-1} of biomass. However, *S. bayanus* KJSK-100 produced 10.55% v/v ethanol from 4.206gL^{-1} biomass. Some correlation was noticed between the specific rate of growth and ethanol productivity. *S. cerevisiae* KJSK-87 was found to give an ethanol productivity of

2.088 $mlL^{-1}h^{-1}$ with 0.05 h^{-1} specific growth rate, *S. cerevisiae* KJSK-96 recorded 2.154 $mlL^{-1}h^{-1}$ with 0.073 h^{-1} specific growth rate and *S. bayanus* KJSK-100 registered 2.197 $mlL^{-1}h^{-1}$ with 0.088 h^{-1} specific growth rate.

All the 27 yeast isolates tested were grouped using Euclidean coefficient (Fig. 5). From the dendrogram, 4 major groups were formed with Euclidean coefficient less than 20. Group 1 differed from group 2 at Euclidean coefficient of 27.4 and Group 3 and 4 differed at Euclidean coefficient of 24.2. But Group 1, 2 and Group 3, 4 were differed at Euclidean coefficient of

40.7. The strains belonging to the same cluster were similar to each other and statistically different from strains in the other clusters. Kinetic variables contributed to the integration of strains in each group are alcohol content, fermentation time, ethanol productivity and specific growth rate. For eg. *S. cerevisiae* KJSK-96 and *C. stellata* KJSK-112 produced 10.63% and 10.06% v/v ethanol respectively, but were included in different groups of 4 and 1. Strains of these groups differed in fermentation time i.e. 48-53 h for group 4 and 95-103 h for group1. Group 4 (*S. cerevisiae* KJSK-57, 87, 96, *S. bayanus* KJSK-100, *H. anomala* KJSK-69, *C. maynoliae* KJSK-58 and yeast isolate KJSK-114) was found to contain highly efficient yeast isolates with very good fermentation kinetics. Industrial strains *S. ellipsoideus* MTCC 180 and CFTRI 101 were grouped into separate group (Group 2). *S. cerevisiae* KJSK-96 and *S. bayanus* KJSK-100 were found to be on par in their growth kinetics.

4.2.5. Osmotic tolerance of the selected yeast isolates

Visible scoring of gas production in Durham's tubes indicated that most of the yeast isolates were able to grow upto 40% sugar concentration (Table 8). The maximum level of osmotolerance was found in *S. cerevisiae* KJSK-96 (upto 70%) followed by *S. cerevisiae* KJSK-37 and *K. marxianus* KJSK-49 (upto 60%). *S. bayanus* KJSK-105, *S. ludwigi* KJSK-108, *C. stellata* KJSK-112 and yeast isolate KJSK-114 were not able to grow when the sugar level is more than 40%. Thee osmotolerant (*S. cerevisiae* KJSK-37, *K. marxianus* KJSK-49 and *S. cerevisiae* KJSK-96) and one sensitive (*S. bayanus* KJSK-105) along with standard *S. ellipsoideus* CFTRI 101 were used for more detailed study for the osmotolerance based on fermentation rate (weight loss due to CO_2 production) and ethanol yield (Table 9). Even though very strong growth was noticed in 40% sugar level, increased fermentation time and reduced alcohol production was noticed in all screened osmotolerant except *S. cerevisiae* KJSK-96. Higher sugar level reduced the weight loss during the fermentation (Fig. 6) and reduced the alcohol production. Very high sugar (60-70°B) levels strongly influenced the alcohol production. The residual sugar and alcohol production was negatively correlated (Fig. 7).

4.2.6. Temperature tolerance of the selected yeast isolates

From Table 10, it was found that the temperature of 25-30°C to be optimum for growth of all the yeast isolates. *S. cerevisiae* KJSK-37, 57, 96, *H. anomala* KJSK-69, *C. intermedia* KJSK-90 and *S. bayanus* KJSK-100 were able to grow at a temperature upto 10°C. Six thermotolerant yeast isolates (*K. marxianus* KJSK-49, *K. thermotolerans* KJSK-111, *C. maynoliae* KJSK-58, *S. cerevisiae* KJSK-87 and *S. ellipsoideus* KJSK-106) were able to tolerate upto 40°C. Based on the preliminary result, 2 psychotolerant (*H. anomala* KJSK-69 and *S. bayanus* KJSK-100) and 2 thermotolerant (*S. cerevisiae* KJSK-87 and *K. thermotolerans* KJSK-111) were selected and used for the detailed study along with standard *S. ellipsoideus* CFTRI 101. From table. 11, it was observed that significant increase in alcohol content was found at low temperature upto 20°C, in spite of reduced weight loss (g of CO_2 production). However, at higher temperature (35°C) sudden reduction in both ethanol and fermentation rate was noticed. Only *K. thermotolerans* KJSK-111 performed better at higher temperature (Fig. 8). But it was failed to grow at 15°C. Only psychrotolerant yeast isolates performed well at low temperatures. Tolerance to a wide range of temperature (15°C –40°C) was found in *K. marxianus* KJSK-49, *C. maynoliae* KJSK-58, *S. cerevisiae* KJSK-87, *S. ellipsoideus* KJSK-106 and *S. ellipsoideus* MTCC-180.

4.2.7. pH tolerance of the selected yeast isolates

From Table 12, it was observed that all yeast isolates tested were found to tolerate the acidic pH range of 5.0 to 3.0. Some were able to grow even upto pH 2.0 (*C. oleophila* KJSK-27, *K. marxianus* KJSK-49 and *C. intermedia* KJSK-90). Detailed study of the selected yeast isolates along with standard (Table 13) indicated no significant variation in fermentation characters in the pH range of 5.0 –3.0. *C. intermedia* KJSK-90 was able to perform well even at pH 2.0. The final pH was reduced from the initial level to a pH of around 3.5. No significant difference in ethanol yield and fermentation rate was noticed between pH 5.0 and 3.5.

4.2.8. Alcohol tolerance of the selected yeast isolates

All yeast isolates studied were able to tolerate higher levels of alcohol upto 8% v/v ethanol concentration (Table 14). *S. cerevisiae* KJSK-96 *S. cerevisiae* KJSK-37 and *C. intermedia* KJSK-90 were able to tolerate upto 16% v/v alcohol. *S. bayanus* KJSK-100, 105, *S.*

ellipsoideus KJSK-101, CFTRI 101, *S. capensis* KJSK-110, *C. stellata* KJSK-112 and yeast isolate KJKS-114 were found susceptible to more than 8% v/v alcohol.

4.2.9. SO_2 tolerance of the selected yeast isolates

All yeast isolates studied, except *C. maynoliae* KJSK-58, were found to tolerate upto 100 ppm SO_2 level (Table 15). Very strong tolerance to SO_2 was noticed in *H. anomala* KJSK-69, *C. stellata* KJSK-70, *S. cerevisiae* KJSK-87 and *C. intermedia* KJSK-90. Only two yeast isolates (*H. anomala* KJSK-69 and *C. intermedia* KJSK-90) were able to tolerate upto a maximum of 300 ppm SO_2.

4.2.10. Production of extra cellular hydrolytic enzymes by the selected yeast isolates

From the Table 16, it was understood that the non-*Saccharomyces* yeasts were found to be the major source of hydrolytic enzymes (Plate.18). *H. anomala* KJSK-69 was found to produce most of the enzymes (polygalacturanase, cellulase, amylase and β -glucosidase). Polygalacturanase, cellulase and amylase were found to be rare enzymes in *Saccharomyces.* Protease enzyme was produced mostly by *Candida* sp. (*C. oleophila* KJSK-29, *C. maynoliae* KJSK-58, *C. stellata* KJSK-70, *C. intermedia* KJSK-90 and *C. stellata* KJSK-112). Esterase and β-glucosidase were found to be produced rarely by both *Saccharomyces* and non-*Saccharomyces.*

4.2.11. Killer toxin production by the selected yeast isolates

Based on interaction among the 26 yeast isolates, five major killer toxin producers were selected and their resistant / sensitive interaction with other yeast isolates were tabulated (Table 17) (Plate 19). *S. cerevisiae* KJSK-57 was found to produce killer toxin against 92% of the yeasts used with broad host range followed by *H. anomala* KJKS-69 with 68%. The percentage of yeasts killed was less than 50% in *Hansenula* sp. KJSK-11 (44%), *S. ellipsoideus* KJSK-106 (40%) and *Candida intermedia* KJSK-90 (32%). Interestingly, all five killer toxin producers were having broad host range not specific to single genus. The genetic basis of killer toxin production was found to be dsRNA for *S. cerevisiae* KJSK-57 and *C. intermedia* KJSK-90, based on dsRNA recovered during total genomic DNA isolation. No plasmid DNA was recovered during total DNA isolation for *H. anomala* KJSK-69 and *Hansenula* sp. KJSK-11. The

results suggested that the genetic basis was related with chomosomal DNA. However, *S. ellipsoideus* KJSK-106 did not produce dsRNA, but produced only 2μ plasmid, so the genetic origin was not confirmed.

4.2.12. Higher alcohols, esters and aldehydes production by the selected yeast isolates

From GC analysis (Fig 9 and Table 18), it was confirmed that isoamyl alcohol was the major contributor for higher alcohols. Higher amount of n-propanol was found to be produced (29.25 to 58.21 mg/L) by *Candida* sp. than *Saccharomyces* sp (0 to 24.85 mg/L). Six yeast isolates (*S. cerevisiae* KJSK-37, *S. capensis* KJSK-97, *S. ellipsoideus* KJSK-101 and MTCC 180, *Candida stellata* KJSK-70 and *C. intermedia* KJSK-90) were found to produce methanol. Isopropanol was found to be a rare higher alcohol produced.*S. capensis* KJSK-97 was found to produce less amount of (22.43 mg/L) higher alcohols. Total higher alcohol production was found to be more than 100 mg/L in *H. anomala* KJSK-69, *Candida stellata* KJSK-70, 112, *C. intermedia* KJSK-90, *S. cerevisiae* KJSK-99, *S. bayanus* KJSK-100, 105, *S. ellipsoideus* KJSK-101, 106, *K. thermotolerans* KJSK-111 and yeast isolate KJSK-114. Acetaldehyde production ranged from 0-195 mg/L. *S. ellipsoideus* KJSK-106 was found to be the maximum acetaldehyde producer (195 mg/L). Ethyl acetate production by the various yeast isolates ranged from 5 to 15 mg/L.

4.2.13. Organic acids and glycerol production by the yeast isolates during wine fermentation

HPLC analysis showed, no significant change in malic acid (6.03 g/L), tartaric acid (4.72 g/L) and citric acid (0.63 g/L) levels. The amount of succinic acid and acetic acid produced in the fermented must differed with the yeast isolate inoculated (Fig 10 and Table 19). *Candida stellata* KJSK-112 and KJSK-70 were found to produce maximum amount of succinic acid (1.16 and 1.32 g/L respectively). Acetic acid production capacity was found to vary with *Saccharomyces* sp. A maximum of 1.09 g/L was produced by *S. cerevisiae* KJSK-87 followed by *S. cerevisiae* KJSK-37 (1.07 g/L). In general, glycerol production ranged from 7-10 g/L. Thee species of *Candida viz.,* (*C. oleophila* KJSK-29, *C. maynoliae* KJSK-58 and *C. stellata* KJSK-70) were able to produce more than 10 g/L of glycerol. *Torulaspora delbrueckii* KJSK-121 was found to be the lowest (3.37 g/L) producer of glycerol followed by yeast isolate KJSK-95(4.94 g/L).

A principal component analysis (PCA) was carried out using volatile compounds, organic acids, glycerol produced by the preliminarily selected yeast isolates along with *S. ellipsoideus* CFTRI 101 and MTCC 180. Thee principal components (PC1, PC2 and PC3) with more than 10% variance were listed and based on the principal component scores, the yeast isolates were plotted in the graph (Fig. 11). From the graph, it was noticed that most of the *Candida* sp. and *Saccharomyces* sp. were separately grouped, *Saccharomyces bayanus* KJSK-105 and *S. cerevisiae* KJSK-37 were found to be highly deviating from the major groups in both interactions. Type strain *S. ellipsoideus* CFTRI-101 was found to be grouped with the major *Saccharomyces* group.

4.2.14. Selection of wine yeasts based on exclusion tests

Based on oenological characters, stress tolerance, kilter toxin production, hydrolytic enzyme production and other characters listed in section 3.13, the yeasts with undesirable characters were excluded and five efficient wine yeasts were selected (Fig. 12).

4.2.15. Cluster analysis of the yeast isolates

To find out whether the 122 yeast isolates could be grouped by their oenological properties, a cluster analysis was carried out with 7 principal oenological characters (section 3.14). Further clustering was also done by adding other oenological and enzymatic characters (25 characters). The results are shown in Fig. 13 and 14. Here, 4 large groups of yeast isolates were grouped with Euclidean co-efficient (1a, 1b, 2a and 2b).The yeast isolates which belong to the same cluster were found similar to each other and statistically different from the yeast isolates of other clusters. The oenological variables that contributed to the integration of the yeast isolates in each groups were given below. Yeast isolates with less fermentative ability were grouped into 2 and high fermentative yeast isolates were grouped into 1. Group 2a and 2b were separated based on the variable H_2S production. The major variables, which differentiated 1a and 1b, were found to be the tolerance to ethanol (93% non tolerant to 12% ethanol) and H_2S production (50% H_2S producers).

Cluster analysis by 25 oenological and enzymatic characters were found to increase the Euclidean distance during grouping (3.83 to 4.7). The members of the groups were not changed, but only the grouping distance varied. In second type of grouping, *H. anomala* KJSK-69 was grouped separately in 1a cluster with Euclidean distance of 4.6. But in clustering based on 7 major oenological character grouped with Euclidean distance of 1.7, *S. bayanus* KJSK-100, which was selected in exclusion test, was found to be grouped into 1b because of its less alcohol tolerance.

The group 1a (12 yeast isolates) was found to be oenologically superior than other groups. From this cluster analysis, it was found that the major variables which should be considered during wine yeast selection were fermentation rate, H_2S production and ethanol tolerance.

4.3. Studies on the suitability of commercial varieties of banana, papaya and grapes for fruit wine making

4.3.1. Selection of banana variety for wine making

In banana varieties, the total soluble solids (TSS) level was found to be in the range of 12 to 18°B (Table 20a). The pH level was found to be in the range of 4.0 to 5.0. The maximum TSS was observed in Nendran followed by Rasthali and Matti. Red banana and Nendran were found to be less acidic (pH more than 5.0). The wine recovery in Poovan and Karpooravalli was found to be high (more than 70% v/v) followed by Robusta and Peyan.

The data on banana wine composition (Table 20b) revealed that alcohol content was found to be in the range of 6-8% v/v. There was no relationship between titrable acidity and volatile acidity. Red banana wine was found to be having a titrable acidity of 1.49 g/100ml and produced 0.126 g/100ml of volatile acidity. But Karporavalli wine was found to be having titrable acidity of 1.16 g/100ml and produced 0.06 g/100ml volatile acidity only. The residual sugar level ranged from 0.2 to more than 2.0°B. (2.2 °B for Rasthali and 2.4 °B for Nendran). The final pH was found to be slightly reduced from the initial level. No significant difference in reducing sugar level was noticed. Tannin was found to be high in Matti, Red banana, Robusta and Rasthali wines and low in Nendaran and Karpooravalli wines. The organoleptic scores were found to be high for Robusta (17.50) followed by Rasthali (16.75).

4.3.2. Selection of papaya variety for wine making

In papaya varieties, the TSS level was found to range from 10.8 to 12.8 and the pH ranged from 3.8 to 4.2 (Table 21a). Wine recovery was found to be high in CO 7 wine (66.0% v/v) followed by CO 1 (62.3% v/v) and CO 2 (62.7% v/v). Papaya wines (Table 21b) were found to yield 5-7% v/v of ethanol. The titrable acidity of CO 1, CO 2 and CO 3 was found to be less than the others. Residual sugar level was high in CO 4 (5.2°B). Tannin content was comparatively low (400-800 mg/L) than the other fruit wines. The wine colour was found to be independent of pulp colour i.e. most of the carotene's were not extracted well. The organoleptic score indicated that CO 2, CO 3 and CO 7 wine were found to be acceptable.

4.3.3. Selection of grape variety for wine making

In grape varieties, Bangalore blue and Sharad were found to be have more than 18°B sugar. The pH level of most the varieties were found to be acidic (pH 3.8 –3.2). Wine recovery (% v/v) was found to be very high compared to other fruits (Table 22a). Among the wines produced from different varieties of grapes, Bangalore blue was found to be the best with 18.50 score (Table 22b). Alcohol content was high in Sharad wine (8.56% v/v) followed by Bangalore blue (8.23% v/v). The reducing sugar level of grapes was found to be very high (2-9 gL^{-1}) compared to other fruits. Tannin level was also comparatively higher (1000-2000 mgL^{-1}) than other fruit wines. It was found to be low in Muscat wine (972 mgL^{-1}) and very high in Sharad (2122 mgL^{-1}).

In the suitability studies of different commercial varieties of banana, papaya and grapes for wine production, the local availability and fruit yield were also taken into consideration along with biochemical data and organoleptic score for selection.The results clearly indicated that the varieties *viz.,* Robusta, CO 2 and Muscat were found to be more suitable for banana, papaya and grape wine production respectively.

4.4. Standardization of fermentation conditions for fruit wine making

4.4.1. Effect of TSS level on fruit wine production

Increasing TSS level was found to yield increased alcohol production in banana wine production (Table 23). Significant difference in alcohol production was noticed with different yeast strains. No significant difference in residual sugar level and pH was found upto 20°B level. At higher sugar levels, the residual sugar was found to be increased and the pH reduced

slightly. Tannin content was found to be increase with increased alcohol yield. It was found to be high at 24°B (average of 1409 mgL^{-1}). The organoleptic score was found to be maximum in sugar level of 24°B. Volatile acidity was increased markedly at 26°B sugar level.

In papaya wine standardization (Table 24) also increased alcohol production due to increased sugar level was noticed. Sudden increase in residual sugar level, volatile acidity and decrease in pH level was found in the sugar level of more than 24 °B. Here also tannin content increased with increased alcohol production. The organoleptic score was found to be maximum at 24°B sugar level.

In grape wine (Table 25), increased alcohol production upto 24°B was noticed. At 26°B, both *S. cerevisiae* KJSK-96 and *S. bayanus* KJSK-100 were found to show increasing trend in ethanol production and volatile acidity increased after 22°B level. The pH level was also suddenly reduced after 22°B. Tannin content was found to be increased with alcohol produced upto 4000 mgL^{-1}. From organoleptic score, 22°B was found be the optimum for grape wine production.

The optimized TSS level for banana wine from Robusta and papaya wine from CO 2 was 24°B. For grape wine from Muscat, it was optimized as 22°B level.

4.4.2. Effect of pH on fruit wine production

In banana wine standardization, upto pH 3.5, no significant change in alcohol production, titrable acidity, TSS, tannin content was noticed (Table. 26). The pH values of fruit wines were found to be reduced from the adjusted values. No change in reducing sugar level was noticed. From the organoleptic score, pH 4.0 and pH 3.5 were found to yield good banana wine. But during aging, browning of banana wine was noticed in wine produced at pH 4.0 but not in pH 3.5 (Plates 20 and 21).

Difference in wine recovery between different levels of sugar and pH was observed in banana pulp fermentation. The interaction between the sugar amelioration and acidification on banana wine recovery was tabulated (Table 27) and found that 24°B and pH 3.5 yielded the maximum banana wine. During sugar amelioration at 24°B, the banana pomace was precipitated and during acidification at pH 3.5, the pomace was clumped together and the fermented juice

was pushed out from the pulp. Interestingly, precipitation of pectin along with pomace during banana pulp fermentation was also observed as white creamy patches (Plates 22-29)

In papaya wine standardization (Table 28), upto pH 3.5, no significant change in alcohol production, tannin content, residual sugar, volatile acidity was recorded. But above pH 3.5, the volatile acidity, titrable acidity and residual sugar were found to be increased significantly. pH 3.5 was found to give the acceptable organoleptic score. Based on the results of this study, pH level for banana wine and papaya wine was optimized as pH 3.5 (Plates 30 & 31).

4.4.3. Effect of different acidifiers on fruit wine making

Acidification by natural acid fruit products like lime juice and tamarind pulp were tried along with commonly used citric acid. In banana wine standardization (Table 29), tamarind pulp acidification reduced the alcohol yield (6-8% v/v), left more residual sugar (10°BTSS), tannin and recorded very poor organoleptic score (approximately 10 points out of 20) for both banana and papaya fermentation. Acidification by citric acid and lime juice was found to be optimum for banana wine fermentation. Lime juice addition resulted in very good flavour addition apart from acidification and scored more than 18 points in organoleptic evaluation. But in papaya wine (Table 30), lime juice acidification was found to give negative impact on flavour. Based on the results lime juice (or) citric acid for banana pulp acidification (upto pH 3.5) and citric acid for papaya pulp acidification were optimized.

4.4.4. Effect of low temperature on fruit wine making

A general increase in alcohol production during fermentation at 20°C compared to room temperature was noticed in most of the yeast cultures and in all fermented fruits (Tables 31, 32 and 33). *S. ellipsoideus* MTCC 180 was found to be highly sensitive to low temperature. At 15°C fermentation, the reduced alcohol production and increased volatile acidity with no change in titrable acidity was observed. The residual unutilized sugar level was found to be increased at 15°C fermentation. No major change in tannin content was noticed during fermentation at 20°C and room temperature. Organoleptic score was also not statistically significant in most of the cultures

during fermentation at 20°C and room temperature. But an excellent fruit flavour improvement was sensed at low temperature (20°C) fermentation.

4.5.1. Separation of anthocyanin pigments in the fruit wine by TLC

Using upper phase of butanol acetic acid water (BAW), thee major pigments were separated by TLC. Based on spectral characteristics (Table 34) *viz.*, colour in visible light and UV light, Rf values and absorption maxima in both UV and visible range, the pigments were identified as malvidin-glycoside complexes (Mv-3G, red purple), peonidin (Peo, magenta) and malvidin (Mv, purple) (Plate.32).

4.5.2. Changes in anthocyanin pigments during aging

From the Muscat grape skin anthocyanin composition, it was found that the malvidin was the major colour compound in grapes skin (Table 35). But during wine production, only 45% of Mv-3G, 19.2% Peo and 36.7% Mv was released from skin to wine. The major contributors to wine colour were found to be Mv-3G and Mv. In no skin contact time (W0), some quantities of Mv-3G was found to be released. During aging Mv-3G reduced to 30% level. Peo was found to be reduced to 12.8% and Mv was reduced to 69.5%. During aging, Mv was found to be more stable than other pigments. HPLC analysis of the pigments, eluted from TLC, expressed the intactness of the major pigment Mv-3G complex and during aging the reduced Mv-3G was also detected (Fig. 15).

Treatments for more colour extraction (SC_2 to SC_8) facilitated more peonidin extraction. But during heating (H_{10} –H_{50}), peonidin was destroyed. Increased skin contact time resulted in the slight increase in Mv-3G and Mv. But during heat treatment for 10 minutes, more than 72% increase in Mv-3G was found (Fig. 16). But more than 10 min. (H_{20} –H_{50}), the increase was found to be comparatively low. Malvidin level was found to be increased to 40.4% during heating for 10 min. During aging (3 month), the extracted colour was retained up to 52.5%. The colour retention in heat extraction (H_{10}) after aging (0.38 Mv 3G: 0.08 Peo: 0.54 Mv) was found to be comparable to fresh wine without any treatment (0.42 Mv 3G: 0.17 Peo: 0.48Mv) (Plate. 33).

4.5.3. Effect of skin contact time on colour extraction

From the result (Table. 36), it was noticed that yeast isolates used for wine production were also found to be differed in the colour extraction ability. With 2 days skin contact time, yeast isolates *S. cerevisiae* KJSK-96 (Y_3) and *S. ellipsoideus* KJSK-106 (Y_5) were found to extract more colour (more than 0.2 OD at 420 nm). Brightness was found to be increased with increase skin contact time. The hue value was well balanced during increased skin contact. But tannin content was found to be increased up to three fold from 651 to 1815 mgL^{-1}.

4.5.4. Effect of heating time on colour extraction

In this study also, the difference in colour extraction by different yeast isolates was noticed (Table 37). More than 78% increase in colour extraction was observed in 10 min (Fig. 17) heat treatment (0.5 to 0.73 OD at 520nm). But with more than 10 min. heat treatment, the increase in colour extraction was found to be very low (for 10 min. to 30 min only 5.3% increase in brightness noticed)(Plate 34). Tannin content was not increased as much as skin contact time (From 537 to 750 mgL^{-1} for 10 min heating and 1000 mgL^{-1} for 50 min heating).

4.5.5. Colour stability during aging in Muscat grape wine

Increased skin contact time was found to be related with increased hue (0.8 to 1.2) during aging. In increased heat treatment, the hue value was maintained up to 6 months. During organoleptic evaluation, increased skin contact time scored less than the increased heating time. The maximum score was found to be recorded by the wine obtained with the fermentation of the must exposed to treatment for 10 min (Table. 38).

4.6. Yeast population dynamics during co-inoculation in grape and banana wine fermentation

During grape and banana wine fermentation, development of native yeast (contaminants) was noticed even when pasteurized with 100ppm SO_2. To study their role in wine fermentation and to avoid these contaminants, thee yeast isolates with broad-spectrum killer toxin production were used. The killing percentage of these isolates against yeast isolates studied were 92% for *S. cerevisiae* KJSK-57, 68% for *H. anomala* KJSK-69 and 40% for *S. ellipsoideus* KJSK-106. Their population dynamics during wine fermentation upto 15 days and their effect on the native contaminants were studied using molecular marker technique. Distinct molecular markers were

developed for the above thee yeast isolates by PCR-RAPD polymorphism using primer M13 (Plate 35). The protocol for direct colony PCR was also standardized. Ten µl of yeast cell lysate (tooth pick swapped yeast cell biomass suspended with 100 µl distilled water and heated upto 95°C for 5 min in thermal cycler) was used for PCR amplification.

PCR-RAPD pattern for *S. cerevisiae* KJSK-57 was designated as 'A' type pattern, *H. anamola* KJSK-69 as 'B' type pattern and *S. ellipsoideus* KJSK-109 as 'C' type pattern. In uninoculated control 3 days after pasteurization, the development of contaminants was observed, which reached the maximum level on 8 days after pasteurization (13 $x10^6$ viable yeast cells ml^{-1}). It was found to be similar to C pattern *(Saccharomyces* genus specific pattern) indicated as '+' (Tables 39 and 40) and designated as 'C^{1}' which was contaminating the yeast belonging to the genus *Saccharomyces.* In the uninoculated control of banana pulp, contamination was detected after 5 days.

In general the population density build-up in grapes was found to be higher ($x10^6$ viable cell/ml) than banana fermentation ($x10^5$ viable cells/ml). When inoculated with *S. cerevisiae* KJSK-57 alone, a high density 'A' pattern population of $321x10^6$ was found on 8th day in grape wine fermentation and $47.45x10^5$ at 5^{th} day in banana wine fermentation. After that, slow decline of viable cells were noticed (Fig 18 and 19).

In *H. anomala* KJSK-69 inoculation, 'B' type and 'C' type pattern population build-up was found, which reached the maximum (B $147x10^6$; C^1 $100.5x10^6$) on 8^{th} day in grape must fermentation. This dominance of 'C^{1}' pattern indicates the build-up of uninoculated native *Saccharomyces* population during fermentation. This was also noticed in banana pulp fermentation but in lesser scale (B 51.6 $x10^6$: C^1 32.2 x 10^6). In *S. ellipsoideus* KJSK-106 inoculation, maximum contaminants were found on 5^{th} day (34.24 x 10^6 $cellsml^{-1}$). Most of the contaminants were found to be similar with the banding pattern of *Candida* sp. in diversity studies with M13 primer. 'C' type population pattern reached maximum on 8^{th} day (159.5 $x10^6$ $cells.ml^{-1}$). Similar case was also noticed in banana pulp fermentation but in reduced scale.

During co-inoculation of *S. cerevisiae* KJSK-57 and *H. anomala* KJSK-69, the contaminants were totally eliminated after 8^{th} day. The maximum population build-up of both was recorded on 8^{th} day (A $180x10^6$: B $70.95x10^6$).

In banana must fermentation; contaminants were only detected on 5^{th} day. Co-inoculation of *S. cerevisiae* KJSK-57 and *S. ellipsoideus* KJSK-106 resulted in very low population build-up in 'C' type pattern. (A $183x10^6$: C $14.85x10^6$) on 8^{th} day. After 8^{th} day, C type population reduced significantly. This was also found in banana must fermentation.

During co-inoculation of all the three, contaminants were totally eliminated after 8^{th} day in grape must fermentation and after 5^{th} day in banana pulp fermentation. A and B type population were found to be dominated, but C type population was fully eliminated after 8^{th} day in both grape wine (A $115.5x10^6$: B 55.9x106: C $0x10^6$) and banana wine (A $12.45x10^6$: B $13.75x10^6$: C $0x10^6$) fermentation.

4.7. Standardization of methodology for mini scale production of banana, papaya and grape wine

Based on above standardized fermentation conditions for banana wine (pH 3.5 using citric acid/ lime juice, TSS 24°B and temperature 20°C), papaya wine (pH 3.5 using citric acid, TSS 24°B and temperature 20°C) and grape wine (TSS 22°B, temperature 20°C, 2 days skin contact time and 10 min heating), working experience with fruit wine making and the knowledge of wine making from the review of literature, the overall methodology for fruit wine production was standardized (Plates. 36-41) (Fig. 20, 21 and 22). Activation of yeast starter culture was standardized at 42°C for 1 h 20 min for vigorous initiation of fermentation. The repeated mini scale fruit wine production in water siphoned 5 liter model and semi automated bioreactor ensured the reproducibility of the lab scale results in larger volumes (Plates 42-45). Banana wine production under semi solid-state fermenter model was found to give promising results for commercialization of this technology (Plates 46 and 47).

4.8. Composition of phenolic compounds in fruits wine

More than 47 peaks with different retention time (RT) were detected in HPLC analysis of phenolic compounds in fruit wines (Fig. 23,24 and 25.). Among this, 22 phenolic compounds were identified and quantified. Unknown peaks were named as peak A to Z and expressed as gallic acid equivalents. The total phenolic compounds composition in banana wine was found to be higher (348.08 mgL^{-1}) than other two wines (236.2 mgL^{-1} for grape

wine and 110.9 mgL^{-1} for papaya wine) (Table 41). In banana wine, more than 19 new peaks were detected which were not eluted in the other two wines.

The major phenolic compounds in banana wine were found to be syringic acid (73.8 mgL^{-1}), caffeic acid (34.58 mgL^{-1}) and unidentified peak G (44.22 mg gallic acid equivalent /L), which is not present in both papaya and grape wine (Fig. 26). In papaya wine, the major phenolic compounds detected were t-ferulolyl tartaric acid (22.62 mgL^{-1}), epicatechin (20.16 mgL^{-1}) and gallic acid (11.44 mgL^{-1}). Malvidin –3-0 glu was found to be the major phenolic compound in grape wine followed by gallic acid (35.64 mg/L) and epicatechin (11.20 mgL^{-1}). Gallic acid was found to be very low for banana wine (9.02 mgL^{-1}) than the other types (11.44 mgL^{-1}) for papaya wine and 35.64 mgL^{-1} for grape wine). P- coumaric acid (15.75 mgL^{-1}) and quercetin (21.75 mgL^{-1}) were found to be very high in banana wine than the others. Kampferol, t-caftaric acid and t-ferulolyl tartaric acid were not detected in banana wine. Epicatechin was found to be high (20.16 mgL^{-1}) for papaya wine. *trans*-resveratrol was detected only in grape wine (0.39 mgL^{-1}).

Total phenolic compounds were found to be increasing from 455 mgL^{-1} to 951 mgL^{-1} with increase in contact time from 2 days skin contact time to 8 days skin contact time (Table 42). During heating more phenolic compounds extraction was detected (455 mgL^{-1} for control to 1050 mgL^{-1}) for 50 min heating at 70°C. Catechin and cafferic acid level was found to be increasing due to increased skin contact time (Fig. 27). But during heating, the above two compounds denatured fully and the t-ferulolyl tartaric acid was found to be increasing with increase in the skin contact time but decreasing during heating. P-coumaric acid was found to be increasing with both increased skin contact time and heating (Fig. 28). Procyanin was not changed during both heating and increased skin contact time. Gallic acid level was found to be increasing heavily (37.2-105.7 mgL^{-1} for 8 days) due to increased skin contact time. But the increase was not significant during heating (37.2 –60 mgL^{-1} for 50 min heating.)

The major pigment malvidin–3-o-glu was found to be increasing from 350 mgL^{-1} to 706 mgL^{-1} during increased skin contact time. During heating sudden increase was noticed for 10 min (767.22 mgL^{-1}) followed by slight increase over a period of 50 min heating (Fig. 29). The other pigments delphinidin and cyanidin levels were found to be increasing during increased skin contact time. No major change was noticed in petunidin, peonidin, myrcetin and quercitin. *trans*

–Resveratrol level was found to be increasing from 0.41 mgL^{-1} to 3.65 mgL^{-1} during increased skin contact time. *trans*- Resveratrol, quercetin and isorhamnetin were found to be degraded during heating.

4.9. *trans* – Resveratrol content in fruit wine

HPLC analysis showed (Fig. 30) that the *trans*-resveratrol, an anticancer compound, was found to be at 0.667 mgL^{-1} in Muscat grape wine, trace in Robusta banana wine and not detected in CO 2 papaya wine (Table 43), when the treated samples for increased colour extraction in grape wine were analyzed for the *trans* –resveratrol content, it was found that increased skin contact time also increased the *trans* –resveratrol level in the grape wine. In the treatment SC_8 with 8 days skin contact time, a four-fold increase in *trans*–resveratrol content was recorded. But in heating, a heavy loss of *trans*–resveratrol level was observed. At H_{40} (70°C for 40 min), the above anticancer compound was found to be fully destroyed (Fig. 31). However, when the cluster stem was added to the must and subjected to alcoholic fermentation for 15 days, a drastic increase in the trans-resveratrol level from 0.67mgL^{-1} to 3.8 mgL^{-1} was noticed.

CHAPTER V
DISCUSSION

There has been a considerable increase in the production of fruits in India during the last few years and there are possibilities for its further increase. Most of the fruit growers and retailers are left with no option than to discard over ripened fruits when the production is surplus. Very few fruits are produced and marketed in India. Huge post harvest losses due to the perishable nature of banana, papaya and grape fruits call for a processing technology for the production of value added products from the surplus fruit. The production of fruit wines will help to utilize more quantities of fruits especially unmarketable surplus which otherwise go as a waste (Satyavati, *et al.*, 1972). Since the fruit wine industry has good export market potential, the industry will strengthen the economy of fruit growers and generate employment opportunities besides providing a health nourishing and stimulating drink. Highly efficient wine yeast will be a key player for high quality fruit wine production through fermentation. Isolation and selection of best wine yeast from various sources have been carried out by different researchers (Okafor, 1978 and Heard, 1999). Potential palm wine yeast germplasm of our country should be explored for their effective fermenting capacity and the best strain should be selected for quality fruit wine production (Opara *et al*., 1986, Okagbue, 1988, Uzochukwu *et al.*,1999 and Zarozoso *et al.*, 2000).

5.1. Wine yeast selection

Several authors have shown that using different starter culture and indigenous yeast, wine with significantly different chemical composition could be produced (Zoecklein *et al.,* 1997). Recent studies have clearly elucidated the impact of non-*Saccharomyces* yeast on the sensory characters of wine (Ciani and Picciotti, 1995). Hence, in the present investigation, fermentative yeasts were isolated from western parts of Tamil Nadu particularly from the base of Western Ghats (which is one of the biggest biodiversity centres in India).

There has been no simple and effective procedure for selecting wine yeasts for industrial use. There is a general agreement among experts that selected yeast should show low production

of volatile acidity, high tolerance to ethanol and high ethanol producing capacity according to the quantity of total fermentable sugars in the must, good fermentation rate, growth at high temperature, resistance to sulfur dioxide, low sulfur dioxide production, low hydrogen sulfide production, flocculation after fermentation, low foaming, killer phenotype, low acetaldehyde production, good glycerol production and limited production of higher alcohols (Tipper and Schmitt, 1991, Stratford, 1992 and Romano and Suzzi, 1993).

Among the various criteria used for selection, initiation of fermentation will give an idea about the activeness of fermentative yeast. Strains which take long time for fermentation are unfavourable, because most of them cannot ferment the sugar completely and as a result the wine produced are sweet. Also, longer time taken for fermentation is an indication of low growth rate. Residual sugar level in fermented must will give an idea about its sugar exhaustion ability i.e., the capacity to completely ferment the total amount of sugars present in the must (Thornton, 1991).

In the present study, one hundred and twenty two isolates were grouped into 4 groups and their positive relationship between the fermentation rate and ethanol yield were investigated based on the report of Atputharajah *et al.* 1989. The biochemical basis for this relationship is the mode of sugar break down by yeasts (Amerine *et al*,1980 and Boulton *et al.* 1996) as indicated below.

$$\underset{(100\%)}{C_6H_{12}O_6} \xrightarrow{\text{Yeast}} \underset{(50\%)}{C_2H_5OH} + \underset{(50\%)}{CO_2\uparrow}$$

Premature flocculation of yeast cells hampers the completion of fermentation (Bellal *et al.*, 1995), where as failure of the cells to flocculate at the end of fermentation process necessitates the use of expensive centrifugation or filtration to remove the cells. Most of the yeast isolates had a high fermentative rate, particularly the yeast, *Saccharomyces* sp. isolated from palmyra juice / toddy samples had very high fermentative capacity (alcohol production). On the other hand in the yeast isolates obtained from wild flowers, nectars and fruits, low fermentative non-*Saccharomyces* were predominating. (Low final pH is also an indication of higher acetic acid production by the yeast isolates).

A significantly positive relationship between the fermentation rate and the production of H_2S was observed. The strains with higher fermentation rate produced more H_2S (Iranzo *et al.*, 1998). Based on blackening of PbAcO impregnated paper, H_2S production was scored visually as low, medium and high. Very high level of variation in the intensity of blackening was noticed. Even commercial strains tested also produced more H_2S. Compared to *Saccharomyces*, non-*Saccharomyces* yeasts were found to produce less H_2S. Most of the yeast isolates, even commercial strains were also found to flocculate prematurely. A large number of reports have clearly explained the role of cell wall components in the formation of cellular flocs (Nishihara *et al.* 1987). It is suggested that flocculation at the end of fermentation is highly desirable.

Foaming is not only based on yeast isolates used but also mostly by the medium in which it grows. More vigorous fermentation (stuck fermentation) leads to more foaming. This stuck fermentation can be reduced by low temperature fermentation and low nitrogen availability. Most of the non-*Saccharomyces* yeasts were found to adhere with glasses because of its surface growth (top yeasts), which was not observed in bottom yeasts (*Saccharomyces*).

In the present investigation, most of the yeasts possess an autolytic capacity of 10% v/v alcohol in model wine but were able to produce more than 10% v/v alcohol in other conditions. This may be due to the sudden exposure of young yeast cells in model wine, compared to the adaptation of cells to gradual increase in alcohol during fermentation. This gives an idea about the cell wall tolerance (Martinez –Rodriquez and Polo, 2000) to alcohol but not to the real autolysis taking place after alcoholic fermentation. Alternatively, alcohol and acids in model wine might be having synergistically deleterious effect on cell wall.

Even though well-trained persons were not involved in our sensory evaluation the bouquet was evaluated in comparison with export quality red wine (Port wine). This was generally correlated with more ethanol production.

S. cerevisiae the principal wine yeast was not recognized as a significant producer of extra cellular enzymes, although a few strains have recently been reported to degrade polygalacturamic acid (Iranzo, *et al.*, 1998). This pectic enzyme degrade the pectin in the must,

increasing its clarification rate and preventing the disappearance of aesthetically important substrates such as anthocyanin and precursors of varietal characters. Nowadays, these enzymes are obtained on an industrial scale from *Aspergillus niger* and although they are purified, they always have a considerable amount of unwanted residual mycotoxic activity (Pariza and Foster, 1983 and Pariza and Johnson, 2001).

In the present study, most of the non-*Saccharomyces* isolates produced more hydrolytic enzymes than *Saccharomyces.* This result was supported by Charoenchai *et al.* 1997 and Sanchez *et al.* 1984. Polygalacturanase was found to be a rare enzyme in *Saccharomyces,* whereas *Hansenula anomala* KJSK-69 produced most of the hydrolytic enzymes including pectinase. Contrary to the above results, Kay (1988) concluded that more than 30% of wine yeast were capable of producing polygalacturanase enzyme. Bilinski *et al.* (1987) suggested that it was difficult for *Saccharomyces* strains to show their phenotype in the plate tests. Now-a-days scientists are using co-inoculation of non-*Saccharomyces* with *Saccharomyces* to see the effect on wine aroma. It is very difficult to find a yeast strain with both oenological and enzymatic characters (Iranzo *et al.,* 1998).

From the growth kinetic studies of the present investigation, no relation was found between the biomass yield and the ethanol production (Ciani and Picciotti, 1995). Fermentation rate was found to be positively correlated with ethanol yield. Instability in fermentation rate was noticed in most of the cultures. Ethanol yield and productivity will give an idea about the efficiency of sugar utilization and ethanol conversion. The highly efficient sugar converters can be used for industrial ethanol production (Cysewski and Wilke, 1978, Athanasiadi *et al.,* 2001).

Different yeast strains used, produced different combination of secondary metabolites (Mateo *et al.,* 1992). The volatile compounds of wine greatly varied due to the influence of yeast strain (Steger *et al.,* 2000 and Soles *et al.,* 1982). The acetate esters, aldehydes and acids of ethanol and the higher alcohols are often the volatiles providing the major aroma to the freshly fermented wine. Burning taste and an unpleasant aroma are the characteristics of isoamyl alcohol (Ciani and Picciotti, 1995). Isobutanol and n-propanol are less objectionable. These compounds, when present in wine at sensorial detection levels, have negative quality connotations. Isoamyl alcohol was found to be a key higher alcohol, which differed heavily in

the selected yeast isolates. Large amount of ethyl acetate and acetic acid produced by yeast isolates are considered undesirable for wine making. Most of the selected yeast isolates produced more higher alcohol than the type strains. The difference in organic acid production by yeast isolates might be due to the difference in the activities of alcohol and aldehyde dehydrogenases (Shimadza and Watanabe, 1981).

No variation in tartaric acid and malic acid production was noticed. Sponholz (1993) suggested that the tartaric acid which is not metabolized by yeast is the most stable organic acid found in wine. But, Rodriquez and Thorntion (1990) showed that yeast strains have the ability to ferment malic acid. However, in the present investigation, no variation in malic acid level was observed in the HPLC analysis. The reason might be that most of the deacidification capacity was linked with *Schizosaccharomyces* genus (Ethiraj and Suresh, 1978). Lactic acid production by some yeast isolates were also noticed which might be due to the lactic acid bacteria (Liu, 2002).

Quantitatively glycerol is the major end product of fermentation after ethanol and CO_2. Glycerol may contribute to the fullness, smoothness and body of wines (Eustace and Thornton, 1986). Significant differences were detected in the glycerol production by different wine yeasts. It has been suggested that the ability of different yeast strains to produce different yield of glycerol may be related to difference in the activity of glycerol 3- phosphate dehydrogenase (Michnick *et al.* 1997). Glycerol plays an important role in the organoleptic properties of wine. Due to the favorable impact of glycerol on wine quality, the benefits of increasing glycerol production to improve the sensory character of wine which is lacking in body have been emphasized (Rankine and Bridson,1971). Radler and Schulz (1982) differentiated *S. cerevisiae* species based on glycerol production. The amount of glycerol needed to produce a detectable increase in sweetness was 5.2 g/L. (Noble and Bursick, 1984). Most of the yeast strains selected in the present study were able to produce 7-9 g/L of glycerol.

Glycerol and ethanol together share for the sweetness and viscosity (Lubbers *et al.,* 2001). The results of the present investigation indicated that *Candida* sp was able to produce more glycerol than *Saccharomyces.* This is in conformity with the finding of Ciani and Piccioti (1995). Another aspect of glycerol production is its increased extent of osmolarity which leads to osmotic stress tolerance (Michnick *et al.,* 1997 and Myres *et al.,* 1997). A slight

increase in glycerol production in wine can be achieved by selecting yeast strain with high glycerol production and by optimizing fermentation condition.

5.2. Stress tolerance of wine yeasts

There are several kinds of stress conditions which affect wine production and industrial preparation of the yeast cells to be inoculated into the must. Heat shock stress has been widely studied in the yeast strain (Ivorra *et al.,* 1999). Although this stress condition is not usually found during the fermentation process, especially wine with temperature control system (Attfield,1997). Hyper osmolarity is another important stress for wine yeasts (Boulton *et al.,* 1996). Sugar starvation during final fermentation and increasing ethanol concentration leads to development of resistance against hyper osmolarity (Hallesworth, 1998).

In the present study, *S. cerevisiae* KJSK-96 was found to ferment vigorously in more than 50% w/v sugar and even up to 80% sugar level. This may be due to the high level of glycerol production, stress protein expression etc.(Puig and Perez-ortin, 2000). In visible screening, up to 50% sugar tolerance was noticed the selected yeast isolates. When the fermentation rate was studied, tolerance level was limited and the fermentation time was prolonged even with more than 20°B sugar level. Ethanol production was inhibited at high sugar level. This detrimental effect on ethanol production was described by Gough *et al.* (1996).

Fruits used in the present study contained generally less than 20°B TSS. So osmotolerance more than 30°B cannot be taken into consideration. Mostly, grapes grown in hot region and Mediterranean countries were reported to have more than 30°B sugar level. To ferment these fruits, an osmotolerant yeast is needed. Among the standard practices for fruit wine production, sugar amelioration was a common practice to increase TSS level. The osmoloterant yeasts selected in the present study can be used for industrial ethanol production from molasses (more then 40% sugar).

Low temperature fermentation was practiced to produce high flavour wine. But low temperature fermentation will be requiring more time (Bakoyianis *et al*., 1998). Temperature tolerance of the yeast isolates was found to be related with their geographical area of collection. The yeast isolates identified in the present study as tolerant to low temperature include *H. anomala* KJSK-69 from apple (temperate fruit), *S. bayanus* KJSK-100 from

Marthandam, Kanyakumari and *S. cerevisiae* KJSK-87 from Kambam, which were cooler zones. High temperature tolerant *K. thermotolerans* was isolated from Tuticorin district which is one of the dry zones of Tamil Nadu.

A general increase in alcohol content at low temperature upto 20°C was noticed. (30°C-10% v/v to 20°C –12% v/v). This may be due to the fact that the low temperature slowdown the fermentation process (Argiriou, *et al.* 1996). Increased fermentation time also increased the contact time of the substrate to active yeast cell *i.e.* lag phase may be extended. So the active cell (active enzyme) contact time with substrate (sugar) was increased, which leads to the increased production of alcohol. In high temperatures sudden decline in alcohol content was noticed. This may be due to stuck fermentation (Ivorra *et al.* 1999). High temperature tolerant isolates can be used for distilleries in tropical zones to reduce the cooling cost (Slapack *et al.*, 1988).

No impact on fermentation rate and ethanol yield was noticed in between pH 3-5. During selection itself, yeasts with the ability to grow at pH 3.5 were selected. This might be a reason for low pH tolerance of the yeast isolates tested. There is no need to use a pH less than 3.5 for wine yeast selection, because most of fruits studied were having pH more than 3.5 (Grape, papaya and banana). Some non-*Saccharomyces* sp. (*Candida* sp) were found to tolerate a pH of 2.0. This yeast can be used for fermenting acidic fruit juices (orange, pineapple etc.). Irrespective of the initial pH (5-3.5) the final pH tends to decrease (towards pH 3.5 level). This may be due to aerobic fermentation leading to the acetic acid production (Ciani and Ferraro, 1997) and the favourable environment for fermentation. This was not found in the case of fruit wine during standardization, where the water siphoning carried out for 15 days reduced the O_2 availability ultimately leading to less acetic acid production. The strategy adopted by *S. cerevisiae* for acidic pH tolerance is largely through high levels of proton and acid extrusions (Piper *et al*., 2001).

Most of the selected yeasts were found to produce more than 10% alcohol. But during screening for alcohol tolerance, most of them were not able to grow at 10% v/v alcohol concentration. The reason may be due to double stress induced by initial sugar level (20%) and 10% v/v alcohol whereas in the former case there was a gradual increase in alcohol with decreasing sugar level during fermentation. Both alcohol and sugar may have synergistic effect on growth of yeast

Mostly non – *Saccharomyces* were found to tolerate more SO_2 (upto 200 ppm) than *Saccharomyces*. Some workers used 300 ppm SO_2 for screening. Only 100 ppm SO_2 was used for screening, because for most of the fruit wine production 100 ppm SO_2 was used for pasteurization (Perez – Coello *et al*. 1999). The stationary phase of wine fermentation is an ideal stage to express enzymes that improve the floral and fruit aroma of wine (Fuge *et al*., 1994 and Riou *et al*., 1997). During stationary phase of microvinification, most of the stress responsive elements (STRE) with oenological importance were expressed (Puig and Perez-ortin, 2000).

5.3. Characterization of wine yeast

Traditionally, yeasts are identified by morphological and physiological criteria which are both laborious and time consuming (Kregar Van Rij 1984, Barnett *et al.,* 1990 and Kurtzmann and Fell, 1998). In morphological characterization, some yeast isolates were not stained with lacto phenol cotton blue. This may be due to the nature of cell wall composition. Instead of chitin in cell wall (Ascomycetes and Basidiomycetes), some of yeasts belonging to hemi-ascomycetidiae contain mannan and β -glucose (Burnett, 1968).

Some yeast cultures were found to take more than 5 weeks for sporulation. Chain of cells was also sometimes mistaken as pseudomycelium formation. *Hansenula* was found to be very small ($1/10^{th}$ size of other yeast). *S. ludwigi* was found to be very big in size and mostly *Candida* cells were fully elongated.

There is a demand for suitable kits for the identification of yeast based on purely physiological and biochemical characters, the results of which are easy to interpret, reliable and fast (Velezquez *et al.* 2001).During initial selection itself, glucose was used for fermentation of yeast isolates. There was a possibility of missing non-glucose fermenting wine yeast. In nature, more than 25% of yeast isolates were starch utilizers (Hongpattarakere and Kitti aun, 1995). However, in the present investigation only two out of twenty four isolates were found to be starch utilizers. This reduced number may be due to selection of fermentative yeast using glucose. Not even a single isolate of *Schizosaccharomyces* sp., which is a common yeast and potent ethanol producer, could be isolated in the present study. The reason may be the pH (3.5) used for isolation, because *Schizosaccharomyces* prefer a pH of 4.5 –5.0 (Kregar Van Rij, 1984).

No single chemotaxonomic and genetic technique has been proved to be adequate. Use of multiple yeast identification techniques such as cellular long chain fatty acid analysis, orthogonal filed alternate gel electrophoresis, co-enzyme Q analysis, G+C values and physiological and genetic techniques are more reliable. DNA based technique have the advantage of being independent of gene expression.

Techniques such as chromosome karyotyping, PCR amplification using random and specific primer, RFLP and sequence of specific genes have been used to identify various groups of yeasts (Mozina *et al.* 1997, Heyford and Jespersen, 1999). The RAPD-PCR analysis was considered the most appropriate method for yeast identification due to the low cost of the analysis. For molecular level characterization, RAPD-PCR polymorphism and yeast plasmid profile were studied. For this, a new methodology for total genomic DNA isolation with very high intact DNA recovery has been standardized. Normally cell biomass was harvested by centrifugation of large volume of broth. Yeast cell wall is very hard to break because of its chitinous nature and hence enzymatic breakdown of cell wall using zymolyase is used to release the DNA from cell (Adams *et al.* 1997). However, in the present investigation, yeast biomass was directly harvested from agar plate streak growth. The biomass yield was very high compared to liquid culture (Nigam, 2000). Like plant DNA extraction by liquid N_2 grinding (freeze cracking of cell wall), freezed yeast biomass paste was ground well in liquid N_2 followed by dissolving it in buffer and precipitating with ethanol, which gave maximum DNA recovery. Using above standardized protocol, 18S, 23S rRNA were also isolated which is an indication of good RNA recovery. Using this methodology, 2μ circular plasmid and dsRNA plasmid were also isolated. The protocol standardized above, is independent of cell wall composition. So it can be used for DNA isolation from any type of microorganisms.

5.4. Yeast genetic diversity

A number of different strategies based on the analysis of DNA polymorphism have been used to differentiate oenological yeast strains. RAPD– a PCR based technique is one of the powerful tool to characterize the best industrial isolate and to check the quality of its fermented product. It is also used for ecological researches to study the intra specific and inter specific diversity of the indigenous yeast isolates (Versavaud *et al*., 1995 and Pataro *et al.*, 2000).

Using M13 and RF2 primers, yeast isolates *viz*., *Saccharomyces*, *Kluvyveromyces* and *Candida* were differentiated up to genus level by RAPD-PCR analysis (Suzi *et al.,* 2000). For strain level differentiation, DNA restriction pattern was commonly used (Querol and Ramon, 1996). In the present study, strain level differentiation was established using ITS and OPD5 primers. The reason might be that ITS primer is having the sequence 'GTATGT' which is highly conserved with intron splice region (Barros- Lopes *et al.,* 1996 and Guerra *et al*., 2001).

Simple biochemical tests do not provide sufficient evidences to distinguish between wine yeast strains (Querol *et al*., 1992). In the present study ,only twelve strains of *Saccharomyces* was identified based on biochemical methods. But using RAPD – PCR, 14 strains of *Saccharomyces* were identified based on homology in banding pattern and were recharacterized and grouped under *Saccharomyces.* This is in agreement with the reports of Vaughan – Martini and Martini (1995 and Wendland, 2001), who stated that many yeast strains were wrongly classified based on morphological and biochemical characters.

Identification of yeast at species and strain level (depending on various techniques and primers used) by PCR mediated methods have been reported by different workers (Baileras – Couto *et al*., 1994, Van der Vossen *et al.,* 1998 and Paramataftki *et al.,* 2000). RAPD – PCR based distinct banding pattern can be used as a rapid identification technique for yeasts (Eghi *et al*., 1998). Normal morphological and biochemical characterization will take 2-3 months for identification. The high level polymorphism among yeast isolates can also be used as a powerful tool to analyze the indigenous microflora during spontaneous must fermentation (Vezinhet *et al*., 19920 and Polsinalli *et al*., 1996).

Versavaud *et al.* (1995) showed significant correlation between geographical origin and the genetic relationship among yeast isolates. In the present study, 100% genetic similarity was established between *S. cerevisiae* KJSK – 87 and 96 which were isolated from places which are located geographically closer on the base of western Ghats.

Due to the importance of yeast biodiversity to the wine industry, a comprehensive long–term research programme has been launched by several researches from the Wine and Fermentation Technology Division at ARC infinite – Nietvoorbij Research Institute and the Institute for Wine Biotechnology at the University of Stallenbosch. As part of this programme

Straus *et al.* (2001) studied the natural distribution and genetic diversity of non-*Saccharomyces* strains in the vine yards of the Western cape in South Africa. To date no extensive geographical survey of the yeast strains has been done in India. There is a need to study the biodiversity of wine yeast in India (which is one of the major biodiversity centre of the World) to boost Indian fermentation industry.

5.5. Suitability of fruit varieties for wine making

Yeast strain *S. ellipsoideus* CFTRI 101 was used as a reference culture for screening new yeasts as well as to study the suitability of fruit varieties for wine making studies, because of its ability to produce consistently acceptable quality wine product (Venkataramu *et al.,* 1979).

The term wine recovery instead of juice recovery was used because the pectinase enzyme was added only after pulping followed by fermentation (Revis *et al.,* 1968). No separation of juice from pulp was carried out before fermentation (Amerine *et al,*1980). In banana varieties, tannin content was found to be high and comparable to grapes. Tannin is mostly related with bitterness (Amerine *et al.,* 1980 and Peynaud, 1984). But very good taste was sensed during organoleptic evaluation. In the present study, Robusta variety of banana was selected for further standardization studies because of its excellent fruity aroma and good taste.

Contrary to the above result of papaya varietal screening, low tannin content with very high bitterness leading to poor organoleptic score was observed. Initially, wine making with ripen papaya fruits was good with fruity aroma, but bad taste. When papain removed fruits were used it gave better quality wine. So, the later was used for further screening which yielded good fruity aroma, but bitterness still persisted at lower level. During fermentation, carotenoid pigments from the pulp of papaya were not extracted well. Because carotenoid pigments may be complexed mostly with pomace. Considering yield, juice recovery, local availability and organoleptic score, CO 2 papaya and Robusta banana varieties were found suitable for wine production.

In grapes, Muscat variety was found to have low tannin (low bitterness) compared to others. This might be the reason for its very high suitability for table purposes. Only locally available varieties of grapes were used for wine making. Even though Bangalore blue gave best wine, which is well standardized for wine making (Sreekantiah and Johar, 1996),

Muscat variety was selected for the present study because Muscat is grown in more than 90% of grape growing area of Tamil Nadu and mostly used for table purpose. Moreover any disease/ pest incidence in Muscat variety will lead to poor quality fruits and lose the market value. Hence, it necessitates the development of a technology for value added product like wine production. Under these circumstances, the grape variety Muscat was selected for standardization of fermentation conditions. The Muscat grapes grown in Thondamuthur area (Coimbatore district) was found to have less titrable acidity than Bangalore blue. High titrable acidity is attributed to higher levels of tartaric acid and malic acid (Peynaud, 1984).

5.6. Standardization of fermentation conditions for fruit wine making

In the standardization procedures of most fruit wine production, fruit pulp was diluted (1:1 or 1:2) with water followed by enzymatic extraction of juice (Schanderl and Koch 1957, Joshi *et al.*, 1990 and Chauhan *et al.*, 1993). The extracted juice was further used for fermentation. The process of enzymatic juice extraction is not much practised in banana and papaya fruits (Ahmed, 1996). These fruit juices were bound tightly to the pulp and cannot be separated easily by squeezing. In the present study, a new concept of fruit juice extraction was standardized which is discussed later in this section. The degree of ripening plays an important role in juice yield. During 100% ripening, the total solids are fully converted into free soluble solids which is in available form for microbial fermentation (Ramana and Jayaraman, 1994 and Ahmed, 1996). So over ripened fruits are highly suitable for wine production (Bhajipale *et al.*, 1998).

Fruit pulp dilution will reduce the fruit wine quality, because pulp of most of the fruits are contains less than 16°B sugar level. Dilution of the pulp not only leads to dilution of native sugar level, but also fruit aroma, taste etc. This might be the main reason for grape wine quality for must which is not diluted normally. Jarezyk and Wzorek (1977) stressed the need for sweetening of fruit juice because of the low sugar content in majority of fruit juices. Sugar amelioration of more than 20°B sugar level was reported to give good quality fruit wine (Amerine *et al*,1980). Normally, cane sugar is used for sugar amelioration to get desired level of alcohol production (Schanderl and Koch, 1957). Sugar level of 18-26°B was tried for standardization in the present study. Alcohol content was found to be increasing upto 24°B sugar level which is in agreement with the report of Joshi *et al.* (1990). For banana and papaya, pulp

ameliorated with 24°B gave maximum ethanol yield and organoleptic score. These results are supported by Singh *et al.* (1998), but in grapes 22°B has recorded good organololeptic score which is in agreement with Tewari *et al.* (1987). TSS content of fruit wine was found to bear an inverse relationship with alcohol content in fruit wine (Kotecha *et al.*, 1995).

Fermentation with high sugar content leads to precipitation of pomace leaving clear fermented supernatant and precipitation of white pasty substance along with pomace. Precipitate was analysed and found to be pectin. This was supported by the findings of Amerine (1960) that the decrease in pectin level (in lots not treated with pectic enzyme) was due to the precipitation of pectin during alcohol production.

Later during pH standardization, it was found that the fruit pulp was clumped together at pH 3.5 leaving transparent fermented juice out. From this result, a new concept called "Biophysical separation" for fruit juice extraction and clarification was developed. In this method, the pulp was clumped together at pH 3.5 and TSS 24°B. During fermentation by inoculated yeast, CO_2 produced, forced out the juice from the pulp leaving soft leavened pomace clump (porous pomace clump like bread). This biophysical separation of clarified juice from pulp may be due to the synergetic effect of high alcohol produced under acidic condition. Increased wine recovery was also noticed due to the interaction between pH and sugar levels. This new concept of biophysical separation was performed well in wine extraction from banana pulp than papaya pulp.

Acidity in banana and papaya was mainly due to ascorbic acid (Vitamin C) (Nemestnikov *et al.*, 1988) while in grape, tartaric acid and malic acids are the major acids. Not much change in ethanol production was noticed during the change of pH between 5.0- 3.5 which was found to be in agreement with Singh *et al.* (1998). But pH 3.5 was found to give good wine recovery and high organoleptic score in both banana and papaya wine (Mir *et al.*, 1988). But this standardized pH was contrary to the earlier reports in which pH range of 4.5 –5.0 was optimized for maximum ethanol production from other fruit juices (Neelar, 1987 and Cheema, 1989).

For grape wine, no pH standardization was carried out because the pH of Muscat grape must itself was approximately 3.5, which was well standardized as highly suitable for grape wine production (Amerine *et al,*1980). In banana wine production, pH 4.0 and pH 3.5 gave maximum organoleptic scores. But in the case of grape wine, with an optimum pH of 4.0 browning was noticed during aging which lowered the organoleptic score (Gautam and Chundavat *et al.,* 1998). The pH standardized for banana wine as 3.4 was in agreement with Harold (1984), for best wine production with better storage life.

For acidification, food grade citric acid was normally used. Natural acidic fruit juices like lime juice and tamarind pulp were also tried along with citric acid. Like vermouth production, a concept of both acidification and aroma enhancement was developed by adding lime juice and tamarind pulp (Joshi *et al.,* 1991 and Attri *et al.,* 1994). Tamarind juice is a popular drink in some parts of West Asia and Syria (Mukinda *et al.,* 1988, Lingappa *et al.,* 1993). Lime juice acidification gave better flavour in banana wine, but for papaya both lime and tamarind gave negative impact on flavour. These fruit extracts provided bitter taste. But addition of sugar might have balanced the taste and favourably influenced various sensory characters (Attri *et al.,* 1994 and Joshi *et al.,* 1997).

Initially adjusted pH was found to decreased during the process of fermentation. This pH decrease is in agreement with earlier reports (Karni *et al.,* 1977 and Patel *et al.,* 1977). This might be due to rapid production of fermentation metabolites such as acetic acid, phenolic compounds, esters and CO_2 (Deacon 1984; Beech and Carr, 1977 and Ogbonna, 1989). After fermentation, different levels of titrable acidity and sourness was organoleptically found. Similar observation was made by Amerine *et al.* (1980), suggesting that this might be due to the buffering capacity of the wine and related amount of various acids influencing the acidity. At same titrable acidity, the order of sourness was malic, tartaric, citric and lactic acid. But at the same pH, the order was malic acid, lactic acid, citric acid and tartaric acid for improved quality.

Fruit wines were produced by fermentation at 20°C temperature. Under this low temperature fermentation, increased alcohol production and good flavour were noticed. Fruit

flavour enhancement was also noticed at low temperature fermentation. This might be due to the reduction of higher alcohol production and increase in proportion of ethaylacetate in the total volatiles (Argiriou *et al.,* 1996, Bakoyianis *et al.,* 1998 and Fundira *et al.,* 2002). Slowing down of fermentation was noticed at 15°C due to restricted yeast growth. During fermentation at room temperature (28-35°C), stuck fermentation also happened some times (Cramer *et al.,* 2002). This might be due to increase in fermentation and growth rate, due to the increased rate of metabolism (Brownsel *et al.,* 1989 and Stokes, 1971). Temperature maintenance is also an important factor for fruit wine fermentation. Contrary to this, Twari *et al.* (1987) standardized a temperature of 30°C as optimum for alcohol fermentation of saccharified banana peels and fruit juices.

At high temperature, glycerol production was also increased, which is one of the sweetening compound in wine (Arigiriou *et al.,* 1996). This might be the reason for less organoleptic score difference between room temperature and 20°C fermented fruit wine. In tropical countries like India high temperature tolerant, low higher alcohol, high ethyl acetate producing yeast should be selected (Regodon *et al.,* 1997) for better quality fruit wine production. This is the area for further research. In banana and grape wine, higher level of reducing sugar noticed lead to production of sweet wine which may be due to complete conversion of disaccharide into monosaccharide and improper utilization of sugars (Kulkarni *et al.,* 1980). According to Amerine *et al.* (1965), a score of 13 or above out of 20 may be considered commercially acceptable and with 17 points and over as superior quality. Though well trained persons were not used for sensory evaluation, the fermented products with high organoleptic score obtained in the present study could be considered as fruit wine of high sensory qualities as well as commercial acceptability.

5.7. Colour stability in Muscat grape wine

In wine making, anthocyanin pigments play a vital role in imparting colour. Pigment extraction depends on the grape variety (Almela *et al.,* 1996), yeast strain used (Dallas *et al.,* 1996) and environmental conditions. The grape, hybrid Muscat (hybrid between an American Bailey and European variety Muscat hanburg (Negi and Randhawa, 1980) was found to contain the following pigments *viz.*, malvidin 3- mono glucoside, malvidin 3, 5- diglucoside acylated with p- coumaric acid, peonidin 3-5- diglucoside, malvidin, petunidin, delphinidin glucoside in a decreasing order of concentration (Harborne, 1967, Somers, 1971, Somers, 1977 and Yokotsuka and Nishino,

1990). Several solvents like 1% HCl, butanol–HCl and formic acid-HCl were tried but the separation was successful in BAW,. Even though several workers separated upto 10 pigments (Matsudomi *et al*, 1977), only 3 major pigments were separated in the present study using upper phase of BAW in TLC. The inability to separate more pigments as reported by many workers might be due to single dimensional TLC instead of 2 dimensional chromatography and HPLC used by other workers (Bakker *et al*., 1986).

Among the 3 pigments separated, Mv–3G was the major one, the intensity of which was decreased during aging (Cordoves and Sanjose, 1995). Similar results were recorded by Bakker *et al*. (1998). The pigment extraction is influenced by the β - glucosidase enzyme (Petrez – Gonzalez *et al.* 1993) as well as acetaldehyde production by yeast strains (Dallas *et al.*, 1996). This might be the reason for the maximum extraction of Mv– 3G, since the yeast strains used were having low enzyme activity and high acetaldehyde production. There are different techniques like heating, SO_2 addition, more ethanol production and increased skin contact time available for extraction of pigments (Leone *et al.*, 1984; Mazza and Miniati, 1993; Bakker and Timberlake (1997) and Geo *et al.*, 1997).

With increased skin contact time, bitterness was increased resulting in less organoleptic score. This increase in bitterness was found to be due to more tannin release. Even though more tannin release was found in both treatments, bitterness was very low in heat treatment. HPLC analysis of the samples revealed the presence of more gallic acid during increased skin contact time than heating. The presence of more gallic acid was found to impart bitterness to the wine there by lowering the organoleptic value. Ethiraj and Suresh (1978) optimized the heating time as 70°C for 30 min for more colour extraction. In the present study, maximum colour was extracted with relatively less tannin content and high organoleptic score at 70°C for 10 min heating.

5.8. Yeast population dynamics during fruit wine fermentation

Indigenous yeast fermentation was seen in fruit must pasteurized with 100 ppm SO_2 in uninoculated control. So the possibility of indigenous yeast domination during fruit pulp inoculated with selected wine yeast was doubtful. This is in agreement with the earlier findings that growth of

indigenous yeast is suppressed but not limited by sulphite addition (Fleet and Heard, 1993). The growth of indigenous yeast can be highly undesirable as they may inflict in appropriate sensory properties to the wine (Sponholz, 1993, Ramirez *et al.*, 1999 and Ubeda and Briones, 2000). The risk of wine spoilage by indigenous yeast growth can be reduced by heavy inoculum load of highly active wine yeast as starter culture in the presence of higher concentration of SO_2 (Fleet and Heard, 1993). Killer yeast may provide an alternative method for preventing the unwanted yeast growth.

During wine yeast selection, three wine yeasts with broad killer activity towards tested yeast strains were used for this study. Killer toxin is highly pH sensitive. At lower pH, the activity will be reduced (Yap *et al*., 2000). To overcome this pH sensitivity, the killer sensitive reaction was tested in medium buffered with pH 3.5 (standardized pH for fruit wine making). Yeast with broad killer activity towards indigenous yeast of wine fermentation can be used as super killer wine yeast to avoid indigenous counteracts. Three killer toxin producers (Two *Saccharomyces* and one non–*Saccharromyces*) with broad host range were investigated to study the effect on indigenous population and within them. The non-*Saccharomyces* isolate *H. anomala* KJSKH 69, was found to be neutral to *Saccharomyces cereviae* KJSK – 57 but *Saccharomyces ellipsoidous* KJSK – 106 was highly sensitive. This intraspecific difference in killer performance was well documented (Walker *et al.*, 1995).

The rapid enumeration and identification of yeast species during fruit must fermentation would be highly useful in fruit wine making because it could furnish, especially in the early stage of fermentation, quantification in fermentation, the composition and dynamics of the yeast population which were co-inoculated. PCR based technology have been shown to be the most suitable tool for rapid yeast identification (Granchi *et al*., 1999). In this study, a PCR based yeast colony identification protocol (PYCIP) using M13 primer has been developed and applied to enumerate the population dynamics of the co-inoculated broad spectrum killer toxin producing wine yeasts and indigenous yeasts. Well-standardized specific molecular profile (RAPD – PCR banding pattern using M13 primer) was applied to follow the population structure of the co-inoculated wine yeasts (Caruso *et a.*, 2002).

From PYCIP data, it was realized that added wine yeast starter culture did not completely dominate the fermentation. In general, during the banana pulp fermentation, the indigenous yeast load was found to be very low and needed more than 8 days to build -up indigenous yeast population. Yeast population in grape wine fermentation was found to be higher than the banana pulp fermentation. It is therefore speculated that the nutritional and chemical composition plays an important role in inhibiting yeast strains (Eghi *et al*., 1998).

In initial fermentation, irrespective of sensitive, resistant and neutral, all inoculated yeasts were predominant. Most of the initial stage contamination, the banding pattern correlated with non–*Saccharomyces* predominantly *Candida* sp. pattern. This non–*Saccharomyces* growth was recorded upto 5^{th} day, after which that the population was reduced. This may be due to the increased alcohol production and alcohol sensitiveness of non- *Saccharmoyces*. After 8^{th} day, general decline in yeast communities was noticed. This decline in wine yeast could be attributed to the lack of nutrition (Pataro *et al*., 2000). In *H. anomala* KJSK – 69 inoculated control, a banding pattern similar to *Saccharomyces* was observed in the population which was predominant. M13 primer differentiated the yeast isolates upto generic level. So there is a need for strain specific primer for differentiating indigenous *Saccharomyces* yeast. In co-inoculation, *Saccharomyces cerevisiae* KJSK- 57 was found to be very effective suppressor of other indigenous *Saccharomyces* species.

5.9. Phenolic compounds of fruit wines and its medical importance

Phenolic compounds greatly influence the organoleptic attributes of fruit wine and are considered very important for production of quality wines. Phenolic compounds are normally determined by reduction of the folin-ciocalteu reagent, estimation of phosphomolybdenum blue produced in the presence of tetra alkylammonium salt and expressed as gallic acid equivalent (Forteza *et al.,* 1995). For separation of individual phenolic compounds in fruit wine, HPLC separation is normally used. For this, a low concentration of acetic acid (or) formic acid (or) phosphoric acid (or) perchloric acid was used (Brenna *et al.,* 1998, Andrade *et al.,* 2001 and Gaulajac *et al.,* 2001). Very good elution of most of the phenolic compounds were obtained by phosphoric acid. For unidentified peaks, no further identification was possible, since no standardization for any of these are available (Escribano- Bailon *et al.,* 1992). Oxidizable phenols greatly influence the colour, flavour, clarity and stability of fruit wine (de Gaulejac *et al.,* 2001). Many of phenolic compounds found in

grapes contribute to astringency, bitterness and harshness of novel wines. The disappearance of much of bitterness and astringency during aging is attributed to oxidation and condensation of these compounds which results in formation of coloured polymers.

Phenolic compounds present in the fruits include phenolic acids (p-coumaric acid, cinnamic acid and caffeic acid), stilbenes (*trans*-resveratrol) and flavanoids (catechin, epicatechin and quercetin) which have medicinal values as antioxidant, platelet aggregation inhibitor, antimutagenic, anti proliferation of cancer cell and decreased risk of cardio vascular diseases (Daminaki *et al.,* 2000). Phenolic acids (Caffeic acid and p-coumaric acid) were found to be very high in banana wine. In papaya wine t-ferulolyl tartaric acid and t-caftaric acid were higher than grape wine. Compared to grape wine, total phenolic acid content in banana wine was found to be very high. Satue –Gracia *et al.* (1999) correlated the caffeic acid and *p*- coumaric acid content with LDL-oxidation. Antioxidant nature of ferulic acid and caffeic acid was well explained by Meyer *et al.,* (1998) and Fukumoto and Mazza (2000).

Fruit wines are rich source of flavanoids. Most abundant flavonoid found in grape wine was quercetin followed by myricitin. (Merida *et al.,* 1991). Quercetin was found to be very high in banana wine. These flavonoids are very good antioxidants which prevent oxidation of human LDL (Pignatelli *et al.,* 2000). Very high quantity of epicatechin in papaya is recognized as medically valuable because of its antioxidant activity (Waterhouse, 2002). Most of the flavonoids (Kaemphenol, quercetin, myricetin, catechol) were reported as reducing agents (free radical terminator), metal chelator, O_2 quenchers, anticancer, anti inflamatry, antiallergic and cardio protective compounds (Hertog *et al.,* 1993, Tiwari 2001 and Gorinstein, 2002).

Proanthocyanins (procyanin, delphinidin, cyanidin, petunidin and peonidin) found in all the three fruit wines are having the ability for complex formation with protein and metalic ions. They act as antioxidants, radical scavengers and their presence in the diet will protect against chronic diseases and confer cardioprotecive ability to reduce ventricular arrhythmia (Haslass, 1996; Gronback *et al.,* 1998 and Sato *et al.,* 1999). Above mentioned polyphenol compounds can be included in the already recognized antioxidants like vitamin C and E, carotenoids, tocopherol etc. (Pratt, 1992).

Phenolic compounds are responsible for the astringency, bitterness and harshness in fruit wine taste. In banana wine, high tannin content was not correlated with bitterness. The reason

might be very low concentration of catechin, epicatechin and gallic acid, which are the compounds responsible for astringency and bitterness (Robichand and Noble, 1990 and Thorngate and Singleton, 1994). But in papaya wine, presence of very high epicatechin and gallic acid might be the reason for bitterness.

Browning was found to be a major problem in banana wine. Before blending the fruits, KMS was added to inhibit polyphenol oxidase enzyme which is responsible for browning (Waterhouse, 2002). This browning also continued during storage. The reason for browning might be the presence of more tartaric esters of hydroxy cinnamic acids (caffeic acid, feruoryl tartaric acids) which are the substrates for polyphenol oxidase enzyme (Cheynier *et al.,* 1986, Rigaud, 1991, Du Plessis, 1973, Machiex *et al.,* 1991 and Arogba, 2000).

trans- resveratrol is a stilbene, a natural substance which is produced by plants as stress metabolite and also as constitutive defence agent such as phytoalexin (Sotheeswaran and Pasupathy, 1993). Research on resveratrol has extended due to interest in the prevention of cancer and heart diseases, since resveratrol inhibits cellular events associated with tumors initiation, promotion and progression. This compound is also active as anti mutagen, antioxidant and has anti fungal properties. The antioxidant effects of resveratrol could be related with its high ability to inhibit oxidation of LDL. Interest in resveratrol can be seen also from the number of related papers in the filed of biology, medicine and chemistry (140 papers in 2000, 46 reports in 1999, 23 reports in 1998, 6 reports in 1995) (Nakagawa *et al.,* 2001, Dobiasova *et al.,* 2002, Gamoh and Nakashima, 1999 and Ragionne *et al.,* 1998).

Muscat red wine was found to contain *trans*-resveratrol, but was very trace in banana wine and not at all detected in papaya wine. During increased skin contact time and cluster stem contact, the *trans*- resveratrol level was increased due to the higher resveratrol content in grape skin (Goldberg *et al.,* 1995). More than 3 mg/L of *trans*- resveratrol was recovered due to cluster stem addition during fermentation. This was very much higher than the earlier report by Bavaresco *et al.* (2000) who observed the *trans*–resveratrol increase due to cluster stem addition. But during heating (70°C), *trans*–resveratrol was totally degraded. This is the first report in resveratrol research that heat pasteurization and thermovinification affect the chemical nature of *trans*-resveratrol. So chemical pasteurization and filter sterilization were recommended.

During vinification of red grapes, the duration of the maceration step together with effect of other parameters such as temperature, stirring of the fermentation medium and the wine making process have a considerable influence on the final phenolic content of wine (Gao *et al.,* 1997). In Muscat grape wine, the decrease in the level of anthocyanin was noticed during aging. This could be explained by the formation copolymer between quinone of caffeic acid and anthocyanin (Cheynier *et al.,* 1994), co-pigmentation (Alonso *et al.,* 1986) and acetaldehyde mediated condensation (Thorngate and Singteton, 1994).

In the present study increased skin contact time and thermovinification were tried for more colour extraction. During increased skin contact time, the hydroxy benzoic acids (gallic acid, epicatechin, caffeic acid and coumaric acids) were extracted more (Gil Munoz *et al.,* 1999).

Temperature strongly influenced the phenol extraction (Gil- Munoz *et al.,* 1995) during 3-4 days of alcoholic fermentation. Thermovinification not only released more anthocyanin pigments, but also extracted more phenolic compounds (Fischer *et al.,* 2000). Eventhough both increased skin contact time and thermovinification increased the total phenol content, less bitterness was reported in thermovinification. This might be due to more epicatecting catechin and gallic acid, found in the skins and seeds, which are responsible for the bitterness obtained during increased skin contact time (Kovac *et al.,* 1995).

5.10. Need for fruit wine based industry and their impact on Indian economy

The tradition of natural food, drink and herbs are so deeply implanted in Indian's gastronomic tradition. Wine is far more suitable choice than the high grade alcoholic drinks that are consumed despite their unsuitability considering the climatic condition in most part of the country. From the social stand point, there is a stigma attached to alcoholic consumption. Wine is therefore questionable, because it is an alcoholic beverage. On the other hand, alcohol consumption is widespread in India.

In evolution, when the vertebrates consumed the fruits and subsequently dispersed the seeds, the sugars present in the fruit pulp provided an energetic (Dutilh and Kramer, 2000) incentive for vertebrates and served as a substrate for fermentation by yeasts. Fruit eating animals (i.e. frugivores) should then inevitably consume ethanol on a regular basis. The occurrence of ethanol in ripe and fermenting fruits indicates sustained historical exposure of all animal frugivores to this

psycho active and addictive compound (Dudley, 2000). The human brains exposure to dietary consumption of ethanol may have been a consequence of regular fruit eating habits from historical period to modern man.The historical ecology of humanoid diets was consistent with the ingestion of ethanol, and modern human might possess the metabolic equipment necessary to obtain hermetic advantage from ethanol at suitably low concentration. The aspect of more Alcohol dehydrogenase (ADH) secretion in liver to degrade ethanol establish a strong dietary association with ethanol (Sullivan and Hagen, 2002). However, this is insufficient to justify, as ADH is also distributed in both unicellular and multicellular taxa.

The fruit wines have been in use and were part of the diet of ancient people. Evidence date back as far as Indus valley civilization. These are nutritious unlike distilled liquour. Their consumption in moderate amount is good for health. This can be produced from various fruits like grapes, banana, papaya, apple etc. The production of fruit wine will help to utilize more quantities of fruits especially unmarketable surplus fruits which otherwise go as a waste (Joshi *et al*., 1990). There exists a market for such products in our country and they can also be exported to earn foreign exchange. As globalization gets more wide spread, goods and services move freely across the borders. With the economic growth that results from liberalized world markets, people everywhere are moving towards higher standard of living, better quality and good variety of processed food products (Malhotra, 2001). Fruit wine can be considered as the drink of 21st century. Since fruit wine industry has good export market potential, the industry will strengthen the economy of fruit growers and generate employment opportunities besides providing a health nourishing stimulative drink (Joshi *et al.*, 1999).

Numerous epidemiological studies suggest a systemic reduction in cardiovascular risk and in overall mortality due to low levels of ethanol consumption (Lieber, 2000, Dudley, 2002). Most of the research suggested that the anticancer, antinflamatary, antiallergic and antioxidant activity of fruit wine is related with its total polyphenol content (Mannino *et al.,* 1998, German and Walzem, 2000 and Psarra *et al.*, 2002).Are phenolic acids and polyphenols from the fruit wine the only health benefit? Are fruits and vegetable rich in phenolics sufficient enough for the above mentioned health benefits? Soleas *et al*. (1997) reasoned out that the fermentation and increased alcohal content lead to polyphenol liberation and solubilization such that they are more likely to be much more bioavailable than in solid food stuff.

In India, majority of teenagers consume soft drinks as an important feature of their life style. Consuming soft drinks in between meals spoils the adolescents appetite for regular meals but provides substantial proportion of daily calorie. However, it doesnot meet out the nutritious requirement of the body. Most of the soft drinks include sucrose, a source of energy and some provide vitamin C. It seems more reasonable to give special attention to educate teenagers to take up food with various nutrients and give special emphasis to new age drinks like ready to serve (RTS) fruit beverages and fruit wines which are the best appetizers and good refreshers with additional nutritive (Annexure I) and medicinal value (Sadana and Khanna, 1998, Bhutani, 1989, Cordoba and Sagarbieri, 2000, Cao *et al.*, 2001, Bamforth, 200, Teisari *et al.*, 2002).

CHAPTER - VI

SUMMARY

India, one of the largest producers of banana and papaya with highest productivity in grape cultivation, needs a promising processing technology to overcome post-harvest losses and increase the economy of fruit growing belt of India. Very few processed preparations from the above fruits like juice, jelly, canned fruits, nectar etc. are marketable primarily due to difficulty in retaining the characters like colour, flavour, texture of fruit during processing and poor shelf life. Fruit wine making is one of the best option for production of value added product with desirable characters such as long time storage and native fruit flavour. Highly efficient wine yeasts will be the key player for high quality fruit wine production through fermentation. The hidden wealth of indigenous yeasts is not studied well in India, which is one of the biggest biodiversity centers. Based on this, a research programme studies entitled "**Selection of suitable yeast cultures and standardization of fermentation conditions for wine making from banana, papaya and grapes**" was carried out at *Azolla* Laboratory, TNAU, Coimbatore. The results are summarized as bellow.

- A total of 122 yeast strains were isolated from various samples collected from several parts of Tamil Nadu and they were analysed for their oenological characters, enzymatic characters, stress tolerance, higher alcohol, esters, aldehydes, organic acids and glycerol production.

- Among the 122 isolates, twenty four efficient yeast isolates were selected based on oenological characters *viz.,* time taken for initiation of fermentation, high fermentation rate, high fermentation vigour, less titrable and volatile acidity, less pH reduction, low residual sugar level, fermentation purity, flocculent capacity, no H_2S production, low foam production, non-adherence with glass, autolytic capacity and desirable bouquet, in comparison with three standard commercial yeasts (*S. ellipsoideus* CFTRI 101, MTCC 180 and *S. cerevisiae* (baker's yeast).

- The twenty four yeast isolates, obtained by preliminary screening, were identified based on morphological and physiological characters. A new protocol for yeast total genomic DNA isolation, independent of cell wall composition with high intact DNA recovery was standardized. Using this protocol, 2 μ circular plasmid and dsRNA (responsible for killer toxin production) were also recovered. Their genetic diversity was analyzed by RAPD-PCR and found that the isolates of *Saccharomyces* formed a single cluster with 72% similarity.

- The fermentation kinetics studies (*viz.*, specific growth rate, ethanol productivity, ethanol yield, sugar utilization and uniform rate of fermentation) indicated that *S. cerevisiae* KJSK-57, 87, 96, *S. bayanus* KJSK-100, *H. anomala* KJSK-69, *C. aynoliae* KJSK-58 and the yeast isolate KJSK-114 were the highly efficient fermenting yeast cultures.

- Osmotic, temperature, pH, ethanol and SO_2 tolerance of the twenty four selected yeast isolates were studied and observed that most of the yeast isolates were able to grow upto 40% w/v sugar concentration. The maximum level of sugar tolerance, upto 70% w/v, was found in *S. cerevisiae* KJSK-96. Higher sugar level was found to reduce the alcohol production. Temperature of 25-30°C was found to be optimum for all. *S. cerevisiae* KJSK-37, 57, 96, *H. anomala* KJSK-69, *C. intermedia* KJSK-90 and *S. bayanus* KJSK-100 were able to grow upto 10°C. In higher temperature, sudden reduction in both ethanol and fermentation rate was noticed. All the yeast isolates were found to tolerate the acidic pH range of 5.0 to 3.0. Upto 8% v/v ethanol tolerance was observed in most of the yeast isolates studied. Only two yeast isolates (*H. anomala* KJSK-69 and *C. intermedia* KJSK-90) were able to tolerate upto a maximum of 300 ppm SO_2.

- Non–*Saccharomyces* yeasts were found to be the major source of hydrolytic enzymes. Among them, *H. anomala* KJSK-69 was found to produce most of these enzymes (polygalacturanase, cellulase, amylase and β-glucosidase). These hydrolytic enzymes were found to be rare in *Saccharomyces.*
- Five major killer toxin producing yeasts (*S. cerevisiae* KJSK-57, *H. anomala* KJSK-69, *Hansenula* sp. KJSK-11, *S. ellipsoideus* KJSK-106 and *C. intermedia* KJSK-90) with

broad host range were identified. Among them *S. cerevisiae* KJSK-57 and *H. anomala* KJSK-69 were found to produce killer toxin against 92% and 68% of the yeast isolates tested respectively. The genetic basis of killer toxin like dsRNA and chromosomal origin was also established.

- Among the higher alcohols produced by the selected yeast isolates, isoamyl alcohol was the major contributor. Higher amount of n-propanol was found to be produced by *Candida* than *Saccharomyces* sp. *S. ellipsiodeus* KJSK-106 was found to be the maximum producer of acetaldehyde (195 mg/L).

- In fermented must, the production of succinic acid and acetic acid differed significantly with different yeast isolates studied. Acetic acid production capacity was found to vary with *Saccharomyces* sp. Among all the isolates, *S. cerevisiae* KJSK-37 produced the maximum of 1.07 g/L.

- Glycerol production ranged from 7-10 g/L in most of the yeast isolates studied. *Candida* sp. was able to produce more than 10g/L of glycerol.

- Based on the above selection studies, exclusion tests and cluster analysis were carried out and five efficient yeast isolates *viz.*, *S. cerevisiae* KJSK-57, *H. anomala* KJSK–69, *S. cerevisiae* KJSK-96, *S. bayanus* KJSK-100 and *S. ellipsoideus* KJSK-106 were selected for standardizing fruit wine production.

- Among the various commercial varieties tested for fruit wine production, Robusta for banana wine, CO 2 for papaya wine and Muscat for grape wine were found to be more suitable for fruit wine production.

- Fermentation conditions (TSS, pH, temperature and acidifier) were standardized for quality wine production. TSS of 24 B° was standardized for both banana and papaya wine making and for grape wine 22 B° was found to give superior quality wine. Acidification of fruit pulp upto pH 3.5 was optimized for fruit wine production and acidification using lime juice and citric acid yielded quality banana wine, whereas lime juice acidification was found to give negative impact on papaya wine production.

- For banana fermented juice extraction and clarification without pectinolytic enzymes, a new concept called "Biophysical separation" was developed by adjusting pH 3.5 and sugar level 24°B during banana wine fermentation. Interaction between pH and sugar level was noticed in banana wine recovery. pH 3.5 and 24 B° sugar level gave the maximum wine recovery through biophysical separation.

- Low temperature (20°C) fermentation was found to enhance fruit flavour, which was sensed during organoleptic evaluation.

- Three major anthocyanin pigments *viz.* malvidin glucoside complexes, peonidin and malvidin were separated from Muscat grape wine and their stability during aging was also studied.

- Yeast isolates used for wine production were also found to differ in colour extraction. More than 78% increase in colour extraction was noticed in 10 min. heating (70°C) of grape must. But increased skin contact time led to drastic increase in tannin level.

- Based on above standardized fermentation conditions, working experience with fruit wine making and the knowledge of wine making from the review of literature, an overall methodology for fruit wine production was standardized. A pilot model semi-solid state fermenter was designed, which was found to give promising results for commercialization of the standardized technology.

- To prevent the risk of unwanted indigenous yeast growth during fermentation, three killer toxin producing yeasts *viz. S. cerevisiae* KJSK-57, *S. ellipsoideus* KJSK-106 and *H. anomala* KJSK-69 were co-inoculated in both banana and grape must fermentation and their population dynamics during fermentation was studied by a PCR based yeast colony identification protocol (PYCIP). The results revealed that, in banana pulp fermentation, the yeast population build-up was found to be lower than grapes. Low indigenous yeast population load was observed in banana wine fermentation. *S. cerevisiae* KJSK-57 was found to be a superior killer yeast strain dominating in all the fermentation conditions studied.

- More than 47 peaks were detected during HPLC analysis of phenoloic compounds present in the fruit wine, among which 22 phenolic compounds were identified and quantified. The total phenolic compounds present in the banana wine was found to be higher (348.08 mg/L) than the other two wines (236.2 mg/L for grape wine and 110.9 mg/L for papaya wine). Phenolic acids (p-coumaric acid and caffeic acid) predominated in banana wine, stilbenes (*trans*-resveratrol) in grape wine and flavonoids (catechin, epicatechin, quercetin) in papaya, which were reported to have medicinal values as antioxidant, antiplatelet formation, aggregation inhibitor, antimutagenic, anticancer and decreased risk of cardio -vascular diseases.

- An anticancer compound called *trans*-Resveratrol was found to be present at 0.667 mg/L in Muscat grape wine. Increased skin contact time and the cluster stem contact during fermentation were found to increase the *trans*-resveratrol level. *trans*-Resveratrol was found to be increased from 0.67 mgL^{-1} to 3.8 mgL^{-1} during fermentation with cluster stem, which was totally destroyed during colour extraction by heating.

Table 19. Organic acids and glycerol production by the selected yeast isolates

Yeast isolates	Succinic acid (g/L)	Acetic acid (g/L)	Glycerol (g/L)
Hansenula sp. KJSK-11	0.283	0.625	7.433
Candida oleophila KJSK-29	0.929	0.196	10.613
Saccharomyces cerevisiae KJSK-37	0.400	1.075	7.830
Kluyveromyces marxianus KJSK-49	0.250	0.325	6.713
Saccharomyces cerevisiae KJSK-57	0.341	0.894	8.230
Candida maynoliae KJSK-58	0.994	0.394	10.423
Hansenula anomala KJSK-69	0.221	0.400	6.963
Candida stellata KJSK-70	1.165	0.296	10.423
*Saccharomyces cerevisiae*KJSK-87	0.540	1.098	6.963
Candida intermedia KJSK-90	0.996	0.468	10.81
Yeast isolate KJSK-95	0.584	0.566	4.936
Saccharomyces cerevisiae KJSK-96	0.422	0.741	7.293
Saccharomyces capensis KJSK-97	0.383	0.782	7.803
Saccharomyces cerevisiae KJSK-99	0.279	0.923	7.413
Saccharomyces bayanus KJSK-100	0.501	0.733	8.500
Saccharomyces ellipsoideus KJSK-101	0.399	0.945	7.946
Saccharomyces bayanus KJSK-105	0.889	0.093	7.290
Saccharomyces ellipsoideus KJSK-106	0.469	0.580	7.780
Saccharomyces ludwigi KJSK-108	0.326	0.864	7.303
Saccharomyces capensis KJSK-110	0.228	0.693	8.120
Kluyveromyces thermotolerans KJSK-111	0.546	0.839	8.166
Candida stellata KJSK-112	1.324	0.541	8.383
Yeast isolate KJSK-114	0.356	0.707	6.940
*Torulaspora delbrueckii*KJSK-121	0.377	0.516	3.370
Saccharomyces ellipsoideus CFTRI 101	0.466	0.787	8.433
Saccharomyces ellipsoideus MTCC 180	0.520	0.841	7.190
SEd	0.16	0.14	1.45
CD (0.05)	0.32	0.29	2.91

Table 2. Oenological characterization of the native yeast isolates

Yeast isolates	Residual sugar intensity (Brix)	Final pH	Initiation of fermentation* (hrs. after inoculation)	Fermentation rate (g of CO_2 produced /day)	Fermentation vigour (alcohol content % v/v)	Titrable acidity (g/L of tartaric acid)	Volatile acidity (g/L of acetic acid)	Fermentation purity	Flocculent capacity[a]	SH_2 production[b]	Less foam production [a]	Adherance to glass[a]	Autolytic capacity[a]	Bouquet [a]
KJSK-1	7.6	3.44	3.30	2.00	6.13	5.40	0.81	0.132	+	+	+	-	+	-
KJSK-2	17.2	3.50	-*	0.67	1.22	7.02	0.31	0.254	+	-	+	-	+	-
KJSK-3	18.0	3.47	-	0.77	1.32	5.61	0.18	0.136	+	+	+	-	+	-
KJSK-4	9.2	3.46	7.00	1.50	5.43	4.93	0.34	0.077	+	-	-	-	+	-
KJSK-5	17.6	3.41	13.30	0.87	1.02	5.59	0.81	0.794	+	-	+	-	+	-
KJSK-6	14.0	3.42	-	0.76	2.86	6.10	0.22	0.077	-	-	+	+	+	-
KJSK- 7	12.85	3.46	-	0.85	3.12	6.52	0.21	0.067	+	-	+	-	+	-
KJSK- 8	10.4	3.38	-	0.74	2.63	4.93	0.68	0.259	-	-	+	+	+	-
KJSK-9	15.4	3.29	-	0.80	2.15	5.86	0.41	0.191	-	-	+	+	+	-
KJSK-10	12.0	3.35	-	0.91	3.85	6.15	0.35	0.091	+	-	+	-	+	-
KJSK- 11	2.0	3.35	4.30	3.43	8.82	4.63	0.44	0.050	+	--	+	-	-	+
KJSK-12	7.2	3.04	6.30	1.47	6.34	6.85	0.89	0.140	-	+	+	+	+	-
KJSK- 13	8.0	3.30	8.00	1.37	5.57	6.01	0.91	0.163	+	+	+	-	+	-
KJSK- 14	13.6	3.36	5.00	0.87	3.34	4.84	0.12	0.036	+	-	+	-	+	-
KJSK-15	12.8	3.50	4.30	1.70	2.99	5.71	0.62	0.207	+	+	+	-	+	-
KJSK-16	8.0	3.32	9.30	1.67	6.32	5.28	0.78	0.123	+	-	-	-	+	-
KJSK- 17	9.2	3.12	10.00	1.37	6.87	6.51	0.81	0.118	-	+	-	+	+	-
KJSK-18	18.4	3.45	-	0.90	1.04	5.85	0.41	0.394	+	-	+	-	+	-
KJSK- 19	11.2	3.43	3.00	1.50	3.68	5.08	0.23	0.063	+	+	+	-	+	-
KJSK- 20	16.8	3.42	4.30	1.13	1.59	5.47	0.81	0.516	+	-	+	-	+	-
KJSK-21	7.8	3.32	7.30	1.47	5.56	5.38	0.41	0.074	-	+	+	+	+	-
KJSK-22	16.0	3.37	-	0.73	2.07	7.06	1.12	0.541	-	-	+	-	+	-
KJSK- 23	5.8	3.08	-	2.17	7.67	5.44	0.16	0.021	+	++	-	-	+	-
KJSK-24	15.6	3.47	-	0.67	2.12	6.04	0.18	0.085	-	-	+	+	+	-
KJSK- 25	7.6	3.08	12.30	1.27	5.47	6.60	0.41	0.075	-	++	+	+	+	-
KJSK- 26	18.8	3.44	13.00	1.00	1.19	4.95	0.28	0.235	+	-	+	-	+	-
KJSK-27	14.8	3.45	-	1.00	2.28	4.24	0.19	0.079	+	+	+	-	+	-
KJSK-28	10.2	3.22	-	1.23	4.63	6.15	0.13	0.028	+	+	+	-	+	-
KJSK- 29	4.8	3.23	10.30	3.00	7.84	6.03	0.57	0.073	+	-	+	-	+	-
KJSK-30	16.8	3.48	-	0.62	2.08	5.78	0.41	0.197	-	-	+	-	+	-
KJSK- 31	9.2	3.41	-	1.43	5.15	6.30	0.22	0.043	-	+	+	+	+	-
KJSK- 32	8.6	2.98	6.30	1.43	5.80	6.82	0.51	0.087	+	++	+	-	+	-
KJSK-33	9.8	3.27	6.30	1.33	4.34	5.53	0.41	0.094	+	+	+	-	+	-
KJSK-34	14.5	3.38	-	0.67	2.50	4.57	0.26	0.104	-	+	+	+	+	-
KJSK- 35	18.2	3.28	11.30	0.97	1.82	5.58	0.81	0.445	-	+	+	+	+	-
KJSK-36	13.6	3.47	-	0.73	3.63	6.03	0.10	0.028	+	-	+	-	+	-
KJSK- 37	1.2	3.45	5.00	3.76	8.03	4.95	0.42	0.052	+	-	+	-	-	+
KJSK- 38	14.0	3.42	-	0.77	2.63	3.55	0.21	0.080	-	-	+	-	+	-
KJSK-39	18.2	3.27	14.00	0.90	1.84	7.33	0.11	0.060	+	-	+	-	+	-

* Initiation of fermentation 15 hours after inoculation was not accounted.

Table 2. (Cont...)

Yeast isolates	Residual sugar intensity (Brix)	Final pH	Initiation of fermentation (hrs. after inoculation)	Fermentation rate (g of CO_2 produced /day)	Fermentation vigour (alcohol content % v/v)	Titrable acidity (g/L of tartaric acid)	Volatile acidity (g/L of acetic acid)	Fermentation purity	Flocculent capacity	SH_2 production	Less foam production	Adherance to glass	Autolytic capacity	Bouquet
KJSK-40	18.0	3.44	13.30	0.87	1.51	6.10	0.68	0.450	+	-	+	-	+	-
KJSK- 41	15.8	3.42	-	0.78	2.14	5.78	0.31	0.145	-	-	+	+	+	-
KJSK-42	9.2	3.48	-	1.49	5.68	5.60	0.35	0.062	+	+	+	-	+	-
KJSK- 43	18.8	3.21	-	0.80	0.74	6.52	0.12	0.162	-	-	+	+	+	-
KJSK- 44	10	3.38	-	0.96	4.82	4.99	0.16	0.033	+	-	+	-	+	-
KJSK-45	9.2	3.15	7.00	1.30	5.16	5.17	0.19	0.019	-	+	+	+	+	-
KJSK-46	14.0	3.27	-	0.89	3.17	7.06	0.46	0.145	-	-	+	+	+	-
KJSK- 47	13.6	3.12	6.30	1.27	3.95	6.00	0.32	0.081	-	++	+	+	+	-
KJSK-48	9.0	3.34	8.30	1.37	5.93	5.56	0.41	0.069	+	+	+	-	+	-
KJSK- 49	5.2	2.96	-	2.03	7.59	5.86	0.26	0.033	-	+	-	-	+	-
KJSK- 50	15.0	3.39	14.30	0.90	2.45	6.19	0.35	0.143	+	-	+	-	+	-
KJSK-51	10.2	3.22	9.30	1.30	4.72	6.30	0.12	0.025	+	+	+	-	+	-
KJSK-52	10.4	3.45	-	0.83	4.07	5.83	0.08	0.020	+	-	+	-	+	-
KJSK- 53	9.0	3.21	10.30	1.23	4.88	6.04	0.05	0.010	+	+	+	-	+	-
KJSK-54	18.6	3.42	-	0.70	0.78	6.24	1.12	1.436	+	-	+	-	+	-
KJSK- 55	15.2	3.49	-	0.17	2.13	4.95	0.03	0.014	+	+	+	-	+	-
KJSK- 56	14.0	3.45	11.30	1.23	2.58	5.79	0.07	0.027	+	+	+	-	+	-
KJSK-57	0.6	3.30	6.00	3.73	8.99	4.80	0.38	0.042	+	-	+	-	+	+
KJSK-58	0.0	3.48	6.00	3.87	10.58	5.40	0.41	0.039	+	++	-	-	+	+
KJSK- 59	18.8	3.38	-	0.73	0.25	6.51	0.04	0.160	+	+	+	-	+	-
KJSK-60	11.6	3.33	-	1.03	5.43	5.79	0.18	0.033	+	+	+	-	+	-
KJSK- 61	13.2	3.42	6.00	1.33	3.61	5.61	0.13	0.036	-	-	+	+	+	-
KJSK- 62	17.0	3.48	-	1.32	1.45	5.21	0.23	0.159	+	-	+	-	+	-
KJSK-63	11.8	3.45	6.00	1.30	5.04	5.61	0.31	0.062	+	-	+	-	+	-
KJSK-64	12.4	3.35	4.00	1.43	4.54	6.16	0.21	0.046	-	+	+	-	+	-
KJSK- 65	12.0	3.42	10.30	1.17	4.68	7.06	0.16	0.034	+	+	+	-	+	-
KJSK-66	8.8	3.46	-	1.23	5.91	6.41	0.63	0.107	+	+	+	-	+	-
KJSK- 67	9.6	3.19	10.30	1.17	4.68	5.43	0.12	0.026	+	+	+	-	+	-
KJSK- 68	14.8	3.47	-	0.67	3.05	3.61	0.22	0.072	+	+	+	-	+	-
KJSK-69	0.2	3.41	9.30	3.67	10.09	4.92	0.41	0.041	+	-	+	-	+	+
KJSK-70	0.0	3.49	9.30	2.90	11.07	5.65	0.81	0.073	+	-	-	-	-	-
KJSK- 71	17.6	3.24	-	0.80	1.50	7.39	0.48	0.320	-	-	+	+	+	-
KJSK-72	11.4	3.28	10.30	1.23	4.99	5.11	0.56	0.112	+	+	+	-	+	-
KJSK- 73	18.6	3.44	-	0.87	0.88	5.04	0.71	0.807	+	+	+	-	+	-
KJSK- 74	8.8	3.27	6.00	1.33	4.54	5.58	0.49	0.108	+	+	+	-	+	-
KJSK-75	9.2	3.42	-	1.63	4.62	5.80	0.48	0.104	+	++	+	-	+	-
KJSK-76	8.0	3.40	3.30	1.43	4.27	6.07	0.86	0.201	+	+	+	-	+	-
KJSK- 77	12.2	3.47	4.00	1.30	4.13	5.38	0.15	0.036	+	+	+	-	+	-
KJSK-78	10.6	3.48	6.00	1.20	4.22	5.62	0.18	0.043	+	+	+	-	+	-
KJSK- 79	9.8	3.44	-	1.03	1.06	5.16	0.31	0.292	+	-	+	-	+	-

Table 2. (Cont...)

Yeast isolates	Residual sugar intensity (Brix)	Final pH	Initiation of fermentation (hrs. after inoculation)	Fermentation rate (g of CO_2 produced /day)	Fermentation vigour (alcohol content % v/v)	Titrable acidity (g/L of tartaric acid)	Volatile acidity (g/L of acetic acid)	Fermentation purity	Flocculent capacity	SH_2 production	Less foam production	Adherance to glass	Autolytic capacity	Bouquet
KJSK- 80	10.4	3.35	3.30	1.73	4.68	5.23	0.41	0.088	+	+	+	+	+	-
KJSK-81	8.2	3.42	-	0.768	1.96	6.43	0.51	0.260	-	-	+	+	+	-
KJSK-82	10.8	3.45	15.30	1.03	3.16	5.16	0.31	0.098	+	-	+	-	+	-
KJSK- 83	6.0	3.26	-	1.215	3.63	7.38	0.31	0.085	+	-	+	-	+	-
KJSK-84	8.4	3.41	-	0.724	2.07	6.08	0.41	0.198	-	-	+	-	+	-
KJSK- 85	10.4	3.48	-	0.892	2.42	5.16	0.23	0.095	-	-	+	-	+	-
KJSK- 86	9.2	3.36	-	0.676	1.86	5.71	0.71	0.382	-	-	+	+	+	-
KJSK-87	1.2	3.37	9.00	3.90	10.85	4.92	0.39	0.036	+	-	+	-	+	+
KJSK-88	13.8	3.42	-	1.02	3.81	5.38	0.24	0.055	-	-	+	-	+	-
KJSK- 89	15.2	3.28	15.00	0.76	1.89	6.63	0.23	0.122	-	+	+	-	+	-
KJSK-90	0.4	3.32	-	3.33	11.83	5.04	0.81	0.068	+	++	-	+	-	-
KJSK-91	12.0	3.23	4.00	1.567	4.29	5.08	0.78	0.182	+	-	+	-	+	-
KJSK- 92	17.2	3.27	10.00	1.26	1.70	5.73	0.41	0.241	+	++	+	-	+	-
KJSK- 93	16.0	3.21	-	0.64	1.56	7.8	0.43	0.276	-	-	+	-	+	-
KJSK-94	14.6	3.42	-	0.89	2.54	4.15	0.46	0.181	+	+	+	-	+	-
KJSK-95	1.2	3.35	4.30	3.36	10.84	6.00	0.81	0.075	+	+++	-	-	+	-
KJSK- 96	0.0	3.31	4.00	3.39	10.95	5.97	0.61	0.056	+	-	+	-	-	+
KJSK-97	4.2	3.34	4.30	3.20	8.80	5.77	0.20	0.023	+	-	-	-	+	+
KJSK- 98	6.8	3.31	4.30	3.57	6.73	5.56	0.24	0.036	+	++	-	-	+	-
KJSK- 99	2.2	3.37	3.30	3.47	948	6.30	0.58	0.061	+	-	+	-	-	+
KJSK-100	0.4	3.29	2.30	4.40	10.50	6.39	0.20	0.019	+	-	+	-	+	-
KJSK-101	5.2	3.46	6.30	3.30	8.80	6.06	0.55	0.063	+	-	+	-	+	-
KJSK- 102	4.6	3.37	6.00	3.43	6.50	5.80	0.43	0.066	+	+	-	-	+	-
KJSK-103	14.8	3.02	8.00	0.892	2.54	5.62	0.86	0.339	+	+	+	-	+	-
KJSK- 104	1.6	3.43	4.00	3.40	7.35	6.43	0.35	0.048	+	+	-	-	+	+
KJSK- 105	2.0	3.25	3.00	3.16	9.84	4.42	0.37	0.038	+	-	-	-	+	-
KJSK-106	0.2	3.43	8.30	3.40	11.78	5.88	0.72	0.061	+	-	+	-	+	+
KJSK-107	2.4	3.31	6.30	3.53	6.08	6.55	0.16	0.026	+	++	+	-	+	-
KJSK- 108	2.8	3.37	7.00	3.47	8.84	5.94	0.42	0.048	+	++	-	+	+	-
KJSK-109	10.6	3.32	5.30	3.20	5.41	5.61	0.78	0.144	+	+++	+	-	+	-
KJSK- 110	0.6	3.30	5.30	3.70	10.69	6.34	0.65	0.064	+	-	-	-	-	+
KJSK- 111	3.2	3.39	5.30	3.00	8.79	4.68	0.32	0.036	+	++	-	-	+	-
KJSK-112	0.2	3.33	4.00	3.80	11.80	6.39	0.46	0.039	+	++	-	+	-	+
KJSK-113	2.0	3.32	3.30	3.43	6.27	5.73	0.86	0.137	+	+	+	-	+	-
KJSK- 114	0.6	3.27	5.30	3.56	10.88	5.70	0.43	0.040	+	++	-	-	-	-
KJSK-115	11.6	3.21	8.30	1.23	5.01	5.88	0.58	0.116	+	+	+	-	+	-
KJSK- 116	12.8	3.51	3.00	1.53	4.72	6.15	0.41	0.087	+	+	+	+	+	-
KJSK- 117	13.0	3.25	3.30	0.57	3.99	6.04	0.81	0.203	+	+	+	+	+	-

Table 2. (Cont...)

Yeast isolates	Residual sugar intensity (Brix)	Final pH	Initiation of fermentation (hrs. after inoculation)	Fermentation rate (g of CO_2 produced /day)	Fermentation vigour (alcohol content % v/v)	Titrable acidity (g/L of tartaric acid)	Volatile acidity (g/L of acetic acid)	Fermentation purity	Flocculent capacity	SH_2 production	Less foam production	Adherance to glass	Autolytic capacity	Bouquet
KJSK-118	18.8	3.24	-	0.20	0.60	5.73	0.86	1.433	-	-	+	+	+	-
KJSK-119	14.6	3.34	-	0.71	2.46	7.05	0.58	0.236	-	-	+	+	+	-
KJSK- 120	12.8	3.25	-	0.80	2.86	6.42	0.49	0.171	+	-	+	-	+	-
KJSK-121	4.8	3.30	3.30	3.43	8.93	5.43	0.84	0.094	+	-	-	-	-	+
KJSK- 122	12.2	3.50	4.00	1.13	3.93	5.92	0.51	0.130	+	+	+	+	+	-
S.ellipsoideus CFTRI 101	3.2	3.31	5.30	3.03	8.89	5.01	0.38	0.043	+	++	+	-	+	+
S.ellipsoideus MTCC 180	6.8	3.32	5.30	2.83	7.68	5.04	0.41	0.053	+	++	+	-	+	-
S. cereviriae (Baker's yeast)	5.6	3.39	5.30	3.23	6.08	5.58	0.82	0.134	+	+++	-	-	+	-
SEd	1.319	0.0816	-	0.414	0.9404	0.5003	0.0814	0.0152	-	-	-	-	-	-
CD (0.05)	2.717	0.1681	-	0.852	1.9369	1.0304	0.1675	0.0324	-	-	-	-	-	-

a – '+' positive '-' negative character

b – based on intensity of blackening scored as + -less, ++ -high, +++ - very high SH_2 producers.

Table 18. Higher alcohols, esters and aldehydes produced by the selected yeast isolates

Yeast isolates	Methanol (mg/l)	Isopropanol (mg /l)	n-propanol (mg/l)	Isobutanol (mg/l)	Isoamyll alcohol (mg/l)	**Total higher alcohol (mg/l)**	Acetal-aldehyde (mg/l)	Ethyl acetate (mg/l)
Hansenula sp. KJSK-11	0.0	0	24.26	6.95	33.86	**65.07**	32.16	5.62
Candida oleophila KJSK-29	0.0	0	21.46	18.12	42.45	**82.03**	30.73	8.23
Saccharomyces cerevisiae KJSK-37	9.56	16.59	6.19	15.94	33.90	**82.18**	0.00	6.28
Kluyveromyces marxianus KJSK-49	0.00	2.23	9.13	15.92	47.67	**74.95**	28.23	6.85
Saccharomyces cerevisiae KJSK-57	0.0	0	6.71	14.21	53.92	**74.84**	29.42	7.59
Candida maynoliae KJSK-58	0.0	0	58.21	22.57	4.98	**85.76**	46.42	15.20
Hansenula anomala KJSK-69	0.0	0	31.52	26.21	68.61	**126.34**	29.36	8.97
Candida stellata KJSK-70	1.46	0	51.10	37.5	48.33	**138.39**	25.67	11.09
*Saccharomyces cerevisiae*KJSK-87	0.0	0	0.0	21.61	51.59	**73.20**	27.63	7.35
Candida intermedia KJSK-90	4.91	0	29.25	50.13	44.06	**128.35**	35.16	11.87
Yeast isolate KJSK-95	0.0	0	7.82	30.46	56.73	**95.01**	25.93	13.45
Saccharomyces cerevisiae KJSK-96	0.0	0	12.96	18.49	42.42	**73.87**	32.31	5.13
Saccharomyces capensis KJSK-97	5.34	4.84	0	0	12.25	**22.43**	0.00	7.26
Saccharomyces cerevisiae KJSK-99	0.0	0	17.48	40.57	67.78	**125.83**	12.41	7.38
Saccharomyces bayanus KJSK-100	0.0	0	22.24	25.30	67.36	**114.90**	31.63	14.92
Saccharomyces ellipsoideus KJSK-101	0.99	0	24.85	33.42	62.41	**121.67**	28.38	12.41
Saccharomyces bayanus KJSK-105	0.0	0	20.43	68.32	15.63	**104.38**	0.00	8.44
Saccharomyces ellipsoideus KJSK-106	0.0	0	22.89	23.12	86.86	**132.87**	195.26	8.21
Saccharomyces ludwigi KJSK-108	0.0	0	21.56	31.42	92.63	**145.61**	85.15	6.25
Saccharomyces capensis KJSK-110	0.0	0	18.21	25.63	52.41	**96.25**	105.24	12.85
Kluyveromyces thermotolerans KJSK-111	0.0	0	31.57	17.36	58.21	**107.14**	84.21	9.46
Candida stellata KJSK-112	0.0	0	39.65	71.42	58.78	**169.85**	0.00	12.72
Yeast isolate KJSK-114	0.0	0	11.15	48.21	92.41	**151.77**	92.67	11.26
*Torulaspora delbrueckii*KJSK-121	0.0	0	15.21	46.32	12.83	**74.36**	20.41	9.85
Saccharomyces ellipsoideus CFTRI 101	0.0	0	11.07	15.77	48.37	**75.21**	98.42	6.32
Saccharomyces ellipsoideus MTCC 180	9.00	0	9.21	10.27	30.67	**59.15**	87.31	7.22
SEd	-	-	2.90	2.62	3.15	-	14.00	1.17
CD (0.05)	-	-	5.98	5.32	6.52	-	28.83	3.43

Table 9. Fermentation characteristics of the selected osmotolerant yeast isolates

Sugar level (Brix)	Yeast isolates	Fermentation time (days)	% ethanol v/v	Weight loss (g of CO_2 produced/100ml)	Residual sugar (g/100ml)
20B°	*S. cerevisiae* KJSK-37	3	10.65	9.86	3.71
	K. marxianus KJSK-49	5	10.21	9.42	2.84
	S. cerevisiae KJSK-96	3	10.23	9.89	2.20
	*S.bayanus*KJSK-105	6	9.31	9.38	3.69
	S. ellipsoideus KJSK-101	5	8.89	9.52	4.33
30B°	*S. cerevisiae* KJSK-37	5	12.63	11.07	6.24
	K. marxianus KJSK-49	9	13.85	11.28	8.21
	S. cerevisiae KJSK-96	5	14.37	12.41	3.08
	S.bayanus KJSK-105	8	9.26	10.62	12.62
	S. ellipsoideus KJSK-101	7	9.21	8.69	10.18
40B°	*S. cerevisiae* KJSK-37	10	11.48	10.21	18.14
	K. marxianus KJSK-49	17	10.21	9.42	19.55
	S. cerevisiae KJSK-96	7	18.92	18.02	5.37
	S.bayanus KJSK-105	22	4.18	3.42	31.16
	S. ellipsoideus KJSK-101	15	6.25	7.16	24.41
50B°	*S. cerevisiae* KJSK-37	21	6.21	7.48	33.76
	K. marxianus KJSK-49	26	5.82	4.59	37.75
	S. cerevisiae KJSK-96	18	23.63	22.47	7.21
	S.bayanus KJSK-105	-	-	-	-
	S. ellipsoideus KJSK-101	25	4.26	3.81	39.62
60B°	*S. cerevisiae* KJSK-37	-	1.6	1.24	55.73
	K. marxianus KJSK-49	-	-	-	-
	S. cerevisiae KJSK-96	23	12.41	10.42	36.71
	*S.bayanus*KJSK-105	-	-	-	-
	S. ellipsoideus KJSK-101	-	-	-	-
70B°	*S. cerevisiae* KJSK-37	-	-	-	-
	K. marxianus KJSK-49	-	-	-	-
	S. cerevisiae KJSK-96	28	2.13	1.86	63.22
	S.bayanus KJSK-105	-	-	-	-
	S. ellipsoideus KJSK-101	-	-	-	-

		SEd	CD (0.05)	SEd	CD (0.05)	SEd	CD (0.05)	SEd	CD (0.05)
Culture		0.28	0.56	0.61	1.23	10.93	22.09	1.53	3.14
Brix		0.31	0.61	0.55	1.10	9.77	19.75	1.19	2.43
Culture x Brix		0.68	1.37	1.22	2.48	21.86	44.19	2.66	5.44

Temperature	Yeast isolates	Fermentation time (days)	% ethanol v/v	Weight loss (g of CO_2 produced/100ml)	Residual sugar (Brix)
40°C	*H. anomala* KJSK-69	-	-	-	20.0

	S. bayanus KJSK-100	-		-		-		20.0	
	S. cerevisiae KJSK-87	22		1.22		2.13		15.8	
	K. thermotolerans KJSK-111	18		5.31		6.41		8.6	
	S. ellipsoideus CFTRI-101	23		1.67		1.98		16.2	
35°C	*H. anomala* KJSK-69	12		2.35		3.12		12.4	
	S. bayanus KJSK-100	11		4.69		5.61		6.0	
	S. cerevisiae KJSK-87	9		7.03		7.89		2.2	
	K. thermotolerans KJSK-111	7		8.18		9.23		0.6	
	S. ellipsoideus CFTRI-101	11		6.42		6.84		3.6	
30°C	*H. anomala* KJSK-69	2		10.25		9.81		0.2	
	S. bayanus KJSK-100	2		10.16		8.89		0.2	
	S. cerevisiae KJSK-87	3		10.64		9.21		0.8	
	K. thermotolerans KJSK-111	3		8.27		8.12		1.2	
	S. ellipsoideus CFTRI-101	2		8.85		7.52		0.8	
25°C	*H. anomala* KJSK-69	3		11.76		10.02		0.0	
	S. bayanus KJSK-100	3		12.09		10.58		0.0	
	S. cerevisiae KJSK-87	5		11.14		11.45		0.0	
	K. thermotolerans KJSK-111	8		10.92		11.41		0.4	
	S. ellipsoideus CFTRI-101	5		9.34		9.52		0.8	
20°C	*H. anomala* KJSK-69	5		12.45		9.41		0.8	
	S. bayanus KJSK-100	5		12.39		9.35		0.2	
	S. cerevisiae KJSK-87	9		12.01		9.26		0.4	
	K. thermotolerans KJSK-111	18		8.13		7.70		3.8	
	S. ellipsoideus CFTRI-101	11		9.62		8.41		2.2	
15°C	*H. anomala* KJSK-69	12		10.86		8.32		3.2	
	S. bayanus KJSK-100	14		10.33		8.04		4.0	
	S. cerevisiae KJSK-87	21		9.85		7.57		4.8	
	K. thermotolerans KJSK-111	-		-		-		19.8	
	S. ellipsoideus CFTRI-101	17		7.21		6.55		7.4	
10°C	*H. anomala* KJSK-69	23		5.6		4.21		11.6	
	S. bayanus KJSK-100	24		3.4		3.15		14.2	
	S. cerevisiae KJSK-87	28		1.1		2.20		16.8	
	K. thermotolerans KJSK-111	-		-		-		19.8	
	S. ellipsoideus CFTRI-101	-		-		-		20.0	
		SEd	CD (0.05)	SEd	CD (0.05)	SEd	CD (0.05)	SEd	CD (0.05)
Culture		0.26	0.59	0.58	1.16	8.25	17.15	3.04	6.11
Temperature		0.29	0.65	0.52	1.08	7.47	15.63	3.03	6.10
C x T		0.63	1.34	1.17	2.33	17.26	38.21	6.79	13.65

Table 13. Fermentation characteristics of the selected acid tolerant yeast isolates

pH	Yeast isolates	Fermentation time (days)	Final pH	% ethanol v/v	Weight loss (g of CO_2 produced/100ml)	Residual sugar (Brix)
5.0	*C. oleophila* KJSK-29	3	3.25	7.36	6.41	5.6

	C. intermedia KJSK-90	3	3.16	10.25	8.28	2.4
	H. anomala KJSK-69	3	3.61	10.86	9.21	0.8
	S. ellipsoideus KJSK-106	2	3.68	11.23	8.96	1.2
	S. ellipsoideus CFTRI 101	3	3.52	8.46	7.51	3.2
4.5	*C. oleophila* KJSK-29	3	3.22	7.25	6.38	6.2
	C. intermedia KJSK-90	3	3.28	10.16	9.36	1.2
	H. anomala KJSK-69	3	3.56	10.71	9.41	0.4
	S. ellipsoideus KJSK-106	2	3.51	11.02	9.67	0.0
	S. ellipsoideus CFTRI 101	3	3.48	8.62	7.21	2.8
4.0	*C. oleophila* KJSK-29	3	3.10	7.08	6.21	6.8
	C. intermedia KJSK-90	3	3.15	10.12	9.41	0.4
	H. anomala KJSK-69	2	3.41	11.32	9.86	0.2
	S. ellipsoideus KJSK-106	2	3.58	11.01	9.41	0.6
	S. ellipsoideus CFTRI 101	3	3.45	8.65	7.36	3.0
3.5	*C. oleophila* KJSK-29	3	3.18	7.61	6.86	5.8
	C. intermedia KJSK-90	3	3.22	10.15	9.26	0.6
	H. anomala KJSK-69	2	3.41	11.36	9.82	0.0
	S. ellipsoideus KJSK-106	2	3.38	11.25	9.48	0.2
	S. ellipsoideus CFTRI 101	3	3.46	9.06	8.07	2.6

Table 13. Fermentation characteristics of the selected acid tolerant yeast isolates (Contd...)

pH	Yeast isolates	Fermentation time (days)	Final pH	% ethanol V/V	Weight loss (g of CO_2 produced/100ml)	Residual sugar (Brix)
3.0	*C. oleophila* KJSK-29	5	2.81	7.41	6.78	5.2
	C. intermedia KJSK-90	5	2.76	10.36	9.36	0.8
	H. anomala KJSK-69	5	3.06	11.13	10.06	1.2
	S. ellipsoideus KJSK-106	5	2.97	11.23	9.78	1.4
	S. ellipsoideus CFTRI 101	6	3.01	8.68	7.32	3.4
2.5	*C. oleophila* KJSK-29	8	2.46	6.20	5.21	7.0
	C. intermedia KJSK-90	5	2.47	9.46	8.36	3.2
	H. anomala KJSK-69	12	2.55	8.13	7.21	5.2
	S. ellipsoideus KJSK-106	15	2.71	8.01	7.06	4.0
	S. ellipsoideus CFTRI 101	15	2.56	7.25	6.21	5.8
2.0	*C. oleophila* KJSK-29	11	2.12	3.45	2.86	12.8
	C. intermedia KJSK-90	7	2.03	5.62	4.21	9.6
	H. anomala KJSK-69	-	2.00	-	-	-
	S. ellipsoideus KJSK-106	-	2.00	-	-	-
	S. ellipsoideus CFTRI 101	-	2.00	-	-	-

	SEd	CD (0.05)	SEd	CD (0.05)	SEd	CD (0.05)	SEd	CD (0.05)	SEd	CD (0.05)
Culture	0.21	0.42	0.05	0.10	0.22	0.47	3.58	7.83	2.82	5.73
pH	0.21	0.42	0.05	0.10	0.21	0.45	0.45	9.51	2.86	5.79
Culture x pH	0.47	0.95	0.12	0.23	0.52	10.03	10.30	18.15	6.02	12.15

Table 7. Fermentation kinetics of the selected yeast isolates

Selected isolates	Fermentation time (h)	Starting sugar (g/L)	Residual sugar (g/L)	Sugar utilized (g/L)	Ethanol (% v/v)	Yeth (mL/g) (Ethanol yield)	Rate of fermentation in hourly intervals (g of CO_2 produced /hr interval)*					Fermentation rate (g of CO /day)	Biomass (g/L	Specific growth rate (h^{-1})	Ethanol Productivity ml/L/hr
							I	II	III	IV	V				
Hansenula sp. KJSK-11	102	200	20.6	179.4	9.32	0.520	0.126	0.145	0.166	0.094	0.157	3.433	2.472	0.024	0.913
Candida oleophila KJSK-29	91	200	43.8	156.2	7.51	0.481	0.128	0.107	0.066	0.115	0.100	3.000	2.952	0.032	0.825
Saccharomyces cerevisiae KJSK-37	98	200	18.3	181.7	8.82	0.485	0.356	0.098	0.133	0.115	0.400	3.766	3.296	0.034	0.900
Kluyveromyces marxianus KJSK-49	59	200	85.6	114.4	7.54	0.659	0.092	0.056	0.066	0.036	0.085	2.033	3.320	0.056	1.277
Saccharomyces cerevisiae KJSK-57	53	200	18.3	181.7	9.99	0.550	0.217	0.196	0.166	0.121	0.257	3.733	2.972	0.056	1.885
Candida maynoliae KJSK-58	63	200	12.3	187.7	10.5	0.559	0.128	0.098	0.066	0.142	0.100	3.866	2.642	0.042	1.667
Hansenula anomala KJSK-69	56	200	22.3	177.7	10.78	0.607	0.112	0.138	0.133	0.126	0.142	3.666	2.400	0.043	1.925
Candida stellata KJSK-70	97	200	28.4	171.6	10.07	0.587	0.468	0.098	0.333	0.052	0.114	2.900	1.668	0.017	1.038
*Saccharomyces cerevisiae*KJSK-87	51	200	14.7	185.3	10.65	0.575	0.120	0.089	0.066	0.136	0.142	3.000	2.560	0.050	2.088
Candida intermedia KJSK-90	73	200	36.2	163.8	10.63	0.645	0.250	0.032	0.000	0.142	0.100	3.333	2.526	0.035	1.456
Yeast isolate KJSK-95	97	200	14.1	185.9	10.34	0.556	0.052	0.315	0.000	0.073	0.100	3.633	3.260	0.034	1.065
Saccharomyces cerevisiae KJSK-96	48	200	11.9	188.1	10.34	0.550	0.132	0.148	0.017	0.100	0.100	3.933	3.500	0.073	2.154
Saccharomyces capensis KJSK-97	102	200	42.1	157.9	8.86	0.561	0.089	0.076	0.033	0.094	0.085	3.200	2.864	0.028	0.868
Saccharomyces cerevisiae KJSK-99	68	200	31.6	168.4	9.17	0.545	0.126	0.107	0.133	0.137	0.085	3.566	2.546	0.037	1.348
Saccharomyces bayanus KJSK-100	48	200	13.8	186.2	10.55	0.567	0.112	0.106	0.100	0.137	0.100	4.400	4.206	0.088	2.197
Saccharomyces ellipsoideus KJSK-101	54	200	43.1	156.9	8.89	0.567	0.067	0.089	0.066	0.084	0.085	3.300	3.160	0.059	1.646
Saccharomyces bayanus KJSK-105	67	200	26.7	173.3	9.24	0.533	0.121	0.096	0.133	0.073	0.114	3.400	2.044	0.031	1.379
Saccharomyces ellipsoideus KJSK-106	49	200	32.5	167.5	10.7	0.639	0.123	0.153	0.133	0.115	0.0171	3.533	2.726	0.056	2.183
Saccharomyces ludwigi KJSK-108	91	200	26.1	173.9	9.34	0.537	0.156	0.256	0.400	0.047	0.071	3.466	3.066	0.034	1.026
Saccharomyces capensis KJSK-110	85	200	16.4	181.6	10.67	0.588	0.126	0.113	0.166	0.110	0.085	3.000	2.120	0.025	1.255
Kluyveromyces thermotolerans KJSK-111	78	200	41.7	158.3	8.79	0.555	0.142	0.065	0.033	0.084	0.142	3.800	2.382	0.031	1.127
Candida stellata KJSK-112	93	200	24.8	175.2	10.06	0.574	0.113	0.156	0.100	0.121	0.171	3.433	2.988	0.032	1.081
Yeast isolate KJSK-114	73	200	18.2	181.8	10.06	0.553	0.123	0.326	0.100	0.131	0.00	3.566	2.640	0.036	1.378
*Torulaspora delbruechii*KJSK-121	95	200	16.4	183.6	8.72	0.475	0.251	0.081	0.000	0.178	0.213	2.133	2.360	0.025	0.917
Saccharomyces ellipsoideus CFTRI 101	113	200	46.5	153.5	8.59	0.560	0.096	0.072	0.330	0.084	0.114	3.033	3.026	0.027	0.760
Saccharomyces ellipsoideus MTCC 180	98	200	68.7	131.3	7.03	0.535	0.098	0.152	0.166	0.047	0.071	2.833	3.200	0.033	0.717
S. cererisae (*baker's yeast)*	63	200	57.6	142.4	6.93	0.487	0.112	0.098	0.100	0.063	0.071	2.833	4.260	0.068	1.108
SEd	1.03	-	-	6.55	0.49	0.02	-	-	-	-	-	0.24	0.35	0.004	0.25
CD (0.05)	2.12	-	-	13.50	1.01	0.05	-	-	-	-	-	0.50	0.72	0.008	0.51

* reading were taken 24h after inoculation.

Table 23. Effect of total soluble solids on banana wine making

Treatments		Alcohol content (%)	Titrable acidity (g tartaric acid /100ml)	Volatile acidity (g acetic acid /100 ml)	TSS (Brix)	pH	Reducing sugar (g/L)	Tannins (mg/L)	Organoleptic score
S_0	Y_1	6.83	0.88	0.046	0.8	4.12	2.82	656	14.25
	Y_2	7.13	0.68	0.052	1.2	4.06	1.26	852	14.25
	Y_3	6.91	1.16	0.065	2.2	4.10	1.15	856	13.75
	Y_4	7.41	0.85	0.043	2.2	4.08	1.85	688	14.00
	Y_5	7.86	1.06	0.065	0.2	3.98	1.56	468	13.75
	Y_6	6.86	0.85	0.043	0.6	4.02	3.21	675	12.25
	Y_7	6.16	1.06	0.043	0.2	4.02	1.80	645	14.50
S_1	Y_1	9.06	0.98	0.024	1.0	3.96	2.46	825	15.50
	Y_2	9.01	0.87	0.064	0.2	4.12	1.63	654	16.00
	Y_3	8.87	1.05	0.052	1.2	4.12	1.28	887	16.50
	Y_4	9.16	1.06	0.063	0.2	4.07	2.16	821	15.75
	Y_5	8.15	0.98	0.072	1.2	4.08	1.63	726	16.50
	Y_6	7.15	0.88	0.045	0.8	3.98	3.16	754	14.50
	Y_7	8.73	1.08	0.056	1.2	4.16	1.96	621	14.50
S_2	Y_1	10.12	1.26	0.078	0.6	4.05	3.21	876	16.50
	Y_2	9.67	0.97	0.096	0.2	4.07	1.82	975	15.75
	Y_3	10.13	1.24	0.082	0.8	3.98	2.15	924	16.00
	Y_4	10.11	0.85	0.088	0.8	3.98	2.08	826	18.00
	Y_5	8.47	1.02	0.068	3.8	4.02	1.82	624	15.75
	Y_6	7.78	0.96	0.063	4.2	4.08	3.18	851	15.00
	Y_7	10.15	1.16	0.086	0.8	4.07	2.05	986	15.25

Table 23. Effect of total soluble solids on banana wine making (Contd...)

Treatments		Alcohol content (%)	Titrable acidity (g tartaric acid /100ml)	Volatile acidity (g acetic acid /100 ml)	TSS (Brix)	pH	Reducing sugar (g/L)	Tannins (mg/L)	Organoleptic score
S_3	Y_1	10.65	1.08	0.097	1.8	3.85	3.26	926	16.50
	Y_2	10.08	1.03	0.105	0.8	3.88	2.16	1076	16.50
	Y_3	11.02	1.26	0.102	1.2	3.82	3.26	1426	18.00
	Y_4	10.42	0.98	0.084	4.2	3.68	2.18	1056	18.50
	Y_5	9.41	1.06	0.086	4.2	4.06	1.98	896	16.00
	Y_6	8.13	1.08	0.126	2.8	3.92	3.86	1564	18.50
	Y_7	10.68	1.02	0.079	4.2	3.98	2.07	1021	18.00
S_4	Y_1	10.93	1.21	0.156	2.6	3.76	3.56	1268	16.00
	Y_2	10.14	1.06	0.126	4.2	3.82	2.15	1824	15.50
	Y_3	11.26	1.26	0.099	1.2	3.96	4.25	1543	18.50
	Y_4	11.12	1.02	0.089	0.8	3.82	3.25	1241	18.50
	Y_5	10.81	1.12	0.132	1.8	3.96	3.21	1249	18.00
	Y_6	8.84	1.06	0.098	4.8	3.85	3.38	1254	18.50
	Y_7	10.4	1.12	0.078	2.2	3.9	2.38	1486	18.50
S_5	Y_1	9.52	2.16	0.326	8.4	3.86	4.63	927	14.25
	Y_2	9.86	2.86	0.286	8.6	3.96	3.82	1126	15.25
	Y_3	12.08	3.2	0.285	4.2	3.82	7.81	2268	18.50
	Y_4	10.68	2.65	0.185	5.2	3.76	6.31	1158	16.75
	Y_5	10.02	3.45	0.385	4.2	3.82	4.86	1165	15.75
	Y_6	8.26	2.82	0.306	6.6	3.82	5.63	856	17.50
	Y_7	9.82	2.62	0.213	5.2	3.92	4.56	1126	16.25
SxY	SEd	0.4152	0.1125	0.0061	0.6814	0.1341	0.4161	102.62	0.8641
	CD	0.9263	0.2642	0.0126	1.8263	0.2862	0.9821	263.41	1.9312

Table 24. Effect of total soluble solids on papaya wine making

Treatments		Alcohol content (%)	Titrable acidity (g tartaric acid /100ml)	Volatile acidity (g acetic acid /100 ml)	TSS (Brix)	pH	Reducing sugar (g/L)	Tannins (mg/L)	Organoleptic score
S_0	Y_1	5.82	0.86	0.042	3.8	4.24	1.25	210	11.50
	Y_2	5.27	0.78	0.038	2.0	4.46	0.98	286	10.75
	Y_3	5.15	0.73	0.056	2.6	4.28	1.62	362	10.50
	Y_4	5.92	0.92	0.047	1.2	4.37	1.42	252	11.50
	Y_5	6.17	0.68	0.039	0.8	4.4	1.45	186	12.50
	Y_6	6.20	0.76	0.087	0.2	4.28	1.68	242	10.50
	Y_7	4.28	0.92	0.063	4.2	4.33	1.24	286	10.50
S_1	Y_1	8.23	0.83	0.049	4.2	4.11	1.42	186	11.50
	Y_2	8.78	0.82	0.041	4.2	4.3	1.48	306	12.50
	Y_3	8.62	0.76	0.072	2.0	4.14	1.76	372	13.75
	Y_4	8.26	0.98	0.068	1.8	4.28	1.58	358	12.50
	Y_5	8.38	0.72	0.043	2.2	4.36	1.38	192	10.75
	Y_6	7.86	0.78	0.083	5.0	4.21	1.82	263	13.50
	Y_7	8.13	0.98	0.068	2.2	4.31	1.28	293	12.50
S_2	Y_1	9.06	0.92	0.052	4.0	4.13	1.46	225	12.75
	Y_2	9.46	0.86	0.073	0.4	4.31	1.46	295	14.50
	Y_3	9.15	0.68	0.068	2.2	4.12	1.72	420	15.00
	Y_4	9.06	0.96	0.063	2.2	4.23	1.92	346	13.50
	Y_5	9.7	0.73	0.072	2.8	4.24	1.63	207	12.50
	Y_6	8.01	0.83	0.092	4.2	4.22	1.63	258	13.25
	Y_7	8.41	1.07	0.09	3.8	4.26	1.46	296	14.50

Table 24. Effect of total soluble solids on papaya wine making (Contd...)

Treatments		Alcohol content (%)	Titrable acidity (g tartaric acid /100ml)	Volatile acidity (g acetic acid /100 ml)	TSS (Brix)	pH	Reducing sugar (g/L)	Tannins (mg/L)	Organoleptic score
S_3	Y_1	9.25	0.9	0.063	2.6	4.06	1.38	306	15.50
	Y_2	9.82	0.98	0.082	2.4	4.23	1.82	288	14.75
	Y_3	9.71	0.82	0.086	0.8	4.06	1.96	426	14.75
	Y_4	9.42	1.03	0.096	2.2	4.20	2.07	386	14.00
	Y_5	9.87	0.79	0.08	2.2	4.27	1.67	213	14.75
	Y_6	8.45	0.99	0.098	6.2	4.17	1.72	296	14.25
	Y_7	9.68	1.32	0.093	1.8	4.21	1.82	312	14.50
S_4	Y_1	9.42	0.96	0.058	3.2	4.05	1.85	320	16.25
	Y_2	10.16	0.92	0.086	4.2	4.20	1.87	328	15.00
	Y_3	10.03	0.99	0.107	2.2	3.97	2.52	432	15.25
	Y_4	9.68	1.17	0.111	4.8	4.16	2.03	572	15.50
	Y_5	10.61	0.92	0.083	1.6	4.24	1.96	346	14.50
	Y_6	8.61	0.98	0.096	5.8	4.19	1.98	322	13.75
	Y_7	10.13	1.36	0.123	4.2	4.18	1.98	363	15.25
S_5	Y_1	8.26	1.07	0.092	8.2	3.86	2.65	325	15.50
	Y_2	9.23	1.21	0.109	6.2	3.98	3.58	316	15.25
	Y_3	10.13	1.17	0.132	4.6	3.86	3.06	422	15.00
	Y_4	9.46	1.72	0.198	6.8	3.80	2.36	568	15.25
	Y_5	9.26	1.02	0.113	4.6	4.01	2.78	425	15.00
	Y_6	8.43	1.12	0.126	7	3.87	3.06	637	14.50
	Y_7	9.76	1.48	0.183	6.2	3.91	3.21	516	13.75
SxY	SEd	0.6312	0.1263	0.0216	0.8163	0.0413	0.4312	102.64	0.9638
	CD	1.4831	0.2426	0.0531	1.7263	0.1302	0.7806	321.46	1.8929

Table 25. Effect of total soluble solids on grape wine making

Treatments		Alcohol content (%)	Titrable acidity (g tartaric acid /100ml)	Volatile acidity (g acetic acid /100 ml)	TSS (Brix)	pH	Reducing sugar (g/L)	Tannins (mg/L)	Organoleptic score
S_0	Y_1	8.82	0.63	0.047	0.6	3.35	3.5	1315	15.75
	Y_2	6.67	0.69	0.036	1.6	3.63	6.05	1126	17.25
	Y_3	8.28	0.83	0.046	2.4	3.45	4.3	1019	14.50
	Y_4	8.98	0.78	0.076	0.4	3.56	6.3	1924	18.00
	Y_5	6.94	0.69	0.041	0.2	3.56	3.55	1152	16.50
	Y_6	7.26	0.78	0.058	1.2	3.63	3.65	1216	16.25
	Y_7	7.12	0.75	0.053	1.8	3.42	3.8	614	16.25
S_1	Y_1	8.93	0.78	0.058	1.6	3.23	4.3	1681	17.75
	Y_2	8.66	0.72	0.041	3	3.31	4.3	1142	17.00
	Y_3	8.99	0.84	0.063	3.4	3.34	4.6	1315	17.25
	Y_4	9.71	0.82	0.079	1.2	3.66	2.3	1963	18.25
	Y_5	8.26	0.72	0.049	1.2	3.46	3.8	1145	16.25
	Y_6	8.57	0.86	0.059	2.6	3.46	3.55	1815	18.25
	Y_7	7.93	0.79	0.074	1.2	3.68	4.6	915	16.50
S_2	Y_1	9.88	0.76	0.06	2.2	3.18	2.05	1671	17.50
	Y_2	9.77	0.8	0.048	3.2	3.23	8.1	1261	18.50
	Y_3	9.41	0.9	0.072	4	3.32	3.9	1615	17.00
	Y_4	10.67	0.87	0.091	1.2	3.67	4.55	1315	18.25
	Y_5	9.43	0.75	0.06	2.6	3.38	4.55	1641	18.00
	Y_6	8.33	0.87	0.063	2.8	3.51	4.3	1361	18.50
	Y_7	9.86	0.81	0.08	3.2	3.46	3.15	1214	18.00

Table 25. Effect of total soluble solids on grape wine making (Contd...)

Treatments		Alcohol content (%)	Titrable acidity (g tartaric acid /100ml)	Volatile acidity (g acetic acid /100 ml)	TSS (Brix)	pH	Reducing sugar (g/L)	Tannins (mg/L)	Organoleptic score
S_3	Y_1	10.61	0.97	0.076	2.4	3.24	4.3	1820	18.50
	Y_2	10.18	0.96	0.063	4.8	3.16	6.95	1421	17.50
	Y_3	10.73	0.96	0.098	4.8	3.12	6.2	1816	18.50
	Y_4	11.57	0.95	0.098	1.4	3.41	3.6	1215	18.00
	Y_5	10.68	0.87	0.072	4	3.12	4.8	1925	18.50
	Y_6	8.88	0.91	0.073	7.8	3.42	5.6	1814	18.00
	Y_7	10.11	0.82	0.096	2.2	3.34	4.8	1824	18.00
S_4	Y_1	12.1	1.21	0.92	3.2	2.96	5.3	1360	18.00
	Y_2	11.31	1.06	0.109	5.2	2.91	6.4	1846	15.75
	Y_3	12.22	0.98	0.112	4.8	2.86	9.1	1263	18.00
	Y_4	12.11	0.98	0.118	3.8	3.32	3.8	1625	16.75
	Y_5	11.12	0.99	0.106	4.2	2.86	7.6	1814	14.50
	Y_6	9.41	0.97	0.088	4.8	3.12	6.4	1125	18.25
	Y_7	11.6	1.03	0.106	4.8	3.23	5.35	1162	18.50
S_5	Y_1	10.82	1.26	0.181	6.6	3.06	6.3	1316	16.00
	Y_2	10.12	2.82	0.216	2.2	3.06	7.3	1512	14.50
	Y_3	12.68	1.17	0.365	5.6	3.01	6.05	1521	16.50
	Y_4	11.26	10.02	0.207	5.8	3.12	4.6	1216	14.00
	Y_5	10.23	1.07	0.126	5	2.96	9.1	1656	16.00
	Y_6	2.62	0.99	0.191	6.2	3.48	7.05	1691	16.25
	Y_7	10.68	1.1	0.262	6	3.42	8.65	1100	17.75
SxY	SEd	0.3214	0.2615	0.0132	0.3841	0.076	1.832	182.42	0.8561
	CD	0.6816	0.4521	0.0362	0.7463	0.018	4.216	372.16	1.9315

Table 29. Effect of different acidifiers on banana wine making

Treatments		Alcohol content (%)	Titrable acidity (g tartaric acid /100ml)	Volatile acidity (g acetic acid /100 ml)	TSS (Brix)	pH	Reducing sugar (g/L)	Tannins (mg/L)	Organoleptic score
A_0	Y_1	8.41	1.23	0.132	6.8	4.26	3.26	1026	16.75
	Y_2	8.63	1.41	0.185	8.2	4.07	2.18	975	16.25
	Y_3	9.24	1.67	0.103	6.8	3.98	3.21	1028	17.75
	Y_4	8.14	1.81	0.123	4.6	4.12	3.87	1152	16.25
	Y_5	8.47	1.41	0.086	6.8	3.92	3.45	987	17.75
	Y_6	8.42	1.86	0.152	4.8	4.07	4.56	1021	16.25
	Y_7	8.56	1.86	0.107	6.8	3.94	4.62	1078	16.00
A_1	Y_1	9.23	1.48	0.166	4.2	3.42	3.62	1426	18.50
	Y_2	9.24	1.62	0.189	4.0	3.38	4.12	1125	17.00
	Y_3	10.86	2.08	0.158	4.2	3.48	5.62	1262	17.25
	Y_4	10.15	1.96	0.206	5.0	3.41	4.86	1456	18.50
	Y_5	10.16	1.63	0.182	6.4	3.40	6.42	1124	18.75
	Y_6	9.15	1.76	0.183	6.0	3.42	7.23	1127	18.25
	Y_7	10.83	1.67	0.123	1.2	3.43	6.75	1028	16.00

Table 29. Effect of different acidifiers on banana wine making (Contd...)

Treatments		Alcohol content (%)	Titrable acidity (g tartaric acid /100ml)	Volatile acidity (g acetic acid /100 ml)	TSS (Brix)	pH	Reducing sugar (g/L)	Tannins (mg/L)	Organoleptic score
A_2	Y_1	9.26	1.83	0.212	4.2	3.40	6.82	1428	18.00
	Y_2	9.27	2.01	0.207	4.2	3.41	8.62	1072	18.25
	Y_3	9.41	2.12	0.198	6.8	3.45	6.82	1360	17.50
	Y_4	9.28	2.01	0.216	4.3	3.38	4.35	1241	18.00
	Y_5	10.21	2.12	0.283	4.6	3.28	8.36	998	18.50
	Y_6	9.46	1.96	0.237	6.2	3.36	8.87	1846	18.00
	Y_7	10.56	2.18	0.219	3.2	3.42	8.23	1521	17.75
A_3	Y_1	7.18	3.86	0.583	8.9	3.21	2.16	4156	8.75
	Y_2	6.81	4.15	0.426	10.6	3.16	3.18	6843	10.75
	Y_3	7.81	3.98	0.541	8.2	3.34	1.67	5861	10.50
	Y_4	6.46	4.07	0.632	10.7	3.28	2.15	7260	11.25
	Y_5	4.15	4.21	0.515	12.8	3.41	2.18	7451	13.50
	Y_6	7.86	2.83	0.416	10.8	3.20	2.75	6283	9.25
	Y_7	6.83	3.68	0.453	10.2	3.36	3.19	4263	10.25
AxY	SEd	0.4163	0.1316	0.0098	0.7213	0.1241	0.4216	58.63	0.8526
	CD	0.8914	0.3263	0.0185	1.7641	0.3163	0.8913	230.41	1.9124

Table 30. Effect of different acidifiers on papaya wine making

Treatments		Alcohol content (%)	Titrable acidity (g tartaric acid /100ml)	Volatile acidity (g acetic acid /100 ml)	TSS (Brix)	pH	Reducing sugar (g/L)	Tannins (mg/L)	Organoleptic score
A_0	Y_1	9.08	0.87	0.082	6.2	4.12	1.65	286	14.5
	Y_2	9.18	0.73	0.096	4.1	4.06	1.72	198	12.5
	Y_3	9.13	0.65	0.108	5.2	4.11	1.90	326	11.3
	Y_4	9.21	0.78	0.132	5.4	4.28	1.58	216	12.8
	Y_5	9.38	0.71	0.072	6.2	4.26	2.63	298	12.3
	Y_6	8.62	0.83	0.191	8.2	4.15	1.85	426	12.8
	Y_7	9.07	0.96	0.107	1.8	4.32	2.08	216	13.5
A_1	Y_1	9.26	1.08	0.163	5.2	3.46	1.58	312	14.8
	Y_2	9.46	1.76	0.197	5.0	3.38	1.56	326	15.5
	Y_3	9.43	1.42	0.132	4.8	3.43	1.49	310	13.5
	Y_4	9.36	1.38	0.163	5.8	3.41	1.38	358	15.5
	Y_5	9.63	1.48	0.107	4.8	3.43	1.82	307	15.5
	Y_6	9.13	1.58	0.126	3.2	3.46	1.42	348	15.8
	Y_7	9.23	1.27	0.138	4.8	3.52	1.43	482	15.8

Table 30. Effect of different acidifiers on papaya wine making (Contd...)

Treatments		Alcohol content (%)	Titrable acidity (g tartaric acid /100ml)	Volatile acidity (g acetic acid /100 ml)	TSS (Brix)	pH	Reducing sugar (g/L)	Tannins (mg/L)	Organoleptic score
A_2	Y_1	9.18	1.32	0.232	5.4	3.23	1.69	327	10.5
	Y_2	9.32	1.58	0.241	6.4	3.42	2.63	263	10.5
	Y_3	9.28	1.67	0.117	4.2	3.39	1.63	365	12.5
	Y_4	8.76	1.48	0.182	6.2	3.28	1.85	323	11.5
	Y_5	9.14	1.57	0.128	6.4	3.40	1.92	390	12.5
	Y_6	9.07	1.81	0.182	4.2	3.26	1.45	268	10.5
	Y_7	9.16	1.68	0.154	4.6	3.44	1.85	497	12.5
A_3	Y_1	3.32	3.52	0.426	6.8	3.16	1.86	1072	10.3
	Y_2	6.72	4.83	0.563	10.8	3.20	2.53	2318	10.5
	Y_3	8.15	2.58	0.326	8.2	3.17	3.28	1892	10.3
	Y_4	7.86	3.18	0.407	10.2	3.07	5.13	986	12.5
	Y_5	6.84	4.36	0.315	11.2	2.96	4.07	2831	10.5
	Y_6	7.02	3.07	0.286	10.2	3.32	2.16	1967	12.8
	Y_7	6.23	2.81	0.186	12.0	3.01	3.07	1077	10.5
AxY	SEd	0.5263	0.1326	0.0311	0.7341	0.0562	0.3163	92.63	0.9362
	CD	1.2316	0.2912	0.0741	1.62560	0.1126	0.6152	196.14	1.9632

Table 20a. Suitability of commercial varieties of banana for wine making

Variety	TSS (B°)	pH	Wine recovery (%)
Poovan	11.6	4.30	71.13
Robusta	14.4	4.82	66.00
Rasthali	16.2	4.16	60.33
Karpooravalli	12.4	4.89	70.12
Red banana	13.0	5.21	65.30
Peyan	12.4	4.02	64.63
Padathi	14.2	4.07	60.26
Nendran	18.0	5.12	51.42
Matti	16.6	4.23	58.21
SEd	0.69	0.07	2.55
CD	1.48	0.15	5.49

Table 20b. Wine composition

Variety	Alcohol content (%)	Titrable acidity (g tartaric acid /100ml)	Volatile acidity (g acetic acid /100 ml)	TSS (Brix)	pH	Reducing sugar (g/L)	Tannins (mg/L)	Organoleptic score
Poovan	6.36	1.26	0.106	1.2	3.76	1.10	937	14.50
Robusta	7.10	0.91	0.032	1.8	3.84	1.34	1920	17.50
Rasthali	7.86	0.92	0.086	2.2	4.25	1.43	1890	16.75
Karpooravalli	6.21	1.16	0.062	0.8	3.12	1.41	766	14.25
Red banana	6.65	1.49	0.126	0.6	3.79	1.40	1913	15.00
Peyan	6.02	1.36	0.113	1.2	3.67	1.42	1025	13.50
Padathi	6.86	1.40	0.108	0.8	3.92	1.63	1261	14.00
Nendran	8.23	0.96	0.052	2.4	4.18	1.81	826	14.25
Matti	8.12	1.42	0.096	0.2	3.73	1.38	1938	14.50
SEd	0.32	0.14	0.01	0.69	0.12	0.31	93.91	0.79
CD	0.69	0.30	0.01	1.48	0.26	0.66	201.48	1.70

Table 21a. Suitability of commercial varieties of papaya for wine making

Variety	TSS (B°)	pH	Wine recovery (%)
CO 1	12.03	4.22	62.67
CO 2	12.33	3.97	62.33
CO 3	12.66	4.21	56.66
CO 4	12.03	4.22	61.00
CO 5	10.67	3.88	57.33
CO 6	12.67	3.81	52.00
CO 7	12.67	3.89	66.00
SEd	0.54	0.04	1.75
CD	1.17	0.09	3.76

Table 21b. Wine composition

Variety	Alcohol content (%)	Titrable acidity (g tartaric acid /100ml)	Volatile acidity (g acetic acid /100 ml)	TSS (Brix)	pH	Reducing sugar (g/L)	Tannins (mg/L)	Organoleptic score
CO 1	6.60	0.73	0.043	3.80	3.80	2.13	420	13.00
CO 2	7.12	0.92	0.068	1.80	3.96	1.96	606	15.50
CO 3	7.63	0.86	0.032	1.80	3.96	2.51	826	14.50
CO 4	5.91	1.21	0.086	5.20	3.81	1.41	614	10.00
CO 5	5.13	1.21	0.145	2.00	4.06	1.72	673	10.50
CO 6	5.21	1.41	0.152	3.00	3.96	1.81	862	10.50
CO 7	7.24	1.21	0.096	2.60	4.02	2.14	626	14.50
SEd	0.47	0.12	0.026	0.85	0.07	0.22	71.51	0.86
CD	0.99	0.27	0.056	1.83	0.15	0.48	153.40	1.86

Table 22a. Suitability of commercial varieties of grape for wine making juice composition

Variety	TSS (B°)	pH	Juice / Wine recovery (%)
Bangalore blue	18.20	3.81	88.20
Paneer	14.00	3.41	92.30
Thompson seedless	13.40	3.28	94.20
Alguish	14.60	3.91	91.00
Sharad	18.20	3.86	92.30
SEd	0.411	0.0628	2.81
CD	0.882	0.1347	6.03

Table 22b. Wine composition

Variety	Alcohol content (%)	Titrable acidity (g tartaric acid /100ml)	Volatile acidity (g acetic acid /100 ml)	TSS (Brix)	pH	Reducing sugar (g/L)	Tannins (mg/L)	Organoleptic score
Bangalore blue	8.23	0.52	0.082	3.20	3.51	9.38	1072	18.50
Paneer	7.66	0.73	0.048	3.00	3.39	3.72	972	16.50
Thompson seedless	6.62	0.85	0.086	4.20	3.68	4.41	1262	13.25
Alguish	6.24	1.79	0.148	5.80	3.32	2.12	1620	16.00
Sharad	8.56	0.68	0.116	2.80	3.41	9.82	2122	16.25
SEd	0.278	0.120	0.013	0.454	0.066	1.239	102.40	0.834
CD	0.597	0.258	0.027	0.979	0.147	2.657	228.30	1.8533

Table 26. Effect of pH on banana wine production

Treatments		Alcohol content (%)	Titrable acidity (g tartaric acid /100ml)	Volatile acidity (g acetic acid /100 ml)	TSS (Brix)	pH	Reducing sugar (g/L)	Tannins (mg/L)	Organoleptic score
P_0	Y_1	8.76	1.08	0.208	2.8	4.07	3.56	986	16.25
	Y_2	8.14	0.96	0.216	2.2	4.01	3.86	1023	15.50
	Y_3	9.48	1.12	0.312	2.8	4.01	3.81	1213	16.50
	Y_4	9.36	1.04	0.214	2.6	3.98	3.62	1123	14.25
	Y_5	9.45	1.13	0.246	1.2	4.09	3.42	988	13.50
	Y_6	8.84	0.98	0.215	2.8	4.14	3.16	976	14.75
	Y_7	9.01	1.16	0.221	1	3.96	3.31	1072	13.75
P_1	Y_1	9.12	1.23	0.214	2.6	3.86	3.41	1524	18.00
	Y_2	9.25	1.32	0.248	2.8	3.93	3.72	1492	18.00
	Y_3	10.42	1.63	0.218	2.2	3.84	3.63	1436	18.75
	Y_4	9.28	1.62	0.286	1.2	3.88	3.49	1146	16.50
	Y_5	9.62	1.45	0.214	2.4	3.86	3.48	1023	18.50
	Y_6	8.26	1.42	0.281	2	3.72	3.58	952	17.25
	Y_7	9.28	1.45	0.241	2.8	3.78	3.82	1221	18.00

Table 26. Effect of pH on banana wine production (Contd...)

Treatments		Alcohol content (%)	Titrable acidity (g tartaric acid /100ml)	Volatile acidity (g acetic acid /100 ml)	TSS (Brix)	pH	Reducing sugar (g/L)	Tannins (mg/L)	Organoleptic score
P_2	Y_1	9.26	1.52	0.212	2.2	3.46	3.68	1486	18.50
	Y_2	9.85	1.38	0.21	1.2	3.52	3.52	1467	18.00
	Y_3	10.45	1.68	0.318	2.6	3.37	3.72	1562	19.00
	Y_4	10.26	1.86	0.281	1.8	3.42	3.67	1492	18.00
	Y_5	9.85	1.6	0.382	1.8	3.43	3.92	1245	18.50
	Y_6	8.82	1.65	0.301	2.2	3.42	3.41	985	18.50
	Y_7	10.38	1.8	0.286	1.8	3.38	3.52	1472	19.00
P_3	Y_1	8.24	1.68	0.262	6.8	3.02	2.16	1521	10.25
	Y_2	7.63	1.58	0.282	7.2	3.12	3.68	1326	8.50
	Y_3	7.51	2.12	0.268	4.6	3.18	3.41	892	12.25
	Y_4	8.21	1.93	0.321	2.8	3.06	3.28	1028	10.50
	Y_5	7.68	1.86	0.314	5.6	2.92	3.12	921	9.50
	Y_6	6.41	1.68	0.316	6.8	3.06	3.69	821	10.25
	Y_7	8.25	1.82	0.327	8.2	2.96	3.68	1016	11.35
PxY	SEd	0.4652	0.1241	0.0032	0.5231	0.1526	0.5243	126.41	0.9826
	CD	1.2631	0.3015	0.0152	1.2653	0.3141	1.0259	324.56	2.1423

Table 28. Effect of pH on papaya wine production

Treatments		Alcohol content (%)	Titrable acidity (g tartaric acid /100ml)	Volatile acidity (g acetic acid /100 ml)	TSS (Brix)	pH	Reducing sugar (g/L)	Tannins (mg/L)	Organoleptic score
P_0	Y_1	8.83	0.42	0.042	1.6	4.06	1.26	361	11.25
	Y_2	9.08	0.53	0.056	0.4	3.96	1.31	358	12.75
	Y_3	9.02	0.49	0.048	0.2	4.16	1.32	368	10.50
	Y_4	8.96	0.32	0.034	1	4.21	1.25	378	11.50
	Y_5	9.74	0.53	0.041	0.6	4.13	1.32	373	11.25
	Y_6	8.42	0.42	0.038	0.2	4.06	1.45	328	10.75
	Y_7	9.56	0.68	0.046	0.2	4.31	1.35	385	10.50
P_1	Y_1	9.24	0.78	0.053	3.2	3.86	1.24	385	15.25
	Y_2	9.89	0.69	0.058	1.8	3.81	1.28	372	15.50
	Y_3	9.28	0.52	0.046	1	3.92	1.28	385	13.50
	Y_4	9.31	0.38	36	1.2	3.78	1.36	398	14.75
	Y_5	9.98	0.55	0.043	1.4	3.92	1.22	385	14.50
	Y_6	8.58	0.56	0.046	3.2	3.89	1.53	362	13.50
	Y_7	10.15	0.65	0.041	0.8	3.82	1.27	415	12.50

Table 28. Effect of pH on papaya wine production (Contd...)

Treatments		Alcohol content (%)	Titrable acidity (g tartaric acid /100ml)	Volatile acidity (g acetic acid /100 ml)	TSS (Brix)	pH	Reducing sugar (g/L)	Tannins (mg/L)	Organoleptic score
P_2	Y_1	9.46	0.83	0.056	2.8	3.42	1.36	360	15.00
	Y_2	10.26	0.73	0.049	0.8	3.38	1.23	398	16.25
	Y_3	9.84	0.76	0.056	2.4	3.36	1.38	389	15.25
	Y_4	9.38	0.61	0.041	1.6	3.42	1.45	385	15.50
	Y_5	10.4	0.72	0.055	2.8	3.42	1.65	422	15.75
	Y_6	8.63	0.82	0.051	3.2	3.32	1.33	375	15.75
	Y_7	10.03	0.86	0.062	1.2	3.48	1.63	434	15.25
P_3	Y_1	6.48	1.86	0.061	4.8	3.12	1.82	285	14.75
	Y_2	5.62	3.28	0.051	8.6	3.06	1.48	262	12.50
	Y_3	8.36	0.98	0.072	4.2	3.02	1.42	376	12.50
	Y_4	5.62	3.56	0.098	9.4	3.07	1.68	306	10.25
	Y_5	10.28	0.86	0.063	1.2	3.12	1.82	432	9.50
	Y_6	8.12	1.26	0.081	3.8	3.08	1.72	351	11.25
	Y_7	6.26	3.68	0.092	4.8	3.12	1.98	392	10.50
PxY	SEd	0.5831	0.1026	0.0316	0.936	0.0523	0.3821	92.64	1.213
	CD	1.2633	0.2314	0.0783	1.924	0.1821	0.8263	251.54	2.567

Table 42. Effect of heating and skin contact time on phenolic compounds extraction in grape wine (mgL^{-1})

Phenolic compounds	SC_0	SC_2	SC_4	SC_6	SC_8	H_{10}	H_{20}	H_{30}	H_{40}	H_{50}
Gallic acid	16.87	37.22	86.51	94.93	105.70	41.51	42.30	45.45	50.95	60.01
t- caftaric acid	1.84	1.17	0.79	1.04	1.21	0.77	0.78	0.79	1.05	1.21
Catechin	-	1.33	2.61	3.27	3.34	-	-	-	-	-
Epicatechin	7.52	16.64	22.31	30.99	32.78	9.97	9.65	8.91	8.05	7.26
Caffeic acid	-	3.71	4.62	4.99	5.77	-	-	-	-	-
t-fernlolyl tartaric acid	3.15	2.92	4.08	8.53	12.99	1.893	0.406	0.23	-	-
Syringic acid	1.03	0.55	0.983	1.56	2.63	1.06	0.96	0.87	0.24	-
P1	-	-	2.42	3.62	3.86	-	-	-	-	-
P2	-	-	4.32	4.44	5.03	-	-	-	-	-
P3	-	-	3.23	4.24	7.44	-	-	3.08	3.43	3.63
p-coumaric acid	2.96	4.44	11.96	12.29	12.94	5.28	9.18	13.86	15.54	18.04
Procyanin	6.80	6.31	3.34	3.74	5.26	6.78	5.84	5.34	3.97	3.58
delphinidin- 3-o glu	2.51	3.48	8.16	8.29	8.93	1.57	1.73	1.80	1.92	2.17
cyanidin – 3-o- glu	0.94	0.77	2.60	2.82	3.53	0.29	0.57	1.58	1.89	2.03
malvidin –3-o- glu	67.44	350.16	706.69	753.96	706.95	767.22	803.92	846.47	879.71	928.27
P4	-	0.90	1.01	1.11	1.33	0.62	0.69	0.75	0.75	0.78
P5	0.54	0.44	0.48	0.44	0.42	0.91	0.98	1.07	1.10	1.14
P6	-	0.28	0.30	0.30	0.32	-	-	0.28	0.30	0.33
P7	0.66	0.35	0.73	1.02	2.14	1.59	1.7	0.83	0.55	0.32

Table 42. Effect of heating and skin contact time on phenolic compounds extraction in grape wine (Contd...)

Phenolic compounds	SC_0	SC_2	SC_4	SC_6	SC_8	H_{10}	H_{20}	H_{30}	H_{40}	H_{50}
petunidin –3-o- glu	0.66	2.10	2.48	2.95	3.41	3.07	3.18	3.38	3.01	2.87
peonidin –3-o-glu	0.26	0.25	0.37	0.43	0.47	0.25	0.33	0.37	0.41	0.47
myrcetin –3-o- glu	1.15	2.01	1.97	2.30	2.47	2.80	2.77	2.41	2.62	2.69
quercitin –3-o-glu	1.56	1.62	1.72	1.76	1.81	2.80	2.78	2.41	2.62	2.69
P8	1.18	1.86	1.92	1.97	1.97	-	-	-	-	-
P9	0.11	-	-	-	-	-	-	-	-	-
P10	0.12	0.34	0.37	0.35	0.37	-	-	-	-	-
P11	0.21	-	-	-	-	-	-	-	-	-
myricetin	0.84	6.88	5.02	5.56	5.19	5.07	5.44	5.73	6.75	8.17
P12	0.32	-	-	-	-	-	-	-	-	-
P13	0.91	-	-	-	-	4.39	4.24	4.34	4.96	5.20
trans –resveratrol	0.41	0.76	1.06	2.57	3.65	-	-	-	-	-
quercetin	7.42	8.31	8.63	8.57	9.21	-	-	-	-	-
kampferal	0.91	0.11	0.13	0.12	0.13	0.11	0.10	0.09	0.12	0.13
isorhamnetin	0.37	0.26	0.37	0.39	0.38	-	-	-	-	-
Total	128.69	455.17	891.183	968.55	951.63	857.953	897.546	950.04	989.94	1050.99

Variance explained by the first three principal components obtained by PCA analysis of the phenolic compounds, extracted during various treatments.

Principal component	Variance explained (%)	Cumulative proportion (%)	Best variable correlations and their loadings.
PC_1	16.02	16.02	Catechin (0.991) ,Delphinidin–3-0 glu(0.984),Epicatechin (0.979),Caffeic acid(0.968),P_1(0.962),P_2(0.958),trans resveratrol (0.945),t-fernolyl tartaric acid(0.911),P_{10}(0.905),Gallic acid(0.877),P_8(0.864),Cyanidin 3-0 glu (0.828),Quercetin (0.814),Isorhamnetin (0.799),P_{13} (-0.798),P_5 (-0.774),P_4 (0.753),Syringic acid (0.727),P_3 (0.707),Quercetin 3-0 glu (-0.665) and P_6 (0.548)
PC_2	11.45	27.47	Malvidin 3-0 glu (0.971),Petunidin 3-0 glu (0.923),Myrietin 3-0 glu (0.911),P_9= (-0.898),P_{11} = (-0.898),P_{12} = (-0.898),Kaempferol (-0.897),Myricetin (0.843),p-coumaric acid (0.775),t-caftarmic acid (-0.753),Peonidin 3-0 glu (0.600) and
PC_3	3.06	30.53	Procyanin (-0.635) and P_7 (-0.770)

Table 34. Spectral characteristics of anthocyanin pigments of Muscat grape skins from TLC elution using Butanol: Acetic Acid: Water (BAW)

Pigment number	Colour		Rf values	Absorption maxima		Identification
	Visible	UV		UV	Visible	
1	Red purple	Dull purple	0.22	282	552	Malvidin glucoside complexes
2	Magenta	Dull magenta	0.56	280	535	Peonidin
3	Purple	Fluorescence	0.73	306	548	Malvidin

Table 35. Changes in anthocyanin pigments during aging in different colour extraction treatments

Wine samples	Initial			After 3 months		
	Absorbance at 520 nm					
	Mv- 3G	Peo	Mv	Mv- 3G	Peo	Mv
Skin color	0.931	0.897	1.323	-	-	-
W_0	0.126	-	-	0.083	-	-
W_1	0.423	0.172	0.485	0.125	0.022	0.337
SC_2	0.465	0.185	0.468	0.197	0.026	0.398
SC_4	0.491	0.493	0.531	0.232	0.078	0.411

SC_6	0.518	0.635	0.586	0.240	0.105	0.437
SC_8	0.587	0.722	0.627	0.265	0.136	0.506
H_{10}	0.726	0.124	0.681	0.381	0.081	0.539
H_{20}	0.761	0.095	0.726	0.397	0.091	0.585
H_{30}	0.763	0.021	0.722	0.401	0	0.618
H_{40}	0.782	0	0.731	0.408	0	0.637
H_{50}	0.789	0	0.738	0.425	0	0.690
SEd	0.012	0.006	0.013	0.008	0.004	0.015
CD	0.045	0.018	0.052	0.036	0.015	0.046

Table 38. Colour stability in grape wine during aging

Wine samples	Initial		3 months		6 months		Organoleptic score
	Brightness	Hue	Brightness	Hue	Brightness	Hue	
W_0	0.0856	0.958	0.031	0.985	0.024	0.987	16.00
SC_2	0.4443	0.794	0.282	1.037	0.1411	1.257	17.25
SC_4	0.4539	0.823	0.291	1.086	0.1483	1.326	16.75
SC_6	0.5230	0.857	0.313	1.123	0.1562	1.353	15.50
SC_8	0.5295	0.817	0.318	1.128	0.1536	1.421	13.25
H_{10}	0.793	0.827	0.574	0.953	0.314	0.937	18.50
H_{20}	0.828	0.889	0.623	0.927	0.326	0.943	18.00
H_{30}	0.873	0.992	0.627	0.915	0.328	0.956	18.00
H_{40}	0.978	0.988	0.656	0.938	0.336	0.932	17.50
H_{50}	1.076	0.973	0.673	0.952	0.417	0.896	17.00
SEd	0.013	0.027	0.011	0.024	0.006	0.017	0.12
CD	0.026	0.059	0.025	0.046	0.018	0.038	0.26

Table 39. Yeast population dynamics during co-inoculation in grape wine fermentation

Sl. No	Yeast inoculated		Yeast population ($x10^6$)				
			Initial	after 3 days	after 5 days	after 8 days	after 15 days
1.	Uninoculated control	O	-	0.00018+0.000078	0.552+0.293	5.28+7.92	0.054+1.299
2.	*S. cerevisiae* KJSK-57	O	0.002675+0	2.34+0.78	0+9.45	0+0	0+0
		A	0.104	28.05	179.5	321	63
3.	*H. anomala* KJSK -69	O	0	2.065+1.55	23.6+23.8	20.1+100.5	1.925+36.3
		B	0.086	17.05	87.75	147	38.45
4	*S. ellipsoideus* KJSK-106	O	0.00525	4.005	34.24	26.85	6.675
		C	0.2045	13.77	73.6	159.5	82.3
5	***Coinoculation of***	O	0.00405+0	1.625+0.32	7.8+0.641	6.45+0	0
	S. cerevisiae KJSK-57 and	A	0.081	5.525	25.35	180	43.4
	H. anomala KJSK-69	B	0.07695	2.925	47.9	70.95	12.6
6	***Coinoculation of***	O	0	2.16	2.475	0	0
	S. cerevisiae KJSK-57 and	A	0.0649	12.35	84.1	183	59.45
	S. ellipsoideus KJSK-106	C	0.0531	7.02	12.35	14.85	1.525
7	***Coinoculation of***	O	0	4.9	9.55	0	0
	S. cerevisiae KJSK-57	A	0.029	9.1	71.6	115.5	46.15
	H. anomala KJSK-69 and	B	0.034	7.7	95.45	55.9	24.85
	S. ellipsoideus KJSK-106	C	0.027	6.3	14.3	0	0

O- Other native yeasts
A- *S. cerevisiae* KJSK-57
B- *H.anomala* KJSK-69
C- *S. ellipsoideus* KJSK-106

Table 40. Yeast population dynamics during co-inoculation in banana wine fermentation

Sl. No	Yeast inoculated		Yeast population ($x10^5$)				
			Initial	after 3 days	after 5 days	after 8 days	after 15 days
1.	Uninoculated control	O	-	-	0.00076+0.00068	0.0211+0.0351	0.2925+0.4875
2.	*S. cerevisiae* KJSK-57	O	0	0+4.835	2.035+0	-	0
		A	1.18	8.51	79.45	44.3	1.86
3.	*H. anomala* KJSK -69	O	0	0+0.1285	12.6+12.8	2.15+32.2	0+0.388
		B	0.73	5.01	75.85	51.6	1.035
4	*S. ellipsoideus* KJSK-106	O	0	0.445	18.8	0.9075	0.0697
		C	0.68	8.456	64.75	11.1	0.8565
5	***Coinoculation of***	O	0	0	0.965+2.42	0	0
	S. cerevisiae KJSK-57 and	A	0.4485	4.125	12.50	14.95	0.6505
	H. anomala KJSK-69	B	0.3315	3.730	24.05	6.42	1.515
6	***Coinoculation of***	O	0	0	3.365	0.95	0
	S. cerevisiae KJSK-57 and	A	0.4415	2.415	32.8	32.3	0.809
	S. ellipsoideus KJSK-106	C	0.488	1.785	7.855	4.76	0.0207
7	***Coinoculation of***	O	0	0.1405	0	0	0
	S. cerevisiae KJSK-57	A	0.3315	2.110	17.63	12.45	0.78
	H. anomala KJSK-69 and	B	0.2805	1.965	19.65	13.75	0.973
	S. ellisoideus KJSK-106	C	0.408	1.401	4.145	0	0

O-Other native yeasts
A- *S. cerevisiae* KJSK-57
B- *H.anomala* KJSK-69
C- *S. ellipsoideus* KJSK-106

Table 37. Effect of heating on colour extraction in grape wine production

Treatment		A_{420}	A_{520}	Brightness (420 +520)	Hue (420/520)	Tannin content
H_0	Y_1	0.1799	0.2491	0.4289	0.7222	537.2
	Y_2	0.1980	0.2550	0.4529	0.7770	526.6
	Y_3	0.2366	0.2860	0.5226	0.8271	648.2
	Y_4	0.2317	0.2802	0.5118	0.8270	650.6
	Y_5	0.1990	0.2534	0.4524	0.7853	653.2
	Y_6	0.1907	0.2326	0.4233	0.8204	568.5
	Y_7	0.1792	0.2513	0.4305	0.7135	518.1
H_{10}	Y_1	0.3405	0.4190	0.7595	0.8125	756.4
	Y_2	0.2690	0.2740	0.5430	0.9875	596.3
	Y_3	0.3365	0.3775	0.7160	0.9025	724.1
	Y_4	0.3445	0.4095	0.7540	0.8410	787.2
	Y_5	0.3750	0.4300	0.8050	0.8720	796.4
	Y_6	0.2965	0.2915	0.5880	1.0160	622.4
	Y_7	0.3010	0.3110	0.6120	0.9675	616.3
H_{20}	Y_1	0.3395	0.4095	0.7110	0.8290	831.3
	Y_2	0.2885	0.3145	0.6020	0.9180	611.8
	Y_3	0.3800	0.4295	0.7745	0.9005	752.7
	Y_4	0.3510	0.4310	0.7820	0.8145	768.3
	Y_5	0.3755	0.4410	0.8160	0.8520	821.4
	Y_6	0.2905	0.2860	0.5265	1.0165	706.2
	Y_7	0.3120	0.3200	0.6320	1.0050	711.7

Table 37. Effect of heating on colour extraction in grape wine production (Contd...)

Treatment		A_{420}		A_{520}		Brightness (420 +520)		Hue (420/520)		Tannin content	
H_{30}	Y_1	0.3805		0.4110		0.7915		0.9255		746.9	
	Y_2	0.2260		0.2690		0.4950		0.8410		638.1	
	Y_3	0.3795		0.4180		0.7975		0.9080		786.5	
	Y_4	0.4035		0.4640		0.8675		0.8685		721.6	
	Y_5	0.3990		0.4615		0.8605		0.8665		731.7	
	Y_6	0.2810		0.2965		0.5775		0.9410		689.3	
	Y_7	0.3285		0.3485		0.6770		0.957		713.4	
H_{40}	Y_1	0.4620		0.5075		0.9840		0.9380		753.6	
	Y_2	0.3240		0.3885		0.7420		0.9090		736.8	
	Y_3	0.4870		0.5360		1.0455		0.9505		857.0	
	Y_4	0.4455		0.5220		1.0060		0.9390		864.3	
	Y_5	0.4815		0.5090		0.9980		1.0080		876.4	
	Y_6	0.3120		0.3855		0.7045		0.9320		736.5	
	Y_7	0.3275		0.3775		0.7220		0.9115		746.1	
H_{50}	Y_1	0.5485		0.5870		1.1490		0.9565		1026	
	Y_2	0.2950		0.3490		0.6670		0.9095		926.3	
	Y_3	0.4770		0.5525		1.0745		0.9460		1112.1	
	Y_4	0.5175		0.5815		1.1230		0.9300		1044.8	
	Y_5	0.5295		0.5595		1.0975		0.9610		1073.2	
	Y_6	0.2880		0.3640		0.6845		0.8780		946.2	
	Y_7	0.3150		0.3810		0.7190		0.8855		985.3	
		SEd	CD	SEd	CD	SEd	CD	SEd	CD	SEd	CD
S		0.005	0.009	0.006	0.013	0.012	0.024	0.029	0.059	12.14	24.50
Y		0.005	0.010	0.007	0.014	0.013	0.026	0.032	0.064	13.11	26.46
S x Y		0.013	0.026	0.017	0.034	0.032	0.064	0.077	0.156	32.11	64.82

Table 31. Effect of low temperature on banana wine production

Treatments		Alcohol content (%)	Titrable acidity (g tartaric acid /100mL)	Volatile acidity (g acetic acid /100 mL)	TSS (Brix)	pH	Reducing sugar (g/L)	Tannins (mg/L)	Organoleptic score
T_0	Y_1	10.08	1.36	0.126	2.8	3.46	3.85	936	16.25
	Y_2	9.72	1.26	0.132	1.6	3.36	4.42	865	17.75
	Y_3	10.32	1.32	0.126	1.0	3.38	3.62	956	17.50
	Y_4	11.12	1.29	0.146	0.2	3.28	4.26	987	18.00
	Y_5	9.85	1.46	0.156	0.8	3.48	3.86	971	18.50
	Y_6	8.86	1.41	0.126	2.2	3.43	3.46	927	17.00
	Y_7	10.25	1.28	0.138	0.2	3.28	3.85	978	18.50
T_1	Y_1	10.62	1.21	0.151	2.8	3.52	4.12	921	17.00
	Y_2	9.87	1.32	0.148	1.8	3.32	3.86	906	18.25
	Y_3	10.21	1.62	0.147	0.8	3.31	3.87	924	18.00
	Y_4	11.26	1.28	0.142	0.2	3.32	4.31	992	18.50
	Y_5	10.26	1.51	0.163	0.2	3.41	3.51	988	18.50
	Y_6	9.03	1.38	0.135	1.6	3.45	4.38	969	18.25
	Y_7	8.13	1.37	0.156	3.6	3.47	3.63	956	17.50
T_2	Y_1	9.86	1.18	0.163	3.4	3.53	4.63	986	17.00
	Y_2	9.32	1.08	0.151	2.2	3.42	3.26	912	18.00
	Y_3	6.17	1.21	0.172	6.2	3.56	5.21	872	16.00
	Y_4	10.13	1.13	0.186	1.8	3.44	4.63	923	18.50
	Y_5	6.83	1.12	0.148	6.8	3.54	3.92	692	14.25
	Y_6	7.31	1.38	0.172	6.4	3.51	3.42	648	16.25
	Y_7	5.16	1.21	0.142	8.2	3.56	4.21	628	12.50
T_{xY}	SEd	0.25	0.13	0.007	0.56	0.11	0.42	108	0.92
	CD	0.53	0.27	0.015	1.63	0.24	0.89	276	2.06

Table 33. Effect of low temperature on grape wine production

Treatments		Alcohol content (%)	Titrable acidity (g tartaric acid /100mL)	Volatile acidity (g acetic acid /100 mL)	TSS (Brix)	pH	Reducing sugar (g/L)	Tannins (mg/L)	Organoleptic score
T_0	Y_1	11.4	1.92	0.112	2.6	3.42	7.2	1251	18.00
	Y_2	11.2	1.86	0.126	2.2	3.46	9.7	1352	18.25
	Y_3	11.3	1.62	0.124	0.8	3.42	7.8	1642	18.00
	Y_4	11.3	1.63	0.163	0.2	3.21	6.3	1682	18.50
	Y_5	10.8	1.70	0.182	0.8	3.36	6.8	1641	18.00
	Y_6	8.7	1.72	0.121	3.2	3.48	5.8	1563	17.50
	Y_7	11.7	1.81	0.182	0.2	3.26	7.1	1862	18.50
T_1	Y_1	12.1	1.73	0.183	2.4	3.47	8.2	1621	18.50
	Y_2	11.8	1.68	0.216	2.8	3.51	10.5	1422	18.50
	Y_3	10.7	1.63	0.138	1.4	3.36	6.1	1563	18.00
	Y_4	11.8	1.68	0.128	0.6	3.26	4.8	1721	18.50
	Y_5	9.3	1.86	0.126	2.4	3.42	6.2	1523	17.75
	Y_6	8.9	1.96	0.164	2.8	3.40	7.1	1721	16.75
	Y_7	6.8	1.68	0.163	3.6	3.47	8.6	1215	16.25
T_2	Y_1	11.6	1.62	0.212	3.2	3.56	11.6	1326	18.50
	Y_2	10.8	1.42	0.286	4.2	3.53	18.3	1415	18.00
	Y_3	6.3	1.56	0.172	7.0	3.51	10.3	1126	15.75
	Y_4	11.2	1.72	0.212	1.0	3.42	5.2	1842	18.00
	Y_5	5.8	1.92	0.263	7.6	3.41	8.6	1241	15.25
	Y_6	5.1	1.82	0.321	7.2	3.52	9.2	1362	13.50
	Y_7	3.2	1.86	0.156	9.8	3.51	10.3	1142	14.00
T_{xY}	SEd	0.46	0.12	0.026	0.31	0.052	1.92	152.4	0.65
	CD	0.97	0.37	0.072	0.70	0.086	5.63	422.6	1.74

Table 32. Effect of low temperature on papaya wine production

Treatments		Alcohol content (%)	Titrable acidity (g tartaric acid /100mL)	Volatile acidity (g acetic acid /100 mL)	TSS (Brix)	pH	Reducing sugar (g/L)	Tannins (mg/L)	Organoleptic score
T_0	Y_1	10.80	0.82	0.056	0.8	3.41	1.62	289	12.50
	Y_2	9.70	0.87	0.048	0.2	3.40	1.56	271	11.00
	Y_3	10.10	0.85	0.063	0.2	3.28	1.69	335	12.50
	Y_4	8.96	0.86	0.073	0.2	3.31	1.52	267	13.50
	Y_5	9.26	0.87	0.063	0.6	3.42	1.58	326	14.00
	Y_6	8.24	0.92	0.098	1.2	3.41	1.68	315	14.00
	Y_7	9.26	0.85	0.065	0.2	3.26	1.86	322	14.50
T_1	Y_1	10.60	0.83	0.073	1.2	3.38	1.67	326	13.25
	Y_2	10.20	0.81	0.052	0.2	3.41	1.72	318	13.00
	Y_3	8.90	0.63	0.071	1.0	3.46	1.53	267	10.00
	Y_4	9.42	0.92	0.107	0.2	3.45	1.61	307	14.00
	Y_5	8.39	0.76	0.071	2.4	3.43	1.76	312	13.00
	Y_6	8.63	0.87	0.063	2.8	3.37	1.71	301	14.00
	Y_7	6.31	0.72	0.070	3.2	3.42	1.52	256	11.25
T_2	Y_1	9.80	0.76	0.071	2.6	3.48	2.81	267	11.75
	Y_2	10.20	0.56	0.051	1.2	3.31	2.93	328	13.50
	Y_3	5.80	0.57	0.051	6.8	3.52	1.85	242	10.25
	Y_4	9.31	0.81	0.082	0.8	3.42	2.86	316	14.25
	Y_5	4.82	0.61	0.056	10.8	3.51	2.12	268	10.75
	Y_6	4.21	0.56	0.076	10.2	3.48	1.98	251	9.50
	Y_7	2.38	0.69	0.051	8.2	3.38	2.12	253	10.00
$_{TxY}$	SEd	0.52	0.13	0.02	0.63	0.031	0.41	56.4	0.56
	CD	1.31	0.27	0.056	1.35	0.069	0.87	112.3	1.34

Table 43. *trans* –Resveratrol content in fruits wine

Wine samples		*trans*- Resveratrol content (mgL^{-1})
Grape wine		0.667
Banana wine		0.008
Papaya wine		0.0
Grape wine		
A. Skin contact time (days)		
	SC_0	0.086
	SC_2	0.672
	SC_4	1.621
	SC_6	2.412
	SC_8	2.737
B. Heating (70°C) time (min.)		
Control	H_0	0.626
	H_{10}	0.129
	H_{20}	0.065
	H_{30}	0.028
	H_{40}	0.0
	H_{50}	0.0
C. Cluster stem addition (15 days contact time)		3.798
SEd		0.204
CD		0.423

Table 36. Effect of skin contact time on colour extraction in grape wine production

Treatment		A_{420}	A_{520}	Brightness (420 +520)	Hue (420/520)	Tannin content
SC_0	Y_1	0.0580	0.0619	0.1199	0.9369	278.3
	Y_2	0.0350	0.0435	0.0785	0.8005	281.5
	Y_3	0.0540	0.0567	0.1106	0.9552	293.1
	Y_4	0.0454	0.0520	0.0975	0.8802	290.1
	Y_5	0.0420	0.0474	0.0894	0.8914	281.0
	Y_6	0.0347	0.0409	0.0756	0.8491	263.5
	Y_7	0.0312	0.0401	0.0672	0.7782	258.2
SC_2	Y_1	0.1794	0.2545	0.4339	0.7055	651.5
	Y_2	0.1980	0.2486	0.4244	0.7964	621.3
	Y_3	0.2401	0.2809	0.5050	0.3546	663.1
	Y_4	0.2258	0.2856	0.5114	0.7914	643.0
	Y_5	0.2061	0.2559	0.4620	0.8052	648.2
	Y_6	0.1888	0.2191	0.4079	0.8627	651.3
	Y_7	0.1693	0.2399	0.4092	0.7054	631.2
SC_4	Y_1	0.1863	0.2781	0.4644	0.6703	1521.3
	Y_2	0.2203	0.2561	0.4747	0.8601	1421.2
	Y_3	0.2487	0.3016	0.5503	0.8250	1621.2
	Y_4	0.2302	0.2825	0.5127	0.8152	1537.6
	Y_5	0.2062	0.2430	0.4491	0.8486	1586.3
	Y_6	0.1908	0.2281	0.4189	0.8397	1531.2
	Y_7	0.1932	0.2464	0.4395	0.7847	1531.7
SC_6	Y_1	0.2194	0.2927	0.5120	0.7495	1851.4
	Y_2	0.2158	0.2703	0.4861	0.7993	1621.5
	Y_3	0.2582	0.3071	0.5652	0.8406	1786.3
	Y_4	0.2176	0.2927	0.5102	0.7443	1781.3
	Y_5	0.2365	0.2865	0.5230	0.6905	1856.2
	Y_6	0.2132	0.2448	0.4580	0.8725	1682.1
	Y_7	0.2080	0.2564	0.4644	0.8122	1721.3
SC_8	Y_1	0.2864	0.3069	0.5133	0.6729	1815.2
	Y_2	0.2260	0.2766	0.5032	0.8190	1631.2
	Y_3	0.2379	0.3094	0.5472	0.7249	1868.2
	Y_4	0.2272	0.2904	0.5176	0.7818	1845.1
	Y_5	0.2351	0.2919	0.5270	0.8056	1931.2
	Y_6	0.2062	0.2571	0.4633	0.8022	1631.5
	Y_7	0.2174	0.2592	0.4766	0.8396	1721.4

	SEd	CD	SEd	CD	SEd	CD	SEd	CD	SEd	CD
S	0.002	0.005	0.006	0.012	0.005	0.011	0.043	0.087	20.29	41.20
Y	0.003	0.007	0.007	0.014	0.007	0.013	0.051	0.104	24.01	48.74
S x Y	0.007	0.015	0.016	0.032	0.015	0.029	0.114	0.232	53.70	109.01

Table 41. Phenolic compounds composition in fruits wine (mgL^{-1})

Retention Time(min)	Phenolic compound	Banana wine	Papaya wine	Grape wine
5.80	Peak A	21.78	-	-
7.70	Peak B	8.58	-	-
8.03	Peak C	-	0.13	0.74
8.2	Peak D	7.92	0.48	-
10.10	Peak E	3.08	4.40	-
10.6	Peak F	-	6.82	-
11.7	Peak G	44.22	-	-
16.17	Peak H	1.10	-	-
17.8	Peak I	6.16	-	-
19.5	gallic acid	9.02	11.44	35.64
20.8	Peak J	12.98	-	-
23.6	Peak K	0.57	-	-
24.1	Peak L	-	1.91	-
26.3	t-caftaric acid	-	2.36	1.886
28.9	Peak M	2.23	-	-
32.1	Peak N	2.30	-	-
34.0	catechin	-	-	2.88
36.5	epicatechin	1.47	20.16	11.20
39.2	caffeic acid	34.58	-	6.46
42.0	t-ferulolyl tartaric acid	-	22.62	6.24
45.4	syringic acid	73.8	-	1.14
50.0	Peak O	4.2	-	-
51.0	p-coumaric acid	15.75	1.05	5.40
53.0	procyanidin	11.2	11.3	8.44
54.4	delphimidin 3-o-glu	4.25	4.00	4.75
56.6	cyanidin c-o-glu	0.40	0.72	1.08
58.9	cyanidin c-o-glu	1.94	-	-

Table 27. Effect of total soluble sugar levels and acidification on wine recovery in banana (% v/v)

Treatments		B0	B1	B2	B3	B4	B5
P_0	Y_1	31.41	42.57	51.57	56.71	66.45	59.85
	Y_2	31.00	42.43	50.86	58.71	66.07	67.57
	Y_3	29.57	32.71	50.57	60.28	59.57	65.57
	Y_4	30.25	35.00	45.86	61.71	62.86	64.43
	Y_5	33.85	37.85	50.28	59.85	61.85	65.71
	Y_6	25.28	33.86	53.42	63.29	67.28	67.00
	Y_7	23.86	35.85	51.14	56.71	63.14	64.43
P_1	Y_1	36.56	41.57	49.57	64.57	65.14	67.35
	Y_2	33.28	45.86	53.71	57.85	63.43	68.17
	Y_3	31.28	36.57	54.00	56.14	64.28	64.43
	Y_4	38.71	34.00	40.42	68.14	64.29	61.85
	Y_5	38.42	36.99	53.14	60.56	63.00	65.14
	Y_6	31.86	36.57	56.71	64.86	68.14	70.71
	Y_7	30.42	36.14	53.00	57.43	66.15	61.85
P_2	Y_1	39.57	42.71	49.00	64.14	66.71	65.14
	Y_2	39.57	48.57	55.86	63.00	71.57	66.00
	Y_3	36.85	42.71	50.28	63.14	64.45	63.99
	Y_4	38.71	47.99	53.14	69.71	73.57	70.99
	Y_5	48.71	49.85	51.14	60.57	71.71	70.86
	Y_6	42.87	48.86	58.14	68.28	70.86	68.14
	Y_7	37.71	42.86	57.86	64.85	64.38	65.25
P_3	Y_1	47.86	53.14	51.28	60.87	68.28	66.00
	Y_2	48.71	51.87	53.57	62.43	69.57	64.57
	Y_3	50.14	53.71	53.99	61.28	64.71	63.57
	Y_4	58.28	69.71	67.42	71.43	71.85	70.43
	Y_5	55.85	57.42	62.85	70.42	68.28	66.43
	Y_6	48.85	54.57	58.86	65.71	70.14	68.00
	Y_7	45.28	51.57	54.28	65.71	67.85	64.40
P_4	Y_1	35.46	39.28	35.43	34.14	39.14	34.29
	Y_2	32.71	38.57	33.14	33.28	34.00	43.85
	Y_3	32.56	28.30	34.57	36.58	31.43	33.71
	Y_4	41.25	37.85	40.14	45.83	51.42	51.85
	Y_5	39.71	37.42	37.71	41.42	40.57	42.14
	Y_6	36.71	41.86	40.00	43.57	38.43	45.14
	Y_7	36.00	39.57	42.14	38.86	38.14	42.86

	SEd	CD
B	0.424	0.836
T	0.458	0.903
P	0.387	0.763
BT	1.122	2.212
TP	1.024	2.019
BP	0.948	1.8695
BTP	2.508	4.946

Table 41. Phenolic compounds composition in fruits wine (mgL^{-1}) (Contd...)

Retention time	Phenolic compound	Banana wine	Papaya wine	Grape wine
60.3	malvidin 3-o-glu	16.75	8.25	120.0
61.9	Peak P	0.73		
63.3	Peak Q	1.25		
64.4	Peak R	0.62		
66.8	petunidin 3-o-glu	2.76	1.78	2.8
68.6	peonidin 3-o-glu	15.2	2.48	2.85
70.0	myrcetin 3-o-glu	-	-	3.80
71.0	quercitin 3-o-glu	-	-	6.80
72.0	Peak S	2.95	-	-
73.7	Peak T	1.00	-	2.12
76.0	Peak U	7.84	-	-
77	Peak V	2.20	-	-
78	myricitin	0.19	1.64	3.08
80	Peak W	2.76	-	-
81	trans-resveratrol	-	-	0.39
81.7	quercetin	21.75	7.95	7.65
83.2	Peak X	4.53	0.54	-
84.7	kampferol	-	0.69	0.69
89.6	Isorhnatin	0.02	0.21	0.20
	Total	348.08	110.93	236.236

Variance explained by the first three principal components obtained by PCA analysis of the phenolic compounds, present in the fruit wines

Principal component	**Variance explained (%)**	**Cumulative proportion (%)**	**Best variable correlations and their loadings.**
PC_1	**64.8%**	**64.8%**	**Caffericacid(-0.933),Cyamidin3-0glu(0.933),Epicatechin (0.771),Isorhamnatin (0.972),Myricetin (0.946),*p*-coumaric acid] (-0.886),Peonidin (-0.977,)Quercetin (-0.985),Syringic acid (-0.979),t-caftaric acid (-0.928),Peak C (0.770),Peak D (-0.991),Gallic acid (0.715),Peak X (-0.997)**
PC_2	**35.1%**	**99.9%**	**Procyanin (-0.776), Velphinidin (0.929),Peak t (0.975), Malvidin (0.799)t-fernolyl tartaric acid (0.956) , petunidin (-0.823),Peak E (-0.914)**

Table 3. Morphological characterization of the selected yeast isolates

Yeast isolates	Colony morphology	Morphology of the vegetative cells and reproduction	Pseudomycelium formation and sporulation
KJSK-11	Whitish colony, butyrous colony margin was entire, produced pleasant odour.	Small spheroidal cells, occured singly, pair, monopolar budding.	Branched pseudohyphae, hat shaped ascospores (1-4 /ascus)
KJSK-29	Streak culture was creamy coloured, raised growth radiating from the centre	Slender, cylindrical cells occured singly, pair and short branched Pseudomycelium also present.	Pseudomycelium with branched chain of elongated blastospores. No ascus formation
KJSK-37	Streak culture was butyrous, slightly brownish, raised, smooth dull colonies.	Globose cells, present singly monopolar budding	No pseudomycelium formation. Four globular ascospores / ascus.
KJSK-49	Brownish –cream, flat, smooth, shiny colonies with entire margin.	Spherical cells reproduction by monopolar budding, occured singly.	No pseudomycelium production. 1-4 spherical ascospores/ ascus in sporulation media.
KJSK-57	The streak culture was butyrous on malt agar medium. Creamy, slightly raised, smooth and glossy colonies.	Cells were globose, single or in pair. Reproduction by monopolar budding.	No pseudomycelium formation. Four globular ascospores / ascus were formed.
KJSK-58	The streak culture was yellowish, soft colonies.	Cells were globose, multi lateral budding.	No pseudomycelium formation. Short chair of globose cells were present. No ascus formation
KJSK-69	Tannish -white colony, butyrous, colony margin were entire, producing pleasent odour.	Small spheroidal cells, occured singly, in pairs, Monopolar budding	Hat shaped spores (1-4/ascus). Branched psendohyphae was also produced.
KJSK-70	Streak culture was greyish white, glossy smooth colonies.	Cells were ovoid, multilateral budding, cells were arranged in star like configuration.	No pseudomycelium was formed. No ascus formation.

Table 3. (Contd...)

Yeast isolates	Colony morphology	Morphology of the vegetative cells and reproduction	Pseudomycelium formation and sporulation
KJSK-87	Butyrous, creamy, slightly raised, smooth, glossy colonies	Cells were globose, in pairs or in short chain, monopolar budding.	Rudimentary pseudohyphae was found on the corn meal agar. Four globular ascospores / asci were formed.
KJSK-90	The streak culture was creamy, mycelial bounded.	Cells were ovoid to cylindrical, in chain and bipolar budding.	Pseudomycelium was in tree like manner, producing ovoid blastospores. No ascus formation.
KJSK-95	Whitish colony, colony margin were entire, raised smooth colony	Spheriodal cells, occured singly or in pairs. Monopolar budding.	No pseudomycelium formation. No ascospore formation
KJSK-96	Streak culture was butyrous, creamy, raised, smooth, glossy colonies.	Globose cells, single or in pairs, monopolar budding.	No pseudomycelium formation. Four globose ascospores / ascus.
KJSK-97	Butyrous, slightly brownish, raised, with light striation, dull colonies.	Globular cells, chain of cells, monopolar budding.	Pseudomycelium was not produced. Four ascospores / ascus was found.
KJSK-99	Butyrous, slightly brownish, raised, dull colonies	Globular cells, elongated cells were also seen, chain of cells, monopolar budding	Psedomycelium was not found. Four ascospores / ascus were produced.
KJSK-100	Butyrous, slightly brownish, smooth with striation, dull colonies	Globose cells, elongated also found, single short chain, monopolar budding.	2-4 globular ascospores / ascus. Pseudomycelium was not seen.
KJSK-101	Butyrous, creamy, raised, smooth and glossy colonies	Ellipsoidal cells, single monopolar budding.	Four globular ascospores /ascus. Pseudomycelium was not produced.
KJSK-105	The streak culture was butyrous, slightly brownish, raised, smooth with light striations, dull colonies.	Cells were elongated, ellipsoidal in pairs and short chain, monopolar budding.	Pseudomycelium was not found. Four ellipsoidal ascospores /ascus was found.

Table 3. (Contd...)

Yeast isolates	Colony morphology	Morphology of the vegetative cells and reproduction	Pseudomycelium formation and sporulation
KJSK-106	Butyrous, creamy, raised, smooth, glossy colonies	Cells were ellipsoidal, single, monopolar budding.	Pseudomycelium was not found. Four globular ascospores /ascus was found.
KJSK-108	Creamy coloured, smooth, semi-glossy low convex with an irregular border.	Broad oval shaped with a swelling in the middle, shiny, reproduction by bipolar budding.	Pseudomycelium was found like branched chain of elongated cells. Spheroidal 4 ascospores /ascus was found in two groups.
KJSK-110	The streak culture was butyrous, creamy, slightly raised, glossy colonies.	Cells were globose, single, monopolar budding.	Pseudomycelium was absent. Asci containing 1-4 ascospores were found.
KJSK-111	Butyrous, creamy coloured, shiny and smooth colonies. The margin was fingered with Pseudomycelium.	The cells were spherical, reproduced by monopoloar budding, singly elongated cells were also seen.	Pseudomycelium was found and branched. The blastospores were arranged in clusters. 1-4 spheroidal ascospores with lipid globules / ascus.
KJSK-112	Streak culture was butyrous, cream colored, flat, shiny, smooth, the margins were entire.	Cells were round, budded multilaterally and occured in clusters.	Pseudomycelium with chain of globose blastospores. No ascus formation in sporulation media.
KJSK-114	Streak culture was butyrous, cream coloured, raised shiny smooth and the margin was entire.	Cells were round, buds bilaterally and occured in chain	Pseudomycelium with chain of globose in blastospores. No ascus formation.
KJSK-121	The streak culture was off-white, smooth and glossy with an entire margin.	Cells were globose, in pair reproduction by monopolar budding.	No pseudomycelium was formed. Formation of 2 ascospores /ascus.
Saccharomyces ellipsoideus CFTRI 101	Butyrous, creamy, raised, smooth, glassy colonies.	Cells were ellipsoidal, single, monopolar budding.	No peudomycelium formation. Four ascospores/asci was found.

Table 4. Fermentation of sugars by the selected yeast isolates

Yeast isolates	Glucose	Galactose	Sucrose	Maltose	Lactose	Raffinose
KJSK-11	+ w	+	+	+	-	-
KJSK-29	+	+	+	+	-	+
KJSK-37	+	+	+	+	-	+
KJSK-49	+	+	+	-	-	+
KJSK-57	+	+	+	+	-	+
KJSK-58	+	-	+	-	-	+
KJSK-69	+	-	+	+	-	-
KJSK-70	+	-	+	-	-	+
KJSK-87	+	+w	+	+	-	+
KJSK-90	+	-	+	-	-	+
KJSK-95	+	+	+	-	-	+
KJSK-96	+	+	+	+	-	+
KJSK-97	+	-	+	-	-	+
KJSK-99	+	-	+	-	-	+
KJSK-100	+	-	+	+	-	+
KJSK-101	+	+	+	+	-	+
KJSK-105	+	-	+	+	-	+
KJSK-106	+	-	+	+	-	+
KJSK-108	+	-	+	-	-	+
KJSK-110	+	-	+	-	-	+
KJSK-111	+	-	+	-	-	+
KJSK-112	+	+s	+	-	-	+
KJSK-114	+	+s	+	+	-w	-
KJSK-121	+	-	+	+	-	+
Saccharomyces ellipsoideus CFTRI 101	+	-	+	+	-	+

+ Very strong +s strong +w weak -w very weak - No growth

Table 5. Assimilation of carbon compounds and nitrate by yeast isolates and their growth in vitamin free medium at 37°C

Yeast isolates	Assimilation of carbon compounds																		Assimilation of nitrate	Growth in vitamin free medium	Growth at 37°C
	Galactose	Sucrose	Maltose	Cellobiose	Trehalose	Lactose	Raffinose	Soluble strach	D-xylose	L-Arabinose	D-ribose	L-Rhamnose	Erythritol	Ribitol	D-Mannitol	Succinc acid	Citric acit	Inositol			
KJSK-11	+	+	+	-	+	-	+	-	+	+	+	-	+	-	+	+	+	-	+	+	+
KJSK-29	+	+	+	+	+	-	-	-	+	+	+	-	-	+	+	+	+	+	-	-	-
KJSK-37	+	+	+	-	+	-	+	-	-	-	-	-	-	-	+	+	-	-	-	+	-
KJSK-49	+	+	-	-	-	+	+	-	+	+	+	-	-	+	+	+	+	-	-	-	+
KJSK-57	+	+	+	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	+	-
KJSK-58	+	+	-	-	-	-	+	-	-	-	+	-	-	-	+	+	-	-	-	-	+
KJSK-69	+	+	+	+	+	-	+	+	+	+	+	-	+	-	+	+	+	-	+	+	+
KJSK-70	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
KJSK-87	+	+	+	-	+	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	+
KJSK-90	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	+	-	-	-
KJSK-95	+	+	+	+	+	-	+	-	+	+	+	-	+	-	+	+	+	-	+	+	+
KJSK-96	+	+	+	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-
KJSK-97	+	+	+	-	-	-	+	-	-	-	-	-	-	-	+	+	-	-	-	-	-
KJSK-99	+	+	+	-	+	-	+	-	-	-	+	-	-	-	+	-	-	-	-	-	-
KJSK-100	+	+	+	-	+	-	+	-	+	+	+	-	-	-	+	-	-	-	-	-	-
KJSK-101	+	+	+	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-
KJSK-105	+	+	+	-	+	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-
KJSK-106	+	+	+	-	-	-	+	-	-	-	-	-	-	-	+	+	-	-	-	-	+
KJSK-108	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
KJSK-110	+	+	+	-	+	-	+	-	-	-	-	-	-	-	+	+	-	-	-	-	-
KJSK-111	+	+	+	-	+	-	+	-	-	-	-	-	-	+	+	+	-	-	-	-	+
KJSK-112	-	+	+	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-
KJSK-114	+	+	+	-	-	+	-	-	+	+	+	-	-	-	+	+	+	+	-	-	-
KJSK-121	+	+	+	-	+	-	+	-	+	-	-	-	-	-	+	+	-	-	-	-	-
Saccharomyces ellipsoideus CFTRI 101	+	+	+	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-		+

+ Positive growth - No growth

Table 16. Production of extra cellular hydrolytic enzymes by the selected yeast isolates

Yeast isolates	Polygalacturonase	Cellulase	Amylase	β-glucosidase	Protease	Esterase
Hansenula sp. KJSK-11	+	-	-	-	-	-
Candida oleophila KJSK-29	+	-	-	-	+	-
Saccharomyces cerevisiae KJSK-37	-	-	-	-	-	-
Kluyveromyces marxianus KJSK-49	+	-	-	-	-	-
Saccharomyces cerevisiae KJSK-57	-	-	-	-	-	-
Candida maynoliae KJSK-58	-	-	-	-	+	+
Hansenula anomala KJSK-69	+	+	+	+	-	-
Candida stellata KJSK-70	-	-	-	-	+	-
Saccharomyces cerevisiae KJSK-87	-	-	-	-	-	-
Candida intermedia KJSK-90	-	+	+	-	+	-
Yeast isolate KJSK-95	-	-	-	-	+	-
Saccharomyces cerevisiae KJSK-96	-	-	-	-	-	+
Saccharomyces capensis KJSK-97	-	-	-	-	-	-
Saccharomyces cerevisiae KJSK-99	-	-	-	-	-	-
Saccharomyces bayanus KJSK-100	-	-	-	-	-	-
Saccharomyces ellipsoideus KJSK-101	-	-	-	-	-	-
Saccharomyces bayanus KJSK-105	-	-	-	+	-	-
Saccharomyces ellipsoideus KJSK-106	-	-	-	-	-	-
Saccharomyces ludwigi KJSK-108	-	-	-	-	-	-
Saccharomyces capensis KJSK-110	-	-	+	-	-	-
Kluyveromyces thermotolerans KJSK-111	+	-	-	-	-	+
Candida stellata KJSK-112	-	-	-	-	+	-
Yeast isolate KJSK-114	-	+	-	-	-	+
Torulaspora delbrueckii KJSK-121	-	-	-	-	-	-
Saccharomyces ellipsoideus CFTRI 101	-	-	-	-	-	+
Saccharomyces ellipsoideus MTCC 180	-	-	-	-	-	-

'+' – Visible exogenous enzyme production in agar plates

'-' – No visible exogenous enzyme production in agar plates

Table 6. Identification of the selected yeast isolates based on morphological and biochemical characters

Yeast isolates	Identification of the culture	Designation of the isolate
KJSK-11	*Hansenula* sp.	*Hansenula* sp. KJSK-11
KJSK-29	*Candida oleophila*	*Candida oleophila* KJSK-29
KJSK-37	*Saccharomyces cerevisiae*	*Saccharomyces cerevisiae* KJSK-37
KJSK-49	*Kluyveromyces marxianus*	*Kluyveromyces marxianus* KJSK-49
KJSK-57	*Saccharomyces cerevisiae*	*Saccharomyces cerevisiae* KJSK-57
KJSK-58	*Candida maynoliae*	*Candida maynoliae* KJSK-58
KJSK-69	*Hansenula anomala*	*Hansenula anomala* KJSK-69
KJSK-70	*Candida stellata*	*Candida stellata* KJSK-70
KJSK-87	*Saccharomyces cerevisiae*	*Saccharomyces cerevisiae* KJSK-87
KJSK-90	*Candida intermedia*	*Candida intermedia* KJSK-90
KJSK-95	Unknown	*Yeast isolate* KJSK-95
KJSK-96	*Saccharomyces cerevisiae*	*Saccharomyces cerevisiae* KJSK-96
KJSK-97	*Saccharomyces capensis*	*Saccharomyces capensis* KJSK-97
KJSK-99	*Saccharomyces cerevisiae*	*Saccharomyces cerevisiae* KJSK-99
KJSK-100	*Saccharomyces bayanus*	*Saccharomyces bayanus* KJSK-100
KJSK-101	*Saccharomyces ellipsoideus*	*Saccharomyces ellipsoideus* KJSK-101
KJSK-105	*Saccharomyces bayanus*	*Saccharomyces bayanus* KJSK-105
KJSK-106	*Saccharomyces ellipsoideus*	*Saccharomyces ellipsoideus* KJSK-106
KJSK-108	*Saccharomyces ludwigi*	*Saccharomyces ludwigi* KJSK-108
KJSK-110	*Saccharomyces capensis*	*Saccharomyces capensis* KJSK-110
KJSK-111	*Kluyveromyces thermotolerans*	*Kluyveromyces thermotolerans* KJSK-111
KJSK-112	*Candida stellata*	*Candida stellata* KJSK-112
KJSK-114	Unkown	Yeast isolate KJSK-114
KJSK-121	*Torulaspora delbrueckii*	*Torulaspora delbrueckii* KJSK-121

Table 8. Osmotic tolerance of the selected yeast isolates

Yeast isolates	Sugar concentration (% w/v)				
	30	40	50	60	70
Hansenula sp. KJSK-11	+++	++	+	-	
Candida oleophila KJSK-29	+++	+++	++	-	-
Saccharomyces cerevisiae KJSK-37	+++	+++	++	+	-
Kluyveromyces marxianus KJSK-49	+++	+++	++	+	-
Saccharomyces cerevisiae KJSK-57	+++	++	++	-	-
Candida maynoliae KJSK-58	+++	+++	+++	-	-
Hansenula anomala KJSK-69	+++	++	++	-	-
Candida stellata KJSK-70	+++	++	++	-	-
Saccharomyces cerevisiae KJSK-87	+++	++	+	-	-
Candida intermedia KJSK-90	+++	+++	++	-	-
Yeast isolate KJSK-95	+++	++	+	-	-
Saccharomyces cerevisiae KJSK-96	+++	+++	+++	++	+
Saccharomyces capensis KJSK-97	+++	++	+	-	-
Saccharomyces cerevisiae KJSK-99	+++	++	+	-	-
Saccharomyces bayanus KJSK-100	+++	+++	++	-	-
Saccharomyces ellipsoideus KJSK-101	+++	+++	+	-	-
Saccharomyces bayanus KJSK-105	+++	+	-	-	-
Saccharomyces ellipsoideus KJSK-106	+++	++	+	-	-
Saccharomyces ludwigi KJSK-108	+++	+	-	-	-
Saccharomyces capensis KJSK-110	+++	+++	+	-	-
Kluyveromyces thermotolerans KJSK-111	+++	++	+	-	-
Candida stellata KJSK-112	+++	++	-	-	-
Yeast isolate KJSK-114	+++	++	-	-	-
*Torulaspora delbrueckii*KJSK-121	+++	++	+	-	-
Saccharomyces ellipsoideus CFTRI 101	+++	++	+	-	-
Saccharomyces ellipsoideus MTCC 180	+++	++	+	-	-

*"+++" - Very strong
"++" - Strong
"+" - Weak
"-" - No growth

Table 10. Temperature tolerance of the selected yeast isolates

Yeast isolates	Temperature (°C)						
	10	15	20	25	30	35	40
Hansenula sp. KJSK-11	-	-	+	+++	+++	+	-
Candida oleophila KJSK-29	-	-	++	+++	+++	+	-
Saccharomyces cerevisiae KJSK-37	+	+++	+++	+++	+++	+	-
Kluyveromyces marxianus KJSK-49	-	++	+++	+++	+++	+++	++
Saccharomyces cerevisiae KJSK-57	+	+++	+++	+++	+++	+	-
Candida maynoliae KJSK-58	-	+	+++	+++	+++	+++	++
Hansenula anomala KJSK-69	+	+++	+++	+++	+++	+	-
Candida stellata KJSK-70	-	-	+	+++	+++	-	-
Saccharomyces cerevisiae KJSK-87	-	+	+++	+++	+++	+++	++
Candida intermedia KJSK-90	+	+++	+++	+++	+++	-	-
Yeast isolate KJSK-95	-	+	+++	+++	+++	-	-
Saccharomyces cerevisiae KJSK-96	+	++	+++	+++	+++	+	-
Saccharomyces capensis KJSK-97	-	+	++	+++	+++	-	-
Saccharomyces cerevisiae KJSK-99	-	+	++	+++	+++	-	-
Saccharomyces bayanus KJSK-100	+	+++	+++	+++	+++	+++	-
Saccharomyces ellipsoideus KJSK-101	-	-	++	+++	+++	-	-
Saccharomyces bayanus KJSK-105	-	-	+	+++	+++	-	-
Saccharomyces ellipsoideus KJSK-106	-	++	+++	+++	+++	+++	+
Saccharomyces ludwigi KJSK-108	-	-	+	+++	+++	-	-
Saccharomyces capensis KJSK-110	-	-	+	+++	+++	-	-
Kluyveromyces thermotolerans KJSK-111	-	-	++	+++	+++	+++	++
Candida stellata KJSK-112	-	-	+	+++	+++	-	-
Yeast isolate KJSK-114	-	++	+++	+++	+++	-	-
*Torulaspora delbrueckii*KJSK-121	-	-	+	+++	+++	-	-
Saccharomyces ellipsoideus CFTRI 101	-	++	+++	+++	+++	+	-
Saccharomyces ellipsoideus MTCC 180	-	+	+++	+++	+++	+++	+

*"+++" - Very strong
"++" - Strong
"+" - Weak
"-" - No growth

Table 12. pH tolerance of the selected yeast isolates

Yeast isolates	pH levels						
	2.0	**2.5**	**3.0**	**3.5**	**4.0**	**4.5**	**5.0**
Hansenula sp. KJSK-11	-	+	+++	+++	+++	+++	+++
Candida oleophila KJSK-29	++	+++	+++	+++	+++	+++	+++
Saccharomyces cerevisiae KJSK-37	-	+	++	+++	+++	+++	+++
Kluyveromyces marxianus KJSK-49	++	+++	+++	+++	+++	+++	+++
Saccharomyces cerevisiae KJSK-57	-	+	+++	+++	+++	+++	+++
Candida maynoliae KJSK-58	-	-	++	+++	+++	+++	+++
Hansenula anomala KJSK-69	-	+	++	+++	+++	+++	+++
Candida stellata KJSK-70	-	+	+	+++	+++	+++	+++
Saccharomyces cerevisiae KJSK-87	-	+	++	+++	+++	+++	+++
Candida intermedia KJSK-90	++	+++	+++	+++	+++	+++	+++
Yeast isolate KJSK-95	-	+	++	+++	+++	+++	+++
Saccharomyces cerevisiae KJSK-96	-	+	+++	+++	+++	+++	+++
Saccharomyces capensis KJSK-97	-	+	++	+++	+++	+++	+++
Saccharomyces cerevisiae KJSK-99	-	+	+++	+++	+++	+++	+++
Saccharomyces bayanus KJSK-100	-	+	++	+++	+++	+++	+++
Saccharomyces ellipsoideus KJSK-101	-	-	++	+++	+++	+++	+++
Saccharomyces bayanus KJSK-105	-	+	++	+++	+++	+++	+++
Saccharomyces ellipsoideus KJSK-106	-	+	+++	+++	+++	+++	+++
Saccharomyces ludwigi KJSK-108	-	-	++	+++	+++	+++	+++
Saccharomyces capensis KJSK-110	-	+	++	+++	+++	+++	+++
Kluyveromyces thermotolerans KJSK-111	-	+	++	+++	+++	+++	+++
Candida stellata KJSK-112	-	-	+	+++	+++	+++	+++
Yeast isolate KJSK-114	-	+	++	+++	+++	+++	+++
*Torulaspora delbrueckii*KJSK-121	-	+	+++	+++	+++	+++	+++
Saccharomyces ellipsoideus CFTRI 101	-	+	+++	+++	+++	+++	+++
Saccharomyces ellipsoideus MTCC 180	-	+	++	+++	+++	+++	+++

*"+++" Very strong
"++" Strong
"+" Weak
"-" No growth

Table 14. Alcohol tolerance of the selected yeast isolates

Yeast isolates	Ethanol concentration in the medium (% v/v)					
	6	**8**	**10**	**12**	**14**	**16**
Hansenula sp. KJSK-11	+++	+++	+++	+++	+++	-
Candida oleophila KJSK-29	+++	+++	+	-	-	-
Saccharomyces cerevisiae KJSK-37	+++	+++	+++	+++	+++	++
Kluyveromyces marxianus KJSK-49	+++	+++	++	-	-	-
Saccharomyces cerevisiae KJSK-57	+++	+++	+++	+++	++	-
Candida maynoliae KJSK-58	+++	+++	++	-	-	-
Hansenula anomala KJSK-69	+++	+++	+++	+++	++	-
Candida stellata KJSK-70	+++	+++	+++	+++	+	-
Saccharomyces cerevisiae KJSK-87	+++	+++	++	-	-	-
Candida intermedia KJSK-90	+++	+++	+++	+++	+++	++
Yeast isolate KJSK-95	+++	+++	+	-	-	-
Saccharomyces cerevisiae KJSK-96	+++	+++	+++	+++	+++	+++
Saccharomyces capensis KJSK-97	+++	+++	++	-	-	-
Saccharomyces cerevisiae KJSK-99	+++	+++	+++	+++	+	-
Saccharomyces bayanus KJSK-100	+++	++	+	+	-	-
Saccharomyces ellipsoideus KJSK-101	+++	+++	-	-	-	-
Saccharomyces bayanus KJSK-105	+++	++	-	-	-	-
Saccharomyces ellipsoideus KJSK-106	+++	+++	+++	+++	++	-
Saccharomyces ludwigi KJSK-108	+++	+++	++	-	-	-
Saccharomyces capensis KJSK-110	+++	++	-	-	-	-
Kluyveromyces thermotolerans KJSK-111	+++	+++	++	-	-	-
Candida stellata KJSK-112	+++	++	-	-	-	-
Yeast isolate KJSK-114	+++	++	-	-	-	-
*Torulaspora delbrueckii*KJSK-121	+++	+++	+++	+++	++	-
Saccharomyces ellipsoideus CFTRI 101	+++	++	-	-	-	-
Saccharomyces ellipsoideus MTCC 180	+++	+++	+++	++	-	-

*"+++" - Very strong
"++" - Strong
"+" - Weak
"-" - No growth

Table 15. SO_2 tolerance of the selected yeast isolates

Yeast isolates	SO_2 concentration (ppm)					
	50	100	150	200	250	300
Hansenula sp. KJSK-11	+++	+++	+	-	-	-
Candida oleophila KJSK-29	+++	+++	+++	++	-	-
Saccharomyces cerevisiae KJSK-37	+++	+++	+++	+	-	-
Kluyveromyces marxianus KJSK-49	+++	+++	+++	++	-	-
Saccharomyces cerevisiae KJSK-57	+++	+++	+++	++	-	-
Candida maynoliae KJSK-58	++	-	-	-	-	-
Hansenula anomala KJSK-69	+++	+++	+++	+++	+++	++
Candida stellata KJSK-70	+++	+++	+++	+++	++	-
*Saccharomyces cerevisiae*KJSK-87	+++	+++	+++	+++	+	-
Candida intermedia KJSK-90	+++	+++	+++	+++	++	+
Yeast isolate KJSK-95	+++	+++	-	-	-	-
Saccharomyces cerevisiae KJSK-96	+++	+++	+	-	-	-
Saccharomyces capensis KJSK-97	+++	+++	++	+	-	-
Saccharomyces cerevisiae KJSK-99	+++	+++	+++	+	-	-
Saccharomyces bayanus KJSK-100	+++	+++	+++	++	+	-
Saccharomyces ellipsoideus KJSK-101	+++	+++	-	-	-	-
Saccharomyces bayanus KJSK-105	+++	+++	+++	++	-	-
Saccharomyces ellipsoideus KJSK-106	+++	+++	+++	++	-	-
Saccharomyces ludwigi KJSK-108	+++	+++	+++	+	-	-
Saccharomyces capensis KJSK-110	+++	+++	+++	+	-	-
Kluyveromyces thermotolerans KJSK-111	+++	+++	+++	++	-	-
Candida stellata KJSK-112	+++	+++	+++	+	-	-
Yeast isolate KJSK-114	+++	+++	+++	+	-	-
*Torulaspora delbrueckii*KJSK-121	+++	+++	+++	++	-	-
Saccharomyces ellipsoideus CFTRI 101	+++	+++	+++	+	-	-
Saccharomyces ellipsoideus MTCC 180	+++	+++	+++	+	-	

*"+++" - Very strong
"++" - Strong
"+" - Weak
"-" - No growth

Table 17. Resistant/sensitive interaction of the selected yeast isolates with killer yeasts at pH 3.5

Killer yeast isolates (streak) / **Selected yeast isolates (Lawn)**	*Hansenula anomala* KJSK-69	*Saccharomyces cerevisiae* KJSK-57	*Candida intermedia* KJSK-90	*Saccharomyces ellipsoideus* KJSK-106	*Hansenula* sp. KJSK-11
Hansenula sp. KJSK-11	+	+	-	-	-
Candida oleophila KJSK-29	+	+	+	-	-
Saccharomyces cerevisiae KJSK-37	-	+	+	-	-
Kluyveromyces marxianus KJSK-49	-	+	-	+	+
Saccharomyces cerevisiae KJSK-57	+	-	-	-	-
Candida maynoliae KJSK-58	-	+	-	+	-
Hansenula anomala KJSK-69	-	-	-	-	-
Candida stellata KJSK-70	-	-	-	-	-
*Saccharomyces cerevisiae*KJSK-87	+	+	-	-	-
Candida intermedia KJSK-90	-	+	-	-	+
Yeast isolate KJSK-95	+	+	+	+	+
Saccharomyces cerevisiae KJSK-96	+	+	-	-	-
Saccharomyces capensis KJSK-97	+	+	-	-	-
Saccharomyces cerevisiae KJSK-99	+	+	-	-	-
Saccharomyces bayanus KJSK-100	+	+	+	+	+
Saccharomyces ellipsoideus KJSK-101	+	+	-	+	-
Saccharomyces bayanus KJSK-105	+	+	+	+	+
Saccharomyces ellipsoideus KJSK-106	+	+	+	-	+
Saccharomyces ludwigi KJSK-108	+	+	-	-	+
Saccharomyces capensis KJSK-110	+	+	-	-	-
Kluyveromyces thermotolerans KJSK-111	-	+	+	+	+
Candida stellata KJSK-112	+	+	-	+	+
Yeast isolate KJSK-114	+	+	+	+	+
*Torulaspora delbruechii*KJSK-121	+	+	-	-	+
Saccharomyces ellipsoideus CFTRI 101	-	+	-	-	-
Saccharomyces ellipsoideus MTCC 180	-	+	-	+	-
Total killed	17	23	8	10	11
Percentage killed (%)	68	92	32	40	44
Genetic basis of killer toxin production	Chromosomal DNA	ds RNA	ds RNA	-	Chromosomal DNA

Table 1. Isolation of the yeast cultures from various sources for fruit wine production

S.No	Source	Location	Yeast isolates
1.	Over ripened mango fruits	Modaikad, Kanyakumari District	KJSK-1, 2
2.	Over ripened plum fruits	Palamuthir Nilayam, Coimbatore	KJSK-3, 4,5, 6,7,8,9, 10 and 11
3.	Cotton flowers	Central Institute for Cotton Research (CICR), Coimbatore	KJSK-12, 13,14,15, 16, 17, 18 and 19
4.	Banana flower nectar	Palayankottai, Thirunelveli District	KJSK-20, 21, 22, 23,24, 25 and 26
5.	Banana flower nectar	Orchard, TNAU, Coimbatore	KJSK-27, 28, 29, 30, 31, 32 and 33.
6.	Over ripened pineapple fruits	Palamuthir Nilayam, Coimbatore	KJSK-34, 35 and 36
7.	Over ripened pomegranate fruits	Fruits Market, Madurai.	KJSK-37 and 38
8.	Honey bee (*Apis indica)* gut	Orchard, TNAU, Coimbatore	KJSK-39 and 40
9.	Rice milky endosperm	Paddy breeding Station, TNAU, Coimbatore	KJSK-41, 42, 43 and 44
10.	Cucurbitaceous flowers	Farmers field, Ramathapuram District	KJSK-45, 46, 47 and 48
11.	Over ripened pear fruits	Palamuthir Nilayam, Coimbatore	KJSK-49, 50 and 51.
12.	Over ripened Bangalore blue grapes	Palamuthir Nilayam, Coimbatore	KJSK-52, 53, 54 and 55
13.	Over ripened Muscat grapes	Grapes Orchard, Thondamuthur, Coimbatore	KJSK-56, 57, and 58
14.	Over ripened Guava fruits	Horticultural Research Station, Yercaud.	KJSK-59, 60, 61, 62 and 63
15.	Wild flowers	Kuttralam	KJSK-64, 65 and 66
16.	Over ripened orange fruits	Palamuthir Nilayam, Coimbatore	KJSK-67
17.	Over ripened apple fruits	Palamuthir Nilayam, Coimbatore	KJSK-68, 69, 70 and 71

Table 1. (Contd...)

S.No	Source	Location	Yeast isolates
18.	Over ripened papaya fruits	Orchard, TNAU, Coimbatore	KJSK-72, 73 and 74
19.	Over ripened plum fruits	Fruit market, Madurai	KJSK-75
20.	Over ripened butter fruits	Horticultural Research Station, Yercaud	KJSK-76, 77 and 78
21.	Gladiolus flowers, wild flowers nectar	Horticultural Research Station, Yercaud	KJSK-79, 80, 81, 82 and 83
22.	Palmyra juice samples (without addition of lime)	Mondaikad, Kanyakumari District	KJSK-84, 85, and 86
23.	Over ripened Muscat grapes	Kambam, Theni district	KJSK-87 and 88
24.	Palmyra juice samples (lime added)	Pulliyankudi, Tiruneveli District	KJSK-89 and 90
25.	Indigenous toddy samples (from palmyra juice)	Manavalakurichi, Kanyakumari District	KJSK-91, 92, 93 and 94
26.	Indigenous toddy samples (from palmyra juice)	Vasudevanallure, Tirunelveli District	KJSK-95, 96, 97 and 98
27.	Indigenous toddy samples (from coconut juice)	Marthandum, Kanyakumari District	KJSK-99, 100 and 101
28.	Sugarcane juice samples	Vasudevanellure, Pulliankudi and Rajapallayam	KJSK-102, 103, 104 and 105
29.	Indigenous toddy samples	Vallanadu, Tuticorin District	KJSK-106, 107, 108, 109, 110 and 111
30.	Wild flowers nectar	Western Ghats, Thenkasi	KJSK- 112, 113, 114, 115, 116, 117 and 118
31.	Wild flowers nectar	Horticultural Research Station, Ooty, Nilgiris	KJSK-119 and 120
32.	Indigenous toddy samples (from palmyra juice)	Viruthunagar	KJSK-121 and 122
33.	Type strain	Yeast culture collection, CFTRI, Mysore	*Saccharomyces ellipsoideus* CFTRI-101
34.	Type strain	Microbial type culture collection (MTCC), Chandigarh.	*S. ellipsoideus* MTCC 180
35.	Commercial baker's yeast	Iyer & Co Bakery, Coimbatore	*S. cereivisiae*

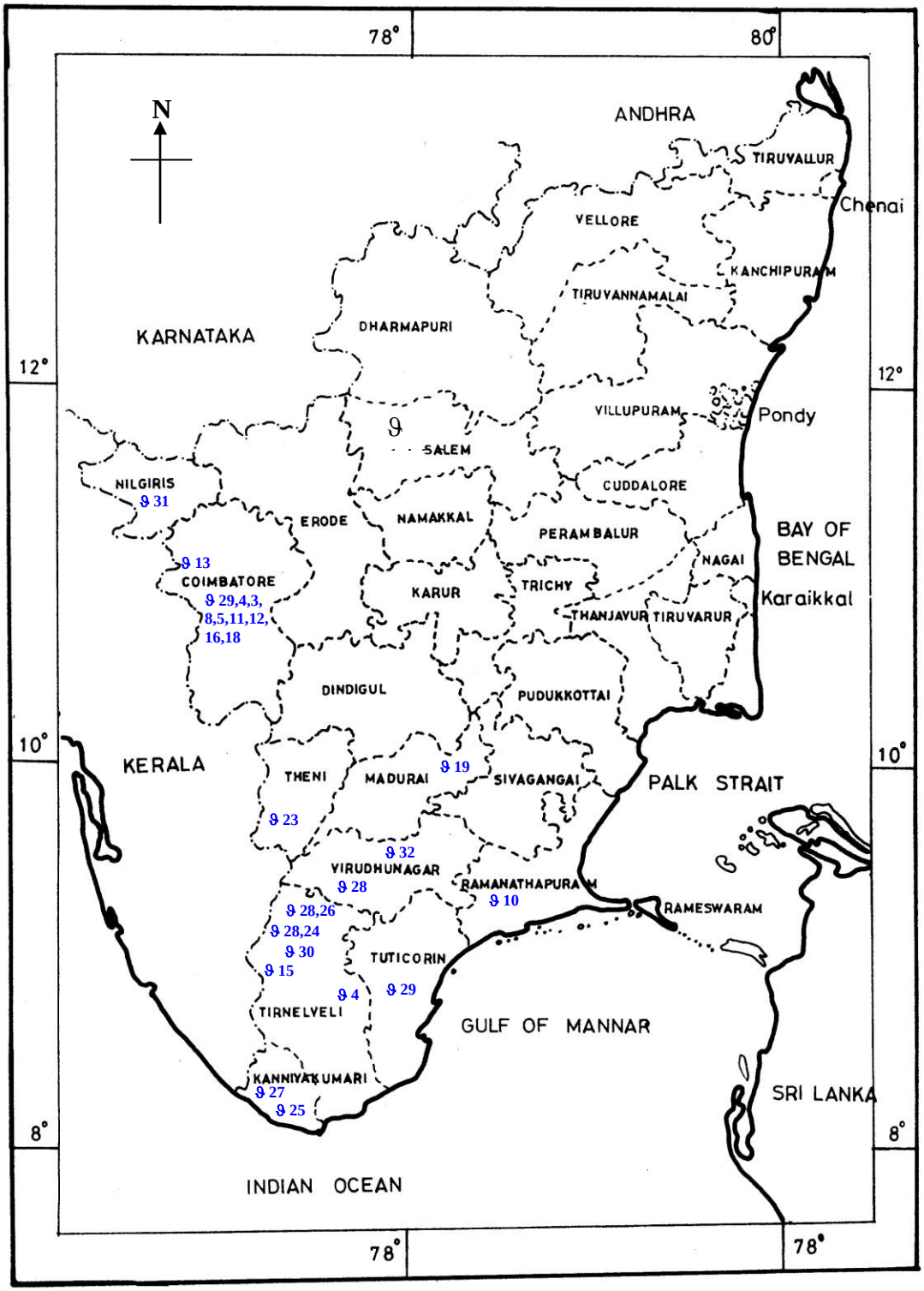
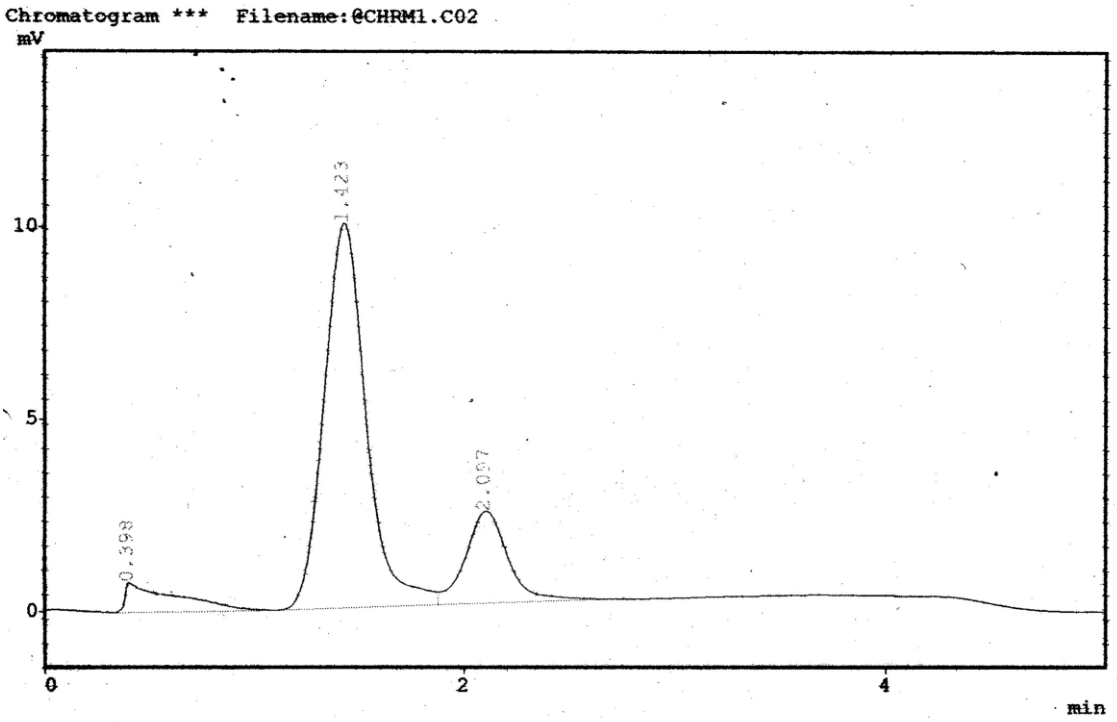


Fig. 1. Locations of samples collected from Tamil Nadu, India



Peak No.	Retention time (min.)	Compound
1	0.398	Water
2	1.423	Ethanol
3	2.097	Isopropanol

Fig. 2. Gas chromatogram of ethanol and internal standard isopropanol

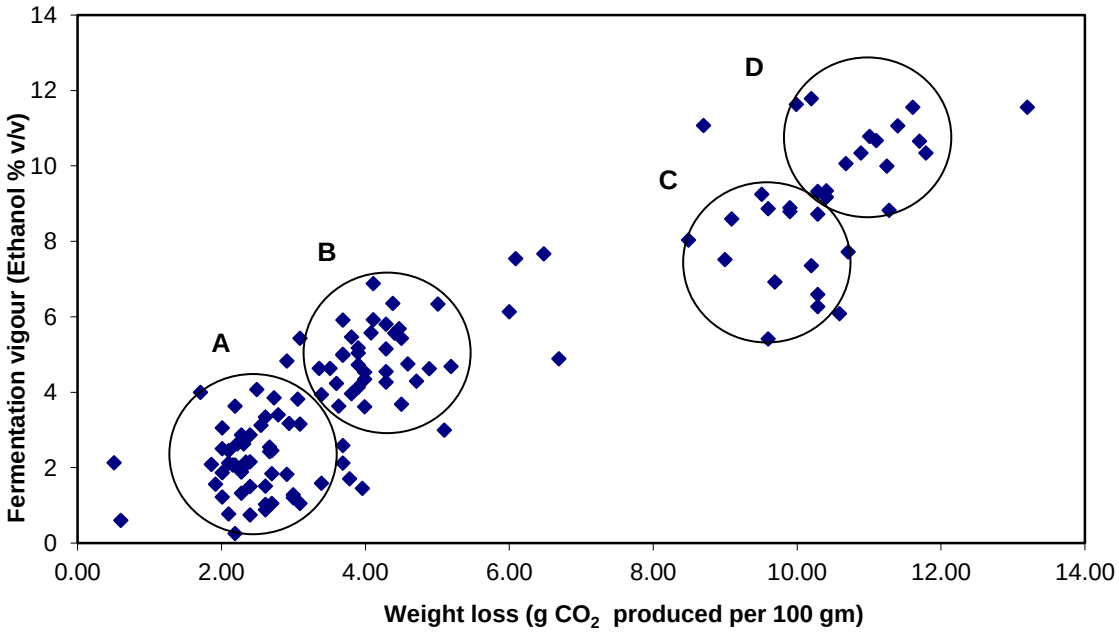
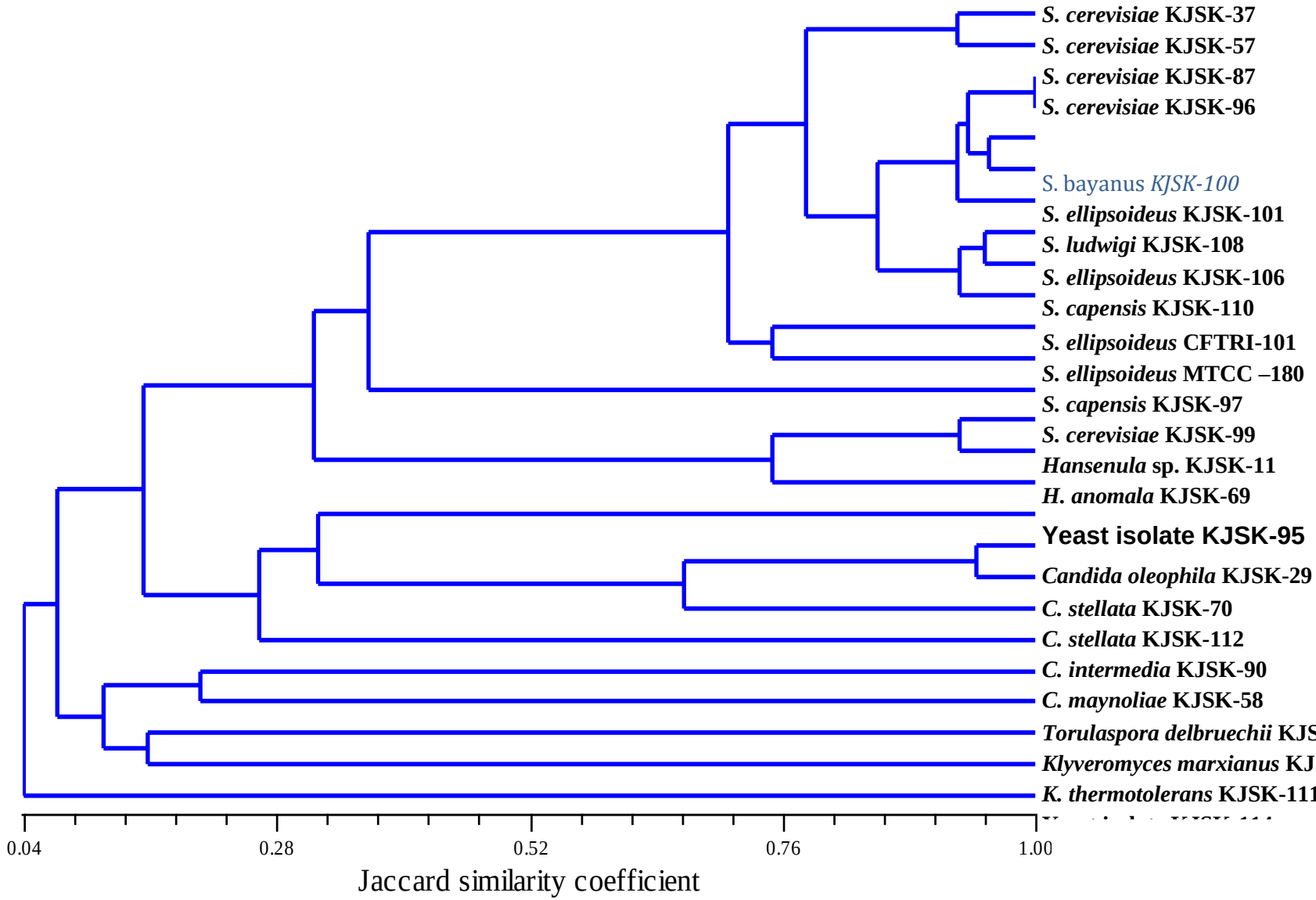
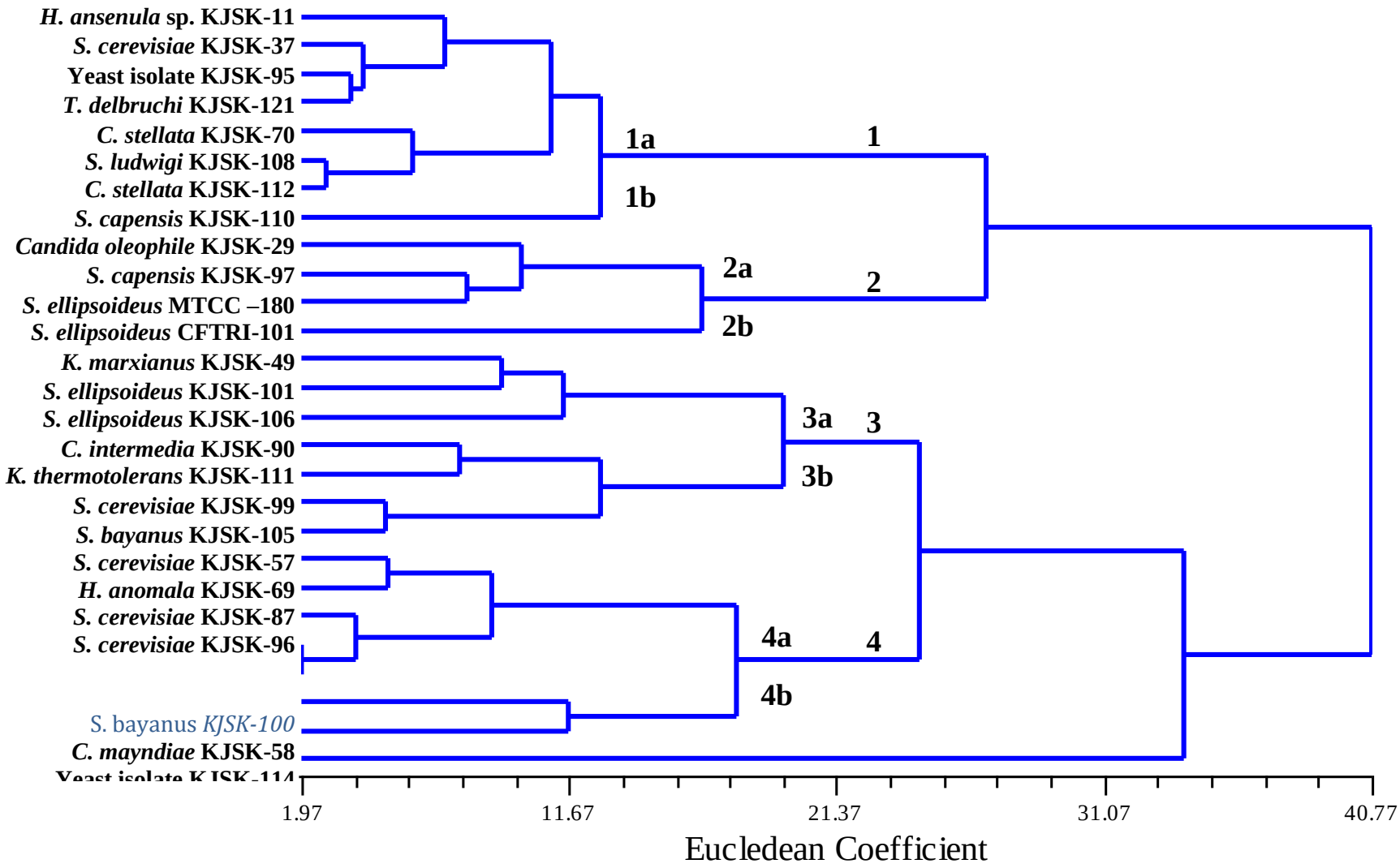


Fig.3. Grouping of yeast isolates based on fermentation rate and fermentation vigour


S. cerevisiae KJSK-37
S. cerevisiae KJSK-57
S. cerevisiae KJSK-87
S. cerevisiae KJSK-96
S. bayanus KJSK-100
S. ellipsoideus KJSK-101
S. ludwigi KJSK-108
S. ellipsoideus KJSK-106
S. capensis KJSK-110
S. ellipsoideus CFTRI-101
S. ellipsoideus MTCC –180
S. capensis KJSK-97
S. cerevisiae KJSK-99
Hansenula sp. KJSK-11
H. anomala KJSK-69
Yeast isolate KJSK-95
Candida oleophila KJSK-29
C. stellata KJSK-70
C. stellata KJSK-112
C. intermedia KJSK-90
C. maynoliae KJSK-58
Torulaspora delbruechii KJSK-12
Klyveromyces marxianus KJSK-4
K. thermotolerans KJSK-111
0.04
0.28
0.52
0.76
1.00
Jaccard similarity coefficient


H. ansenula sp. KJSK-11
S. cerevisiae KJSK-37
Yeast isolate KJSK-95
T. delbruchi KJSK-121
C. stellata KJSK-70
S. ludwigi KJSK-108
C. stellata KJSK-112
S. capensis KJSK-110
Candida oleophile KJSK-29
S. capensis KJSK-97
S. ellipsoideus MTCC –180
S. ellipsoideus CFTRI-101
K. marxianus KJSK-49
S. ellipsoideus KJSK-101
S. ellipsoideus KJSK-106
C. intermedia KJSK-90
K. thermotolerans KJSK-111
S. cerevisiae KJSK-99
S. bayanus KJSK-105
S. cerevisiae KJSK-57
H. anomala KJSK-69
S. cerevisiae KJSK-87
S. cerevisiae KJSK-96
S. bayanus KJSK-100
C. mayndiae KJSK-58
1a
1b
1
2a
2b
2
3a
3b
3
4a
4b
4
1.97
11.67
21.37
31.07
40.77
Eucledean Coefficient

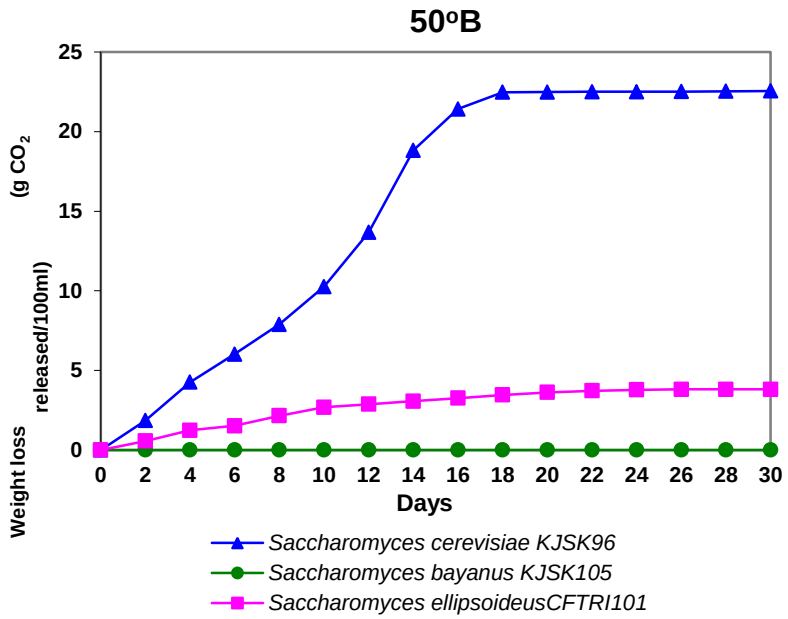
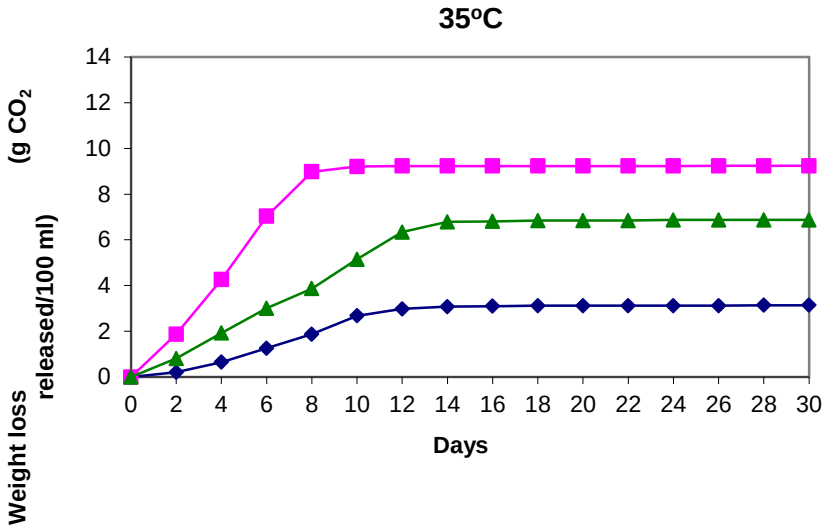
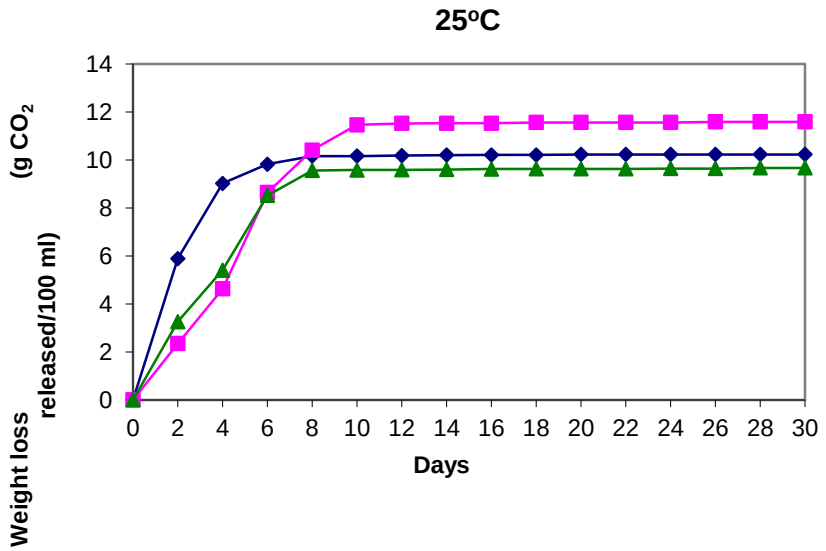


Fig. 6. Sugar tolerance of the selected osmotolerant yeast isolates


35ºC
Weight loss (g CO_2 released/100 ml)
0
2
4
6
8
10
12
14
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
Days


25ºC
Weight loss (g CO_2 released/100 ml)
0
2
4
6
8
10
12
14
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
Days

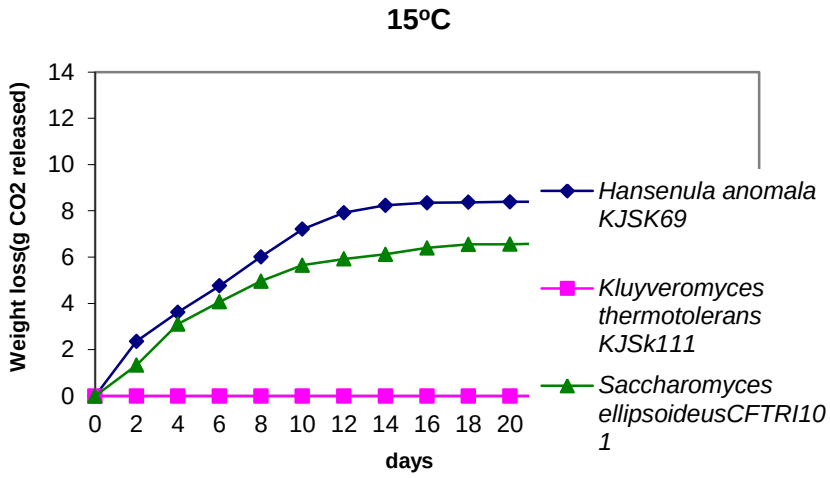
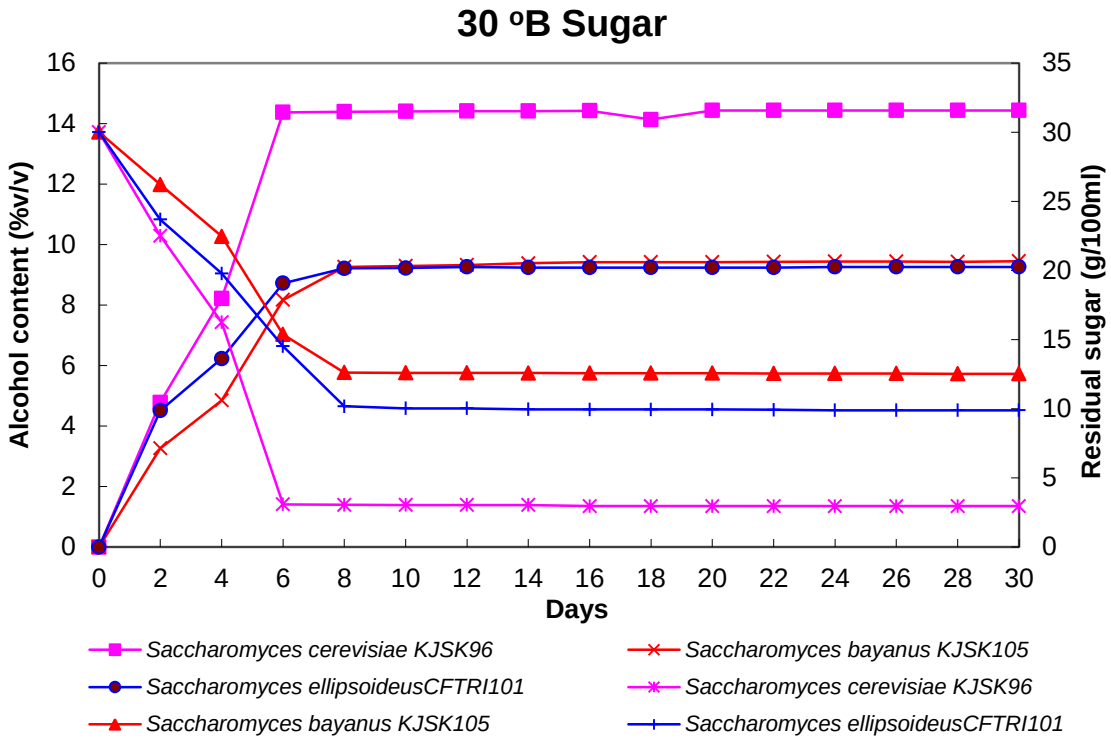


Fig. 8. Temperature tolerance of the selected yeast isolates


30 ºB Sugar
Alcohol content (%v/v)
Residual sugar (g/100ml)
Days
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
0
2
4
6
8
10
12
14
16
0
5
10
15
20
25
30
35
Saccharomyces cerevisiae KJSK96
Saccharomyces ellipsoideusCFTRI101
Saccharomyces bayanus KJSK105
Saccharomyces bayanus KJSK105
Saccharomyces cerevisiae KJSK96
Saccharomyces ellipsoideusCFTRI101

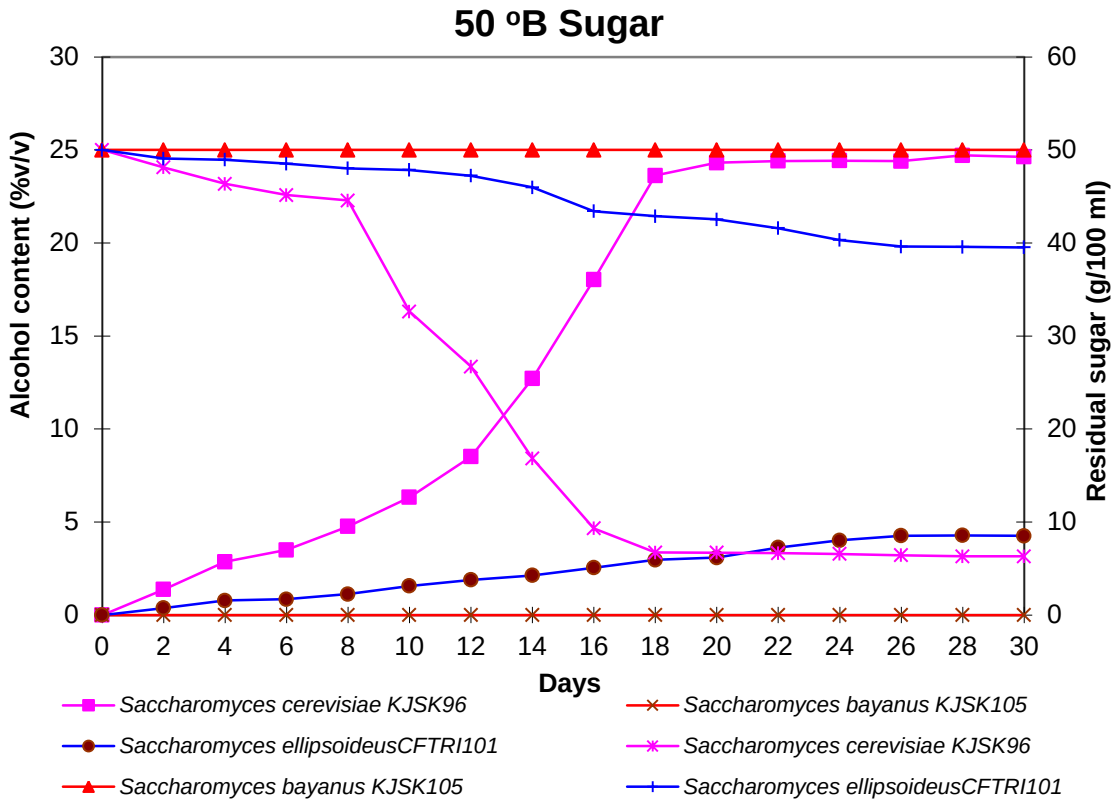
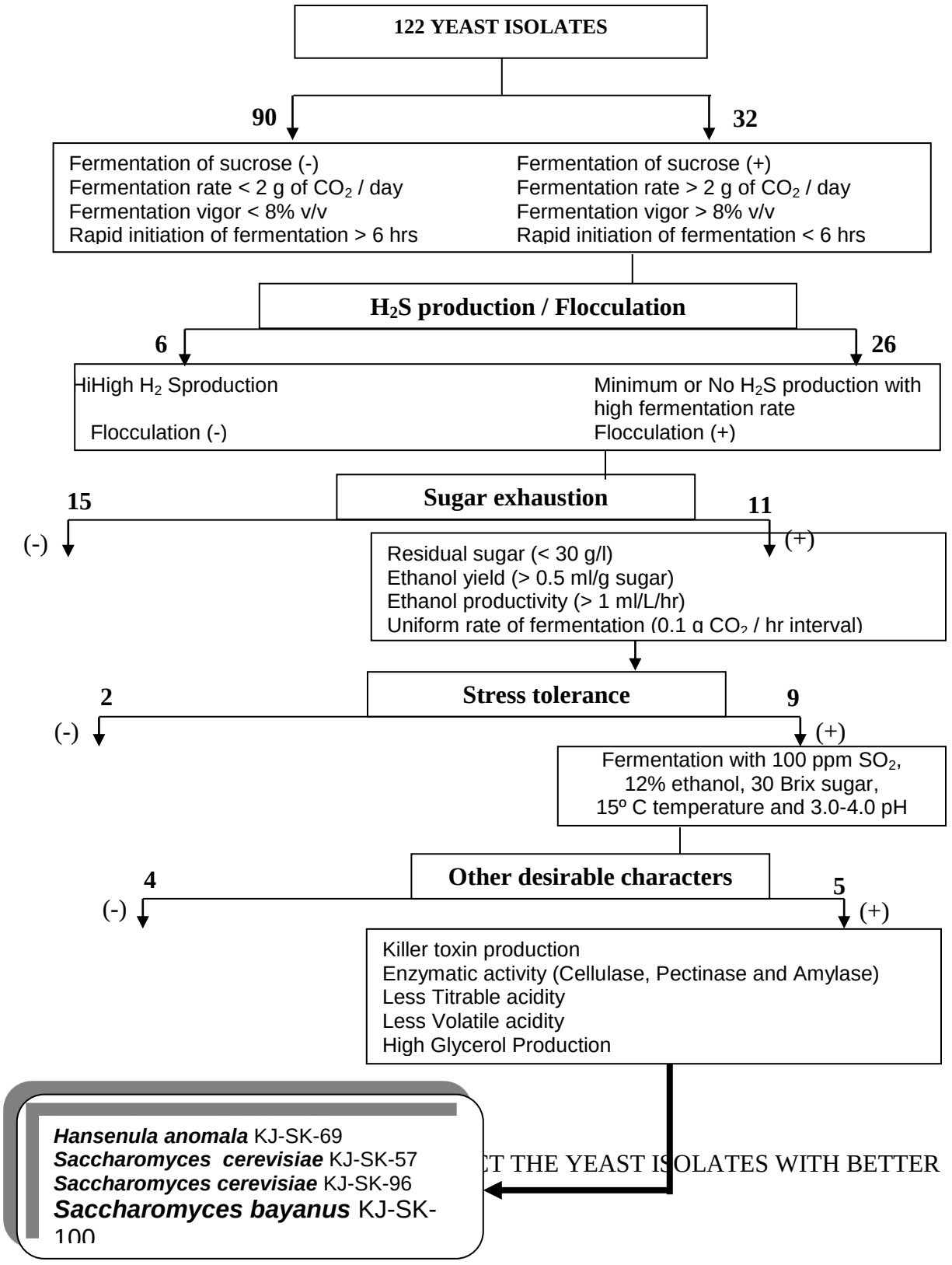
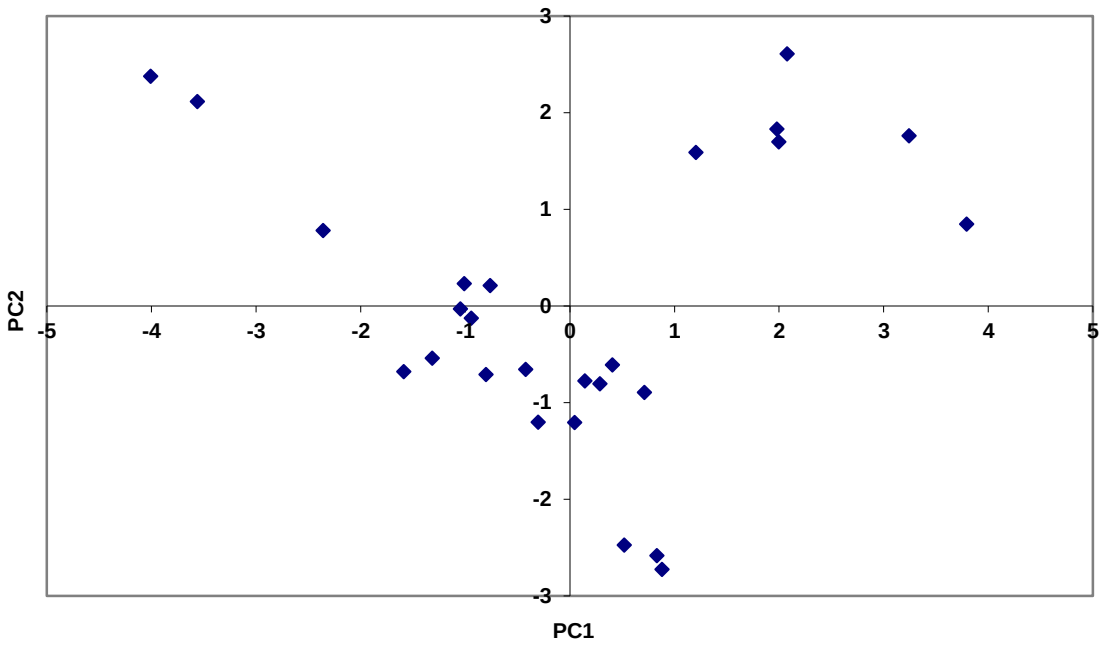
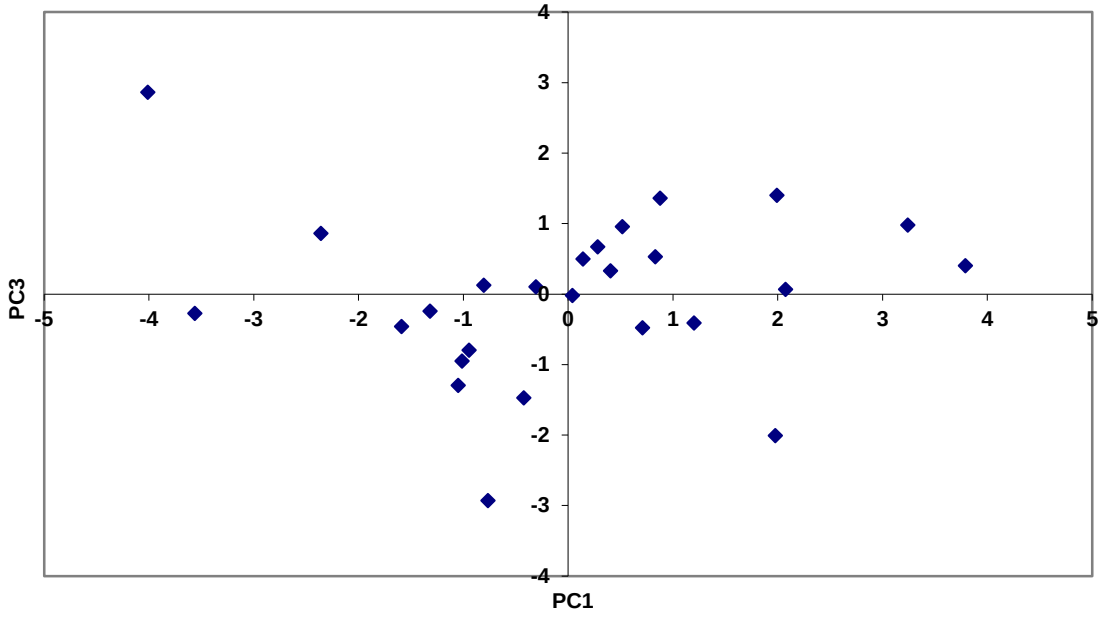


Fig. 7. Interaction between the residual sugar and alcohol production in osmotolerant yeast isolates

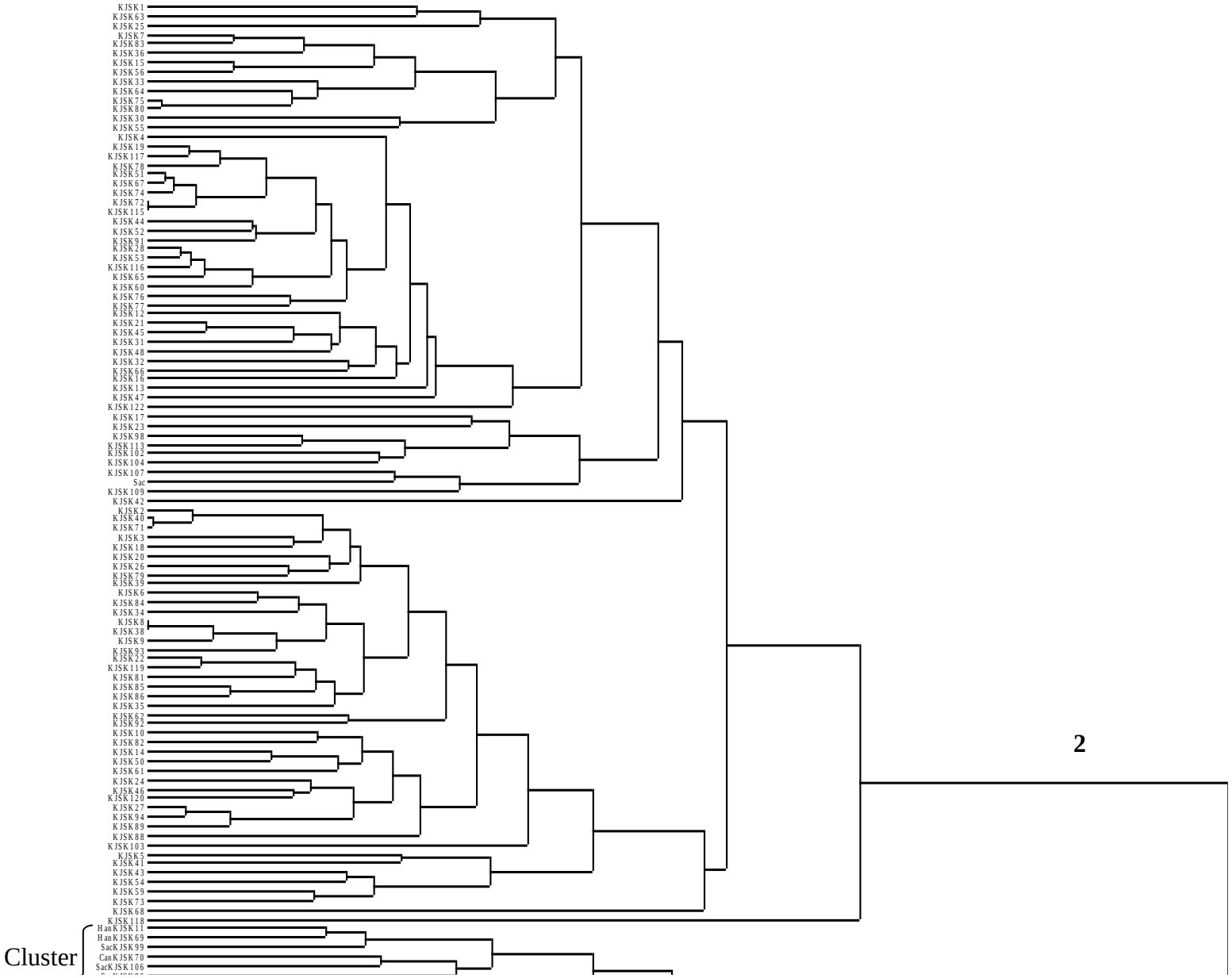

122 YEAST ISOLATES
90
32
Fermentation of sucrose (-)
Fermentation rate < 2 g of CO_2 / day
Fermentation vigor < 8% v/v
Rapid initiation of fermentation > 6 hrs
Fermentation of sucrose (+)
Fermentation rate > 2 g of CO_2 / day
Fermentation vigor > 8% v/v
Rapid initiation of fermentation < 6 hrs
H_2S production / Flocculation
6
26
HiHigh H_2 Sproduction
Flocculation (-)
Minimum or No H_2S production with high fermentation rate
Flocculation (+)
Sugar exhaustion
15
11
(-)
(+)
Residual sugar (< 30 g/l)
Ethanol yield (> 0.5 ml/g sugar)
Ethanol productivity (> 1 ml/L/hr)
Uniform rate of fermentation (0.1 g CO_2 / hr interval)
Stress tolerance
2
9
(-)
(+)
Fermentation with 100 ppm SO_2,
12% ethanol, 30 Brix sugar,
15º C temperature and 3.0-4.0 pH
Other desirable characters
4
5
(-)
(+)
Killer toxin production
Enzymatic activity (Cellulase, Pectinase and Amylase)
Less Titrable acidity
Less Volatile acidity
High Glycerol Production
Hansenula anomala KJ-SK-69
Saccharomyces cerevisiae KJ-SK-57
Saccharomyces cerevisiae KJ-SK-96
Saccharomyces bayanus KJ-SK-100
CT THE YEAST ISOLATES WITH BETTER

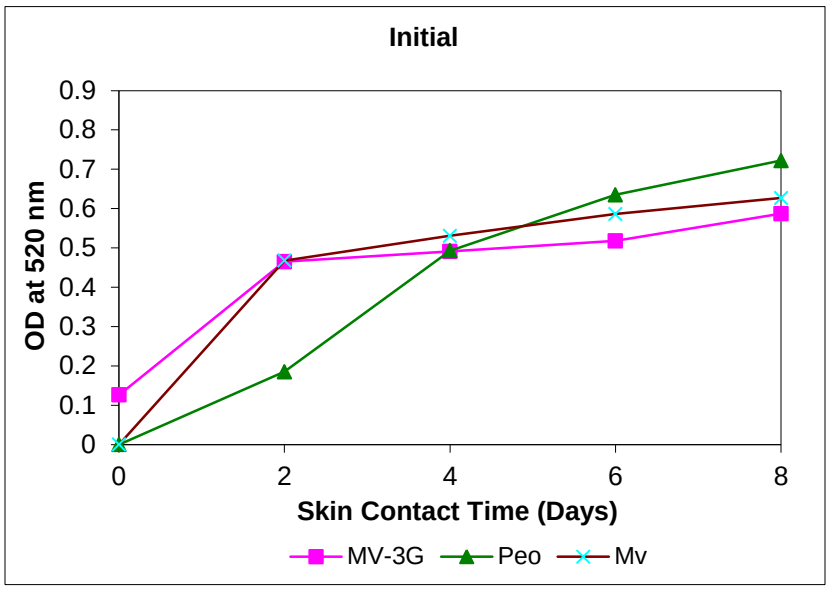


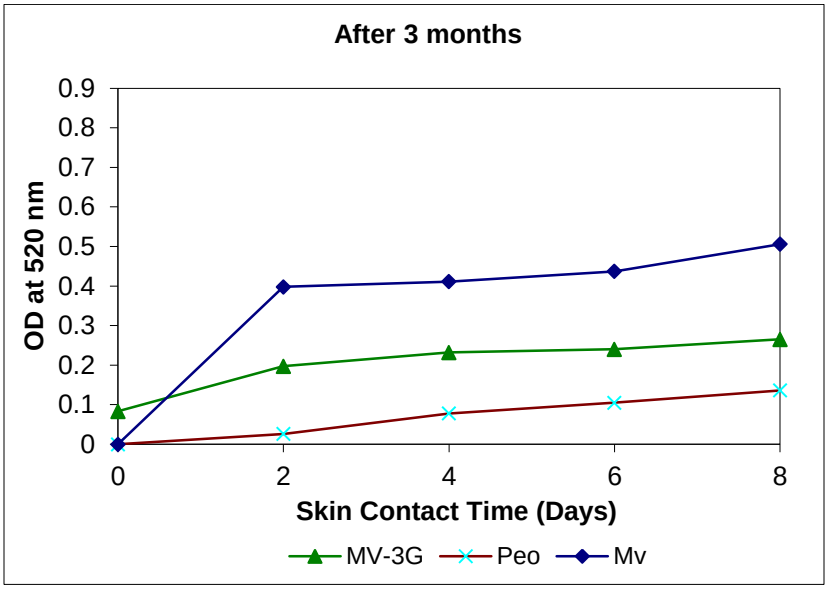


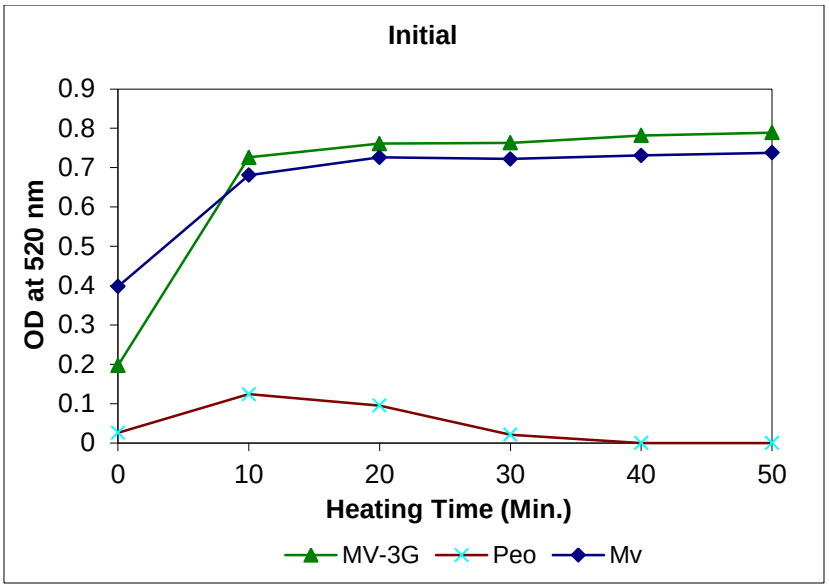
Principal component	Variance explained (%)	Variables highly correlated with the axis and their loadings.
PC1	33.09	n-Propanol (0.828), Succinic acid (0.797) Acetic acid (-0.696), Ethyl acetate (0.666) Isobutanol (0.626)
PC2	21.28	Isopropanol (0.643), Iso amyl alcohol (-0.744) Methanol (0.701), Acetaldehyde (-0.620)
PC3	13.29	Glycerol (0.705)

Fig. 11. Variation in the first three principal components obtained by PCA analysis of the volatile compounds, organic acids and glycerol produced by the selected wine yeasts


KJSK1
KJSK63
KJSK25
KJSK7
KJSK83
KJSK36
KJSK15
KJSK56
KJSK33
KJSK64
KJSK75
KJSK80
KJSK30
KJSK55
KJSK4
KJSK19
KJSK117
KJSK78
KJSK51
KJSK67
KJSK74
KJSK72
KJSK115
KJSK44
KJSK52
KJSK91
KJSK28
KJSK53
KJSK116
KJSK65
KJSK60
KJSK76
KJSK77
KJSK12
KJSK21
KJSK45
KJSK31
KJSK48
KJSK32
KJSK66
KJSK16
KJSK13
KJSK47
KJSK122
KJSK17
KJSK23
KJSK98
KJSK113
KJSK102
KJSK104
KJSK107
Sac
KJSK109
KJSK42
KJSK2
KJSK40
KJSK71
KJSK3
KJSK18
KJSK20
KJSK26
KJSK79
KJSK39
KJSK6
KJSK84
KJSK34
KJSK8
KJSK38
KJSK9
KJSK93
KJSK22
KJSK119
KJSK81
KJSK85
KJSK86
KJSK35
KJSK62
KJSK92
KJSK10
KJSK82
KJSK14
KJSK50
KJSK61
KJSK24
KJSK46
KJSK120
KJSK27
KJSK94
KJSK89
KJSK88
KJSK103
KJSK5
KJSK41
KJSK43
KJSK54
KJSK59
KJSK73
KJSK68
KJSK118
HanKJSK11
HanKJSK69
SacKJSK99
CanKJSK70
SacKJSK106
2
Cluster
1a


Initial
OD at 520 nm
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
2
4
6
8
Skin Contact Time (Days)
MV-3G
Peo
Mv


After 3 months
OD at 520 nm
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
2
4
6
8
Skin Contact Time (Days)
MV-3G
Peo
Mv



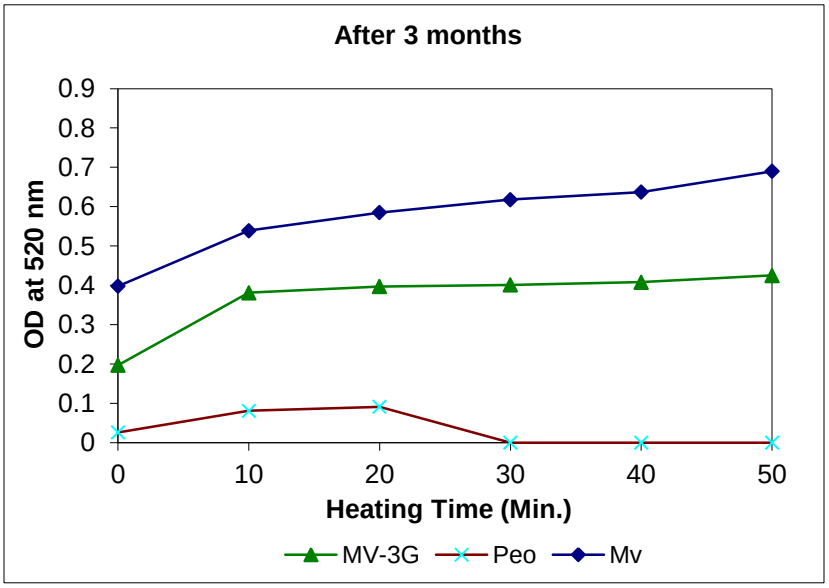
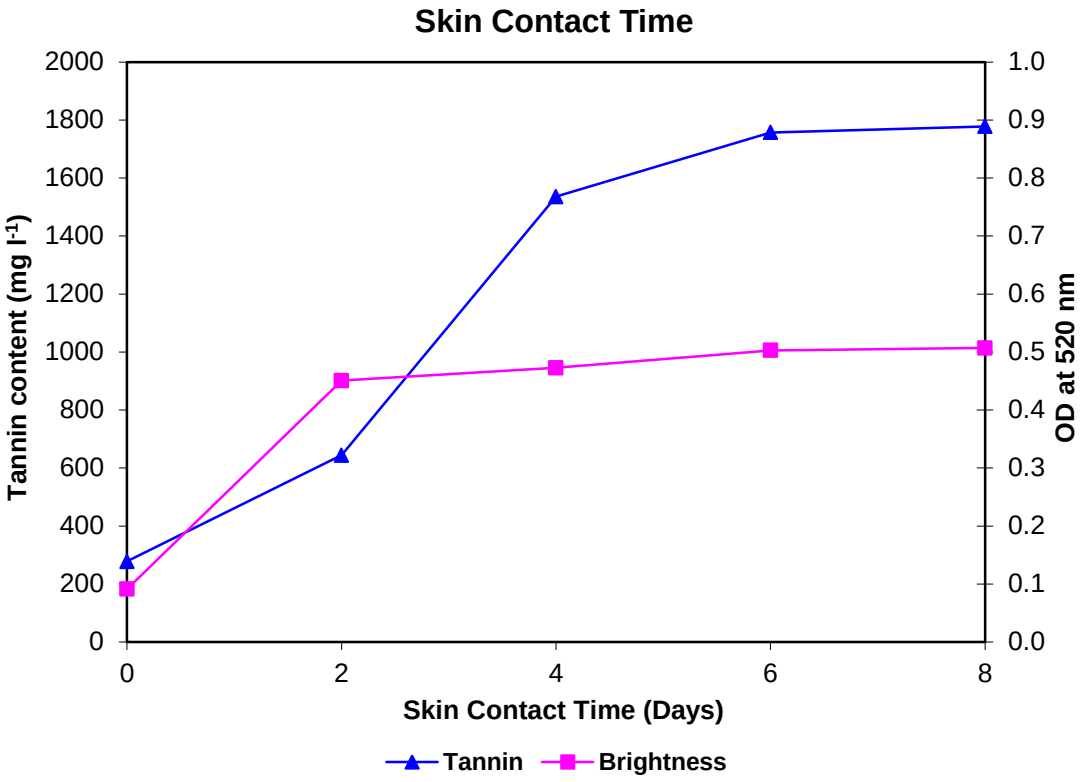


Fig. 16. Changes in anthocyanin pigments during aging in different color extraction treatments


Skin Contact Time
Tannin content (mg l-1)
OD at 520 nm
Skin Contact Time (Days)
Tannin
Brightness

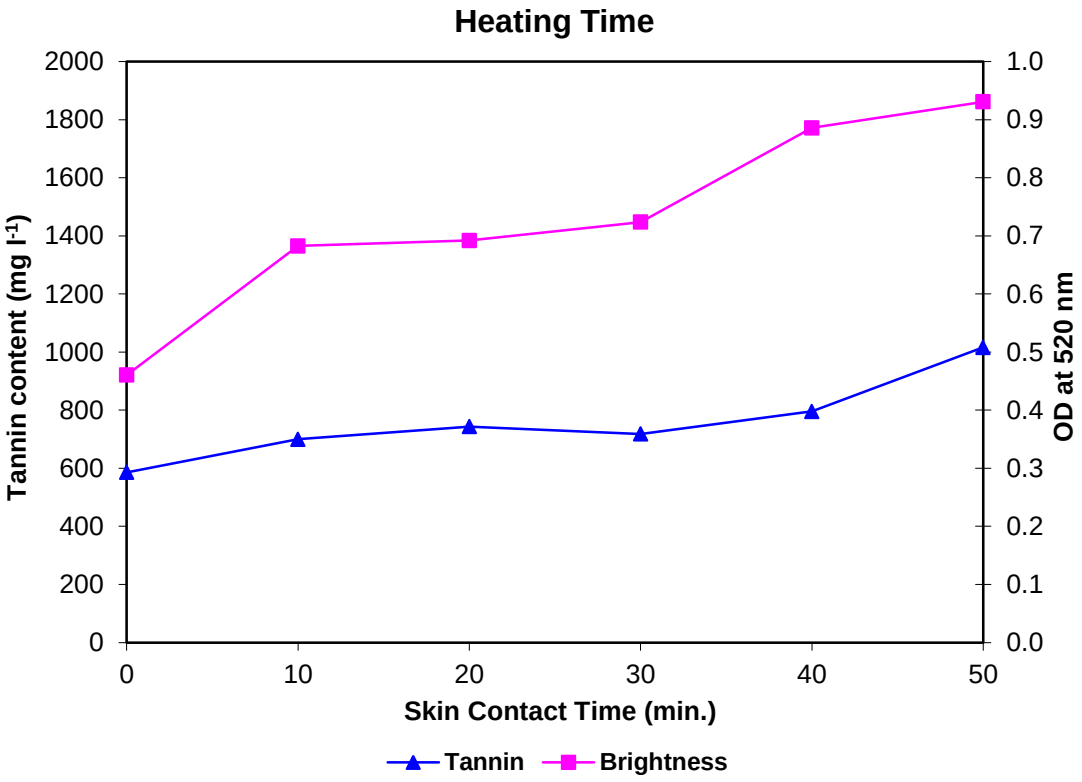
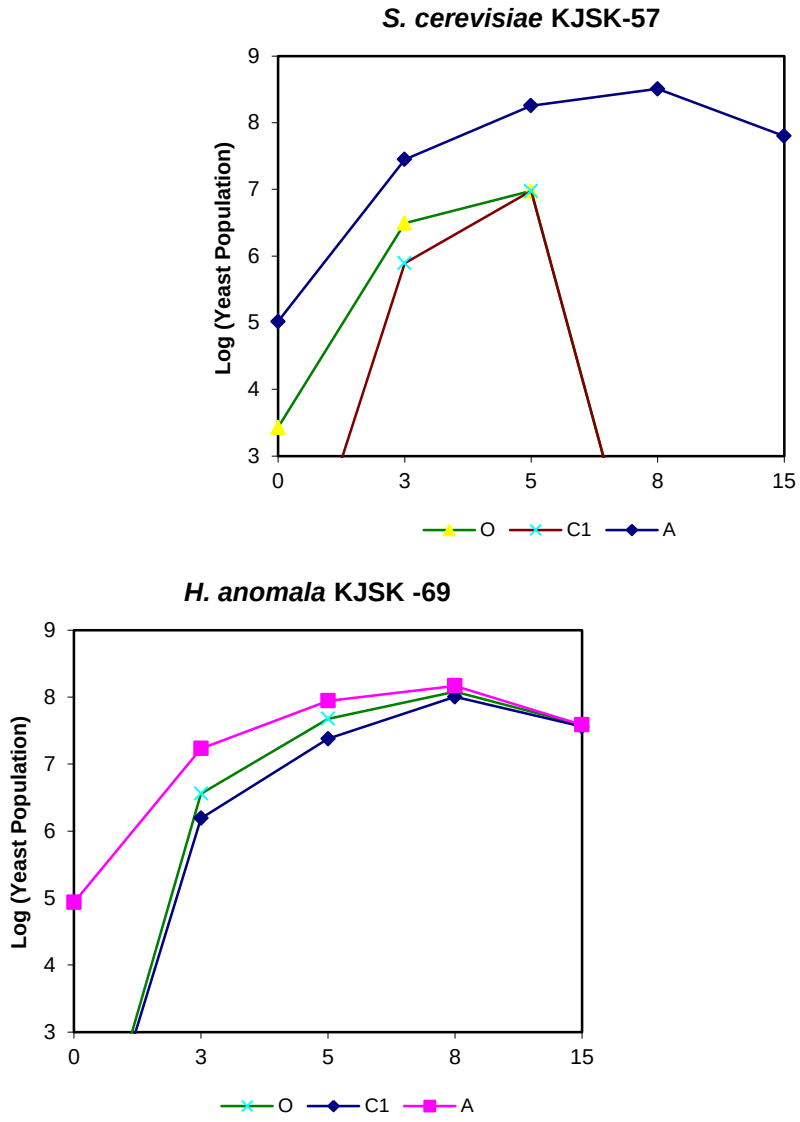
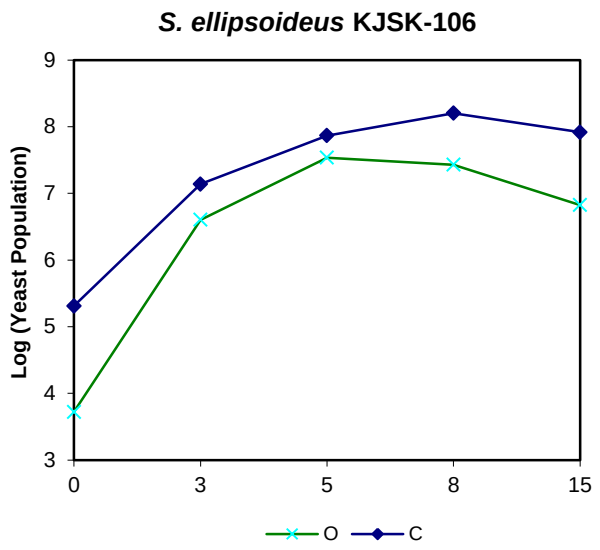
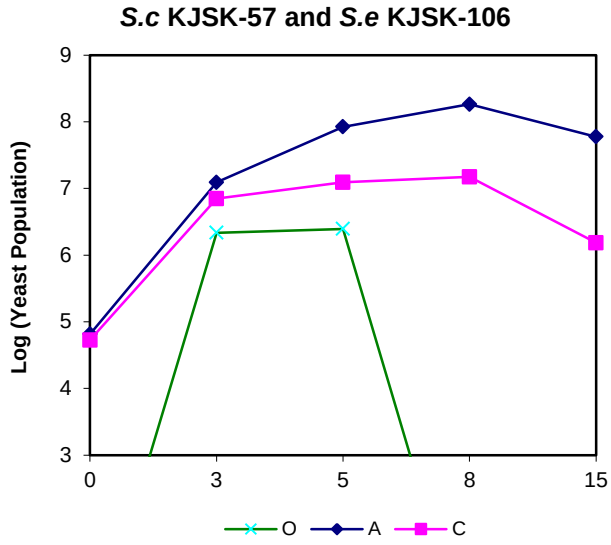

Heating Time
Tannin content (mg l-1)
OD at 520 nm
Skin Contact Time (min.)
Tannin
Brightness

Fig. 17. Effect of skin contact and heating time on colour and tannin extraction in grape wine production




S. ellipsoideus KJSK-106
Log (Yeast Population)
9
8
7
6
5
4
3
0
3
5
8
15
O
C


S.c KJSK-57 and S.e KJSK-106
Log (Yeast Population)
9
8
7
6
5
4
3
0
3
5
8
15
O
A
C

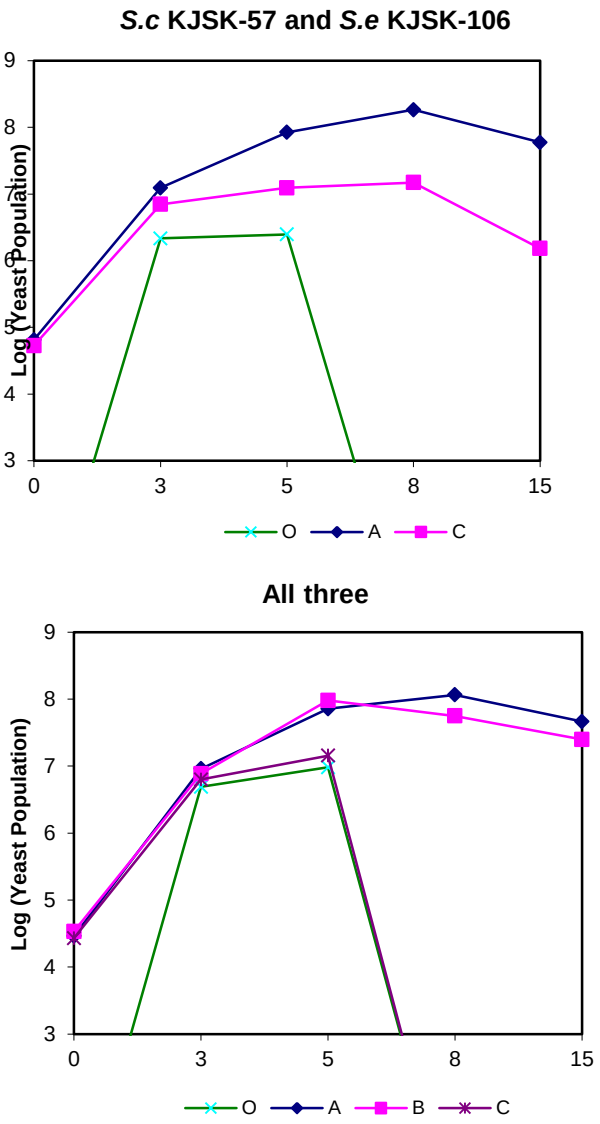
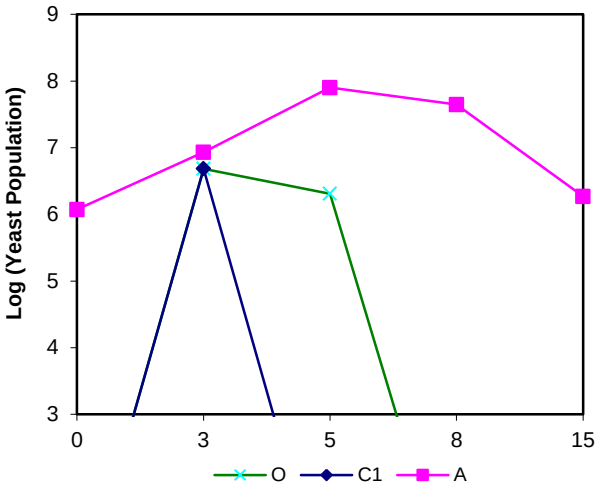
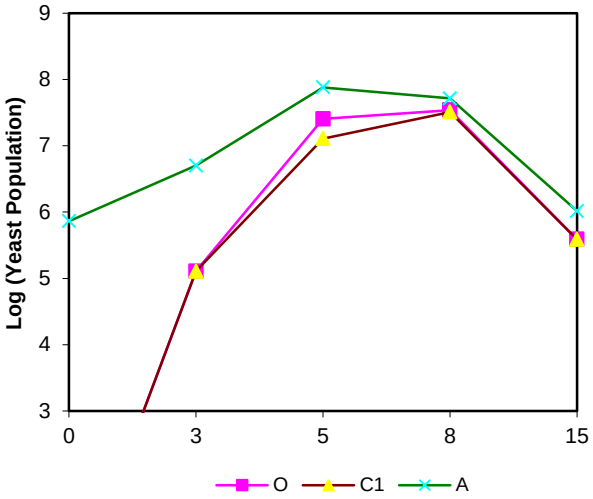


Fig. 18. Yeast population dynamics during co-inoculation in grape wine fermentation

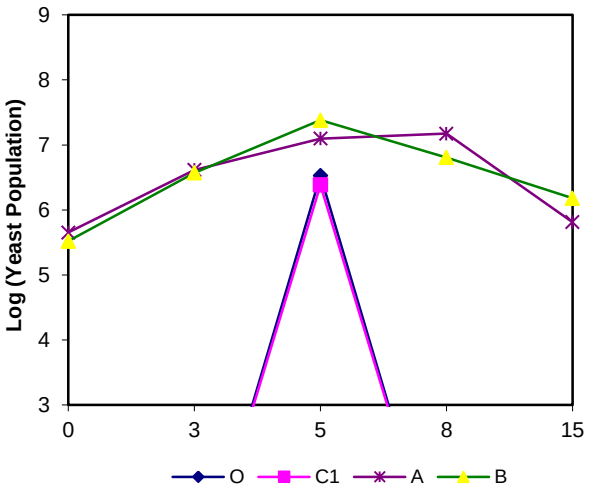
***S. cerevisiae* KJSK-57**

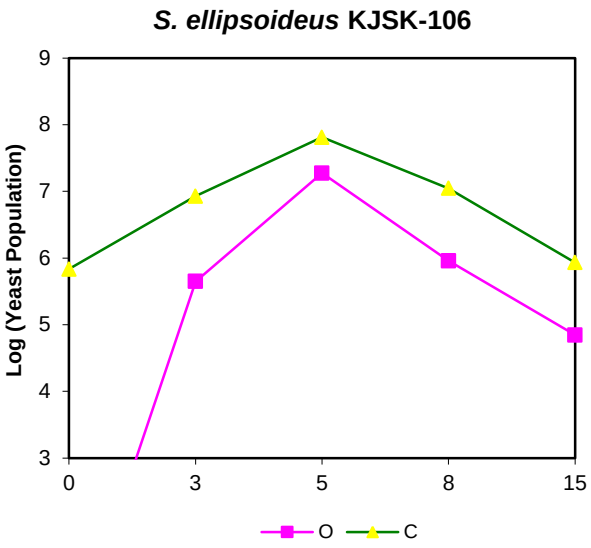


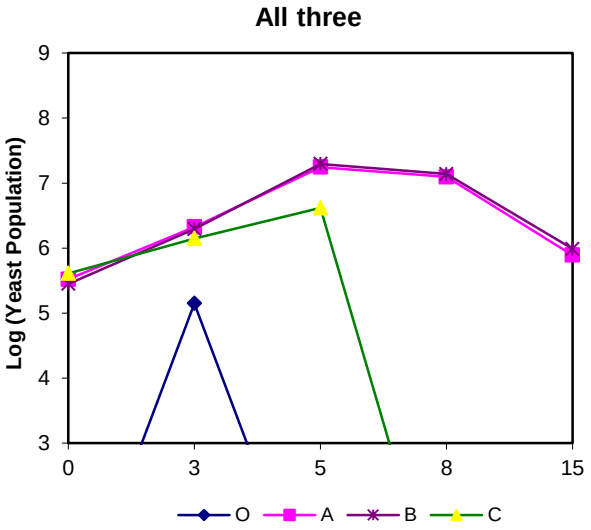
***H. anomala* KJSK -69**



***S.c* KJSK-57 and *H.a* KJSK-69**




S. ellipsoideus KJSK-106
Log (Yeast Population)
9
8
7
6
5
4
3
0
3
5
8
15
O
C


All three
Log (Yeast Population)
9
8
7
6
5
4
3
0
3
5
8
15
O
A
B
C

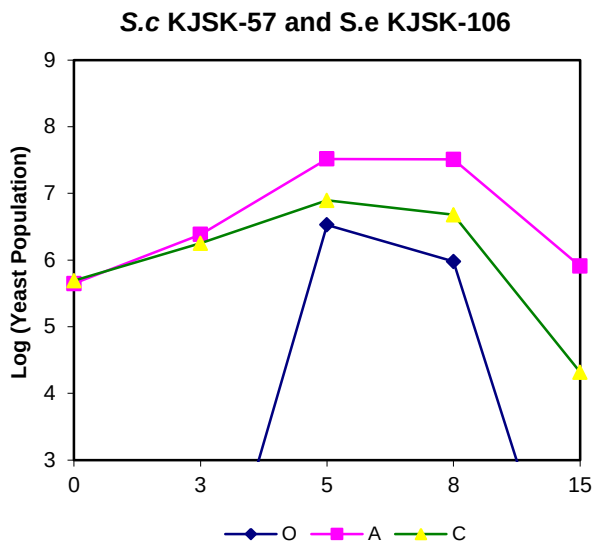
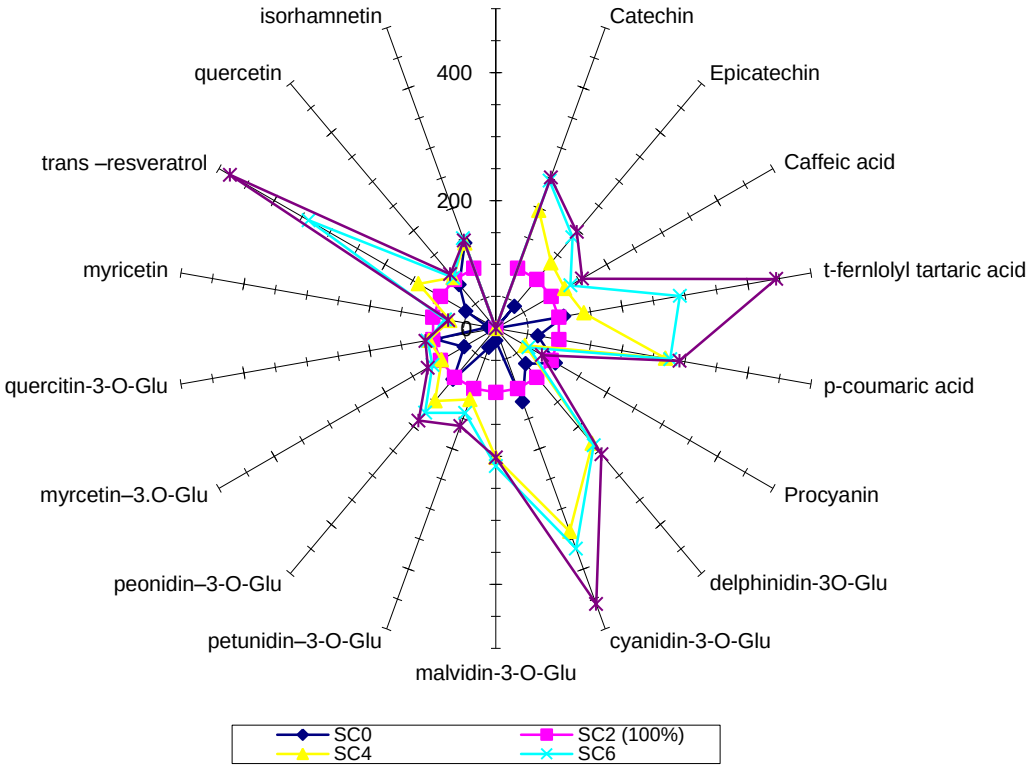


Fig. 19. Yeast population dynamics during co-inoculation in banana wine fermentation


isorhamnetin
Catechin
quercetin
Epicatechin
400
trans –resveratrol
Caffeic acid
200
myricetin
t-fernlolyl tartaric acid
0
quercitin-3-O-Glu
p-coumaric acid
myrcetin–3.O-Glu
Procyanin
peonidin–3-O-Glu
delphinidin-3O-Glu
petunidin–3-O-Glu
cyanidin-3-O-Glu
malvidin-3-O-Glu
SC0
SC2 (100%)
SC4
SC6

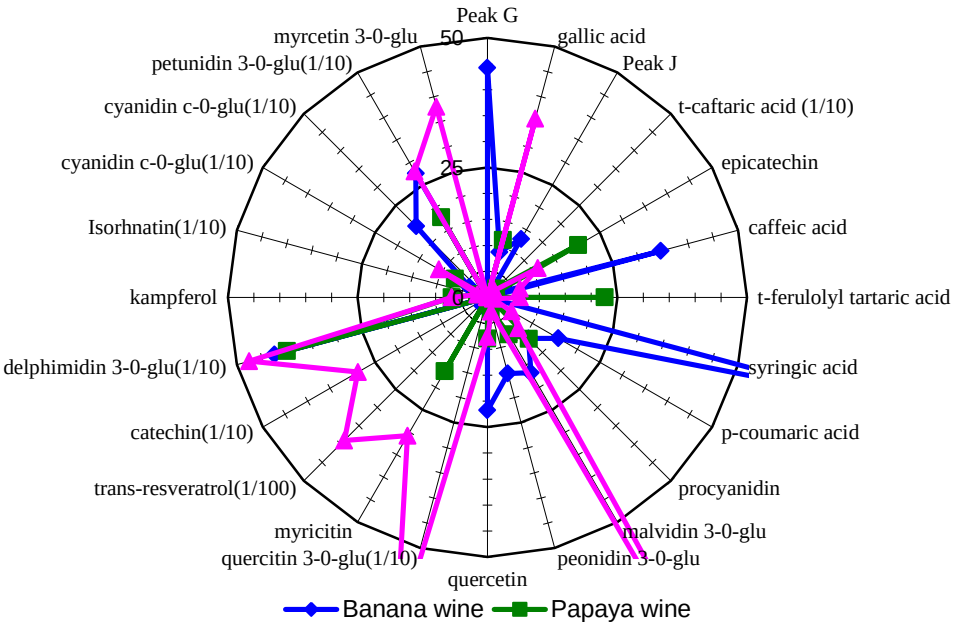


Fig. 26. Principal phenolic compounds present in the banana, papaya and grape wines

APPENDIX

I. Yeast Extract Malt Extract Agar (Wickerham, 1951)

Yeast extract	-	3.0g
Malt extract	-	3.0g
Peptone	-	5.0g
Glucose	-	5.0g
Demineralized water	-	1000ml
Agar	-	20.0g
pH	-	5.6

(+ antibiotic chloramphenicol 20 μg /ml)

II. Yeast Extract Peptone Dextrose Agar (Adams *et al.*, 1997)

Yeast extract	-	10g
Peptone	-	20g
Glucose	-	20g
Agar	-	20g
Dematerialized water	-	1000ml

III. Model wine (Martinez – Rodriguez *et al.* 2001)

Ethanol	-	10% v/v
Tartaric acid	-	4.0 g l^{-1}
Malic acid	-	3.0 g l^{-1}
Acetic acid	-	0.1 g l^{-1}
Potassium sulfate	-	0.1 g l^{-1}
Magnesium sulfate	-	0.025 g l^{-1}
PH	-	3.0 (adjusted with (0.1 N NaOH))

IV. Restricted growth medium (Herman, 1971)

Yeast extract	-	0.05 g
Peptone	-	0.05 g
Glucose	-	0.25 g
Agar	-	5.0 g
Demineralized water	-	250 ml

V. Corn meal agar (Bernhardt, 1946)

Added 15.5 g yellow corn meal to in 300 ml water, stirred and heated in water bath at 60°C for 1 hour and then filtered through paper. The volume of the filtrate is made up to 300 ml and 3.8 g agar is added.

VI. Composition (l^{-1}) of the medium of chemically defined media for taxonomic study of yeasts (Kregar Van Rij, 1984)

Ingredients	Yeast morphology agar	Yeast nitrogen base for carbon assimilation tests	Yeast carbon base for nitrogen assimilation tests	Vitamin –free yeast base for vitamin requirement test
Nitrogen sources				
Ammonium sulphate	3.5g	5g	none	5g
Asparagine	1.5g	none	none	none
Carbon sources				
Dextrose	10g	none	10g	10g
Amino acids				
L-Histidine monohydrochloride	10mg	10mg	1mg	10mg
DL-Methionine	20mg	20mg	2mg	20mg
DL-Tryptophan	20mg	20mg	2mg	20mg
Vitamins				
Biotin	20 µg	20 µg	20 µg	none
Calcium pantothenate	2, 000 µg	2, 000 µg	2, 000 µg	none
Folic acid	2 µg	2 µg	2 µg	none
Inositol	10,000 µg	10,000 µg	10,000 µg	none
Niacin	400 µg	400 µg	400 µg	none
p- Aminobenzoic acid	200 µg	200 µg	200 µg	none
Pyridozine hydrochloride	400 µg	400 µg	400 µg	none
Riboflavin	200 µg	200 µg	200 µg	none
Thiamine hydrochloride	400 µg	400 µg	400 µg	none
Compounds supplying trace elements				
Boric acid	500µg	500µg	500µg	500µg
Copper sulphate	40 µg	40 µg	40 µg	40 µg
Potassium iodide	100 µg	100 µg	100 µg	100 µg
Ferric chloride	200 µg	200 µg	200 µg	200 µg
Manganese sulphate	400 µg	400 µg	400 µg	400 µg
Sodium molybdate	200 µg	200 µg	200 µg	200 µg
Zinc sulphate	400 µg	400 µg	400 µg	400 µg
Salts				
Potassium phosphate monobasic	0.85 g	0.85 g	0.85 g	0.85 g
Potassium phosphate dibasic	0.15g	0.15g	0.15g	0.15g
Magnesium sulphate	0.5g	0.5g	0.5g	0.5g
Sodium chloride	0.1g	0.1g	0.1g	0.1g
Calcium chloride	0.1g	0.1g	0.1g	0.1g
Agar	18g	none	none	none
Amount of final medium from 100g dehydrated medium	2.8 l	14.6 l	8.5 l	5.9 l
Amount of dehydrated medium per liter of finished medium	35.0 g	6.7 g	11.7 g	16.7 g

VII. Liquid culture medium (Iranzo *et al.* 1998)

Glucose	-	10g
Peptone	-	5g
Yeast extract	-	3g
Deminerialized water	-	1000ml

VIII. TE buffer

10mM Tris –cl (pH 7.4)

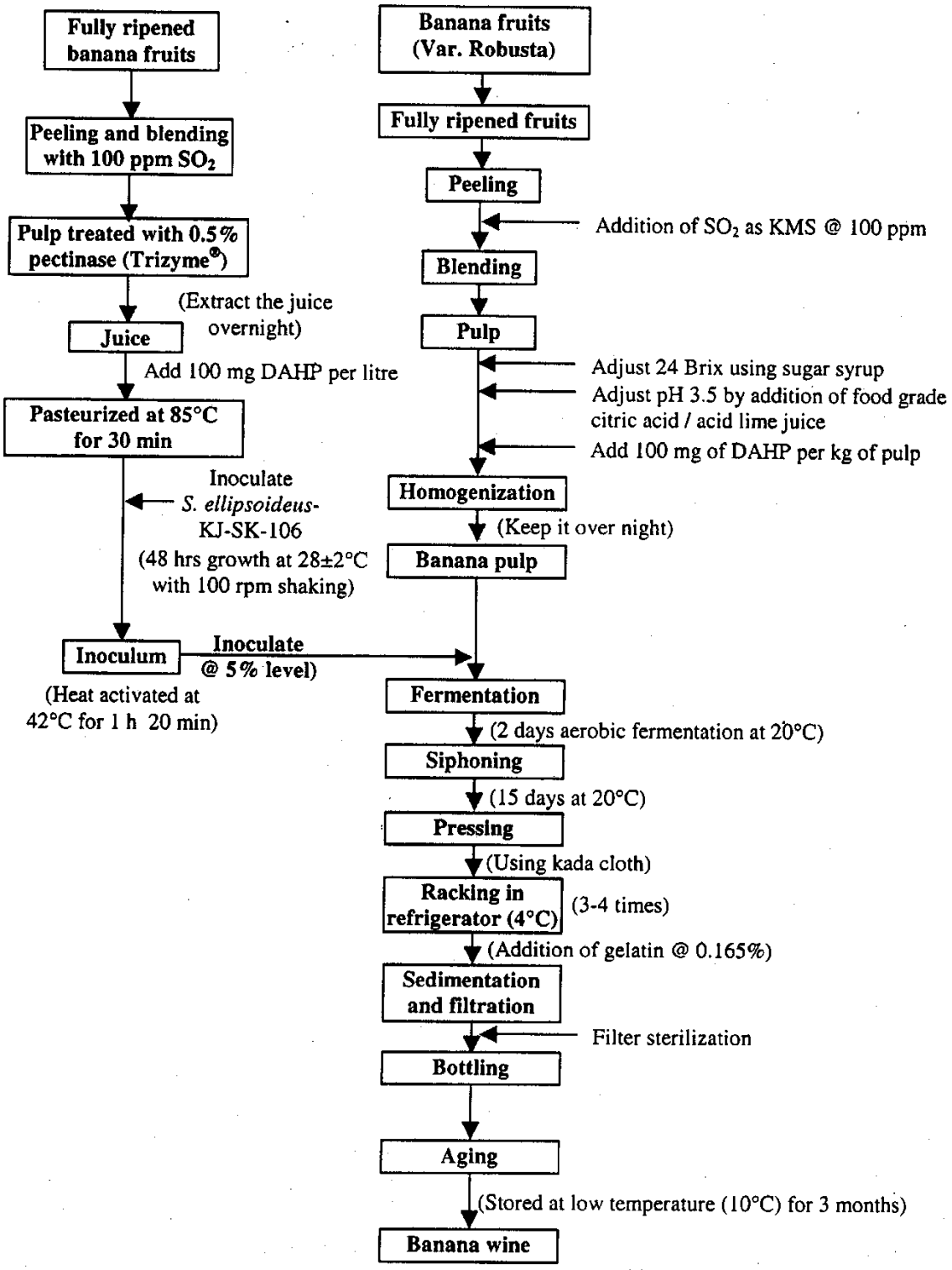
1mM Na_2 EDTA

IX. Organoleptic Score Card (Amerine *et al.*, 1980)

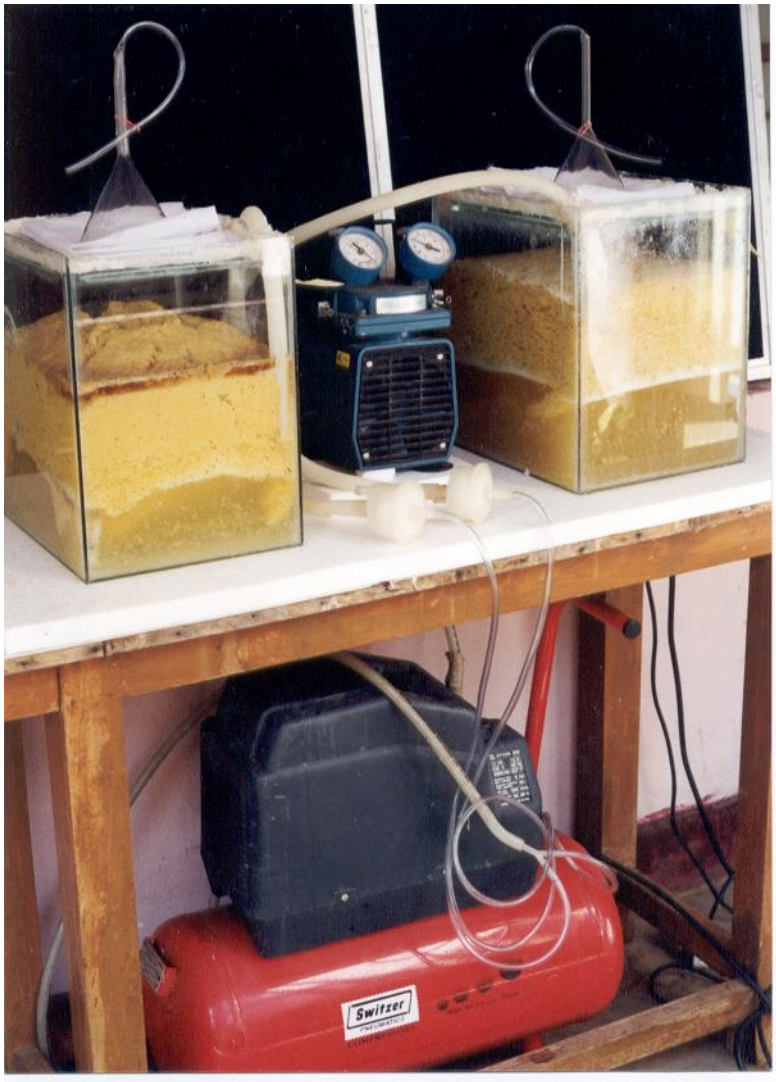
S.No	CHARACTERISTICS	Rating	
1.	Clarity Mildly cloudy	2.0 1.5 1.0 0.5	Very cloudy
2.	Colour Very attractive	2.0 1.5 1.0 0.5	Not attractive
3.	Aroma Very good	4 3 2 1	Very poor
4.	Free from acetic odour Not pungency	2.0 1.5 1.0 0.5	Pungency
5.	Total acid to taste Tolerable	2.0 1.5 1.0 1.5	In tolerable
6.	Astringency Sufficient	2.0 1.5 1.0 0.5	High
7.	Body Clear	1.0 0.75 0.50 0.25	Interfering substances
8.	Sugar Sufficient	1.0 0.75 0.50 0.25	Not sufficient
9.	Characteristic fruit flavour Flavour	2.0 1.5 1.0 0.5	No flavour
10.	Overall acceptability Very good	2.0 1.5 1.0 0.5	Very poor

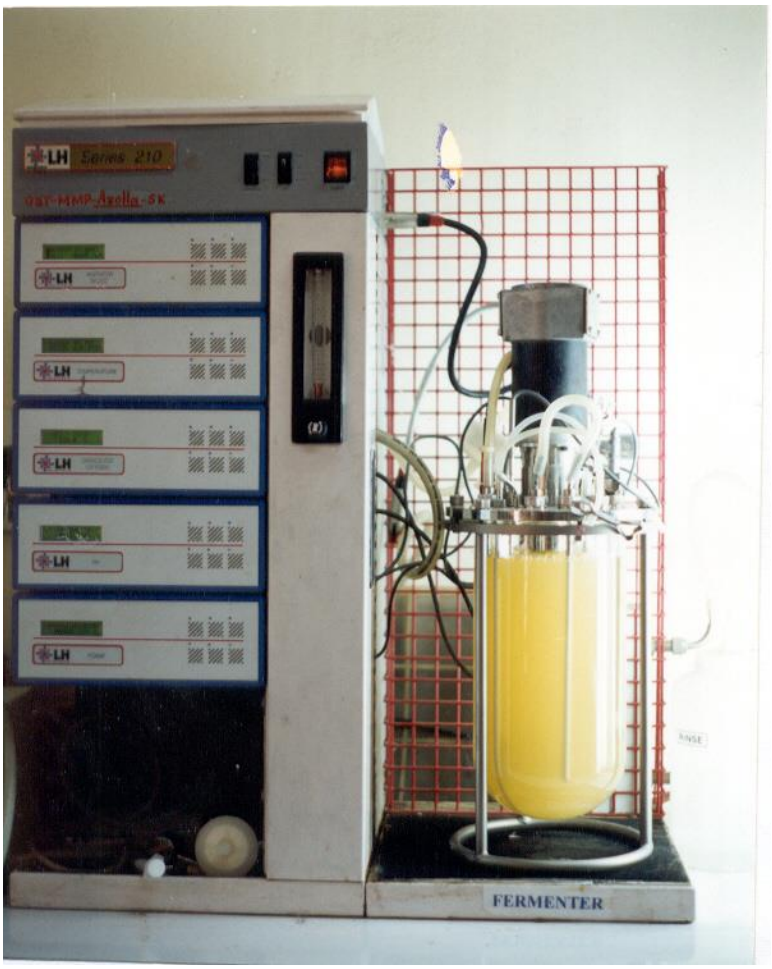
X. Nutritive values of fruits used

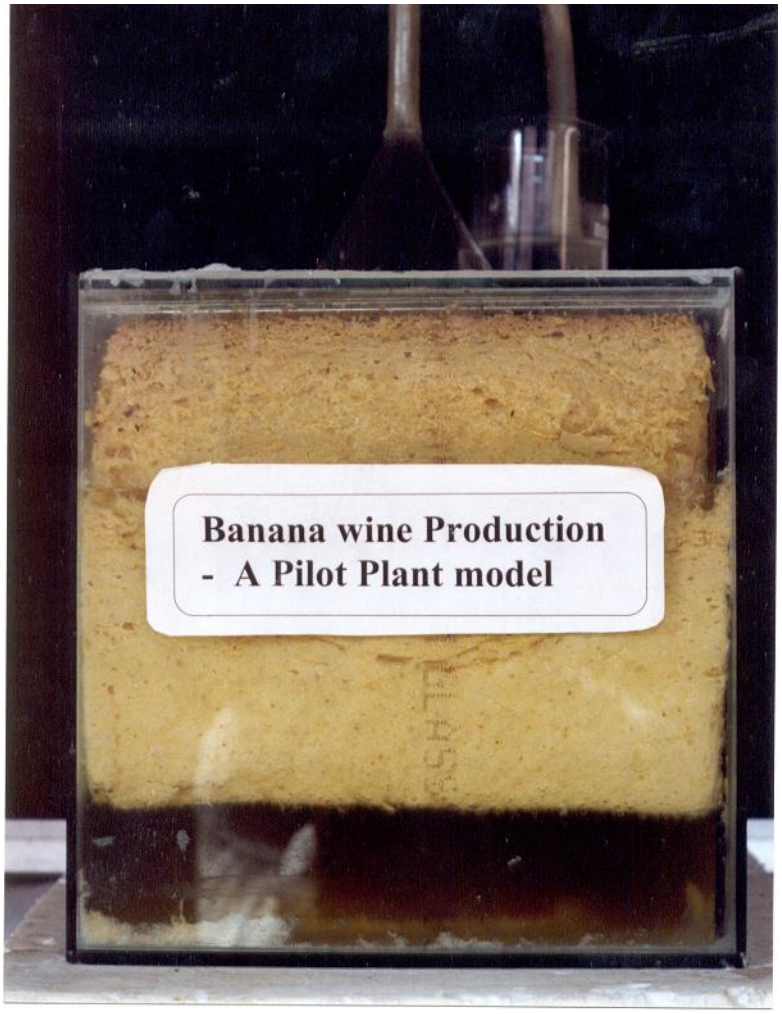
Components	Grapes	Banana	Papaya
Edible protein (%)	95.00	71.00	75.00
Moisture (g)	82.2	70.10	90.80
Protein (g)	0.6	1.20	0.60
Fat (g)	0.4	0.30	0.10
Minerals (g)	0.9	0.80	0.50
Fibre (g)	2.8	0.40	0.80
Carbohydrate (g)	13.1	27.20	7.20
Energy (Kcal)	58.00	116.00	32.0
Ca (mg)	20.00	17.00	17.0
P (mg)	23.00	36.00	13.0
Fe (mg)	0.5	0.90	0.50
Carotene (μg)	3.00	78.00	666.00
Thiamine (mg)	0.04	0.05	0.04
Riboflavin (mg)	0.03	0.08	0.25
Niacin (mg)	0.2	0.50	0.2
Vitamin C (mg)	1.00	7.00	57.00

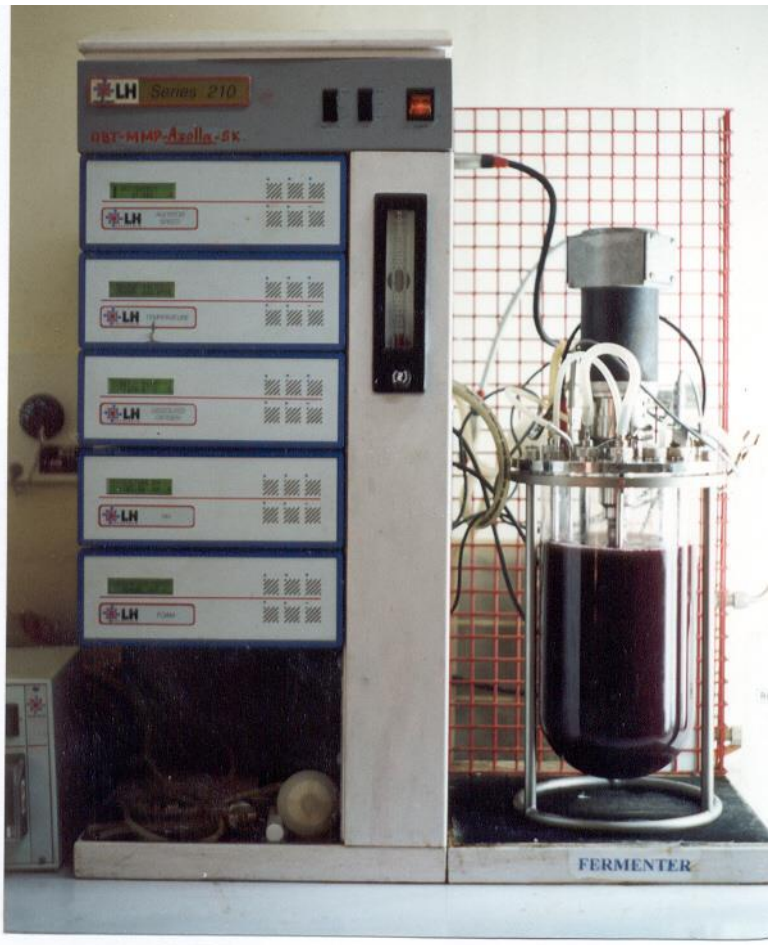

Fully ripened banana fruits
Peeling and blending with 100 ppm SO_2
Pulp treated with 0.5% pectinase (Trizyme®)
(Extract the juice overnight)
Juice
Add 100 mg DAHP per litre
Pasteurized at 85°C for 30 min
Inoculate
S. ellipsoideus-KJ-SK-106
(48 hrs growth at 28±2°C with 100 rpm shaking)
Inoculum
Inoculate @ 5% level)
(Heat activated at 42°C for 1 h 20 min)
Banana fruits (Var. Robusta)
Fully ripened fruits
Peeling
Addition of SO_2 as KMS @ 100 ppm
Blending
Pulp
Adjust 24 Brix using sugar syrup
Adjust pH 3.5 by addition of food grade citric acid / acid lime juice
Add 100 mg of DAHP per kg of pulp
Homogenization
(Keep it over night)
Banana pulp
Fermentation
(2 days aerobic fermentation at 20°C)
Siphoning
(15 days at 20°C)
Pressing
(Using kada cloth)
Racking in refrigerator (4°C)
(3-4 times)
(Addition of gelatin @ 0.165%)
Sedimentation and filtration
Filter sterilization
Bottling
Aging
(Stored at low temperature (10°C) for 3 months)
Banana wine


B. O. D. INCUBATOR
DELUXE AUTOMATIC
MAIN
HEATING
OFF
ON


Switzer


LH Series 210
FERMENTER


Banana wine Production
- A Pilot Plant model

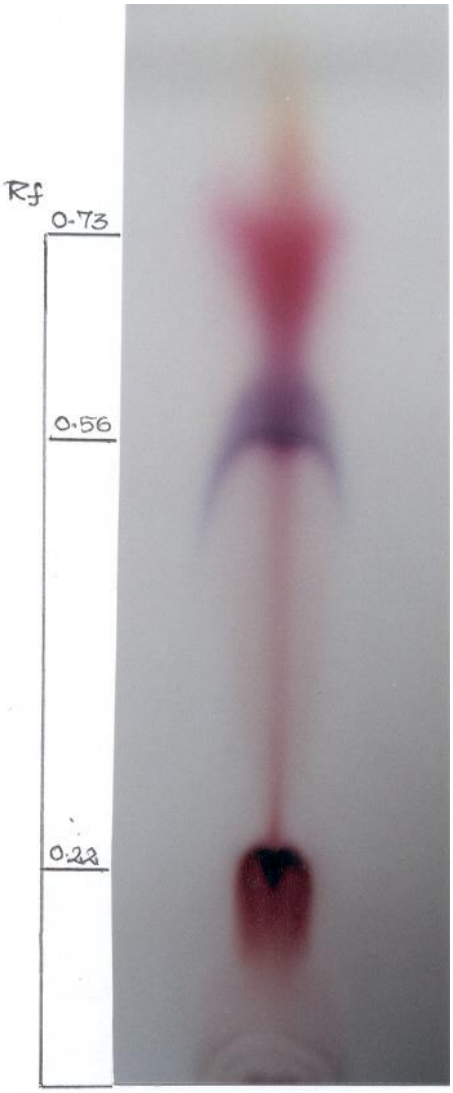

LH Series 210
FERMENTER

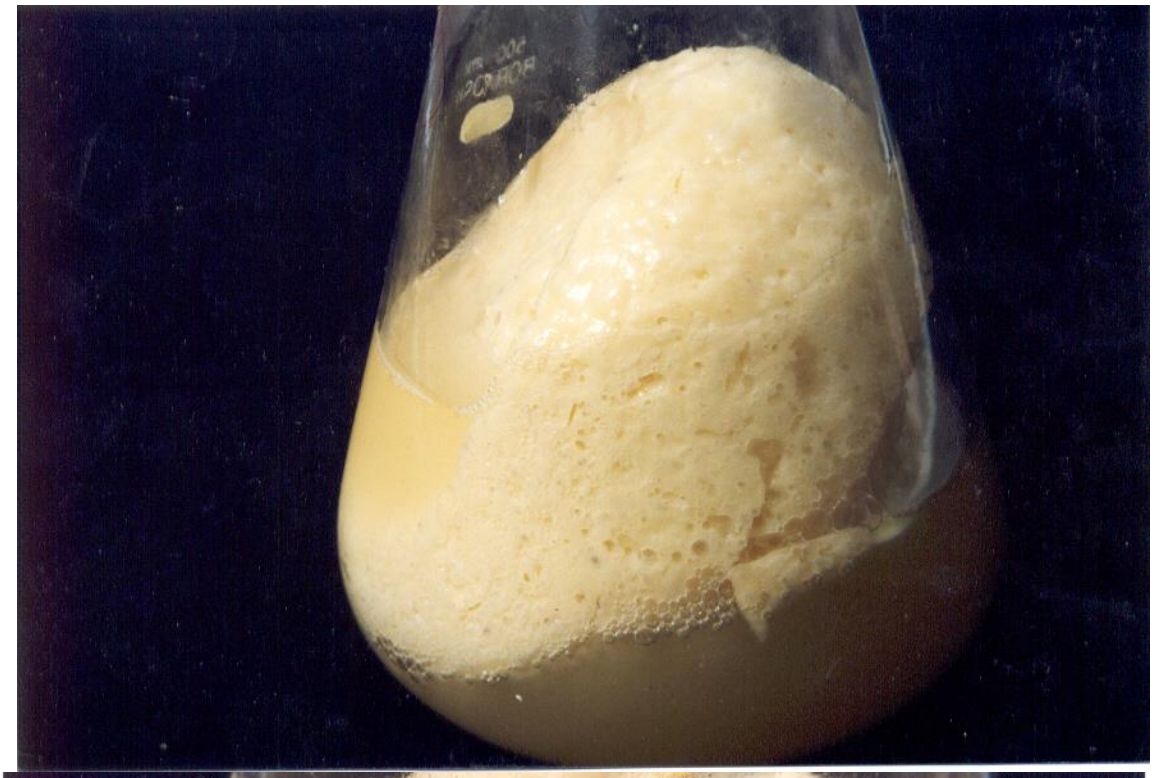

BOROSIL
4060

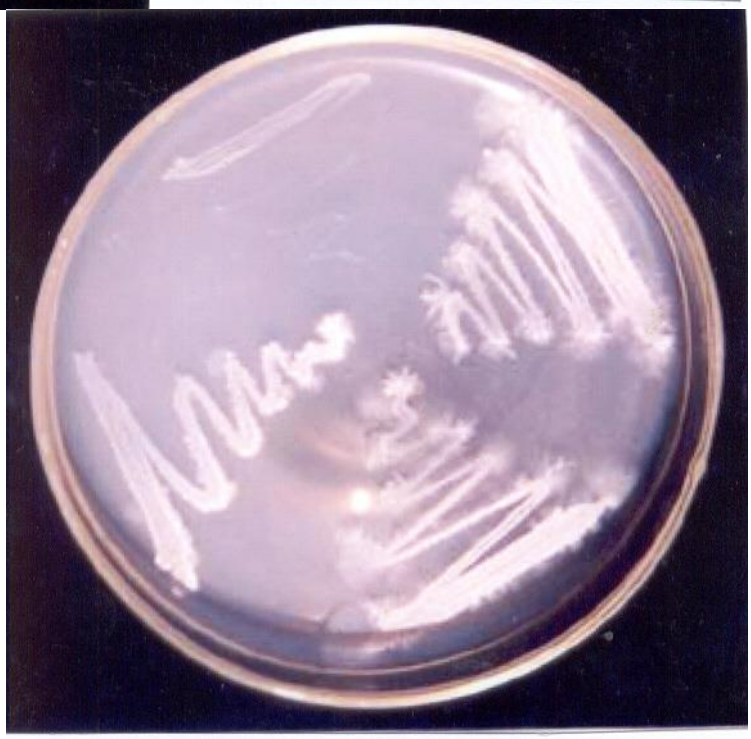

GRAPE WINE
Var: Muskat

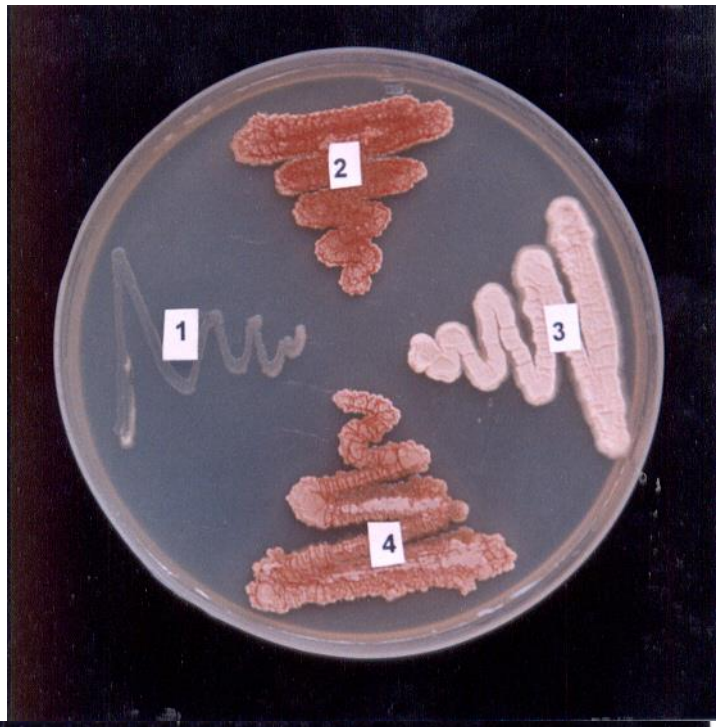

PAPAYA WINE
(CO-2)
67/01

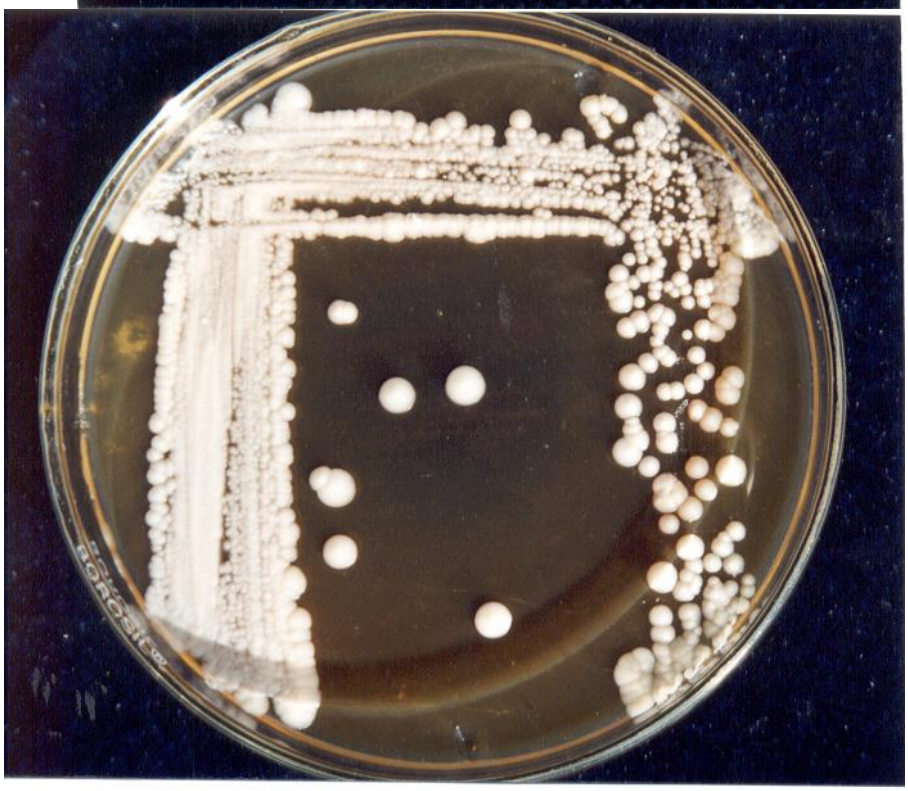

ANANA WINE
ar:Robusta

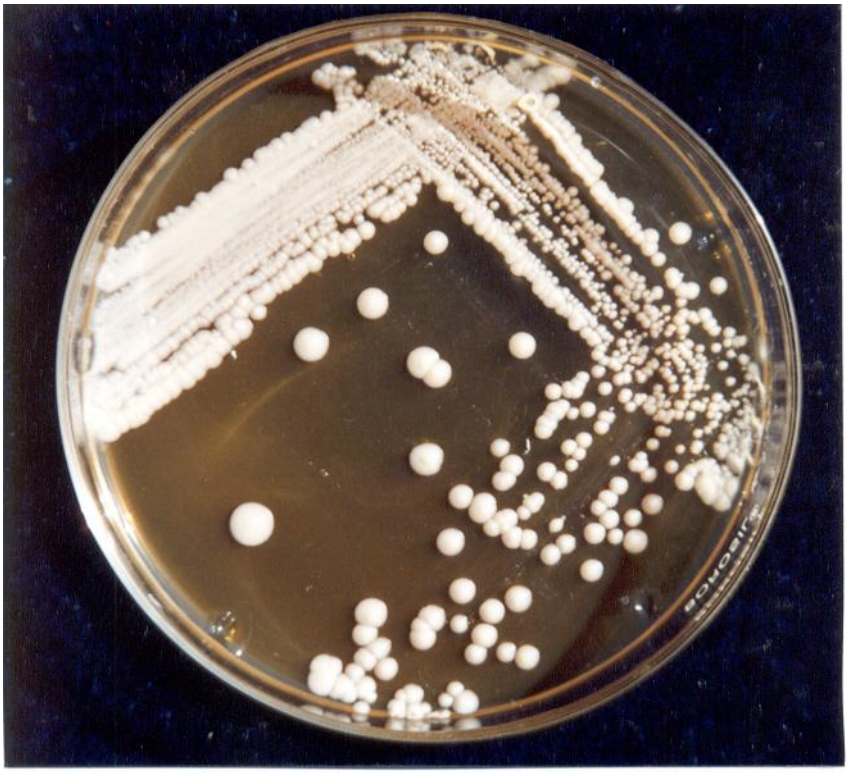

Rf
0.73
0.56
0.22

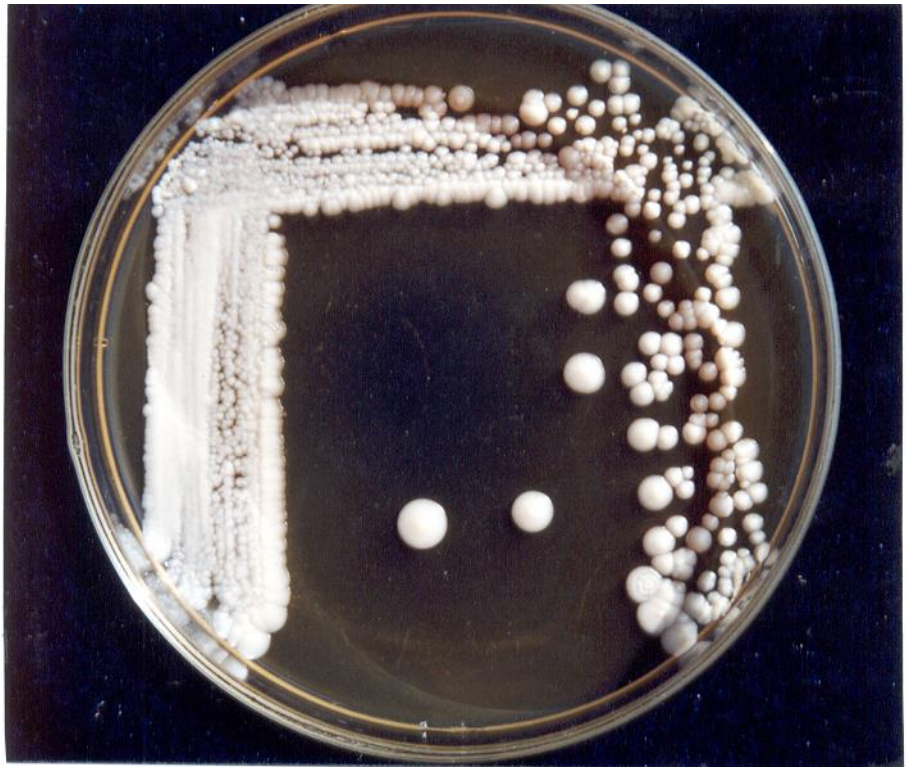


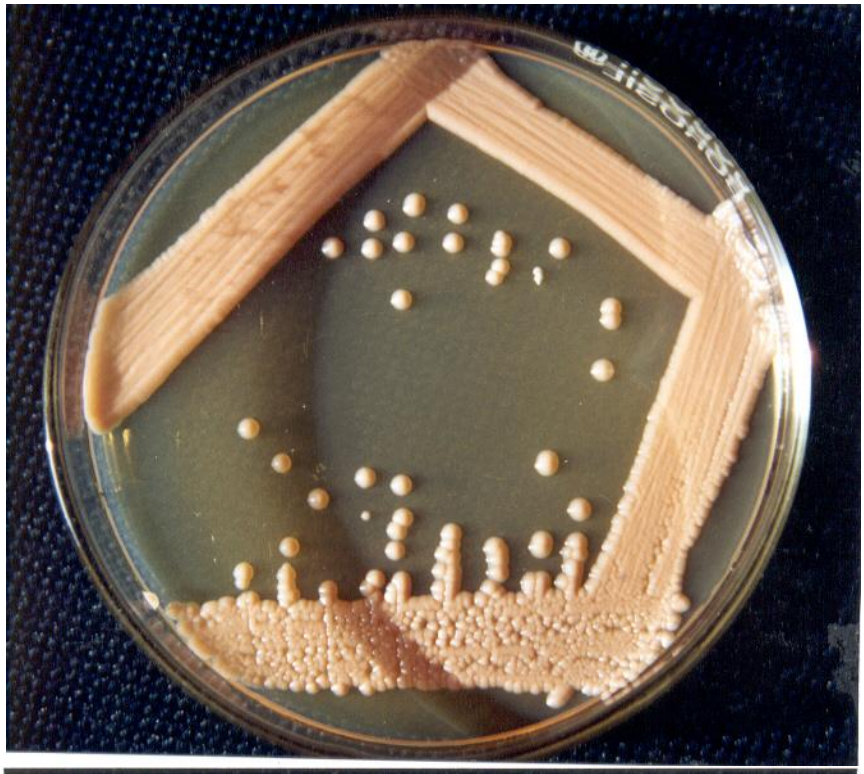


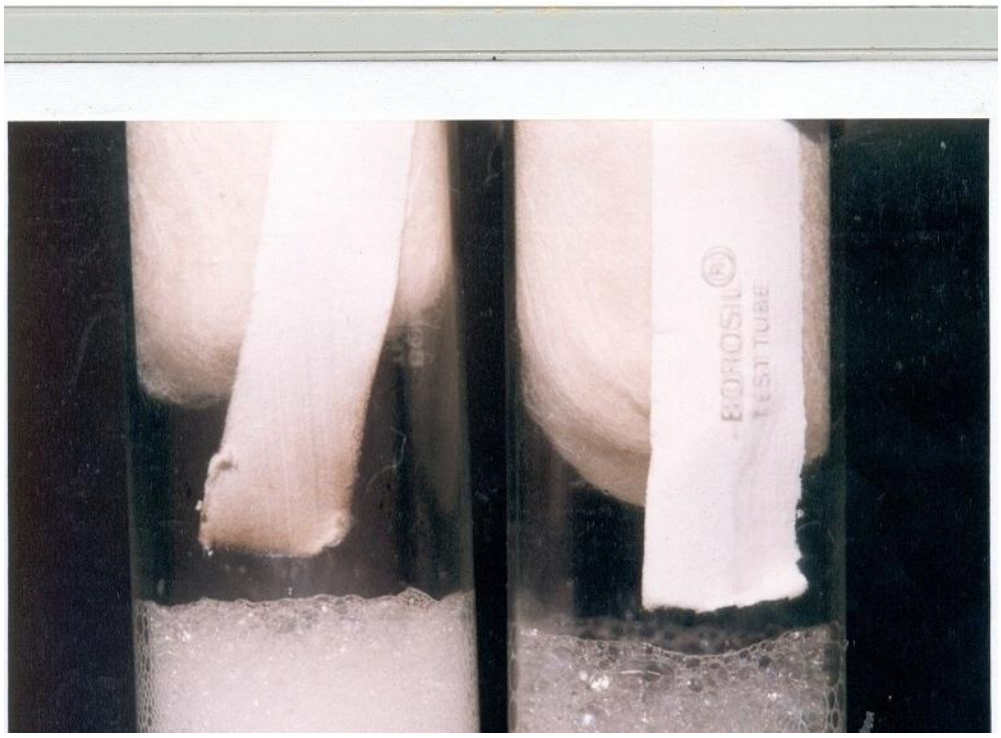

2
1
3
4

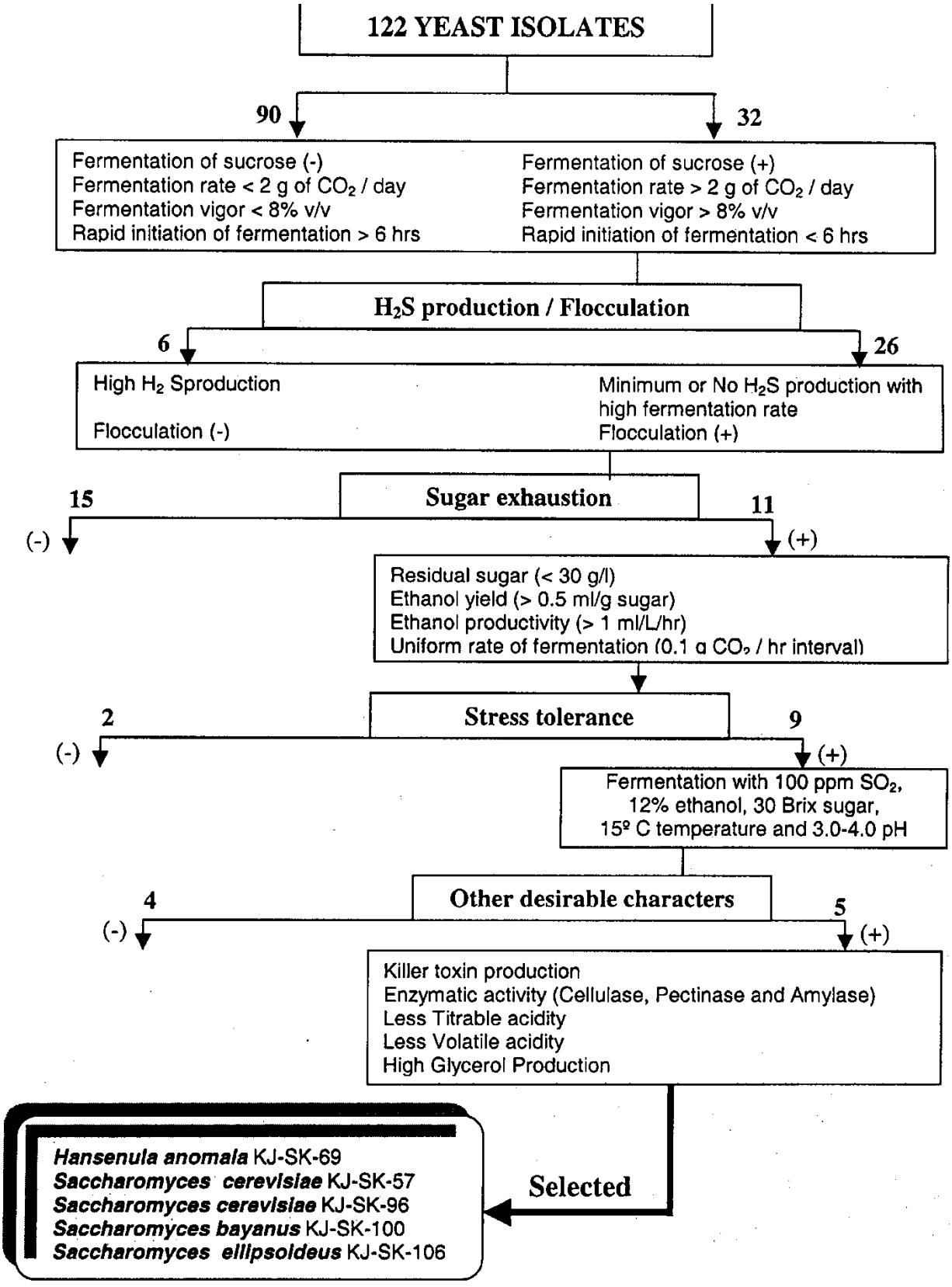

PYREX
BORCAM

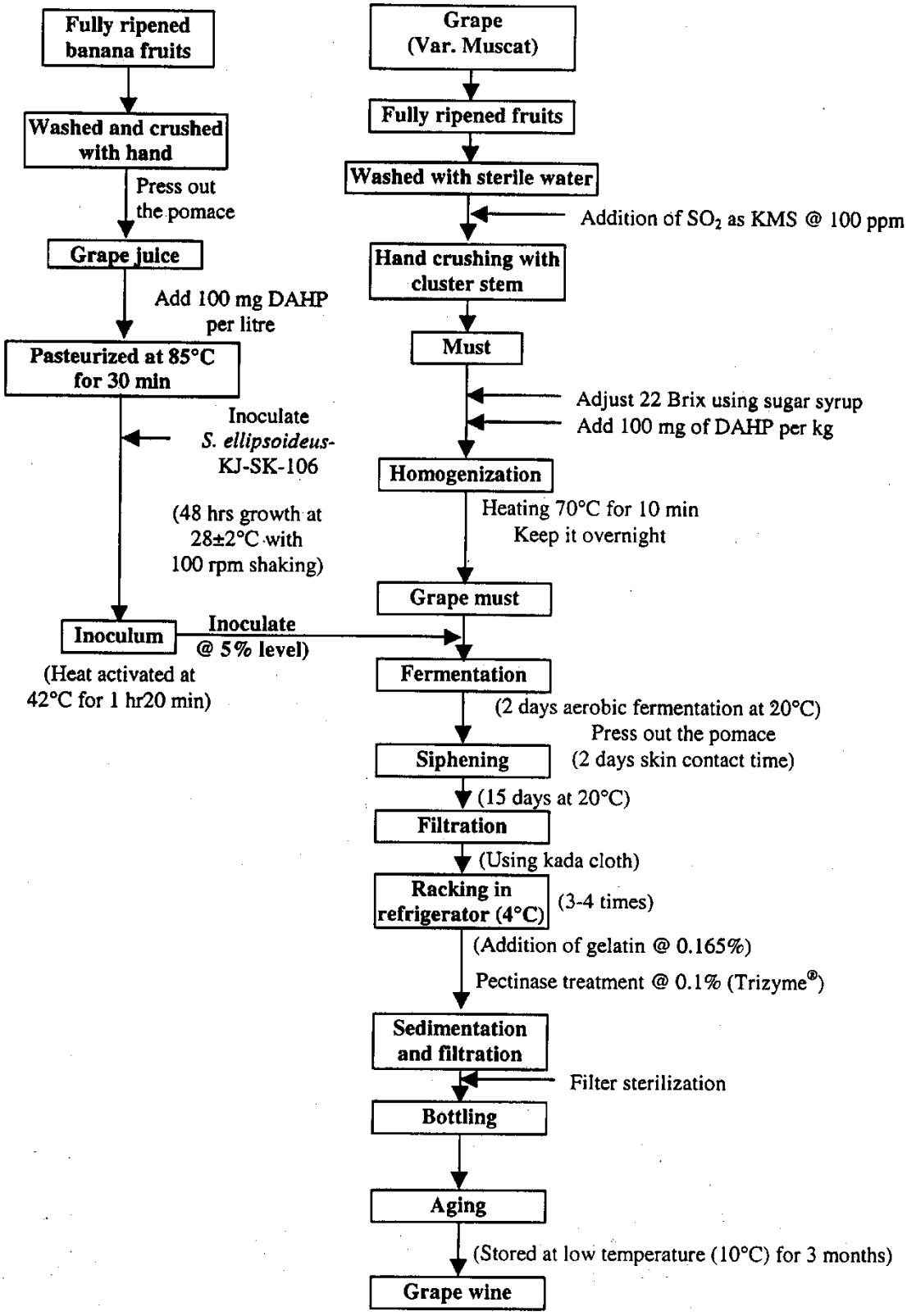







PYROSIL®
TEST TUBE


122 YEAST ISOLATES
90
32
Fermentation of sucrose (-)
Fermentation rate < 2 g of CO_2 / day
Fermentation vigor < 8% v/v
Rapid initiation of fermentation > 6 hrs
Fermentation of sucrose (+)
Fermentation rate > 2 g of CO_2 / day
Fermentation vigor > 8% v/v
Rapid initiation of fermentation < 6 hrs
H_2S production / Flocculation
6
26
High H_2 Sproduction
Flocculation (-)
Minimum or No H_2S production with high fermentation rate
Flocculation (+)
Sugar exhaustion
15
11
(-)
(+)
Residual sugar (< 30 g/l)
Ethanol yield (> 0.5 ml/g sugar)
Ethanol productivity (> 1 ml/L/hr)
Uniform rate of fermentation (0.1 g CO_2 / hr interval)
Stress tolerance
2
9
(-)
(+)
Fermentation with 100 ppm SO_2, 12% ethanol, 30 Brix sugar, 15º C temperature and 3.0-4.0 pH
Other desirable characters
4
5
(-)
(+)
Killer toxin production
Enzymatic activity (Cellulase, Pectinase and Amylase)
Less Titrable acidity
Less Volatile acidity
High Glycerol Production
Selected
Hansenula anomala KJ-SK-69
Saccharomyces cerevisiae KJ-SK-57
Saccharomyces cerevisiae KJ-SK-96
Saccharomyces bayanus KJ-SK-100
Saccharomyces ellipsoideus KJ-SK-106


Fully ripened banana fruits
Washed and crushed with hand
Press out the pomace
Grape juice
Add 100 mg DAHP per litre
Pasteurized at 85°C for 30 min
Inoculate S. ellipsoideus-KJ-SK-106
(48 hrs growth at 28±2°C with 100 rpm shaking)
Inoculum
Inoculate @ 5% level)
(Heat activated at 42°C for 1 hr20 min)
Grape (Var. Muscat)
Fully ripened fruits
Washed with sterile water
Addition of SO2 as KMS @ 100 ppm
Hand crushing with cluster stem
Must
Adjust 22 Brix using sugar syrup
Add 100 mg of DAHP per kg
Homogenization
Heating 70°C for 10 min
Keep it overnight
Grape must
Fermentation
(2 days aerobic fermentation at 20°C)
Press out the pomace
(2 days skin contact time)
Siphening
(15 days at 20°C)
Filtration
(Using kada cloth)
Racking in refrigerator (4°C)
(3-4 times)
(Addition of gelatin @ 0.165%)
Pectinase treatment @ 0.1% (Trizyme®)
Sedimentation and filtration
Filter sterilization
Bottling
Aging
(Stored at low temperature (10°C) for 3 months)
Grape wine