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Production of Grape wine by the use of yeast, *Saccharomycese cerevisiae*

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ABSTRACT

Present study was done for production of Grape Wine from yeast, Saccharomycese cerevisiae.The production was done in Abhilashi Institute of Life Sciences. Fresh grapes for wine production were taken from nearby shops.Rotten grapes were also taken for isolation of Saccharomycese cerevisiae.Wine is traditionally an alcoholic beverage arising from the fermentation of fruit juice by the action of yeast.Wine was prepared from grape juice as a raw material and Saccharomycese cerevisiae was isolated from rotten part of grapes under laboratory conditions.To study the fermentation process,sample was drawn after an interval of 5 days and microbiological and biochemical characterization was done.For microbial investigation,samples drawn after intervals of 5 days were grown on specific media by serial dilution technique.The microflora grown on specific media was identified by staining techniques.The predominant microorganism identified was yeast, Saccharomycese cerevisiae.It increases gradually due to its tolerance power towards low pH ,high acidity and ethanol content.Biochemical analysis includes pH,total acidity,reducing sugar,ethanol content etc.In the investigation of biochemical aspects,it was concluded that the pH of sample was decreased whereas the titrable acidity of sample was increased during fermentation process. Wine was prepared after 21 days of fermentation.

KEYWORDS : Grape Wine, Saccharomycese cerevisiae, serial dilution technique, fermentation.

INTRODUCTION

Grape wine is perhaps the most common fruit juice alcohol.Because of the commercialization of the product for industry,the process has received most research attention.(Bhalla et al.,2004)

The production of grape wine involves the following basic steps:crushing the grapes to extract the juice,bulk storage and maturation of the wine in a cellar,clarification and packaging. Although theprocess is fairly simple,quality control demands that the fermentation is carried out under controlled conditions to ensure a high quality product.(Yarrow,1988)

The distinctive flavor of grape wine originates from the grapes as raw material and subsequent processing operations. The grapes contribute trace elements of many volatile substances(mainly terpenes) which give the final product,the distinctive fruity character.In addition,they contribute non-volatile compounds(tartaric and malic acids) which impact on flavor and tannins which give bitterness and astringency.The latter are more prominent in red wines as the tannin components are located in the grape skins.(Vullo et al.,2005)

Although yeasts are the principle organisms involved,filamentous fungi,lactic acid bacteria,acetic acid bacteria and other bacterial groups all play a role in the production of alcoholic fruit products. Normal grapes harbour a diverse microflora,of which the principle yeasts(Saccharomycese cerevisiae)involved in desirable fermentation are in the minority. Lactic acid bacteria and acetic acid bacteria are also present.The proportions of each and total numbers present are dependent upon a number of external environmental factors including the temperature,humidity,stage of maturity,damage at harvest and application of fungicides.It is essential to ensure proliferation of the desired species at the expense of the non-desired ones.This is achieved through ensuring fermentation conditions as such to encourage Saccharomycese species.(Bajaj et al.,2011)

The fermentation may be initiated using a starter culture of Saccharomycese cerevisiae,in which case the juice is inoculated with populations of yeast of 10^6 to 10^7cfu/ml juice.This approach produces a wine of generally expected taste and quality.If the fermentation is allowed to proceed naturally,utilizing the yeasts present on the surface of the fruits,the end result is less controllable but produces wines with a range of flavor characteristics.It is likely that natural fermentations are practiced widely around the world,especially for home production of wine.In natural fermentations,there is a progressive pattern of yeast growth.Several species of yeast,including Kloeckera,Hanseniaspora,candida and Metschnikowia are active for the first two to three days of fermentation.The build up of end products(ethanol) is toxic to these yeasts and they die off,leaving Saccharomycese cerevisiae to continue the fermentation to the end. Saccharomycese cerevisiae can tolerate much higher levels of ethanol (upto15%v/v or more) than the other species who only tolerate upto 5% or 8% alcohol.(Aidoo,1994)

Because of its tolerance of alcohol, Saccharomycese cerevisiae dominates wine fermentation and is the species that has been commercialized for starter cultures.(Adams,1990)

Grape proteins are responsible for the appearance of haziness in white wines during storage after bottling.The quantity and relative proportion of soluble proteins vary during and after the alcoholic fermentation,as does their heat instability in wine.Grape constituting a large proportion of the total proteins in the wine,seems to play a major role in protein haze formation.The release of yeast polysaccharides is related to an increased heat stability of total wine protein,despite the increase in the relative proportion of their most unstable component.Therefore,the hazing potential of a white wine seems to be affected by variations in the relative proportions of its macromolecular components occurring in the early stages of winemaking.(Vincenzi et al.,2011)

White wines are stabilized by removing the heat unstable proteins through zirconia treatment. Effective treatment was achieved by enclosing zirconia pellets into a metallic cage submerged in the wine. With this method, the wine could be treated with the adsorbent for the time required for protein stabilization and then removed without further manipulation. Zirconia treatments of three unstable wines partially or fully stabilized them without detectable modifications of their physicochemical parameters and colours, apart from the removal of metals and some acids, particularly when wines were treated for long times and with high dosages of the adsorbent. A simple and inexpensive zirconia regenertation method was also developed.(Marangon et al.,2011)

Flavonoid composition of wine is related to wine sensory properties. Differences in the grape berry flavonoid profile were created by exposing bunches to varying levels of sunlight intensity through canopy manipulation.Grapes produced under shaded canopy conditions had reduced anthocyanins and skin tannins but little effect on seed tannins was observed.Pigmented polymers and tannins in wines are related to berry flavonoid composition(anthocyanins,skin and seed tannins and their ratios).Higher concentrations of anthocyanins and skin tannins in berries,coupled with a lower concentration of seed tannins were associated with higher wine quality.The ratio anthocyanins ,skin tannins/seed tannins is proposed as an indicator of flavonoid com-

position,wine colour and wine quality.Excessive canopy shade was detrimental to berry and wine composition and intensified sensory detection of 'straw' and 'herbaceous' characters in the wines.(Ristic et al.,2010)

Potassium (K) is essential for wine growth and yield. Excess potassium levels in grape berries may have a negative impact on wine quality mainly because it decreases free tartaric acid resulting in an increase in the pH of grape juice, must and wine. This necessitates pH adjustment during the vinification process and tartaric acid addition in wine. High potassium concentration may also lead to excessive loss of the additional tartaric acid by precipitation as potassium bitartarate and as a consequence, pH adjustment becomes more difficult and expensive. Ensuring naturally low potassium levels in the berry will help reduce costs of input and waste management at the winery. Potential vineyard management options to manipulate berry potassium accumulation includes selective use of rootstock/scion combination, canopy management and irrigation strategies. However, the impact of these practices on determining the optimum potassium concentrations requires careful caliberation of production parameters and the desirable grape juice and wine quality in relation to tissue potassium concentration. (Mpelasoka et al.,2008)

There is impact of closure type and storage conditions on the composition, colour and flavor properties of wine. The wines were bottled under a screw cap closure, two different natural corks, a synthetic closure and in a glass ampoule. In addition, the effect of storage orientation was investigated. The bottled wines were stored under controlled temperature and humidity conditions. Various analysis were carried out on replicate bottles from each treatment, including sulfurdioxide and ascorbic acid concentration, sensory analysis of appearance and aroma attributes and spectral measures. The largest treatment effect resided with the nature of the closure. Wines sealed with the synthetic closure were relatively oxidized in aroma, brown in colour and low in sulfurdioxide compared to wines held under the other closures. A struck flint/rubber (reduced) aroma was discernible in the wines sealed under the screw caps or in glass ampoules. Wines sealed under natural bark corks in this study showed negligible reduced characters. The bottle orientation during storage under the conditions of this study had little effect on the composition and sensory properties of the wines examined.(Oakey et al.,2005)

Malolactic fermentation is inhibited by Saccharomycese during alcoholic fermentation under low and high nitrogen conditions. Malolactic fermentation was induced in fermenting synthetic grape juice or synthetic wines inoculated with different commercial strains of Saccharomycese cerevisiae. Oenococcus oeni was generally inhibited in wines that contained higher concentrations of total sulfurdioxide although many yeast strains only inhibited the bacteria during fermentation under high nitrogen conditions. Yeast produced higher amounts of sulfurdioxide during fermentation under high nitrogen conditions suggesting that nitrogen affected the malolactic fermentation by influencing yeast sulfurdioxide production.However,the production of sulfurdioxide by yeast did not always account for the inhibition of Oenococcus oeni,suggesting the presence of other inhibitory mechanisms. (Osborne et al,2006)

The concentration of urea in wine is not routinely measured in laboratories but has been examined in studies of yeast metabolism and the formation of ethyl carbamate, a known carcinogen. For alcoholic beverages that may contain high levels of urea,steps have been taken to reduce the concentration of urea and therefore prevent ethyl carbamate production. Methods for the determination of urea in wine can be grouped into three categories that indicate how selectivity of urea is achieved, those based on colour-forming reactions, enzymatic hydrolysis and chromatographic separation. The two dominant methods used by research groups over the past fifteen years for the determination of urea in wine are based on the urea/ammonia test kit available from Boeringer Mannheim/R-Biopharm and the reaction of urea with 1-phenyl-1,2-propanedione-2-oxime,both are time consuming and labour intensive,but involve relatively straightforward and well established procedures.(Paul ,2006)

The colour properties and pigment profiles of Saccharomycese cerevisiae and Saccharomycese bayanus wines were compared at 8 days and 387 days after yeast inoculation. Grape anthocyanin concentration was found to be lower in Saccharomycese bayanus wines than in Saccharomycese cerevisiae wines at day 8 and 387 but Saccharomycese bayanus wine exhibited greater wine colour density. The anthocyanin concentration did not correlate with wine colour density. The levels of pigmented polymers and sulphurdioxide non-bleachable pigments were found to be higher in Saccharomycese bayanus wine at day 387,demonstrating that the formation of stable pyranoanthocyanins and pigmented polymers was enhanced by Saccharomycese bayanus yeast. Electrospray mass spectrometry was used for the compositional analysis of pyranoanthocyanins and direct and ethyl-bridged linked condensation dimers of malvidin 3-o-glucoside (MvG) with flavanols (catechin or epicatechin).The data demonstrated that the formation of acetaldehyde-mediated pigments was enhanced by the use of the Saccharomycese bayanus yeast.The compositional analysis suggested that the differences in colour properties and pigment profiles of Saccharomycese cerevisiae and Saccharomycese bayanus wines were largely due to the greater production of acetaldehyde-mediated pigments by the use of Saccharomycese bayanus yeast.(Yoji et al.,2007)

Flavonol composition of red and white wines determined by high-performance liquid chromatography. Flavonols and their glycosides are important components in wine because of their impact on colour, taste and health properties. In the red wines,quercetin and myricetin dominated the flavanol profile and quercetin-3-glucuronide was by far the most abundant glycoside present. White wines had considerably lower levels of flavonols and their glycosides, in most cases, being below the detection limit of the high-performance liquid chromatography method.Contrary to literature reports of rutin in wine,rutin was not found in any of the wines we analysed and spiking experiments showed rutin rapidly degraded to the aglycon quercetin. Furthermore,changes in elution order for some quercetin glycoside standards were observed depending upon the acid used in the mobile phase.(Parker et al.,2008)

There is formation of temperature gradients in large and small scale red wine fermentations during cap management. Red wine fermentation are not a homogenous mixture in terms of both density (grape solids vs liquid) and temperature distribution. Winemakers practice cap management techniques to facilitate the extraction of flavor and colour but also to achieve a more uniform temperature throughout the fermentation tank. A thermocouple with the temperature difference of 12^0C between the centre of the cap and the liquid of fermentations at both scales, is placed at defined positions in the tank to yield temperature profiles for a virtual cross-section through each fermentation. A 1600-L tank fitted with in place temperature sensors allowed nearly instantaneous determination of temperature cross-sections and revealed that a thorough pumpover was needed to reduce temperature differences to $< 5^0$ C. Temperature gradients can be large in red wine fermentations, regardless of scale, varying with the must and fermentation management. Large temperature differences and high temperatures through and during fermentation have implications for yeast performance, colour, flavor compound extraction, modification and stability.(Schmid et al,2009)

Wines made with indigenous or 'wild' yeast are perceived to be more complex by showing a greater diversity of flavours, however, the chemical basis for the flavor characteristics is not yet defined. Pairs of wine were prepared from the same must and subjected to similar fermentation conditions in the wineries of origin, except for the mode of inoculation. Reference wines were made by inoculation with a Saccharomycese cerevisiae starter culture whereas companion wines were allowed to undergo fermentation with the indigenous microflora. Of all wine chemicals analysed, only yeast-derived volatile fermentation products showed significant differences between the yeast treatments. Inoculated wines were associated with the esters ,ethyl hexanoate and 3-methylbutyl acetate and formed a clear cluster by principle component analysis. By comparison with inoculated wines, 'wild' yeast fermented wines showed high variability in volatile compounds that contribute to wine aroma with higher concentrations of 2-methylpropanol,2-methylbutanoic acid,ethyl 2-methylpropanoate,ethyl decanoate and ethyl dodecanoate potentially being sensorially important.(Varela et al,2009)

Botrytis cinerea, the causal agent of botrytis bunch rot, is an important disease of grapewines worldwide, with canopy management

and the prophylactic use of fungicides being the most common control methods. The latter has resulted in fungicide resistance and is increasingly raising concerns regarding residues in wine and effects on human and environmental health. Research-led alternatives to this practice are beginning to emerge, including a range of biotic and abiotic treatments that induce wine resistance to Botrytis cinerea and inundative applications of biological control agents such as Trichoderma, Bacillus, Ulocladium and Streptomyces species. Also, habitat manipulation techniques that aim to improve the effectiveness of naturally occurring biological control are being developed using mulches brought into the vineyard, as well as mulched cover crops. These can accelerate decomposition of botrytis mycelium and sclerotia on the vineyard floor in winter. The challenges of these different and the prospects for habitat manipulation for this fungal disease are discussed. Extensive tables on synthetic fungicides,biofungicides,essential oils and plant extracts effective against Botrytis cinerea are included.(Walter et al.,2010)

MATERIAL AND METHODS

Microorganism used:

Saccharomycese cerevisiae .

Collection of sample:

Rotten grapes were collected from local market.

Recovery of isolates:

To isolate microorganisms from the grape sample,1ml of rotten grape juice was suspended into test tube containing 9ml distilled water.

Isolation: It was done in three steps-

1) Serial dilution
2) Spread plate method
3) Streak plate method

1) Serial dilution:

One ml of sample from test tube was transferred into9ml of normal saline(0.85%) blank and further dilutions were made upto $10^{-6}$by adding 1ml from previous dilution to next dilution.Serially diluted sample was then inoculated on the Yeast Extract Mannitol Agar media plates by plating out 0.1ml of 10^{-2} ,10^{-4} and 10^{-6}dilutions.

2) Spread plate method:

Using a sterile glass rod,0.1ml of each dilution was spreaded on the Yeast Extract Mannitol Agar media plates and then observed the characteristics of colonies after incubating petriplates for 72 hrs at 21^0C. Studies were done on colony morphology with naked eyes and with the help of magnifying glass.Following features were studied:

*Colour of the colonies (creamish or whitish)
*Elevation of the colonies (raised ,flat or wavy)
*Opacity of the colonies (opaque or translucent)

3) Streak plate method:

From the colonies obtained on the Yeast Extract Mannitol Agar media plates,single separate colony was picked and inoculated on fresh Yeast Extract Mannitol Agar media slants.

Method of fermentation:

General flow chart:-

Selection of grapes (Mature and undamaged grapes)
⇩
Crushing (Traditionally manual but now crushers are used)
⇩
Removal of skins (By standing, filteration or centrifugation)
⇩
Clarification
⇩
Fermentation
⇩
Ageing
⇩
Maturation and Bottling
⇩
Packaging and storage

a) Selection of grapes :

As already discussed, mature and undamaged grapes are selected for fermentation.

b) Crushing :

The grapes were handpicked and transferred to the crusher.The crusher punchers the grapes and transfers it to a de-juicer which seperates the pulp from the juice.While the skin,the stems and other remains from the crushing are used as manure,the juice is sent for fermentation.

c) Removal of skins :

The skins also determines the colour of the wine. Maceration (the time spent while skins and seeds are left with the juice) will go on for a few hours or a few weeks. Pressing will then occur. One way to press the grapes is to use a 'bladder press' which is a large cylindrical container that contains bags that are inflated and deflated several times, each time gently squeezing the grapes until all the juice has run free, leaving behind the rest of the grapes. Solids can also be separated from juice through the use of a centrifuge.

d) Clarification:

The grape juice is first chilled in e combination of stainless steel tanks and oak barrels and then fermented by adding yeast.This process is called the first fermentation of wine and it takes about 8 weeks for clarification of juice.

e) Fermentation:

The clarified juice is transferred to a fermentation tank where fermentation either begins spontaneously or it is induced by the addition of a starter culture. Traditionally, fermentation was carried out in large wooden barrels or concrete tanks. Modern wineries now use stainless steel tanks as these are more hygienic and provide better temperature control. White wines are fermented at 10^0 C to 18^0 C for about 7 to 14 days. The low temperature and slow fermentation favours the retention of volatile compounds.The fermentation can be from naturally occurring yeast on the skin of the grape or using a starter culture of Saccharomycese cerevisiae .

f) Ageing:

Seperation may not immediately occur(especially for red wines),since skins and stems are an important source of 'tannins' which affect wine's taste and maturity through ageing.

g) Maturation and Bottling:

The first fermented wine is further stored in tanks or oak barrels for 6-8 months for maturation. Once the mature wine is ready, it is stabilized through cold treatment. After testing the stability of the wine, it is then filtered to screen the balance fine particles. The filtered wine is then packed in bottles, which are washed internally and externally with double filtered water to remove bacteria and germs.

h) Packaging and storage:

Traditionally wine was delivered to the point of sale in casks. The product is traditionally packed in glass bottles with corks made from the bark of the cork oak (Quercus suber).The bottles should be kept out of direct sunlight. During storage, wines are prone to non-desirable microbial changes.Yeasts, lactic acid bacteria, acetic acid bacteria and fungi can all spoil or taint wines after the fermentation process is completed.

GENERAL PROCEDURE FOR WINE PRODUCTION

Steps involved :

Production of yeast (Saccharomycese cerevisiae)
⇩
Production of broth (Yeast Extract Mannitol)
⇩
Inoculation of culture in broth
⇩
Preperation of Must
⇩
Fermentation
⇩
Ageing
⇩
Storage

a) Production of yeast (Saccharomycese cerevisiae):
Some rotten grapes were taken and skin of grapes was peeled out. Then the grapes were crushed and diluted with distilled water. Serial dilution was done and spreaded 1ml of each dilution on Yeast Extract Mannitol Agar media petriplates. Incubated the plates for 72 hrs at 21^{0}C.After incubation, whitish colonies appeared on the media in the petriplates. Streaked the appeared white colonies on Yeast Extract Mannitol Agar media slants for future use.

b) Production of broth (Yeast Extract Mannitol):
Prepared 500ml of broth by dissolving following constituents:

Peptone-2.5g,Beef extract-1.5g,NaCl-2.5g,Yeast extract-5g,Mannitol-5g,Distilled water-500ml.

c) Inoculation of culture in broth:
Inoculated the loopful culture of Saccharomycese cerevisiae in the broth kept in the flask. Keep this flask on orbital shaker for 3 days.

d) Preperation of Must:
The grapes are crushed and the liquid is separated. Sterilised this must by pasteurization. To avoid contamination, potassium metadisulphide/carbondioxide fumes was added in the must.

e) Fermentation:
500ml of must is added in 500ml of broth. Incubated the broth for 4-5 days at 20-28^{0}C in an incubator. After 4-5 days of incubation, added 8g of sucrose .Again incubated for 21 days.

f) Ageing:
After 21 days of incubation, wine is prepared which is kept in wooden vessel for 1-5 months for aroma and flavour development.

g) Storage:
Product is filtered and pasteurized at 60^{0}C for 2 minutes. Then the wine was bottled. Wine contains 10-18% alcohol.

RESULTS AND DISCUSSION

RESULTS:

Various tests had been performed to check wine quality. These are as follows:

1) Test for pH:
The pH measures with a pH meter,an instrument that determines pH quickly and easily.It represents the active acidity of the wine .If the pH of a wine is too high ,say 4.0 or above, the wine becomes unstable with respect to microorganisms. Low pH inhibits microorganism growth. Tartaric acid is sometimes added to fermenting grape juice in California to ensure that an acceptable final pH can be realized, since some acid is lost during fermentation thus reducing the total acidity and raising the pH.

Table No.1 - pH of wine after regular intervals of time period

Time period (in days)	pH
1st day	6.34
5th day	5.80
10th day	5.30
15th day	4.10
20th day	3.33

2) Test for acidity:
Acidity was estimated by the Titration method by the use of sodium hydroxide as titrant against the diluted fermented juice. Add phenolphthalein as an indicator in the fermented juice.

Total acidity =

$$\frac{\text{Volume of alkali used (ml) X Normality of alkali X 7.5}}{\text{Weight of sample in gram}}$$

Table No.2 – Acidity of wine after regular intervals of time period

Time period (in days)	Initial reading (0.1N NaOH)	Final reading (0.1N NaOH)	Volume used (0.1N NaOH)	Acidity
1st day	0	44.5 ml	44.5 ml	3.375
5th day	0	53.8 ml	53.8 ml	4.035
10th day	0	59.9 ml	59.9 ml	4.492
15th day	0	67.1 ml	67.1 ml	5.032
20th day	0	80.4 ml	80.4 ml	6.030

3) Test for sugar:
Phenol sulphuric acid test is used for the determination of total percentage of sugar in fermented juice by the use of calorimeter.

Table No.3 – Sugar concentration of wine after regular intervals of time period

Time period (in days)	Sugar concentration (%)
1st day	34.54
5th day	29.43
10th day	24.67
15th day	21.23
20th day	17.54

4) Test for ethanol:
The quantitative estimation of ethanol in the fermented product is done by the potassium dichromate method.

Table No.4 – Ethanol concentration of wine after regular intervals of time period

Time period (in days)	Ethanol concentration (%)
1st day	5.53
5th day	7.40
10th day	10.23
15th day	13.48
20th day	15.37

DISCUSSION:

Wine is safe and nutritionally beneficial.The acidity in grapes is primarily due to malic acid,which does not form a precipitate like acid in grapes.For this reason grapes wine does not require cold stabilization. It is important to note that malic acid is not biologically stable.Lactic acid bacteria can metabolize it.

In present study wine was prepared from grape juice as a raw material and Saccharomycese cerevisiae was isolated from rotten part of grapes under laboratory conditions.To study the fermentation process, sample was drawn after an interval of 5 days and microbiological and biochemical characterization was done.

For microbial investigation, samples drawn after intervals of 5 days were grown on specific media by serial dilution technique. The microflora grown on specific media was identified by staining techniques. The predominant microorganism identified was yeast, Saccharomycese cerevisiae.It increases gradually due to its tolerance power towards low pH ,high acidity and ethanol content.

Biochemical analysis includes test for pH, test for total acidity, test for reducing sugar, test for ethanol content etc. In the investigation of biochemical aspects, it was concluded that the pH of sample was decreased whereas the titrable acidity of sample was increased during fermentation process.

Fermentation and post-fermentation processing of grapes wine is somewhat similar to the production of white table wines. After SO_2

addition, clarification and sweetening, the must is ready for fermentation. Various kinds of fermenters such as wood, plastic and stainless steel are available. However, stainless steel tanks with temperature control should be preferred to conduct fermentation. Temperature control during fermentation is crucial to the preservation of delicate fruit flavours in the resulting wine. The fermentation temperature commonly employed by the winemakers ranges between 50^0F to 70^0F . Generally, lower temperatures ranging from 50^0 F to70^0 F yield favourable results. Many strains of yeasts in dried and pure culture form are available to the winemakers. Wine was prepared after 21 days of fermentation.

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REFERENCES

Adams,M.R.(1990) Tropical aspects of fermented foods.Trends in Food Science and Technology, vol.1:141-144. | Aidoo,K.E.(1994) Applications of biotechnology to indigenous fermented foods.Proctechno,vol.12:83-94. | Bajaj, B.K.,Bajpai ,P.(2011) Selection and characteristics of yeast for desirable fermentation characteristics .vol.2:107-109. | Bhalla,T.C.,Thakur,S.(2004) Characterisation of some traditional fermented foods and beverages of Himachal Pradesh.Indian journal of Traditional Knowledge.vol.3:325-335. | Marangon ,M.,Najafpour,G.(2011) White wines stabilized by removing the heat unstable proteins through zirconia treatment.vol.17:28-35. | Mpelasoka ,S.,Marian,I.(2008) Potassium nutrition in grapewines.vol.9:154-168. | Oakey ,H.,Lott,R.V.(2005) The impact of closure type and storage conditions on the composition,colour and flavor properties of wine.vol.11:369-377. | Osborne,P.,Odunfa,S.A.(2006) Inhibition of malolactic fermentation by Saccharomycese during alcoholic frmentation under low and high nitrogen conditions.vol.12:69-78. | Parker,M.,Panek,A.D.(2008) Flavonol composition of red and white wines determined by high performance liquid chromatography.vol.14,153-161. | Paul,S.(2006) The determination of urea in wine.vol.12:97-106. | Ristic,R.,Potter M.M.(2010) Flavonoid composition of wine related to wine sensory properties.vol.16:369-388. | Schmid,F.,Sanni,A.I.(2009) Formation of temperature gradients in large and small scale red wine fermentations during cap management.vol.15:249-255. | Varela,C.,Steinkraus,K.H.(2009) Discovering a chemical basis for differentiating wines made by fermentation with 'wild' indigenous and inoculated yeasts.Role of yeast volatile compounds.vol.15:238-248. | Vincenzi ,G.,Van,A.G.(2011) Nutritive value and wholesomeness of fermented foods.Journal of Agriculture and Food Chemistry.vol.18:576-578. | Vullo,D.L.,Washman,M.B.(2005) A simple laboratory exercise for ethanol production by baker's yeast.Journal of Food Science Education.vol.15:68-78. | Walter,M.,Potter,M.M.(2010) Food Sciences.AVI Publishers.vol.6:354. | Yarrow,D.(1988) Methods for the isolation ,maintainance and identification of yeast.The yeast-a taxonomic study.vol.7:77-100. | Yoji ,Y.,Chaudhary,K.(2007) Bioconversion of starch to ethanol in a single step process by co-culture of amylolytic yeast and Saccharomycese cerevisiae.Bioresources Technology,vol.72:261-266. |