Autochthonous yeasts: Role in vinification and aging of *Cabernet-Sauvignon*

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Abstract— *Selection of the appropriate autochthonous yeasts assures the maintenance of the unique enological characteristics, which could be considered representative of an enological region. The evaluation of yeasts of the Indian Geographical origin on fermentation was carried out using Cabernet Sauvignon. The organic acid profiling of wines indicated a decrease in tartaric and malic acids with a concomitant increase in lactic and succinic acids. Non-anthocyanin transformation studies indicated the increase in monomeric forms (except coumaric, catechin, quercetin) in wines. Principal component analysis model developed was capable of classifying the volatile compounds with respect to yeast and aging, thus indicating that the volatile profile varied with yeast treatment and aging of wine. Sensory analysis of wines revealed that all wines were organoleptically accepted. Thus autochthonous Saccharomyces cerevisiae strains exhibited desired enological properties equivalent to the commercial S.cerevisiae.*

Keywords— Autochthonous, yeasts, Saccharomyces cerevisiae, Wine,Aging, CabernetSauvignon, phenolics, aroma, India.

I. INTRODUCTION

Wine is a product of series of biochemical transformations, which begins during the ripening of grapes, throughout alcoholic fermentation and aging (Romano et al., 2003).Wine composition is determined by the grape variety, geographical and viticultural conditions of grape cultivation, microbial ecology and winemaking practices (Nardi, 2006; Robinson *et al*., 2011).

Yeasts are the heart of biochemical interaction with the must. The diversity of native yeast strains, in the spontaneous fermentation of grape must, can produce wines with different qualities and peculiar flavours. Therefore, production of wine using the selected yeast culture is the common enological practice followed, to produce wine with desirable organoleptic properties and assure the homogeneity of successive vintages (Nardi, 2006).

The most preferred species for global wine production is *Saccharomyces cerevisiae.* Recently, there has been an increase in the use of autochthonous yeasts for fermentation. Autochthonous yeasts are inferred to be more competitive as they are better acclimated to the environmental conditions and dominate the fermentation process. Selection of the appropriate autochthonous yeasts assures the maintenance of the unique enological characteristics, which could be considered representative of an enological region (Nardi, 2006; Aponte & Blaiotta,2016; Tristezza *et al*., 2012).

Wine style is determined by the relative concentrations of volatile and non-volatile compounds extracted from grapes, transformed during fermentation and maturation, and synthesized de-novo by yeast and bacteria during fermentation. The choice of yeast strain represents a low-cost opportunity, amongst the myriad of grape and wine processing options, to broaden the spectrum of wine flavour profiles. Therefore, the choice of yeast strain for red wine fermentation requires consideration of relative impacts on volatile and non-volatile wine components (Holt *et al*., 2013).

Thus, evaluation of the influence of autochthonous yeast on vinification is crucial, to know the potential differences in enological characters of yeast and produce desirable wine. However, limited literature is available on enological properties of Indian yeast isolates and its role in aging of wine. Therefore, the present study was aimed to evaluate, the impact of yeasts of the Indian Geographical origin on fermentation of *Cabernet Sauvignon*, so as to select the yeast strain, which assures the maintenance of unique properties of wine, which could be considered representative of Indian geographical region.

Aging of wine increases the complexity of wine aroma as a consequence of processes involved in its maturation(Pereira *et al*., 2010). The development of post-fermentative flavour during the aging of wine in glass bottles was studied with respect to the variation in the aromatic and phenolic profile of wine.

II. MATERIALS AND METHODS

2.1 Yeast

S.cerevisiae (AAV2) with Accession No. KF551990, *S.cerevisiae* (ITB) with Accession No FN393977 and *S.cerevisiae* (101) with Accession NoKT862833, were isolated from grape, toddy and wine respectively, in Karnataka, India. As reported in our previous study, these yeasts were screened for ethanol tolerance and production (Archana et al (2015). *S.cerevisiae* (AAV2) and *S. cerevisiae (ITB)* were molecularly identified by amplification of the D1/D2 region of large subunit 28S rDNA gene (Archana *et al.*, 2015)and *S. cerevisiae* (101) was identified by the amplification of ITS region. The enological characteristics of this *S.cerevisiae* strains were further studied in comparison with commercial strain *S. cerevisiae* (Lalvin 71B-1122).

2.2 Fermentation conditions

The impact of yeast strains *S.cerevisiae* (AAV2), *S.cerevisiae* (ITB), *S.cerevisiae* (101) and commercial *S.cerevisiae* (COM) strain on fermentation, was evaluated using the grape variety *Cabernet Sauvignon* harvested from Grover Vineyard, Bengaluru. Preparation of the must involves destemming and crushing of the grapes, analysis of pH and Total Soluble solids. This was followed by the addition of the SO_2(98ppm) and was then subjected to cold maceration (18h). The grape must prepared, was inoculated with *S.cerevisiae* strains (inoculum of 10^6cfu/ml) and fermented along with the grape skin, in 5l glass carboys under microaerobic conditions at 20°C for 21 days, followed by filtration and decantation. Aliquots were withdrawn every 5^{th} day till 21 days. Following analysis for pH, Total soluble solids (TSS), Total sugars (Dubois *et al*., 1956), Reducing sugars (Miller, 1959), Total polyphenols (Singleton *et al*., 1999) and Alcohol estimation (Caputi *et al*., 1968) were carried out. The wine samples were then aged in glass bottles for a year and analysed for the above-mentioned parameters. The experiment was carried out in triplicates.

2.3 Enumeration of yeast and bacteria

Aliquots were withdrawn every 5^{th}day were plated to study the growth of yeast and lactic acid bacteria during fermentation. For yeast, plating was done in Sabouraud Dextrose Chloramphenicol Agar and incubated at 30ºC. Native lactic acid bacteria count was obtained by plating samples on MRS agar with Cycloheximide and incubated at 37 ºC.

2.4 Organic acid profile

Wine samples filtered through 0.45µ PTFE membrane was used for organic acid analysis. The chromatographic system (LC10A, Shimadzu) coupled with UV detector was used. The analysis was performed on reverse phase C18 column, using an injection volume of 20µL and detection at 210nm. The mobile phase employed was 0.008 M sulphuric acids, at a flow rate of 1ml min^{-1} in an isocratic mode of elution (Jayaprakasha & Sakariah, 2000)

2.5 Non -anthocyanin transformations

Wine samples were filtered with 0.45µPTFE membrane filters prior to High-pressure liquid chromatography (HPLC) analysis. The system was equipped with an auto-injector and Photodiode array detector (Waters, Milford). The analysis was performed on RP C-18 column, using an injection volume of 20µL, followed by the detection at the range of 210-420nm. The mobile phases employed were A (Methanol 100 %) and B (0.5% Acetic acid). The gradient program followed was: 0-28.6 min, 90-40 % B, 28.6-30 min 40-90% B equilibrated till 35 mins, at a flow rate of 1ml min^{-1} (Ivanauskas *et al*., 2008)

2.6 Alcohol Analysis

Qualitative analysis of the alcohol, was carried out in Perkin Elmer Clarus 580 gas chromatography equipped with Flame Ionization detector (FID) using the Zebron Wax plus capillary column containing (polyethylene glycol). The temperature program followed was: 40°C (1min hold) to 70°C at a rate of 5 °C min^{-1} and to 220°C at a rate of 25°C min^{-1} for 3 minutes. The carrier gas was nitrogen, with the flow rate of 1 ml min^{-1}

2.7 Profiling of volatiles

Aroma compounds in wine were extracted using liquid-liquid extraction method. The samples extracted were analysed by Perkin Elmer Clarus 580 gas chromatography equipped with Flame Ionization detector (FID) using the Carbowax column.

The temperature program followed was: 40°C (3 min hold) to 180°C at the rate of 3°C min^{-1} and to 240°C at the rate of 20°C min^{-1} (10 min hold time). Nitrogen was used as the carrier gas with the flow rate of 1 ml min^{-1}.The esters analysed was further confirmed by GC-MS (Ivanova *et al*., 2012).

2.8 Sensory Evaluation

Sensory analysis was carried out for young and aged wines, to evaluate the flavour and their acceptance by trained panelists. Selection of panelists for the sensory evaluation of wines was on the basis of interest and availability. A group of 10 panelists were trained for descriptive sensory analysis. The panelistswere presented with samples of 30ml at room temperature (25°C). Quantitative Descriptive Analysis (QDA) was followed to quantify the intensities of different attributes of wines.

2.9 Statistical analysis

The experimental data obtained for various parameters were subjected to Analysis of Variance (ANOVA). In the case of significant difference, the mean separation was accomplished by Tukey‘s test. Principal component analysis (PCA) was employed to establish the differentiation criteria as a function of yeast strains, time and their volatile profile. Statistical analysis was performed using the software Statistica (5.5 StatSoft Tulsa, OH, USA).

III. RESULT AND DISCUSSION

3.1 Physicochemical properties of wine

Alcoholic fermentation was monitored by studying the total soluble solids (ºBrix) of the must. The total soluble solids in the initial grape must was 22°Brix, which reduced to 5°Brix during alcoholic fermentation and increased to 8°Brix on aging of wines(Table1). The value of pH gradually decreased from 3.9 to 3.7. The decrease in pH indicated the production of acids during fermentation (Ndip et al., 2001).On aging of wines, pH slightly increased which could be due to the utilization of malic acid by *S.cerevisiae* strains (Redzepovic *et al*., 2003).The *S.cerevisiae* (AAV2)strain produced 12.92% (v/v) of alcohol, which was slightly higher than the alcohol produced by the other strains. There was no significant difference in alcohol, during aging of wines. Phenolic compounds contribute to the organoleptic properties of wine (Monagas *et al*., 2005). Yeast influences the extraction of phenolic compounds; depending on their alcohol producing capacity (Monagas *et al*., 2007).The initial concentration of polyphenols in must was 1305µg GE/ml. The concentration of total polyphenols increased on fermentation in all the wines.

TABLE 1
PHYSICOCHEMICAL PROPERTIES OF WINE, FERMENTED BY NATIVE *S.CEREVISIAE* STRAINS IN COMPARISON WITH COMMERCIAL *S.CEREVISIAE*

Attributes	Initial Must	AAV2		ITB		101		COM	
		Young Wines	Aged Wines	Young Wines	Aged Wines	Young Wines	Aged Wines	Young Wines	Aged Wines
pH	3.92±0.06	3.72±0.04	4.25±0.02	3.82±0.05	4.22±0.04	3.78±0.05	4.2±0.03	3.71±0.02	4.1±0.04
Total Soluble Solids(°Brix)	22±0.08	5±0.08	8±0.16	5.6±0.2	8±0.03	5±0.08	8±0.02	6 ±0.2	8±0.06
Total Sugars (mg/ml)	180.4±0.4	3.78±0.08	2.43±0.04	4.09±0.02	2.1±0.3	3.54±0.03	2.32±0.04	4.23±0.03	3.44±0.08
Reducing Sugars (mg/ml)	164.8±0.4	2.84±0.03	ND	3.08±0.03	ND	1.24±0.02	ND	1.77±0.02	ND
Alcohol (%v/v)	ND	12.98±0.4	12.5±0.2	11.21±0.4	11±0.08	12.25±0.2	12±0.3	11.2±0.3	11.2±0.3
Total Polyphenol (µg GE/ml)	1305±0.2	1662±0.4	2019±0.2	1422±0.1	2049±0.4	1401±0.6	1989±0.04	1430±0.3	1634±0.3

Note: - AAV2 – S. cerevisiae; ITB- S. cerevisiae; 101– S. cerevisiae; COM- Commercial S. cerevisiae

3.2 Enumeration of yeast and bacteria during fermentation

During fermentation, the growth of yeast was monitored every 5th day for 21 days. All the *S.cerevisiae* strains exhibited increased growth rate until the 10th day, after which its viability decreased. The native lactic acid bacterial count was low, which further declined on fermentation. The decrease in the growth after ten days may be due to the production of alcohol in wine. Similar growth kinetics was observed in earlier studies by Ndip *et al.* (2001); Rai *et al*. (2010).

3.3 Organic Acids

Tartaric, malic, citric, succinic and lactic acids were detected in all the wines (Table 2). Tartaric and malic acids were the predominant acids at the onset of fermentation. The concentration of tartaric and malic acids decreased in young and aged wines. The decrease in tartaric and malic acids, could result from numerous possible reactions such as the enzyme-catalysed or microorganism initiated reactions, salt precipitation and oxidation–reduction reactions. (Lamikarna, 1997; Torija *et al*., 2003; Redzepovic *et al.* 2003).

TABLE 2
VARIATION OF ORGANIC ACIDS IN WINE (mg/ml)

Organic acids	Initial must	Young wines				Aged wines			
		101	ITB	AAV2	COM	101	ITB	AAV2	COM
Citric	0.149±0.07	0.109±0.061[a]	0.125±0.007[a]	0.139±0.002[b]	0.113±0.007 [b]	0.046±0.309[a]	ND	0.030±0.010[a]	0.050±0.030[a]
Lactic	ND	0.219±0.010[a]	0.182±0.009[a]	0.296±0.004[a]	0.230±0.006 [a]	0.018±0. 026[a]	0 .052±0. 034[b]	0.291±0. 012[b]	0.232±0. 008[b]
Malic	1.342±0.020	1.312±0.086[a]	1.314±0.012[b]	1.291±0.007[b]	1.269±0.092 [b]	1.198±0.014[a]	1.219 ±0.015[a]	1.258 ±0.006[b]	1.243 ±0.038[b]
Succinic	ND	0.803±0.013[a]	1.089 ±0.041[b]	1.178±0.052[b]	1.228±0.052 [b]	ND	ND	ND	ND
Tartaric acid	3.795±0.009	1.464±0.022[a]	1.596 ±0.111[a]	2.114 ±0 .033[b]	2.302 ±0 .010[b]	1.141 ±0.015[a]	1.087 ±0.047[a]	1.164 ±0.015[b]	1.490±0.075[b]

Lactic acid increased on fermentation in all the wines. On aging, the concentration of lactic acid remained constant in wines inoculated with AAV2 and Commercial strain but decreased in wines inoculated with ITB and 101.Succinic acid increased on the 21st day of the fermentation, but on aging it was not detected. The concentration of citric acid decreased on fermentation and during aging of wine. Lamikarna (1997) has reported a decrease in tartaric and malic acids, with the concomitant increase in lactic and succinic acids after seven months of aging.

3.4 Non –anthocyanin transformations in wine

In the present study, (Table 3) the analysis of young wine samples indicated the increase in monomeric forms of non-anthocyanin (except coumaric and quercetin) in all wines, with the significant variation between the *S.cerevisiae* strains. Hydroxybenzoic acids detected in wines were gallic, syringic and vanillin. Gallic acid concentration in wine depends on its extraction from grape seeds during maceration, fermentation processes and its esterification with methanol and ethanol (Monagas, *et al*., 2007; Hernandez *et al*., 2007). Monagas *et al.* (2007) also reported that the concentration of vanillic acid varied with the yeast strains and grape varieties. On aging of wines, the concentration of hydroxybenzoic acids increased. According to Monagas *et al*. (2005), during aging of wines, hydroxybenzoic acids exhibited different trends according to grape variety and considering the variety *Cabernet Sauvignon*, there is a slight increase in hydroxybenzoic acids. Resveratrol increased on aging, in all the wines except, in wines fermented by *S.cerevisiae* strain I TB. Extraction of resveratrol correlates with an increase in ethanol (Pezet & Cuenat, 1996). The evolution of resveratrol during aging of wines did not follow definite trends (Monagas *et al*., 2005).

The concentration of hydroxycinnamic acids increased during aging. According to Somers *et al.* (1987), enzymatic hydrolysis of tartaric acid esters occurs during wine aging in stainless steel tanks and oak barrels. Monagas *et al*. (2005) reported the progressive increase in p-coumaric acid during aging. However, there was no significant increase in the total concentration of hydroxycinnamic acids with aging in the bottle, owing to the interconversion of the different forms.(Monagas *et al*.,2005). Except catechin, flavonoids increased on aging in all wines. These are in concordance with the study carried out by Monagas *et al*. (2005). The decrease in flavanols (catechin) may be due to its role as co-pigment of anthocyanin, thus stabilizing the colour of red wines (Monagas *et al*. 2005; Kammerer &Carle, 2009).Overall, the variation of the phenolic profile brought about by autochthonous yeasts was similar to the commercial *S. cerevisiae.*

TABLE 3
CONCENTRATION (mg/100ml) OF NON-ANTHOCYANIN POLYPHENOL COMPOUNDS IN WINE

Phenolics compounds	Initial must	Young wines				Aged wines			
		101	ITB	AAV2	COM	101	ITB	AAV2	COM
Caffeic	0.423±0.018^{a}	0.864±0.560^{a}	1.515±0.437^{b}	1.423±0.159^{b}	2.793±0.066^{c}	2.357±0.099^{b}	1.714±0.144^{a}	2.279±0.204^{b}	3.157±0.128^{c}
Catechin	1.201±0.206^{a}	2.910±0.951^{a}	4.942±0.036^{c}	4.885±0.025^{bc}	4.726±0.116^{b}	4.917±0.019^{c}	4.208±0.014^{b}	4.715±0.111^{c}	3.571±0.324^{a}
Caumaric	1.463±0.055^{a}	0.586±0.0357^{a}	1.154±0 .085^{b}	1.762±0.064^{c}	0.563±0.057^{a}	0.732±0.026^{a}	1.544±0.178^{c}	1.890±0.069^{d}	1.228±0.034^{b}
Epicatechin	3.876±0. 054^{a}	8.160±.0135^{a}	10.520±0.171^{b}	12.570±.099^{c}	6.762±1.420^{a}	10.206±0.160^{b}	14.495±0.049^{c}	14.359±0.057^{c}	7.294±0.296^{a}
Ferulic	0.142±0.058^{a}	0.195±0.072^{a}	0.705±0.065^{b}	0. 198±0.108^{a}	0.221±0.028^{a}	0.205±0.011^{a}	0.382±0.031^{b}	0.253±0.048^{ab}	0.353±.097^{ab}
Gallic	1.907±0.016^{a}	3.631±0.100^{b}	3.364±0.181^{b}	3.645±0.061^{b}	2.643±0.155^{a}	4.716±0.147^{b}	4.140±0.691^{a}	4.678±0.061^{b}	4.175±0.114^{a}
Kaempherol	ND	0.302±0.012^{c}	0..224±0.010^{a}	0.255±0.015^{b}	0.279±0.005^{bc}	ND	ND	0.243±0.102^{b}	0.273±0.007^{c}
Myricetin	0.923±0.141^{a}	1.315±0.013^{a}	1.196±0.187^{a}	1.437±0.037^{a}	1.381±0.025^{a}	1.520±0.013^{c}	1.315±0.009^{a}	1.544±0.021^{c}	1.414±0.010^{b}
Quercetin	0.210±0.009^{a}	0.173±0.012^{a}	0.550±0.005^{c}	0.351±0.018^{b}	0.357±0.004^{b}	0.418±0.009^{b}	0.511±0.005^{c}	0.337±0.101^{a}	0.326±0.021^{a}
Resveratrol	0.253±0.031^{a}	0.505 ±0.011^{b}	0.757±0.023^{c}	0.852±0.019^{d}	0.343±0.022^{a}	0. 878±0.031^{c}	0.551±0.011^{a}	0.961±0.019^{d}	0.813±0.011^{d}
Sinapic	0.240±0.019^{a}	0.585±0.038^{c}	0.447±0.022^{b}	0.581±0.016^{c}	0.316±0.008^{a}	0.453±0.041^{a}	0.654±0.074^{b}	0.686±0.030^{b}	0.446±0.030^{a}
Syringic	0.231±0.018^{a}	0.627±0.048^{b}	0.926±0.099^{c}	0.882±0.017^{c}	0.390±0.034^{a}	1.135±0.102^{b}	0.912±0.098^{b}	0.988±0.103^{b}	0.470±0.049^{a}
Vanillin	0.145±0.088^{a}	0.438±0.043^{a}	0.719±0.015^{c}	0.728±0.016^{c}	0.628±0.025^{b}	0.808±0.014^{b}	0.786±0.046^{b}	0.801±0.024^{b}	0.355±0.125^{a}

Note: - AAV2 – S.cerevisiae; ITB- S.cerevisiae; 101– S.cerevisiae; COM- Commercial S.cerevisiae.
Data as Mean ± Standard Deviation (SD) for n=3.
Mean values in a row with different superscripts within young and aged wines,for different yeast strains are significantly different at p≤0.05 (Tukey's test). ND= Not Detected

3.5 Alcohol profile

Qualitative analysis of alcohol in wine, carried out by Gas Chromatography revealed the presence of isobutanol, ethanol, isoamyl alcohol and isopropyl alcohol. Ethyl alcohol was the major alcohol produced by all the isolates. Similar results have been recorded in our previous study (Archana *et al*., 2015). There was no significant difference between young and aged wines. Garde-Cerdan&Ancin-Azpilicueta (2006) reported that greater alcohol level favored the extraction of volatile compounds during aging of wine.

3.6 Volatile profiling of wines

In the present study, the volatile profile analysis of the wines by GC-MS led to the identification of 32 compounds (Fig.1). The differences in the volatile profile of the wines fermented by the different *S.cerevisiae* strains appear to be quantitative, which is in agreement with previous studies (Mateo *et al*., 2001; Patel & Shibamoto, 2002; Romano *et al*., 2003; Torrens *et al*., 2008).

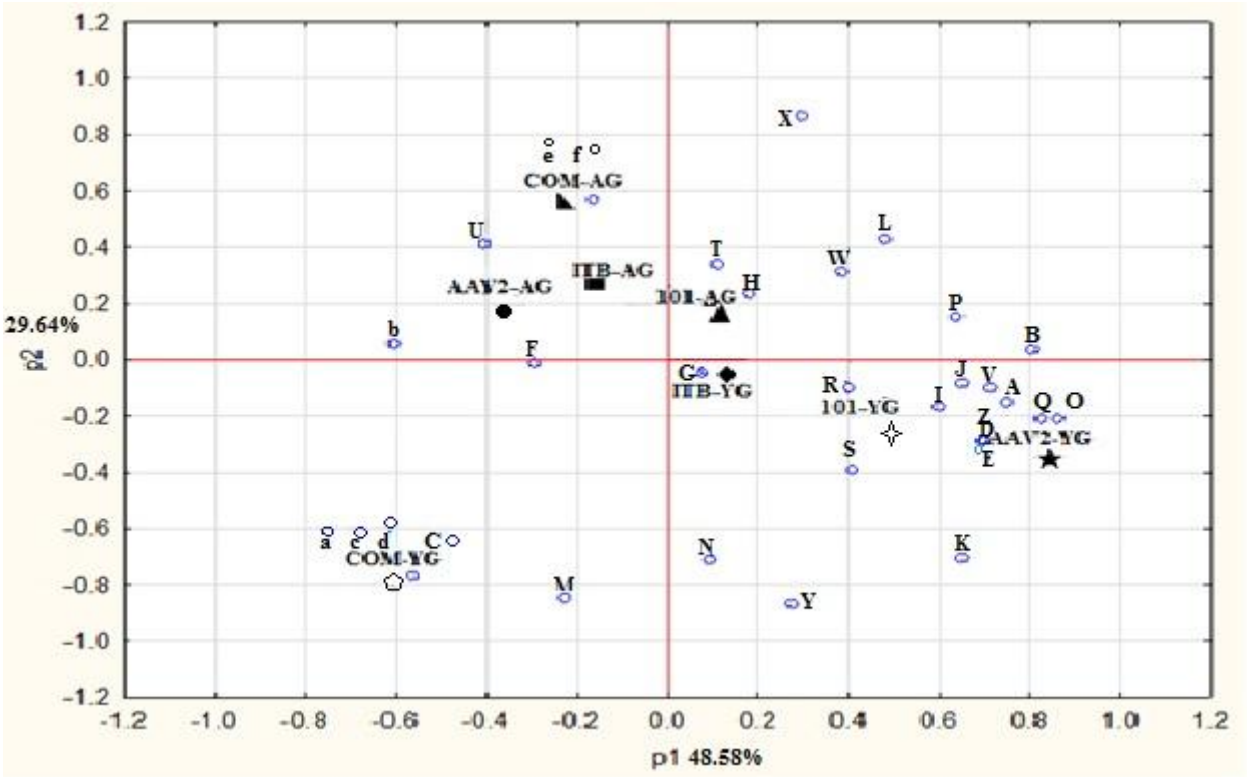

FIG 1. A: PRINCIPAL COMPONENT ANALYSIS OF VOLATILE COMPOUNDS PRESENT IN WINE FERMENTED BY NATIVE S. CEREVISIAE STRAINS IN COMPARISON WITH THE COMMERCIAL STRAINS.

AAV2-YG ★., AAV2-AG ●., ITB- YG ◆., ITB-AG ■, 101-YG ✧., 101-AG ▲ COM-YG ⬠., COM-AG ◣ [AG= aged wine and YG= young wine].**A**:-3-Methyl-1-pentanol.,**B:** 1-Hexanal.,**C**: Propanoic acid-2-methyl., **D**: 2-Isopropyl-5-methyl-1-heptanol., **E**: 1-Propanol 3 ethoxy., **F**: Acetic acid., **G**: Octonoic acid , ethyl ester.**,** **H**: Gamma butyrolactone., **I:** Butanoic acid.**,** **J**: Hexanoic acid 2-methyl., **K**: 3-Methyl thio propanol.**,** **L**: Hexanoic acid., **M**: Benzyl alcohol., **N**: Phenyl ethanol., **O**: Heptanoic acid., **P:** Octanoic acid., **Q**: Propanoic acid, 2-methyl, propyl ester., **R**: Benzeneacetaldehyde., **S**:Benzeneacetic acid., **T**: Decanoic acid., **U**: Ethyl hydrogen succinate., **V**: Dodecanoic acid., **W**: Hexadecanoic acid., **X:** Octadecanoic acid**.,** **Y**: Tryptophol., **Z**: Succinic acid diethyl ester., **a**:Ethyl palmitate**.,** **b**: Tyrosol., **c**:Ethyl heptanoate**.,** **d**:Ethyl decanoate., **e**:Ethyl hexanoate., **f:** Ethyl lactate.

Alcohols are formed by the degradation of amino acids, carbohydrates and lipid (Jiang &Zhang, 2010). The volatile fraction contained 3 methyl pentanol,3 ethoxy propanol,2 isopropyl 5 methyl 1 heptanol, 3 – methyl thio propanol, benzyl alcohol, phenyl ethanol and tryptophol. Phenyl ethanol present in all the wines exhibited floral rose-like aroma. 3-ethoxypropanol and 2-isopropyl 5 methyl1 heptanol were detected only in the wines fermented with AAV2. All of these forms of alcohol detected decreased on aging. Tyrosol a derivative of phenyl ethanol was detected in all wines.

Ethyl octanoate, produced by yeast, have pleasant sweet aromas. The ethyl ester concentrations decrease during aging, due to spontaneous hydrolysis. Ethyl octanoate was detected in the wines fermented with *S.cerevisiae* ITB and AAV2. One of the major volatile compounds produced during fermentation by yeast is monoethyl succinate, which was detected in all wines. It's concentration increased on aging. Diethyl succinate was detected in all wines. Esters of succinic acid contribute to pleasant acidic taste (Patel &Shibamoto, 2003). Phenylacetate and Isobutyl propionate were responsible for honey-like and light floral aromas. Other esters detected were: Ethyl palmitate, ethyl heptanoate, ethyl hexanoate, ethyl lactate and ethyl decanoate.

The fatty acid composition of wine is dependent on the must composition and fermentation conditions. Except commercial strain, acetic acid was detected in all the wines. Acetic acid was not detected on the aging of wines. Isobutyric acid, butanoic acid, 4 hydroxybutyric acid, 2 methyl hexanoic acid, hexanoic acid, heptanoic acid, octanoic acid, capric, palmitic, stearic and lauric acids were detected in wine. On aging of wines, the variation in the fatty acid profile did not follow a specific pattern. Phenylacetaldehyde is one of the important aldehyde detected in wines, fermented by 101 and ITB, which contributes to honey- like aroma. Gamma-butyrolactone that is responsible for cheesy aroma was detected in all the wines.

Principal component analysis was performed to evaluate the impact of yeasts and aging of wine on its volatile composition. The principal axis 1 and 2 accounted for 48.58 %, 29.64% of the variance respectively. Overall, principal axis accounted for 78.22% variance of the total data matrix. This variance indicated that the PCA model developed is valid and is capable of explaining the relationships between variables. In the PCA plot (Fig.2), Sample 101-AG, which occupied 1stquadrant, was associated with decanoic acid and gamma-butyrolactone. Other volatile compounds clustered in the 1st quadrant were hexadecanoic acid, hexanoic acid, octanoic acid, 1- hexanal and octadecanoic acid. Samples AAV2- AG, ITB-AG, and COM-AG occupied the 2nd quadrant. (AAV2- AG) was associated with tyrosol, monoethyl succinate, and ITB-AG was present adjacent to AAV2- AG. The aged wine COM-AG was clustered with ethyl hexanoate and ethyl lactate. Acetic acid was also associated with the 2nd quadrant. The wine model inoculated with Commercial *S.cerevisiae* (COM-YG) occupied the 3rd quadrant and was clustered with ethyl palmitate,ethyl heptanoate, ethyl decanoate, propanoic acid 2methyl and benzyl alcohol. Samples AAV2-YG, SC101- YG and ITB-YG occupied 4th quadrant. AAV2-YG was associated with 2isopropyl5 methyl-1-heptanol, 1-propanol 3 ethoxy, succinic acid diethyl ester,propanoic acid, 2-methyl, propyl ester, heptanoic acid,3 methyl 1 pentanol, dodecanoic acid, hexanoic acid 2 methyl and butanoic acid.Sample SC 101- YG was associated with Benzeneacetaldehyde and ITB-YG was associated with octanoic acid ethyl ester. Other volatile compounds associated with the 4th quadrant were benzene acetic acid, phenyl ethanol, tryptophol, 3-methyl-thio propanol. The influence of yeast strains on wine sensory properties has been linked to acetate and ethyl esters, which in red wines, play a role in the perception of red and fruity aromas (Holt *et al*., 2013).

The PCA model developed is capable of classifying the volatile compounds with respect to yeast and aging, thus indicating that the volatile profile varied with yeast and aging of wine. Torrens *et al*. (2008) reported that discriminant function analysis performed could clearly distinguish the wines obtained from different yeast strains and also that the yeast strain quantitatively affects the final chemical and volatile composition of cava base wines. Perirera *et al*. (2010) studied the aroma profiles in different types of aged Madeira wines by Principal component analysis, which led to the identification of the main sources of variability present and inferred the relationships between the identified compounds and wine characteristics. A study carried out by Varela *et al*. (2009) revealed that inoculated wines formed clear clusters by principal component analysis when compared to the indigenous fermented wines which showed high variability in volatile compounds that contribute to the wine aroma. Tristezza *et al*. (2012) reported that PCA performed, clearly differentiated the indigenous *S.cerevisiae* strains with technological, chemical and aromatic properties for the production of Negroamaro wines.

3.7 Sensory Analysis

The trained panelists followed Quantitative descriptive analysis to quantify the intensity of different attributes of wines. The data was processed, and the average score was calculated for each descriptor in wine. The impact making flavours such as fruity and floral flavours were detected in all the wines. Alcohol, acidity, and astringency were also detected. No significant variation was observed between the yeast treatments (Fig.2). In the present study, astringency reduced on the aging of wines. This is in concordance with the study carried out by Chira *et al*. (2011).Torrens *et al*. (2008) performed discriminant analysis to evaluate the sensory profile of wine fermented by different yeast strains, which classified the wines fermented by the three yeast strains with high fruity character and allowed the correct classification of 100 % of these wines. Sensory analysis of wines indicated that all wines were organoleptically accepted.

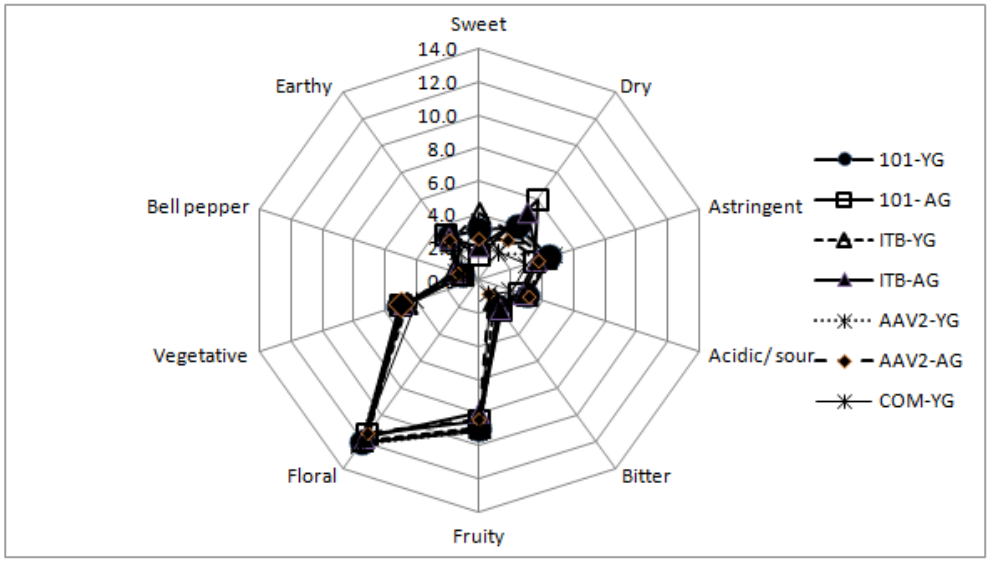

FIG 2. SENSORY PROFILE OF WINES

IV. CONCLUSION

S.cerevisiae strains isolated from the Indian geographical origin exhibited ideal enological properties, with wines being rated equivalent to the commercial *S.cerevisiae* produced wine. The flavour profile of the wines produced, highlights the importance of the wine yeasts. Further, autochthonous yeasts can be refined to produce tailor-made wine with specified flavours required by the wine enthusiasts. Thus, selection of the appropriate autochthonous yeasts is the most crucial aspect in the production of wine with unique enological characteristics of any given region.

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REFERENCES

[1] Aponte, M. & Blaiotta, G. (2016). Selection of an autochthonous *Saccharomyces cerevisiae* strain for the vinification of *"Moscato di Saracena",* a Southern Italy(Calabria Region) *passito*wine. *Food Microbiology*, 54, 30-39.

[2] Archana, K.M., Ravi, R. & Anu,-Appaiah, K.A. (2015). Correlation between ethanol stress and cellular fatty acid composition of alcohol producing non- *Saccharomyces* in comparison with *Saccharomyces cerevisiae* by multivariate techniques. *Journal of Food Science and Technology*, 52, 6770-6776.

[3] Caputi, A., Ueda, M. & Brown, T. (1968). Spectrophotometric determination of ethanol in wine. *American Journal of Enology and Viticulture*, 19, 160-165.

[4] Chira, K., Pacella, N., Jourdes, M. & Teissedre, P.L. (2011). Chemical and sensory evaluation of Bordeaux wines (Cabernet-Sauvignon and Merlot) and correlation with wine age. *Food Chemistry*, 126, 1971-1977.

[5] Differential malic acid degradation by selected strains of Saccharomyces during alcoholic fermentation.*International Journal of Food Microbiology*, 83(1), 49-61.

[6] Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.& Smith, F. (1956). Colorimetric method for determination of sugars and related substances.*Analytical chemistry*, 28, 350-356.

[7] Garde-Cerda'n, T. &Anci'n- Azpilicueta, C. (2006). Review of quality factors on wine ageing in oak barrels.*Trends in Food Science and Technology*, 17, 438-447.

[8] Hernandez, T., Estrella, I., Pérez-Gordo, M., Alegría, E. G., Tenorio, C., Ruiz-Larrrea, F.& Moreno-Arribas, M. V. (2007). Contribution of malolactic fermentation by *Oenococcus oeni* and *Lactobacillus plantarum* to the changes in thenon-anthocyanin polyphenolic composition of red wine. *Journal of Agricultural and Food Chemistry*, 55, 5260-5266.

[9] Holt, H., Cozzolino, D., McCarthy, J., Abrahamse, C., Holt, S., Solomon, M., Smith, P., Chambers, P.J. & Curtin, C. (2013). Influence of yeast strain on Shiraz wine quality indicators, *International Journal of Food Microbiology*, 302-311.

[10] Ivanauskas, L., Jakstas, V., Radusiene, J., Lukosius A. & Baranauskas, A. (2008). Evaluation of phenolic acids and phenylpropanoids in the crude drugs. *Medicina (Kaunas)*, 44(1), 48-55.

[11] Ivanova, V., Stefova, M., Stafilov,T.,Vojnoski, B., Bíró. I., Bufa, A. & Kilár, 1. F. (2012).Validation of a method for analysis of aroma compounds in red wine using liquid-liquid extraction and GC-MS. *Food Analytical Methods*, 5, 1427–1434.

[12] Jayaprakasha, G.K. & Sakariah, K.K. (2000). Determination of (-) Hydroxycitric acid in commercial samples of *Garcinia cambogia* extract by liquid chromatography with ultraviolet detection. *Journal of Liquid Chromatography & Related Technologies*, 23(6), 915-923.

[13] Jiang, B., & Zhang, Z. (2010). Volatile compounds of young wines from *Cabernet Sauvignon*, *Cabernet Gernischet* and *Chardonnay* varieties grown in Loess Plateau Region of China. *Molecule*, 15, 9184-9196.

[14] Kammerer, D.R. & Carle, R. (2009). Evolution of polyphenols during vinification and wine storage, *Functional Plant Science and Biotechnology*, 3(1), 46-56.

[15] Lamikanra, O. (1997) Changes in organic acid composition during fermentation and aging of Noble Muscadine Wine. *Journal of Agriculture and Food Chemistry*, 45, 935-937.

[16] Mateo, J.J., Jimenez, M., Pastor, A. & Huerta, T. (2001). Yeast starter cultures affecting wine fermentation and volatile. *Food Research International*, 34, 307-314.

[17] Miller, G.L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical chemistry*, 31, 426-428.

[18] Monagas, M., Bartolomé, B. & Gómez-Cordovés, C. (2005). Evolution of polyphenols in red wines from *Vitis vinifera* L. during aging in the bottle : II. Non-anthocyanin phenolic compounds. *European Food Research and Technology*, 220, 331–340.

[19] Monagas, M., Gomez-cordoves, C. & Bartolome, B.(2007). Evaluation of different *Saccharomyces cerevisiae* strains for red winemaking. Influence on the anthocyanin, pyranoanthocyanin and non-anthocyanin phenolic content and colour characteristics of wines. *Food Chemistry*, 104, 814–823.

[20] Nardi, T. (2006). Molecular approaches for the individuation and characterization of technological and quality traits. Ph,D theisis. Universita'DegliStudi di Padova.

[21] Ndip, R.N., Akoachere, J.F.K.T., Dopgima, L.L. & Ndip, L.M. (2001). Characterization of yeast strains for wine production. *Applied Biochemistry and Biotechnology*, 95, 209–220.

[22] Patel, S. & Shibamoto, T. (2002). Effect of different strains of *Saccharomyces cerevisiae* on the production of volatile components in Napa Gamay wine and Petite Sirah wine. *Journal of Agricultural and Food Chemistry*, 50, 5649-5653.

[23] Patel, S., & Shibamoto, T. (2003). Effect of 20 different yeast strains on the production of volatile components in symphony wine. *Journal of Food Composition and Analysis*, 16, 467-476.

[24] Pereira, A.C., Reis, M.S., Saraiva, P.M., & Marques, J.C. (2010). Aroma aging trends in GC-MS profiles of liqueur wines. *Analytica Chimica Acta*, 659, 93–101

[25] Pezet, R. & Cuenat, P. (1996). Resveratrol in Wine: Extraction from skin during fermentation and post-fermentation standing of must from Gamay grapes. *American Journal Enology and Viticulture*, 47, 287–290.

[26] Rai, A.K., Prakask, M., & Anu-Appaiah, K.A. (2010). Production of garcinia Wine: Changes in biochemical parameters, organic acids, and free sugars during fermentation of Garcinia must. *International Journal of Food Science and Technology*, 45, 1330-1336.

[27] Redzepovic, S., Orlic, S., Majdak, A., Kozina, B., Volschenk, H. & Viljoen-Bloom, M. (2003). Differential malic acid degradation by selected strains of Saccharomyces during alcoholic fermentation. *International Journal of Food Microbiology*,83(1), 49-61.

[28] Robinson, A.L., Boss, P.K., Heymann, H., Solomon, P.S. & Trengove, R.D. (2011). Influence of yeast strain, canopy management, and site on the volatile composition and sensory attributes of *Cabernet Sauvignon* wines from Western Australia. *Journal of Agriculture and Food Chemistry*,59, 3273–3284.

[29] Romano. P., Fiore, C., Paraggio, M., Caruso, M. & Capece, A. (2003). Function of yeast species and strains in wine flavour.*International Journal of Food Microbiology*,86, 169–180.

[30] Singleton, V.L., Orthofer, R. &Lamuela-Raventos, R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means ofFolin–Ciocalteau reagent. *Methods in Enzymology*, 299, 152–178.

[31] Somers, T.C., Verette. E. &Pocock,K.F. (1987). Hydroxycinnamate Esters of *Vitis vinifera*: Changes during White Vinification, and Effects of Exogenous Enzymic Hydrolysis. *Journal of the Scienceof Food and Agriculture*, 40, 67-78.

[32] Torija, M.J., Beltran, G., Novo, M., Poblet, M., Rozès,N., Mas, A. & Guillamón, J.M. (2003). Effect of organic acids and nitrogen source on alcoholic fermentation: study of their buffering capacity.*Journal of Agricultural and Food Chemistry*, 51, 916-922.

[33] Torrens, J., Urpí,P., Riu-Aumatell, M., Vichi, S., Lopez-Tamames, E. & Buxaderas, S. (2008). Different commercial yeast strains affecting the volatile and sensory profile of cava base wine. *International Journal of Food Microbiology*, 124, 48–57.

[34] Tristezza, M., Vetrano, C., Bleve, G., Grieco, F., Tufariello, M., Quarta, A., Mita, G., Spane, G. & Grieco, F. (2012). Autochthonous fermentation starters for the industrial production of Negroamaro wines.*Journal of Industrial Microbiology and Biotechnology*, 39, 81-92.

[35] Varela, C., Siebert, T., Cozzolino, D., Rose, L., Mclean, H.&Henschke, P. A. (2009). Discovering a chemical basis for differentiating wines made by fermentation with 'wild' indigenous and inoculated yeasts: role of yeast volatile compounds. *Australian Journal of Grape and Wine Research*, 15, 238-248.