Preparation of Mango (*Mangifera indica* L.) Wine Using a New Yeast-Mango-Peel Immobilised Biocatalyst System

Sadineni VARAKUMAR, Kondapalli NARESH and Obulam Vijaya Sarathi REDDY

Department of Biochemistry, Sri Venkateswara University, Tirupati, India

Abstract

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The preparation of mango wine by yeast-mango peel immobilised biocatalyst system by repeated batch fermentation was conducted and compared to free cells fermentation at 15, 20, 25, and 30°C. The operational stability of the biocatalyst was good as the ethanol concentrations (76.0–96.0 g/l) and productivities (1.53–3.29 g/l/h) were high, showing the suitability of the biocatalyst for even low temperature winemaking. The concentration of ethyl acetate was not above 40 mg/l in all cases, and higher alcohols were low (< 330 mg/l) in wine with immobilised cells indicating an improvement in the product compared to free cells fermentation. Amyl alcohols were proved to be temperature dependent and decreased with the decrease in temperature (262.48–146.83 and 239.74–184.34 mg/l) in the case of fermentation batches with immobilised and free cells, respectively, from 30°C to 15°C. Sensory evaluation revealed fruity aroma (7.9 ± 0.73), fine taste (7.7 ± 0.24), and the overall improved quality of the wines produced by the immobilised system.

Keywords: *Saccharomyces cerevisiae*; immobilisation; Mango peel biocatalyst; sensory evaluation

Immobilisation systems are applied to enzymes, microbial, animal, and plant cells and are intended to confine or localise the intact cells into a defined space in such a way that they retain their activities over a long period of time. Immobilised cells technology offers several important advantages comparatively to the fermentation using free cells such as higher cell densities per unit bioreactor volume that results in very high fermentation rates, the reuse of the same biocatalysts for prolonged periods, and the development of continuous processes that may be operated beyond the nominal washing-out flow rate (Pilkington *et al*. 1998). For industrial wine production, the selection of a suitable support for the cell immobilisation is important because a number of factors are known to influence the cell-support interactions such as the nature of support and microbial cell, environmental conditions and hence further research is needed to obtain cells immobilised on a support that is more hygienic, cheap, abundant in nature, and suitable for low temperature fermentation. To satisfy these prerequisites, various natural supports have been proposed for ambient and low-temperature wine making such as fruit pieces like apples, pears, raisin berries (Kourkoutas *et al*. 2001; Mallios *et al*. 2004; Tsakiris *et al*. 2004), potato pieces (Kandylis & Koutinas 2008), cork pieces (Tsakiris *et al*. 2010) water melon rind pieces and sugarcane pieces (Reddy *et al*. 2008, 2010). Furthermore, cell immobilisation was applied for the production of a wide variety of fermented beverages such as beer, probiotic milk, and fermented cheese. Even though a very good number of natural immobilisation supports were tried for wine-making or for other fermented beverages, their usage was limited due to their abundance and cost effectiveness.

Winemaking is one of the most ancient technologies and is now one of the most commercially prosperous biotechnological processes. Even though the grapes are the main raw material used

for the wine production, there is an increasing interest in the search for indigenous fruits such as orange, apple, mango, and also palm sap that are cheap and readily available for wine making in such countries where grapes are not abundantly available (Reddy & Reddy 2005).

Mango (*Mangifera indica* L.) is one of the most important tropical fruits of India, accounting for 54.2% of the total mangoes produced world-wide and is considered as 'the king of fruits'. It is highly perishable seasonal fruit and is processed into various products like slices, nectar, jams and pickles. However the production of wine from mango, which has a high carbohydrate content (16–18% w/v), is one of the alternative ways to exploit and convert the surplus production into a valuable product (Kumar *et al*. 2009), and it has been proved that mango wine contains bioactive molecules which impart antioxidant activity to the wine (Varakumar *et al*. 2011). In the processing of mango, peel is a major by-product and represents a serious disposal problem. The use of mango peels for the production of biogas and dietary fiber has been described; however, the studies on peels are scarce. Their use as animal feed is known, although they can also be used for obtaining more valuable products like good quality pectins (Pedroza-Islas *et al*. 1994; Kumar *et al*. 2010).

Mango peel is rich in dietary fiber, antioxidant phytochemicals such as carotenoids, polyphenols, anthocyanins, and volatile compounds (Ajila *et al*. 2007). It is a safe and inexpensive material, comprising an interesting new support for cell immobilisation for wine fermentation. The preparation of wine or any other beverage using cells entrapped in mango peel has not been attempted yet, and it is a very attractive proposition because of its full compatibility in the wine production. Therefore, the aim of the present study was to investigate the suitability of immobilised cells entrapped in mango peels for mango wine fermentation at various temperatures, as well as the influence of the immobilised biocatalyst on the volatile composition of the produced wines.

MATERIAL AND METHODS

Yeast strain and inoculum preparation. The ethanol producing yeast used in the experimens, *Saccharomyces cerevisiae*, was a generous gift from Prof. Roberto Ambrosoli, University of Turin, Italy. The culture was maintained on MPYD agar slants containing Maltose 3, Peptone 5, Yeast extract 3, Dextrose 10, and agar 20 (g/l) (Himedia, Mumbai, India), stored at 4°C and subcultured regularly. The inoculum was prepared according to Kumar *et al*. (2009).

Preparation of mango juice. Ripe mango (*Mangifera indica* L.) fruits cv. Rumani were procured from the local fruit market in Tirupati, Andhra Pradesh, South India, and were processed and homogenised. The juice obtained was sterilised by autoclaving at 115°C for 10 min and then subjected to analysis for total soluble solids, sugars (total and reducing), total acidity, and pH. The final concentration of sugar was adjusted to ~20% (w/v) with commercial glucose, and pH to 3.8 using tartaric acid.

Yeast cell immobilisation. The method used for yeast cell immobilisation on mango-peel pieces was similar that described by Reddy *et al*. (2010). In brief, mango peel from cv. Banginapalli was obtained by peeling off the fruits manually and the ideal ones were selected, cut into small pieces (3 × 5 cm, 200 g), and sterilised by autoclaving at 121°C for 15 minutes. These pieces were taken into a 1000 ml glass cylinder and fermented with 400 ml of yeast cells inoculums with optical density (O.D.) of 1 at 590 nm, and then allowed to ferment for 12 hours. The fermented broth was decanted to remove the unimmobilised yeast cells. The biocatalyst prepared by this method was used for the repetitive batch fermentation and the biocatalyst was washed twice with 200 ml mango juice after each batch of fermentation.

Batch fermentations. Repeated batch fermentations were carried out with 100 g of mango peel biocatalyst per 1000 ml of mango juice in a glass cylinder for fermentation. The fermentation was carried out separately at various temperatures (15, 20, 25, and 30°C) and no stirring was performed during any stage of the fermentation. The end point of the fermentation was detected by measuring the residual sugars content as less than 2 g/l. The fermented liquid was decanted and the support was washed twice with 200 ml of the medium that was used for wine production. The volume of the biocatalyst in the bioreactor and volatiles were determined in all fermentations performed and the effect of temperature was monitored during the repeated fermentations.

Enumeration of immobilised cells. The determination of immobilised cells on wet mango

peel pieces and of free cell concentrations were carried out by the method of Reddy *et al*. (2008) with sterilised Ringer's solution. The immobilisation on the mango peels was confirmed before and after repeated batches by Scanning Electron Microscope (JEOL Model JSM-840A; JEOL USA Inc., Peabody, USA) .

Viability determination. For the viability determination, 100 µl of appropriate dilutions of the cultures were plated (in triplicate) on MPYD agar plates. The plates were incubated at 30°C until the appearance of colonies (1–3 days), and the number of colony forming units (CFU) per ml of cell culture was determined.

Determination of sugars, glycerol, and acidity. Total reducing sugars were determined spectrophotometrically using dinitrosalicylic acid (DNS) method (Miller 1959) while glycerol was determined enzymatically by glycerol kinase method (Wieland 1959) on diluted samples employing the commercial kit from Megazyme International Ireland (Wicklow, Ireland). Total acidity was estimated by the titration of samples with 0.1M NaOH previously standardised using standard oxalic acid while the values were expressed as tartaric acid equivalents, and volatile acidity by the titration with 0.1M NaOH of distillates obtained by steam distillation of wine samples by Ripper method (Zoeklein *et al*. 1990) and the results were expressed as acetic acid (g/l).

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

Scanning electron microscopy (SEM). The immobilised biocatalyst was washed and fixed with glutaraldehyde in phosphate buffer at 4°C for 4 h and dehydrated by using a series of graded alcohol and dried at a critical point in a Hitachi HCP-2 (Hitachi Koki Co., Ltd., Tokyo, Japan) with CO_2. It was then coated for about 90 s with a thin layer of platinum using an automated sputter coater (Polaton, Watford, UK) and the samples were then scanned under SEM (Hitachi S520; Hitachi, Tokyo, Japan) at various magnifications at the Indian Institute of Chemical Technology (IICT), Hyderabad, India.

Sensory evaluation. The final beverage was evaluated by 15 expert panelists, males and females of 25–45 years of age, including students and staff. The panelists were selected for participation on the basis of their preference for dry (< 5 g/l of sugar) beverage, interest and availability. Randomised refrigerated (10°C) samples of 50 ml were served in clear tulip shaped glasses coded with a 3-digit random code. Distilled water was provided for rinsing the palate during the testing. The evaluations took place in the mornings between 9:00 and 10:00 a.m. and were conducted at room temperature (22–24°C) under white light. The mango wines were evaluated for their appearance, aroma, taste and general acceptability according to the 9-point Hedonic scale (Dias *et al*. 2007). This scale consists of the comparison, punctuation, and classification of foods and beverages of the same class or origin according to their qualities and defects.

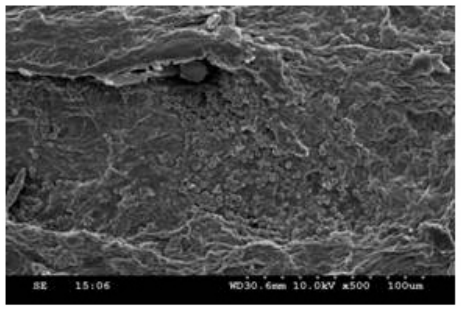
Statistical analysis. All the experiments were carried out in triplicate and the mean value and standard deviation were presented. Student's *t*-test was used to compare the mean values. The data were analysed by one-way analysis of variance (ANOVA) using SPSS, Version 12.0 (SPSS Inc., Chicago, USA).

RESULTS AND DISCUSSION

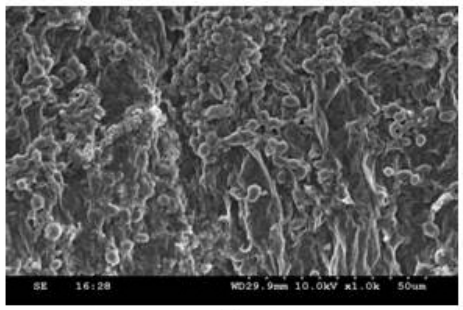
The mango cultivar Rumani, a low-priced fruit is abundantly available locally during the season with a juice yield of 56.0 ± 5.2 ml/kg, medium pectin content (8–10% w/w), and with a total sugar content of 14.3 to 15.5% (data not shown in tables). The total sugar content in all the trials was adjusted to ~20% with commercial glucose. However, wines from other high-priced cultivars like Banginapalli and Alphonso were also tried (Kumar *et al*. 2009; Reddy & Reddy 2009). In the present study, the cv. Rumani was selected to exploit the low-priced mango fruits to produce a good quality wine which would be profitable to the farming community.

Of the seven different cultivars of mango peels studied as the immobilisation support in mango wine production, the peels of cv. Banginapalli (pectin content of 15.2% w/w) imparted better aroma and taste (data not given); hence the data on wines produced from Banginapalli peel as an immobilisation support only were discussed in this paper. Several researchers employed fruits pieces, grape by-product like skins as supports for the cell immobilisation to develop products with better taste and aroma; this was due to the transfer of some of their aroma constituents into the wine. The use of the above mentioned fruit pieces other than mango peels may impart aroma and flavour to the wine, however, the peels from Banginapalli mango cultivar had intensified the unique aroma of the mango fruit as well as the colour, flavour, and taste of the final wine.

For the immobilisation of yeast cells, pieces of mango peel were mixed with a liquid culture of yeast biomass and allowed to ferment for 8 hours. Around this time, about $3.4 \times 10^8 \pm 1.0 \times 10^6$ yeast cells were attached per gram of mango peel pieces. The prepared biocatalyst was washed and used for 12 repeated batch fermentations of mango juice for wine-making at room and low temperatures. The stability and productivity in the repeated batch fermentations and the leached out free cells concentrations are shown in Table 1. The morphology of the mango peel surface after the immobilisation of yeast cells and their existence or attachment on the fibers of mango peel (biocatalyst) was proved by the electron micrographs (Figure 1A and B). The predominance and proliferation of the yeast cells within the biocatalyst tissue structure could be viewed at higher magnification (Figure 1C). Cells immobilised on mango peel were found to be suitable for wine-making at ambient temperatures and the biocatalyst appeared to have a good operational stability. An effective immobilisation of yeast cells on mango peel biocatalyst was proved by the ability to perform successive repeated batch fermentations for ~5 months without any significant loss of the biocatalytic activity at different temperatures (15–30°C); although the support was washed after each batch to remove free cells, it showed yeast cells densely and homogenously adhered to the surface of the carrier support. The adhesion of *S. cerevisiae* is essentially dependent upon electrostatic interactions between the support and the normally negatively charged cell surface, and cell immobilisation on the peel pieces may take place either by natural entrapment into the porous pectin-cellulosic material of mango peel, or due to physical adsorption by electrostatic forces or covalent binding between the cell membrane and the carrier support (Tsakiris *et al.* 2004).



(A)



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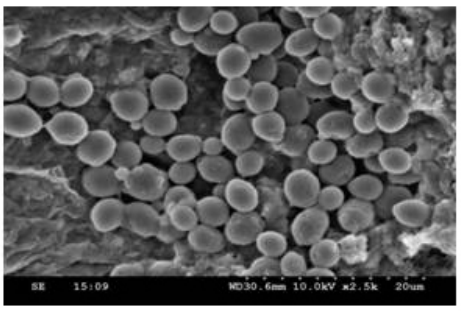

(C)

Figure 1. Electron micrographs showing the surface of mango peel immobilised with yeast at various magnifications at (A) 500×, (B) 1000× and (C) 2500×

Repeated batch fermentation

Repeated batch fermentations were conducted with entrapped and free cells separately at different temperatures (15, 20, 25, and 30°C). All the fermentations were carried out using mango peel supported biocatalyst with the same initial concentration of sugar ~20% (w/v) (Table 1). The residual sugar content was very low (ranging from none to 0.8 g/l), indicating that the biocatalyst was very active and suitable for alcoholic fermentation and the resultant wine

Table 1. Effect of the use of immobilised yeast on fermentation parameters at different temperatures

Temperature (°C)	Repeated batches	Initial sugar (%)	Fermentation time (h)	Residual sugar (g/l)	Sugar conversion (%)	Ethanol			Free cell concentration (g/l)	Acidity (g/l)	
						concentration		productivity (g/l/h)		total	volatile
						(% v/v)	(g/l)				
30(C)	3	20.8	74	0.9	95.7	11.0	88.0	1.19	6.2	2.1	0.15
30	R1	20.4	61	0.2	99.0	11.8	94.4	1.55	3.1	2.9	0.20
30	R2	20.2	43	tr	99.5	11.9	96.0	2.23	4.1	2.4	0.14
30	R3	20.6	28	tr	99.5	11.5	92.0	3.29	4.4	3.1	0.12
25(C)	3	20.1	52	1.1	94.5	10.0	80.0	1.54	5.6	3.2	0.19
25	R4	20.4	43	0.2	99.0	10.1	80.8	1.88	3.1	2.9	0.17
25	R5	20.9	47	0.3	98.6	10.0	80.0	1.70	4.8	4.6	0.11
25	R6	20.5	39	tr	99.5	10.5	84.0	2.15	5.0	4.0	0.24
20(C)	3	20.6	71	1.4	93.2	9.42	75.3	1.10	5.5	3.5	0.26
20	R7	20.1	52	0.8	96.0	10.7	85.6	1.65	3.0	2.1	0.14
20	R8	20.7	50	0.4	98.1	10.1	80.8	1.62	4.3	3.6	0.16
20	R9	21.1	35	tr	99.5	10.0	80.0	2.29	4.7	4.5	0.20
15(C)	3	20.3	64	1.7	91.6	9.50	76.0	1.19	4.1	3.5	0.17
15	R10	20.4	52	0.4	98.0	9.95	79.6	1.53	2.8	2.6	0.20
15	R11	20.1	40	0.4	98.0	9.50	76.0	1.90	3.6	2.9	0.18
15	R12	20.5	31	tr	99.5	9.70	77.6	2.50	3.9	2.4	0.20

C – control, fermentation batches with free yeast cells; R – repeated batch fermentations with biocatalyst; tr – trace

contained alcohol concentrations similar to dry and table wines – 9.5–12% (v/v). It was found that the temperature mainly affected the fermentation rate. At 15°C, the fermentations were completed in 72 h which is less time that is required for the natural fermentation of mango juice, while at 30°C it took only 40 hours. Both higher alcohols and ethanol productions were higher than in fermentations with free cell batches and were significantly affected by temperature ($P < 0.05$). At low temperatures (15 and 20°C), an improvement of the fermentation time and productivity were observed after the first two batches. This may be probably due to the adaptation of the immobilised yeast cells to the mango peel matrix.

Wine and ethanol productivity was slightly reduced after the first three repeated batches. This may be due to the difficulty in nutrient transfer, since there is a decrease in the mango peel biocatalyst and therefore, the yeast cells were not uniformly spread throughout the mango peel. Therefore, the first and second batches were carried out with 400 ml, and the subsequent batches with 300 ml. The volume of the mango peel pieces was weighed after every batch and a slight decrease in weight was observed up to 4 repeated batches. This decrease was probably due to the utilisation of the peel sugar by the yeast cells. The peel pieces volume remained stable after the seventh or eighth batch and was not disrupted significantly and remained intact throughout the fermentation experiments, which was mainly due to the unfermentable residual ligno-cellulosic and pectin matrix of the peel pieces. It was observed that the viability of yeast cells was high (> 90%) in the biocatalyst at the end of the fermentation when compared to conventional fermentation. This may be due to the tolerance of the immobilised yeast cells to various stresses like ethanol concentration and heat shock. The yeast population increased during the repeated batch fermentations and the enumeration of immobilised viable cells after immobilisation revealed a yeast cell population of 6.42 CFU/g of mango peel biocatalyst, the amount of cells retained on the biocatalyst being about threefold higher than the amount of free cells in the broth. As the cell number increased, the decrease of the surface on the immobilised material led to the detachment of few yeast cells from the immobilising support and a subsequent growth in the medium solution, which initially was devoid of yeast cells. The appearance of the yeast cells was observed in the medium after

25 h of fermentation. The detached cell biomass concentrations ranged from 2.8 to 5.0 g/l for the entire duration of the experiment at different temperatures, being in agreement with the results obtained on wine produced by yeast cells entrapped in corn starch gel (Kandylis *et al.* 2008). But, the concentrations of the detached cells in the wine in the present study were lower when compared to those in the wines produced by immobilised sugarcane pieces (Reddy *et al.* 2010). However the cell mass in the immobilised pieces was maintained constant. It may be probably due to the new cells which were also adsorbed onto the support. A two fold increase in the fermentation rate was observed with immobilised cells at low temperature (15°C) and this, in turn, shortened the fermentation time when compared to the free cells.

Total and volatile acidities were in the ranges of 2.1 to 4.6 and 0.11 to 0.26 (g/l), respectively, which were within the normal limits of dry wines (4–6 g of tartaric acid/l). In the present study, the fermentation temperature and immobilisation support did not affect the volatile acidity and total acidity. Kourkoutas *et al.* (2001) reported that there was a little increase of total acidity due to the transfer of apple acids to the wine prepared with yeast immobilised on apple pieces, but there was no increase in the total acidity throughout the study, However they observed that total acidity was lowered slightly as the temperature dropped from 9 to 1°C. This reduction can be attributed to the increase of crystallisation of tartrate salts with the decrease in temperature. The chemical analyses of the mango wine showed that the produced wine was similar to dry table wines with respect to the alcohol and residual sugar contents (Table 1) as it is generally known that dry wines contain residual sugars generally below 1.5 g/l consisting mostly of pentoses such as arabinose, rhamnose, and xylose (Soleas *et al.* 1997).

Volatile by-products

As mango peel pieces proved to be a suitable support for wine-making, particularly easy to use for immobilisation, the study of the aroma through the determination of the most abundant volatile by-products in the wine was essential. The major compounds defining the overall volatile effects on wine aroma are acetaldehyde, ethyl acetate, and higher alcohols such as 1-propanol, isobutyl alcohol, and amyl alcohols. The effects of temperature and the immobilisation technique on the concentrations of these compounds in the produced wines are summarised in Table 2.

Higher alcohols

Higher alcohols or fusel alcohols are the largest group of aroma compounds in alcoholic beverages and are secondary products of alcoholic fermentation. Fusel alcohols have a strong pungent smell and taste. Although they exhibit a harsh, unpleasant aroma at the concentrations generally found in wine, below 350 mg/l they usually contribute to the desirable complexity of wine. The principal higher alcohols produced by yeast are the aliphatic alcohols such as *n*-propanol, isobutanol (2-methyl-1-propanol), active amyl alcohol (2-methyl-1-butanol), isoamyl alcohol (3-methyl-1-butanol) and the commonly account for about 50% of the aromatic constituents of wine.

Among the higher alcohols, propanol and isobutanol were significantly decreased with the decrease in temperature (Table 2). The formation of higher alcohols was decreased with the decrease in temperature and the products formed with low concentrations of higher alcohols are of good quality (Mallouchos *et al.* 2003a). The results from this study are comparable with earlier immobilisation studies using watermelon pieces, quince fruit, and pear pieces (Kourkoutas *et al.* 2001; Reddy *et al.* 2008). The higher alcohols formation varies during fermentations and is mainly dependent on the yeast strain and fermentation conditions.

The concentration of amyl alcohols decreased significantly with the decrease in temperature, which is a positive factor for the wine quality, as they are considered as off-flavours (Mallios *et al.* 2004). In general, low temperature greatly reduced the amount of higher alcohols. These results show that the product is of improved quality because of low concentrations of higher alcohols. These observations are in agreement with the results of Kourkoutas *et al.* (2001) contained with wines from apple pieces as the immobilising agent.

Ethyl acetate

Ethyl acetate is one of the important volatile compounds and its presence imparts a significant effect on the organoleptic characteristics of the

Table 2. Effect of the use of immobilised yeast on volatile compounds at different temperatures

Temperature (°C)	Batch	Acetaldehyde (mg/l)	Ethyl acetate (mg/l)	1-Propanol (mg/l)	Isobutanol (mg/l)	Amyl alcohols (mg/l)	Total higher alcohols†	Methanol (mg/l)	Total volatiles‡	Glycerol (g/l)
30	R1–3	30.18 ± 1.2^{a}	27.75 ± 2.4^{bc}	12.88 ± 0.8^{b}	50.43 ± 1.5^{c}	262.48 ± 3.8^{h}	325.79 ± 2.4	154.67 ± 2.4^{e}	383.72 ± 4.9	8.9 ± 1.2^{f}
25	R4–6	31.19 ± 0.8^{a}	27.97 ± 2.3^{bcd}	16.28 ± 1.7^{c}	51.08 ± 0.4^{d}	233.68 ± 3.6^{f}	301.04 ± 1.4	147.2 ± 3.8^{d}	360.2 ± 5.6	7.2 ± 1.1^{e}
20	R7–9	33.10 ± 0.5^{b}	26.89 ± 0.9^{d}	15.25 ± 2.0^{c}	47.90 ± 1.4^{bc}	201.54 ± 5.6^{c}	264.69 ± 1.8	128.95 ± 4.9^{c}	324.68 ± 5.5	5.6 ± 1.2^{c}
15	R10–12	38.54 ± 1.2^{c}	29.31 ± 0.8^{d}	19.52 ± 1.2^{e}	42.61 ± 0.5^{a}	146.83 ± 4.9^{a}	208.96 ± 3.1	129.13 ± 1.6^{bc}	276.81 ± 4.4	4.1 ± 1.4^{b}
30(C)	1–3	36.71 ± 0.5^{c}	29.16 ± 1.6^{b}	21.53 ± 0.5^{g}	49.34 ± 2.6^{e}	239.74 ± 6.1^{g}	310.61 ± 1.8	114.24 ± 4.7^{a}	376.48 ± 3.6	7.1 ± 0.7^{e}
25(C)	4–6	38.38 ± 1.1^{d}	22.21 ± 1.4^{a}	20.75 ± 0.9^{f}	47.40 ± 2.1^{c}	216.17 ± 4.9^{e}	284.32 ± 1.3	115.26 ± 2.5^{ab}	344.91 ± 4.1	6.4 ± 0.5^{d}
20(C)	7–9	36.16 ± 1.3^{c}	31.52 ± 1.3^{bc}	17.63 ± 1.4^{d}	45.21 ± 1.6^{b}	203.42 ± 3.4^{d}	266.26 ± 1.7	126.32 ± 5.5^{c}	333.94 ± 3.9	4.5 ± 1.2^{b}
15(C)	10–12	37.87 ± 0.6^{c}	38.67 ± 1.7^{cd}	12.44 ± 1.8^{a}	41.56 ± 1.1^{a}	184.34 ± 4.6^{b}	238.34 ± 2.6	123.64 ± 4.8^{c}	314.88 ± 5.5	3.9 ± 0.7^{a}

†sum of 1-propanol, isobutanol and amyl alcohols (mg/l); ‡sum of acetaldehyde, ethyl acetate and total higher alcohols, excluding methanol; C – control, fermentation batches with free yeast cells; R – repeated batch fermentations with biocatalyst; values not sharing a common superscript letter differ significantly at $P \leq 0.01$ Duncan's multiple range test (DMRT)

wine. It is the most important and abundant ester in wines which is considered to contribute at low concentrations (50–80 mg/l) to wine olfactory complexity having a positive impact on the wine quality, and only at concentrations > 120 mg/l may it spoil the bouquet with an unpleasant, pungent tang. And in addition, it was found that any factor that decreases the speed of fermentation like temperature, pH, and low oxygen conditions simultaneously increases the amount of ethyl ester and acetaldehyde (Kandylis *et al*. 2008). In the present study also the ethyl acetate concentration increased with the decrease in temperature. Ethyl acetate concentrations in wine produced with apple pieces as the immobilising agent was relatively high up to 150 mg/l (Kourkoutas *et al*. 2001). However, in the present study the concentration of ethyl acetate was < 50 mg/l, and there was no indication of vinegar odour in the final product; on the contrary, it had a fruity aroma and a fine taste.

Other components

Acetaldehyde is one of the most important carbonyl compounds formed during degradation of sugars by yeasts and constitutes more than 90% of the total aldehyde content in wine. At low levels, it gives a pleasant fruity aroma, but at high concentrations (> 100 mg/l) it possesses a pungent irritating odour, which is undesirable for table wines which were poorly appreciated by the wine tasters. The acetaldehyde concentration in wines usually ranges from 13 to 40 mg/l. Low acetaldehyde concentrations were detected in the present study with a maximum of 38 mg/l (Table 2). However, its concentration may reach 75 mg/l (Mallios *et al*. 2004) or up to 115 mg/l (Kandylis *et al*. 2008) in some batches, which could be due to either incomplete fermentation, or the presence of SO_2 in the grape must used may reveal relatively high amounts of acetaldehyde in the finished wines (Romano *et al*. 1994). The differences in acetaldehyde content in wines could also be attributed to the effect of temperature and immobilisation on the activity of pyruvate decarboxylase and alcohol dehydrogenase, which are implicated in the biosynthesis of acetaldehyde by yeasts (Tsakiris *et al*. 2010).

Glycerol is the major fermentation product after ethanol and carbon dioxide in wines. Glycerol is a non-volatile and has no direct impact on the

aromatic characteristics of wine. However, it has a favourable effect on wine quality by contributing sweetness, fullness, and smoothness to the wine. Glycerol is naturally found in wines and its concentrations in wines vary between 1 and 10 g/l. Glycerol production is influenced by many factors like the yeast strain, fermentation temperature, sulphur dioxide concentration, agitation time, and pH levels. In the present study, the glycerol concentrations in batches with immobilised cells ranged from 4.4 g/l to 8.9 g/l, however, it was low in batches with free cells and ranged there from 3.9 g/l to 7.19 g/l (Table 2). It was observed that the glycerol concentration in all the fermentation batches with immobilised cells on mango peel decreased with the decrease in temperature, showing that the fermentation temperature plays an important role in glycerol formation. However, the glycerol concentration obtained in the present study with mango wine was lower when compared to the glycerol concentration with grape wine made with immobilised cells (11.9–14.9 g/l) and free cells (10.2–12.8 g/l) (Balli *et al*. 2003), but it was higher when compared to mango wine with glycerol concentrations of about 6.94 g/l (Kumar *et al*. 2009). The increased glycerol concentration in the wines produced by immobilised yeast on mango peel could be attributed to the nature of the supports, immobilisation, and yeast strain.

Methanol is not a major constituent in wines and has no direct sensory effect. The amount of methanol found in wine is primarily generated by the enzymatic breakdown of pectins. The methanol content in the present study ranged from 113.4 mg/l to 154.6 mg/l; however, in the traditional grape wine fermentation the usual range of methanol content is below 100 mg/l. Unlike most fruits, grapes are low in pectin. As a result, grape wine generally has the lowest methanol content among fermented beverages. In the first 4 batch fermentations, methanol concentrations in wines produced by immobilised cells (141.64–154.67 mg/l) were higher than in those produced byf free cells (114.24–126.32 mg/l) as expected. This could be attributed to the fact that the mango peel contained pectin substances, which after enzyme hydrolysis might release methanol. After that, a reduction in methanol concentration was observed and the methanol content of the wines produced from 5th batch fermentations of must by immobilised cells remained at low levels similar to those of wines produced by free cells. The methanol concentration, in general, was not affected by the reduction in the incubation temperature (Table 2). Similarly, the formation of methanol was not affected by the immobilisation of cells as its formation was not due to metabolic activity of the yeast.

Sensory evaluation

After the chemical analyses, the beverage was subjected to sensory analysis to assess its acceptance among the consumers. Table 3 presents notes attributed to the beverage by 15 trained tasters, designated in the Hedonic scale of nine points (1 = dislike extremely; 9 = like extremely). The average values were recorded for the four evaluated attributes of which the aroma is the one with a slightly higher value, followed by the taste, appearance, and overall acceptance, with respective notes of 7.9, 7.7, 7.6, and 7.5. The tests indicated some improvement in aroma and taste of the wines produced by using cells immobilised on mango peels, particularly at low temperatures, when compared to wines produced by free cells (Table 3). This can be attributed to the reduction of amyl alcohols, which are off-flavour compounds, at lower temperatures and therefore to an increase in the proportion of other aroma compounds in total volatiles. Mallouchos *et al*. (2003b) reported that wines produced by cells immobilised on grape skins have a better fruity aroma. Similar results were also reported by García-Romero *et al*. (1999) who found a considerable improvement in the wine sensory profile when fermentations were carried out in contact with the skins of Airen white wine grapes because of the transfer of the precursors of volatile compounds like esters, aldehydes, and alcohols into the wine. The wines produced by immobilised yeast biocatalyst showed fine clarity at the end of fermentation with low free cell concentrations as well as characteristic pleasant soft aroma and fruity taste.

Table 3. Effect of the use of immobilised yeast on sensory characteristics

Attribute	Wine from free yeast cells	Wine from immobilised yeast on mango peels
Appearance	5.6 ± 0.82	7.6 ± 0.54 ($P < 0.0243$)
Aroma	6.1 ± 0.25	7.9 ± 0.73 ($P < 0.0156$)
Taste	6.9 ± 0.81	7.7 ± 0.24 ($P < 0.1763$)
General acceptance	6.7 ± 0.67	7.5 ± 0.61 ($P < 0.2009$)

CONCLUSIONS

Yeast-mango-peel immobilised biocatalyst can be a good and effective system for wine fermentation at both low and room temperatures, as the wines produced by this procedure had a potentially better aroma than those obtained by free cell fermentation. The biocatalyst is economical, food grade, and does not need special pretreatment before use. Mango peels, which otherwise may pollute the environment, can be beneficially used as an alternative cell immobilisation support. This first study on the use of mango peel as an immobilisation support for yeast during wine making showed the potentialities of this process. The results obtained open the possibilities of applying this process also to other fermented beverages.

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Corresponding author

Prof. Dr. O. Vijaya Sarathi Reddy, Sri Venkateswara University, Department of Biochemistry, Tirupati-517 502, A.P. India
tel. +91 877 228 94 95, e-mail: ovsreddy@yahoo.com