8.0 SUMMARY AND CONCLUSIONS

- Seven cultivars of ripe mangoes were screened that were grown locally and based on their juice yields and economic feasibility, the cultivars, Banganapalli and Rumani were used in further studies.

- Nine wine yeast strains were screened for mango wine fermentation and based on the fermentation kinetics, S. cerevisiae (UCD 522), S. bayanus (UCD 595), S. bayanus (S.B), S. cerevisiae (S.C) and S. cerevisiae gave promising results at all conditions and selected for further studies.

- The alcohol (%) in wines were is in the range of 10.2-13.8 %. The high ethanol production was found in wine produced from the S. cerevisiae (UCD 522) (12.8%), S. bayanus (UCD 595) (11.8%), S. bayanus (S.B) (11.5 %) and lower alcohol (8.8%) in wine from S. cerevisiae (3045).

- The methanol concentrations in wine produced are in the range of 121-168.5 mg/L and the acetaldehyde concentration is ranged from 65.6-87.8 mg/L. The ethyl acetate concentrations were in the range from 12.4 to 22.4 mg/L. These variations observed could be due to the fermentation temperature, mango cultivar used and also to the yeast strain employed.

- Isobutanol in the mango wine from different cultivars was in the range of 56.2-84.1 mg/L; n-propanol (24.6-45.3mg/L) iso and act-amyI alcohols (100.4-119.6 mg/L) and the concentration of glycerol was ranged from 4.5-7.5 g/L.

- The total polyphenolic compounds present in the mango wine were in the range of 202.7-537.4mg/L. The highest were in wine from Alphonso (537 mg/L) and the lowest was in Totapuri (202.7 mg/L).

- The glycerol concentration and Hunters colour measurements were good and even after fining. For fining trials, the combination of bentonite+PVPP at a concentration of Bn 0.5g/L+PVPP 0.7g/L was finalised for mango wine-making.

- The co-fermentations were performed along with S. cerevisiae as main fermenting yeast and or with M. pulcherrima and T. delbrueckii (IIHR and NCIM). S. cerevisiae showed the highest fermentative ability in pure culture with the production of 11.9% (v/v) ethanol. Mixed culture fermentations along
with the main wine yeast also produced higher concentrations of ethanol ranging from 11.04 to 11.53% (v/v).

- In mixed culture of *M. pulcherrima* and *S. cerevisiae*, *M. pulcherrima* multiplied up to 6.7 log cfu/mL. After maximum growth, it did not show the stationary phase and a decline phase was observed. However, it survived up to 14 days. *M. pulcherrima* proliferated to 3.4 log cfu/mL, followed by a rapid decline and disappearance in the mixed culture.

- The amounts of higher alcohols produced in mono culture of *S. cerevisiae*, *M. pulcherrima* and *T. delbrueckii* strains (NCIM and IIHR) were 359.70, 164.68, 301.58 and 290.26 (mg/L) respectively, whereas the levels of 350.62, 323.61 and 324.94 (mg/l) were observed for simultaneous co-fermentations in *S. cerevisiae + M. pulcherrima*, *S. cerevisiae + T. delbrueckii* (NCIM) and *S. cerevisiae + T. delbrueckii* (IIHR), respectively. Both strains of *T. delbrueckii* showed similar capability to produce higher alcohols in mono cultures.

- Wine co-fermented with *M. pulcherrima* was acceptable with a high score for overall acceptability (8.5), colour (7.1) and taste (6.2) followed by *T. delbrueckii*. There was no significant sensorial difference observed between the two *T. delbrueckii* strains. However, wine fermented with pure *S. cerevisiae* showed the lowest sensory attributes.

- In all the wines after malolactic fermentation, there was an increase in pH by about 0.3-0.9 units as compared to the wine inoculated with yeast (S.C or UCD 522 or UCD 595) alone. However there were no significant differences between the treatments in the concentration of glycerol (5.91-6.88 g/L), free SO₂ (8.2-10.8 mg/L), and ethanol. (11.5-12.5% v/v).

- Alcohol fermentations was finished in all the treatments between 10 to 12 days in simultaneous inoculations, the yeast populations were not altered by bacterial inoculation. The viable yeast cell population grew from 3.5 to 6.9 log CFU/mL and the bacterial growth to 7.1 log CFU/mL over the first 6 days. L-malic acid was completely degraded by this time in all the treatments.
In sequential inoculation, the yeast cells reached their maximum growth and the viable yeast cell population decreased from 8.2 to 5.6 log CFU/mL. The bacterial growth increased to its maximum of 7.8 CFU/mL and decreased at the end of MLF, over a period of 9 days. L-Malic acid was completely degraded by this time in all the sequential treatments also.

The acetaldehyde and ethyl acetate contents were in the range of 14.1-42.5 and 26.6-41.1 (mg/L) respectively.

The concentrations of n-propanol, isobutanol and amyl alcohols were in the range of 12.6-13.4, 40.4-50.1 and 241.4-269.0 (mg/L) respectively. The total higher alcohols concentration was in the range from 350.9 to 372.4 mg/L.

Irrespective of the yeast strain used, there was a significant (p<0.05) differences observed between the control (no *O. oeni* added) and simultaneous and sequential treatments.

Wine produced with simultaneous treatment irrespective of the yeast strain used showed better sensorial influence on flavor, fruity aroma and overall acceptability hence this method is suitable for MLF.

Immobilization of yeast cells was carried out on mango peel from seven different cultivars however; further work was carried out only with *Banginapalli* peel because of its abundance.

Repeated batch fermentations were conducted with entrapped and free cells separately at different temperatures (15, 20, 25 and 30°C).

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 than that required for the natural fermentation of mango juice, while at 30°C it took only 40 h.

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 the dry wines (4-6 g/L) showing that fermentation temperature and immobilization support did not affect the volatile acidity and total acidity.

Among the higher alcohols, propanol and isobutanol were significantly decreased with decrease in temperature. 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. The higher alcohol formation varied during fermentations and was mainly dependent on yeast strain and fermentation conditions.

- In the present study, the glycerol concentrations in batches with immobilized cells ranged from 4.4 to 8.9 g/L, however, it was low in batches with free cells, ranged from 3.9 to 7.19 g/L. It was observed that the glycerol concentration in all the fermentation batches with immobilized cells on mango peel was decreased with decrease in temperature, showing that the fermentation temperature plays an important role in glycerol formation.

- Sensory evaluation indicated improvement in aroma and taste of the mango wines produced by immobilization particularly at low temperatures, when compared to mango wines produced from free cells.

- Yeast-mango-peel immobilized biocatalyst can be a good and effective system for mango wine fermentation at both low and room temperatures, as the mango wines produced by this had a potentially better aroma than that obtained from free-cell fermentation. The biocatalyst is economical, food grade and does not need special pretreatment before their use.

- The highest total carotenoids were present in the peel of Alphonso (8.62±0.52) followed by Sindhura (6.38±0.92 mg/100g) and the lowest was found in Raspuri (1.48±0.15 mg/100g). However, the highest anthocyanins were found in Sindhura (6.23±0.07) followed by Alphonso (3.65±0.05) and Rumani (2.13±0.13 mg/100g).

- Among the carotenoids analysed in puree, β-carotene was the major carotenoid ranging from 65.4 to 94.1%, followed by lutein (4.5-29.4%), violaxanthin (0.6-3.8%) and neoxanthin (0.7-3.1%). The relative highest total carotenoid levels (µg/100 g) in puree were found in Sindhura (5810), followed by Alphonso (5720), Rumani (3970), Banginapalli (3955), Totapuri (1920), Raspuri (1080) and Neelam (980) is the poor source of carotenoids with respect to the mango purees studied.
The total carotenoids in the mango wines were in the range of 578-4330 µg/100g and the highest amount of total carotenoids from mango wine was in Alphonso (4330), followed by Sindhura (4101), Banginapalli (2943), Rumani (2857), Totapuri (690), Raspuri (634), and Neelam (578 µg/100g).

Though all the wines are in yellow visually in terms of colour, there were little differences among the wines in b* parameter (yellowness). Positive values of a* and b*, as observed in this work, attributed to the carotenoids present in the wine. C* (Chroma) is a parameter that indicates the contribution of a* (redness) and b* (yellowness) and Chroma of the wines were ranging from 10.33 to 16.05 (Table 6.3.). Alphonso wine was with larger chroma (16.05) than Totapuri wine (10.33) and Hue angle (colour, h°) was ranging from 77.6 to 82.69°. Overall, wine from Alphonso, Sindhura, Banginapalli and Rumani (mean hue angle value = 81.35°) were slightly more orange than the wines from other cultivars.

Results indicate that the xanthophylls were degraded more than β-carotene (hydrocarbon carotenoid). Among xanthophylls, lutein was degraded more ranging from 78.7 to 93.9%, followed by neoxanthin (26.8-83.3%) and violaxanthin (50-74.3%), irrespective of the mango cultivar used. It is suggestive from the above that the degraded products of carotenoids might contribute to the wine aroma.

The free-radical scavenging potentials of the mango wine were analyzed by the in vitro DPPH method. The scavenging potentials were in the ranged from 81-91% at 100 µM total carotenoid concentration, where as the standard antioxidant showed 97%.

The mango wine exhibited 31.7, 48.3 and 66.4% protection against rat LDL oxidation in vitro at the end of 2, 4 and 6h incubation at100 µM concentration respectively.

The serum glucose levels and total proteins remained unaltered and there were no significant differences observed between groups.
The serum bilirubin, urea, uric acid and creatinine (mg/dl) concentrations were significantly increased in EtOH treated group; however there were no significant differences observed in other groups when compared to PFC.

There was a significant (P<0.05) induction in triglycerides concentrations in EtOH treated group when compared with PFC and other groups.

The serum HDL-C concentrations were increased in all the experimental groups when compared to PFC, however the highest concentration was found in DMW group. However, there was significant reduction in the total cholesterol and LDL-C in RW treated group, followed by MW group.

Significantly increased levels of serum marker enzymes viz., AST, ALT, ALP, GGT, LDH and AMY have been observed in EtOH treated group when compared to other groups.

There was a significant decrease in SOD, CAT, GPx, GR, and GST activities in both liver and kidney of EtOH treated group; similarly there was also a significant reduction in the non-enzymatic antioxidants viz., reduced glutathione and vitamin C.

There was a significant elevation in lipid peroxidation in the liver and kidney of EtOH treated group.

The histological sections revealed that significant degeneration and congestions were observed in liver and kidney slides of EtOH treated group.

MW and or DMW can be considered safe as it did not cause any lethality or adverse changes in the general behavior and also no detrimental effects caused by these beverages in biochemical and histopathological changes in rat model was observed. This shows the non toxic nature of the mango wine and or dealcoholised mango wine. Thus these may be safely administered for further in vivo studies to study its antioxidant activities in humans.

From the studies on mango wine production, it would be worthwhile to exploit mango pulp/juice to wine. No reports are available on the mango wine production by applying multistarter yeasts, applying malolactic bacteria (O. oeni) and also in preparation of yeast-mango peel biocatalyst and hence this would be the first report of
the kind. The volatile profile and composition of mango wine was comparable to that of grape wine can be recommended for the commercial production because of its economic feasibility and also because of its non-toxic nature. However, scale-up studies are warranted for better output and commercial production of mango wine before going to industrial scale.