



Full Length Research Paper

Pre-fermentation supplementation of mango must with ammonium and primary amino nitrogen influences mango wine chemistry

Helene Maisonnave^a, Aldegunda Matunda^b, E. Ken Hurley^c, Ann Sandbrook^c, Sean O'Keefe^c, Amanda C. Stewart^{c*}

^aESIROI, Reunion Island and Indian Ocean Engineering School, Food Engineering Department, Reunion Island, France

^bSokoine University of Agriculture, Morogoro, Tanzania

^cDepartment of Food Science and Technology, Virginia Polytechnic Institute and State University
Corresponding Author Email: amanda.stewart@vt.edu 540.231.0868, fax: 540.231.9293.

ABSTRACT

Mango wine is a shelf-stable value-added product that can be produced from fresh mango, mango juice or mango concentrate. Mango wine production techniques including cultivar selection, yeast strain selection and pressing conditions have been investigated, but no prior work has examined the effect of nitrogen supplementation on mango wine fermentation performance. After sugar, yeast assimilable nitrogen (YAN) is the most important nutrient required for yeast growth and metabolism during fermentation. In this study, the effect of supplementation of mango must with YAN on fermentation kinetics, residual sugar in mango wine, and residual yeast assimilable nitrogen in mango wine were determined. Nitrogen sources investigated included diammonium phosphate at two concentrations (100 mg/L and 200mg/L) and Feraid O at two concentrations (100mg/L and 200mg/L). Initial YAN in mango must was 70mg/L, and increasing YAN concentration with either exogenous nitrogen source led to increased fermentation rate. Supplementation to the same initial YAN concentration of approximately 100 mg/L YAN with Feraid O resulted in lower residual sugar in mango wine as compared to supplementation to the same initial YAN concentration with DAP. Conversely, residual YAN concentration in mango wine was higher when Feraid O was the source of exogenous nitrogen.

Keywords: Mango wine, Yeast assimilable nitrogen, Fermentation, Tropical fruit wine

INTRODUCTION

Mangoes are a climacteric fruit, with limited shelf life once fully ripe. Fermentation of fruit by *Saccharomyces* yeast to produce an alcoholic beverage (winemaking) is a long standing traditional means of fruit preservation. Mango wine is a value-added product with relatively long shelf-life that can be made from fresh mangoes, mango juice, or mango concentrate. Prior research has investigated mango cultivars suitable for wine production (Akubor 1996, Li, Chan et al. 2012, Garg, Kumar et al. 2015) and mango wine production

techniques such as yeast strain selection, malolactic fermentation, juice extraction procedures, maceration with enzyme preparations, and fermentation conditions including temperature and aeration (Reddy and Reddy 2005, Kumar, Prakasam et al. 2009, Reddy and Reddy 2009, Li, Yu et al. 2011, Reddy and Reddy 2011, Li, Chan et al. 2012, Li, Yu et al. 2012, Sadineni, Kondapalli et al. 2012, Li, Lim et al. 2013, Varakumar, Naresh et al. 2013, Li, Chan et al. 2014). However the effect of nitrogen supplementation in mango

wine production has not been previously investigated.

In addition to sugar, yeast assimilable nitrogen (YAN) is required for yeast growth and metabolism during fermentation (Bell and Henschke 2005). The main forms of YAN in fruit juice are primary amino nitrogen (PAN) and ammonium ions. Nitrogen present in protein or other plant cell structures cannot be assimilated by yeast and should not be included in measurements of YAN concentration (Bell and Henschke 2005). Lack of sufficient yeast assimilable nitrogen in fruit juice or must pre-fermentation can result in incomplete fermentation (Mendes-Ferreira, Mendes-Faia et al. 2004) and/or the production of undesirable aroma compounds during fermentation (Wang, Bohlscheid et al. 2003, Mendes-Ferreira, Barbosa et al. 2009, Ugliano, Fedrizzi et al. 2009, Mendes-Ferreira, Barbosa et al. 2010), translating to poor wine quality and incomplete valorization of the fruit. Due to the many factors involved in fermentation performance, defining a universal minimum concentration of yeast assimilable nitrogen required for wine fermentation has proven challenging, and remains an area of current research (Wang, Bohlscheid et al. 2003, Bohlscheid, Fellman et al. 2007, Bohlscheid, Osborne et al. 2011, Childs, Bohlscheid et al. 2012). Suggested minimum concentrations for grape wine fermentation range from 140 mg/L to over 400 mg/L YAN pre-fermentation (Butzke 1998, Bisson and Butzke 2000, Ugliano, Kolouchova et al. 2011). Observed YAN values in grape and apple range from 20 mg/L to over 700 mg/L (Butzke 1998), but very little information on YAN concentration in other fruits such as mango is currently available. Therefore measurement of YAN pre-fermentation is generally required to determine whether a deficiency exists. In wine production, yeast assimilable nitrogen deficiencies are often corrected through pre-fermentation addition of food grade diammonium phosphate (DAP), an inexpensive and widely available product used to increase ammonium ion concentration in juice or must pre-fermentation. Yeast nutrient supplements containing sources of PAN (generally made from yeast hydrolysate) are also commercially available and gaining broader application in the wine industry. The purpose of this study was to compare the effects of addition of DAP and a commercial PAN supplement, Fermaid O, on sugar depletion and yeast assimilable nitrogen concentration over the time course of mango must fermentation.

MATERIALS AND METHODS

Preparation of mango pulp and must

Mango processing, pressing, and amelioration

Mangoes, cv. Hayden grown in Mexico, were purchased

at a supermarket in Blacksburg, VA (Kroger, Cincinnati, OH). Mangoes were sorted, to remove under-ripe, over-ripe, and bruised fruits. Sorted fruits were washed with water, rinsed and peeled. The seed and core were removed and the flesh of the fruit was cut into pieces of approximately 2 cm³. Pectinase (Scottzyme Pec5L, Scott Laboratories, Petaluma, CA, USA) was added to fruit to improve juice yield upon pressing. The commercial pectinase preparation PEC5L was diluted to a 10% v/v solution in cool water and the diluted solution was then added to juice to a final concentration of 13 µL diluted solution in 1L juice. Potassium metabisulfite, an antioxidant and antimicrobial routinely used in wine production, was added to a final concentration of 1 g/3.8 L juice. This mixture was stored for 24 hrs at 4°C. The pulp was then pressed using a 10L wooden basket press with cheesecloth inserted inside the basket to improve pulp straining. The pulp was then diluted 1:5 pulp: water by volume, and the sugar concentration of the must was ameliorated with white cane sugar (sucrose) to reach 20°Brix. Addition of relatively large amounts of water and sugar, while not acceptable in most grape wine production regions, is common practice in the production of many industrial fruit wines.

Mango must nitrogen supplementation

Two nitrogen supplementation products were used: Diammonium phosphate (DAP) (Lallemand, Montreal, Canada) and Fermaid O (Lallemand, Montreal, Canada). The musts were supplemented at 100 mg/L and 200 mg/L added DAP and 100 mg/L and 200 mg/L added Fermaid O. Each treatment level was fermented in triplicate.

Inoculation and fermentation conditions

Active dry yeast strain EC 1118 (Lalvin, Lallemand, Montreal, Canada) was employed in this experiment. The ability of EC 1118 to ferment at low temperature, its strong competitive character, and excellent alcohol tolerance make this yeast an appropriate strain for a wide range of applications such as fruit wines, ciders or sparkling wines. Due to its strong fermentation characteristics and neutral contribution to wine aroma, this yeast strain is often used in wine research. The final concentration of the yeast was 0.3g/L juice. Active dry yeast was rehydrated in 10 times its mass in warm water (35-37°C) for 20 min before adding to juice. Airlocks were placed on fermentation vessels after inoculation to prevent oxygen ingress into juice while allowing CO₂ egress. Fermentation vessels were then placed in a 20°C temperature controlled chamber and allowed to ferment without agitation or aeration, to simulate commercial wine production conditions.

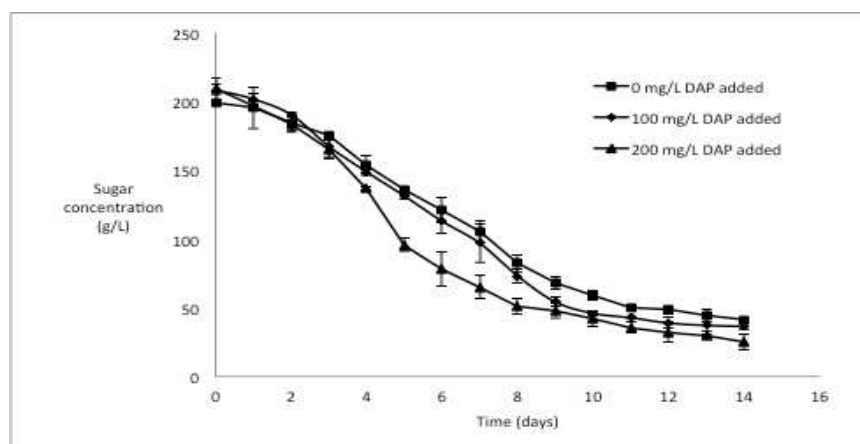


Figure 1. Sugar concentration vs. time as a function of the concentration of diammonium phosphate (DAP) added to mango must pre-fermentation. Error bars represent standard error of the mean over n=3 replicate fermentations.

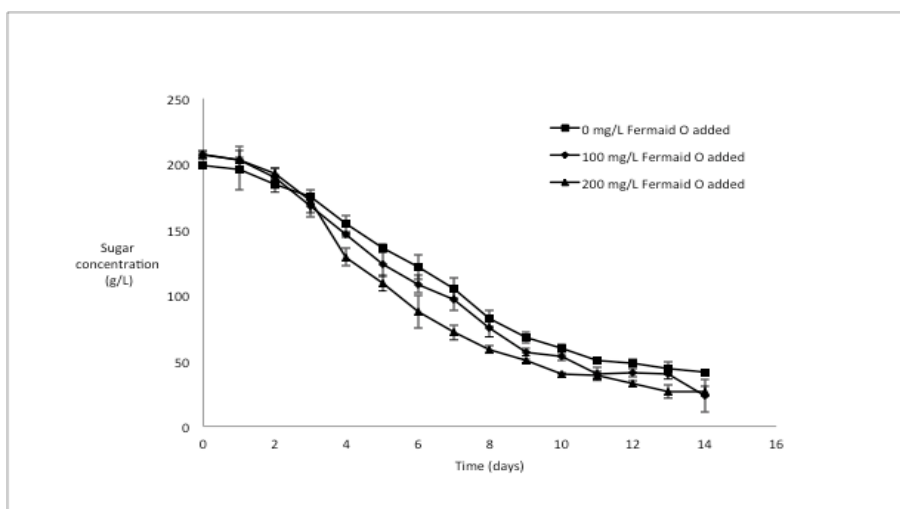


Figure 2. Sugar concentration vs. time as a function of the concentration of Fermaid O added to must pre-fermentation. Error bars represent standard error of the mean over n=3 replicate fermentations.

Determination of physical and chemical parameters

Yeast assimilable nitrogen (YAN) concentration was determined via measurement of primary amino nitrogen (PAN), and free ammonium ions using commercially available kits (K-PANOPA and K-AMIAR, respectively; Megazyme, Wicklow, Ireland). Sugar concentrations were determined via assays for sucrose, D-glucose, and D-fructose using commercially available kits (Sucrose, D-glucose and D,fructose; Boehringer Mannheim; R-BIOPHARM; Germany). The temperature of each flask was recorded daily.

Statistical analyses

Statistical analyses of data were performed using Prism

v6.0e (GraphPad Software, LaJolla, CA). Data for final residual sugar and residual YAN concentration were analyzed by one-way ANOVA followed by Fisher's Least Significant Difference Test. Differences in initial YAN concentration between the DAP and Fermaid O addition were assessed via t-test. Significance was defined as $p < 0.05$.

RESULTS AND DISCUSSION

The initial YAN concentration in the mango must used in this study was 70 ± 2 mg/L. As illustrated in Figure 1, addition of increasing concentrations of diammonium phosphate (DAP) from 0 to 200 mg/L DAP added to mango must pre-fermentation resulted in increasing fermentation rate, as monitored by sugar

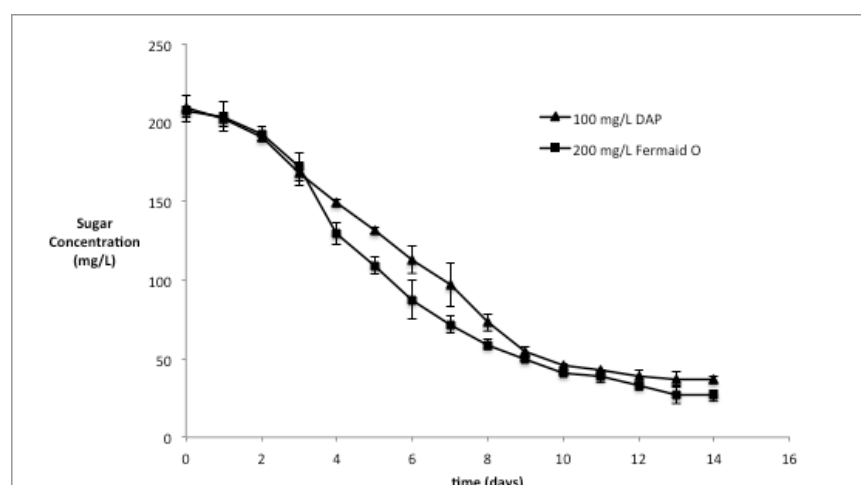


Figure 3. Sugar concentration vs. time as a function of nitrogen source (DAP vs. FO, initial YAN not significantly different, $p=0.3197$. Error bars represent standard error of the mean over $n=3$ replicate fermentations.)

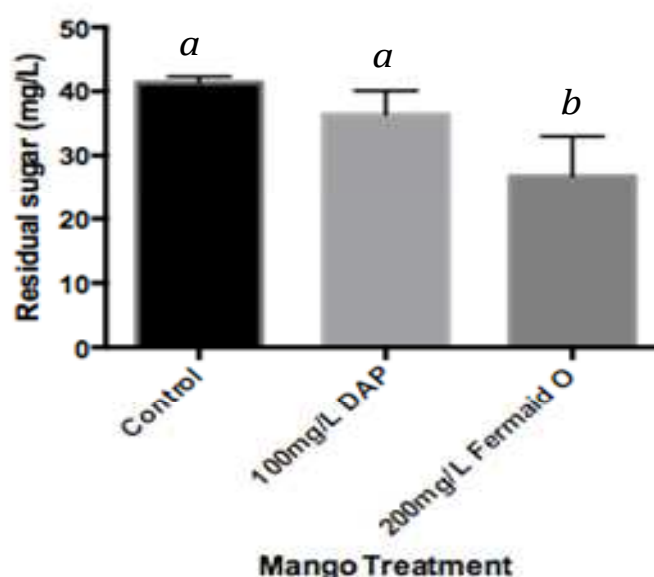


Figure 4. Residual sugar concentration as a function of nitrogen source (DAP vs. FO, where initial YAN not significantly different, $p=0.3197$)

consumption. These results are in accordance with prior research investigating the addition of DAP to grape must. (Mendes-Ferreira, Mendes-Faia et al. 2004)

Similar to the effect of DAP additions shown in Figure 1, addition of Fermaid O at 100mg/L and 200mg/L resulted in increasing fermentation rate, as shown in Figure 2.

In order to examine the effect of the exogenous nitrogen source (DAP vs. Fermaid O) on mango fermentation kinetics and final sugar concentration in

mango wine, sugar consumption during mango fermentation with 100mg/L DAP added (final average initial YAN of 96 ± 6.4 mg/L), was compared to mango fermentation with 200mg/L Fermaid O added (final average initial YAN of 99 ± 4.5 mg/L). This allowed for direct comparison of the effect of different nitrogen sources on sugar consumption during mango wine fermentation without differences in the initial total YAN concentration ($p=0.3197$). This comparison is illustrated in Figure 3.

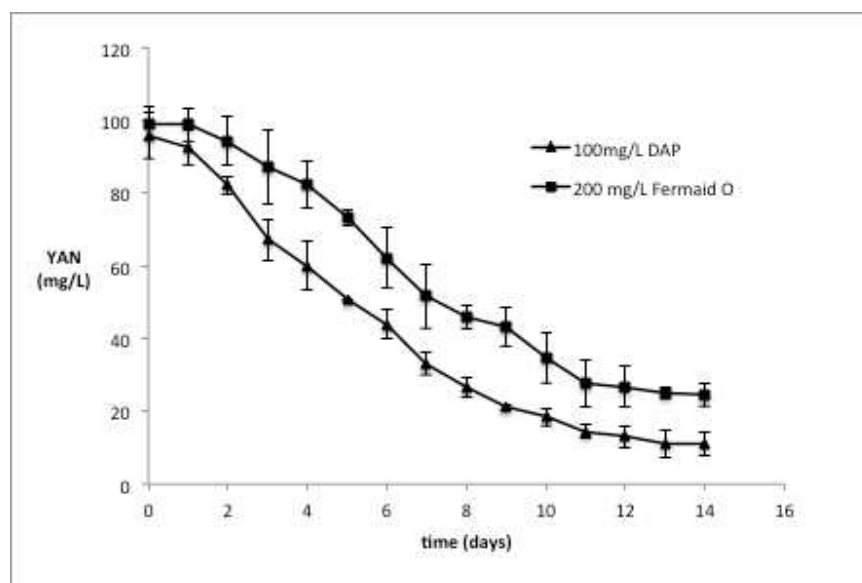


Figure 5. YAN concentration vs. time as a function of nitrogen source (DAP vs. FO, initial YAN not significantly different, $p=0.3197$. Error bars represent standard error of the mean over $n=3$ replicate fermentations.)

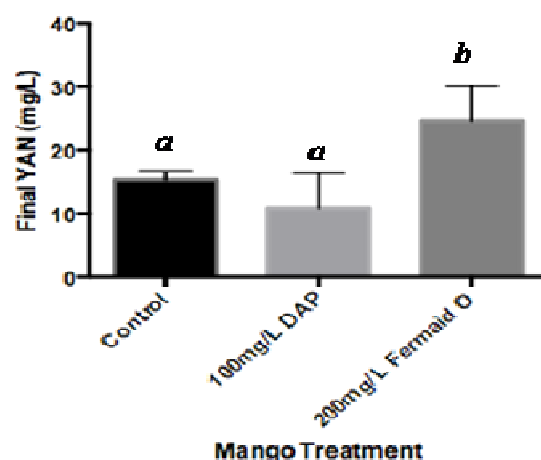


Figure 6. Residual YAN concentration as a function of nitrogen source (DAP vs. FO, initial YAN not significantly different, $p=0.3197$, no difference between treatments)

Residual sugar concentrations of the mango wines are reported in Figure 4. For wines which had the same initial total YAN concentration pre-fermentation, addition of Fermaid O resulted in lower residual sugar concentration in mango wine than addition of DAP. Addition of DAP did not result in different residual sugar than the control, with no added nitrogen source.

Figure 5 represents the YAN concentration over the time course of fermentation for the two sources of exogenous nitrogen (DAP and FO). Although the initial YAN concentration for the two conditions was not

significantly different, the DAP was consumed more quickly than the Fermaid O, particularly early on in the fermentation. Figure 6 illustrates that the final YAN concentration in the mango wine, after fermentation, was higher when Fermaid O was the source of exogenous nitrogen than when DAP was the source of exogenous nitrogen. Taken together, these results indicate that for more complete fermentation (lower residual sugar concentration in wine), Fermaid O is a better choice as a source of exogenous nitrogen when compared to DAP. However, when added to achieve the same initial YAN

concentration of approximately 100 mg/L, residual YAN in wine is higher when Fermaid O was the source of exogenous nitrogen. Wine makers should be aware that this excess residual nitrogen may serve as a source of nutrients for spoilage bacteria, and should take measures to discourage unwanted microbial growth, such as maintaining pH < 3.4, exclusion of oxygen from the headspace above the wine, and addition of appropriate concentrations of antimicrobial agents such as sulfur dioxide.

ACKNOWLEDGEMENTS

The authors would like to thank USAID for student support, and also the ESIROI internship program for providing funding for Ms. Maisonnavé's internship at Virginia Tech.

Abbreviations

DAP – diammonium phosphate
KMB – potassium metabisulfite
PAN – primary amino nitrogen
YAN – yeast assimilable nitrogen
FO – Fermaid O

REFERENCES

- Akubor PI (1996). "The suitability of African bush mango juice for wine production." *Plant Foods for Human Nutrition* 49(3): 213-219.
- Bell SJ, PA Henschke (2005). "Implications of nitrogen nutrition for grapes, fermentation and wine." *Australian Journal of Grape and Wine Research* 11(3): 242-295.
- Bisson LF, CE Butzke (2000). "Diagnosis and rectification of stuck and sluggish fermentations." *American Journal of Enology and Viticulture* 51(2): 168-177.
- Bohlscheid JC, JK Fellman, XD Wang, D Ansen, CG Edwards (2007). "The influence of nitrogen and biotin interactions on the performance of *Saccharomyces* in alcoholic fermentations." *J. Applied Microbiol.* 102(2): 390-400.
- Bohlscheid JC, JP Osborne, CF Ross, CG Edwards (2011). "Interactive Effects Of Selected Nutrients And Fermentation Temperature On H₂S Production By Wine Strains Of *Saccharomyces*." *J. Food Quality* 34(1): 51-55.
- Butzke CE (1998). "Survey of yeast assimilable nitrogen status in musts from California, Oregon, and Washington." *Ame. J. Enol. and Viticulture* 49(2): 220-224.
- Childs BC, JC Bohlscheid, CG Edwards (2012). "Sugar-Dependent Nitrogen Demands of *Saccharomyces* and Ramifications for Microbial Wine Spoilage." *Ame. J. Enol. and Viticulture* 63(3): 449A-449A.
- Garg N, S Kumar, P Yadav, A Dikshit (2015). Evaluation of Commercial Mango Cultivars of North India for Wine Preparation. Global Conference on Augmenting Production and Utilization of Mango: Biotic and Abiotic Stresses. H. Ravishankar, N. Garg and M. Mishra. 1066: 207-211.
- Kumar YS, RS Prakasam, OVS Reddy (2009). "Optimisation of fermentation conditions for mango (*Mangifera indica* L.) wine production by employing response surface methodology." *Intl. J. Food Sci. and Technol.* 44(11): 2320-2327.
- Li X, LJ Chan, B Yu, P Curran, SQ Liu (2012). "Fermentation of three varieties of mango juices with a mixture of *Saccharomyces cerevisiae* and *Williopsis saturnus* var. *mrakii*." *Intl. J. Food Microbiol.* 158(1): 28-35.
- Li X, LJ Chan, B Yu, P Curran, SQ Liu (2014). "Influence Of *Saccharomyces Cerevisiae* And *Williopsis Saturnus* Var. *Mrakii* On Mango Wine Characteristics." *Acta Alimentaria* 43(3): 473-481.
- Li X, S L Lim, B Yu, P Curran, SQ Liu (2013). "Impact of Pulp on the Chemical Profile of Mango Wine." *South African J. Enol. and Viticulture* 34(1): 119-128.
- Li X, B Yu, P Curran, SQ Liu (2011). "Chemical and Volatile Composition of Mango Wines Fermented with Different *Saccharomyces cerevisiae* Yeast Strains." *South African J. Enol. and Viticulture* 32(1): 117-128.
- Li X, B Yu, P Curran, SQ Liu (2012). "Impact of two *Williopsis* yeast strains on the volatile composition of mango wine." *International Journal of Food Science and Technology* 47(4): 808-815.
- Mendes-Ferreira A, C Barbosa, V Falco, C Leao, A Mendes-Faia (2009). "The production of hydrogen sulphide and other aroma compounds by wine strains of *Saccharomyces cerevisiae* in synthetic media with different nitrogen concentrations." *J. Industrial Microbiol. and Biotechnol.* 36(4): 571-583.
- Mendes-Ferreira A, C Barbosa, A Ines, A Mendes-Faia (2010). "The timing of diammonium phosphate supplementation of wine must affects subsequent H₂S release during fermentation." *J. Applied Microbiol.* 108(2): 540-549.
- Mendes-Ferreira AA, Mendes-Faia C, Leao (2004). "Growth and fermentation patterns of *Saccharomyces cerevisiae* under different ammonium concentrations and its implications in winemaking industry." *J. Applied Microbiol.* 97(3): 540-545.
- Reddy LVA, OVS Reddy (2005). "Production and characterization of wine from mango fruit (*Mangifera indica* L.)." *World J. Microbiol. and Biotechnol.* 21(8-9): 1345-1350.
- Reddy LVA, OVS Reddy (2009). "EFFECT OF ENZYMATIC MACERATION ON SYNTHESIS OF HIGHER ALCOHOLS DURING MANGO WINE FERMENTATION." *J. Food Quality* 32(1): 34-47.
- Reddy LVA, OVS Reddy (2011). "Effect of fermentation conditions on yeast growth and volatile composition of wine produced from mango (*Mangifera indica* L.) fruit juice." *Food and Bioprocess Processing* 89(C4): 487-491.
- Sadineni V, N Kondapalli, VSR Obulam (2012). "Effect of co-fermentation with *Saccharomyces cerevisiae* and *Torulaspora delbrueckii* or *Metschnikowia pulcherrima* on the aroma and sensory properties of mango wine." *Annals of Microbiol.* 62(4): 1353-1360.
- Ugliano M, B Fedrizzi, T Siebert, B Travis, F Magno, G Versini, PA Henschke (2009). "Effect of Nitrogen Supplementation and *Saccharomyces* Species on Hydrogen Sulfide and Other Volatile Sulfur Compounds in Shiraz Fermentation and Wine." *J. Agric. and Food Chem.* 57(11): 4948-4955.
- Ugliano M, R Kolouchova, PA Henschke (2011). "Occurrence of hydrogen sulfide in wine and in fermentation: influence of yeast strain and supplementation of yeast available nitrogen." *J. Industrial Microbiol. and Biotechnol.* 38(3): 423-429.
- Varakumar S, K Naresh, PS Variyar, A Sharma, OVS Reddy (2013). "Role of Malolactic Fermentation on the Quality of Mango (*Mangifera indica* L.) Wine." *Food Biotechnol.* 27 (2): 119-136.
- Wang XD, JC Bohlscheid, CG Edwards (2003). "Fermentative activity and production of volatile compounds by *Saccharomyces* grown in synthetic grape juice media deficient in assimilable nitrogen and/or pantothenic acid." *J. Applied Microbiol.* 94(3): 349-359.