International Food Research Journal 19(4): 1633-1639 (2012)
Journal homepage: *http://www.ifrj.upm.edu.my*

Optimizing process conditions for palm (*Borassus flabelliffer*) wine fermentation using Response Surface Methodology

Ghosh, S., Chakraborty, R. and *Raychaudhuri, U.
Centre for Medicinal Food & Applied Nutrition, Department of Food Technology & Biochemical Engineering, Jadavpur University, Kolkata 700032, India

Article history

Received: 19 October 2011
Received in revised form: 8 December 2011
Accepted:8 December 2011

Keywords

Central Composite Design (CCD)
ethanol
fermentation
palm wine
Response Surface Methodology (RSM)

Abstract

Palm juice from palm tree (*Borassus flabelliffer*) is a seasonal and low priced drinking juice in many of the countries like India. In our present study it has been used for palm wine production. Using a batch fermentation process, *Saccharomyces cerevisiae* (NCIM 3045) was cultivated in palm juice and different physical parameters such as temperature, pH and time have been varied to maximize the yield of wine. The fermentation process was standardized by statistical methods. Response surface methodology (RSM) based on the 23 factorial central composite design (CCD) was applied to determine the optimum conditions for the maximum yield of ethanol with the variation of temperature and pH. The highest yield of ethanol concentration of was obtained at 32^0C and pH 5.5 after 48 h of fermentation. The model showed that the value of R^2 (0.9973) was high and p- value of interaction of variance was < 0.005. Hence the model can be said to be of high significance. Highest concentration of ethanol obtained by fermentation was found to be 82.3 g/l.

Introduction

Wine is a fermented beverage made from various fresh fruits and vegetables. For the global fruit wine industry, Europe and China has taken the place for the largest fruit wine industry (Wang *et al*., 2004). In Asia India has been taking an important role for the production of the palm wine, produced by alcohol fermentation of palm juice which has been collected from the palmyra palm tree (Jirovetz *et al*., 2001). The palmyra palm (*Borassus flabelliffer*), a multipurpose tree of great utility, occurs extensively in states of Tamil Nadu, West Bengal and other places in India (Davis *et al*., 1987). This plant has a commercial and medicinal value. Various parts of the plant body like leaves, timber, fruits serve different purposes in our life. The palm sugar can be produced from the palm juice by boiled down into syrup or crystallized into a palm sugar called jaggery in Hindi (Rao *et al*., 2009). Toddy is also another fermented product produced from palm juice. The juice that has been collected without lime being added to the receptacle is called toddy. Toddy is considered as a non-beverage by modern medicine because of its yeast and other atmospheric contaminated organism content, but it is drunk by individuals occupying the lower socioeconomic classes in India. Toddy is used as a leavening agent in bread making and in "appam," a type of pancake made with rice flour (Davis *et al*., 1987). The plant possesses stimulant, anti-laprotic, diuretic, antiphlogistic properties. It has been claimed that the fruit contains some medicinal properties and acts as stomachic, sedative and laxative. The juice contain sugars, proteins, lipids, vitamin A, B-complex, vitamin C and others minerals (Barh *et al*., 2008). It has anti-inflammatory effects (Nadkarni 1954; Vaidyaratnam 1994; Kapoor 2000). It has also been reported to possess immunosuppressant properties (Revesz *et al*., 1999). The recent investigation has been carried out to find the effect of ethanolic extract of male flowers (inflorescences) of *Borassus flabellifer* for its anti-inflammatory activity in rodents (Paschapur *et al*., 2009).

The fermentation can be carried out with the help of wine yeast (*Saccharomyces cerevisiae*) to catalyze the rapid and efficient conversion of sugar to alcohol. The activity of the yeast is influenced by temperature and pH of the environment. Temperature controls yeast metabolism and growth rate (Torija *et al*., 2003). The carbon and nitrogen source utilization depend on the temperature which influences the production of ethanol. Effects of pH on yeast vary depending on strain specificity. Metal toxicity and inhibition of yeast activity depend on the pH of the medium (da-Silva *et al*., 2009).

The objective of this paper was to optimize the

*Corresponding author.
Email: *utpal31@hotmail.com*

fermentation conditions for production of palm wine from Indian palm juice (*Borassus flabellifer*). The statistical model i.e. Response Surface Method (RSM) was used in this study to see the aggregated effect of several variables such as temperature, pH and time and to seek optimum conditions for a multi-variable system. The Central Composite Design (CCD) (Murthy *et al*., 2000; Ambati *et al*., 2001) was used for predicting the optimization of the different factors that play an important role in ethanol production.

Materials and Methodology

Chemicals

Dextrose (GR), KH2PO4, K2HPO4, MgSO4. $7H_2O$, $FeSO_4$. $7H_2O$, and Urea, were all purchased from Merck, India. Glucose, Yeast extract and Peptone were purchased from (Himedia, India), DNS reagent was of Merck, India.

Microorganism and culture preparation

A stock culture of *Saccharomyces cerevisiae* (NCIM 3045) was procured from National Chemical Laboratory (NCL) Pune, India. The culture media consisted of 0.3 malt extract, 1.0 glucose, 0.3 yeast extract and 0.5 peptone all in g/100 mL. The organisms were grown at a temperature of 30^0C and pH 6.5. The incubation period was 48 h. After incubation the culture was stored at 4^0C in a refrigerator.

Preparation of fermentation media

The palm juice was collected from rural areas of West Bengal, India. It was preserved at -50^0C in an ultra low temperature freezer (Model C340, New Brunswick Scientific, England). For fermentation, carbon, nitrogen and other trace elements were added to palm juice in appropriate quantities. The composition of the fermentation media (g/100 ml) was- palm juice 100 ml, glucose 1g, Urea 0.3g, KH_2PO4 0.05g, K_2HPO_4 0.05g, $MgSO_4$. $7H_2O$ 0.05g, $FeSO_4$. $7H_2O$ 0.001g. Fermentation was carried out in a 250 ml flask. The pH of fermentation medium was adjusted by ammonium solution (1:1) and 2(N) HCl solution as per experiment before autoclaving. After autoclaving, 100 ml of fermentation media was inoculated with the yeast culture, the concentration of yeast cells in OD was 1.3. The temperature was also maintained at different range as per experiment. The incubation time was 3 days under semi-anaerobic conditions. The samples were withdrawn at appropriate time intervals for the analysis.

Estimation of ethanol, sugar and protein concentration

A 5 ml aliquot of fermented sample was centrifuged (Remi C-24, Mumbai, India) at 10000 rpm for 10 min. The supernatant solution was used to determine the ethanol concentration by Gas Chromatography (Perichrom SGE D11, column BP1-dimethyl polysiloxane). The absorbance of the sugar solution was determined using a spectrophotometer (Model 2800, Hitachi, Japan) at 540 nm by DNS method (Miller 1959). Protein was estimated by Lowry method at 650 nm (Wilson *et al*., 2000).

Estimation of biomass concentration

The biomass concentration was determined by the dry weight method. The cells were separated by centrifuging at 3500 rpm for 20 min consecutively twice with distilled water. The cells were dried at 65^0C for 2 days. Dilutions of the culture were also made and the absorbance was measured. The calibration curve correlating absorbance and dry weight gave a straight line (Raychaudhury *et al*., 2003).

Total soluble solid

To determine the TSS (% w/v) using a refractometers (Models 0–32 ^{0}Brix, Erma, Japan) having resolution 0.1 ^{0}Brix (% w/v) for each.

Estimation of metals

Raw juice sample was centrifuged at 10000 rpm for 10 min; the supernatant solution was used to determine the concentration of Zn and Cu by atomic absorption spectrophotometer (Chemito AA 203, Ireland).

Estimation of ascorbic acid

Ascorbic acid content was measured using the 2,6-dichlorophenol-indophenol spectrophotometric method (Rangana 1986).

Response surface methodology

Response surface methodology (RSM), an empirical modeling technique was used to estimate the relationship between a set of controllable experimental factors and observed results (Lee *et al*., 2003; Li *et al*., 2002). It is one of the most popular statistical optimization techniques in the field of food science and technology because of its comprehensive theory, responsibly high efficiency and simplicity (Arteaga *et al*., 1994; Gacula 1984). In the RSM, the Central Composite Design (CCD) is a most common experimental design which has equal predictability in all directions from the center (Liu *et al*., 1998; Reddy *et al*., 2000). The CCD are optimized designs

Table 1. Interactions of the independent variables as shown by the central composite design matrix

Run	X_1(^{0}C)	X_2	X_3(h)	Ethanol(g/l)	
				Observed	Predicted
1	32 (0)	5.50 (0)	48 (0)	79.02	79.83
2	34 (+1)	4.50 (-1)	36 (-1)	38.90	36.68
3	32 (0)	7.50 (+2)	48 (0)	07.63	06.31
4	32 (0)	5.50 (0)	48 (0)	82.30	79.83
5	30 (-1)	4.50 (-1)	60 (+1)	48.11	45.80
6	32 (0)	5.50 (0)	48 (0)	79.02	79.83
7	34 (+1)	6.50 (+1)	36 (-1)	38.20	40.26
8	32 (0)	5.50 (0)	72 (+2)	58.52	59.18
9	28 (-2)	5.50 (0)	48 (0)	07.23	06.92
10	32 (0)	5.50 (0)	48 (0)	79.02	79.83
11	32 (0)	5.50 (0)	24 (-2)	33.56	33.15
12	30 (-1)	6.50 (+1)	60 (+1)	35.11	37.08
13	36 (+2)	5.50 (0)	48 (0)	26.45	27.01
14	30 (-1)	6.50 (+1)	36 (-1)	23.00	22.66
15	34 (+1)	4.50 (-1)	60 (+1)	48.20	48.29
16	32 (0)	5.50 (0)	48 (0)	80.36	79.83
17	32 (0)	3.50 (-2)	48 (0)	09.89	11.45
18	30 (-1)	4.50 (-1)	36 (-1)	25.14	26.20
19	32 (0)	5.50 (0)	48 (0)	79.02	79.83
20	34 (+1)	6.50 (+1)	60 (+1)	48.00	46.69

*Experimental results were average of three replications

for fitting quadratic models and the number of experimental points in the CCD is sufficient to test statistical validity of the fitted model and lack of fit of the model (Arteaga *et al*., 1994).

Central Composite Design was used in the fermentation optimization of the palm wine production from the palm juice. The three different parameters, temperature, pH and time were chosen as main independent variables and designated as X_1, X_2, and X_3 respectively. Each parameter was studied at three different levels (−1, 0, +1). All parameters were taken at a central coded value considered as zero. The ranges of alpha values of these variables used in the experimental design are given in Table 1. The ethanol concentration (Y, g/l) was used as dependent output variable. With the three independent variables 20 experimental sets were chosen to optimize the parameters. The minimum and maximum ranges of parameters were investigated and the full experimental plans with respect to their values in actual and coded form are listed in Table 1. This resulted in an empirical model that related the response measured in the independent parameters. All tests were performed in triplicates and the data represented is a mean of the three. The coefficients of the polynomial model were calculated using the following equation-

$$Y = \beta 0 + \sum_{i=1}^{n} \beta i\, Xi + \sum_{i<j}^{n} \beta ij\, Xi\, Xj + \sum_{j=1}^{n} \beta jj\, Xj2$$

where Y represent the predicted response, i and j are linear and quadratic coefficients respectively, β is the regression coefficient, n is the number of variables studied in the experiments.

In our present study, the statistical software Design expert, (Version 7.1.6, Sat-Ease, Inc, USA) was used for regression analysis of the data obtained and to estimate the significance of each coefficients of the regression equation. The fit of the regression model attained was determined by adjusted coefficient (Radj). Appropriate model significance was determined by Fischer's F-test. The three dimensional graphical representation and their respective contour plots were determined by the interaction of dependent variable and the independent variables.

Results and Discussion

The palm wine fermentation was carried out by controlling various physical factors which were important for production of ethanol. The composition of the palm juice was analyzed and found as 16.80 Brix, reducing sugar 8.88 g/L, protein 0.66 g/L, ascorbic acid 20.11 mg/L and metals Cu and Zn 0.0013 and 0.0001 g/L respectively, these results more or less similar with reported results by Rao *et al*, (2009) and Bhar *et al*. (2008). In this study, first the temperature was optimized while when pH was kept constant (the unadjusted pH of raw juice was 6.1-6.5) and on the other hand, pH was optimized under optimized temperature conditions. The palm juice contain total soluble solid was 16.8 Brix, it was considered as total sugar it means that total sugar concentration of the palm juice was 168 g/L (Naknean *et al*., 2010). During the fermentation initial sugar concentration was 168 g/L and again 10 g/L dextrose was added for enhance the initial yeast growth. After optimizing temperature and pH, these two factors was interacted to each other with time were studied by the RSM method at the same composition fermentation medium.

Temperature optimization in palm wine fermentation

In batch fermentations, the fermentation process is influenced by the temperature. But temperature tolerance for growth of yeast and fermentation is strongly strain dependent (Rousseau *et al*., 1992). In the palm wine fermentation, initially, ethanol production was high at 34^{0}C upto 24 h but after 48 h, highest ethanol was obtained at 32^{0}C was 76.7 g/L. The reason was that after a period of time this high temperature (34^{0}C) inactivated the yeast cell. Therefore, initially the rate of ethanol production at 34^{0}C was higher than 32^{0}C, but gradually the yeast activity increased at 32^{0}C with time. It was reported that ethanol producing yeast could grow rapidly at temperature 25-33^{0}C and again ethanol production was high at 30-37^{0}C (Ozcelik *et al*., 1996). At 36^{0}C

and 28^{0}C, it was not favorable temperature for our yeast strain therefore at these stress conditions ethanol productions was lowest. At 30^{0}C, the yeast cell was moderately activated and ethanol concentration was gradually increased with time upto 48 h. Therefore the yeast cells were very much affected by temperature. Temperature controls the cell viability, growth rate, exponential phase, enzyme activity and membrane function (Torija *et al*., 2003).

pH optimization in palm wine fermentation

To study the influence of pH on palm wine fermentation, experiments were carried out at different pH values to optimize the conditions for maximum enzyme activity of *Saccharomyces cerevisiae* for palm wine production. The growth of biomass and ethanol production was highly affected by variations in pH. The highest ethanol was obtained at pH 5.5 was 80.5 g/L, at temperature 32^{0}C and incubation time was 48 h, this result also supported by Bennett *et al*., 1998 (Law *et al*., 2011). The ethanol production at pH 3.5 was lowest. At pH 6.5, ethanol concentration was higher than pH 4.5. On the third day, the ethanol production rate slowed down with respect to the second day for all the pH values studied. Prior research work (Narendranath *et al*., 2001) states that the yeast growth was affected by environmental pH which was controlled by acetic acid or lactic acid produced by yeast. In such cases, consumption of sugar and production of ethanol were reduced along with decrease in the growth of yeast. The increase in biomass production is dependent on both pH and temperature. The maximum biomass (6.7 g/L) was obtained at pH 5.5 and temperature 32^{0}C for 48 h. In both cases of pH 3.5 and pH 4.5, biomass increase was comparatively lower but steady throughout. It may be due to the presence of sufficient nutrients in the fermentation media till the third day as the growth rate was low. On the other hand for pH 5.5 and pH 6.5, the rate of biomass production significantly decreased after the second day due to high initial growth and later of nutrient deficiency in the medium.

RSM analysis for the palm wine fermentation

Statistical significance of palm wine fermentation model is explained by analysis of variance (ANOVA). Nature of fit of the regression model is determined by the adjusted co-efficient of determination (R^2_{adj}). The high value of R^2_{adj} 0.9948 indicates the goodness of fit of the regression equation. The predicted co-efficient of determination (R^2_{pred}) value was 0.9838. The R^2_{pred} will decrease when there are too many insignificant values in the model. As per thumb rule, these values should be within 0.2 of each other. Here the difference was less than 0.2 and so the model was significant. The probability of p-value for models of less than 0.05 indicate that models were significant, p-value less than 0.0001 indicate the models were highly significant and p-value greater than 0.1000 indicate the models were not significant. So our model p value was <0.001 it was significant. The words lack of fit refers to the fact that the simple linear regression model may not adequately fit the data. There is evidence that the simple linear regression model is not appropriate because the treatment means do not appear to have a straight-line relationship with the amount of the treatment factor. If the p-value for lack of fit of model is significant ($p<0.05$), then a more complex model would be required (Banik *et al*., 2007). Our p value for lack of fit of model was insignificant it indicted that our experimental model system was statistically significant.

Table 2. Statistical significance of regression coefficient of palm wine production

Factor	Coefficient	Standard Error	F-value	p-value
Intercept	79.83	0.75		
Temperature	5.02	0.47	113.61	<0.0001
pH	-1.29	0.47	7.43	0.0213
Time	6.51	0.47	190.56	<0.0001
Temperature x pH	1.78	0.67	7.13	0.0235
Temperature x Time	-2.00	0.67	8.93	0.0134
pH x Time	-1.29	0.67	3.77	0.0807
Temperature x Temperature	-15.72	0.38	1747.44	<0.0001
pH x pH	-17.74	0.38	2225.48	<0.0001
Time x Time	-8.42	0.38	501.16	<0.0001

Applying the multiple regression analysis on the experiment, the response variables and the test variables are related by following second order polynomial equation:

$$Y = +79.93 + 5.02\,X_1 - 1.29\,X_2 + 6.51\,X_3 + 1.78\,X_1X_2 - 2.00\,X_1X_3 - 1.29\,X_2X_3 - 15.72\,X_1^2 - 17.74\,X_2^2 - 8.42\,X_3^2$$

Table 2 shows the response of the variables temperature, pH, time, temperature^2, pH^2, time^2 and temperature x pH, temperature x time were significant with p-value of less than 0.05. For pH x time, p-value was > 0.05 and therefore this value was insignificant. But most of the values were significant, and thus the whole model was significant. Actually temperature, pH and time were linear term of the model and had high influence on palm wine fermentation therefore their p value were significant (Table 2). But effect of these three variables in interactive term of the model i.e. (temperature x pH, temperature x time) here significant, but p value for (pH x time) was 0.0807 hence insignificant. It means that 91.93 %, of the model was affected by this interactive relationship.

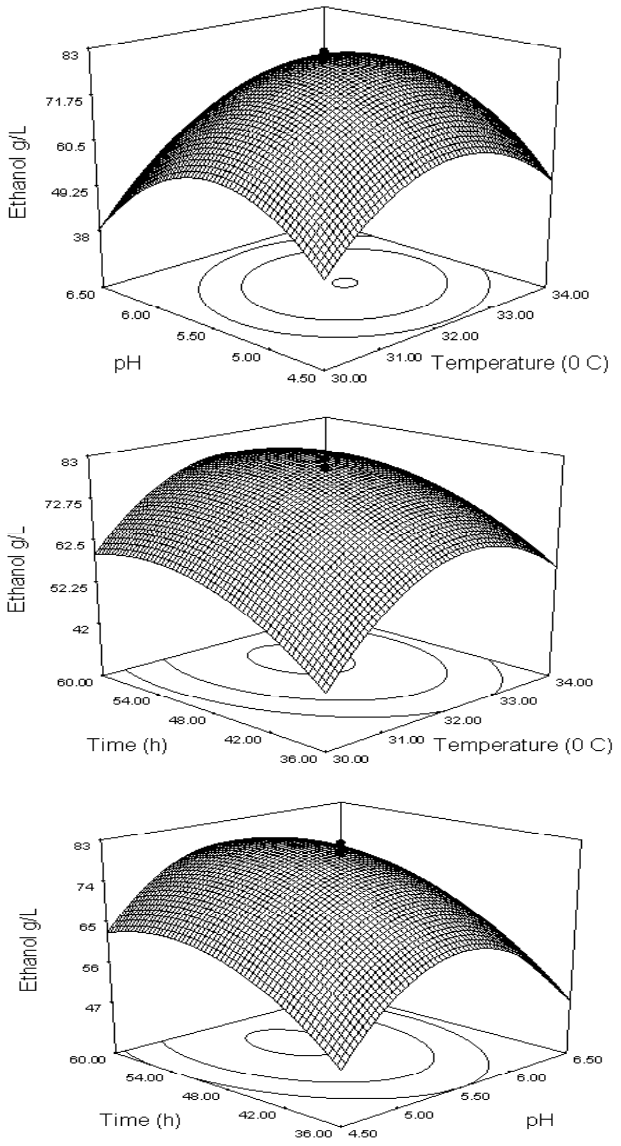

Figure 1(a-c). 3D Response surface and contour plot showing the effect of pH, temperature and time on the production of ethanol by *Saccharomyces cerevisiae*

It can be said that pH and time have effect on model but have been statistically low than the other relationships. And the quadratic term of the model, the temperature^2, pH^2 and time^2 are shown in Table 2, their p value were highly significant i.e. 0.0001. It means that the temperature, pH and time play a significant role in ethanol production in terms of quadratic relationship.

Interaction of factors and optimization of palm wine fermentation

The response surface and the contour plot show the conditions for ethanol production. The interaction between the two variables and their optimum level can be determined from Figure 1a which shows the interaction between the two variables i.e. temperature and pH. It was found that with increase of pH and temperature, ethanol production is increased. But beyond a certain limit, upto temperature 32^{0}C and pH 5.5 with increase in temperature and pH, ethanol concentration is decreased. Actually wine yeast is very particular for their optimum condition (Torija *et al*., 2006). This optimum condition is also varying with strains. The midpoint of the curve showed that maximum production of ethanol was obtained at pH 5.5 and temperature 32^{0}C.

Figure 1b shows the interaction between temperature, time and their effect on ethanol production. The temperature 32^{0}C and 48 h are the optimum conditions for highest ethanol yield. Longer incubation time with high temperature also decreases the production of ethanol in the fermentation. Again at optimum temperature for long time incubation the ethanol concentration was also decreased because ethanol was utilized by the yeast. It was indicated that at 32^{0}C optimum temperature after 48 h incubation maximum ethanol production could be attained.

Figure 1c shows the interaction between pH and time and the optimum pH for the highest ethanol production was 5.5. Low pH and long time incubation decreased the production level. The enzyme activity was influenced by surrounding pH. The hexokinase and alcoholdehydrogenase these two enzymes present in yeast which are most important for glycolysis and ethanol production. Both the enzyme activity was influenced by pH (Kalinman 1975; Kumar *et al*., 2004). But *in vivo*, these enzyme activities vary depend upon mutant strain also. According to the response surface graph, at pH 5.5 and 48 h incubation time ethanol produced was the highest. From the experiments, the highest yield of ethanol production was obtained as 82.3 g/l with pH-5.5, temperature 32 ^{0}C and time 48 h.

Conclusions

A cheap source of raw material, palm juice was used for ethanol production. The effect of temperature and pH with time on the palm wine fermentation was studied. Optimum pH and optimum temperature enhance fermentation with Saccharomyces cerevisiae and as a result increase ethanol production. The statistical optimization of palm wine production was carried out using RSM based on the 23 factorial CCD. The experimental design and regression analysis was done to study the effect of temperature, pH and time on palm wine fermentation. It was found that temperature, pH and time in terms of linear and quadratic relationship significantly influenced the ethanol production from

palm juice. The experimental values and predicted values (Table 1) were close to each other; hence optimum conditions for this particular fermentation with this strain of *Saccharomyces cerevisiae* (NCIM 3045) were temperature 32^{0}C, pH 5.5 and time 48 h.

Acknowledgements

The research work is financially supported by the Centre for Advanced studies (CAS I) programme under University Grants Commission (UGC), Govt. of India and Dept. of Food Processing Industries & Horticulture, Govt. of West Bengal, India, Some facilities have been provided by the centre for Medicinal Food & Applied Nutrition of Jadavpur University, India.

References

Ambati, P. and Ayyanna, C. 2001. Optimizing medium constituents and fermentation conditions for citric acid production from palmyra jaggery using response surface method. World Journal Microbiology Biotechnology 17(4): 331–335.

Arteaga, G. E., Li-Chan, E., Vasquez-Arteaga, M. C. and Nakai, S. 1994. Systematic experimental designs for product formula optimization. Trend Food Science Technology 5: 243–254.

Banik, R. M., Santhiagu, A. and Upadhyay, S. N. 2007. Optimization of nutrients for gellan gum production by *Sphingomonas paucimobilis* ATCC-31461 in molasses based medium using response surface methodology. Bioresour Technology 98: 792–79.

Barh, D. and Mazumdar, B. C. 2008. Comparative Nutritive Values of Palm Saps Before and after Their Partial Fermentation and Effective Use of Wild Date (*Phoenix sylvestris* Roxb.) Sap in Treatment of Anemia. Research Journal of Medicine and Science 3(2): 173-176.

Brand-Williams, W., Cuvelier, M. E. and Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. Lebensmittel Wissenschaft und Technologie 28: 25-30.

Davis, T. A. and Johnson, D. V. 1987. Current utilization and further development of the Palmyra Palm (*Borassus flabellifer* L., Areeaceae) in Tamil Nadu State, India, Economic Botany 41(2): 247-266.

da-Silva, S. M., de Oliveira, S. L., Leite, C. A. O., do Prado, R. S., de Faria, F. P., Oliveira, R. C. N. and da-Silva, F. M. S. M. 2009. Effect of pH, dextrose and yeast extract on cadmium toxicity on Saccharomyces cerevisiae PE-2. Ciência e Tecnologia Alimentos 29(2): 295-299.

Gacula, M. C. and Sing, J. 1984. Statistical Methods in Food and Consumer Research. Academic Press inc, London

Jirovetz, L., Buchbauer, G., Fleischhacker, W. and Ngassoum, M. B. 2001. Analysis of the aroma compounds of two different palm wine species ('Matango and Raffia') from Cameroon using SPMEGC- FID, SPME-GC-MS & olfactometry. Ernahrung/Nutrition67-71.

Kalinman, P. J. 1975. Acid-Base catalysis in the yeast alcoholdehydrogenase reaction, The Journal of Biological Chemistry 250: 2573-1975.

Kapoor, L. D. 2000. Handbook of Ayurvedic medicinal plants: Herbal reference library. CRC Press, p 82, USA, Florida.

Kumar, D. P., Tiwari, A. and Bhat, R. 2004. Effect of pH on the Stability and Structure of Yeast Hexokinase A Acedic amino acid residues in the cleft region are critical for the opening and the closing of the structure. The Journal Biological Chemistry 279: 32093–32099.

Law, S. V., Abu Bakar, F., Mat Hashim, D. and Abdul Hamid, A. 2011. Popular fermented foods and beverages in Southeast Asia. International Food Research Journal 18: 475-484.

Lee, H., Song, M. and Hwang, S. 2003. Optimizing bioconversion of deproteinated cheese whey to mycelia of *Ganoderma lucidum*. Process Biochemistry 38: 1685–1693

Li, C., Bai, J., Cai, Z. and Ouwang, F. 2002. Optimization of a cultural medium for bacteriocin production by *Lactococcus lactis* using response surface methodology. Journal of Biotechnology 93: 27–34

Liu, B. L. and Tzeng, Y. M. 1998. Optimization of growth medium for the production of spores from *Bacillus thuringiensis* using response surface methodology. Bioprocess Engineering 18: 413–418.

Miller, G. L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry 31: 426-428.

Murthy, M. S. R. C., Rakshit, T. S. S. K. and Kosugi, Y. 2000. Statistical optimization of lipase catalyzed hydrolysis of methyloleate by response surface methodology. Bioprocess Engineering 22: 35–39.

Narendranath, N. V., Thomas, K. C. and Ingledew, W. M. 2001. Effects of acetic acid and lactic acid on the growth of *Saccharomyces cerevisiae* in a minimal medium. Journal Industrial Microbiology Biotechnology 26: 171–177.

Nadkarni, K. M. 1954. Indian Materia Medica, Ed 3rd, Vol. 4, p 2571-2575, Popular Book Dept, Bombay, India.

Naknean, P., Meenune, M. and Roudaut, G. 2010. Characterization of palm sap harvested in Songkhla province, Southern Thailand. International Food Research Journal 17: 977-986.

Ozcelik, F and Denli, Y. 1996. Sarap mayalarinin teknolojik ozellikleri, Gida 24(6): 385-389

Paschapur, M. S., Patil, M. B., Kumar, R. and Patil, S. R. 2009. Evaluation of anti-inflammatory activity of ethanolic extract of *Borassus flabellifer* L. male flowers (inflorescences) in experimental animals. Journal of Medicinal Plants Research 3(2): 49-54.

Rangana, S. 1986. Hand Book of Food Analysis and Quality control of fruit and vegetables product. Ed 2nd, Tata McGraw-Hill: city.

Rao, P. V. K. J., Das, M. and Das, S. K. 2009. Changes in

physical and thermo-physical properties of sugarcane, palmyra-palm and date-palm juices at different concentration of sugar. Journal of Food Engineering 90: 559–566.

Raychaudhury, B., Chakraborty, R. and Raychaudhuri, U. 2003. Modeling and simulation of diffusional mass transfer of glucose during fermentative production of pediocin AcH from *Pediococcus acidilactici* H. Biochemical Engineering Journal 16: 237-243.

Reddy, P. R. M., Mrudula, S., Ramesh, B., Reddy, G. and Seenayya, G. 2000. Production of thermostable pullulanase by *Clostridium thermosulfurogenes* SV2 in solid-state fermentation: optimization of enzyme leaching conditions using response surface methodology. Bioprocess Engineering 23: 107–112

Revesz, L., Hiestand, P., La Vecchia, L., Naef, R., Naegeli, H.U. and Oberer, L. 1999. Isolation and synthesis of a novel immunosuppressive 17 alpha-substituted dammarane from the flour of the Palmyrah palm (*Borassus flabellifer*). Bioorganic and Medicinal Chemistry Letters 9: 1521-1526.

Rousseau, S., Rouleau, D., Yerushalmi, L. and Mayer, R. C. 1992. Effect of temperature on fermentation kinetics of waste sulfite liquor by *Saccharomyces cerevisiae*. Journal of Chemical Technology and Biotechnology 53: 285-291.

Torija, M. J., Beltran, G., Novo, M., Poblet, M., Manuel, J, Mas, G. A. and Roze, N, 2003. Effects of fermentation temperature and *Saccharomyces* species on the cell fatty acid composition and presence of volatile compounds in wine. International Journal of Food Microbiology 85: 127– 136.

Vaidyaratnam, P. S. 1994, Reprint 2002. Variers Arya Vaidya Sala - Indian Medicinal Plants A Compendium of 500 species, Vol. 4, p. 293-296, Orient Longman: Chennai, India.

Wang, D., Xu, Y., Hu, J. and Zhao, G. 2004. Fermentation kinetics of Different Sugar by Apple Wine. The Journal of the Institute of Brewing 110: 340-346.

Wilson, K. and Walker, L. 2000. Practical Biochemistry : Principles and Technique. Cambrige University Press. Cambrige