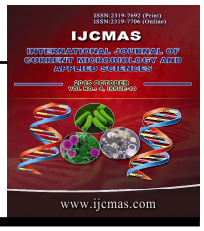
Int.J.Curr.Microbiol.App.Sci (2015) 4(10): 487-496

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 10 (2015) pp. 487-496**
http://www.ijcmas.com



Original Research Article

Determination of Efficient Fermentation Inhibitor of the tapped Inflorescence Sap of *Caryota urens* in Sri Lanka

Ranganathan Kapilan*

Department of Botany, University of Jaffna, Jaffna, Sri Lanka
**Corresponding author*

Keywords

Caryota urens, Commercial lime, Fermentation inhibitor, Hal bark, Sweet sap

A B S T R A C T

The study was aimed to determine the effect of various substances on inhibition of fermentation of the sweet sap of *Caryota urens* (Kithul) and to recommend the best fermentation inhibitor. Pieces of fresh bark of Hal (*Vateria copallifera*), Kakata (*Careya arborea*), commercial lime and leaves of Kohomba (Neem - *Azadirachta indica*) at the same rate (weight - 15g / six litre pots) were added into each collecting pot before they were hung on the tree around the tapped inflorescence. The samples of kithul sap were collected from the pots at equal time intervals and analyzed for reducing sugars, total sugars, pH, alcohol content, number of yeast and bacterial cells and protein content. The mean alcohol contents of the kithul sap observed with kakata, kohomba and control were significantly higher than that of Lime and hal bark after 45 hours. The pH values of kithul sap treated with lime were significantly higher than that of other treatments. The mean number of the bacterial and yeast cells in the sap were significantly lower in all the treatments compared to the control. Amount of reducing sugar remained unchanged in all the test saps, except kakata. Commercial lime and hal bark both could be recommended as efficient fermentation inhibiters of sweet kithul sap. Protein content of the limed and hal treated saps did not change significantly from that of control, after 60 hours. Kahata bark and leaves of kohomba are not recommended because of their inability to inhibit the accumulation of alcohol in the sap.

Introduction

The main source of crude sugar (jaggery) in Sri Lanka is the sweet sugary sap obtained from the inflorescence of the Coconut (*Cocos nucifera*), Palmyrah (*Borassus flabellifer*) and Kithul (*Caryota urens*) palms. The young inflorescences of these palms are tapped to obtain the sweet sugary phloem sap that is called as sweet toddy. This non-fermented sap is a famous summer drink among the local public. This sap can be processed to produce sweeteners such as treacle, sause, syrup and jaggery. If fermentation by the airborne bacteria and fungi is allowed, the collected phloem sap will be converted into intoxicating fermented beverages such as toddy and alcohol (Ranasinghe *et al.*, 2012; Somasiri *et al.*, 2011).

Caryota urens is one of the underutilized

palm categorized under family Arecaceae. *Caryota* species are widely distributed in the rain forests of the tropical Australasian countries such as Indonesia, India, Sri Lanka, Malaysia, Philippines, Australia and New Guinea (Dalibard, 1999). This palm has a special popular synonym as Kithul in Sri Lanka. Generally, kithul palm reaches a height of 40 to 60 feet (15 to 20 meter) under favorable growing conditions. Bipinnate leaves of kithul can grow as much as 7-8 meters in length (Trimen, 1998). The leaflets of this palm are very special, looks like the fancy tail of a fish, thereby getting the popular English name, Fish-Tail palm (Molagoda, 1945; Trimen, 1998). Non-fermented *Caryota urens* sap and sap based products such as sugar, jiggery and treacle are famous for health promoting properties. All the sap products have a very high demand because of their natural origin and the organic food production techniques. However, these products are very expensive due to the scarcity in production and the higher demand due to the specific taste and aroma and due to the potential medicinal value (Ranasinghe *et al.*, 2012).

Generally the products of *C. urens* have anti-diabetic, anti-ageing and anti-rheumatic properties. The sap and treacle of *C. urens* have α-glucosidase inhibition activity and thus proves the existence of anti-diabetic properties (Ranasinghe *et al.*, 2012). However, the significant importance of the medical properties of *C. urens* sap have not been determined scientifically yet.

Sap of *Caryota urens* is nutritionally rich and contains diverse simple sugars such as sucrose, glucose and fructose (Somasiri *et al.*, 2011). When a comparative testing analysis of raw sugars was done by the Fairchild Botanical Gardens in Florida, kithul jaggery topped the list, among those of highest quality and taste (Somasiri *et al.*, 2013). Kithul sap based products provide a significant source of income to the large number of people living along forest fringes and isolated rural areas. Export potential of the kithul sap has been tested with success in the world consumer market. Therefore, scientific information of functional properties of fresh *C. urens* sap would help to improve the value, quality and most preferable consumer taste of these products.

Though the fresh saps of the palms are free of fermentation and highly charged with sugar, they become rich in alcohol and acids by the natural fermentation process. Several methods are practiced in Sri Lanka to prevent such fermentation taking place in the kithul sap in order to get the sweet sap. Lining the inside of the pot with fresh lime, placing hal bark (*Vateria copallifera*), placing kakata bark (*Careya arborea*) and placing the leaves of kohomba (*Azadirachta indica*) in a clean pot before it is used for collecting sap, are the most common methods used to reduce fermentation, in Sri Lanka (Chandrasekhar *et al.*, 2012; Dalibard 1999; Kalaiyarasi *et al.*, 2012; Thevendirarajah *et al.*, 1997). Though the usage of such substances reduces considerable amount of fermentation, some of them might change the original taste of the sweet toddy and the jaggery to a great extent.

This results in poor consumer affinity towards sweet toddy and its products. Thus the substance that can inhibit fermentation of the sweet sugary sap of *C. urens* effectively without changing the quality and the original taste needs to be determined. The objective of the study was to determine the effect of various substances used in Sri Lanka to inhibit fermentation of the sweet sugary sap of *Caryota urens* (Kithul) and to recommend the best fermentation inhibitor.

Materials and Methods

Plant material

Caryota urens (Kithul) palm growing widely in the diverse regions of Sri Lanka was selected. Twenty to twenty five years old, matured male and female *Caryota* trees were chosen randomly in three different locations of the north, north central and western regions of Sri Lanka.

Methodology

To study the effect of lime, the inside of the sterile collecting pot was lined with a thin coating of commercial lime (15 grams/ 6 Litre pot) before it was hung on the kithul tree. Pieces of fresh bark of hal and kahata and leaves of kohomba at the rate of 15 grams/ 6 litre pot were put in each case into the sterile collecting pot before use. Control pot without the addition of any substances was also maintained. In each treatment there were three replicates used.

Measurements

The samples were collected after from the palm trees 15 hours, 30 hours, 45 hours and 60 hours and analyzed in the laboratory for reducing sugars, total sugars, pH, alcohol, number of yeast cell and bacterial cells and protein content. Assay of sugars was done using reducing test with copper reagent (Theivendirarajah *et al.,* 1977). Alcohol present in the sample was bubbled into a mixture of K dichromate and H_2SO_4. The colour change in the dichromate solution was read colorimetrically (Atputharajah, 1986; Shamala and Sreekantiah, 1988; Theivendirarajah *et al.,* 1977). The percentage of alcohol was then determined using standard calibration curve. Yeast cell counts were made by viable plate count on glucose peptone yeast extract agar medium (Kalaiyarasi *et al.,* 2013; Kumuthini Chrystopher and Theivendirarajah 1988a; Nguyen *et al.,* 2012), while bacterial cells were counted by the same method but on nutrient agar medium (Kapilan and Arasaratnam, 2010; Kapilan and Vasanthy Arasaratnam, 2011; Kumuthini Chrystopher and Theivendirarajah, 1988b). After the selection of the fermentation inhibition substance (lime), protein content of the non-fermented and fully fermented were used to measure the protein content (Kalaiyarasi *et al.,* 2013). Into ten labeled tubes 0.2 to 2.0 mL of standard bovine serum albumin solution was taken and the total volume was made up to 2.0 mL with distilled water. Then each protein standard solution and test solution was taken and mixed with 1mL working bicinchoninic acid (BCA) / $CuSO_4$ solution. The mixtures were incubated at 37^{o}C for 40 min for the colour to develop (Borse *et al.,* 2006, Ghosh *et al.,* 2012). The colour developed was measured in a spectrophotometer (Spectronic 21D) against the reagent blank at 562nm. The reagent blank was prepared similar to protein standards but with distilled water instead of standard protein solution. Standard curve was drawn between the concentrations of protein and absorbance value (Atputharajah *et al.,* 1986; Babasaheb *et al.,* 2007; Kalaiyarasi *et al.,* 2013).

Statistical analysis

Statistical analyses were performed using R statistical package version 2.15.3 (R Development Core Team, 2011). Data sets were checked for the parametric assumptions of normality (Shapiro-Wilk and Kolmogorov-Smirnov tests) and homogeneity of variances (Bartlett's test). Box plots were used for identifying outliers from the data set that were removed before the statistical analysis. When necessary to meet the assumptions of normality and

homogeneity of variance, the data were transformed, either by log transformation or square root transformation. The data were analyzed using ANOVA. Tukey's multiple comparison test was used to determine significant differences at $p \leq 0.05$.

Results and Discussion

The mean alcohol contents of the *Caryota* sap observed with kakata, kohomba and control were significantly higher than that of commercial ime and hal bark ($p < 0.05$, Figure 1) after 45 hours. This confirms that lime and hal bark inhibit fermentation of the *Caryota* sap because of their basic pH range. The pH values of *Caryota* sap observed with hal, kakata, kohomba and control were significantly lower than that of lime ($p < 0.05$, Figure 2). The pH of the limed sap was comparatively very high (12–13) throughout the experiment. At higher pH values, majority of the microbes will either be killed or their activity would be greatly inhibited (Ghosh *et al.,* 2012). Most of the enzymes involved in fermentation are generally active at neutral pH. Highly acidic or basic pH of the media will eventually inhibit the enzyme actions (Kapilan and Vasanthy Arasaratnam, 2010b). Hal bark might have some anti microbial activities that is responsible for the low production of alcohol.

The mean number of yeast cells in the *Caryota* sap observed with hal, kakata, kohomba and lime were significantly lower than that of control ($p < 0.05$, Figure 3). There was a very low amount of yeast cells present in the limed pot even after 60 hours. Though yeast cells were present in almost all treatments, their amount did not show an exponential growth. There might be some biochemical reactions taking place between the fermentation inhibitory material added and the specific content in the *Caryota* sap and these results in the production or activation of some antibiotic substances in the sap. Some antibiotics substances might get activated / produced in the *Caryota* sap because of the presence of the inhibitory substances and this antibiotic inhibits the microbial growth. In kohomba and kakata treated saps, yeast cells had an increasing trend towards the end of the experiment. However, the number of yeast cells was very much reduced in the kohomba and kakata treated pots.

The mean number of the bacterial cells in the *Caryota* sap observed with hal, kakata, kohomba and commercial lime were significantly lower than that of control throughout the experiment ($p < 0.05$, Figure 4). In kohomba treated saps only there was an increase in the number of bacterial cells with the time. The estimate of bacteria in these samples might not be a true estimation because of the particular medium used for their isolation of bacteria (Kapilan and Arasaratnam, 2010).

Amount of total sugar in the *Caryota* sap showed a decreasing trend with time throughout the experiment, in all the samples (Figure 5). As the sugary substances in the sap, might get oxidized if the pans are kept open to the environment. There are no significant differences in the total sugar contents of the different saps treated with diverse fermentation inhibitory substances. Interaction between these inhibitory agents with the specific component of the *Caryota* sap might have converted some biomolecules into sugars. Fluctuation of the sugar content in the sap might be due to the utilization of sugar for energy, conversion of sugar into other substances and the influence of airborne bacteria and yeasts (Michael *et al.,* 1988; Somasiri *et al.,* 2011). There was no significant difference in the amount of reducing sugar, between the saps treated with hal bark and commercial lime, all the time tested (Figure 6).

Table.1 Amount of protein present in the fermented *Caryota* toddy and saps treated with lime and Hal bark (60 hours after collection)

Samples of *Caryota* sap analyzed	**Mean protein content (g /100 mL)**
Fermented *Caryota* sap	0.496 (SD – 0.13)
Limed *Caryota* sap	0.407 (SD – 0.14)
Hal treated *Caryota* sap	0.421 (SD – 0.16)

Figure.1 Changes of amount of alcohol of the *Caryota* sap when it is treated with different fermentation inhibitory substances such as hal bark, kakata bark, kohomba leaves and commercial lime

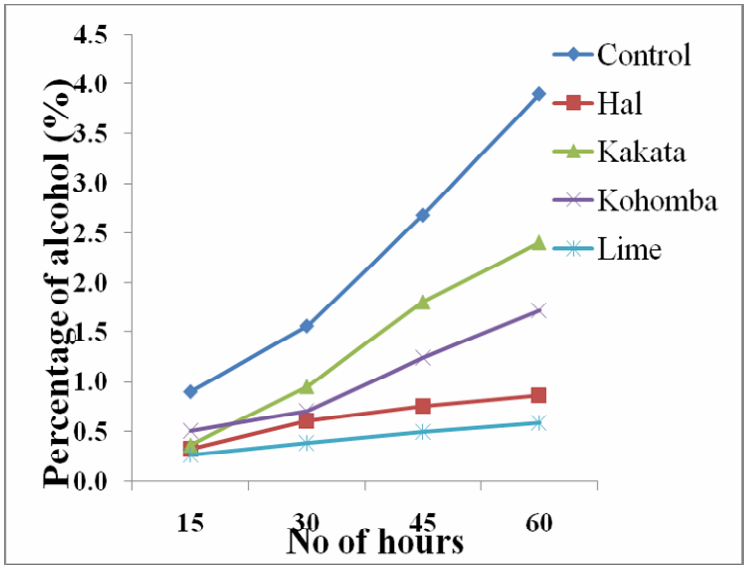


Figure.2 Changes of pH of the *Caryota* sap when it is treated with different fermentation inhibitory substances such as hal bark, kakata bark, kohomba leaves and commercial lime

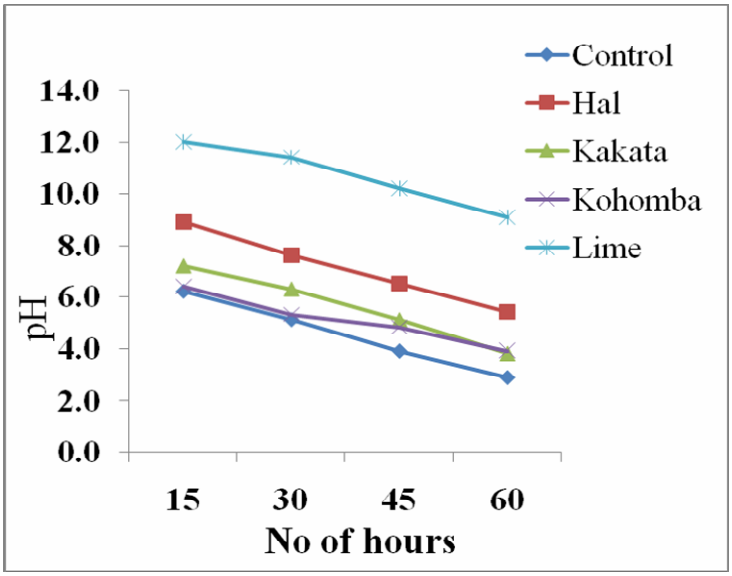


Figure.3 Changes of number of yeast cells / mL in the *Caryota* sap when the sap is treated with different fermentation inhibitory substances such as hal bark, kakata bark, kohomba leaves and commercial lime

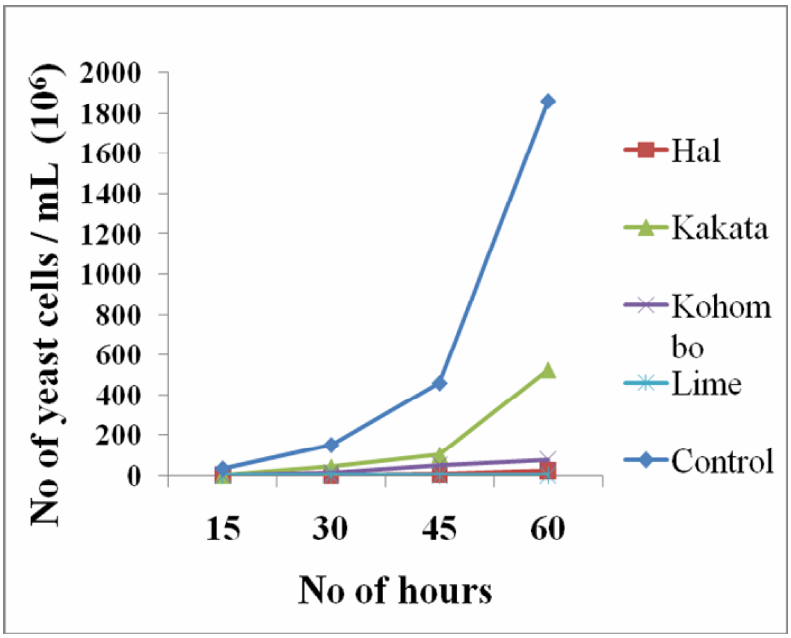


Figure.4 Changes of number of bacterial cells / mL in the *Caryota* sap when the sap is treated with different fermentation inhibitory substances such as hal bark, kakata bark, kohomba leaves and commercial lime

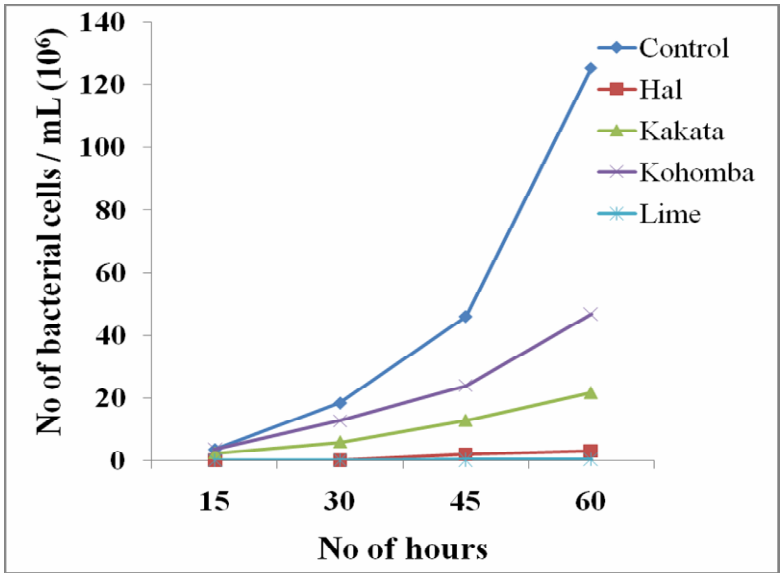


Figure.5 Changes in the amount of reducing sugar (%) of the *Caryota* sap when treated with different fermentation inhibitory substances such as hal bark, kakata bark, kohomba leaves and commercial lime

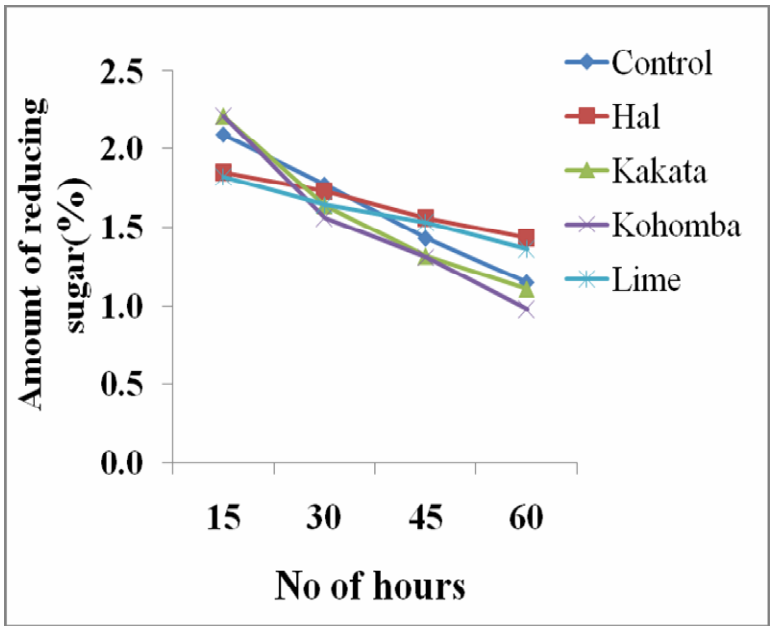
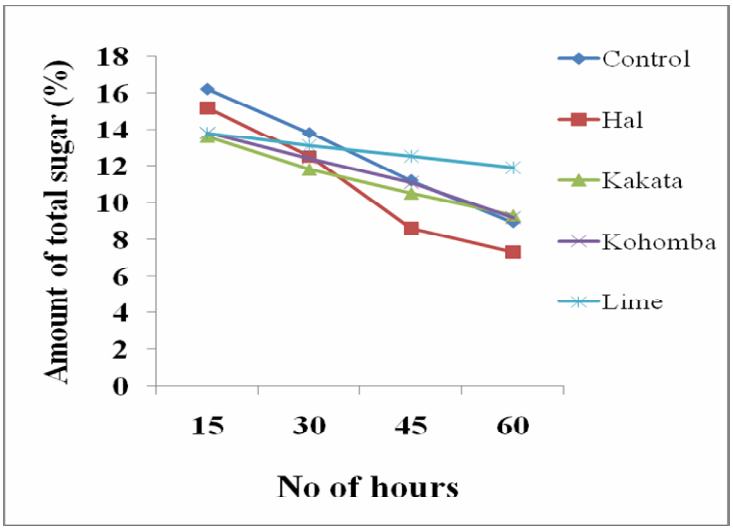


Figure.6 Changes in the amount of reducing sugar (%) of the *Caryota* sap when treated with different fermentation inhibitory substances such as hal bark, kakata bark, kohomba leaves and commercial lime



There was no significant difference in the amount of reducing sugar in the saps treated with hal bark and commercial lime all the time. Generally sugars in the sap have a reducing trend with time as most of the sugars are utilized by the microbes for their energy needs.

Protein content of the saps treated with lime and hal bark are significantly lower than that of fermented *Caryota* sap (Table 1) after 60 hours. This may be due to the limitation of microbial growth and the lower number of spores present in the sap that has fermentation inhibitors. The amount of protein content of the sweet sap decreases with time when lime or hal are added as fermentation inhibitors. Considerable amount of proteins would be provided from the microbial organisms that are accumulated in the sap. When there are no fermentation inhibitors, there will be an accumulation of acids and alcohol in the fermenting pans that will again start to inhibit the microbial growth, during fermentation.

Because of the high sucrose content in the fresh sap of Caryota urens, yeast initiates the fermentation of sucrose, glucose, and ferments these sugars during later stages as the fermenting pan is open followed by the fermentation of trace sugars (Nguyễn thị Minh Ngọc *et al.*, 2014; Nguyen *et al.*, 2012). This could be due to the higher content of sugars, minerals and vitamins in the kithul sap (Kalaiyarasi *et al.*, 2013). Mode of action of inhibition of fermentation is a multifactor effect that depends on factors like substance type, amount of microbes present in the sap, rate of oozing of sap and the biochemical reaction between the substance and the microbes (Somasiri *et al.*, 2011). Season of sap collection, environmental condition, sex of the palm tree from which the sap is collected (Amount of sugar is comparatively higher in the female tree), quality and the surface of the inhibitory substance are some other factors that could influence the inhibition of fermentation (Ranasinghe *et al.*, 2012; Shamala and Sreekantiah, 1988; Somasiri *et*

al., 2011). Caryota sap naturally has an antimicrobial effect. Lime and hal bark have a trend of inhibiting microbial growth in the sweet sap of Caryota. However kakata bark and kohomba leaves are not recommended as efficient inhibitors because of their inability to inhibit the accumulation of alcohol and maintain higher sugar content.

In conclusion, Lime is the most effective, cheapest and easily available substance that inhibits fermentation of sweet sugary sap of kithul. However, hal bark could also be used as effectively as lime to inhibit the fermentation of the sweet sugary sap of kithul. Therefore, lime and hal bark both could be recommended as efficient fermentation inhibiters of sweet kithul sap. Kahata bark and leaves of kohomba are not recommended because of their inability to inhibit the accumulation of alcohol and to maintain higher sugar content in the sap.

Acknowledgement

The authors sincerely thank Robika Kailayalingam, Subajini Mahilrajan and Srithayalan Srivijeindran of Palmyrah Research Institute, Kaithady, Jaffna, Sri Lanka and the staff of the water board, Negombo for preserving the samples and providing facilities to analyze the samples.

Reference

Atputharajah, J.D., Widanapathirana, S., Samarajeewa, U. 1986. Microbiology and biochemistry of natural fermentation of coconut palm sap. *Food Microbiol.*, 3: 273–280.

Babasaheb, B.B., Lingamallu, J.M.R., Kulathooran, R.B.R. 2007. Chemical composition of volatiles from coconut sap (neera) and effect of processing. *Food Chem.,* 101: 877–880.

Borse, B.B., Rao, L.J.M., Ramalakshmi, K., Raghavan, B. 2007. Chemical composition of volatiles from coconut sap (neera) and effect of processing. *Food Chem.,* 101(3): 877–880.

Chandrasekhar, K., Sreevan, S., Seshapani, P., Pramodhakumari, J. 2012. A Review on palm wine. *Int. J. Res. Biol. Sci.*, 2: 33–38.

Dalibard, C. 1999. Overall view on the tradition of tapping palm trees and prospects for animal production. Livestock Research for Rural Development, 11, 1999; Online-edition, www.lrrd.org/lrrd11/lrrd11.htm.

Development Core Team, R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. 2011: http://www.R-project.org

Ghosh, S., Chakraborty, R., Raychaudhuri, U. 2012. Optimizing process conditions for palm (*Borassus flabelliffer*) wine fermentation using Response Surface Methodology. *Int. Food Res. J.*, 19: 1633–1639.

Jansz, E.R., Jeyaraj, E.E., Abeyratne, D.J., Premaratne, I.G. 1975. Control of sulphide formation in coconut toddy, *J. Natn. Sci. Coun. SriLanka,* 3: 1–10.

Kalaiyarasi, K., Sangeetha, K., Rajarajan, S. 2013. A comparative study on the microbial flora of the fresh sap from cut inflorescence and fermented sap (toddy) of *Borrassus flabellifer* Linn (Palmyrah tree) and of *Cocos nucifera* Linn (Coconut tree) to identify the microbial fermenters. *Int. J. Res. Pure Appl. Microbiol.*, 3(3): 43–47.

Kapilan, R., Arasaratnam, V. 2010. A Novel *Bacillus pumilus* strain for alkaline

xylanase production at and above 40°C. *Ceylon J. Sci (Bio Science)*, 39(1): 61–70.

Kapilan, R., Vasanthy Arasaratnam, 2011. Paddy husk as support for solid state fermentation to produce xylanase from *Bacillus pumilus*. *Rice Sci.*, 18(1): 36–45.

Kumuthini Chrystopher, R., Theivendirarajah, K. 1988a. Palmyrah palm wine 1: Microbial and Biochemical changes. *J. Natn. Sci. Coun. SriLanka*, 131: 141–12.

Kumuthini Chrystopher, R., Theivendirarajah, K. 1988b. Palmyrah palm wine II: Improvements in alcohol production. *J. Natn. Sci. Coun. Sri Lanka*, 131: 147–157.

Michael, O., Eze, A., Uzoechi Ogan, 1988. Sugars of the unfermented sap and the wine from the oil palm, *Elaeis guinensis*, tree. *Plant Foods Human Nutr.*, 38: 121–126.

Molagoda, T.W. 1945. The kitul palm. Tropical agriculturist, peradeniya, Colombo, Vol. 101, Pp. 251–259.

Nguyễn thị Minh Ngọc, Nguyen Phuoc Minh, Dong Thi Anh Dao, 2014. Different processing conditions affect palm (THOT NOT) wine fermentation. *Am. J. Res. Commun.*, 2(1): 142–157. www.usa-journals.com.

Nguyen, V.T., Nguyen, M.T., Neáng, T. 2012. Isolation and screening of yeast strains from palm juice collecting at Tri Ton, An Giang province. *Can Tho Sci. J.*, 22: 203–212.

Nwachukwu, I.N., Ibekwe, V.I., Nwabueze, R.N., Anyanwu, B.N. 2006. Characterization of palm wine yeast isolates for industrial utilization. *Afr. J. Biotechnol.*, 5: 1725–1728.

Opara, C.C., Ajoku, G., Madumelu, N.O. 2014. Palm wine mixed culture fermentation kinetics. *Greener J. Phys. Sci.*, 3: 28–37.

Ranasinghe, P., Premakumara, G.A.S., Wijeyarathna, C.D., Ratnasooriya, W.D. 2012. Antioxident avctivity of *Caryota urens* L. treacle. *Trop. Agricult. Res.*, 23(2): 117–125.

Shamala, T.R., Sreekantiah, K.R. 1988. Microbiological and biochemical studies on traditional Indian palm wine fermentation. *Food Microbiol.*, 5: 157–162.

Somasiri, H.P.P.S., Premakumara, G.A.S., Mahanama, K.R.R. 2011. Free amino acid profile of Kithul palm *Caryota urens* L. treacle and its potential application in authentication of treacle types, Proceedings of the 12th Government Food Analysts Conference.

Theivendirarajah, K. 2008. Palmyrah palm – a monograph.

Theivendirarajah, K., Dassanayake, M.D., Jeyaseelan, K. 1977. *Ceylon J. Sci. (Bio.Sci.)*, 12(2): 146–150.

Trimen, H. 1998. A handbook to the flora of Ceylon, Vol 4. Dulau & Co., London.