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Research Article

# Efficient fermentation inhibitor of sweet phloem sap of Palmyrah (*Borassus flabellifer* L.) in Sri Lanka

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## Abstract

This study was aimed to determine the effect of various substances on inhibition of fermentation of the sweet phloem sap of palmyrah palm (*Borassus flabellifer* L.) and to recommend the best fermentation inhibitor. Pieces of fresh bark of hal (*Vateria copallifera*), bark of kahata (*Careya arborea*), leaves of kohomba (neem - *Azadirachta indica*) and commercial lime powder at the same rate (w/v 10g / six litre pots) were added into each collecting pot before use. The samples of sap were collected at equal time intervals and analyzed for reducing sugars, total sugars, pH, alcohol content, number of yeast and bacterial cells. The pH values of palmyrah saps added with hal, kahata, kohomba and control (without the addition of any substances) were significantly lower than that of lime ($p < 0.05$). The pH was very high (10 -13) in limed sap and remained the same throughout the experiment. The alcohol content of the palmyrah sap treated with lime was significantly lower than the other treatments. Bacterial and yeast cells were found to increase in all the treatments except the one with limed pot, where there were significantly low numbers of yeast and bacterial cells recorded. Amount of total and reducing sugars present in the tested saps show a decreasing trend throughout the experiment whereas in the limed sap, sugar content remain unchanged. Lime could be recommended as efficient fermentation inhibitor of the sweet sugary sap of palmyrah. Hal bark, kahata bark and leaves of kohomba cannot be recommended as efficient fermentation inhibitors because of their poor ability to inhibit the alcohol production and microbial growth.

**Keywords:** *Borassus flabillifer*; fermentation; inhibitor; lime; hal bark; sweet phloem sap.

## Introduction

*Borrassus flabellifer* (Palmyrah palm) is widely distributed naturally in the tropical and subtropical regions. They are the members of family Palmae and spread all over in large area of the south asian countries such as India, Sri Lanka, Philippines, Indonesia and Malaysia [18, 19]. Even though all the palm tree parts are economically very important, the main products are sweet sugary sap that is used as the raw material for toddy production [5, 19]. The sap derived from tapping of the inflorescence of the tree is called toddy, a fermented native intoxicating beverage of most of these countries. The sap on fermentation by air borne yeast and bacteria becomes palm wine and is one of the famous popular alcoholic beverages among the local people of these countries. Fresh unfermented sap is referred to as pathaneer, that is consumed immediately as a refreshing non-alcoholic drink or it is concentrated by heating to produce sugar, treacle, jaggery and other products [4, 6, 16, 18]. The fresh palmyrah palm sap is very sweet and has exceptional nutritive value because of the minerals such as Ca, Fe, Zn, Cu, P, niacin and diverse vitamins present in excessive amount [18].

The main source of crude sugar (Jaggery) in Sri Lanka is the sweet sugary sap obtained from the tapped inflorescence of the Palmyrah (*Borassus flabellifer*), Coconut (*Cocos nucifera*) and Kithul (*Caryota urens*) palms. This sap is free of fermentation, sterile and highly charged with sugar. Unless special precautions

are taken, fermentation by yeast and bacteria leads to accumulation of alcohol and acids. Several methods are practiced in SriLanka to prevent such fermentation taking place in the sap [9, 10, 11]. Lining the inside of the pot with fresh commercially available lime, placing hal bark (*Vateria copallifera*), placing kakata bark (*Careya arborea*) and placing the leaves of kohomba - Neem (*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.

Lining the inside of the pot with lime is the usual method that is used to obtain unfermented toddy from the Palmyrah, Coconut and Kithul palms in SriLanka. Though the usage of such substances reduce considerable amount of fermentation, they might change the original taste of the sweet toddy and the jaggery to a considerable extent. This results in poor consumer affinity towards sweet toddy products [18,19]. Thus the efficient substance is needed to inhibit fermentation taking place in the sweet sugary saps of palm trees in order to get the sweet toddy with the original taste. The objective of the study is to study the effect of various substances used in Jaffna, to prevent fermentation taking place in the palmyrah sap and to recommend the cheapest and easily available substance, which prevents fermentation efficiently.

## Materials and Methods

### Plant Material

Palmyrah palm growing widely in the northern region of Sri Lanka was selected. Matured male and female palm trees were chosen randomly in three different areas of the northern region.

### Methodology

To study the effect of lime, the inside of the collecting pot was lined with a thin coating of lime (10 grams / 6 litre) before it was hung on the coconut tree. Pieces of fresh bark of hal, kahata and leaves of kohomba at the rate of 10 grams / 6 L pot were put in each case into the collecting pot before use. Control pot without the addition of any substance 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. Assay of sugars was done using reducing test with copper reagent. 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 [13]. 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, while bacterial cells were counted by the same method but on nutrient agar medium [15, 18]. 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 [5, 6. 7, 8]. Into ten labelled 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 [16, 19]. 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 [1, 2, 3].

### Statistical analysis

Statistical analyses were performed using R 2.15.3 [16]. 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 $p \leq 0.05$ Significant differences of the measured parameters between the treatments and the control were noted [17].

## Results and Discussion

The mean alcohol contents of the palmyrah sap treated with hal, kakata, kohomba and control were significantly higher than that of Lime ($p < 0.05$, Figure 1) after 30 hours. This may be due to the higher pH

range maintained by lime that inhibits fermentation, thus inhibits alcohol production. At higher pH values, majority of the microbes will either be killed or their activity is greatly inhibited [6, 20]. 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 action [7, 8, 20]. The pH values of palmyrah sap observed with hal, kakata, kohomba and control were significantly lower than that of lime ($p < 0.05$, Figure 2). The pH range of the sap was found to be very high (10 - 13) in lime and remained at that value throughout the experiment.

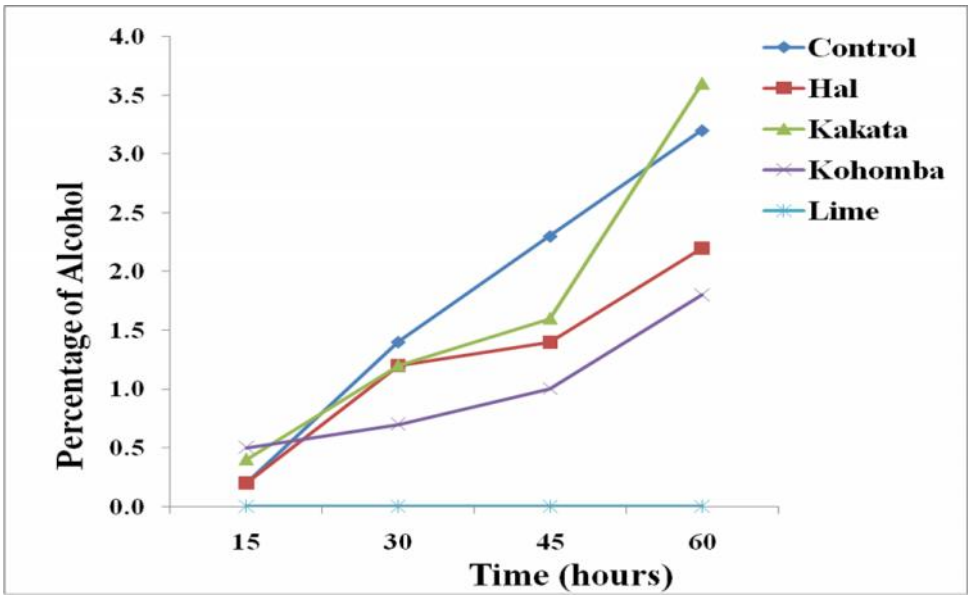


**Figure 1**: Changes of amount of alcohol of the palmyrah sap when it is treated with different fermentation inhibitory substances

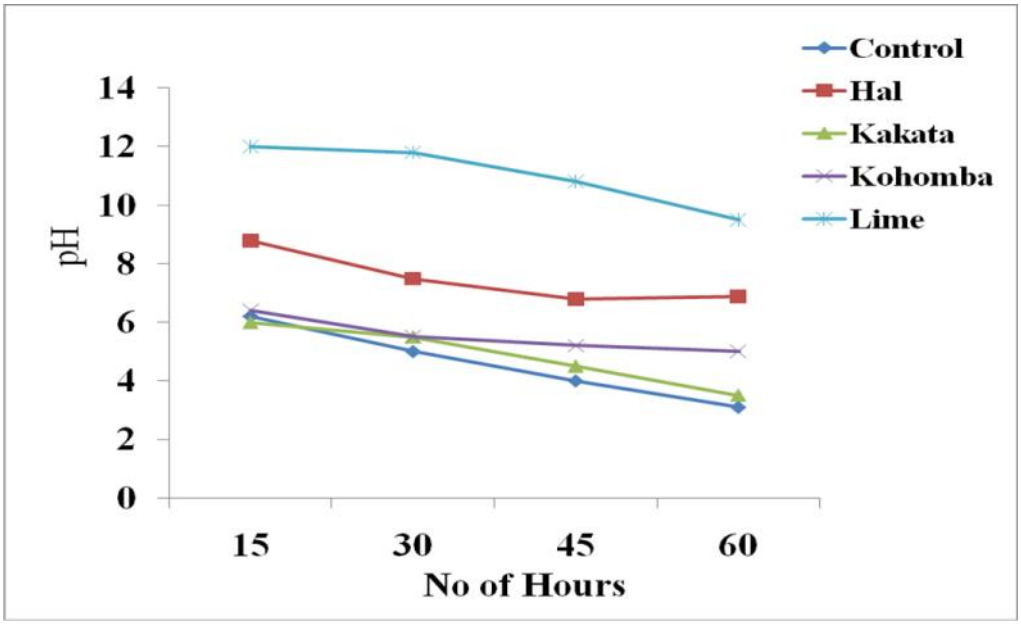


**Figure 2**: Changes of pH of the Palmyrah sap when it is treated with different fermentation inhibitory substances

The number of yeast cells in the palmyrah sap treated with hal, kakata, kohomba and lime were significantly lower than that of control ($p < 0.05$, Figure 3). There was significantly lower amount of yeast cells found in the limed pot even after 60 hours. In kohomba treated sap, there were very less number of yeast cells initially and then there was a trend of increasing yeast cells after 45 hours. However, towards the end (60 Hours), the number of yeast cells were very much reduced in the kohomba treated pots. All the inhibitory substances are capable of inhibiting the yeast growth or able to delay the multiplication of yeast cells.

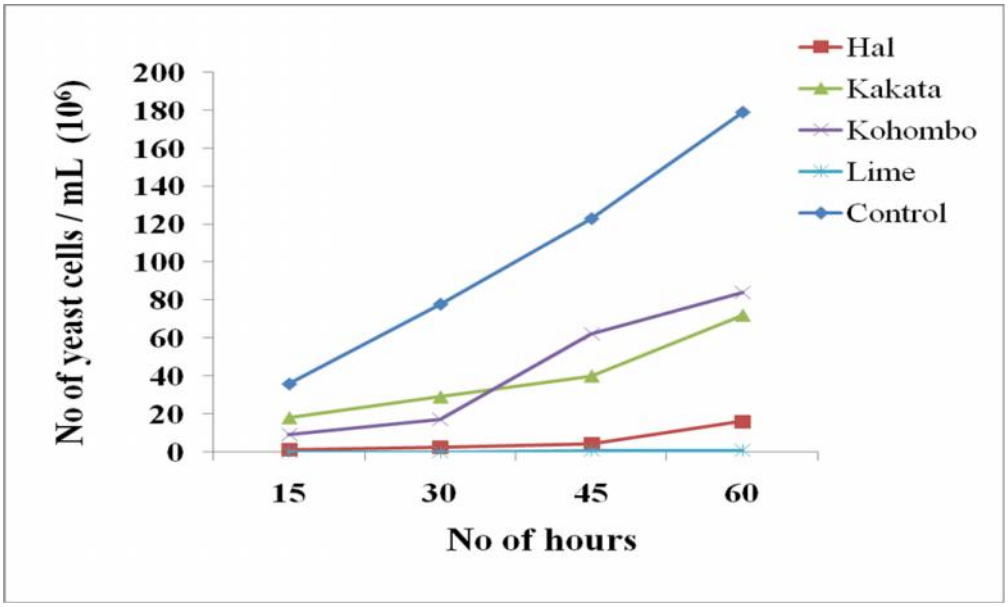


**Figure 3**: Changes of number of yeast cells / mL in the palmyrah sap when the sap is treated with different fermentation inhibitory substances

The mean number of the bacterial cells in the palmyrah sap treated with hal, kakata, kohomba and lime were significantly lower than that of control after 30 hours ($p < 0.05$, Figure 4). Bacterial cells present after 15 hours were in order of $10^7 – 10^8$ cells per ml in all the treatments except with lime. Number of bacterial cells was very much lower ($1.12 * 10^4$ cells /ml) in lime and this number gradually decreased with the time. Multiplication of the bacterial population was also very much controlled in kakata and hal barks too. The estimate of bacteria in these samples might not be a true estimation because of the media composition might get altered for the isolation because of the biological substances added. [14].

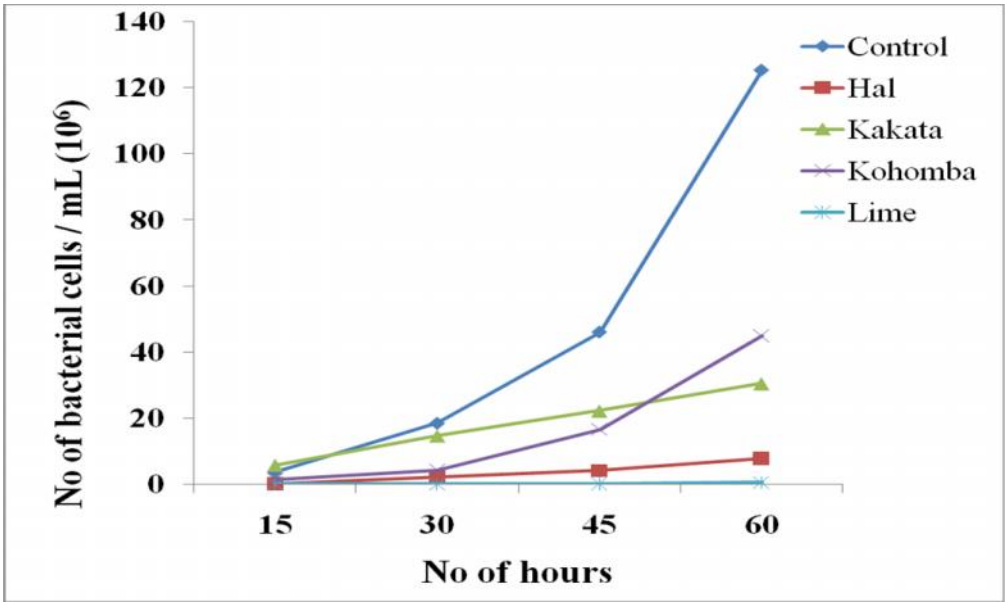


**Figure 4**: Changes of number of bacterial cells / mL in the Palmyrah sap when the sap is treated with different fermentation inhibitory substances

Amount of total and reducing sugars in the tested saps show a decreasing trend throughout the experiment. This may be due to the utilization of sugars by the microbes growing in the sap. With slight fluctuation, the total and reducing sugar contents remained more or less same in the limed saps (Figure 5). As the amount of microbes growing in the limed sap is very low, the rates of decrease in the sugar contents were comparatively low in the limed saps. 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 [11, 16]. Saps treated with fermentation inhibitory substances did not show any significant difference in the amount of reducing sugar among them at all the time (Figure 6).

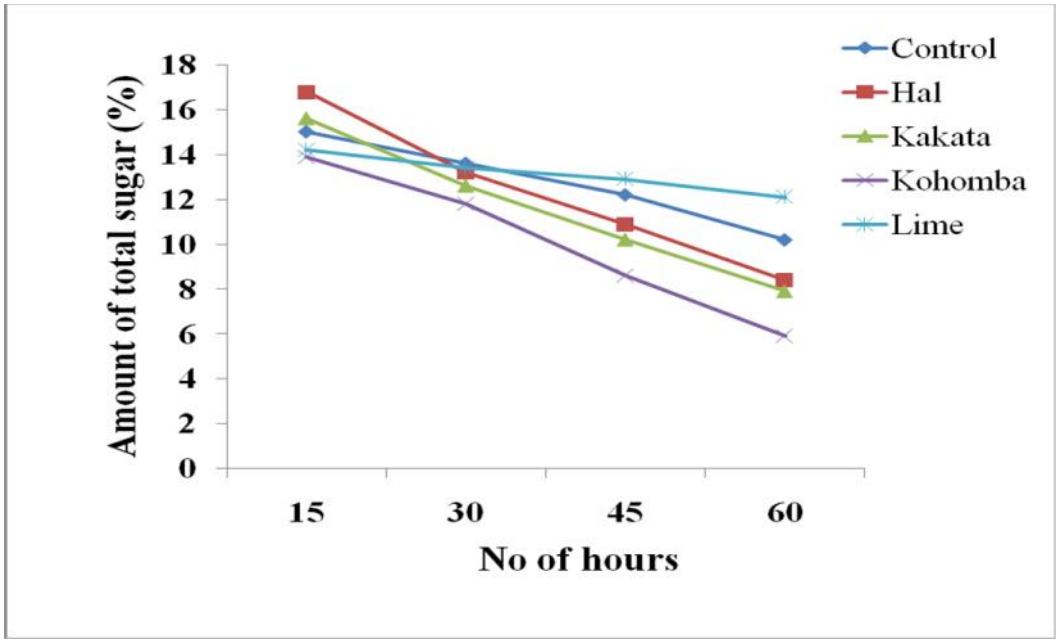


**Figure 5**: Changes in the amount of total sugar (%) of the Palmyrah sap when treated with different fermentation inhibitory substances

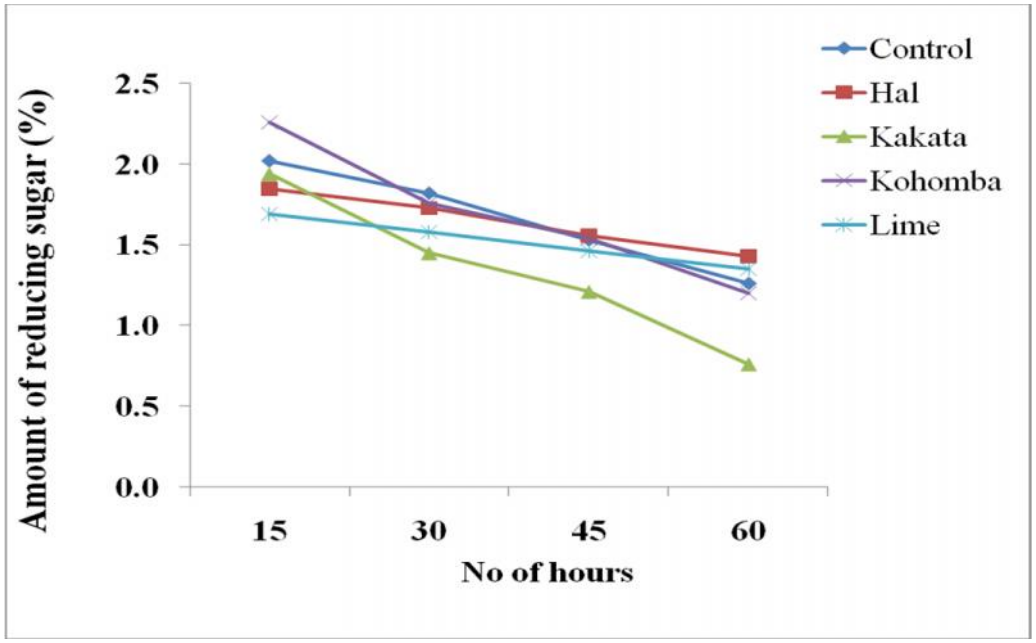


**Figure 6**: Changes in the amount of reducing sugar (%) of the Palmyrah sap when treated with different fermentation inhibitory substances

There is a significant difference in the protein content between the fermented and fermentation inhibited palmyrah saps ($p < 0.05$, Table 1) after 60 hours of hanging the pot on the tree. The protein content of the sap decreases when lime is used as a fermentation inhibitor. The proteins in the microbial body will involve in the count of protein amount. If fermentation is allowed, the production of acids and alcohols from sugar might start to inhibit the growth of microbes, at a later stage [6]. Since microbial growth and fermentation are very much controlled in the limed sap, the amount of protein present in the limed sap is comparatively lower than the fermented sap.

**Table 1**: Amount of protein present in the fermented palmyrah toddy and limed palmyrah sugary sap (60 hours after collection)

| Samples analyzed | Mean protein content (g /100 mL) |
|---|---|
| **Fermented sap** | 0.291 (SD- 0.031) |
| **Limed sap** | 0.242 (SD- 0.012) |

The microorganisms present in the toddy of palmyrah (*Borassus flabellifer*) are *Candida glabrata, Candida pseudotropicals*, *Pichia anghophorae* and *Saccharomyces* chevalieri [14, 16]. Because of the high sucrose content in the fresh sap of *Borassus flabillifer*, *Schizosaccharomyces japonicus* initiates the fermentation of sucrose, glucose, and *Saccharomyces chevaleri* ferments sucrose and glucose during later stages as the fermenting pan is open followed by the fermentation of trace sugars by *Pichia membraneafaciens*, *Candida glabrata* [16].

The microbial flora is richer in the sap and toddy of *Borassus flabillifer* compared to the *Cocos nucifera* [9, 10]. This could be due to the higher content of sugars, minerals and vitamins in the sap of *Borassus flabellifer* than in the sap of *Cocos nucifera* [16, 17]. 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 [12,19]. 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 [5, 9, 10].

## Conclusion

Lime is the most effective and easily available substance which prevents fermentation taking place in the sweet sugary sap of palmyrah, in Sri Lanka. It must be emphasized that hal could also be used as effectively as lime to preserve sweet toddy of Palmyrah, for 30 hours. Kakata bark and kohomba leaves are not recommended because of their poor ability to inhibit fermentation. Very large scale multicentre studies should be done to confirm this finding.

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