International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
Coden: IJARQG(USA)

Research Article

SOI: http://s-o-i.org/ 1.15/ijarbs-2-12-6

Optimization of the usage of commercial lime for the inhibition of fermentation of sweet sugary saps of *Borassus flabellifer* and *Caryota urens*

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Abstract

The study was aimed to optimize the usage of commercial lime to inhibit the fermentation of the sweet sugary saps of *Borassus flabellifer* L. (Palmyrah) and *Caryota urens* (Kithul) and to recommend the appropriate concentration for the rural industrial application. Different concentrations (0, 1, 2, 3, 4, 5, 6, 7 and 8 grams/ liter (w/v)) of commercial lime was applied the inside of the each sterile collecting pan before they were hung on the palmyrah and kithul palms around the tapped inflorescence. The sap samples of each palm trees were collected separately from the pans after 60 hours and analyzed for reducing sugars, total sugars, pH, alcohol content, number of yeast and bacterial cells and protein content. Increasing concentration of lime decreases the microbial count and accumulation of alcohol significantly in the saps of *Borassus flabellifer* and *Caryota urens*. Therefore, the commercial lime effectively inhibits the fermentation of sweet sugary saps of *Borassus flabellifer* and *Caryota urens*. The study recommends the usage of three grams of commercial lime / liter of *Caryota* sap and two grams commercial lime / liter of *Borassus* sap to completely inhibit the natural fermentation process without affecting much of the natural taste of the respective saps and their products.

Keywords: *Borassus flabellifer*; *Caryota urens*; commercial lime; concentration; sweet sap.

Introduction

The sweet sugary saps obtained from the inflorescences of the Palmyrah (*Borassus flabellifer*), Coconut (*Cocos nucifera*) and Kithul (*Caryota urens*) palms are of great economic importance in the area where these palms are growing in the world. The sap derived from tapping the inflorescence of the palm is called toddy, a fermented native intoxicating beverage. The sap on fermentation by air borne yeast and bacteria becomes palm wine and is one of the famous popular alcoholic beverages in the life among the local people of the most of the South Asian countries where these palms grow extensively (Chandrasekhar et al., 2012). The fresh unfermented palm sap is very sweet and has exceptional nutritive value because of the existence of substantial amount of minerals and vitamins (Sharmila et al., 1988, Theivendirarajah, 2008). The fresh sap is consumed immediately as a refreshing non-alcoholic drink or it can be processed to produce diverse food items such as treacle, sause, syrup and jaggery (Chandrasekhar et al., 2012). 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 saps of the palms have anti-diabetic, anti-ageing and anti- rheumatic properties. *Borrassus* and *Caryota* sap based products provide a significant source of income to a huge population living in the rural areas of the developing countries where these palms are widely spread (Ranasinghe et al., 2012, Somasiri et al., 2011). Therefore, scientific knowledge of the functional properties of fresh saps of

these palms would help to improve the value, quality satisfactory consumer taste of their products.

Borrassus flabellifer (Palmyrah palm) is one of the widely distributed palm of tropical and subtropical regions. They are categorized under family Palmae and spread all over the south Asian countries such as India, Sri Lanka, Philippines, Indonesia and Malaysia (Sharmila et al., 1988, Theivendirarajah, 2008). *Caryota urens* (Kithul palm) is 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 generally reaches a height of 15 to 20 meters under favorable growth conditions. 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).

If fermentation by the airborne bacteria and fungi is allowed, the collected phloem sap of the palms will be converted into intoxicating fermented beverages such as toddy and alcohol (Ranasinghe et al., 2012, Somasiri et al., 2011, Chandrasekhar et al., 2012). 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 saps of the palms in order to get the sweet sugary sap which is of great economical importance. Lining the inside of the pot with fresh commercial lime in a sterile clean pot before it is used for collecting sap, is the most efficient method that has been in practice to inhibit fermentation, in Sri Lanka (Chandrasekhar et al., 2012, Dalibard 1999, Kalaiyarasi et al.2012, Thevendirarajah et al., 1997). Though the usage of lime efficiently inhibits the microbial fermentation mainly because of its higher alkaline nature, appropriate concentration of the lime is not used in the application. Usage of higher concentration of lime might change the original taste and the acceptable quality of the sweet toddy and the jaggery to a great extent. This results in poor consumer affinity towards sweet toddy and its products. If the application of lime is in low concentration, then sap might be used as a source for the entry and growth of airborne yeasts and bacteria and this will lead to accumulation of alcohol in the sap. Thus the appropriate concentration of commercial lime that can efficiently inhibit the fermentation of the sweet sugary saps of *Borassus flabellifer* and *Caryota urens* without changing the consumable quality and the original taste, needs to be determined. Therefore, the objective of the study was to optimize the usage of commercial lime to inhibit the fermentation of the sweet sugary saps of *Borassus flabellifer* L. (Palmyrah) and *Caryota urens* (Kithul) and to recommend the appropriate concentration for the rural industrial application.

Materials and Methods

Plant Material

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

Methodology

To determine the effective concentration of lime, different concentrations of commercial lime (0, 1, 2, 3, 4, 5, 6, 7 and 8 grams/ liter) were applied at the inner surface of each sterile clean collecting pot before they were hung on the palm trees of each type. Control pots without the addition of any substances were also maintained. In each treatment there were three replicates used for each palm. After 60 hours, the saps were collected separately and the following parameters were measured.

Measurements

The samples were analyzed in the laboratory for reducing sugars, total sugars, pH, alcohol, number of yeast cells and bacterial cells and protein content. Assay of sugars was done using reducing test with copper reagent (Theivendirarajah, 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, Theivendirarajah, 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 1988, 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). After the 60 hours saps of each pan were subjected to the tasting panel contained 25 people in order to test the acceptable customer consuming quality. The terms used to measure the taste were excellent, very good, good, poor and bad.

Statistical analysis

Statistical analyses were performed using R statistical package version 2.15.3 (R Development Core Team, 2011). The data were analyzed using ANOVA. Tukey's multiple comparison test was used to determine the significant differences at $p \leq 0.05$ (Zar, 1999).

Results and Discussion

Addition of more concentrated commercial lime might affect the quality and taste of the sweet sap and its products. The values of amount of alcohol, pH, number of yeast and bacterial cells, total and reducing sugar amount in the fermented palm toddy and saps treated with different concentrations of lime after 60 hours, are shown in Table 1 (*Borassus*) and Table 2 (*Caryota*).

Effect of lime concentration on *Borassus* sap fermentation

With the increasing concentration of lime, microbial count reduces and the amount of alcohol produced by fermentation drops. However, the amount of alcohol present in the *Borassus* sap with the concentration of one gram / liter (w/v) was significantly lower than that of control ($p < 0.05$). At this concentration, the bacterial and yeast cell counts were significantly lower than that of control ($p < 0.05$). When the concentration of lime was raised to two grams / liter, there was no alcohol in the sap. Beyond this concentration neither microbial growth nor alcohol production was witnessed. Satisfactory consumer taste was obtained at this concentration of sap. Beyond the concentration of two grams / liter of commercial lime, the taste of the sap was slightly bitter and not satisfactory. Therefore it was decided to recommend the usage of two grams lime / liter of *Borassus* sap, to inhibit the natural fermentation process completely. The mean number of yeast cells in the *Borassus* sap observed with two grams / liter of commercial lime was significantly lower than that of lower lime concentrations and the non-limed control ($p < 0.05$). There was a very low amount of yeast cells present in the limed pot treated with two grams / liter of lime, even after 60 hours. Though yeast cells were present in the saps treated with lower concentrations of commercial lime, the amount of yeast found in the saps treated with the higher lime concentrations was almost none. The mean number of the bacterial cells in the *Borassus* sap maintained at the concentration of one grams of commercial lime / liter sap, were significantly lower than the saps kept at one gram / liter of the concentrations of lime ($p < 0.05$). The estimation of bacteria in might not be a true estimation sometimes because some microbes might have been killed during the culturing process and the particular medium used for the isolation of bacteria (Kapilan and Arasaratnam 2010).

Effect of lime concentration on *Caryota* sap fermentation

With the increasing concentration of lime, bacterial and yeast count reduces and the amount of alcohol produced by fermentation drops. However, alcohol is still present in the *Caryota* sap with considerable amount of bacteria and yeast cells when a concentration of 2 grams / liter (w/v) lime was used to inhibit fermentation. When the concentration of lime was raised to 3 grams / liter, alcohol present in the sap was very low. Beyond the concentration of 3 grams / liter of lime, the taste of the sap was not satisfactory. Therefore it was decided to recommend the usage of 3 grams lime / liter of *Caryota* sap, to inhibit the natural fermentation process completely.

Table 1: Amount of alcohol, pH, number of yeast and bacterial cells, total sugar and reducing sugar in the fermented *Borassus* toddy and saps treated with different concentrations of lime (60 hours after collection from tree)

Lime concentration (g / L)	Alcohol percentage (%)	pH	No of yeast cells (10^6)	No of bacterial cells (10^6)	Total sugar (%)	Reducing sugar (%)
0	3.4	4.8	7302	817	15.7	1.17
1	0.9	10.9	14	36	16.4	1.43
2	0	12.1	0	0	17.8	1.84
3	0	12.3	0	0	17.2	1.96
4	0	12.7	0	0	16.9	1.82
5	0	12.9	0	0	15.9	1.89
6	0	12.8	0	0	17.3	1.74
7	0	12.5	0	0	15.5	1.69
8	0	12.6	0	0	16.4	1.75

The mean number of yeast cells in the *Caryota* sap observed with two grams / liter of commercial lime was significantly lower than that of lime concentration one gram / liter and the non-limed control ($p < 0.05$). There was a very low amount of yeast cells present in the limed pot treated with two grams / liter of lime, even after 60 hours. The mean number of the bacterial cells in the *Caryota* sap maintained at the concentration of two grams of commercial lime / liter sap, were significantly lower than the saps kept at lower concentrations than two grams of lime ($p < 0.05$).

Table 2: Amount of alcohol, pH, number of yeast and bacterial cells, total sugar and reducing sugar in the fermented *Caryota* toddy and saps treated with different concentrations of lime (60 hours after collection from tree)

Lime concentration (g / L)	Alcohol percentage (%)	pH	No of yeast cells (10^6)	No of bacterial cells (10^6)	Total sugar (%)	Reducing sugar (%)
0	3.9	4.5	9452	1596	16.3	0.97
1	2.9	11.7	189	149	14.4	1.34
2	0.7	12.4	0.004	0.0003	13.8	1.42
3	0	12.6	0	0	13.0	1.46
4	0	12.6	0	0	12.9	1.42
5	0	12.9	0	0	12.8	1.39
6	0	13.1	0	0	13.1	1.44
7	0	12.9	0	0	13.5	1.42
8	0	13.2	0	0	13.6	1.45

The pH values of the limed saps were comparatively very high (12 - 13) throughout the experiment than the non-limed control. At higher pH values, important microbes will be killed or their activity would be greatly inhibited (Ghosh et al., 2012). Enzymes involved in the microbial fermentation are generally active at neutral pH. Highly acidic or basic pH of the media will inhibit the enzymatic reactions (Kapilan and Vasanthy Arasaratnam, 2010). There might be some biochemical reactions taking place between the commercial lime and the specific ingredient(s) of the palm saps and the end products might activate some antibiotic substances in the sap. There might be a chance of accumulation of certain antibiotics in the sap that might inhibit the microbial growth

.

Amount of total sugar in the *Caryota* sap showed a increasing trend with the increasing higher concentrations of lime (Kapilan et al., 2015b and Ranganathan Kapilan, 2015). Interaction between the lime with the specific component of the saps might have converted some biomolecules into sugars at lower concentrations of lime. Fluctuation of the sugar content in the sap with different concentrations of lime 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).

Table 3: Amount of protein present in the fermented toddy and unfermented sugary saps (60 hours after collection) of *Borassus* and *Caryota*

Samples analyzed	Mean protein content (g /100 mL)
Fermented *Borassus* sap	0.289 (SD- 0.029)
Limed sap (2g/L)	0.256 (SD- 0.022)
Fermented *Caryota* sap	0.298 (SD- 0.031)
Limed sap (3g/L)	0.244 (SD- 0.012)

Protein contents of both the *Palmyrah* and *Caryota* saps treated with lime are significantly lower than that of the respective fermented saps (Table 3) after 60 hours. The varying amount of proteins may be due to the limitation of microbial growth and the lower number of spores present in the saps where fermentation is arrested. The amount of protein content of the sweet saps was lower when higher concentrations of lime were used to inhibit fermentation. Considerable amount of proteins would be provided from the microbial organisms that are accumulated in the sap at lower concentrations of lime. When there is no inhibition of fermentation (Control), there will be an accumulation of acids and alcohol in the sap because of the microbial function (Ranganathan Kapilan et al., 2015a, b).

Table 4: Interpretation of the tasting panel results obtained after 60 hours

Concentration of the lime in the sap (gL^{-1})	*Borassus* sap	*Caryota* sap
0	R	R
1	R	M
2	M	M
3	M	M
4	M	B
5	B	B
6	B	B
7	B	B
8	B	B

R – excellent, very good, M – Good, B – poor, bad

It is reported that the amount of sugar in the sap is comparatively higher in the female trees. In order to avoid the difference due to sex, sexually mixed palm trees were chosen in this study. Mode of action of inhibition of fermentation is due to the involvement of multiple factors such as substance type, microbial amount present in the sap, sap oozing rate and the biochemical reactions between the sap ingredients and the microbial secretions (Somasiri et al., 2011). Season of the year of sap collection, environmental factors, sex of the palm tree used to collect are among the factors that might influence the inhibition of fermentation (Ranasinghe et al., 2012, Shamala and Sreekantiah, 1988, Somasiri et al., 2011).

Conclusion

Commercial lime effectively inhibits the fermentation of sweet sugary saps of *Borassus flabellifer* and *Caryota urens*. The study therefore, recommends the usage of three grams of commercial lime / liter of *Caryota* sap and two grams commercial lime / liter of *Borassus* sap to completely inhibit the natural fermentation process without affecting much of the natural taste of the respective saps and their products.

Acknowledgments

The author expresses his sincere gratitude to the staff of the Palmyrah research Institute, Jaffna for their immense help in analyzing the samples.

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How to cite this article:
Ranganathan Kapilan. (2015). Optimization of the usage of commercial lime for the inhibition of fermentation of sweet sugary saps of *Borassus flabellifer* and *Caryota urens*. Int. J. Adv. Res. Biol. Sci. 2(12): 60–66.