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Palmyrah (*Borassus flabellifer* L.) Toddy as a Source of Vinegar Production and its Chemical Analysis

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ABSTRACT

Purpose: *Vinegar is a condiment prepared by alcoholic and consequent acetic acid fermentation using different sugary and starchy materials. This can be utilized as a flavoring agent, preservative, medicine, cleaning agent and herbicide. Palmyrah toddy contains around 4-6% of alcohol which is produced in huge amounts during their season. So, this research was designed to increase the utilization of toddy by means of vinegar.*

Research Method: *Vinegar was prepared with toddy as a substrate (5.0% of alcohol) by using Orleans, generator and optimized submerged fermentation processes. The chemical characteristics of vinegar produced from Palmyrah were determined based on SLS 168:1999.*

Findings: *Orleans process significantly ($p<0.05$) reduced the time required to produce the maximum acid (4.01%) contained corn cob and activated bacteria covering gel-like structure. During the generator process over oxidation of ethanol was observed due to the high rate of aeration with the pump.*

Optimized conditions of submerged fermentation was exhibited as 10% inoculum size (5, 10, 20 and 30%), 30°C temperature (28, 30 and 32°C)and with shaking showed 3.96, 4.23 and 4.53% of acid production at 8th, 7th, and 5th day of fermentation respectively. Chemical characteristics of produced palmyrah vinegar complied with SLS 168:1999 as total acidity (4.32%), total solids (4.08 (w/v), alkaline oxidation value (89.6), iodine value (556) and residual ethyl alcohol (0%). The efficiency of acetic acid production by using an optimized submerged fermentation process was 92.4%.

Originality/ Value: *Palmyrah toddy could be used for the production of natural vinegar under optimum conditions, and further field study has to be done to apply this finding at the industrial level.*

Keywords: *Acetic acid, Palmyrah toddy, submerged fermentation, Vinegar*

INTRODUCTION

Generally, synthetic vinegar available in the market does not contain any nutrition or medicinal value. Vinegar produced from different natural substrates called as natural vinegar has been used as a preparation condiment, as a preservative, flavoring, medicine and also a cleaning agent (Baena-Ruano *et al*. 2006; Qiu *et al*. 2010; Budak *et al*. 2014). Natural vinegar can be achieved from different raw materials such as apple, honey, rice, cereals, coconut water and coconut toddy, etc., having a good fermentation potential. In addition, the palmyrah toddy containing alcohol of around 3.5 to 6% (v/v) could be used as a substrate for the production of vinegar, which is a seasonal product and during the peak season of production, manufacturers only preserve the toddy through pasteurization. Nevertheless, the excess could be used for the synthesis of vinegar which leads to boosting the production efficiencies of toddy.

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So, this research was designed with the aim of production of vinegar using Palmyrah toddy as the main source of the substrate and its chemical analysis.

MATERIALS AND METHODS

Sample collection

Palmyrah toddy was collected from Achchuvelly Palm Development Cooperative society and used for this study. Corn cob was collected from a local market in Jaffna.

Isolation, selection and purification of acetic acid bacteria

Conical flasks (250 ml) containing pasteurized toddy were allowed to ferment until it reached optimum acetic acid content with and without corncob at room temperature (28±2 °C). Sample obtained from fermented medium which contained toddy with corn cob was inoculated into nutrient agar plate and incubated at 30 °C for 24 hours then colonies with different morphological characteristics were selected and purified with repeated streaking and named as S1, S2 and S3. Then selected bacterial colonies were inoculated into fermentation medium as toddy and corn cob also control experiment was maintained without bacterial strain.

Selection of best bacteria

Gram staining (Kaiser, 2001) and the Catalase test were used for the selection of strains.

Production of acetic acid using Orleans process

Orleans' process was carried out in three different treatments such as T1, T2, and T3 having toddy as a fermentation medium with triplicates. Corn cob was added to T1, T2 and activated inoculum containing Glucose (10 g), yeast extracts (1.2 g) peptone (0.5 g) and the strain was added to T1, T3. Then allowed to fermentation at room temperature (28 °C±2) and acidity was measured.

Production of acetic acid using Generator process

Fermentation medium containing corn cob was allowed to aerate using a pump that is used in the fish tank. The other flask was incubated without aeration to save as a control. All samples were allowed for fermentation at room temperature (28°C±2) and acidity was measured.

Production of acetic acid using the submerged fermentation process

The effect of temperature (28, 30 and 32 ˚C), shaking (200 rpm) and inoculum size (5, 10, 20 and 30 ml) on formation of acetic acid in submerged fermentation were studied.

Filtration and Pasteurization

The vinegar was filtered using whatman No 1 filter paper and bottled in sterile glass bottle then heated in a water bath at 65°C for 30 minutes.

Efficacy of acetic acid fermentation

Efficacy of acetic acid fermentation was determined according to Theivendirarajah and Chrystopher's method (1986).

Chemical Analysis

Determination of Acidity: The method of SLS: 168:1999 was used. The acidity of the sample was determined as acetic acid (% w/w) by titrating 10 ml of the sample against 0.1 N NaOH using phenolphthalein as an indicator.

Determination of Alcohol content: Alcohol content was determined directly for each sample using the ebulliometer Dujardin-Salleron at room temperature (28 ºC±2) and expressed in terms of percentage (v/v) (Christy *et al*. 2021).

pH: pH was determined by using a digital pH meter (Sension PH 31-Spain) (AOAC 973.41).

Determination of total solids: Total solids of the vinegar was determined by the oven-dry method (SLS: 168:1999).

Determination of permanganate oxidation value, alkaline oxidation value and iodine value:

Vinegar (60 ml) was distilled from a 350 ml flask fitted with a small tap funnel. Water 15 ml was added to the flask during distilled vinegar reaching 45 ml and distilled further 15 ml to give a total volume of 60 ml distillate.

Determination of permanganate oxidation value: Distillated (2ml) was added into the 250ml glass stoppered bottle then 25% sulfuric acid (10ml) and 15ml of 0.02mol/l potassium permanganate were added. They were allowed to stand for 30 minutes. Potassium iodide 10% solution (5ml) was added to the mixture. Liberated iodine was titrated with 0.02 mol/l sodium thiosulfate using starch as an indicator. The blank sample was also carried out simultaneously.

Permanganate oxidation value = 40 (V2- V1)

where,

V1 is the volume, in ml of sodium thiosulfate solution used in the titration, and

V2 is the volume, in ml of sodium thiosulfate solution used in the blank.

Determination of alkaline oxidation value: Distillated (2ml) was added into the 250ml glass stoppered bottle. Then distilled water (100ml), Sodium hydroxide 10% (10ml) and 0.02mol/l potassium permanganate (10ml) were added. They were allowed to stand for 30 minutes. Mixture was acidifying with 25% sulfuric acid (10ml). Titration was done for liberated iodine with 0.02 mol/l sodium thiosulfate using starch as indicator. The blank sample also carried out simultaneously.

Alkaline oxidation value = 8 (V4- V3)

where,

V3 is the volume, in ml of sodium thiosulfate solution used in the titration, and

V4 is the volume, in ml of sodium thiosulfate solution used in the blank.

Determination of iodine value: Distillated (5 ml) was added into the 250 ml glass stoppered bottle. Litmus paper was made just neutral with potassium hydroxide 10mol/l. Potassium hydroxide 1mol/l (10ml) and 0.1 mol/l iodine (10 ml) were added. They were allowed to stand in dark for 15 minutes. Sulfuric acid 25% solution (10 ml) was added into the mixture. Titration was done for liberated iodine with 0.02 mol/l sodium thiosulfate using starch near the endpoint. The blank experiment was carried out simultaneously.

Iodine value = 40 (V6- V5)

where,

V3 is the volume, in ml of sodium thiosulfate solution used in the titration, and

V4 is the volume, in ml of sodium thiosulfate solution used in the blank.

Determination of total phenolic content: The total phenolic content of the vinegar samples was determined using the Folin-Ciocalteu reagent (Singh and Maurya, 2010). The reaction mixture contained 0.5 ml of diluted samples, 2.5 mL of freshly prepared 10 % diluted Folin-Ciocalteu reagent and 2 ml of 7.5% sodium carbonate. Mixtures were kept at ambient conditions for 30 min to complete the reaction. The absorbance at 760 nm was measured. Gallic acid was used as standard and the results were expressed as mg gallic acid (GAE)/ml vinegar.

Statistical analysis

Results were analyzed using one-way ANOVA by complete randomized design. The significant difference between the treatment was analyzed using Turkey comparison and the significant difference between control and treatment was tested using Dunnett comparison in the least significance at 5% level using Minitab 17 software.

RESULTS AND DISCUSSION

The fermentation of vinegar basically consists of two steps, the first one being the anaerobic conversion of fermentable sugars to ethanol by the yeasts, generally Saccharomyces species, and the second step aerobic oxidation of ethanol to acetic acid by the bacteria, usually Acetobacter species (Adams, 1998; Horiuchi *et al*. 2000). Palmyrah toddy is spontaneously fermented sap consisting in the range of 5-6% of alcohol obtained from the young and mature inflorescence of male and female palms (Kumuthini and Theivendirarajah. 1988; Mahilrajan *et al*. 2014). This is an excellent starting material for production of natural vinegar that generally contains more medicinal values than synthetic vinegar available in the market. Corn cob was dried at 50 ˚C for 48 hours, to kill the wild yeast and other microorganisms (San Chiang Tan, 2005). To prevent moisture absorption and further contamination, it was packed using a sterile polythene bag and then used for the isolation of acetic acid bacteria.

Isolation of acetic acid bacteria

Toddy with corn cob showed a significant increase in acetic acid production until 6th day of fermentation and showed 3.27% of acetic acid as the highest production (Figure 01). Toddy used in this study was pasteurized, there were no live bacteria to produce acetic acid. Hence, corn cob was not a sterile material. It had live bacteria and served as an inoculum, which resulted production of acid and formed a thick slime coating around a non-compacting material like corn cob (Figure 02). While in the toddy without corn there was no surface area for the growth of bacteria. Therefore, the production of acetic acid was less, when compared with corn cob added toddy.

Acidity obtained from the medium having toddy inoculated with S1, S3 and control (without bacterial strain) was significantly increased while S2 was significantly decreased with the fermentation period. Besides S3 showed the highest (2.7%) acetic acid production at 7th day of fermentation (Figure 03) further-more it produced jelly-like mass in fermentation medium, among the selected strains.

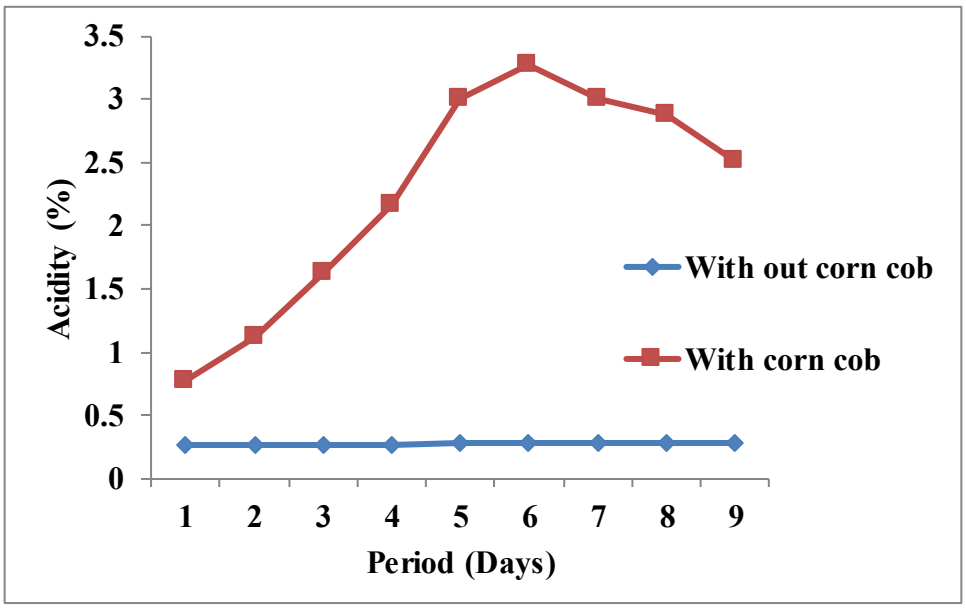

Figure 01: Effect of corn cob on formation of acetic acid

Figure 02: Formation of thick gel like layer in toddy with corncob

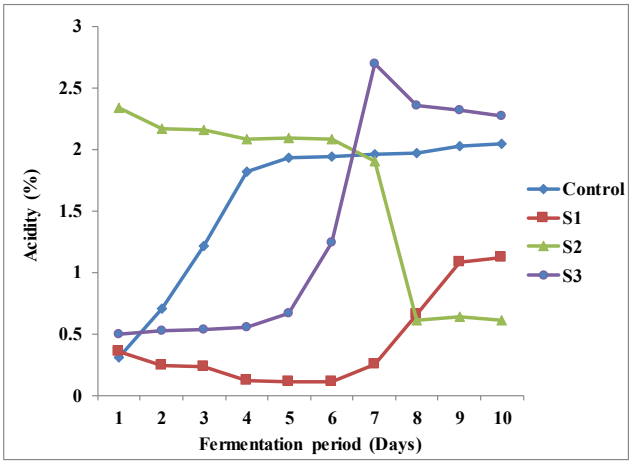

Figure 03: Production acetic acid by selected bacterial strains

Selection of best bacteria

Selected bacterial strain S3 showed pale to off-white colonies and shape were circular on culture plate. Identified as gram negative, ellipsoidal, rods and also catalase test showed positive for catalase test and produced bubbling in the presence of hydrogen peroxide. Acetic acid bacteria are needed aerobic metabolism by oxygen as the terminal electron acceptor and Gram-negative showed pink in colour, ellipsoidal to rod-shaped cells (Gonzalez *et al*. 2004).

Production of acetic acid using Orleans process

The Orleans process was the simple way to make pure wine vinegar (Mitchell 1916), and was stated to be the top method to harvest fine quality table vinegar (Hickey and Vaughn 1954).

Fermentation of toddy with only corncob (T2) showed 3.37% of acetic acid formation and corn cob with activated inoculum (T1) showed significantly the highest acetic acid production (4.01%) at 5th days of fermentation while other without corn cob but contained activated inoculum (T3) showed 3.98% of acetic acid production. Fermentation medium with both corncob and activated inoculum showed a high amount of alcohol when compared with others because that corn cob serves as righteous media for the multiplication of the organism.

Production of acetic acid using Generator process

Mean acetic acid formation was significantly the highest (2.2%) in non-aerated fermentation medium when compared with aerated (0.6%) fermentation medium (Figure 04). During the process both aerated and non-aerated fermentation medium showed a significant decrease in alcohol content. In the aerated flask acetic acid production was less but alcohol content continuously decreased. This may be due to the over oxidation of Acetic acid that aerating pump did not contain any air flow controller. During the over oxidation, acetic acid is easily oxidized to carbonic acid gas and water with loss of aroma and flavour.

Production of acetic acid using the submerged fermentation process

Effect of temperature on the formation of acetic acid

Low or fluctuating temperatures may lead to the slow process and a high temperature can cause the destruction of bacteria. In this experiment, there were no significant differences in acetic acid formation between the fermentation temperature at 28, 30 and 32 ^{o}C. The formation of acetic acid was significantly increased with the fermentation period for all the fermentation temperatures and 4.03, 4.23 and 4.17% of the highest acetic acid was produced respectively (Figure 05). For further study 30^{o}C for selected as the optimum temperature.

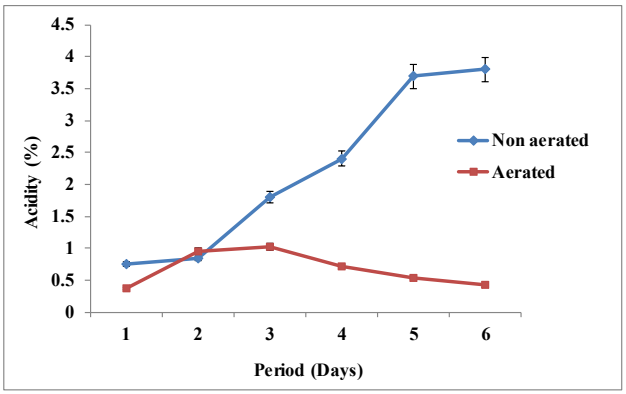

Figure 04: Production of acid during aerated and non-aerated process

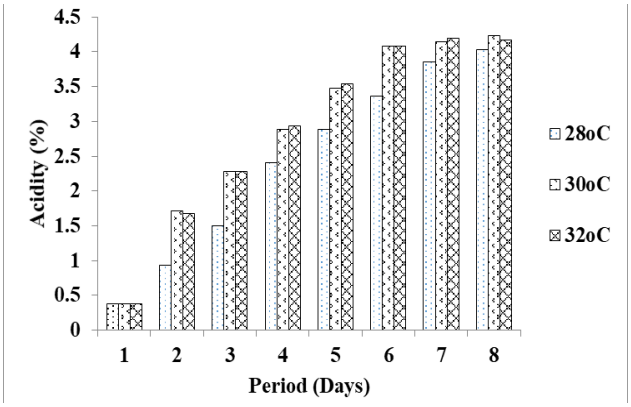

Figure 05: Changes in acetic acid formation at different temperature

Effect of shaking on the formation of acetic acid

The formation of acetic acid was significantly increased with the fermentation period for both shaking and without shaking and produced the highest 3.86, 3.14 % of acetic acid at the 8th day of fermentation respectively (Figure 06).

Effect of inoculum size on formation of acetic acid

There were no significant differences in the formation of acetic acid (3.74%) between the size of the inoculum, whereas, that of acetic acid formation showed a significant difference during the fermentation period. Inoculums size, such as 5, 10 and 20 % showed the same acetic acid production (4.5%) at 8th, 5th and 7th day of fermentation respectively while 30 % inoculums size produced less amount of (4.3 %) of acetic acid when compared with other inoculums size at a 4th day of fermentation (Figure 07). Therefore, inoculums size 10% were selected for further study. This result showed that selected bacteria have the ability to oxidize significant amounts of ethanol under the acidic conditions formed by the presence of acetic acid and agreed with the study of Hickey Richard and Vaughn Reese (1954).

Efficacy of acetic acid fermentation

Generally, efficiency of acetic acid production was increased from Orleans process to submerge process. In this study over oxidation of ethanol takes place during generator process and showed less efficiency when compared with Orleans process because aerated pump that used for this study has a uniform rate of aeration that cannot adjust the follow rate. Submerged fermentation condition was showed 92.4% (Table 01) of efficiency with 10% of activated inoculum under shaking at 30 °C.

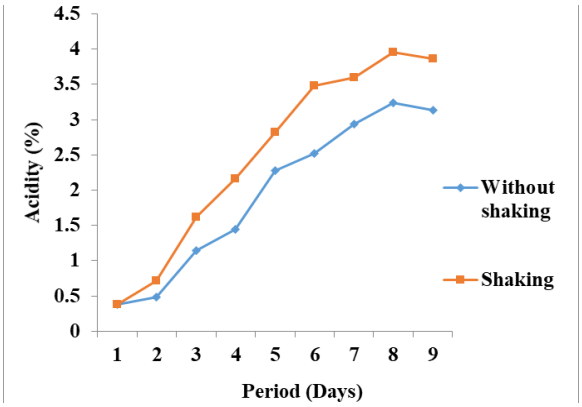

Figure 06: Formation of acetic acid with and without shaking

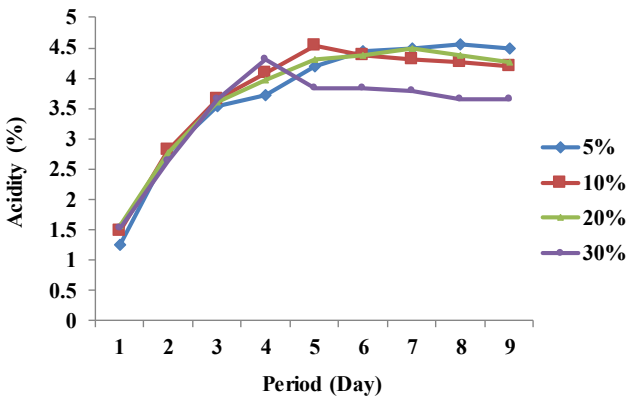

Figure 07: Formation of acetic acid during fermentation with different inoculums size

Table 01: Efficiency of different process of vinegar production

Process	Alcohol (%)	Expected value (v/v)	Observed value (v/v)	Efficiency (%)
Orlean process	4.6	4.51	4.01	88.9
Generator process	5.0	4.9	3.80	77.6
Submerged process	5.0	4.9	4.53	92.4

Table 02: Chemical characteristics of vinegar from Palmyrah, coconut and their requirements

	Palmyrah	Coconut	SLS 168 :1999 requirement
Total acidity (g/100 ml)	4.32	4.9	Min 04
Total solids (g/100 ml)	4.08	4.4	Min 01
Alkaline oxidation value	89.6	243.2	Min 80
Iodine value	556	1896	Min 160
Residual ethyl alcohol (% v/v)	0	0	Max 01
pH	2.85	3.05	-

Chemical Analysis

Required characteristics of coconut toddy vinegar is considered in SLS 168:1999. Based on the standard, chemical analysis of palmyrah vinegar which is obtained from palmyrah toddy, formed, by using optimum submerged condition and commercial coconut vinegar were carried out. All the characteristics of coconut and palmyrah vinegar are listed in the (Table 02). All the results obtained from the analysis of coconut and palmyrah vinegar listed in Table 02 and palmyrah vinegar showed total acidity (4.32%), total solids (4.08 (w/v)), alkaline oxidation value (89.6), iodine value (556) and residual ethyl alcohol (0%) also revealed permanganate oxidation value (428) and total phenolic content (5.54mg/100ml) and besides, complied with SLS 168:1999.

CONCLUSIONS

In this study, acetic acid-producing bacteria were selected by using corncob, based on the acetic acid production, morphological characterization also the formation of gel-like layers in the fermentation medium. Among the three processes of acetic acid production, the submerged process yielded the highest amount of acetic acid (4.53%) at the 5th day of fermentation and showed 92.4% of efficiency. Therefore, palmyrah toddy could be a suitable substrate for the production of natural vinegar with high efficiency and a short fermentation period besides chemical characteristics of vinegar formed using Palmyrah toddy, complied with the SLS.

Competing interests

The authors declare that they have no competing interests.

Authors' contribution

JS- Internal supervisor of the research project; SM External supervisor; SK- Undergraduate research student; SK & SM carried out the research activity on production of vinegar and chemical analysis and carried out statistical analysis; SK, SM &JS - Drafted the manuscript. All authors read and approved the final manuscript.

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REFERENCES

Adams, M.R. (1998) vinegar. In: Wood BJB (ed) microbiology of fermented food. Blackie academic and professional, London, 1: 1-44.

Baena-Ruano, S. Jiménez-Ot, C. Santos-Dueñas, IM. Cantero- Moreno, D. Barja, F. and García-García, I. (2006). Rapid method for total, viable and non-viable acetic acid bacteria determination during acidification process. Process Biochemistry,41(5), pp: 1160-1164. DOI: http://dx.doi.org/10.1016/j.procbio.2005.12.016.

Budak, N.H. Aykin, E. Seydim, AC. Greene, A.K. and Guzel-Seydim, Z.B. (2014). Functional properties of vinegar. Journal of Food Science, 79(5), pp. 757-764. DOI: http://dx.doi.org/10.1111/1750-3841.12434.

Christy, E.J.S.B.A. Mahilrajan, S. Chandrasena, G. and Kapilan, R. Bioethanol production from Palmyrah (Borassus flabellifer) wastes using yeast (2021), J. Natn. Sci. Foundation Sri Lanka 49 (4): pp 609. DOI: http://dx.doi.org/10.4038/jnsfsr.v49i4.10828.

Gonzalez, N. Hierro, M. Poblet, N. Rozes, AM. Guillamon, JM. 2004. Appilcation of molecular methods for the differentiation of acetic acid bacteria in a red wine fermentation. Journal of Applied Microbiology 96. pp: 853-860. https://doi.org/10.1111/j.1365-2672.2004.02220.x

Hickey-Richard, J. Vaughn-Reese, H. (1954). Industrial Fermentation: Chemical Publishing Co. Inc. New York, Chapter 17, Volume 1, pp: 498-535.

Horiuchi, J. I., Kanno, T., and Kobayashi, M. (2000). Effective onion vinegar production by a two-step fermentation system. Journal of bioscience and bioengineering, 90(3): 289- 293.

Kaiser, G. (2001). Prokaryotic cell structure: The Gram Positive cell wall. In: Kaiser G (ed) Doc Kaiser's Microbiology. BC Publishing Company, India, pp. 160–184.

Kumuthini, C.R. and Theivendirarajah, K. (1988). Palmyrah palm wine II: Improvements in alcohol production., J. Natn. Sci. Coun. Sri Lanka, 131, pp: 147-157.

Mahilrajan, S. Kailayalingam, R. Srivijeindran, S. and Navaratnam P. (2014). Improving the quality of palmyarh bottled toddy and its partial clarification. In Proceedings of the Peradeniya Univ. International Research Sessions, Sri Lanka, 4-5 July. p 520.

Mitchell, C.A. Oxon, B.A. FIC. (1916). Vinegar: Its manufacture and examination. Exeter Street, Strand: Charles Griffin and Company. pp: 100.

Qiu, J. Ren, C. Fan, J.and Li, Z. (2010). Antioxidant activities of aged oat vinegar in vitro and in mouse serum and liver. Journal of Science Food and Agriculture, 90(11), pp. 1951-1958. DOI: http://dx.doi.org/10.1002/jsfa.4040.

Singh, D. and Maurya, S, (2010).Quantitative analysis of total phenolic content in Adhatoda vasica nees extract. Int J Pharm Tech Res, 2. 2403-2406.

Sri Lanka Standards Institution, (1999). Coconut vinegar, 168.

Tan, San Chiang, (2005) "Vinegar fermentation". LSU Master's Theses. 1225. https://digitalcommons.lsu.edu/gradschool_theses/1225.

Theivendirarajah, K. and Chrystopher, RK.(1986). Chemical analysis of Palmyarh palm (*Borassus flabellifer*) wine. Vingnanam: Journal of Science, 1,pp:1-7.