Physico-chemical Changes in Palmyra Palm (*Borassus flabellifer*) sap at different temperature

Ronald Valder, Krishna Prasad Nooralabettu*

Abstract— Freshly tapped palm sap is nutritionally rich but contamination during tapping process degrades these components. Changes in physico-chemical characteristics of freshly tapped palm sap were studied at various temperatures. The pH reduce from 7 to 4, which correlates well with the changes in the colour of the palm sap from clear liquid to brownish. Protein content and Vitamin C content dropped by more than half at the end of 25 min, but no changes in lipid content was observed. Sugar hydrolyzed at and above 110°C and sucrose hydrolysed above 80°C at pH 7. Highly reactive monosaccharide formed during disaccharide hydrolysis at elevated temperature was not detectable due to the fragmentation of monosaccharide in the early Milliard reaction. Yeast population reduced by 100 folds and lactic acid bacteria reduced by 20 folds in first 5 minutes of exposure at 60°C of moist heat. Present study gives valuable information for the intervention of the palm sap from fermentation.

Index Terms— Palmyra Palm, sterilization, monosaccharide, *Borassus flabellifer*, disaccharides, Toddy, Palm sap. .

1 INTRODUCTION

Toddy is a popular alcoholic beverage available along coastal Karnataka, India is tapped from the tip of the inflorescence of Palmyra palm (*Borassus flabellifer*) tree. Palm sap is sweet when freshly tapped, but fermentation starts with 6-8 hours due to the contamination as the clear sap flows through the cut inflorescence colonized with microbes and latter when it comes in contact with the contaminated collecting earthen pot and insects [1], [2], [3]. Freshly tapped palm sap is very good media for the microbes to grow and microbes proliferates very fast. Palm sap becomes increasingly heady as the day progresses to the level that a glass of Toddy is intoxicating and detrimental to the society. However, fresh palm sap has nutritional, medicinal, religious and social uses that increase the demand for this beverage. Socio-economic aspect of the section of the society in Mangalore and Udupi district is depends on this industry. Freshly tapped palm sap is clear, less viscous, and sweet with the pH of neutral and nutritionally rich [4]. Fresh palm sap is rich in sucrose and traces of reducing sugar such as glucose and fructose and reduces to more than half by microbes within 24 hours resulting in the production of lactic acid and acetic acid along with the ethanol. These acids reduces the pH of the palm sap to about 5 making the palm sap unacceptable to drinking [5], [6] [7], [8]. In one hand palm sap is nutritionally rich and on the other hand palm wine is a rich source of number of commercially important yeast and lactic acid bacteria [9], [10], [11]. Knowledge of these microbes and thermal degradation of these microbes with minimum hydrolysis of nutritional components gives crucial information to intervene the fermentation of sweet palm sap to produce health drink.

- *First author Ronald Valder is currently pursuing Doctoral degree program in Biotechnology Engineering in Visvesvarayya Technological University, Belgum, India, PH-+0919964277520. E-mail: ronald_bio@pace.edu.in*
- *Corresponding author Krishna Prasad Nooralabettu is currently working as Professor and Head, Department of Biotechnology, P. A. College of Engineering, Mangalore, India, PH-+0919448529048. E-mail: hod_biotech@pace.edu.in*

2 MATERIALS AND METHODS

2.1 Sample Collection

Nine palm (*Borassus flabellifer* L.) trees were identified in Sajipa of Dakshina Kannada District (Karnataka, India). Freshly tapped palm sap was collected at around 6.00 AM over 14 tapping process in the month of December. Under sanitary condition, 50 mL of the samples are collected directly from the palm sap collection earthen pot into a sterile 50 mL sample collection vessels. Temperature of the environment varied from 18 to 24°C and within 30 min samples were transported to the laboratory of the Department of Biotechnology, P. A. College of Engineering, Mangalore, in an insulated container maintained at 4°C until analysis. Samples were immediately filtered using sterile muslin filter cloth and maintained at 4°C until analysis, and if any delay then preserved at –50°C in a deep freezer (Model C340, New Brunswick Scientific, England).

2.2 Chemicals

Analytical grade chemicals were procured from Merck Limited (Mumbai, India), and reagents were prepared as per the current American Chemical Society specifications [14]. Nutrient agar, Yeast Extract Potato Dextrose Agar (YEPDA), Yeast Extract Potato Dextrose Broth (YEPDB), deMan, Rogosa and Sharpe (MRS) Agar, deMan, Rogosa and Sharpe (MRS) broth were procured from Himedia, Mumbai and prepared as per manufacturer's instructions. Media was sterilized by autoclaving at 15 lbs pressure at 121 °C for 15 min and after inoculation plates were incubated at 37 °C in incubator. The colonies are enumerated using digital colony counter (Systronics, Mumbai).

2.4 Sterilization study of the palm sap

To study the effectiveness of moist heat in sterilizing the palm sap without degrading the nutritional components of palm sap, samples were exposed to moist heat of 60, 70, 80, 90, 100, 110 or 120°C for time intervals of 5, 10, 15, 20 or 25 min.

Sterilization was carried out in water bath (Rotek Instruments, Kerala), pressure cooker (Prestige, India) or autoclave (Rotek Instruments, Kerala) in 250 ml capacity flask at varying process parameters. Properly plugged and wrapped flasks were arranged in the water bath, pressure cooker or autoclave with sufficient space to facilitate the moist heat penetration. Palm sap exposed to various temperatures for different intervals of time was then analyzed for total protein content, total lipid content, Vitamin C, reducing sugar, non-reducing sugar, glucose, sucrose, ethanol, mould count and bacterial count to optimize the parameter.

2.3 Proximate analysis during sterelization

At different intervals of experiments, palm sap samples were collected in quadruplicates. Cell free samples were prepared by centrifuging the samples at relative centrifugal force (RCF) of 1681.1 x *g* for 5 min at 4°C (C-24BL/CRP24 model centrifuge, Remi Laboratory Instruments, Mumbai, India). The pH was measured using Portable Glass electrode pH meter (Systronics, Mumbai). Incubation of the samples at 30°C was carried out in an incubator (Rotek Instruments, Kerala). Temperature of the samples was estimated using infra-red thermometer (Quicktemp 826 T4, Austria). Absorbance of the cell free samples was measured in 1 cm Quartz cell at 420 nm with a visible spectrophotometer (Systronics, Mumbai). Total protein content in the samples exposed to moist heat of 60, 70, 80, 90, 100, 110 or 120°C (TPC-60, TPC-70, TPC-80, TPC-90, TPC-100, TPC-110 and TPC-120, respectively) at intervals of 5, 10, 15, 20 or 25 min were estimated by Lowry's method and values were expressed in mg/mL[15]. Total lipid content in palm sap at 60, 70, 80, 90, 100, 110 or 120°C (LPC-60, LPC-70, LPC-80, LPC-90, LPC-100, LPC-110 and LPC-120, respectively) for time intervals of 5, 10, 15, 20 or 25 min were estimated after extraction with chloroform ethanol method followed by reaction with sulfuric acid and vanillin phosphoric acid reagent and values were expressed in percentage (w/v) [16]. Vitamin C content in the samples at 60, 70, 80, 90, 100, 110 or 120°C (VC-60, VC-70, VC-80, VC-90, VC-100, VC-110 and VC-120, respectively) for time intervals of 5, 10, 15, 20 or 25 min were estimated by Redox Titration methods using 2, 4-dinitrophenyl hydrazine (DNPH) dye and standard ascorbic acid, and values were expressed as mg/mL [Reducing sugar in palm sap at 60, 70, 80, 90, 100, 110 or 120°C (RS-60, RS-70, RS-80, RS-90, RS-100, RS-110 and RS-120, respectively) for intervals of 5, 10, 15, 20 or 25 min were estimated dinitrosalicylic acid reagent, and values were expressed in percentage (v/v) [18]. Glucose and Sucrose at 60, 70, 80, 90, 100, 110 or 120°C (GU-60, GU70, GU-80, GU-90, GU-100, GU-110 and GU-120, and SU-60, SU70, SU-80, SU-90, SU-100, SU-110 and SU-120, respectively) for at 5, 10, 15, 20 or 25 min were estimated using High sensitive Glucose and Sucrose Assay kit provided by EMerck, India, and values were expressed in percentage (w/v). Changes in ethanol content in the palm sap was estimated at different intervals of time at different temperature based on the colorimetric reaction of ethanol with sodium dichromate, and values were expressed in percentage (v/v) [19]. Changes in mould count and bacterial count of the palm sap at 60, 70, 80, 90, 100, 110 or 120°C (TMC-60, TMC70, TMC-80, TMC-90, TMC-100, TMC-110 and TMC-120, and MBC-60, MBC-70, MBC-80, MBC-90, MBC-100, MBCU-110 and MBC-120, respectively)were performed as per APHA method and values were expressed in cfu/mL [20]. The samples were collected and analysed in quadruplicate and data was analysed by One way-analysis of variance (ANOVA) using the Fisher's least significant difference (LSD) test to estimate the significant differences between each sample (P≤0.05) using Statgraphics Centurion XV software (Statpoint Technologies Inc., Warrenton, VA, USA).

3 RESULTS

3.1 Changes in physical characteristics of palm sap

Changes in the pH and associated colour change in the palm sap kept at 30°C and exposed to 60, 70, 80, 90, 100, 110 or 120°C of moist heat for 5, 10, 15, 20 or 25 min is illustrated in the figure 1.

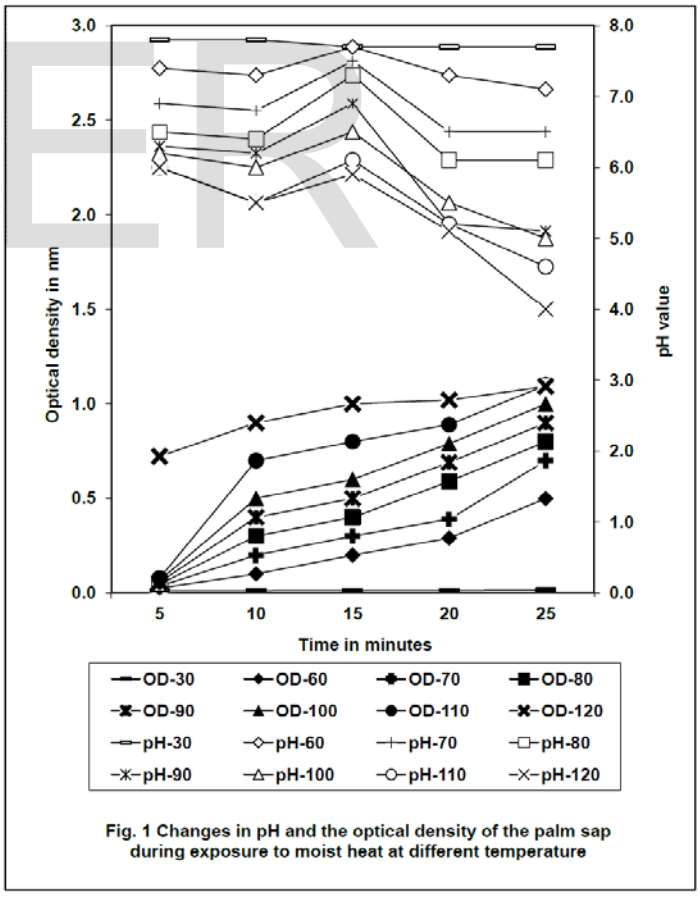

Fig. 1 Changes in pH and the optical density of the palm sap during exposure to moist heat at different temperature

The pH of the fresh samples was ranging from 7 to 7.4 and is near to neutral. At 30°C of incubation, pH of the sample was recorded are 7.8±0.07, 7.8±0.09, 7.7±0.08, 7.7±0.07 and 7.7±0.09, during 5, 10, 15, 20 or 25 min, respectively. During 25

minutes of exposure to moist heat at 60, 70, 80, 90, 100, 110 or 120°C the pH of the palm sap decreased, respectively to 7.1±0.5, 6.5±0.4, 6.1±0.3, 5.1±0.4, 5.0±0.1, 4.6±0.5, and 4.0±0.3. Palm sap incubated at 30°C did not record any significant (p>0.05) level of change in pH during 30 min of incubation, as indicated by One way ANOVA with *post hoc* Tukey's test. Whereas, One way ANOVA with *post hoc* Tukey's test was able to establish a significant (p<0.05) difference in the pH values of the samples exposed to moist heat at 60, 70, 80, 90, 100, 110 or 120°C, in comparison to the fresh samples and the samples incubated at 30°C for 25 min. Absorbance of the fresh palm sap was 0.01±0.004 nm at 420 nm. The sample that was kept in the incubator at 30°C recorded the absorbance of 0.02±0.007, 0.02±0.009, 0.03±0.008 0.03±0.007, and 0.04±0.009 nm during 5, 10, 15, 20 or 25 min of incubation, respectively. When samples were exposed to 60, 70, 80, 90, 100, 110 or 120°C the absorbance of the palm sap was 0.489±0.5, 0.698±0.4, 0.798±0.3, 0.898±0.4, 0.998±0.1, 1.068±0.5, and 1.098±0.3 nm, respectively. Here, overall significant effect of moist heat on the increase in absorbance at all thermal treatment samples remained at 5% level of significance, as indicated by One way ANOVA with *post hoc* Tukey's test.

3.2 Changes in biochemical characteristics of palm sap

Changes in the total protein content in the palm sap kept at 30°C, and those samples exposed to 60, 70, 80, 90, 100, 110 or 120°C temperatures of moist heat for 5, 10, 15, 20 or 25 min is illustrated in the figure 2.

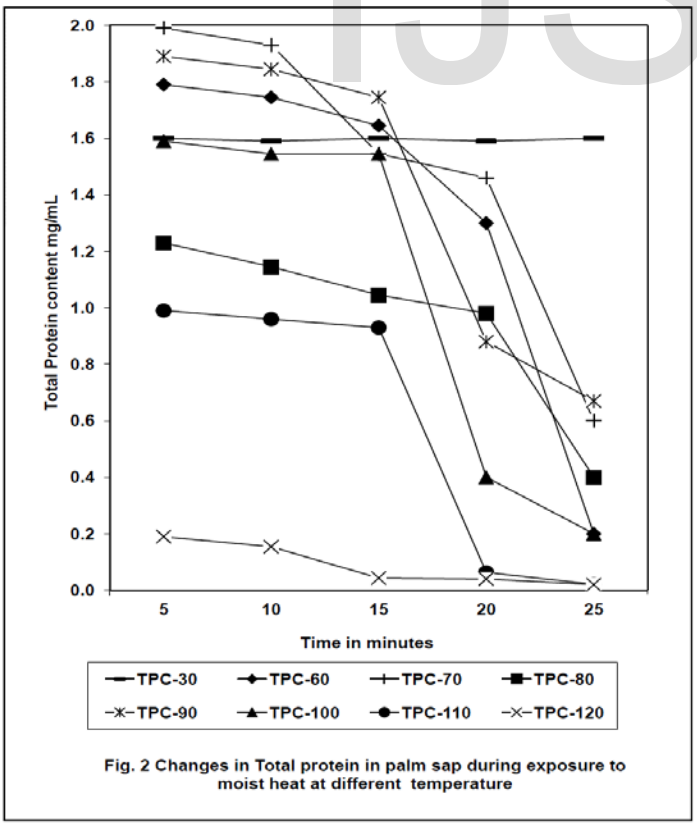

Fig. 2 Changes in Total protein in palm sap during exposure to moist heat at different temperature

Protein content in samples kept at 30°C was 1.60±0.05, 1.56±0.04, 1.44±0.06, 0.9±0.07 and 0.6±0.04 mg/mL, during 5, 10, 15, 20 or 25 min, respectively. On 25 minutes of exposure of palm sap to moist heat at 60, 70, 80, 90, 100, 110 or 120°C, the protein content decreased to 0.20±0.5, 0.60±0.4, 0.40±0.3, 0.67±0.4, 0.20±0.1, 0.02±0.5, and 0.02±0.3 mg/mL, respectively. Total protein content of the sample at 30°C did not vary significantly (p>0.05) during 30 min of incubation, as indicated by One way ANOVA with *post hoc* Tukey's test.

Changes in the Vitamin C and total lipid content in samples storage at 30°C and in samples sterilized at 60, 70, 80, 90, 100, 110 or 120°C for 5, 10, 15, 20 or 25 min is illustrated in the figure 3.

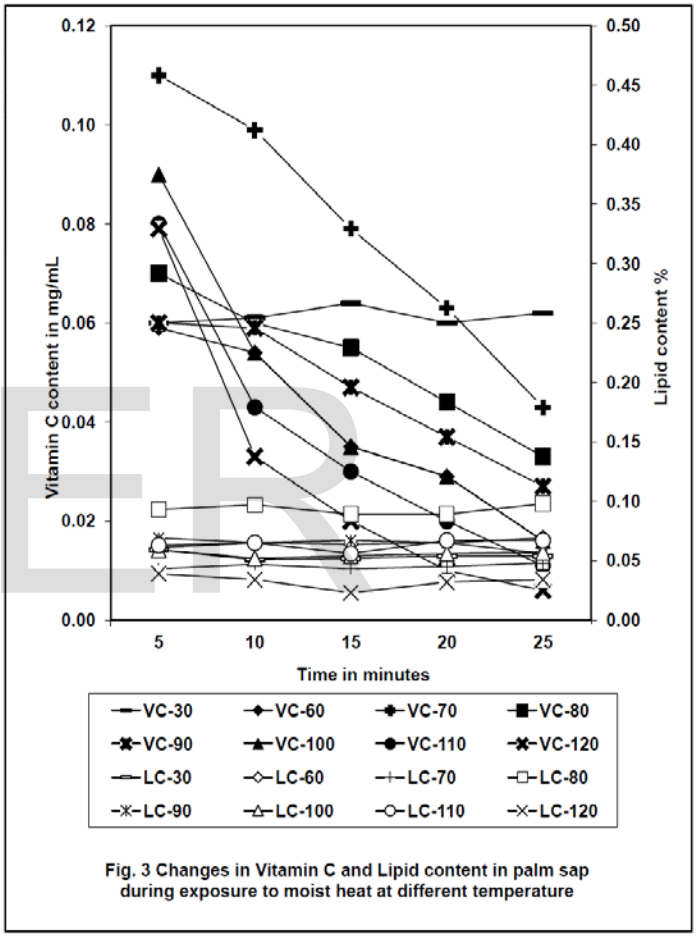

Fig. 3 Changes in Vitamin C and Lipid content in palm sap during exposure to moist heat at different temperature

Vitamin C in palm sap during storage at 30°C remained constant at level of 0.61±0.001 mg/mL during 25 min of storage. Thermal reduced the Vitamin C levels to 0.016±0.5, 0.043±0.4, 0.033±0.03, 0.027±0.03, 0.016±0.01, 0.011±0.05, and 0.003±0.02 mg/mL, respectively. Here, overall significant effect of moist heat on the thermal degradation of protein remained at 5% level of significance, as indicated by One way ANOVA with *post hoc* Tukey's test.Total lipid content of the sample did not vary significantly (p>0.05) during at 30°C for 30 min, as indicated by One way ANOVA with *post hoc* Tukey's test.

Changes in sucrose and glucose in the palm sap kept at 30°C and in those samples exposed to 60, 70, 80, 90, 100, 110 or 120°C temperatures of moist heat for 5, 10, 15, 20 or 25 min is

illustrated in the figure 4. Total sugar content in the fresh palm sap was varying between 09.88±0.08% and 17.32±0.04%. Non-reducing sugar was varying between 8.49±0.06% and 2.64±0.01%, wich is mainly sucrose. Reducing sugar in the palm sap was varing between 1.38±0.03% and 2.64±0.01%, of which percentage of glucose varied from 0.69±0.01% to 01.32±0.06%, and non-glucose reducing sugar varied from 0.53±0.02% to 1.32±0.06%. Total sugar content was 86.41±0.99% of non-reducing sugar Of the various reducing sugar contents of the palm sap samples, 49.84±0.14% of total reducing sugar was glucose and 40.46±3.08% of it is non-glucose. In one hand, more than 85% of sugar content of the palm was lost at and above 110°C, and on the other hand nearly 95% of sugar content of the palm was lost at and above 80°C. Sugar content in the palm sap did not vary significantly (p>0.05) either during 30 min of incubation at 30°C, or during sterelization below or at 100°C for 25 min as indicated by One way ANOVA with *post hoc* Tukey's test. However, One way ANOVA with *post hoc* Tukey's test was able to establish a significant (p<0.05) difference in sucrose in samples exposed to 110 or 120°C for 25 min, in comparison to the samples incubated at 30°C or at or below 100°C.

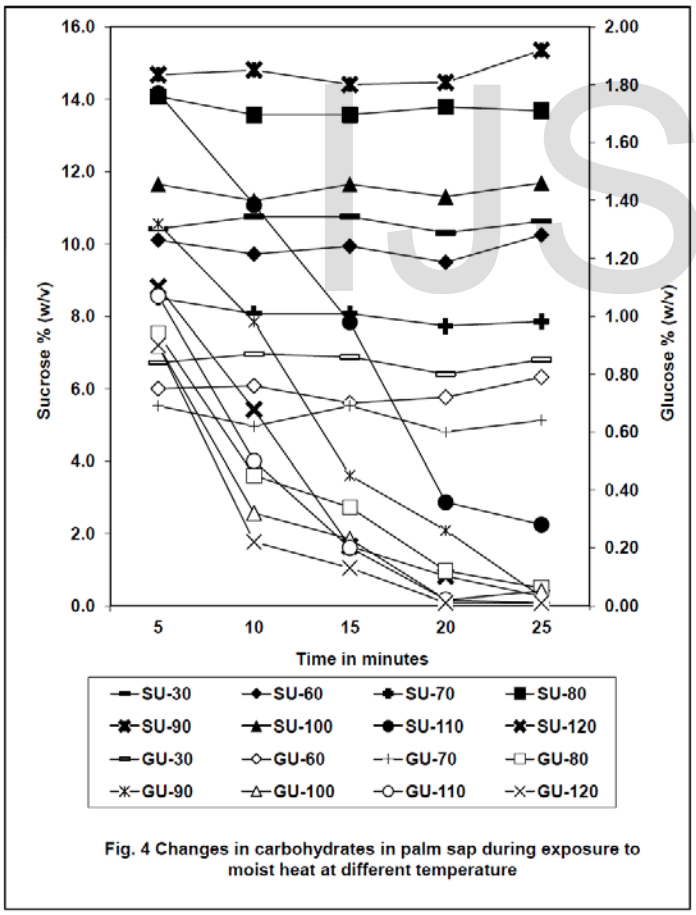

Fig. 4 Changes in carbohydrates in palm sap during exposure to moist heat at different temperature

3.3 Changes in microbial characteristics of palm sap

The growth of microorganisms in fresh palm sap samples at 30°C with and without moist heat treatment was investigated. Samples of palm sap was recorded the initial microbial load of 10^5 cfu/mL on MRS agar and 10^6 cfu/mL on YEPDA agar. Different batch of samples were thermally treated at 60, 70, 80, 90, 100, 110 or 120°C of moist heat for 5, 10, 15, 20 or 25 min. Lactic acid bacterial load reduced by 20 folds at 60°C for 5 min. For the same thermal treatments, yeast populations were reduced by more than 100 folds at 60°C in 5 min. The results of this study on palm sap demonstrate that Yeast population is more sensitive than the lactic acid bacterium in for thermal treatment.

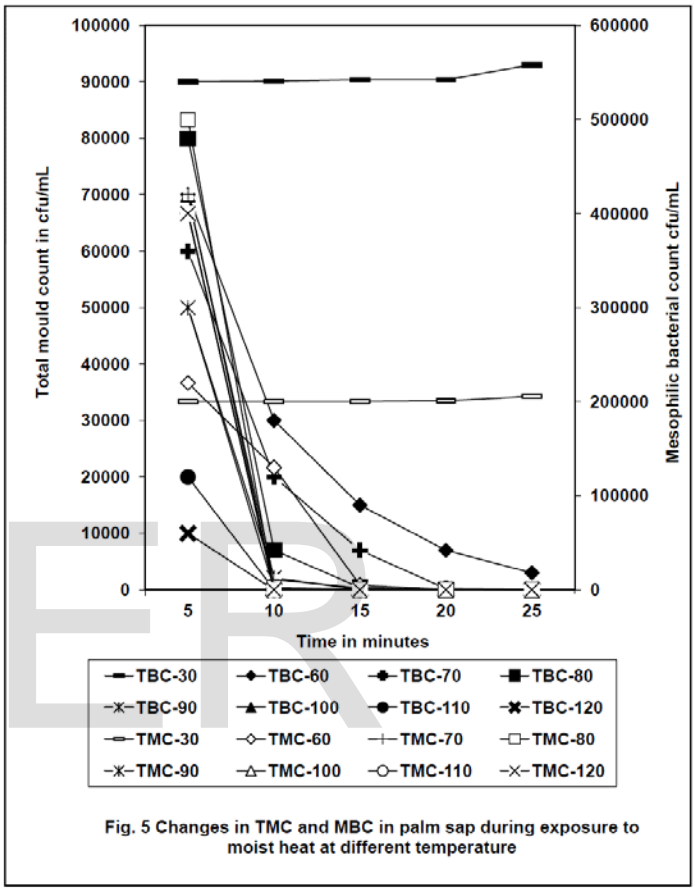

Fig. 5 Changes in TMC and MBC in palm sap during exposure to moist heat at different temperature

4 DISCUSSION

Freshly tapped palm sap collected from nine different palm trees was clear, less viscous, and sweet. During thermal exposure colour of the palm sap changed from colourless to whitish, milkish white, off white, brownish solution, and the absorbance increased from 0.012 to 1.098 nm at 25 min of processing, which was associated with inverse decrease in the pH values from 7.8 to 4.0 through the process. It is interesting to note here that the pH of the medium increased slightly at 15 min of the exposure, which might be due to the decarboxylation and CO_2 evolution from the palm sap. Degradation of proteins into amino acids, and amino acids into amino groups is responsible for Browning colour through Milliard reaction. Increment in absornbance combined with decrement in pH are characteristics of Milliard reaction [21]. Brown colour during Milliard reaction is due to degradation of sucrose due to thermal heat and building up of melanoidins skeleton produced out of degraded sugar product [22], [23]. The pH of the medium is known to influence the colour and overall aroma [24].

Sterilization of palm sap shifted the pH of the medium from neutral to acidic side, and this process depends on the initial pH of the medium. Thermally degraded products of glucose and sucrose in combination with various nitrogenous compound forms a complex that inhibits the growth of microbes, but there are published results that shows that sterilization actually produces growth stimulant specific for yeast [25], [26]. Reports show that temperature of 150°C hydrolyses carbohydrates to reducing sugars in *Arenga pinnata* that affected the overall quality of the sugar and colour of the sap. There are few reports suggest that heating process increases fructose in sap but it increase with increase in acids that may be due to the inversion of glucose to fructose [27]. Published work suggests that at pH 8.3 and dissolved solids of 65% sucrose degraded at 100°C within 8 h, and under same condition glucose and fructose degraded at 80°C within 2 h [28]. However, aqueous reaction conditions leads to the formation of more reactive hydrodynamic degradation of carbohydrates to high reactive monosaccharide such as such glucose and fructose. But these monosaccharide precipitates into large pool of C2, C3, and C4 dicarbonyl compounds by the catalytic activity of highly activity of amino groups formed from the amino acids in the early stage of milliard reaction [27]. In our study even lower temperature of moist heat degraded Vitamin C on long period of exposure to moist heat [29]. However, no palm sap samples registered any change in lipid content and registered any traces of alcohol content during storage at 30oC or during the thaemal treatment. It is interesting to register here that the during thermal treatments yeast populations decreased by more than 100 folds in 60°C for 5 min and lactic acid bacterial load reduced by 20 folds at 60°C for 5 min. The results of this study on palm sap demonstrate that Yeast population is more sensitive than the lactic acid bacterium in for thermal treatment.

5 CONCLUSION

Freshly tapped palm sap is sweet and clear, but microbial activity changes it to milky white and sour. Degradative activity of yeast and lactic acid bacteria can be intervened by thermal treatment by moist heat below, at and above This study showed that pH of the medium reduced during thermal treatment at and above 80°C with the samples more translucent and milkish whit to brownish white. Vitamin C, protein and glucose thermally degraded above 60°C at pH 7. Lactic acid bacteria and yeast in the palm sap destroyed at 60°C at pH 7 for 25 min. Present study gives valuable information for the intervention of the palm sap from fermentation.

REFERENCES

[1] W.K. Amoa-Awua, E. Sampson and K. Tano-Debrah, "Growth of yeasts, lactic and acetic acid bacteria in palm wine during tapping and fermentation from felled oil palm Elaeis guineensis in Ghana," *J Appl Microbiol*, vol. 102, no. 1, pp 599-606, 2007.

[2] P. Naknean and M. Meenune, "Impact of Clarification of Palm Sap and Processing Method on the Quality of Palm Sugar Syrup (*Borassus Flabellifer Linn*.)," *Sugar Tech*, vol. 17, no. 1, pp. 195, 2015.

[3] R. Poché, R. Garlapati, K. Dixon, A. Gendernalik, D. Poché, D. Perry, "The role of Palmyra palm trees (*Borassus flabellifer*) and sand fly distribution in northeastern India, *Journal of Vector Ecology*, vol. 37, no. 1, pp. 148–153, 2012.

[4] D. Barh and B.C. Mazumdar, "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," *Res. J. Medicine & Med. Sci*., vol. 3, no. 2, pp. 173-176, 2008

[5] M. Stringini, F. Comitini, M. Taccari and M. Ciani, "Yeast diversity during tapping and fermentation of palm wine from Cameroon," *Food Microbiol*, vol. 26, no. 1, pp. 415–420, 2009

[6] L. Ouoba, C. Kando, C. Parkouda, H. Sawadogo-Lingani, B. Diawara and J.P. Sutherland, " The microbiology of Bandji, palm wine of *Borassus akeassii* from Burkina Faso: identification and genotypicdiversity of yeasts, lactic acid and acetic acid bacteria,'' *J Appl Microbiol,* vol. 113, no.1, pp. 1428–1441, 2012

[7] J.A. Santiago-Urbina, A.G. Verdugo-Valdez, F. Ruíz-Terán, "Physicochemical and microbiological changes during tapping of palm sap to produce an alcoholic beverage called "Taberna", which is produced in the south east of Mexico," *Food Control*, vol. 33, no. 1, pp. 58–62, 2013

[8] S.Ghosh, R. Chakraborty, A. Chatterjee, and U. Raychaudhuri, "Optimization of media components for the production of palm vinegar using response surface methodology", vol. 120, no. 4, pp. 550–558, 2014

[9] B.Boboye, I.Dayo-Owoyemi, and F.Akinyosoye, "Organoleptic Analysis of Doughs Fermented with Yeasts from A Nigerian Palm Wine (Elaeis guineensis) and Certain Commercial Yeasts," *The Open Microbiology Journal*, vol. 2, no. 1, pp. 115-119, 2008

[10] I. Nwachukwu, V. Ibekwe, R. Nwabueze and B Anyanwu, "Characterisation of palm wine microbial isolates for industrial utilization," *Afr J Biotechnol*, vol. 5, no. 9, pp. 1725-1728, 2008

[11] R. K. chrystopher and K. Theivendirarajah," Palmyrah palm wine 1: Microbial and Biochemical changes," *J Natn Sci Coun SriLanka*, vol. 16, no. 1, pp.131-141, 1988

[12] O. Bassir, "Observation on the fermentation of Palm wine," *West Afr J Biol Appl Chem*, vol. 6,no.1, pp 20-25, 1962

[13] O. Obire, "Activity of Zymomonas species in palm sap obtained from three areas in Edo state, Nigeria," *J. Appl. Sci. Environ. Manage*, vol. 9, no.1, pp. 25-30, 2005

[14] American Chemical Society (ACS), Lab Guide, Washington D.C., American Chemical Society, pp. 98-99, 1999

[15] O.H. Lowry, N.J., Rosebrough, A.L. Farr and R.J. Randall, "Protein measurement with the folin phenol reagent," J Biol Chem, vol. 193, no. 1, pp. 265-275, 1951

[16] E.V. Handel, "Rapid Determination of Total Lipids in Mosquitoe," *J Aru Mosq. Conrnol Asso*c, vol.1, no.3, pp. 302-304, 1985

[17] S. B. Mussa and I. Sharaa, "Analysis of Vitamin C (ascorbic acid) Contents packed fruit juice by UV -spectrophotometry and Redox Titration Methods", *IOSR Journal of Applied Physics,*vol. 6, no. 5, pp. 46-52, 2014

[18] G.L.Miller, "Use of dinitrosalicylic acid reagent for determination of reducing sugar," *Anal. Chem.,* vol. 31, no. pp. 426, 1959

[19] S.V Sumbhate, Satish Nayak, Damodar Goupale, Ajay Tiwari, Rajesh S Jadon, Journal of Analytical Techniques, Vol 1, No 1 (2012).

[20] APHA, Recommended Procedure for the Examination of Seawater and Shellfish, 2nd ed., American Public Health Association, Washington, pp. 12-13, 1982

[21] A. Apriyantono and J.M. Ames, "Xylose-lysine model systems: The effect of pH on the volatile reaction products," *J. Sci. Food Agric*., vol. 61, no. 1, pp. 477-484, 1993.

[22] B. Cammerer and W. Kroh, "Investigation of the influence of reaction conditions on the elementary composition of melanoidins," *Food Chem.,* vol. 53, no. 1, pp. 55-59, 1995.

[23] V.A. Yaylayan and E. Kaminsky, "Isolation and structural analysis of

maillard polymers: Caramel and melanoidin formation in glycine/glucose model system," *Food Chem*., vol. 63, no. 1, pp. 25-33, 1998.
[24] A. Meynier and D.S. Mottram, "The effect of pH on the formation of volatile compounds in meat-related model systems," *Food Chem*., vol. 52, no.1, pp. 361-366, 1995.
[25] R.M. Skirvin, M.C. Chu, M.L. Mann, "Stability of tissue culture medium pH as a function of autoclaving, time and cultured plant material," *Plant cell reports*, vol. 5, no 4, pp. 292-294, 1986.
[26] E.L. Fulmer, A.L. Williams and C. H. Werkman, "The effect of sterilization of media upon their growth promoting properties towards bacteria," *Iowa state Coll jour scie*, vol. 1, pp. 411-417, 1927,
[27] C.W. Ho, W.M. Aida, M.Y. Maskat, H. Osman, "Effect of thermal processing of palm sap on the physico-chemical composition of traditional palm sugar", *Pak j biol sci*., vol. 11, no. 7, pp. 989-995, 2008.
[28]. G. Eggleston and J. R. Vercellotti, "Degradation of Sucrose, Glucose, and Fructose in concentrated aqueous solution under constant pH conditions at elevated temperature", *Journal of Carbohydrate Chemistry,* vol. 19, no. 9, pp. 1305-1318, 2000.
[29] N.C. Igwemmar, S.A. Kolawole, I.A. Imran, "Effect of heating on Vitamin C content of some selected vegetables," IJSTR, vol. 2, no 1, pp. 209-212, 2013.