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Investigation of Some Physicochemical and Microbial Succession Parameters of Palm Wine

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Abstract: The microbial successions, based on some physicochemical parameters of palmwines from Eastern Nigeria during storage were investigated. The wines were investigated when fresh, at 36, 72, 480 and 600 h. The physicochemical parameters investigated included P^H, titrable acidity, soluble solids, percentage moisture, reducing sugar and alcohol contents. The microbial investigation included total aerobic and anaerobic bacterial count, total yeast counts, identification of bacteria and yeast present and their successions. A total of 13 genera of bacteria and 8 species of yeasts were isolated and identified. Significant variations were observed in the physicochemical succession parameters during storage ($p \leq 0.05$).

Key words: Microbial succession, palm wine, physicochemical parameters

INTRODUCTION

Palm wine is an important alcoholic beverage in Africa, where it is consumed by over 10 million people[1]. Palm wine is a beverage produced by fermentations of sugars present in sap of palm trees to ethanol by the yeasts and certain bacteria present. These yeasts and Bacteria have by all means been exposed to varying concentrations of ethanol and acids. The strains that survive to any extent in these wines must have some degree of ethanol tolerance, which is of monumental importance in choosing a yeast strain for industrial ethanol fermentations[2]. This bares the need to rediscover palm wine with a view to understanding the physicochemical changes that these wines undergo over time and their microbial successions. This might lead to discovery of novel bacterial and fungal strains that could have very outstanding industrial applications, especially in the areas of industrial ethanol production.

MATERIALS AND METHODS

Fresh palm wine samples obtained from Raffia palm (Raphia raphia) and Oil palm (Elaensis guineensis) were collected in sterile 2.5 litre sample containers from palm wine tapers in some towns in Eastern Nigeria within 30-60 min of tapping. The sources and codes of the wines are as shown in Table 1. The samples were immediately transported to the laboratory for analysis in iced coolers at 4°C.

Identification of organisms in palm wine: One millilitre of each appropriately aged serially diluted sample was platted out on properly labelled Nutrient Agar (NA) and Saborauds Dextrose Agar (SDA), (oxoid) plates. These were supplanted with chloramphenicol (0.05 mg L^{-1}) for yeasts, while replicate plates were prepared without the chloramphenicol for isolation of bacteria.

Incubation was at 28°C under both aerobic and anaerobic conditions. The morphological and cultural characteristics of the yeasts were studied after Isolation on Glucose yeast Agar (GYA) and Yeast Malt Agar (YMA) (Biolife). Successions of organisms were determined by plating out fresh (within 1 h of taping), at 36, 72 and 480 h old samples, aged at the ambient room temperature.

Isolation and identification of the organisms were by use of standard morphological and physiological tests and identification keys described by Barnett and Kregger[3,4] for yeasts and Bergey and Holt[5] for bacteria.

The tests used in the identification of bacteria included morphology, gram reaction, Acid fast, spore production, motility, aerobic growth, anaerobic growth, catalase, oxidase, production of Acid from glucose, oxidation and fermentation of simple sugars. The tests used in identification of yeasts included; Morphology, surface characteristics on MEA, presence of pseudohyphae, Ascospore formation and vegetative reproduction. Fermentative tests included sugars such as glucose, galactose, sucrose maltose, cellobiose, trehalose, lactose, Raffinose, soluble starch, D- xylose, L- arabinose

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Table 1: Sources and codes for palm wine samples

Sample code	Type of wine	Source
R_1	Raffia palm	Aronta Mbutu, Imo State
R_2	Raffia palm	Obowo, Imo State
P_1	Oil palm	Nsukka Urban, Enugu State
P_2	Oil palm	Ibagwa, Enugu State

and D- Ribose. Other tests included Nitrate assimilation, growth in 10% NaCl +50% glucose in yeast extract, growth at 37°C and growth in 50% w/w glucose yeast extract.

Standard pour plate counts were used to determine bacterial load, while spread plate was used to determine yeast load[6].

Determination of succession parameters: Succession parameters that included levels of reducing sugars[7], pH (pH meter cassio), Levels of ethanol[8] using calcium oxide as dehydrant. Titrable acidity determination was by methods of Abbo[9]. Moisture content and total solids determination was according to AOAC[10]. These analyses were carried out on all the samples, while fresh at 36, 72 480 and 600 h. The results were analysed using the Analysis Of Variants (ANOVA), Nwachukwu and Egbulonu[8].

RESULTS AND DISCUSSION

Succession parameters of palm wine: All the physicochemical parameters tested varied with respect to time. In all the samples the reducing sugar levels decreased with increase in the age of the wines. The reducing sugar level decreased to non traceable levels after 480 h for sample R_1 and P_1 obtained from Aronta and Nsukka respectively. Reducing sugar disappeared from samples P_2(from Obowo) and R_2 (Ibagwa) at 600 h. The oil palm wines revealed higher levels of reducing sugars than the raphia palm wines at 30-60 min after tapping. Sample P_2 revealed the highest amount of reducing sugar 13 mg mL^{-1} while fresh. These values are as shown on Fig. 1.

The levels of Titrable acidity increased with the increasing age of the wines. The oil wines showed higher values than the raffia wines at 600 h. The wines from Ibagwa gave the highest value equivalent to 1.8 g of citric acid per 10 mL Fig. 2.

The moisture levels decreased with increasing age for all the samples. The sample R_2 revealed the highest moisture level of 98.2% followed by 95.7, 95.2 and 93.7 for sample P_1, R_1 and P_2 respectively. At 600 h sample P_2 had the lowest moisture (85.8%) Fig. 3.

The percentage total solids increased with increasing age of the palm wines. 13% for P_2 at 600 h was the highest level of soluble solids recorded for any of the samples tested. While fresh, sample P_2 also exhibited the highest total solids of 5.5% (Fig. 4).

The pH of all the samples decreased with time. While fresh, the highest pH value was 6.4 for sample p_1, while

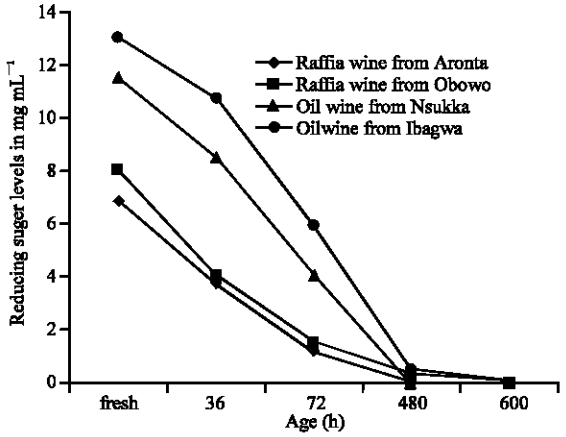

Fig. 1: Levels of reducing sugar in palm wine with time

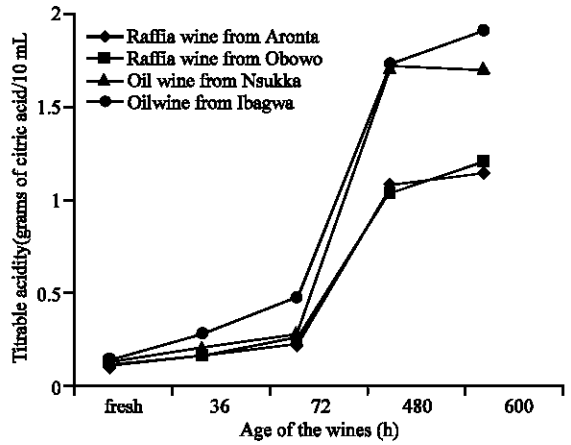

Fig. 2: Levels of titrable acidity in palm wines with age

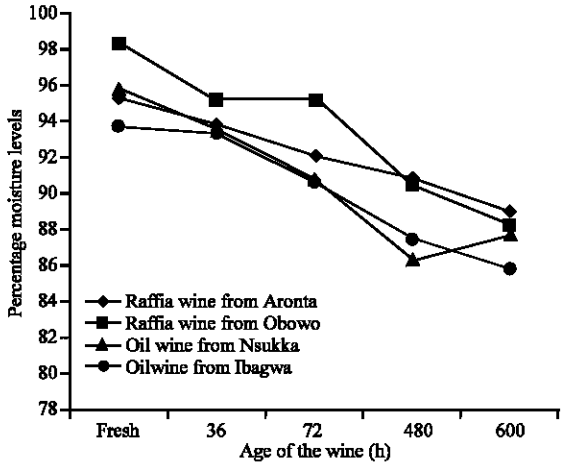

Fig. 3: Percentage moisture levels of palm wines with age

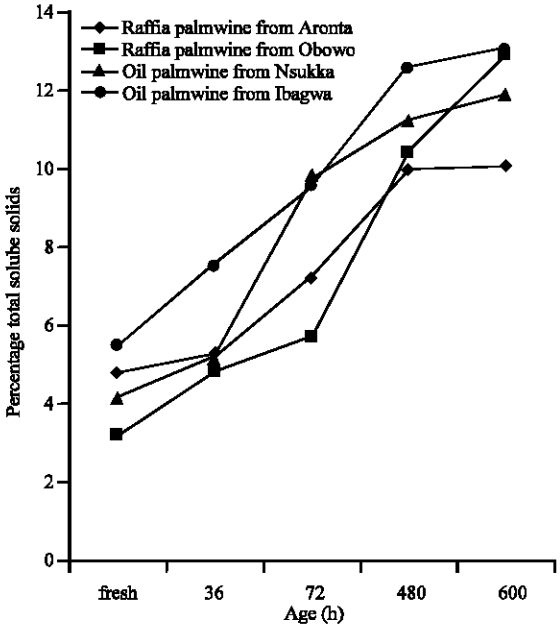

Fig. 4: Percentage total soluble solids in palm wines with age

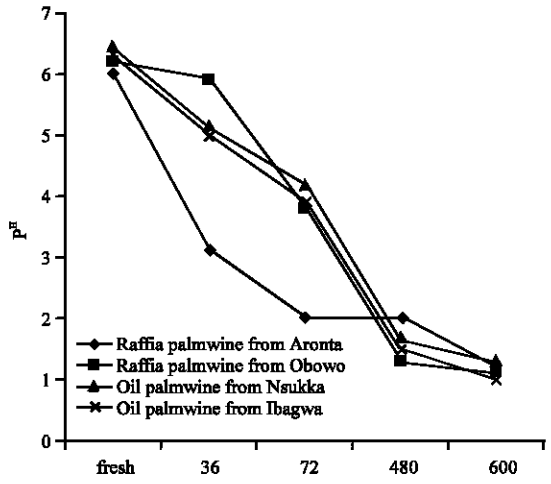

Fig. 5: Changes in the P^H of the palm wines with age

the lowest at that age was 6.0 for sample R_1. The lowest pH recorded was 1.0 revealed by sample P_2 at 600 h. The highest at that age was 1.3 recorded for sample P_1 (Fig. 5).

The quantities of alcohol recorded at various ages of the palm wines varied. The highest level of alcohol recorded was 8.2% v/v recorded for sample P_2 at 72 h. Incidentally the same sample also displayed the lowest level of 2.0% at 600 h. It is worthy of those that all the samples attained their highest alcohol levels at 72 h as shown on Fig. 6.

Successions of microorganisms in palm wine: A total of 13 genera of bacteria and 8 species of yeasts were isolated

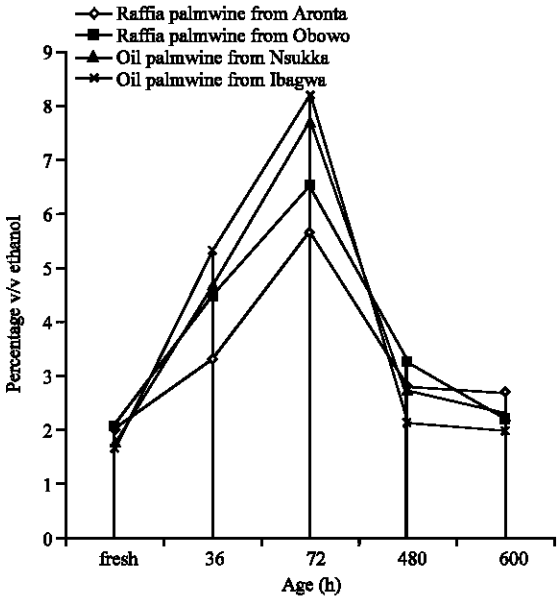

Fig. 6: Changes in percentage ethanol content of palm wines with age

in this study. The variability of the genera isolated decreased with increasing age of all the wines. While fresh, sample R_2 and P_2 showed the highest variability in genera of bacteria of isolated. They contained all 11 different genera of bacteria. The genera of bacteria isolated from the wines are *Lactobacillus*, *Acetobacter*, *Micrococcus*, *Leuconostoc*, *Bacillus*, *Streptococcus*, *Zymomonas*, *Psudomonas*, *Paediococcus*, *Cornybacterium*, *Peptostreptococcus*, *Gluconobacter* and *Chomobacterium* (Table 2).

The species of yeast isolated are *Saccharomyces cerevisiae*, *Saccharomyces globosus*, *Schizosaccharomyces* pombe, Zygo- *Saccharomyces Fermentati*,[a] *Hansenula anomala*, *Pichia Ohmeri*, *Hanseniaspora uvarum*, *Kluveromyces marxianus* and *Zygosaccharomyces fermentati*[b] (Table 2).

The microbial counts of the palm wines showed that aerobic bacteria counts were the highest in all the fresh samples. Sample P_1 had the highest 6.0 $\log_{10}$ cfu mL^{-1}. The highest anaerobic count was 9.8 $\log_{10}$ cfu mL^{-1} at 36 h. All the samples had their highest anaerobic counts at 36 h.

Yeast counts revealed the highest counts in all the samples at 36 h. The overall lowest yeast count was 3.6 $\log_{10}$. cfu mL^{-1} recorded for sample R_1 after 600 h (Table 3).

The results of the succession parameter determinations point to the enormous activities of the microorganisms in palm wine. It also points to the nutritional quality of palm wine as shown by Okafor[11]. The rapid decline in the levels of reducing sugar levels in

Table 2: Genus of bacteria and species of yeast isolated from the palm wine samples with respect to age

Bacteria	Yeast
R_1	
Fresh	
Lactobacillus cornybacterium (a)	*Saccharomyces. cerevisiae*
Micrococcus (B), Bacillus (b)	*Saccharomyces. globosus*
Paediococcus, Leuconostoc	*Schizosaccharomyces pombe*
Acetobacter, Peptostreptococcus	*Zygosaccharomyces Fermentati*
Hansenula anomala	*Pichia ohmeri*
36 h	
Micrococcus	*S. cerevisiae*
Bacillus (b)	*S. globosus*
Lactobacillus	*Z.fermentati (a)*
Leuconostoc	*H. anomala*
Paediococcus	*P. ohmeri*
Acetobacter	
Schizosacharomyces pombe	
Peptostreptococcus	
72 h	
Bacillus	*S. Cerevisae*
Lactobacillus	*S. globosus*
Paediococcus	*Z.Fementati (a)*
Leuconostoc	*P. ohmeri*
Acetbacter	
Peptostreptococcus	
Cornybacterium	
480 h	
Lactobacillus	*S. cerevisiae*
Cornybacterium	*S. globosus*
Leuconostoc	*Z.fermentati (a)*
Peptostreptococcus	*P. ohmeri*
R_2	
Fresh	
Lactobacillus, Acetobacter	*S. cerevisiae, Z. fermentati (b)*
Micrococcus (a), Leuconostoc	*S. globosus*
Bacillus (a), Streptococcus	*P. ohmeri*
Bacillus (b), Zymomonas	*Kluveromyces. marxianus (b)*
Psudomonas, Paediococcus	*H. anomala*
Cornybacterium (a)	*S. pombe*
36 h	
Zymomonas, Acetobacter	*S. cerevisiae, H. anomala*
Bacillus (b), Paediococcus	*S.globosus, H. uvarum*
Cornybacterium (a)	*P. ohmeri*
Lactobacillus	*K. marxianus*
Peptostreptococcus	*S. pombe*
Leuconostoc	
72 h	
Lactobacillus, Paediococcus	*S. cerevisiae*
Cornybacterium (a)	*S. globosus*
Acetobacter	*H. uvarum*
Leuconostoc	
Peptostreptococcus	
480 h	
Lactobacillus, Peptostreptococcus	*S. cerevisiae*
Cornybacterium (a), Paediococcus	*S. globosus*
Leuconostoc	
P_1	
Fresh	
Micrococcus, gluconobacter	*S. cerevisiae, H. uvarum*
Bacillus[a], Zymomonas	*S. globosus,*
Psudomonas, Paediococcus	*S. pombe*
Acetobacter, Lactobacillus	*H. anomala*
Chomobacterium	*P. ohmeri*
36 h	
Acebacter, Leuconostoc	*S. cerevisiae*
Zymomonas, Peptostreptococcus	*S. globosus*
Paediococcus	*S. pombe*
Lactobacillus	*H. uvarum*

Table 2: Continue

Bacteria	Yeast
R_1	
Fresh	
72 h	
Acebacter, Lactobacillus	*S. cerevisiae*
Peptostreptococcus	*S. globosus*
Leuconostoc	*S. pombe*
H. uvarum	
480 h	
Peptostreptococcus	*S. pombe, H. uvarum*
Lactobacillus, Leuconostoc	*S. cerevisiae, S. globosus*
P_2	
Fresh	
Streptococcus, Gluconobacter	*S. cerevisiae,*
Micrococcus, Zynomonas	*S. globosus,*
Bacillus [a], Lactobacillus	*S. pombe*
Psudomonas, Chomobacterium	*H. anomala*
Acetobacter, Cornybacterium [b]	*K. marxianus [a]*
Leuconostoc	*Z. fermentati [a]*
36 h	
Lactobacillus, Acetobacter	*S. cerevisiae*
Leuconostoc, Peptostreptococcus	*S. pombe*
Cornybacterium	*H. anomala H. uvarum*
S. globosus	
72 h	
Leuconostoc	*S. cerevisiae,*
Peptostreptococcus	*S. pombe*
Lactobacillus	*H. uvarum, S. globosus,*
480 h	
Leuconostoc	*S. cerevisiae,*
Peptostreptococcus	*H. uvarum*
Lactobacillus	*S. globosus,*

Key: a and b represents slight differences in morphology

Table 3: Mean microbial counts of the palm wine samples with respect to age at 28°C (log_{10}cfu mL^{-1}) for aerobic, anerobic bacteria and yeast

Age of wines	R_1	R_2	P_1	P_2
Fresh				
Aerobic bact.	4.1	4.8	6.0	5.3
Anaerobic bact.	6.2	7.0	8.3	8.9
Yeast	6.9	6.0	9.5	8.6
36 h				
Aerobic	3.6	3.9	4.3	5.0
Anaerobic	7.0	7.5	8.7	9.8
Yeast	8.5	8.0	10. 3	8.3
72 h				
Aerobic	2.3	2.0	2.1	3.0
Anaerobic	6.6	6.8	7.9	6.7
Yeast	5.1	5.4	8.3	8.4
480 h				
Aerobic	2.0	2.3	2.3	2.5
Anaerobic	3.9	3.4	3.6	4.2
Yeast	4.4	5.0	6.1	6.2
600 h				
Aerobic	1.6	2.0	2.0	2.4
Anaerobic	3.8	4.0	3.6	4.0
Yeast	3.6	3.7	4.9	4.6

all the samples with Age is an indication of its preference by microorganisms for metabolism especially the Yeasts for fermentation[12]. The level of titrable acidity increased steadily, possibly due to the formation of more organic acids by the fermenting bacteria and fungi with time. This explanation is supported by the reduction in pH of all the samples with time as in Okafor[11] and Oyagade[13].

Oyagade[13] however monitored for a few h). That water and Nutrients are used by fermenting organisms to produce acids, Alcohols and biomass' explains why the percentage moisture content declines with time while the total solids kept increasing with time for all the samples.

The alcohol levels of all the wine samples increased with time to peak at 72 h, after which levels diminished for all the samples. It could be as a result of some of the alcohol being converted into more organic acids.

Since synergy between some of these acids e.g., (acetic acid) and ethanol has been established[14], it is possible that their presence in palm wine would potentiate the toxic effects on the palm wine flora. The implication is that medium would be increasingly selective for only organism that can resist these toxic effects.

The organisms isolated after 480 h clearly tolerated these effects at those (pH, titrable acidity, osmotic potential and %v/v alcohol) levels recorded. The increased rate of isolation of Saccharomyces yeasts at 480 h further proves them the most ethanol tolerant organisms available[14].

CONCLUSION

These findings raise very strong hope for the industrial ethanol fermentation industry in Nigeria of reduced bioethanol production costs using modifiable locally available strains.

Further study is at conclusion stage on the genetic modification of these isolated strains and optimizing process for their use in fermenting some locally available substrates to ethanol.

REFERENCES

1. International technology Development Group (ITDG), 2003. Toddy and Palm Wine fermented saps. In practical answers to poverty Bulletin, pp: 1-3.
2. Cassey, G. and W. Ingledew, 1986. Ethanol olerance in Yeasts. Crit. Rev. Microbiol., 13: 219-290.
3. Barnett, J., R. Payne and D. Yarrow, 1990. Yeasts: Characteristics and Identification, 2nd Edn. Cambridge University Press.
4. Kregger Van-Rij, 1987. The Yeasts: A Taxonomic study 3rd Edn. Elsevier Science Oub. Co. Netherlands.
5. Bergey, D.H. and J.G. Holt, 1993. Bergey's Manual of Determinative Bacteriology. The Williams and Wilkins Company Baltimore.
6. Oso, B.A. and M.O. Fawole, 1995. Characterization of Bacterial in: Laboratory Manual for Microbiology. Spectrum Books Ltd. Ibadan Nigeria.
7. Miller, G.L., 1959. Use of Dinitrisalicylic Acid (DNS) reagent for determination of reducing sugars. Analytical Chem., 31: 429-31.
8. Nwachukwu, V. and K. Egbulonu, 2000. Elements of statistical inference. Peace Ent. Co. Owerri, Nigeria.
9. Abbo, E., E. Bahago and E. Ajayi, 2000. Analysis of specific food Commodities. In Practical Manual on Food Technology, Nutrition and Dietetics for school and industries. Dept. of Food Technology, Kaduna Polytechnic Nigeria (Ed.) National Science Forum.
10. AOAC, 1984. Official Method of Analysis, 14th Edn. Washington DC.
11. Okafor, N., 1972. Palm Wine Yeasts from parts of Nigeria J. Sci. Food Agric., 23: 1399-1407.
12. Priest, F. and I. Campbell, 1996. Brewing Microbiology 2nd Edn. Chapman and Hall London Publishers. New York, pp: 196.
13. Oyagade, A., O. Famurewa, J. Oyagade and O. Aringbangba, 2004. Microbial population and survival of some of pathogenic Bacteria in fresh palm wine. Nig. J. Microbiol., 18: 269-276.
14. Ingram, L. and Buttke, 1984. Effects of thanol on Microorganims. Adv. Microbiol. Physiol., 25: 253-300.
14. Pampulha, M.E. and V. Louretro, 1989. Interaction on the effects of Acetic acid and ethanol on inhibition of fermentation in Saccharomyces cerevisiae Biotechnol. Lett., 11: 269-270.