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Zero additives preservation of Raphia palm wine

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ABSTRACT

Palm wine obtained from Raphia palm (*Raphia hookeri*) in Ayingba, Kogi State, Nigeria, was pasteurized through zero addition of preservative and placed on the shelf for 6 months. After 6 months, another sample of palm wine obtained from the same area was fetched and comparative analysis was carried out on both wine samples to find out if there was significant difference in the quality of both samples. The following parameters were analysed: pH, total solids, total acidity, refractive index, alcoholic and sugar contents, ascorbic acid and microbiological analysis. The results showed that there was no significant difference in most of the parameters compared. However, there were significant differences in the alcoholic and sugar contents of the wine samples. This shows that pasteurisation of Raphia palm wine with zero additives is a good alternative to extending its shelf life instead of the use of chemical preservatives that are often not available in the country.

Keywords: Palm wine, shelf life, fermentation, pasteurisation, zero additives.

INTRODUCTION

Palm wine, an alcoholic substance obtained from the sap of a number of species of palm tree, is produced by the natural fermentation of the sap. It is an important beverage in West Africa, India, South Africa, and some other parts of the world. In West Africa, it is commonly obtained from the sap of palm species such as the African Oil palm (*Elaeis guineensis*) and Raphia palm (*Raphia hookeri*) (Uzogara et al., 1990; Uzochukwu et al., 1991; Boboye et al., 2008). The unfermented sap is clean, sweet, colourless syrup containing about 10–12% sugar, which is mainly sucrose (Bashir, 1962; Okafor, 1975a). Upon fermentation by the natural microbial flora, the sugar level decreases rapidly as it is converted to alcohol and other products (Obire, 2005). However, the sap becomes milky-white due to the increased microbial suspension resulting from the prolific growth of the fermenting organisms (Okafor, 1975a,b). Fermentation begins immediately after collection and lasts up to two weeks if not arrested through pasteurisation. The product on complete fermentation is ethanol and water.

Palm wine and beer are two alcoholic beverages that play an important role in local ceremonies in Nigeria (Eluwa et al., 2009). Palm wine is consumed by both men and women including pregnant women. Alcohol is low molecular substance and is therefore capable of crossing the placental barrier and entering the fetus, causing the level of alcohol in the fetus to approximate to that of the mother (Streissguth et al., 1989). Adverse health effects that are associated with alcohol exposed pregnancies include miscarriage,

premature delivery, low birth weight, sudden infant syndrome, and prenatal alcohol–related conditions such as fetal alcohol syndrome. Fetal alcohol syndrome is one of the leading causes of mental retardation, and is directly attributed to drinking during pregnancy. It is characterized by growth retardation, facial dysfunction such as learning abnormalities and lower Intelligent Quotient (IQ) as well as behavioural problems (CDC, 2004).

Palm wine has several nutritional, medical, religious and social uses and these have been reported to have enhanced the demand for this natural product (Fapurusi, 1966; Odeyemi, 1977; Ikenebomeh and Omayuli, 1988; Uzogara et al., 1990; Iheonu, 2000). Other major components of palm wine, apart from sugars include alcohol, organic acids and protein (Bashir, 1968; Van Pee and Swings, 1971; Fapurusi and Bashir, 1972).

In order to lengthen the shelf-life of palm wine, a number of preservation measures have been adopted. These include the use of extract from bark of trees such as *Saccoglottis gabonensis*, *Vernonia amygdalina*, *Euphobia sp*., *Nauclea sp*. and *Rubiacae sp*. (Ogbulie et al., 2007). Sulphite and Benzoate (Levi and Oruche, 1957), pasteurization (Chinarasa, 1968), have all been used for preservation of palm wine. All these attempts have either resulted in change of taste or not completely been able to curb the actions of the fermenting microbes. This study, therefore, aimed at affirming the effectiveness of pasteurization process and use of zero additives preservation of palm wine.

MATERIALS AND METHODS

Fermentation process of palm wine

A number of fermenting organisms have been indentified in the fermentation process by previous studies, and these include yeast (*Saccharomyces cerevisiae* and *Schizosaccharomyces pombe)* and bacteria (*Lactobacillus plantarum* and *L. mesenteroides*) (Okafor, 1975a; Orimaiye, 1997; Nester et al., 2004).

Sucrose, the main sugar in palm wine, is first broken down into monosaccharide by invertase, an enzyme produced by the yeast present in the sap. The monosaccharide is then converted to ethanol through a complex reaction processes catalyzed by various enzymes collectively called zymase.

This process begins with a molecule of glucose being broken down by the process of glycolysis into pyruvate. The reaction is accompanied by the size difference of two molecules of NAD^+ to NADH and a net of two ADP molecules converted to two ATP plus the two water molecules.

Pyruvate is then converted to acetaldehyde and carbon dioxide by an enzyme called pyruvate decarboxylase and requiring thiamine diphosphate as cofactor. The acetaldehyde is subsequently reduced to ethanol by the NADH from the previous glycolysis, which is returned to NAD^+:

$$C_{12}H_{22}O_{11} + H_2O \xrightarrow{Invertase} \underset{(Glucose)}{C_6H_{12}O_6} + \underset{(Fructose)}{C_6H_{12}O_6}$$

$$C_6H_{12}O_6 \xrightarrow{Zymase} \underset{(Alcohol)}{2\,C_2H_5OH} + 2\,CO_2$$

$$CH_3COCOO^- + H^+ \longrightarrow CH_3CHO + CO_2$$

$$CH_3CHO + NADH \longrightarrow C_2H_5OH + NAD^+$$

Collection of samples and pasteurization

Fresh undiluted palm wine samples obtained from Raphia palm (*R. hookeri*) was collected from a traditional palm wine tapper from Ayingba, Kogi State, Nigeria, who was followed straight to the palm tree. The palm wine was collected using a sterilized white plastic container in the month of March 2008. The collected fresh palm wine was filtered to remove just the suspended particles and immediately transferred into five (5) 60 cl bottles filled with 59.8 cl allowing about 2 cm air gap, and corked using a crown corking machine. The bottles and their content were allowed to stand on the table at room temperature for 15 minutes and then pasteurized at 60 ^{o}C for 1 hour. The duration of 15 minutes is ideal to produce enough CO_2 for pasteurization of the palm wine. Excess CO_2 will cause the bottle to explode due to high gaseous pressure. After pasteurization, the samples were stored for 6 months. The samples were labelled $A_1 - A_5$ respectively. After six months, Five (5) bottles (60 cl) of Raphia palm wine freshly produced from the same place were also pasteurized as the previous ones and then analysed. The samples were labelled $B_1 - B_5$ respectively. The entire work lasted for 6 months and two weeks.

Chemical analysis

All the samples of palm wine labelled A_1, A_2, A_3, A_4, A_5, and B_1, B_2, B_3, B_4 and B_5 were analyzed. The pH of the palm wine samples was obtained using a pH meter (320 Model). The meter was first standardized to 4.0 ± 0.02 and 7.0 ± 0.02 by dipping the electrodes in buffer solutions of the pH values. The palm wine sample was then placed in a beaker and the pH measured. The specific gravity of the samples was obtained by the use of specific gravity bottle. The total acidity, a measure of acetic or ethanoic acid, was measured by titrating the samples against 0.01M NaOH, using phenolphthalein as indicator. The percentage acid in each sample of the wine was calculated and recorded. Total solids and refractive indices of the samples were measured at 30 ^{o}C using refractometer. The palm wine samples were placed one after the other between the two lower prisms and the connecting arm was rotated until the critical ray was centred in the eye piece. 95% ethanol was used to clean the prisms before any fresh sample was placed between them by the use of dropping pipette. Alcoholic content of the samples was also determined using distillation method at 64 ^{o}C and 78 ^{o}C for methanol and ethanol respectively. An approximation of the alcoholic strength of the wines was carried out at room temperature using the table of the ratio of the refractive indices and densities of wines (Cooke, 1974). Ascorbic acid (Vitamin C) of the samples was determined using a method described by David (1974), which compares well with spectrophotometric method of Bajaj and Kaur (1981). The sugar content of the samples was measured using Saccharometer.

Microbial analysis

Nutrient agar plates were prepared according to the standard of microbiology techniques as described by Harrigance and McCance (1976). After sterilization, the plates were inoculated with the palm wine samples respectively. The inoculated plates were incubated and gram-negative staining was later done. Microbial counts were made every 24 hours using direct microscopic count method, and the counting of each sample continued until the yeast and bacteria began to grow.

Data analysis

All the data obtained were subjected to statistical analysis using the t-test. Significance of variations in the data was tested at 95% (p=0.05) confidence limit.

RESULTS AND DISCUSSION

Table 1 shows the pH of the palm wines. The average pH of the pasteurized palm wines (PPW) labelled $A_1 - A_5$ was 4.5, while that of the samples $B_1 - B_5$ stood at 4.2. The pH value of sample B is an indication of higher acidity in sample B and probably more alcoholic content in the sample before

pasteurization. The value of pH is enough to make the palm wine unstable with respect to microorganism's activity (Pandell, 1999). The result however shows no significant difference in the pH of the two wine samples. The implication of this is that the pasteurization process arrested microbial activities and thus stopped the degradation of the palm wine.

Table 2 shows the specific gravity of palm wines. The specific gravity values of Sample A and Sample B were 1.0239 and 1.0154 respectively. Although the specific gravity of Sample A is slightly greater than that of Sample B, the result shows a non-significant difference in the mean values, an indication that the contents of the palm wine samples are about the same.

There was also no significant difference in percentage total titrable acid (TTA) of Sample A (0.43%) and that of Sample B (0.44%). This shows that small amount of the ethanol content was oxidized to ethanoic acid. The result of the analysis is shown in Table 3.

There was significant difference in the alcoholic content of the two samples as shown in Table 4 with sample A having higher alcoholic content than sample B. This could be that the palm wine sample A may have fermented appreciably before pasteurization.

The results of the analysis of the total solid and refractive index study given in Table 5 below also show no significant difference between the two samples. This is probably the reason why contents of the palm wine samples are almost the same as shown by the results of the analysis.

The ascorbic acid concentrations in Sample A (8.8%) and Sample B (9.01%) shown in Table 6 depicts that there is no significant difference because the palm wine samples were obtained from palm trees grown on the same soil. There was slight difference in the sugar content of the samples. Sample A contains 7.35% whereas sample B contains 8.20%. The higher sugar content of sample B accounts for the higher alcoholic content of the sample as shown in Table 4. No bacterium of public health significance was identified in the wines after gram staining. Lactic acid bacteria were, however, identified. The result implies that the palm wine sample A was not degraded by microbes after six months.

Table 1: t-test for the difference in pH of two samples of palm wine.

Variables	Mean	SD	Observations	df	t-calculated	t-table (p = 0.05)
Sample A	4.5	± 0.24	5	8	1.464	2.306
Sample B	4.2	± 0.39	5			

Table 2: t-test for the difference in SG of two samples of palm wine.

Variables	Mean	SD	Observations	df	t-calculated	t-table (p = 0.05)
Sample A	1.0239	± 0.02	5	8	0.4891	2.306
Sample B	1.0154	± 0.03	5			

Table 3: t-test for the difference in TTA of two samples of palm wine.

Variables	Mean	SD	Observations	df	t-calculated	t-table (p = 0.05)
Sample A	0.431	± 0.08	5	8	0.146	2.306
Sample B	0.440	± 0.11	5			

Table 4: T-test for the difference in alcoholic content of two samples of palm wine.

Variables	Mean	SD	Observations	df	t-calculated	t-table (p = 0.05)
Sample A	2.508	± 0.64	5	8	4.318	2.306
Sample B	4.038	± 0.45	5			

Table 5: t-test for the difference in total solids and refractive index of two samples of palm wine.

Variables	Mean	SD	Observations	df	t-calculated	t-table (p = 0.05)
			Total Solids			
Sample A	6.5	± 0.59	5	8	0.817	2.306
Sample B	6.2	±0.57	5			
			Refractive Index			
Sample A	1.343	±0.11	5	8	0.0238	2.306
Sample B	1.341	± 0.13	5			

Table 6: t-test for the difference in Ascorbic Acid Concentration and Sugar Content of two samples of palm wine.

Variables	Mean	SD	Observations	df	t-calculated	t-table (p = 0.05)
			Ascorbic Acid Concentration			
Sample A	8.8	±0.19	5	8	0.243	2.306
Sample B	9.01	±0.41	5			
			Sugar Content			
Sample A	7.35	±0.60	5	8	2.451	2.306
Sample B	8.20	±0.49	5			

Conclusion

The study shows that almost all the properties of the palm wines were not affected by the zero additives preservation of the palm wine. Though the alcoholic and sugar contents varied after the storage period, the qualities of the wines did not diminish. These findings proved to a certain extent that pasteurization of local palm wine with zero additives is a good alternative to extending its shelf life. Zero additives preservation will reduce production cost and maintain the quality and nutritional values of the wine. Further studies can, however, be carried out on the preservation of other drinks using the same zero additives method which is safe and does not pose any threat to public health. The stress of procuring chemical additives is also eliminated.

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