
Effect of Food Spices on the Microbiology and Nutritional Status of Fresh Palm Wine

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Abstract

Preservative effects of five food spices (ginger, garlic, alligator pepper, Negro pepper and Guinea pepper) on the microbiological and nutritional status of palm wine were assessed. Six bacteria namely *Staphylococcus aureus*, *E. coli*, *Micrococcus* spp, *Lactobacillus* spp, *Bacillus* spp and *Streptococcus* spp and two yeasts (*Saccharomyces* and *Candida* spp) were isolated from the palm wine. The palm wine was treated with ground spices and stored for 14 days during which the microbiological and sugar content of the palm wine were monitored. There was a decrease in the number of isolates with increase in storage time both for the spice-treated and the control samples. Only *Bacillus* spp, *Lactobacillus* spp and *Saccharomyces cerevisiae* survived in the palm wine after fourteen days of storage. After 14 day, the sugar content of the palm wine treated with ginger was 0.310 g/ml while that for the samples treated with other spices dropped to 0 g/ml. The initial microbial load of ginger-treated sample was 2.27×10^6 cfu/ml, but the count progressively decreased to 2.0×10^4 cfu/ml on the 14th day. The result suggests that treating palm wine with ginger can extend the shelf-life and nutritional quality (sugar content) more than other spices used in the study.

Keywords

Food Spices, Fresh Palmwine, Microbes, Microbiology, Nutritional Status

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1. Introduction

Palm wine, a whitish liquid produced by natural fermentation of the sap of *Elaeis guineensis* and *Raphia hookeri* [1, 2] is consumed throughout the tropics. The unfermented sap is clean, sweet, colourless syrup containing about 10 - 12% sugar, which is mainly sucrose [3, 4]. Palm wine has been shown to contain nutritionally important compounds including amino acids, proteins, vitamins and sugars [5]. Proximate analysis carried out on *E. guineensis* saps revealed the following nutritional ranges: 85.10–87.20% moisture, 0.05–0.23% crude protein, 0.20–2.25% fat and 12.375–14.65% carbohydrate [5]. The freshly tapped sap is often drunk within 1–2 days either as fresh sap or palm wine. It may also be pasteurized and bottled or frozen at industrial level for longer storage or be processed

into different products such as caramel, sugar, spirit, or mixed fruit juice. However, palm wine without any heat treatment is the commonest form of the product in countries like Ghana and Nigeria, where it is served in ceremonies such as traditional festivals, weddings, and funerals. The dregs of palm wine contain yeast sediments and other particles are customarily reserved for the groom in the eastern part of Nigeria because of the long-standing belief that it boosts male sexual-performance.

Generally, both brands of palm wine have several nutritional, medical, religious and social uses which have been reported elsewhere [6, 7, 8, 9 and 1] to have increasingly enhanced the demand for this natural product. Although attempts have

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been made towards the preservation and shelf-life extension of palm wine through bottling, use of chemical additives, addition of plant extracts has greatly affected the organoleptic quality of the product [3, 4, 7, 9, 11 and 12].

Palm wine is produced and consumed in very large quantities in the south eastern Nigeria. It contains nutritionally important components including amino acids, proteins, vitamins and sugars [13]. Increased microbiological studies of the product in the last five years has led to more information on the diversity of *Saccharomyces cerevisiae* strains prevalent in the product [14, 15] and discovery of new microorganisms from the drink [16]. These make palm wine a veritable medium for the growth of a consortium of microorganisms whose growth in turn changes the physico-chemical status of the wine giving rise to competition and successions of microorganisms. The absence of refrigeration in the localities calls for an alternative means of preserving the nutritional and organoleptic qualities of palm wine.

This work aimed at analysing the effect of selected spices as bio-preservatives in improving the shelf-life and nutritional quality of palm wine.

2. Materials and Method

2.1. Source of Material

Raphia palm wine samples were collected from traditional palm wine tappers from Obowo in Imo State. Two spices: ginger (*Zingiber officinale*) and garlic (*Allium sativum*) were sourced from Ginger and Garlic Research program, NRCRI, Umuahia while Alligator pepper (*Aframomum melegueta*), Uda (*Xylopia aethiopica*; negro pepper) and Uziza (*Piper guineense*) seeds were bought from Orie-Ugba market, Ibeku, Umuahia. Laboratory facilities were source from CesLAB Analyticals, Umuahia.

2.2. Sample Preparation

The spices were first processed into powdered form. They were sorted and the good ones were washed in running tap water to remove dust and dirt. They were drained, cut into small bits and dried in the hot air oven at 65°C overnight for four days. They were separately ground into powder and used to treat the palm wine sample. The fresh palm wine was dispersed into pre-sterilized sample bottle of 250 ml capacity. 200 ml of Raphia palm wine were dispensed into pre-sterilized sample bottle of 250 ml and then treated with the ground spices: Alligator pepper, ginger, garlic, uda and uziza respectively. All the treated palm wine samples and the control (containing no spice) were labelled and kept on a shelf at room temperature. Analyses were carried out every 48 hrs for fourteen days.

2.3. Analyses of Palm Wine Samples

2.3.1. Determination of pH

The pH of the palm wine samples was determined using an electric pH meter [17] after calibration with buffer solution of pH 7.0.

2.3.2. Determination of Total Titratable Acidity

The Alkaline Titrimetric Method [17] was used. 10ml of the sample were introduced into a clean dry conical flask and 40ml of distilled water were added to it followed by the addition of 3 drops of phenolphthalein indicator. It was titrated against dilute 0.1ml NaOH solution to a pink end point which persisted beyond 15 seconds. The TTA was calculated as follows:

$$\% \text{ TTA} = 100/v \times N \times \text{Titre}$$

V = volume of sample.

N = Normality of titrant.

2.3.3. Determination of Microbial Load

This was carried out using the method of [18]. The palm wine sample was serially diluted and 0.1 ml aliquot was aseptically taken from suitable dilution and cultured by spread plate technique on Nutrient, MacConkey Sabourand Dextrose Agar (SDA) plates in duplicates. The inoculated plates were labeled, sealed and incubated at 35°C for the isolation of bacterial for 2- 4 days while the SDA plates were incubated at 22°C for 5 days for the isolation of fungi. They were observed daily for growth and the plates were counted accordingly. The isolates were sub-cultured to appropriate media and the pure cultures that developed were stored in agar slants and kept in the refrigerator at 4°C. Gram staining, biochemical and sugar fermentation tests were carried out for the identification of the bacterial isolates [19].

2.4. Lactophenol Cotton Blue Staining

A drop of the cotton blue lactophenol was placed on a clean slide using inoculating wire small piece of the fungal culture was transferred onto the slide. The slide was observed under the microscope and the fungal structures recorded [20].

2.5. Determination of Sugar Content

The sugar content of the palm wine samples was determined using the Anthrone's colorimetric method. 1ml of the sample was mixed with 6ml of Anthrone's reagent in a test tube, corked and boiled in a water bath for 10 minutes. It was cooled in bath of cold water and its absorbance was read at 620nm wavelength. 1ml of standard sugar was treated the same way as described above for the sample. Its absorbance

was also read as the same wavelength. The formula below was used to calculate the sugar content: $D = \text{Dilution factor where applicable}$

$$V_o \text{ sugar} = 100/v \times as/AS \times C \times D$$

V= Volume of sample

as= absorbance of sample

AS = absorbance of standard sugar solution

C= Concentration of standard sugar solution

3. Results

Table 1 shows the six bacteria isolated from the palm wine sample. They are *Bacillus* spp, *Escherichia coli*, *Lactobacillus* spp, *Micrococcus* spp, *Staphylococcus aureus* and *Streptococcus* spp.

Table 1. Identification of bacteria isolates from palm wine samples.

S/N	Colonial Morphology	Cell Shape	Gram reaction	Catalase	Coagulase	Citrates	Oxidase	VP	MR
1.	Smooth and circular, translucent on nutrient agar.	Short, single and separate rods.	-	+	-	-	-	-	+
2.	Raised, irregular white colonies on nutrient agar.	Short rods in chain.	+	+	-	+	-	+	-
3.	Small colourless colonies.	Cocci in chain.	+	-	-	-	-	+	-
4.	Smooth circular and cream colonies on nutrient agar.	Cocci in cluster.	+	+	+	-	-	+	-
5.	Round and yellow colonies on nutrient agar.	Cocci in cluster.	+	+	-	-	-	+	-
6.	Small and smooth white colonies.	Rod	+	-	-	-	-	+	-

Table 1. Continue.

S/N	Colonial Morphology	Cell Shape	Lactose	Glucose	Maltose	Sucrose	Fructose	Isolates
1.	Smooth and circular, translucent on nutrient agar.	Short, single and separate rods.	AG	AG	AG	-	-	<i>Escherichia coli</i>
2.	Raised, irregular white colonies on nutrient agar.	Short rods in chain.	-	A	-	A	-	<i>Bacillus</i> spp
3.	Small colourless colonies.	Cocci in chain.	A	AG	AG	-	A	<i>Streptococcus</i> spp
4.	Smooth circular and cream colonies on nutrient agar.	Cocci in cluster.	AG	AG	AG	A	A	<i>Staphylococcus aureus</i>
5.	Round and yellow colonies on nutrient agar.	Cocci in cluster.	-	-	A	A	A	<i>Micrococcus</i> spp
6.	Small and smooth white colonies.	Rod	A	A	A	A	A	<i>Lactobacillus</i> spp

Keys: + = positive; - = negative; A = Acid production only; AG = Acid and Gas production.

Table 2 shows the yeast isolates (*Saccharomyces cerevisiae* and *Candida* spp) from palm wine.

Table 2. Identification of yeast isolates from palm wine.

S/N	Morphology	Microscopic features	Glucose	Lactose	Maltose	Sucrose	Fructose	Isolates
1.	Round and creamy colonies on SDA.	Single oval cells were seen in pairs.	+	-	+	+	+	<i>Saccharomyces</i> spp
2.	Whitish colonies, not well developed pseudomycellium.	Single round cells were seen.	+	-	+	+	+	<i>Candida</i> spp

Key: + = positive; - = negative

The changes in the microbial load of spice treated palm wine during storage are shown in Table 3. Garlic had the lowest microbial load.

Table 3. Changes in the microbial load of spice treated palm wine during storage (10^6 cfu/ml).

Sample	Days							
	0	2	4	6	8	10	12	14
Control	2.47	3.08	1.84	0.56	0.16	0.06	0.04	0.03
Alligator P	2.30	2.54	1.71	0.43	0.10	0.05	0.03	0.02
Garlic	2.23	2.37	1.69	0.40	0.08	0.05	0.02	0.01
Ginger	2.27	2.42	1.67	0.35	0.08	0.04	0.03	0.02
Negro P	2.23	2.47	1.67	0.43	0.09	0.05	0.03	0.02
Guinea P	2.23	1.43	0.67	0.40	0.08	0.00	0.03	0.02

The survival pattern of microbial isolates is shown in Table 4. Among the bacterial isolates, only Bacillus and Lactobacillus spp were isolated at the 14th day while Sacchoromyces cerevisiae was the only yeast isolated from the samples on the 14th day.

Table 4. The survival pattern of microbial isolates.

Isolates	Days								
	0	2	4	6	8	10	12	14	
Staphylococcus aureus	+	+	-	-	-	-	-	-	
Lactobacillus spp	+	+	+	+	+	+	+	+	
Bacillus spp	+	+	+	+	+	+	+	+	
Streptococcus spp	+	+	-	-	-	-	-	-	
Escherichia coli	+	+	+	-	-	-	-	-	
Micrococcus spp	+	+	-	-	-	-	-	-	
S. cerevisiae	+	+	+	+	+	+	+	+	
Candida spp	+	+	+	-	-	-	-	-	

Keys: + = Present; - = Absent

Figure 1 shows the sugar levels of the spice treated palm wine. Only the ginger-treated palm wine contained sugar (0.310 g/ml) on the 14th day of storage.

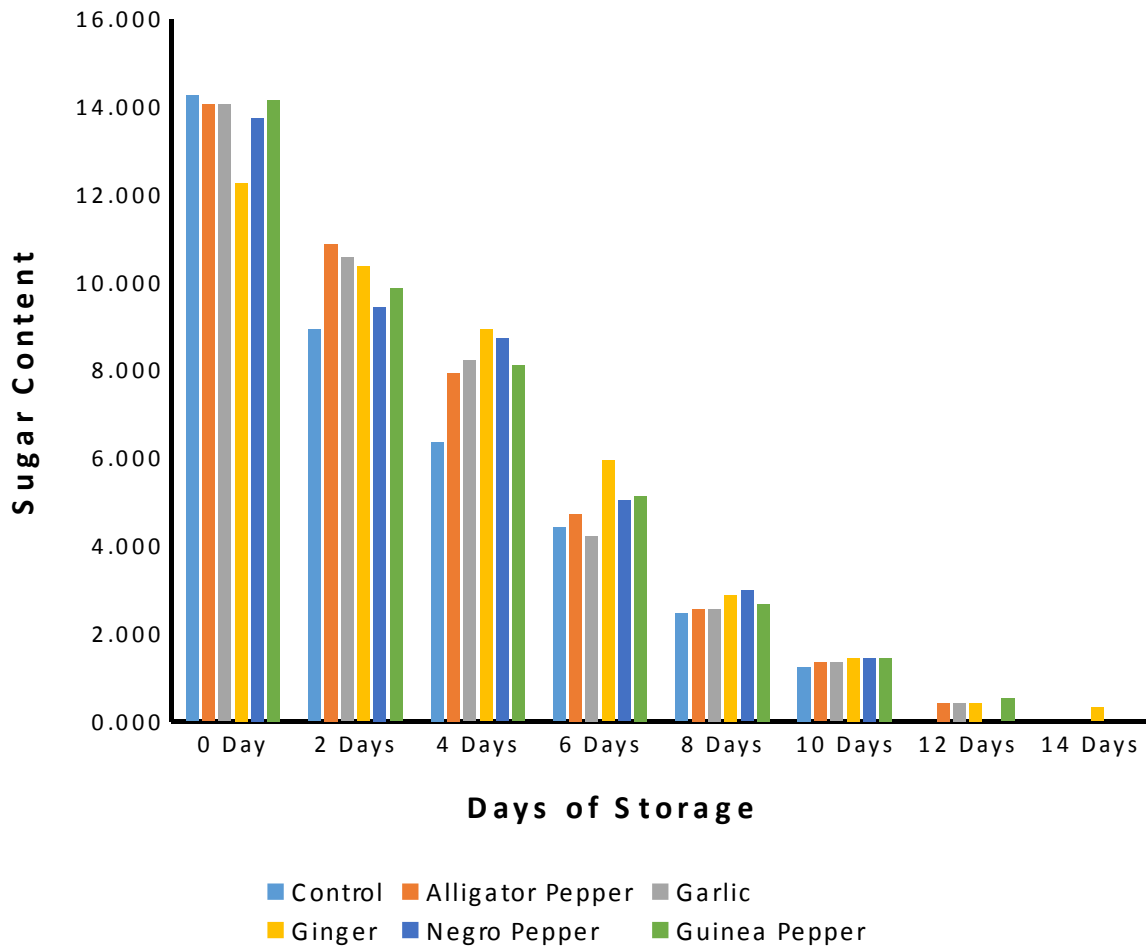


Figure 1. Changes in sugar content of spice-treated palm wine during storage.

Figure 2 shows the effect of the five spices on the pH of palm wine where Guinea pepper has the highest pH value of 3.1 while Garlic has the lowest pH value of 2.1 on the 14th storage day.

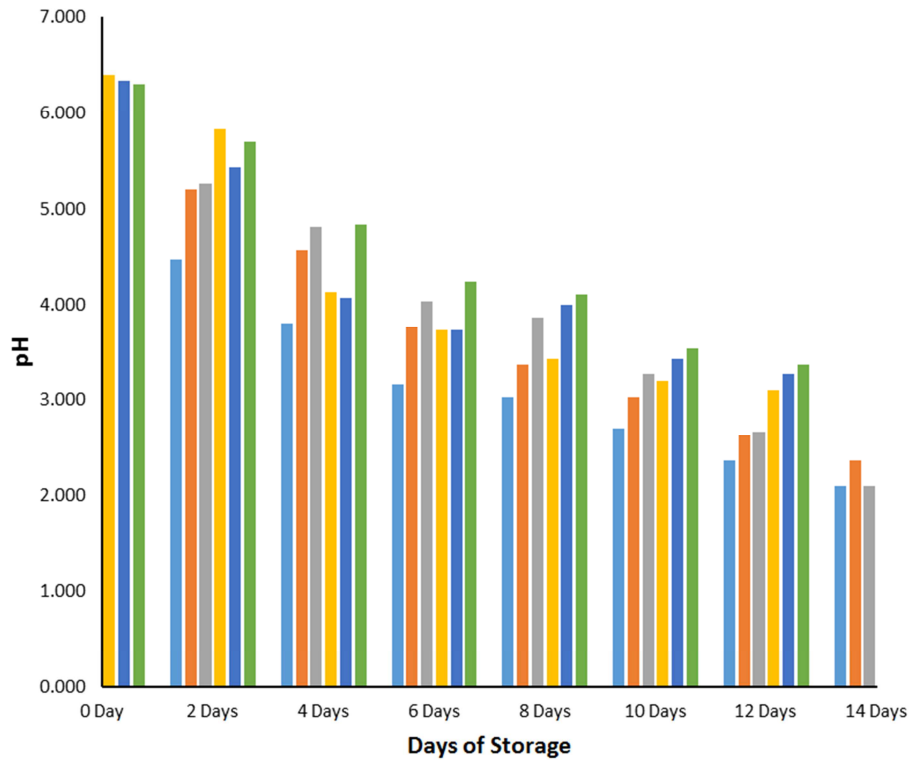


Figure 2. Changes in pH of spice-treated palm wine during storage.

Figure 3 shows the effects of spices on the Titrimetric Acidity (TTA) of the palm wine. The alligator pepper, garlic and guinea pepper have similar result of 0.867 respectively with that of control while negro pepper and guinea pepper was 0.846 and 0.807 respectively after fourteen days.

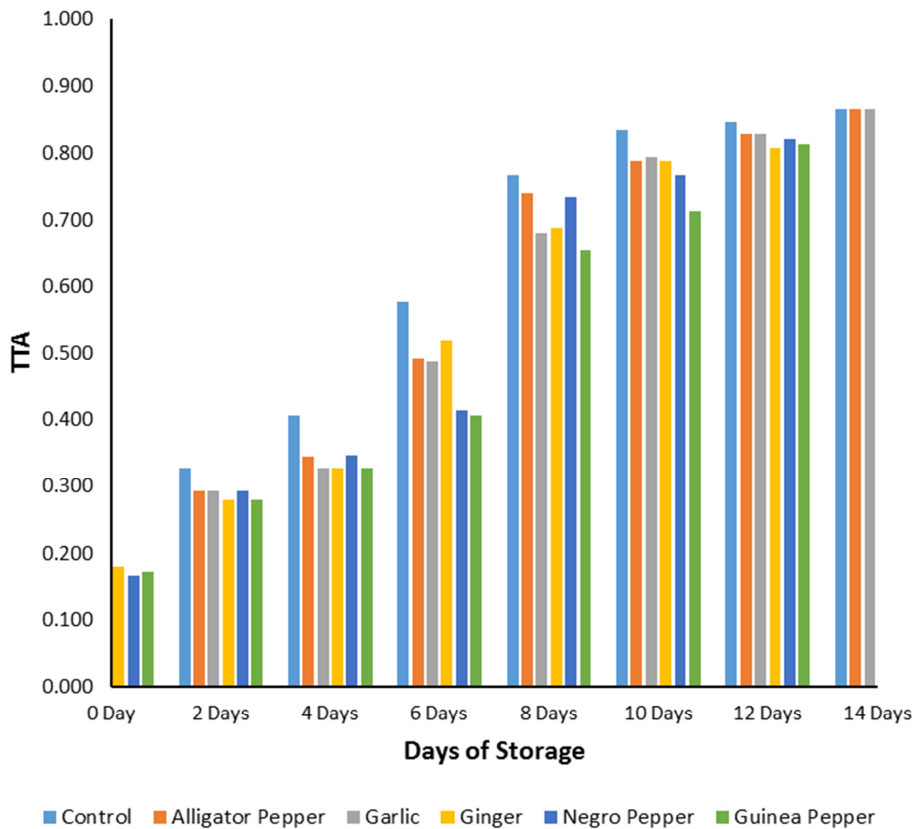


Figure 3. Changes in the Titratable acidity of the spice-treated palm wine during storage.

4. Discussion

Eight bacteria were isolated from the palmwine samples and they include *Staphylococcus aureus*, *Escherichia coli*, *Micrococcus* spp, *Lactobacillus* spp, *Bacillus* spp and *Streptococcus* spp while *Saccharomyces cerevisiae* and *Candida* spp were the two yeasts recovered from the samples. The occurrence of these microbial isolates in the palm wine sample indicated that the palm wine was contaminated probably from the handlers and unhygienic environment. The presence of *E. coli* in the palm wine is expected to be from the water which the handlers use very often to dilute the palm wine and increase the final volumes to boost their sales. The result of the microbial contaminants agrees with the reports of [21, 4 and 22].

A higher bacterial count was observed at the zero day in the samples containing the five spices and also the control. This finding agrees with the reports of [23, 24]. During the second day, the bacteria count increased indicating that the isolates were actively multiplying even in the presence of the spices.

The growth and survival pattern of the bacterial isolates from day 0-14th day shows that *S. aureus*, *Streptococcus* spp and *Micrococcus* spp were eliminated from the samples after 2 days of storage while *E. coli* was eliminated on the 4th day. This result is in agreement with the findings of by [25] while *Bacillus* spp and *Lactobacillus* spp persisted till the end of the fermentation. The elimination of the two pathogens could be due to the production of antimicrobial metabolites such as bacteriocins, H₂O₂, CO₂.

Candida spp was eliminated from the fourth day of the storage while *Saccharomyces* spp was present till the end of the storage. It has been reported that *Saccharomyces* spp is the dominant yeast in palm wine [26, 27 and 25] and this is in agreement with our findings.

Sugar content of the palm wine increased as the storage progressed indicating enzymatic hydrolysis of disaccharides in the palm wine into simple sugars. However, after two days of storage, the sugar content of the control dropped from 14.257 to 8.923 and after 14 days, the sugar content dropped to zero. This indicates that the microorganisms have exhausted the sugar content of the palm wine as there was no spice in it to inhibit the microbes.

By treating the palm wine with different spices, the rate at which the sugar in the palm wine was utilized dropped among the spices. This indicated that the spices had biocidal effect on the isolates to different degrees. Ginger so much extended the sugar content of the palm wine that even after 14 days of storage, the sugar content of the palm wine treated

with ginger was as high as 0.310 g/ml. But after 14 days of storage, the sugar content of the palm wine treated with other spices dropped to 0.00 g/ml just like in the control. This implies that treating palm wine with ginger can extend the shelf-life of the nutritional quality (sugar content) more than other spices used in the study. It was reported that preservation of palm wine could be achieved through inactivation of microbes at about 15 hrs after tapping when the density of these organisms is at its peak [12].

The pH values of the palm wines decreased indicating increase in acidity of the palm wine with time with each of the spices. Treating the palm wine with the five spices had similar results and this is attributed to the activities of *Lactobacillus* spp which is known to produce organic acids like lactic acid responsible for the acidic condition in the palm wine. This finding agrees with the reports of [27, 26 and 24].

The TTA increased from 0.173 to 0.867 after 14 days of storage. Treating the palm wine with alligator pepper, garlic, and ginger had similar result with the control after 14 days. The palm wine treated with Uda and Uziza spices had TTA increased to 0.847 and 0.807 respectively after 14 days of storage and this agree with the findings of [19].

It was also observed that the microbial genera decreased as storage progressed both in the control and treated palm wine. Only *Bacillus* spp, *S. cerevisiae* and *Lactobacillus* spp survived to the end of the storage. The gradual but progressive decrease in the level of the individual isolates as storage progressed could be attributed to the inhibitory effects of the spices as well as nutrient exhaustion and production of anti-microbial substances like ethanol by the yeasts and organic acids by the *Lactobacillus* spp. This study revealed that the palm wine treated with ginger maintained acceptable organoleptic quality for longer time than treatment with other spices used in this study.

5. Conclusion

Palm wine treated with spices (especially ginger) was able to maintain its organoleptic qualities. Based on the microbial counts obtained, loss of viability by the isolate could be associated with the preservative effect of the spices. Using spices as preservatives could however extend the shelf-life of palm wine more. The preservative effect recoded from ginger spice is a welcome development as a possible means of extending the shelf-life of palm wine in the rapidly expanding alcoholic beverage especially in the rural areas where refrigeration is missing to a large extent.

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