

*Full Length Research Paper*

# Effect of storage conditions on the shelf life of locally distilled liquor (Akpateshie)

Zakpaa, H. D. \*, Mak-Mensah, E. E., and Avio, O. A.

Department of Biochemistry and Biotechnology, Faculty of Biosciences, College of Science, Kwame Nkrumah University of Science and Technology, Private Mail Bag, Kumasi, Ghana.

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Locally distilled liquor (Akpateshie) is a beverage which is widely consumed in Ghana. It is produced from different raw materials. The purpose of this research was to determine the presence and levels of certain contaminants in akpateshie from the various sources and to determine effects of ambient temperature, refrigeration, pasteurization and pasteurization in combination with low temperature for the liquor stored for seven weeks. The contaminant microorganisms are spore forming gram positive *Bacillus* spp., responsible for converting alcohol to acetic acid. Total viable count (TVC) reached 25, 120 cfu/100 ml during the first week of storage, under room temperature in the most contaminated sample and thereafter, increased till the end of the storage period. Storage under the other conditions drastically retarded microbial growth. pH decreased from 5.0 to 2.7 at the end of storage period while the titrable acidity increased from 1.10 to 1.63. However, the pH and titrable acidity varied at slower rates in samples stored under other conditions. An increase from 0.02 to 0.04 was recorded for the acetic acid content of S2 (sample obtained from the second production site in the district; New Town). The presence of acetic acid, iron and copper metals were detected in all the samples. The study therefore concludes that the various storage conditions had an appreciable effect on the quality of akpateshie in the order; storage at room temperature followed by the refrigeration, pasteurization, then pasteurization in combination with refrigeration in increasing order of effect and that akpateshie pasteurized and refrigerated is the best for consumption.

**Key words:** Akpateshie, ambient temperature, Ghana, refrigeration, pasteurization.

## INTRODUCTION

Traditional alcoholic beverages have been consumed in Ghana and other West African communities for centuries. Locally distilled liquor, also known as Akpateshie in Ghana and Ogororo in Nigeria, is a spirit drink distilled from palm wine (Odeyemi, 1980). This drink is of historical significance in Ghana and Nigeria because, as a local gin, colonial administrators barred it in an attempt to control the West African liquor trade in the early part of the last century (Isidore, 2001).

Palm wine was originally the drink of choice in southern Ghana, replaced by rum and schnapps during the period of the slave trade (Akyepong, 1996). Men, thus, have come to favor distilled spirits, seen as "hot" or "strong," over palm wine or beer. Drinking distilled spirits was a

sign of prestige in pre-colonial Ghana and as such, a behavior controlled by the elders and the politically powerful. Women did not consume alcohol; young men drank rarely and then only as a result of the beneficence of the rich and the powerful (Akyepong, 1996).

There is a simple way of making distilled liquor without requiring a special monitoring system but this gives a relatively short shelf life period as compared to the industrially prepared liquors (Odeyemi, 1980). Akpateshie is essentially an unflavoured alcohol distillate. The storage materials as well as the conditions affect its alcohol strength with time. The beverage is regarded "flat" if it loses its sharp strength. The Akpateshie has a relatively short shelf life period as compared to the industrially prepared liquors. It can however be aged in order to improve its flavor as well as aesthetic appeal. This is done by storing in the presence of certain plant products like the roots of some plants. This usually imparts

\*Corresponding author. E-mail: [hzakpaa@hotmail.com](mailto:hzakpaa@hotmail.com).

a light yellow or dark brown colour to the liquor and this is popularly known as "bitters". The bitters are known to have some medicinal abilities depending on the type of plant product used during ageing. Apart from the slight differences in flavour, Akpeteshie produced from the different raw materials contain varying levels of ethanol, aldehydes, methanol, higher alcohols (fusel oils), total and volatile acids as well as solids. Pharmacologically, it is known that certain critical levels of these components are toxic to human health.

The production of Akpeteshie was legalized by the Government of the first Republic of Ghana since it was not allowed to be produced by the then colonial administration during the Gold Coast era with the view that the spirits may contain toxic alcoholic components due to lack of scientific quality control (Obot, 2000). Akpeteshie is distilled from fermented palm wine or sugar-cane juice and require a simple apparatus of two tins (usually four-gallon kerosene tins) and copper tubing. The standardized alcohol strength of Akpeteshie today is between 40 and 50% by volume (Akyeapong, 1996). Akpeteshie production, however, has seen no technological advancement since the legalization of its production. The methods employed in its distillation, collection, ageing, preservation and ensuring shelf life stability as well as its bottling have not been well taken care of resulting in its low economic value as compared to the industrially made liquor like Gin. Problems relative to Akpeteshie properties and characteristics such as flavour, colour, aroma and storage conditions with time also pertain to other alcoholic beverages in general. The different liquor-making practices and other important factors bear substantially on particular problems in relation to the liquors which are produced by traditional methods. For example, the industrial liquors in the country have had particular success with the appealing characteristics like flavour, colour, aroma and high shelf life they exhibit since the methods used in their production are technologically controlled and improved.

Akpeteshie production as conducted by most traditional methods does not meet the required standards set by the Ghana Standards Boards (Table 1). The final product is mostly contaminated by the combined action of various unacceptable levels of component substances like fusel oils resulting from inefficient distillation processes and the presence of some acid and alcohol tolerant species of moulds and bacteria resulting from unhygienic methods of liquor production. Some of these organic substances decompose and along with the products of metabolism produced by some of these microbes impart off flavours and cause spoilage of the liquor leading to a product of low quality and shelf life stability. Despite all the above mentioned setbacks, very little effort has been made to ascertain whether, through improved production practices or by various treatment procedures, these characteristics of Akpeteshie can be eliminated or at least reduced to a point where it would have improved taste and stability characteristics.

Research on the levels of contamination of Akpeteshie by product content levels and microbes is essential to design better methods for its production and to yield improved distilled liquors of greater value that will offer competition to the industrially produced ones on the market.

In recent years, there has been the establishment of several local distilleries in the country which employ unscientific and crude methods of producing spirits and as such have flooded the local market with dangerous products with low shelf life. An improvement of these spirits is necessary since they are being overtaken by the industrially distilled liquors which are of high quality, nicely packaged and are less dangerous with high shelf life. As a result, it has become essential for the analysis of locally distilled liquors to be carried out. A qualitative study of Akpeteshie in the northwestern region of Ghana found that consuming this traditional Gin was on the rise among both men and women (LuGinaah and Dakubo, 2003).

There is not a single large scale ethanol distilling industry in Ghana. The distilleries in the country basically buy pure ethanol from the large scale distilling countries like U.S.A., Germany etc. (Paramount Distilleries, Kumasi). They then mix the pure ethanol with distilled water under very hygienic conditions to produce the various kinds of industrially manufactured liquors in the country (Multhauf, 1993).

The main aim of the investigation was therefore, to analyze and establish the levels of the potentially harmful components of Akpeteshie, as specified by the Ghana Standards Board and their effects on shelf life in relation to the processes locally employed in its production. According to the Ghana Standards Board specifications, Akpeteshie should contain no volatile acid (European Commission, 2000).

This research seeks to determine the microbial type and load of Akpeteshie and how they change with time and to determine the levels of some contaminants that pose health hazards if present at high levels, for example, methanol,  $Fe^{2+}$ ,  $Pb^{2+}$ .

## MATERIALS AND METHODS

### Sample collection

Akpeteshie samples were obtained, on the same day, from three different production sites at Kona in the Efigyasekyere district of the Ashanti Region. The alcohol samples to be tested were put in 500 ml plastic containers that had been sterilized with alcohol (80% v/w) under conditions that excluded contamination from external sources.

Three lots from each sample were labeled in 3 groups as sample 1 (S1) from site 1 (Kona Habitat), sample (S2) from site 2 (New Town) and sample 3 (S3) from site 3 (Kona Market), respectively, based on the source of purchase of the mother Akpeteshie sample. Each sample lot was then grouped into four based upon various storage conditions: Akpeteshie stored at room temperature, pasteurized Akpeteshie, refrigerated Akpeteshie and Akpeteshie that has been pasteurized and refrigerated. A fifth sample which

was bottled Gin obtained from Paramount distilleries was used as a control.

### **Sterile practices and conditions**

Petri-dishes were sterilized in a hot oven at 170°C for 2 h (Nester et al., 2004). Micropipette tips and growth media were sterilized in an autoclave at 121°C for 15 min (Nester et al., 2004). Glassware and spatula were prewashed with detergent, dilute HNO<sub>3</sub> (1M) and distilled water.

Inoculations of microorganisms were performed in an inoculation chamber pre-irradiated with ultraviolet light. A burning flame was also used to sterilize open ends of test tubes and inoculation loops during plate streaking.

### **Preparation of growth media**

#### **MacConkey broth**

MacConkey broth was prepared according to the procedure stated by the manufacturer (Mast Group Ltd., Mast House, Derby Road, Bootle, Merseyside, UK) and in agreement with Benson (2002). MacConkey broth is a liquid medium for the detection and enumeration of coliforms by the most probable number (MPN) technique (Childs and Allen, 1953).

#### **Plate count agar (PCA)**

Plate count agar was prepared according to the procedure stated by the manufacturer and in agreement with Prescott et al. (1999). Plate count agar is a medium for performing total viable counts by the pour plate technique.

#### **Eosin methylene blue agar (EMBA)**

Eosin methylene blue agar (EMBA) was prepared according to the procedure stated by the manufacturer. EMBA is a selective differential medium for coliform isolation in aqueous solutions.

#### **Nutrient agar**

Nutrient agar was prepared according to the media manufacturer and in agreement with Benson (2002). It is a general purpose agar for the culture of non-fastidious microorganisms.

### **Detection and isolation of microbial indicator bacteria**

#### **Presumptive test**

Five milliliter volumes of autoclaved MacConkey broth (A multi-component dehydrated powdered medium composed of peptones mixture, lactose, sodium chloride, bile salts and bromo-cresol purple), were placed in three test tubes, sterilized in an autoclave at 121°C for 15 min and arranged in a triplicate rack. The MacConkey broth were incubated with 1ml aliquots of the serially diluted (10, 1, 0.1 ml) Akpeteshie and Gin samples and incubated at 37°C for 48 h to test for the presence of total and fecal coliforms. A control experiment was set up by incubating tubes containing MacConkey broth only.

#### **Enumeration of total viable count (TVC)**

Estimation of TVC bacteria in the Akpeteshie and Gin samples was

done using the pour plate technique. Dilutions of 10<sup>-1</sup> to 10<sup>-5</sup> of the samples were prepared and 1 ml aliquots each of the diluents were pour plated (inoculated) on 10 ml molten plate count agar (PCA) (Micro Essentials Ltd). These were incubated at about 37°C for 4 h and later at 44°C for 44 h. Sterilized Petri-dishes from dilutions containing discrete bacterial colonies were counted and the mean results expressed as the colony forming unit per 100 ml (CFU/100 ml). Colonies were counted using a colony counter.

### **Classification and identification of isolates**

Isolates from the colonies formed for the various samples were subcultured at 4°C on nutrient agar and classified according to their Gram stain employing the Hucker's Gram Staining Technique (Isenberg, 1995).

### **Gram staining and microscopy**

Microbial specimen were stained employing the Hucker's Gram Staining Technique. The slides were then examined under a light microscope using X100 oil immersion objective light microscope to observe the morphological characteristics of the microorganisms on the film.

### **Chemical analysis**

#### **pH determination**

The pH of the samples was determined on a weekly basis using a pH meter (Berkman Instruments, Fullerton, CA). The pH meter was first calibrated with standard buffers at pH 4.0 and 10.0. Each sample was placed in a 50 ml beaker and the bulb of the pH meter dipped into it. The bulb was carefully rinsed with distilled water each time a new reading was to be taken, (AOAC, Official methods of analysis, 1990).

#### **Copper and iron determination**

An atomic mass spectrometer (AAS) was used to determine the levels of copper and iron in the Akpeteshie sample. Standard solutions of copper with concentrations 1.0, 5.0 and 10 ppm were prepared using a CuSO<sub>4</sub> solution. A calibration curve was prepared from the standard solutions following their aspiration into an acetylene-oxygen flame of the AAS. Each of the samples was aspirated in turns into the flame with the copper cathode still in place. The concentration of copper in sample was detected and automatically recorded by an integrated printer attached to the instrument.

The determination of iron was determined by a similar mechanism by replacing the copper cathode light source with an iron cathode light source. Standard solutions of iron with concentrations 1.0, 5.0 and 10 ppm were prepared from a FeSO<sub>4</sub> solution. A calibration curve was drawn by the electronic printer using the standard samples aspirated into the flame in turns, (AOAC, Official methods of analysis, 1990).

#### **Methanol determination**

Exactly 40% ethanol was prepared by diluting 400 ml of pure ethanol in one liter of distilled water. To each of a series of 100 ml volumetric flasks containing 0.2, 0.4, 0.6, 0.8 and 1.0 ml of pure methanol, 40% methanol was added to yield methanol concentrations of 0.2, 0.4, 0.6, 0.8 and 1.0% (V/V), respectively. The content of each flask was thoroughly mixed by inverting the flasks a number of

**Table 1.** Standard parameters of liquor.

Parameter	Standard
Volatile Acids (acetic acid)	Nil
Alcohol Test	40 - 50%
Methanol	Maximum 0.500 mg/L
pH	7 - 8
Iron	Maximum 0.300 mg/L
Copper metal	Maximum 1.000 mg/L
Total acidity	0.6 - 0.9%

Source: Ghana Standards Board, 2009.

times.

With the aid of a 5  $\mu$ l micro syringe, the samples were injected successively into the injection chamber of the automated gas chromatography. The chromatograms were then obtained directly from an integrated recorder.

From the results obtained, a calibration curve was constructed by plotting a graph of Area against concentration (mg/ml). 1  $\mu$ l of the Akpeteshie sample was injected into the instrument under similar conditions as the standards. The area percentage obtained from the chromatograms was extrapolated to obtain the corresponding concentration of the sample on the calibration curve.

#### Titration acidity determination

The titratable acidity of a solution is an approximation of the solution's total acidity. Titratable acidity can be measured by a simple titration method using 0.1 N sodium hydroxide solution. 10 ml of each sample was measured with a 50 ml measuring cylinder into a conical flask and diluted with 30 ml of distilled water. Three drops of phenolphthalein concentration was added and titrated against 0.1 M NaOH. The end point was indicated by a change from a colourless to pink solution. The various volumes of NaOH used in the determination of the titratable acids were recorded and the total acidity calculated as percentage tartaric acid.

#### Acetic acid determination

Acetic acid content of the samples was determined by titration method using 1 M NaOH solution. 20 g of each sample was measured using a measuring cylinder into a 150 ml volumetric flask and diluted with 100 ml distilled water. 25 ml each of the aliquots were measured into a conical flask and diluted with 25 ml of distilled water. Three drops of phenolphthalein concentration was added and titrated with 1 M NaOH. The end point was indicated by a change from a colourless to pink solution. The various volumes of NaOH used in the determination of the titratable acids were recorded.

#### Absolute alcohol determination

A relative density bottle (Merck, Dorset England), was used to determine the specific gravity of the various samples and their corresponding alcohol content were obtained by reference to laboratory tables.

The empty relative density bottle was weighed at room temperature on an electric weighing balance. It was then filled with distilled water and weighed. The relative density bottle was carefully rinsed with a small amount of the first sample and weighed after filling it with the same sample at room temperature. The relative

density bottle was wiped dry with a clean cloth before being weighed. This procedure was repeated for the rest of the samples, rinsing the relative density bottle with small amounts of the particular sample to be weighed.

#### Analysis of data

The data collected was statistically analyzed using a One Way ANOVA (Analysis of Variance). The SPSS statistical software was used to determine the significant difference between the various parameters and that of the standard. A confidence level of 95% was maintained and the results analyzed (Tables 3, 4, and 5).

## RESULTS

From Figure 1, the sample stored at room temperature was the most microbially contaminated sample among the various storage conditions for samples from Kona Habitat (with a TVC of 2,140 cfu/100 ml in the first week followed by the refrigerated sample with 630 cfu/100 ml). The pasteurized sample followed with a TVC of 210 cfu/100 ml. The least contaminated sample (pasteurized and refrigerated) had 72 cfu/100 ml all in the first week. These were lower in contamination than those of their corresponding storage samples from New Town (S2) and Kona Market (S3) groups of samples. One rule of thumb specifies that each 10°C temperature rise roughly doubles reaction rates (Adams and Moses, 1995).

The titratable acidity values for samples from Kona Habitat (S1) in the first week are shown in Figure 2 and were respectively, 1.10 for room temperature storage, 1.08 for refrigeration storage, 0.94 for pasteurization storage and 0.93 for pasteurization and refrigeration storage conditions.

From Figure 3, the pH values of sample from S1 (Kona Habitat) in the first week were respectively, 5.2 for room temperature storage, 5.6 for refrigeration storage, 5.7 for pasteurization storage and 5.8 for pasteurization and refrigeration storage conditions.

Acetic acid content of Akpeteshie from Kona Habitat (S1) in the first week was 0.00% for all storage conditions (Figure 4). The acetic acid levels started to rise to 0.01% in the samples stored at room temperature and refrigeration conditions in the third week and continued to elevate in

**Table 2.** Ethanol, methanol, copper and iron contents of the various samples.

Parameter	Sample from kona habitat (s1)		Sample from new town (s2)		Sample from kona market (s3)		Gin	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Ethanol concentration (%)	20.8	18.9	20.5	17.1	20.5	18.4	45.7	47.9
Methanol concentration (ppm)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
Iron concentration (mg/l)	0.096	0.097	0.102	0.102	0.034	0.031	0.010	0.011
Copper concentration (mg/l)	12.629	12.600	10.152	10.155	10.189	10.183	0.013	0.011

**Table 3.** ANOVA tables for Akpeteshi from Kona Habitat (S1).

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
<b>Total Viable Count (TVC)</b>					
Between groups	85.0	4	11.5	267.22	0.028
Within groups	0.0	110	0.0		
Total (Corr.)	85.0	114			
<b>Titration acidity</b>					
Between groups	69.0	4	39.6	184.32	0.032
Within groups	0.0	110	0.0		
Total (Corr.)	69.0	114			
<b>pH</b>					
Between groups	113.739	4	42.3481	214.67	0.033
Within groups	16.8261	110	0.76567		
Total (Corr.)	130.565	114			
<b>Acetic acid</b>					
Between groups	49.0	4	7.0935	284.17	0.062
Within groups	0.0	110	0.0		
Total (Corr.)	49.0	114			

the subsequent weeks at different rates depending on the extent of microbial contamination. Acetic acid levels remained at 0.00% for sample from Kona Habitat (S1) stored under the other conditions. The Gin sample also maintained a 0.00% acetic acid level under all storage conditions for all the samples analyzed from the various sources (S1, S2 and S3). These results are depicted in Figure 4.

Figure 1 shows the variation in total viable count under the various storage conditions. The sample stored at room temperature was the most microbially contaminated sample under the various storage conditions for samples obtained from New Town (S2), with a TVC of 25,120 cfu/100 ml in the first week followed by the refrigerated sample with 24,190 cfu/100 ml. The pasteurized sample followed with a TVC of 4,070 cfu/100 ml. The least contaminated sample (pasteurized and refrigerated) had 3,034 cfu/100 ml all in the first week. The samples from New Town were the most contaminated as compared to samples under the same storage conditions for Akpeteshie samples from Kona Habitat (S1) and Kona Market (S3).

Titration acidity values of samples from New Town (S2) in the first week were respectively, 1.57 for room tem-

perature storage, 1.55 for refrigeration storage, 1.33 for pasteurization storage and 1.31 for pasteurization and refrigeration storage conditions (Figure 5).

pH values of Akpeteshie samples from New Town (S2) in the first week were respectively, 5.0 for room temperature storage, 5.2, for refrigeration storage, 5.5 for pasteurization storage and 5.6 for pasteurization and refrigeration storage conditions (Figure 6).

Figure 4 shows the comparison of acetic acid produced under the various storage conditions for samples from Kona Habitat, acetic acid level of drink in the first week was 0.02% for the room temperature and refrigeration storage conditions and 0.00% for the pasteurized storage and the pasteurization and refrigeration storage conditions. These, however, showed an increase with time with the room temperature and refrigeration storage samples exhibiting a faster acetic acid level increase rate than the other storage conditions.

The microbial load, variation of acetic acid, pH and total acidity for Akpeteshie from Kona Market (S3) showed a similar trend to that observed for samples from New Town (S2).

Table 2 indicates that the alcohol content of Akpeteshie

**Table 4.** ANOVA tables for Akpeteshie from New Town (S2).

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
<b>Total Viable Count (TVC)</b>					
Between groups	53.0	4	17.5	144.83	0.021
Within groups	0.0	110	0.0		
Total (Corr.)	53.0	114			
<b>Titration acidity</b>					
Between groups	45.4320	4	67.5	113.14	0.043
Within groups	10.445	110	0.068387		
Total (Corr.)	56.0765	114			
<b>pH</b>					
Between groups	71.0467	4	37.5776	124.92	0.049
Within groups	14.7323	110	0.05679		
Total (Corr.)	85.7790	114			
<b>Acetic acid</b>					
Between groups	24.6	4	63.5	89.35	0.019
Within groups	0.0	110	0.0		
<b>Total (Corr.)</b>	24.6	114			

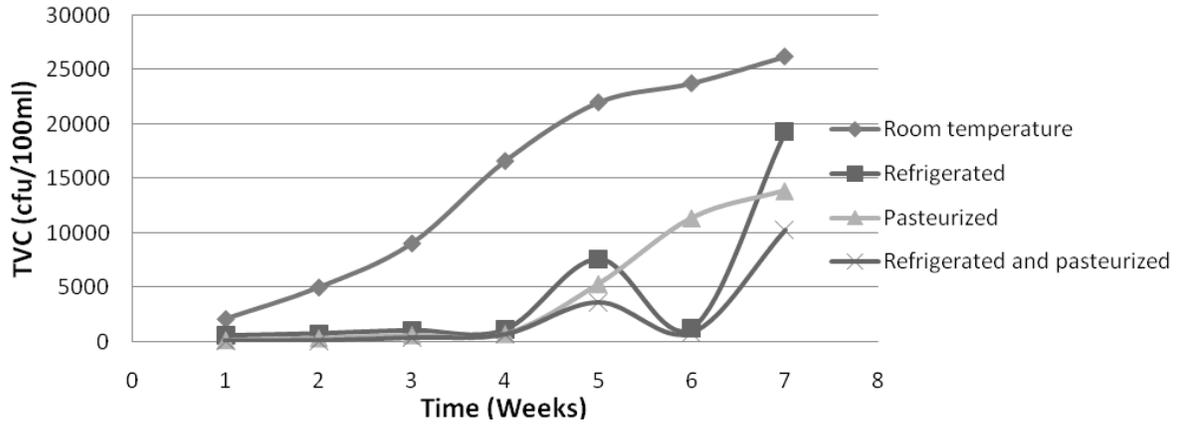
**Table 5.** ANOVA tables for Akpeteshie from Kona Market (S3).

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
<b>Total Viable Count (TVC)</b>					
Between groups	134.765	4	23.9554	174.01	0.046
Within groups	10.093	110	0.03588		
Total (Corr.)	144.858	114			
<b>Titration acidity</b>					
Between groups	64.0	4	59.5	63.33	0.073
Within groups	0.0	110	0.0		
Total (Corr.)	64.0	114			
<b>pH</b>					
Between groups	58.761	4	12.8871	122.67	0.044
Within groups	15.128	110	0.02769		
Total (Corr.)	73.889	114			
<b>Acetic acid</b>					
Between groups	148.656	4	23.337	217.89	0.048
Within groups	9.065	110	0.18782		
Total (Corr.)	157.721	114			

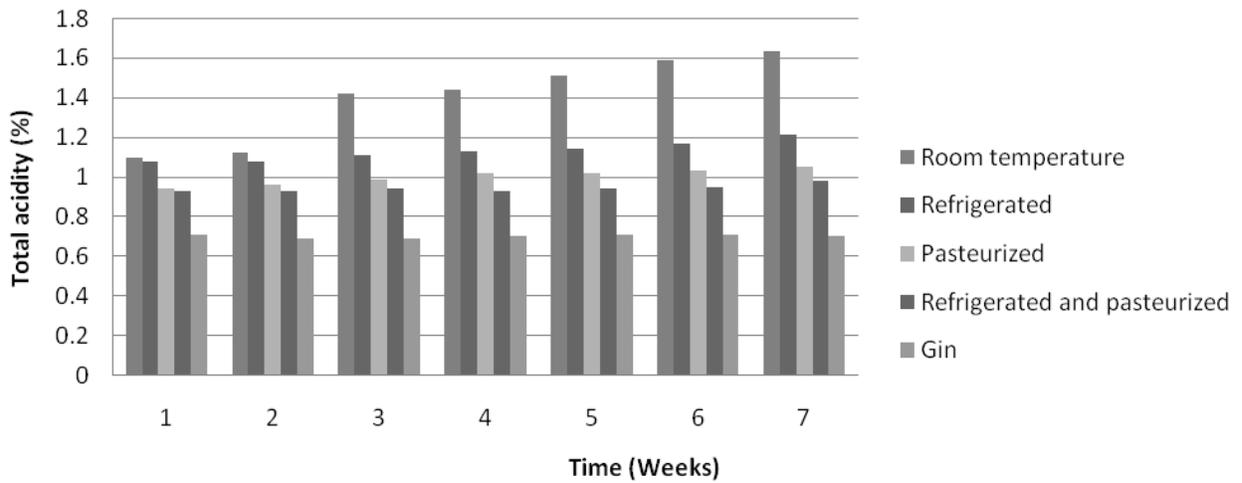
samples S1, S2 and S3 stored at room temperature decreased from 20.8, 20.5 and 20.6% in the first week to 19.5, 18.9 and 19.1%, respectively, in the third week, indicating a respective decrease of about 9.1, 16.6 and 10.7% alcohol content of the samples. From Table 6, there was a significant difference between the alcohol strength of the control, Gin and Akpeteshie samples from Kona Habitat under the various storage conditions (p value of 0.03 was less than the critical value of 0.05). There was also a significant difference between the alcohol strengths of the Akpeteshie samples collected from the

various sites. The average iron content of the samples over seven weeks were 0.097, 0.102 and 0.034 mg l<sup>-1</sup> for samples S1, S2 and S3, respectively. The control gin sample had no determinable iron content.

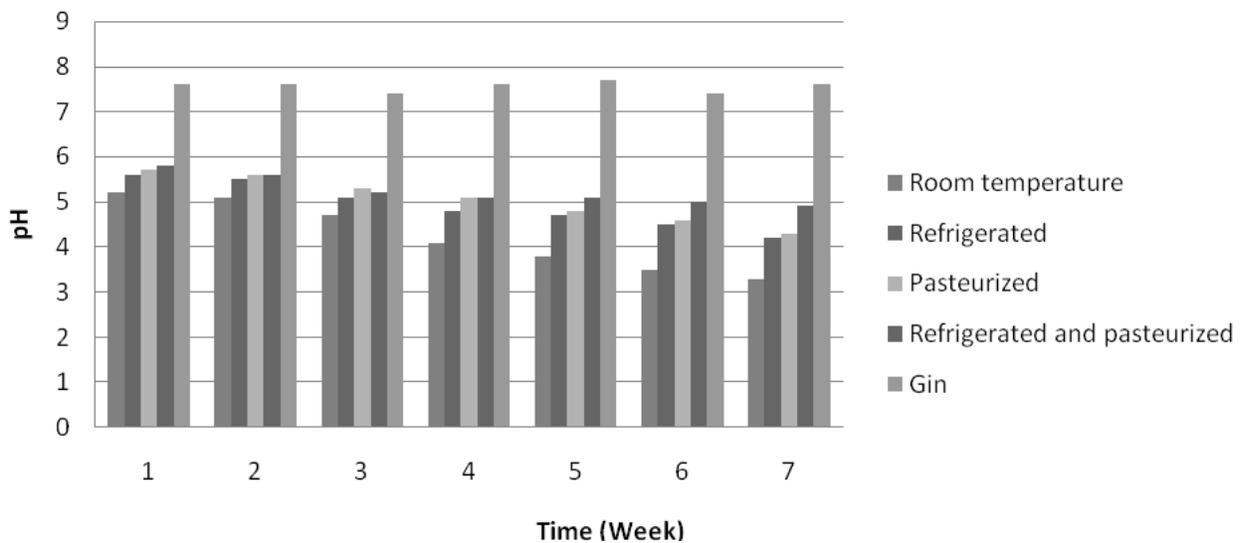
The average copper levels of the samples over seven weeks were 12.611, 10.154, 10.183 and 0.073 mg l<sup>-1</sup>, respectively, (Table 2) for Akpeteshie from Kona Habitat (S1), New Town (S2), Kona Market (S) and the control sample (Gin). Again it can be seen from Table 2 that the iron and copper contents of all the samples remained fairly constant for the seven week period.



**Figure 1.** Variation in the total viable counts (TVC) of Akpeteshie from Kona Habitat (S1) under various storage conditions.



**Figure 2.** Variation in titrable acidity in Akpeteshie from Kona Habitat (S1) under various storage conditions.



**Figure 3.** Variation in pH of Akpeteshie from Kona Habitat (S1) under various storage conditions.

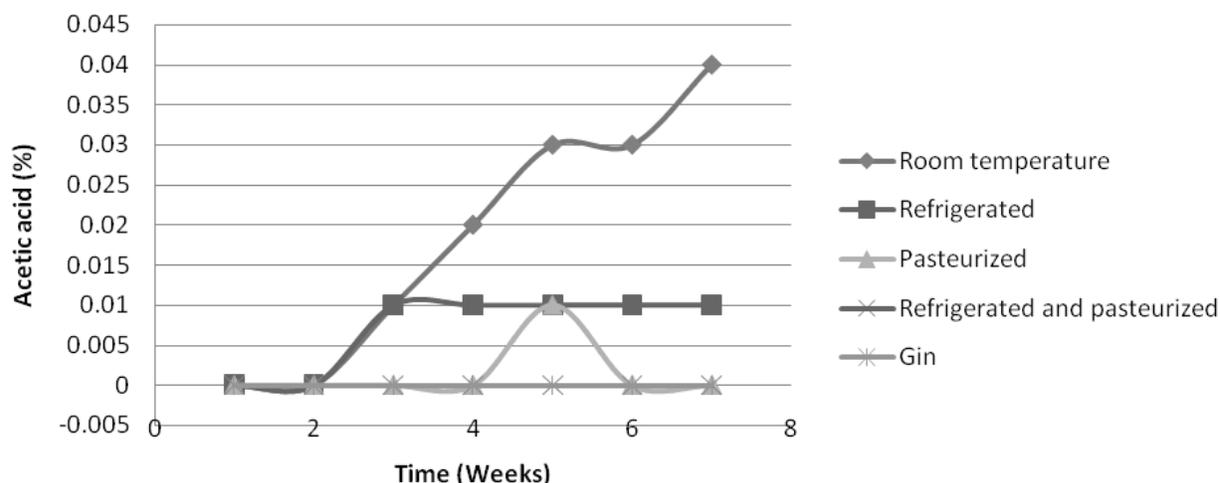


Figure 4. Variation in acetic acid levels of Akpeteshie from Kona Habitat (S1) under various storage conditions.

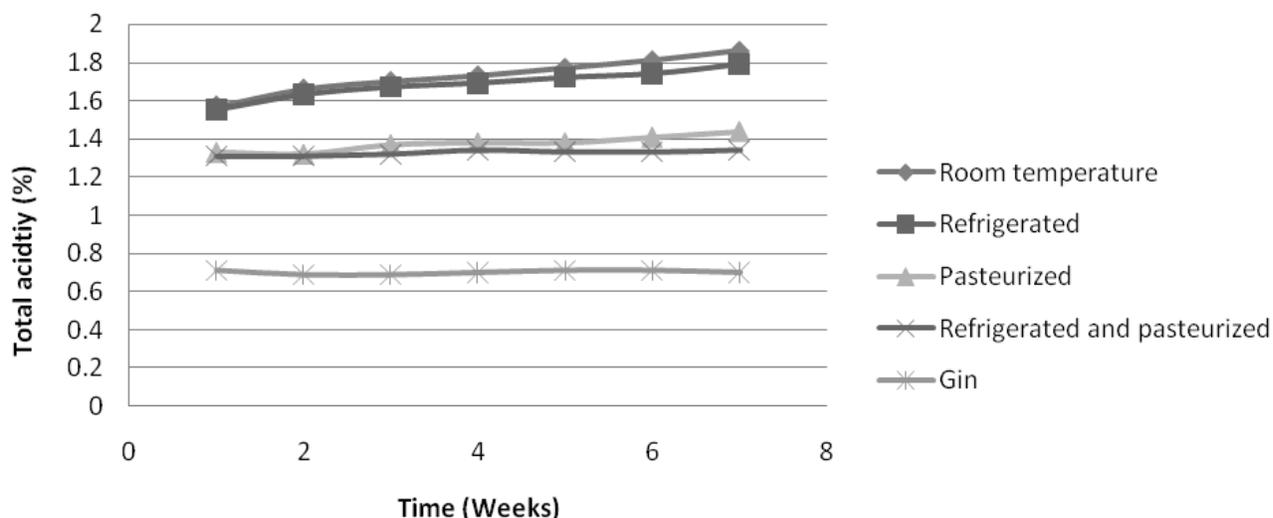


Figure 5. Variation in titrable acidity of Akpeteshie from New Town (S2) under various storage conditions.

## DISCUSSION

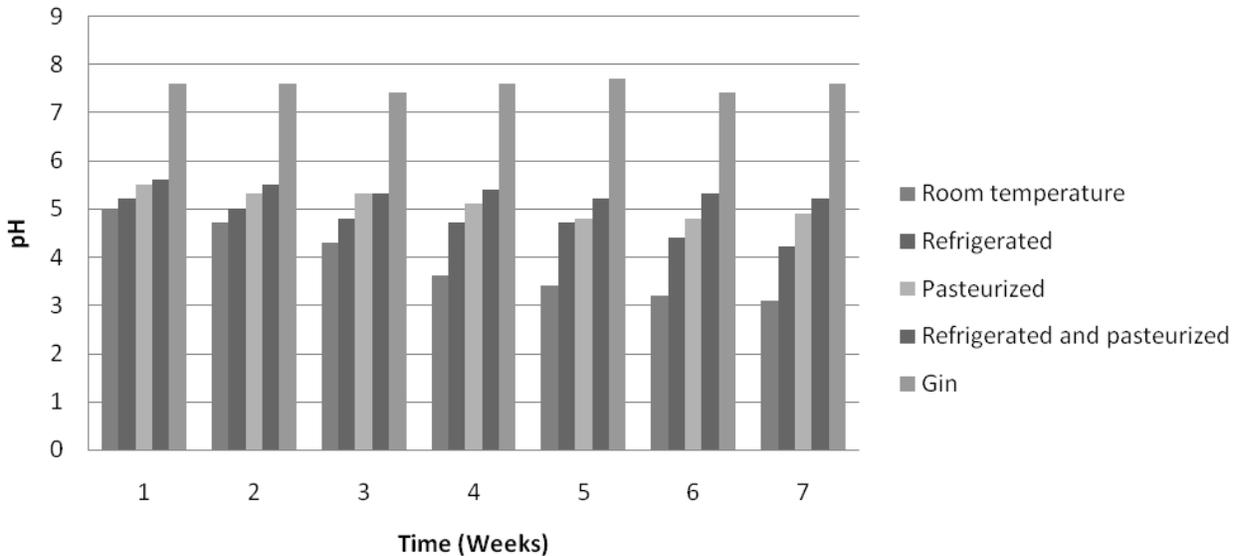
### Total viable count

This study showed that all the Akpeteshie samples from Kona Habitat (S1), New Town (S2) and Kona Market (S3) were extremely polluted with microorganisms as indicated by their Total Viable Count (Figure 1). Ideally, distilled liquors are expected to have no microbial contamination as recommended by the WHO for distilled liquors (WHO, 1993).

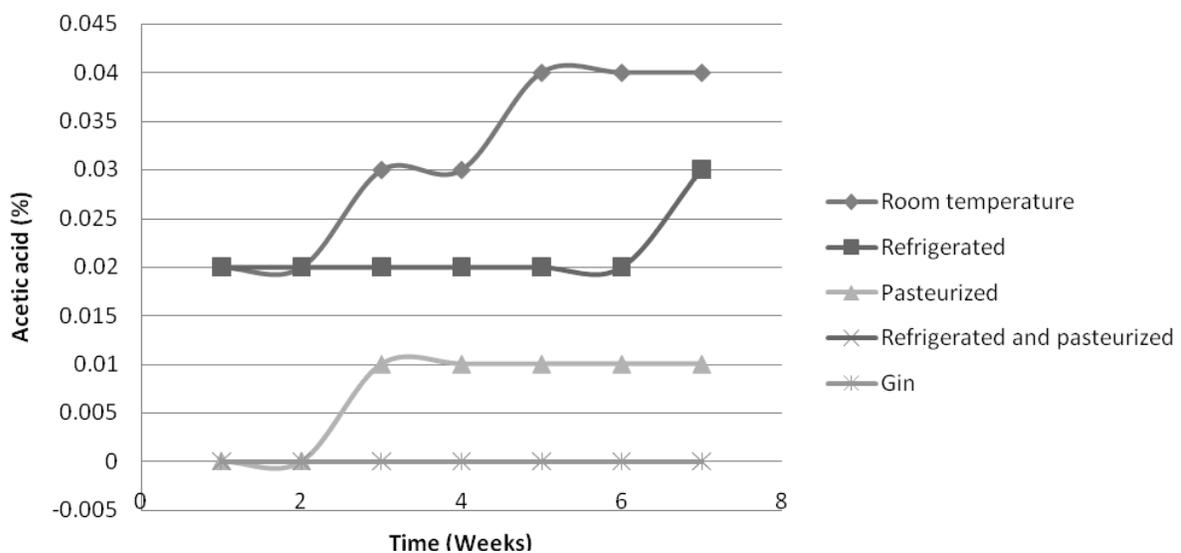
The inefficient fermentation of the substrate under uncontrolled conditions produces Akpeteshie of low alcohol content of about 20.5 to 20.8% (Table 1). The alcoholic strengths of the samples were low with the range of 26.8 to 39.9% previously reported for African distilled liquor (Odunfa and Oyewole, 1998). The range is

also inconsistent with the values given in a report about African traditional beverages from Tanzania (Mosha et al., 1996). This alcohol concentration is not sufficient to destroy contaminating microbes because only solutions of 60-70% alcohol and above can be used as preservatives or antiseptics by rapidly killing vegetative bacteria and fungi by coagulating enzymes and other essential proteins and by damaging lipid membranes (Kister, 1992). The distilling drums, collection containers and the water used to wash them and to cool the vapours during distillation were not stored under hygienic conditions. The environment was also not clean and these can lead to the contamination of the product by microbes, especially those that survive alcohol concentrations lower than 60% (Prescott et al., 1999).

During TVC enumeration, bacteria colonies isolated were identified as gram positive *Bacillus species* which is



**Figure 6.** Variation in pH of Akpeteshie from New Town (S2) under various storage conditions.



**Figure 7.** Variation in levels of acetic acid in Akpeteshie from New Town (S2) under various storage conditions.

widely distributed in soil and air usually as spores and are also capable of converting alcohol to acetic acid. It survives at a temperature of 25 to 30°C and in acidic mediums with pH values of 2.8 to 5.8 (Alcama, 1997).

Low growth rates were exhibited by the microbes in the refrigerated and pasteurized samples relative to the ones stored at room temperature (Figures 1, Tables 3, 4 and 5).

Pasteurization temperatures did not kill all the microbes present but those that survive were generally too damaged to cause any spoilage (Gould and Hurst, 1969). The treatment readily almost kills all yeast and acid tolerant bacteria hence pasteurization reduced the microbial

numbers and growth rate to improve the shelf life of the pasteurized samples.

There was the weekly steady growth of the present micro-organisms at different rates depending on the different storage conditions. There was highest growth rate in the sample stored at room temperature followed by the refrigerated sample, pasteurized sample and the pasteurized and refrigerated sample in that order.

In all the groups, there was a significant difference in the rate of TVC change since they all had a p value less than 0.05 (Tables 3, 4 and 5).

The titrable acidity of a solution is a measure of the hydrogen ions ( $H^+$ ) of all the organic acids present in wine

**Table 6.** ANOVA table for liquor strength.

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups	163.739	4	40.9348	284.52	0.03
Within groups	15.8261	110	0.143874		
Total (Corr.)	179.565	114			

or liquor.

In regards to wine and liquor production; total acidity is given in units of  $\text{g l}^{-1}$  as per tartaric acid equivalent with the acceptable standard level being 0.6 to 0.9% (Lewis et al., 1961). The titrable acidity levels of all the highly contaminated samples from the third week were all higher than the accepted standard indicating the presence of other acids apart from tartaric acid (Figure 5). Volatilization of some of the organic compound and organic acids during distillation may account for the initial increase in pH while the subsequent decrease observed all through the storage period may be associated with the extensive microbial activities which help to break down carbohydrates for the production of organic acids. This may be responsible for gradual increase recorded in the titrable acidity. Similar findings for other food items have been documented (Ogiehor et al., 2003). The increase in pH resulted from the increased production of acids like acetic acid (Tables 3, 4 and 5) produced by the Gram positive *Bacillus* species in metabolizing alcohol to acetic acid. Consequently, the alcohol content of all the samples decreased as the acetic acid levels elevated. This was evident from the tables as the alcohol content of samples stored progressively decreased from the first week to the seventh week. There was an increase in titrable acidity values of all the samples with time since an increase in microbial contamination caused an increased rate of alcohol conversion by the microbes to acetic acid which consequently resulted in higher titrable acidity values of all the samples. The control sample showed no significant change in its titrable acidity values and alcohol content over the seven week period. Samples under each group showed a statistical significance in the rate of titrable acidity change.

The acetic acid levels of the samples may have increased as a result of the conversion of alcohol to organic acids (acetic acid) by the contaminating microbes. The acetic acid level changes in all samples from New Town (S2) and Kona Market (S3) were statistically significant but this was not so for samples obtained from Kona Habitat (S) since it had a p value of 0.062 (Tables 3, 4, and 5).

## pH

Atsriku and Boadu (1991) showed that the various types of Akpeteshie from various sources had pH within the range of 3.86 to 4.04. Akpeteshie analyzed from the

various sources had a pH range between 2.7 for the most contaminated sample, from New Town (S2), under room storage conditions in the seventh week and 5.8 for the least contaminated sample, that from Kona Habitat (S1; pasteurized and refrigerated), in the first week indicating that Akpeteshie is fairly acidic (Figure 3).

There was a decrease in pH values with time for all the samples since an increase in acid produced in solution caused a decrease in pH values. The pH values of the samples were however not in the standard range of 7 to 8 units for distilled liquors. According to Robinson (2000), the optimum pH range for the growth of most *Bacillus* species is 2.8 to 5.8. The acidic pH of the samples ensured the continued existence and growth of the contaminating microorganisms helping to decrease the shelf life of the samples (Crueger and Crueger, 1984). Again, the control sample showed no significant change in its pH values over the seven week period. There was statistical significance, p value less than 0.05 (Tables 3, 4 and 5), in the pH changes of all samples from the various sources, S1, S2 and S3.

## Metals

The average iron contents of the samples were below the critical standard level of 0.300 mg/ml to cause harm to the consumer (Tompsett, 1992). The average iron contents of Aketeshie from Kona Habitat (S1), New Town (S2) and Kona Market (S3) were 0.097, 0.102 and 0.033  $\text{mg l}^{-1}$ , respectively. A daily iron intake of 5 to 10 mg for men and 15 mg for women are the minimum requirements for maintaining normal health (Multhauf, 1993). The control sample had no determinable iron content.

The copper levels varied for the various samples because of the different copper condensing tubes used in distillation at the three different sites. The average copper contents of the samples S1, S2 and S3 were 12.615, 10.154 and 10.186  $\text{mg l}^{-1}$ , respectively (Table 2). The copper concentrations may be possibly traced to the copper stills used for distillation as the major source. It is therefore not atypical to find copper levels of this magnitude in spirits. Levels as high as 5.31  $\text{mg l}^{-1}$  of copper were measured in sherry brandies (Camean et al., 2000) or levels up to 9.2  $\text{mg l}^{-1}$  (Bettin et al., 2002) or up to 14.3  $\text{mg l}^{-1}$  (Nascimento et al., 1999) of copper in Brazilian sugar cane spirits. The presence of copper in the samples may have resulted from the erosion of the corroded portions of the inner lining of the copper

condensing tube by the hot and acidic Akpeteshie during distillation. Work carried out by Tompsett (1992) indicated a daily requirement of copper by an individual to be about  $0.6\text{mg l}^{-1}$ . For all three samples, except the control sample, the amount of copper carried along was far above the threshold level of  $1.000\text{mg l}^{-1}$  to cause harm to the consumer.

## Methanol

Methanol is released to the environment during industrial uses and naturally from volcanic gases, vegetation and microbes. Exposure may occur from ambient air and during the use of solvents. Acute (short-term) or chronic (long-term) exposure of humans to methanol by inhalation or ingestion may result in blurred vision, headache, dizziness and nausea. Birth defects have been observed in the offspring of rats and mice exposed to methanol by inhalation (Burton et al., 2000). The reference dose (RfD) for methanol is 0.5 milligrams per kilogram body weight per day (mg/kg/d) (Dutkiewitz et al., 1987). Methanol is the substance most often associated with the toxicity of distilled liquors. Though some level of methanol was detected in all the samples of Akpeteshie analyzed, the levels were so low to cause any immediate problems to the consumer (Dutkiewitz et al., 1987).

## Conclusion

The unhygienic and uncontrolled fermentation of palm wine and its further inefficient distillation method are factors that result in Akpeteshie contaminated with micro-organisms. The production of acetic acid causes an increase in total acidity values and a decrease in pH values and alcohol levels of Akpeteshie. The Akpeteshie samples contain insignificant amounts of iron and methanol but lethal amounts of copper.

This study has shown the effectiveness of the benefits of low temperature storage and pasteurization in keeping the total quality of Akpeteshie during storage. The Akpeteshie produced at Kona in the Efigyasekyere District of the Ashanti Region loses its quality with time.

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