Conservation of Palm Wine Using Ultraviolet Radiation

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Abstract

Purpose: This study investigated the conservation of palm wine using ultraviolet radiation.

Methodology: The palm wine was conserved by sterilization with ultraviolet rays sterilizer. The time of contact of the rays and the intensity of the rays with palm wine was varied. The microorganisms considered include mould, yeast and bacteria.

Results: Results obtained shows that the microbial load of sterilized palm wine decreased with time of contact with rays. The microbial loads of the wine before and after sterilization were determined. More so, the microbial load further decreased during storage of sterilized samples. In fact, sterilization time of 240 seconds (4 minutes) reduced the mould and yeast from 3.25 × 10⁶ to 0.01 × 10⁶ and then to zero after 10 days of preservation.

Limitations: This research did not consider the effect of stirring on the microbial load reduction.

Contribution: This research has established an ideal sterilization time of 240 seconds (4 minutes) for palm wine sterilization.

Keywords: Palm wine, Ultra-violet radiation, Mould, Microbial load, Bacteria


1. Introduction

Drinkable palm wine is highly consumed in Asia, sub-Saharan Africa, and South America. The sap of numerous palm trees is fermented to generate palm wine. The beverage is made from a variety of palm trees that are grown all over the sub-tropical and tropical regions, with a few breeds coming from climatic regions because of the illiberality of seedling freeze (Reichgelt, West, & Greenwood, 2018). The beverage is popular and known by various aliases throughout the world. Significant uptick in the microbiological analysis of the beverage have led to increased discussion of the variety of Saccharomyces cerevisiae strains that predominate in the beverage (Tapsoba, Legras, Savadogo, Dequin, & Traore, 2015; Tra Bi et al., 2016) and newly identified microorganisms from the drink (Ouoba et al., 2015).

According to numerous reports (Nwaiwu & Itumoh, 2017), the method of hurling the beverage has been established. The palm sap varies according to the location of the palm trees, which has been determined in various reports. Due to the active microorganisms it contains, palm wine is normally a sweet, white liquid that fizzes. In the south-eastern region of Nigeria, the Igbo ethnic group value palm wine highly as their primary alcoholic beverage during ceremonial occasions. Its pale appearance is due to a yeast suspension. Palm wine has replaced human and animal blood in purifying and expiatory ceremonies due to its potency in the development of African traditional religion (Okolo et al., 2019). The beverage is now bottled as industrial production has improved. In Nigeria, the product is typically packaged in beer containers, and most distributors sell the beverage in repurposed table water containers with a top that is not too tightly fitted to prevent foaming (Nwaiwu & Itumoh, 2017).
The upkeep of the wine's stable chemical and physical properties depends heavily on preservation of palm wine. Many traditional preservative methods only make the spoilage agents (microorganisms) inactive, so over time, these organisms may regain their bioactivity and cause the palm wine to spoil. Again, the use of chemical preservation methods has the disadvantage of interfering with the wine's active components, changing the texture, flavor, viscosity, and other characteristics, which defeats the goal of uniqueness. As a result, there is a critical need to investigate alternative wine preservation methods that combine effectiveness and creativity while eliminating deterioration agents and preserving the wine's excipients. So, in an effort to discover the ideal preservation method with a low economic cost and a high success rate, this study was chosen.

2. Literature Review

The primary organisms involved in the beverage's fermentation are yeasts, which are a part of the natural flora of palm palms. No matter where the drink originates from, unless it is kept in cold storage, it always seems to get sour within 24 hours. Palm wine is widely used in African traditional medicine particularly in the treatment of measles, chronic yellow or malaria fever. In Ivory coast, palm wine is said to alloy the pain caused by poisonous bites. Many nursing mothers who drink palm wine believe that it helps the flow of breast milk for their babies as well as having a diuretic effect. The distinctive qualities of palm wine have sparked interest in study into its potential industrial applications and practical uses (Nwachukwu, Ibekwe, Nwabueze, & Anyanwu, 2006).

In Nigeria, Raphia hookeri and Elaeis guineensis are the two trees that are most commonly used to make palm wine. The origin or provenance of these palm trees is a topic of discussion. In freshwater swamps of west and central Africa's freshwater wetlands, the Raphia hookeri tree is the most widely-known. It is produced in India, Malaysia, Singapore, and Nigeria's tropical rain forest, where there are numerous regional variants. The E. guineensis oil palm variety is more prevalent globally. In accordance with a source, the E. guineensis palm tree evolved in the tropical rain forests of West Africa and is now widespread throughout the region, including in Cameroon, Côte d'Ivoire, Ghana, Liberia, Nigeria, Sierra Leone, Togo, Angola, and the Congo. According to Legras, Merdinoğlu, Cornuet, and Karst (2007), some palm fruits were transported to the Americas and then to the Far East during the fourteenth and seventeenth centuries, where they flourished. Given that yeast strains are known to reflect human history (Legras et al., 2007), it is conceivable that the yeast strains identified in palm wine were transported to other countries via plant materials that were brought to those areas.

Naturally occurring yeasts of the genus Saccharomyces ferment the sugar-rich saps that make up palm wine. In the absence of oxygen, sugar is used during fermentation, a metabolic process that yields alcohol and carbon (IV) oxide. The overall equation of reaction is given as Equation (1).

\[ C_6H_{12}O_6 \rightarrow 2 C_2H_5OH + 2 CO_2. \]  

Equation (1)

Anaerobic bacteria like Clostridium or Acetobacterium can convert carbohydrates directly to acetic acid during anaerobic fermentation, eliminating the requirement for ethanol as an intermediate. This equation of reaction is given as Equation (2).

\[ C_6H_{12}O_6 \rightarrow 3 CH_3COOH \]  

Equation (2)

In addition to cells lacking oxygen, yeast and bacteria also exhibit it. Zymology is the field that studies fermentation. This calls for the application of a trustworthy preservation technique that can maintain the flavor and components of the wine while also having a favorable impact on health. According to statistics, about ten million Africans enjoy palm wine, according to Santiago-Urbina and Ruíz-Terán (2014). Due to its all-natural constituents, it is a popular mainstream drink.

Also prevalent in Nigerian palm wine are pathogenic microorganisms as Lactobacillus, Lieuconostoc, Klebsiella, Streptococcus, Racillus, Zymononas, Brevibacterium, Acetobacter, and Sereratia, which
are thought to have originated from the water used by the tappers to dilute the beverage (Amoa-Awua, Sampson, & Tano-Debrah, 2007). The unique flavor of fresh palm wine has been attributed to lactic acid bacteria (Okafor, 2007). The tapper uses a palm wine keg to collect sap; other sources of the yeast and bacterial microflora are the air, knife, and palm tree. As a result of the yeast's fermentative action, the alcohol content rises over time.

Due to the uncontrolled metabolic activities of yeast and bacteria, the beverage has a limited shelf life, which is the key issue with handling it. The food and beverage sectors have employed ultraviolet light to successfully clean surfaces and the air. The nucleic acid bases are the target organelles of the microbes (pyrimidine and thymine bases). The radiation damages DNA, which leads to lethality and the death of the organism. A successful application of UV light to regulate these creatures' metabolic processes will be a particularly welcome outcome (Dibofori-Orji & Ali, 2019; Nwaiwu et al., 2016). There is no reported work - to our knowledge - on the preservation of palm wine using ultraviolet radiation. This study is therefore aimed at establishing standard conditions for palm wine preservation using ultraviolet radiation.

3. Research methodology

3.1 Materials

All reagents used in this research are of analytical grade. Glass wares were sterilized. Instruments were calibrated before use. These glassware included glass test tubes, petri-dishes, pipette, Bijou bottles and McCartney bottles.

3.2 Methods

3.2.1 Microbial Analysis

3.2.1.1 Agar Nutrient

One liter of distilled water was used to dissolve a 28-gram dried nutritional agar. The mixture was then poured into 250-ml bottles, corked, and placed in an autoclave for 15 minutes at 121 °C. The sterile nutrient agar was taken into a sterile petri dish while still liquid, allowed to set, and then dried in the incubator for 15 minutes at 50 °C. For perfect sterility before to use, it was then cultured for 24 hours.

3.2.1.2 Malt Extract Agar

Mycological media differ from bacterial media because of the differences in the requirements of yeast and mould for growth, with fungi having optimum pH, much lower than that of most bacteria. The malt extract agar contains 10 % of citric acid which masks the growth of bacteria grossly. The agar was prepared with the same method as in the preparation of nutrient agar.

3.2.1.3 Total Viable Count (TVC)

The method of pour-plating was used to determine total viable count, which accounted for the number of entire living organisms or clamps of organisms (colonies) in the sample of palm wine in test. Nine milliliters of quarter strength ringers solution (1/4R) was the diluent used, and the dilution factor of $10^{-1}$, $10^{-2}$ were used for preserved sample and $10^{-4}$ in the fresh, unsterilized sample.

Some molten agar medium (0.34) and 2% glucose solution were mixed with the above diluents in a sterile petri-dish, and then incubated at 35 °C for 25 hours for bacterial organisms and at 37 °C for 3 days in the case of yeast and mould. Colonial counts were limited only to those with 30-300 colonies. Duplicate set of plates were incubated at each temperature and average results obtained.

3.2.2 Sample Determination Physiochemical Analysis

The physicochemical properties which were examined include titrable acidity, volatile acidity, alcoholic content, sugar content, pH, moisture content, relative density, total dissolved solid, suspended solid and vitamin C content.
3.2.2.1 Determination of the Titrable Acidity

Titrable acidity is a term for the amount of organic acids in the wine sample being examined. The titration determines the quantity of organic acid content that can be titrated to a pink end point with a dilute alkali solution.

In a sterile 500 ml conical flask, 200 ml of distilled water was placed and then heated. Indicator solution containing 1% aqueous alcohol and phenolphthalein was added in 1 ml. To produce this light pink tint, a 0.1 M NaOH solution was titrated. The boiling neutralized solution was then pipetted with 5 ml of the "must," which was then titrated once again to the end point with the same 0.1 M NaOH solution. The formula used to determine the titrable acidity was tartaric acid as shown in Equation (3).

\[ \text{Tartaric acid} \text{ g/100ml} = \frac{V \times M \times 75 \times 100}{100 \times V} \]

(3)

where  \( V \) = Volume of NaOH (Final reading - Initial reading)

\( M \) = Molarity of NaOH

3.2.2.2 Determination of Volatile Acidity

Volatile acidity measures the acetic acid content of the sample by determining the volume of base (NaOH) that completely neutralized 10 ml of the sample to the end point coloration of pink lasting for about 15 seconds.

600 ml of boiling, deionized water was injected in the outer chamber of a distillation column. 10 ml of the sample was accurately measured into the inner chamber. The sample container was rinsed thoroughly with deionized water into the same inner chamber. The outer chamber was brought to slow boiling and about 40 ml of the distillate collected in a receiving flask. The distillate was quickly titrated with 0.1 M NaOH to a pink coloration end point in 15 seconds using around 1 ml of phenolphthalein indicator, or 2-3 drops. The amount of NaOH used was noted, and the volatile acidity was computed. The volatile acidity was determined using Equation (4).

\[ \text{Acetic acid} \text{ g/100ml} = \frac{V \times M \times 60 \times 100}{100 \times V} \]

(4)

3.2.2.3 Determination of Alcoholic Content

Following the distillation of the sample's alcohol, the specific gravity of the ethanol-to-water distillate was calculated.

A volumetric flask was filled with 25 ml of the sample, and the 2 M of sodium hydroxide was used to neutralize the sample's acid content. The solution was washed into a distillation flask, with repeated rinsing to ensure complete transfer (45 ml of deionized water was used to avoid charring). Anti-foaming agents were added and the flask connected to the condenser with air tight quick fit end. The set-up was distilled at slow boiling to obtain approximately 90 ml of distillate. The temperature of the distillate was adjusted to 200 °C and the volume brought to 100 ml with deionized water. 50 ml of the distillate was measured accurately into the specific gravity bottle and the weight noted. 50 ml of distilled water was also carefully measured into the same bottle, and its weight was also noted. The ratio of the weight of the distillate to the weight of equal volume of distilled water was calculated. The equivalent alcohol percentage composition was calculated from the relation:

0.9999 specific gravity= 0.01% alcohol

3.2.2.4 Determination of Sugar Content

This was determined by direct reading engineering method (DREM) using saccharimeter. The meter contains a glass bulb whose one end is sugar saturated when dipped in container of 10 ml of the sample embedded corresponding to sugar content and the value read out as calibrated in a chart attached to the wall of the bulb.
3.2.2.5 Determination of pH
10 ml of the sample was measured into a 10 ml conical flask. The electrode of the pH meter, cleaned with distilled water and dried was dipped into the flask of the sample. The electrode was agitated to drive away air bubbles. pH value was read out as displayed on the liquid crystal display panel of the meter.

3.2.2.6 Determination of Moisture Content
A weighted evaporating plate was filled with precisely 10 ml of the sample, (W₁). The sample and the dish was weighed (W₂). The evaporating dish with the sample was placed on the ringed water bath, and the moisture evaporated. The dish was dried in a glass desiccator to a constant weight (W₃). The moisture content was determined by the difference in wet weight and dry weight. The moisture content was calculated using Equation 5.

\[ \text{Percentage Moisture} = \frac{W_2 - W_3}{W_2} \times 100 \]  

3.2.2.7 Determination of Relative Density
The relative density of the sample was determined by the gravimetric method, using pycnometer. A pycnometer, prewashed with deionized water and dried in an oven, cooled in a desiccator was weighed, its weight (W₁) was recorded. 5ml of sterilized water was accurately measured into the pycnometer and weighed (W₂). 5ml of the sample was accurately measured into the pycnometer and its weight (W₃) recorded. The relative density was calculated using Equation (6).

The relative density was calculated as the ratio of the weight of sample to the weight of equal volume of water.

\[ \text{Relative Density} = \frac{W_3}{W_1} \]  

3.2.2.8 Counting the Total Amount of Dissolved Solids (TDS)
A 50 ml glass beaker was filled with 10 ml of the sample. To remove air bubbles, the meter proof was dipped into the sample in the beaker and gently stirred. The reading was recorded exactly as it appeared on the meter's liquid crystal display panel. The total dissolved solid was calculated using Equation (7).

\[ \text{Total solids (mg/l)} = \frac{W_2 - W_1}{V} \times 1000 \]  

where
\[ W_1 = \text{Initial weight of dried residue + dish in mg} \]
\[ W_2 = \text{Final weight of the dish in mg} \]
\[ V = \text{Volume of sample used in ml} \]

3.2.2.9 Determination of Suspended Solid
Similar to how moisture content was measured, the suspended solid was measured using the gravimetric method with an evaporating dish and a bath of water. The dish's weight (W₁) and the dish plus 10 ml of the sample were both recorded (W₂). In a water bath, the sample in the dish was evaporated to dry, and its dry weight (W₃) was recorded. The relationship used to compute the suspended solid is given as Equation 8.

Weight of empty dish = W₁
Weight of empty dish + sample = W₂
Weight of sample = W₂ − W₁
Weight of dish + sample after evaporation to dryness = \( W_3 \)

Weight of dry matter (solids) = \( W_2 - W_3 \)

\[
\text{Percentage of solids} = \frac{W_2 - W_3}{W_2 - W_3} \times \frac{100}{1}
\]  

(8)

3.2.2.10 Determination of Vitamin C

This was done by the titrimetric method using standard indophenol solution.

Preparation and standardization of the Indophenol dye:

By dissolving 0.05 g of 2.6-dichlorophenol, an indophenol solution was obtained. Indophenol dye is added to up to 100 ml of distilled water. The filtrate was labeled after filtering. To standardize, 250 ml of water was added to 0.05 g of pure ascorbic acid, which was then dissolved in 10 ml of 20 % metaphosphoric acid. A 50 ml conical flask was filled with 10 ml of this solution, which was then titrated against an Indophenol solution until a faint pink tint that lasted for 15 seconds was attained.

Procedure for determination of ascorbic acid (vitamin C):

A 50 ml measuring cylinder was used to precisely measure 50 ml of the sample into a 100 ml volumetric flask. As a stabilizing agent, 25 ml of 20 % metaphosphoric acid was added. Distilled water was then used to make up the gap. To eliminate sulfur as acetone bisulfate and reduce interference with the titration's end point, 2.5 ml of acetone was added to 10 ml of the solution before it was pipetted into a 50 ml conical flask. After that, the sample was titrated against the Indophenol solution until a 15-second pink colour was formed. The relation was used to calculate the vitamin C content as shown in Equation 9.

\[
100 \text{ ml sample} = \frac{B \times 10}{V} \text{ mg}
\]  

where \( B \) = volume of dye solution used

\( V \) = volume of dye solution in standardization

3.2.3 Physical Examination

3.2.3.1 Sensory Evaluation

This was done by random sampling method. The fresh sample was given to a selected set of people consisting of six men aged 40-60, four men aged below 40 years. The same set of people were used to evaluate the preserved sample and their reports used as measure of the physical sensory evaluation (Ishiwu, Nnanwube, Nkem, & Ezegbe, 2020).

4. Results and Discussion

4.1 Results

4.1.1 Analytical Results

Value for microbial analysis were obtained by multiplying the average number of colonies with dilution factor. The microbial load of fresh unpreserved palm wine sample is shown in Table 1.

\[
C_O = C_n \times \frac{1}{f}
\]

where

\( C_O \) = Original cell count

\( C_n \) = Number of colonies

\( f \) = Dilution factor

Table 1. Microbial load of fresh unpreserved palm wine sample

<table>
<thead>
<tr>
<th>Average number of colonies</th>
<th>Total cell count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mould Yeast</td>
<td>Bacterial</td>
</tr>
<tr>
<td>Yeast, Mould</td>
<td>Bacterial</td>
</tr>
</tbody>
</table>

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The media used were malt agar for moulds and yeast, while standard plate agar fortified with malt extract agar and glucose was used for bacterial total visible count (TVC).

4.1.2 Preserved Sample
Microbial load was measured against period of U.V radiation and a table of count per time was made (Table 2). The variation of microbial count with time of exposure of palm wine sample to U.V. radiation for samples preserved for 10 days is shown in Table 3.

<table>
<thead>
<tr>
<th>Number of colonies</th>
<th>Total count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period of U.V exposure(seconds)</td>
<td>Mould and Yeast</td>
</tr>
<tr>
<td></td>
<td>F=10^{-4}</td>
</tr>
<tr>
<td>0</td>
<td>325</td>
</tr>
<tr>
<td>30</td>
<td>280</td>
</tr>
<tr>
<td>60</td>
<td>325</td>
</tr>
<tr>
<td>90</td>
<td>110</td>
</tr>
<tr>
<td>120</td>
<td>95</td>
</tr>
<tr>
<td>150</td>
<td>45</td>
</tr>
<tr>
<td>180</td>
<td>25</td>
</tr>
<tr>
<td>210</td>
<td>10</td>
</tr>
<tr>
<td>240</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Table 3. Variation of microbial count with time of exposure of palm wine sample to U.V. radiation for samples preserved for 10 Days

<table>
<thead>
<tr>
<th>Period of colonies of exposure</th>
<th>Total count</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEP(seconds)</td>
<td>Mould and Yeast</td>
</tr>
<tr>
<td></td>
<td>Mould and Yeast</td>
</tr>
<tr>
<td></td>
<td>F=10^{-4}</td>
</tr>
<tr>
<td>30</td>
<td>130</td>
</tr>
<tr>
<td>60</td>
<td>85</td>
</tr>
<tr>
<td>90</td>
<td>50</td>
</tr>
</tbody>
</table>
### 4.1.3 Chemical Analysis

The chemical analysis of palm wine after exposure to U.V rays is shown in Table 4 while the variation of properties of palm wine sample with time of exposure to U.V radiation for sample preserved is shown Table 5.

#### Table 4. Chemical Analysis of Palmwine after Exposure to U.V rays

<table>
<thead>
<tr>
<th>Exposure time(seconds)</th>
<th>pH</th>
<th>Turbidity</th>
<th>Ascorbic Acid (mg/l)</th>
<th>Titrable acidity (mg/l)</th>
<th>Relative Density</th>
<th>Volatile acidity (mg/l)</th>
<th>Alcohol content (%)</th>
<th>Total sugar (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.2</td>
<td>0.105</td>
<td>2.3</td>
<td>2.28</td>
<td>1.08</td>
<td>0.7</td>
<td>8.5</td>
<td>19.4</td>
</tr>
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<td>19.4</td>
</tr>
<tr>
<td>120</td>
<td>3.1</td>
<td>0.105</td>
<td>2.3</td>
<td>2.8</td>
<td>1.076</td>
<td>0.7</td>
<td>8.5</td>
<td>19.4</td>
</tr>
<tr>
<td>150</td>
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</table>

#### Table 5. Variation of Properties of Palm wine Sample with Time of Exposure to U.V Radiation for Sample Preserved

<table>
<thead>
<tr>
<th>Time</th>
<th>pH</th>
<th>Turbidity</th>
<th>Ascorbic acid (mg/l)</th>
<th>Titrable acidity (mg/l)</th>
<th>Relative Density</th>
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4.2 Discussion of Results

All electromagnetic rays have energy $E = hf$

Where $f =$ frequency factor characteristic of any electromagnetic ray

$h =$ plank’s constant

This energy is an ionizing energy with high generating power

Table 1 shows the microbial loads prior to exposure of the palm wine to the U.V radiation. Table 2 shows the microbial load after exposure to U.V radiation. A comparison of the two tables shows a remarkable decrease on the microbial loads down the table. Within the first 30 seconds of exposure, the microbial load decreased from $3.25 \times 10^6$ to $2.80 \times 10^6$ for bacterial representing 13.8 % reduction. After 120 seconds (2 minutes) of exposure, the load decreased extensively from $3.25 \times 10^5$ to $0.95 \times 10^5$ for moulds/yeast and $2.5 \times 10^5$ to $0.4 \times 10^4$ bacterial, representing 70.8 % to 84 % respectively. After 240 seconds (4 minutes), the microbial loads were almost very insignificant for moulds/yeast and zero for bacterial.

When the preserved palm wine (post U.V. bottled wine) was kept aside for 10 days and reanalyzed, a surprisingly remarkable observation was made. There was further decrease in the microbial loads. The sample of palm wine exposed for 30 seconds showed a further reduction in loads from $1.3 \times 10^6$ for moulds/yeast, approximately 50 % reduction. This could mean that the moulds/yeast, not killed within the first 30 seconds were reasonably alternated so much that they could not grow on a medium, an indication of bio-inactivity.

The alcohol content diminishes with passing time. It can be said with certainty that ultraviolet light was successful in halting the fermentation of the palm wine. The increase in pH increases susceptibility to general biological degradation when combined with a decrease in sulfur dioxide present in its free or unbound state. In addition, there is a potential environmental element that is to blame for this phenomenon because UV exposure makes it difficult for the enzymes to grow. Also, exposure to UV rays has reduced the substrate content at the palm wine's active sites. According to Karamoko, Moroh, Bouatenin, and Dje (2016), a subsequent decrease in pH and sugar content was accompanied by a rise in titrable acidity and alcoholic rate. The primary source of UV radiation on earth, sunlight, is said to be where the fermentation process takes place. The same trends of microbial load reduction were observed in other bottles, presupposing that the U.V radiation affected microbial activity positively.

Chemical analysis of the samples preserved as shown on Tables 1, 2, 3, and 4 indicated that the values of chemical parameters were virtually the same for fresh and preserved palm wine. This again means that while the ultra violet radiation monitored the microbial bioactivity, it did not in any way affect the nutritional values of the palm wine. This indicates that while UV radiation kept track of the microbial activity, it had no impact on the nutritional content of the palm alcohol. Because it eliminates the endogenous microorganisms (the spoiling agent) and leaves the excipient components alone, ultraviolet light is a useful physical preservation technique for palm wine (the active ingredients).

5. Conclusion

Ultra-violet radiation is an effective way of the physical means of preserving palm wine, because it destroys the endogenous microbial organisms (spoilage agents) while it leaves the excipients factors (active ingredients) undestroyed. This research establishes the determination of the effect of contact time and intensity of radiation on the shelf life of palm wine (time of spoilage of wine).
5.1 Limitation/s and study forward

This research did not consider the effect of stirring on the microbial load reduction. Future studies should consider this factor as well as the combined study of all relevant factors by optimization. There is need to expose the palm wine for a longer time and where possible, the glass tubing through which the palm flows should be provided with constructions at close point to provide a mixing effect. This will ensure a uniform exposure of a given volume of wine to the ultra-violet radiation.

References


