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# MICROBIOTA OF COMMERCIAL PALMWINE SOLD IN LAGOS STATE, NIGERIA

[1] OKORONKWO K.A., [2] ATANDA O.O AND OKORONKWO B.C

[1]DEPARTMENT OF BIOLOGICAL SCIENCES,
EDWIN CLARK UNIVERSITY, KIAGBODO, DELTA STATE, NIGERIA
UGHELLI, DELTA STATE, NIGERIA.

[2]DEPARTMENT OF FOOD SERVICE AND TOURISM, UNIVERSITY OF AGRICULTURE, ABEOKUTA,
ALABATA ROAD, ABEOKUTA, OGUN STATE, NIGERIA.

**ABSTRACT**

This study investigated the presence of pathogenic microbes in commercial palm wine sold in Lagos State, Nigeria. Adulterated palm wine samples were obtained randomly from vendors in three different locations in each of the twenty local government areas. The microbiological quality of the palm wine was assayed by morphological and biochemical characterization of the organisms isolated from the palm wine. The total plate count ranged from 0.68 to 7.17, yeast count from 2.00 to 4.73, mould count 2.03 to 4.92 and coliform count from 2.48 to 4.98 x $10^{10}$cfu/ml respectively while the *staphylococcal*, *salmonella* and *shigella* counts ranged from 1.51 to 4.00 x $10^{8}$ and 2.03 to 4.00 x $10^{1}$cfu/ml. The microbial investigation revealed the presence of pathogenic bacteria's (*Escherichia Coli, Staphylococcus aureus, Salmoella enterica and Shigella dysenteriae)* and yeasts (*Saccharomyces fermentati, Candida tropicalis and saccharomyces cerevisiae)* above permissible levels recommended by the International Commission on Microbiological Specification for Foods (ICMSF) and Standards Organization of Nigeria (SON). This is a source of concern as microbes can be injurious to human health.

**Key Words:** Microbiota, Commercial Palm wine, Lagos State

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## INTRODUCTION

Palm wine is a popular traditional alcoholic beverage consumed by more than 10 million people in West Africa (FAO, 1998). It is a sweet, effervescent drink obtained from the sap of the oil palm (*Elaeis guineensis)* and raphia palm (*Raphia Hookeri).* Palm wine has several nutritional, medicinal, religious and social uses (Faparusi, 1972, Sylva *et al*., 1988; Uzogara *et al*., 1990; Iheonu, 2000) which has enhanced the demand for this natural product. The wine is a rich nutrient medium containing sugars, protein, alcohol and minerals (Ezeagu and Fafunso, 2003). It also contains a dense population of yeasts (Bassir and Maduagwu, 1978). Thus, when it is allowed to stand, fermentation converts the sugars to ethanol and subsequently to acetic acid, leading to loss of sweetness, shortened shelflife and decreased acceptability (Odunfa, 1985).

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Previous studies on commercial palmwine have incriminated several bacterial and yeast flora to be involved in the fermentation process (Faparusi and Bassir, 1972; Okafor, 1975; Eze and Ogun, 1987; Ejiofor *et al*., 1994; Nester *et al.*, 2004). These organisms have also been reported to originate from several sources, which include tapping equipment, packaging materials, the environment, water of questionable quality used for dilution or processing and food handlers who do not observe good hygienic regulations (Faparusi and Bassir, 1972).

One of the most frequent complaints of regular palm wine consumers is the adulteration of the products with water and artificial sweeteners, which sometimes result in diarrhea and abdominal pains (Ghana Review International, 2004). Since commercial palm wine processing in Lagos State is still carried out at small scale level involving the possible use of poor quality water, there is the possibility of contamination of the product with pathogenic organisms.

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## MATERIALS AND METHODS

**Collection and Preparation of Samples:** The commercial palm wine samples were obtained randomly from three different locations in each local government of Lagos State. The samples were collected in pre-sterilized labeled 100ml sample bottles with perforated screw caps to allow for the release of carbon dioxide. Freshly tapped palm wine that was not diluted with water was used as control. The perforated screw caps were plugged with sterile non-absorbent cotton wool and transported to the laboratory in a cooler containing a mixture of salt and ice block to delay fermentation.

## MICROBIOLOGICAL ANALYSIS

The palm wine samples were diluted serially by inoculating 1ml of the sample to 9ml of peptone water. This formed the initial dilution from which subsequent ten fold dilutions were made and used for analysis. Media were sterilized at a temperature of $121^{O}C$ and pressure of $15kg/m^2$ for 15 min for bacteria and 10 min for yeast isolates. The total

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viable bacterial count of the commercial palm wine was determined by diluting the samples decimally and spread plating 1ml aliquot on nutrient agar (Oxoid) and incubating at $30^{O}C$ for 48h (A.0.A.C, 2005).

## Culture Media

The following media were used in culturing the micro-organisms; Nutrient agar, Potato dextrose agar (PDA), MacConkey agar No. 3, blood agar and selenite broth.

## Culture Procedure

All microbiological media used were prepared according to the manufacturer's instructions. The yeast and mould count were determined using PDA (Oxoid) in which 0.01% chloramphenicol had been added to inhibit bacterial growth while the coliform count, *staphylococcal* count, *salmonella* and *shigella* count were determined at different temperature and time using MacConkey agar No. 3 (Oxoid), blood agar (oxoid), *salmonella* and *shigella* agar respectively. The colonies were counted and recorded. The different colonies on the plates were isolated, purified and stored on nutrient agar (LABTEC) slants for further characterization and identification. The bacterial

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classifications were made using series of cultural and biochemical test and compared with Bergey's manual of determinative bateriology (Holt *et al*., 1994). Cultural and morphological identification of bacterial and yeast isolates were based on forms and arrangement of cells, gram stain and motility as compared with Barnett *et al*., 1990.

**Statistical Analysis:** All data were reported as means of triplicates. Analysis of variance (ANOVA)) was used to establish significant differences ($P<0.05$) using SPSS version 10.0 and means separated by Duncans Multiple Range Test.

## RESULTS AND DISCUSSION

**Table 1:** Microbial Counts of Commercial Palm Wine in Lagos State, Nigeria

| Sample Location | Total plate count ($x10^{10}$cfu/ml) | Yeast count ($x10^{10}$cfu/ml) | Mould count ($x10^{10}$cfu/ml) | Coliform count ($x10^{10}$cfu/ml) | Staphylococcal count ($x10^{8}$cfu/ml) | Salmonella and Shiella count ($x10^{1}$cfu/ml) |
|---|---|---|---|---|---|---|
| Fresh Palm Wine | $1.93^{fg}\pm1.12$ | $2.00^{k}\pm2.00$ | $2.03^{o}\pm2.08$ | $4.01^{d}\pm6.73$ | $1.51^{o}\pm1.53$ | $2.53^{k}\pm2.08$ |
| Agege | $5.55^{abc}\pm0.03$ | $4.37^{b}\pm2.39$ | $2.37^{n}\pm1.53$ | $4.03^{d}\pm6.81$ | $2.52^{k}\pm2.52$ | $2.62^{j}\pm1.53$ |
| Ajeromi-Ifelodun | $5.30^{abc}\pm0.01$ | $3.95^{cd}\pm4.51$ | $4.02^{g}\pm2.52$ | $2.96^{f}\pm7.23$ | $2.52^{kl}\pm2.65$ | $2.82^{g}\pm2.65$ |
| Alimosho | $5.35^{abc}\pm0.05$ | $2.60^{gh}\pm2.00$ | $4.52^{d}\pm2.52$ | $4.56^{b}\pm5.51$ | $2.62^{i}\pm2.52$ | $2.87^{f}\pm1.53$ |

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| | | | | | | |
|---|---|---|---|---|---|---|
| Amuwo-Odofin | $0.68^{g}\pm0.58$ | $2.55^{gh}\pm4.73$ | $4.82^{c}\pm2.52$ | $3.63^{e}\pm2.00$ | $2.72^{h}\pm2.65$ | $3.00^{e}\pm1.00$ |
| Apapa Wharf | $6.37^{ab}\pm5.51$ | $3.05^{e}\pm4.04$ | $3.58^{j}\pm1.15$ | $2.77^{g}\pm15.50$ | $2.79^{g}\pm1.00$ | $2.48^{l}\pm2.65$ |
| Badagary | $1.30^{fg}\pm0.02$ | $2.78^{f}\pm7.64$ | $3.48^{k}\pm2.52$ | $2.48^{h}\pm31.77$ | $2.35^{m}\pm1.53$ | $2.31^{n}\pm1.53$ |
| Epe | $1.10^{fg}\pm0.01$ | $2.83^{f}\pm2.52$ | $3.70^{i}\pm1.53$ | $4.09^{cd}\pm7.77$ | $2.87^{f}\pm2.52$ | $2.41^{m}\pm2.52$ |
| Eti Osa | $7.17^{a}\pm0.31$ | $3.88^{d}\pm3.06$ | $4.92^{b}\pm3.00$ | $3.12^{f}\pm12.06$ | $3.03^{d}\pm2.00$ | $2.40^{m}\pm2.52$ |
| Ibeju/Lekki | $5.02^{abcd}\pm0.14$ | $2.01^{k}\pm1.53$ | $3.82^{h}\pm3.00$ | $2.58^{h}\pm4.16$ | $3.93^{b}\pm2.08$ | $2.70^{h}\pm1.53$ |
| Ifako-Ijaye | $4.34^{bcde}\pm0.14$ | $2.11^{j}\pm3.21$ | $3.43^{k}\pm2.52$ | $3.04^{f}\pm5.29$ | $2.96^{e}\pm1.53$ | $2.03^{o}\pm2.00$ |
| Ikeja | $2.55^{efg}\pm0.05$ | $2.62^{gh}\pm2.08$ | $3.00^{l}\pm1.53$ | $4.19^{cd}\pm2.00$ | $3.03^{d}\pm2.52$ | $2.80^{g}\pm1.53$ |
| Ikorodu | $6.77^{a}\pm0.03$ | $2.28^{i}\pm2.00$ | $2.75^{m}\pm2.52$ | $4.94^{a}\pm4.16$ | $2.05^{n}\pm2.00$ | $3.23^{d}\pm2.08$ |
| Kosofe | $5.55^{cdef}\pm0.05$ | $4.73^{a}\pm3.51$ | $5.00^{a}\pm1.00$ | $4.98^{a}\pm6.03$ | $4.00^{a}\pm2.00$ | $3.51^{c}\pm1.53$ |
| Lagos Island | $3.37^{cdef}\pm0.32$ | $2.98^{e}\pm1.15$ | $4.42^{e}\pm15.72$ | $3.06^{f}\pm6.66$ | $2.99^{e}\pm2.00$ | $4.00^{a}\pm1.53$ |
| Lagos Mainland | $4.20^{bcde}\pm0.26$ | $3.05^{e}\pm4.51$ | $4.27^{f}\pm2.65$ | $3.80^{e}\pm1.00$ | $2.57^{j}\pm1.53$ | $2.47^{l}\pm2.00$ |
| Mushin | $1.54^{fg}\pm0.03$ | $4.00^{c}\pm0.58$ | $3.02^{l}\pm1.53$ | $4.28^{c}\pm2.52$ | $2.72^{h}\pm2.65$ | $2.40^{m}\pm1.53$ |
| Ojo | $2.93^{defg}\pm0.31$ | $2.52^{h}\pm2.52$ | $2.99^{l}\pm1.53$ | $3.63^{e}\pm22.81$ | $2.69^{h}\pm1.00$ | $2.34^{n}\pm1.53$ |
| Oshodi-Isolo | $5.61^{abc}\pm0.90$ | $4.43^{b}\pm3.06$ | $4.53^{d}\pm2.08$ | $4.17^{cd}\pm3.51$ | $3.39^{c}\pm2.08$ | $2.65^{i}\pm2.00$ |
| Shomolu | $1.85^{fg}\pm0.05$ | $2.65^{g}\pm2.52$ | $2.72^{m}\pm2.65$ | $4.13^{cd}\pm4.93$ | $2.70^{h}\pm1.53$ | $3.23^{d}\pm2.52$ |
| Surulere | $5.82^{ab}\pm0.06$ | $2.97^{e}\pm2.00$ | $2.77^{m}\pm1.53$ | $2.58^{h}\pm4.93$ | $2.49^{l}\pm2.08$ | $3.77^{b}\pm2.08$ |

Mean values followed by different superscripts within a column are significant ($P<0.05$)

± Standard deviation of three replicates.

There were significant differences ($P<0.05$) between the total plate count, coliform count, yeast count, mould count, *Staphylococcal* count, salmonella and shigella counts in all the local governments.

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**Table 2:** Morphological, Cultural, Biochemical and Carbohydrate Fermentation tests of Bacteria isolates from Commercial Palm Wine in Lagos State, Nigeria.

| Morphological Characteristic | Cultural characteristic | | | Biochemical characteristic | | | | Carbohydrate fermentation test | | | | | | Probable bacteria identity |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Forms and arrangement of cells | GS | M | | Ph | C | Ca | O | GP | I | H | L | G | S | |
| Long slender rods in pairs, while some occurred singly. | - | + | Rose pink colonies | 5.0 | - | - | + | - | + | - | + | + | + | *Escherichia coli* |
| Cocci: occurred singly, in pairs, tetrads, irregular clusters. | + | + | Clear zones on blood agar | 4.0 | + | - | + | + | + | + | - | + | + | *Staphylococcus aureus* |
| Long rods | - | + | Jet black clonies with translucent peripheries. | 4.2 | - | - | - | + | + | - | - | + | + | *Salmonella enterica* |
| Long rods | - | + | Colourles colonies | 5.5 | - | - | - | + | + | - | - | + | + | *Shigella dysenteriae* |

**Key:**

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GS = Gram Stain, M = Motility, C = Coagulase, GP = Gas Produciton, I = Indole, O = Oxidase, H = Haemolysis, L = Lactose, G = Glucose, S = Sucrose, Ca = Catalase, +ve = Positive reaction,-ve = Negataive reaction

**Table 3:** Morphological, Cultural, Biochemical Tests and Carbohydrate Fermentation tests of Yeast Isolates from Commercial Palm Wine in Lagos State, Nigeria.

| Morphological Characteristic | Cultural characteristic | Growth in medium | | | | | Biochemical test | Carbohydrate fermentation test | | | | | Probable bacteria identity |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 25$^0$C | 30$^0$C | 37$^0$C | 40$^0$C | N | | Glucose | Maltose | Sucrose | Lactose | Starch | |
| Lemon shaped colonies, reproduction by budding, No filament. | Cream, butyrous colonies | + | + | - | - | - | | + | - | - | - | - | *Saccharomyces fermentati* |
| Vegetative reproduction by budding, No filaments. | White to cream, butyrous colonies. | + | + | - | - | - | | + | - | + | - | - | *Candida tropicalis* |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Vegetative reproduction by budding. No filaments, medium oval cells. | Creamy, butyrous colonies. | + | + | + | + | - | + | - | + | - | - *Saccharomyces cerevisae* |

**Key:**

+ve = Positive reaction, - ve = Negative reaction, N = Nitrate reduction test

The high bacterial count observed in the study may be attributed to factors such as the environment which include exposure of the foods to air, soil, type of water used in diluting the Palm wine, Post production Operations and Personal hygiene of the food handlers (Kawo and Abdulmumin, 2009; Aboloma, 2008). Exposure of the palm wine to air or dust at the point of sale is likely to increase the counts of the bacteria as virtually most of the bacteria are carried in aerosols, by dust and air (Food and Drug Administration, 2009).

Most of the organisms isolated were coagulase negative with the exception of *Staphylococcus aureus*. *Staphylococcus aureus* is a normal flora of the skin, nose, throat, palm, hairs and mucus membrane and a common etiological agent of septic arthritis (Alice, 1979).

The isolation of coliforms in the samples is an indication of recent faecal contamination. *E coli* is an important member of the coliform group and is part of the normal flora of the intestine of human and vertebrates. Some strains of *E. coli* can cause gastroenteritis, diarrhea and urinary tract infections (Pelczer *et al.,* 1993). Coliforms in the samples which were above the recommended standard of $10^2$ cfu/ml recommended by the International commission on microbiological specification for foods (ICMSF) (1998) and

the standards organization of Nigeria (SON) is of public health concern (Ezeama and Nwankpa, 2002).

Owhe-Ureghe *et al.*, (1993) reported that the possible entry of the pathogens in foods could probably be from the initial raw materials, the processing equipment or the food handlers as they have been reported to be good source of diarrhea and gastro intestinal disturbance for adult and children. Sylvia *et al*., (1991) also reported that the most abundant and consistent organisms found in palm wine are the yeast *saccharomyces* and lactic acid bacteria: *Lecuconostoc* and *Lactobacillus* regardless of the implements used. One of the factors contributing to this is the traditional tapping practice of using implements repeatedly, especially funnels and metal containers for months or years without cleaning the inner surfaces to get rid of microbial deposits. Thus, such receiving vessels contain huge deposits of microorganisms.

*Saccharomyces cerevisae* though an important industrial yeast and ingested in normal diets, may produce fatal complications in immune deficient patients. The increasing incidence of yeast infections has stimulated the development of commercial yeast identification systems. *Candida tropicalis* is probably the third most important yeast pathogen of humans. Deep or

systemic infections by *Candida tropicalis* appear to be increasing and there is some suggestion that the prognosis for a disseminated infection with *Candida tropicalis* is more grave than for *Candida albicans*. The incidence of food borne infection by salmonella continues to be an important problem world-wide. Non typhoidal salmonellae are among the foremost bacterial pathogens implicated in food-borne gastroenteritis (Mead *et al*., 1999).

Stella *et al*., (2008) reported that food handlers constitute a significant health risk in the spreading of S. *typhi* and other *salmonella spp* in Lagos.

The differences in the microbiological quality of commercial palm wine from Lagos State may be due to the fact that palm wine is usually diluted with water of questionable quality, saccharine, Indian hemp and sugar. Similarly, flies deposit substantial amount of pathogenic microbes in the containers as they perch on Palm wine.

The findings of this study will be useful to environmental health officers and the national Agency for Food and Drug Administration and control (NAFDAC) in developing health education campaign messages for palm wine tappers and regular consumers of palm wine in Lagos State. Routine checks must be carried out to ascertain the microbiological quality of palm wine sold in Lagos State, Nigeria in order to prevent disease out break.

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