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Detection and identification of public health important pathogens present in fruit salads sold on Lagos State University campus

Adedayo Emmanuel Ogunware*[1], Odubiyi Opeyemi Esther[2], Pelumi Abimbola Adedigba[1], Adesewa Adeyinka Adekoya[1]

[1]Cell and Tissue Culture Research Laboratory (Drug Discovery Unit), Department of Biochemistry, Lagos State University, Ojo, PMB 0001, LASU Post office, Lagos Badagry Expressway, Nigeria, [2]Department of Microbiology, Lagos State University, Ojo, PMB 0001, LASU Post office, Lagos Badagry Expressway, Nigeria

ABSTRACT

A total of fifteen pre-packaged fruit salad samples containing pineapple, water melon, pawpaw and cucumber sold in the Lagos State University, Ojo Campus was analyzed using culture techniques for its microbial qualities. Five bacteria genera isolates obtained are *Bacillus spp*, Staphylococcus *aureus*, *Pseudomonas spp*, *Escherichia coli*, *Streptococcus* and the three fungi genera isolates are *Aspergillus species*, *Penicillium species*, *and Saccharomyces cerevisiae*. *Escherichia coli* had the highest frequency of (40%) followed *by Streptococcus* with (20%), Staphylococcus, *Bacillus*, *Pseudomonas* has the same frequency of (13%). The total viable count was in the range of 1.6×10^5 cfu/g to $5.6^5 \times 10^5$ cfu/g while the total coliform count ranged from 1.0×10^5 to 3.3×10^5 cfu/g. The fungal count ranged from 1.5×10^5 to 3.4×10^5 cfu/g. This study revealed that fruit salads in the studied area needs proper sanitation practice during processing in order to avoid risks associated with the consumption of contaminated fruits for the consumers.

KEYWORDS: Fruit salads, public health, bacteria, fungi, Lagos state.

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***Corresponding Author:**
Adedayo Emmanuel Ogunware
Email: Adedayoogunware21@gmail.com

BACKGROUND

Majority of consumers patronize street vended fruit salad due to the fact that they are cheaper than whole fruits, convenient to eat and partly because they are easily available. Fruit salad is usually a combination of various fresh fruits such as apples, watermelon, pineapples, cucumber, pawpaw and orange. They are generally sliced into small pieces and packed in small transparent plastic bowls. The salad can be eaten by means of a fork or tooth pick, with or without addition of milk [1]. Fruit salad is low in cholesterol, sodium and saturated fat but high in vitamins A, C and D, manganese, copper and dietary fibers[2]. The fruits used in the preparation of the salad are typically kept on the ground near the slicing tables without any form of protection. Hence the microbiological quality of the prepared fruit salad remains uncertain. Fruits are susceptible to microbial contamination because they are constantly in contact with water, dust and soil and by handling at harvest or during post-harvest processing. Pathogenic microbes may also enter the fruits through damaged surfaces, such as wounds punctures, splits and cuts. Such pathogens may become internalized, survive and grow within the fruit and subsequently become health hazard to consumers. Outbreaks of *Listeriosis* and *Salmonellosis* have previously been associated with the consumption of ready to-eat fruit salad [3]. In Nigeria where street food marketing is very common, there is lack of information on the prevalence of food borne diseases related to the street sold foods. However, microbial studies on such foods in Asia, America, Europe and some African countries have revealed increased bacterial pathogens in fruit salad [4]. In view of the health risk posed by the bacterial pathogens in fruit salad and the increasing demand for such street vended salad, the present study was undertaken to evaluate the microbiological quality of freshly prepared fruit salad sold in Lagos state University, Ojo, Nigeria. Therefore, this work is of utmost importance and the findings will enable people most especially students to appreciate the possible health implications of eating microbiologically unsafe fruit salad.

MATERIALS AND METHODS

Samples Collection

A total of 15 pre-packaged fruit salad samples comprising cucumber, pawpaw, pineapple and water melon were obtained

randomly from Itire market known for vending fruit salad. All the samples were collected wholly in small transparent plastic bowls as sold and transported in ice-packed cooler to the laboratory where they were analyzed within 1 hour after collection.

Sterilization of Materials (Glassware and other Apparatus)

Glass ware such as pipettes, Petri dish, etc. were sterilized using the oven at temperature 250◦C for 3hours or at 180◦C for 8hours. Innoculating loop were sterilized by holding the nichrome wire in the Bunsen flame until it glowed red. Hockey sticks for spreading inoculums on plates were sterilized by dipping in absolute ethanol and flaming to burn off the alcohol. The work bench area in the laboratory was cleaned with cotton wool soaked in alcohol before analyses started.

Apparatus and Materials

The apparatus and materials used in this experiment include;

Petri dish, micropipette tips, test tubes, aluminum foil, Bunsen burner, cotton wool, autoclave, inoculating loop, conical flasks, teat tube racks, measuring cylinder, spirit lamp, Bijou bottles, glass slides, hand gloves, beaker, spatula.

Equipment

Weighing balance, microscope, incubator.

Media and Reagents Used

The media and reagents used include: Nutrient agar, MacConkey agar, Potato dextrose agar, Mannitol salt agar, distilled water, lugol's iodine, safranin, ethanol, crystal violet, kovac's reagent, methylated spirit, Fruit salads obtained from Lagos State University, Ojo campus.

Media Preparation

All the media used in this project were prepared based on the manufacturer's instructions. Depending on the number of plates to be prepared, certain grams of the powder were dissolved in specific litre of distilled water in the conical flask, plugged with cotton wool and aluminum foil and sealed firmly with masking tape. The media were then sterilized at 121◦C for 15 mins in the autoclave. The sterile media was allowed to cool to about 45◦C, and then dispensed into sterile petri dishes following aseptic techniques.

Nutrient Agar

Nutrient agar is a general-purpose nutrient medium used for the cultivation of microbes supporting growth of a wide range of non-fastidious organisms.

Agar of 28g was weighed and dissolved in 1000ml of distilled water, it was then autoclaved at 121◦C for 15 minutes, it was allowed to col and was poured into the petri dishes. The medium is a general-purpose medium for the cultivation of microorganism and supporting the growth of non-fastidious microorganism. The nutrient agar was incubated at 37◦C.

MacConkey Agar

MacConkey agar is a selective and differential used for the isolation and differentiation of non-fastidious of the family Enterobacteriaceae and genus pseudomonas. MacConkey agar of 49.5g was weighed and dissolved in 1000ml of distilled water; it was autoclaved at 121◦C for 15 minutes. It was allowed to cool and then poured into the petri dishes. The agar serves as differential medium for the isolation of coliforms. The MacConkey plates were incubated at 35◦C for 24-48hours.

Potato Dextrose Agar

Potato dextrose agar was weighed and dissolved in 1000ml of distilled water; it was autoclaved at 121◦C for 15 minutes. It was allowed to cool and then poured into petri dishes. The agar serves as a medium for the enumeration of mold and yeasts. The potato dextrose plates were incubated at 31◦C for 24 hours for yeast isolates and 3-5 days for molds.

Mannitol Salt Agar

Mannitol salt agar is commonly used selective and differential growth medium in Microbiology. It encourages the growth of a group of certain bacteria while inhibiting the growth of others, it is selective for most gram negative and some gram-positive Staphylococcus and Micrococcus. It typically contains 1.0g/l of beef extract, 10.0g/l D-Mannitol,75.0g/l of sodium chloride 0.025g/l of phenol red, 3.05g/l of agar ph7.4 at 25 °C . Following manufacturer instruction 110g is dissolved into 1000ml of distilled water and autoclave at 121°C for 15mins and then allowed to cool and poured into Petri dishes which are covered immediately. Once the dishes hold solidified agar, they are turn upside down

Serial Dilution Techniques (SDT)

Serial dilution techniques were applied to thin out high population densities of microorganisms present in the samples to countable numbers. The SDT used was 10-fold serial dilution method.

Procedure for the Serial Dilution Method

The diluents (peptone water) was sterilized by autoclaving at 121◦C for 15minutes.The diluents was allowed to cool at room temperature before inoculation, so as to avoid denaturing of the microorganism present in each sample. Six test tubes each containing 9.0ml of sterile diluents were arranged in a clean test tube rack and labeled 1-10 respectively and 1.0ml was taken from the sample using sterile calibrated pipette 2.0ml and dispensed into test tube 1 to give 10^{-1} dilution. The samples were thoroughly mixed so that the quantity added in a representative sample of the bulk sample to be analysed. From test tube 1,0.1ml was aseptically transferred to test tube 2 to give 10^{-2} dilution. The procedure was repeated subsequently until test tube 6 to

give 10-6 dilution. The dilution factor of 10-5 was taken, plated on the growth media and incubated.

Microbiological Analysis of the Collected Samples

Microbiological analysis included isolation, enumeration and identification of pathogens using standard procedures. Media used for this analysis were the Oxoid brand of nutrient agar, peptone water, potato dextrose agar, MacConkey agar. All the media were prepared according to manufacturers' instruction. For the isolation and enumeration of pathogens in the samples, each fruit salad sample was blended in a sterile blender and 10g of the homogenate constituted in 90ml of sterile peptone water. From there 10-fold serial dilution was performed and 0.1ml of dilutions (10-5) were inoculated on appropriate media using plate technique. Bacterial plates were incubated at 37°C for 24-48h, while the fungal plates were incubated at 28°C for up to 5 days. After incubation both the bacterial and fungal plates were examined for the presence of discrete colonies. Colonies were counted using the colony counter (*Galenkamp, England*) and expressed as colony forming unit per gram (cfu/g) of sample homogenate Specifically, total aerobic counts were performed on nutrient agar, while *E.* coil and fungi were enumerated on potato dextrose agar respectively. MacConkey and Mannitol agar is used to enumerate *Staphylococcus aureus* and non-*E. coli* coliforms respectively. Characteristic discrete colonies on the different media were isolated and purified by repeated sub-culturing on the same media. Pure colonies were stored on agar slants at 4°C for further characterization.

Isolation and Identification of Microorganisms

Bacterial colony of different morphology was streaked on respective media to obtain pure culture. All the plates incubated at 37◦C for 24 hours. Pure bacterial isolates were preserved at 4◦C. The bacterial isolates were recognized on the basis of morphological and biochemical characteristics such as Oxidase test(*Steel,2015*),Indole test*(Macfaddin,2012*), Methyl red test(*Macfaddin,2012)*, Citrate test(*Claus,2014)*and Motility test *(Eklund and Lankford,2015)*. Finally, microorganisms were provisionally identified according to the Bergey's manual of determinative bacteriology and manual for the identification of medical bacteria[5].

Characterization and Identification of Bacterial Isolates

The different growth of microorganisms which appeared on the media were sub-cultured into a basal and selective media to get an axenic culture. Pure cultures of bacterial isolates were identified on the basis of their morphology and biochemical characteristics. The biochemical tests carried out are explained below.

Colonial Morphology

The shape, size, pigmentation, elevation and marginal characteristics of the bacterial species were examined on the selective media after appropriate incubation periods.

Microscopic Examination

Slide preparation of the molds were made, pieces of the young mycelium from the periphery of the culture was made with sterile inoculating loop and put on a glass slide. The cut sections were flamed briefly to melt the agar and later stained with lactophenol cotton. Blue cover slips were placed over them and examined under microscope. The microbial isolates were counted and thereafter sub cultured on fresh agar plates to ensure purity.

Gram Staining Technique

The gram staining techniques was developed by Christian Gram in (1884) to differentiate between the two types of bacteria: The Gram positive and Gram-negative bacteria.

Gram reaction test for each isolate was carried out with the following procedure: Smears of 18-24 hours old cultures of bacterial isolates on clean glass slides were heat fixed and stained with crystal violet for 45seconds.The dye was drained and then fixed with Lugol's iodine for 30seconds.The slides were rinsed under slow running water from a tap, and was then decolorized with70% ethanol for about 10-15 seconds and was rinsed under slow running water. The slides were counter stained with safranin for 30seconds then rinsed, air dried and examined under the microscope using x100 objective lens. The slide was examined under the microscope for Gram reaction and cellular morphology.

Gram positive(+ve) organisms stained blue to purple while Gram negative (-ve) stained pink to red.

Biochemical Test

Oxidase test

The oxidase test is used to identify bacteria that produce cytochrome C oxidase, an enzyme of the bacterial electron transport chain. Few drops of 1% tetramethyl-p-phenylene-diminedrochloride were used to moisten a strip of filter paper unto which the test organism was then smeared with the aid of a sterile inoculating loop. A positive reaction was shown by a deep purple colour appearing within 5-10 secs.

Indole test

This test demonstrates the ability of certain bacteria to decompose the amino acid tryptophan to indole, which accumulates in the medium. Indole production test is important in the identification of Enterobacteria. The result was read after the addition of kovac's reagent. The positive test was indicated by the red layer at the top of the tube after addition of kovac's reagent. The negative result was indicated by the absence of colour change at the top of the tube after the addition of kovac's reagent.

Motility test

This test was carried out using motility indole urease test medium. The medium was inoculated with the different

bacterial isolates by stabbing with a sterile inoculating needle at the center of the medium to over half the depth. The motile organisms grew and spread out from the line of inoculation while the non-motile grew only along the line of inoculation.

Citrate test

Citrate utilizing test is used to determine the ability of bacteria to utilize sodium citrate as its only carbon source and inorganic ammonium dihydrogen phosphate. Simmons citrate agar slightly on the slant by touching the tip of a needle to a colony that is 18 to 24hours incubates at 35◦C to 37◦C for 18 to 24hours. Some organism may require up to 7 days of incubation due to their limited rate growth on citrate medium the development of blue colour; denoting alkalization was observe.

Purification and Maintenance of Isolates

Each discrete colony on a petri dish was transferred using a sterile inoculating loop into plates containing freshly prepared Nutrient agar and were incubated at 37^{0}C for 24-48hrs.After incubation, the colonial morphologies(culture characteristics) of the isolates were recorded and compared with descriptive features contained in the Bergey's manual of determinative bacteriology [6]. The isolates were then preserved on nutrient agar slants and stored in the refrigerator at 4^{o}C.

RESULTS

A total number of 15 samples of fruit salads was bought from different food sellers on Lagos state University Campus. The total viable count ranges from (5.6 × 1.0^5-1.6 × 10^5), Total viable coliform count range from (3.0 × 10^5-1.5 × 10^5) while fungal counts ranged from (3.1 × 10^5-1.5 × 10^5) (Table1). Biochemical test on the samples identified the presence of 5 bacterial isolates namely *Escherichia coli*, *Streptococcus*, *Staphylococcus*, *Bacillus*, *and Pseudomonas* (Table 2).

Of the Five bacterial isolates obtained *Escherichia coli* had the highest frequency of (40%) followed by *Streptococcus* with (20%), *Staphylococcus*, *Bacillus*, *Pseudomonas* has the same frequency of (13%) (Table 3).

Three Fungal organisms isolated were *Aspergillus*, *penicillium* and *Saccharomyces cerevisiae* (Table 4).

DISCUSSION

Fruits are good dietary source of nutrients, micronutrients, vitamins and fiber for human; hence they are very essential for the overall wellbeing of man. The consumption of locally prepared assorted fruits, commonly known as fruit salad, has increased over the years in many regions of the world. In Nigeria, salad is classified as ready-to-eat street food because they are frequently obtained directly from hawkers or street vendors or at roadside kiosks and food stores and consumed instantly in the form they are bought without additional processing like washing, peeling or slicing[1]. Microbiological populations of

Table 1: The microbial count of the fruit salad samples

Sample code	TVC	TCC	TFC
LU1	3.2×10^5	1.8×10^5	1.5×10^5
LU2	2.0×10^5	1.4×10^5	2.3×10^5
LU3	3.0×10^5	2.0×10^5	1.9×10^5
LU4	4.7×10^5	1.3×10^5	2.3×10^5
LU5	5.6×10^5	3.3×10^5	2.7×10^5
LU6	2.3×10^5	2.7×10^5	3.4×10^5
LU7	1.7×10^5	2.2×10^5	2.8×10^5
LU8	3.1×10^5	2.0×10^5	2.1×10^5
LU9	4.2×10^5	1.7×10^5	1.5×10^5
LU10	1.6×10^5	1.0×10^5	1.8×10^5
LU11	3.7×10^5	2.6×10^5	3.1×10^5
LU12	2.5×10^5	2.0×10^5	2.6×10^5
LU13	2.8×10^5	2.2×10^5	2.4×10^5
LU14	3.1×10^5	2.3×10^5	2.0×10^5
LU15	4.1×10^5	3.0×10^5	2.3×10^5

LU: Lagos state University ,TVC: Total viable count ,TCC: Total coliform count ,TFC: Total fungal count

fruit salad was examined and five bacteria genera was isolated *Bacillus*, *Staphylococcus aureus*, *Pseudomonas*, *Escherichia and Streptococcus* and three fungal genera was identified to be *Penicillium spp*, *Aspergillus spp*, *Saccharomyces cerevisiae*.

The presence of these organisms can be linked to a number of factors such as improper handling and processing, use of contaminated water during washing, cross contamination from other fruits or the use of dirty processing utensils like knives and tray, isolated species of bacteria which include *Escherichia coli*, *Bacillus spp* from street sold foods and the presence of these microbes were thought to be as a result of inadequate processing. It is possible that vendors did not wash the fruits properly or they might have used focally contaminated water or wastewater to wash the fruits used in preparing the salad. Improper washing of the component fruits adds these microorganisms into the salad resulting in contamination.

From the results obtained, the total viable count falls within the range of 1.6×10^5 cfu/g, with sample LU5 showing the highest count of 5.6×10^5cfu/g and sample LU10 showing the lowest count of 1.0×10^5cfu/g (Table 1) Which is lower compared to the result of the worked done by *oranusi and olorunfemi*, in 2011 were the mean total aerobic plate count ranges from 2.0×10^6 to 8.2×10^8 on Pineapple and Watermelon obtained from the local market and from 6.0×10^4 to 2.7×10^7 on apple and fruit salads obtained from the University cafeteria. All the samples were contaminated with coliform and fungi with counts ranging from 2.2×10^5 to 4.2×10^6 and 2.0×10^1 to 1.0×10^3 in the samples from the cafeteria, and 2.0×10^5 to 3.5×10^6 and 2.0×10^2 to 1.1×10^3 for samples from the local market[7].

A total number of 5 bacteria and 3 fungi were isolated (Table 2 and 4) Some of the microbes encountered in this study are similar to those reported in similar studies from various countries by *Mahale, 2008, Edward et al.., 2012; and Rashed,2013*[2, 4, 8].

This confirms that fruit salad is highly susceptible to microbial contamination. It is assumed that most of these bacterial isolates are known causes of food borne illnesses and might

Table 2: Biochemical characteristics of the microbial isolates from fruit salads

Sample Code	Colony Morphology	Cell Character	Gram Staining	Indole Test	Oxidase Test	Citrate Test	Motility Test	Probable Identity
LU 1	Mucoid	R	–	+	-	-	+	*E. coli*
LU 2	Yellow, small, and irregular	C	+	-	-	+	-	*Staphylococcus*
LU 3	Mucoid	R	-	+	-	-	+	*E. coli*
LU 4	Mucoid	C	+	+	-	+	-	*Streptococcus*
LU 5	Mucoid	R		+	-	+	-	*E. coli*
LU 6	Yellow, small, and irregular	C	+	-	-	+	-	*Staphylococcus*
LU 7	Large, white mucoid	R	+	-	+	+	-	*Bacillus*
LU 8	Mucoid	C	+	+	-	+	-	*Streptococcus*
LU 9	Mucoid	R	-	-	-	+	+	*Pseudomonas aeruginosa*
LU10	Mucoid	R	-	+	-	-	+	*E. coli*
LU11	Mucoid	R	-	+	-	-	+	*E. coli*
LU12	Grape-like Large white	C	-	+	+	-	+	*Streptococcus*
LU13	Mucoid	R	-	+	-	-	+	*E. coli*
LU14	Mucoid	R	-	-	-	+	+	*Pseudomonas aeruginosa*
LU15	Mucoid	R	+	-	+	+	-	*Bacillus*

Positive (+),Negative (-),Cocci (C),Rod (R),LU: Lagos state University

Table 3: Bacteria isolated and their percentage

Organisms isolated	No of isolated	Percentage of isolates
Escherichia coli	6	40
Staphylococcus	2	13
Streptococcus	3	20
Bacillus	2	13
Pseudomonas	2	13
Total	15	100

From the organisms isolated in the fruit salads samples in Lagos state University Campus.
Escherichia coli was the most frequent organism with six isolates (40%) followed by *Streptococcus spp* with 3 isolates (20%) while *Staphylococcus spp, Bacillus spp* and *Pseudomonas spp* have the same frequency of (13%) respectively.

Table 4: Cultural characterization and morphology appearance of fungi from fruit salads

Sample code	Cultural characteristics	Morphological appearance	Probable identity
LU 4	Grey green colour	Finger like sterigma	*Penicillium species*
LU 11	Greenish, powdery colonies with creamy edges	Septate hyphae	*Aspergillus species*
LU 14	Creamy	Flat, smooth and moist	*Saccharomyces cerevisiae*

LU: Lagos state University

have been introduced into the food by unhygienic processing, Hence, consumers of fruit salad are exposed to these food borne pathogens that has the capacity to cause disease, and the nature of fruit salad is such that no further treatment is required before eating so control of the microbial load or flora is difficult.

Table 3 shows the extent of microbial contamination of the fruit salad studied. Out of the15 samples analyzed,13 samples (87%) were contaminated from the contaminated samples *E. coli* comprised 40%, *Streptococcus* comprised of 20% while *Staphylococcus*, *Bacillus*, *and Pseudomonas* comprised of 13% which is similar to the report by *Edward et al.*. (2012)[2].where 20 samples are analyzed, 18 samples (90%) were contaminated with various kinds of microorganisms. From the contaminated samples, *E. coli* comprised 40% while 55% and 35% of the samples yielded *Staphylococcus aureus* and *Bacillus cereus* respectively. The values of microbial contaminants obtained in this study are less than those reported by Edward *et al*. (2012)[2] in a similar study in Port Harcourt, Nigeria. The differences might be due to disparity in the processing methods and the sanitation of the production area and personal hygiene of the vendors.

The high coliform count is very disturbing with the least count of 1.0 × 10^5cfu/g (Table 1) The presence of *S. aureus* in the fruit salad for human consumption should be checked as count of 10 is suggestive of the possibility of food poisoning occurring. Among the bacteria, *Staphylococcus aureus* which are normal microbiome of the skin and mucous membranes, have the sellers as the likely source of contamination of the fruit salad samples. *Bacillus* might have gained entry by soil contamination due to its ubiquity. Unlike the bacterial isolates, the fungi isolates presented a safer ground since *Saccharomyces cerevisiae* is not pathogenic. However, *Aspergillus spp* is known to be pathogenic to man [9].

CONCLUSIONS

This study shows that already prepared fruit salad can contain some pathogenic organisms that could be harmful to man and pose a severe public health problem on the consumers if not properly handled hygienically.

The vendors, water and inadequate washing of hands and utensils appear to be the major hazard associated with these fruits and must be addressed properly. Vendors and consumers are advised to wash fruits properly before peeling, slicing or cutting; fruit should be handled with clean and sterilized hands, utensils and surfaces and also stored refrigerated if any delay consumption.

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