INVESTIGATION OF FUNGAL LOAD IN MIXED FRUIT SALADS SOLD IN BENIN CITY METROPOLIS

BY

Arerepade TIKO (Miss)

LSC1605560

DEPARTMENT OF MICROBIOLOGY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

BENIN CITY

JUNE, 2021.

INVESTIGATION OF FUNGAL LOAD IN MIXED FRUIT SALADS SOLD IN BENIN CITY METROPOLIS

BY

Arerepade TIKO (Miss)

LSC1605560

A PROJECT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF DEGREE OF B. Sc. (HONS) IN MICROBIOLOGY

Deleted[test]:

Deleted[test]:

JUNE, 2021.

CERTIFICATION

This is to certify that this project report was carried out by **Arerepade TIKO (Miss)** in the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City. under the supervision of Dr. C. E. Oshoma.

Formatted[test]: Font: Bold

Deleted[test]:

_____ _____

Dr. C. E. Oshoma **Date**

(Project Supervisor)

APPROVAL

This project work is accepted in partial fulfillment for the award of Bachelor of Science, B.Sc. (Hons.) in the Department of Microbiology, University of Benin, Benin City.

_____ _____

Prof. S. E. Omonigho **Date**

(Head of Department)

DEDICATION

This work is dedicated to the Almighty God and to my parents, Mr and Mrs Tiko. K. Tiko for their support, prayers and sacrifices made for me.

Formatted[test]: Centered, Tab stops: Not at 14.7 ch + 21.49 ch

ACKNOWLEDGEMENT

I wish to express my gratitude to God Almighty for His faithfulness in my life and his Grace and blessings being sufficient in my life. Lord I am Grateful.

My gratitude also goes to my supervisor, Dr. C.E. Oshoma; Thank you for the assistance, corrections and love you rendered throughout the course of this project work. I am indeed grateful to all my Lecturers in the Department of Microbiology for their academic training.

With unlimited gratitude, I acknowledge my parents, Mr Tiko K Tiko and Mrs Julie Tiko for their love, prayers, sacrifices and financial support throughout this research work.

I truly say a big thank you to my siblings, my sister Tiko Tarelayefa for her care and support.

I also want to thank my very good friends Sean, Beyonce, Happy, Blair, Gift, Tolu, Sammy for being special friends and the best friends indeed.

My sincere appreciation goes to Mr Sean for his patience, guidance and support during the course of carrying out this work.

Finally, I am very grateful to everybody that contributed to the success of this work in one way or the other, whose names were not mentioned. May God bless you all (Amen).

Deleted[test]:

Deleted[test]: A

Deleted[test]: e Sean

TABLE OF CONTENT

Deleted[test]:

LIST OF TABLES

LIST OF FIGURES

Formatted[test]: Space After: 0 pt

Deleted[test]: **CHAPTER ONE: INTRODUCTION** - - - - - - - 1

Aim and Objectives of the Study - - - - - - - 2

CHAPTER TWO: LITERATURE REVIEW - - - - - - 3

ABSTRACT

Fruits are consumed by humans as a source of nutrients which also harbour microorganisms. If not checked can lead to food spoilage. This study was aimed at investigating fungal load in mixed fruit salads sold in Benin City metropolis. Samples of mixed fruit salads were purchased from New Benin, Ring road, Uselu and Ugbowo areas all in Benin metropolis Edo State and transported to the laboratory for investigation. The fruits were store at room temperature (28+2^{o}C) and refrigeration temperature (4+2^{o}C) as control. Samples were stored for 10 d and Fungal analysis was conducted periodically after every 2h. Samples were cultured on potato dextrose agar medium. Changes in pH during storage was determined. Results revealed total heterotrophic fungal counts of samples stored at room temperature, (RT) ranging from 3.50 ± 1.50 x 10^{1} cfu/g (CONTROL) - 17.00 ± 1.00 x 10^{1} cfu/g (UGBOWO). Samples kept under refrigeration/cool temperature, CT, (4^{o}C), revealed the total heterotrophic fungal count to range from 0.50 ± 0.50 x 10^{1} cfu/g (CONTROL) - 8.50 ± 0.50 x 10^{1} cfu/g (RINGROAD). The identified fungi isolates were *Rhizopus* spp, *Penicillium* spp*, Aspergillus niger* and *Fusanum* spp. The isolate with highest frequency of occurrence was *Rhizopus* spp (40%) while the lowest was *Fusarium* spp (10%). The presence of these fungi in the investigated fruit salads suggests the need for stricter hygiene and quality measures and practices to be adopted by fruit salad vendors, and enforced by relevant authority to prevent transmission of diseases and illnesses by potentially pathogenic fungi.

CHAPTER ONE

INTRODUCTION

Over the years, there has been significant increase in the consumption of already prepared fruit salad (Edward *et al.,* 2012). This is due to the fact that it is easily accessible, convenient, nutritious and most especially, cheaper than whole fruits. It is composed of different kinds of fresh fruits which may include; water melon, pineapple, paw-paw, apple, berries, grapes etc. that are cut into small pieces and eaten with or without milk or syrup to give it an extra flavour (Alan, 1999). The nutritional qualities can be said to reflect the nutritional quality of the individual fruits used in its preparation. Generally, they are low in cholesterol, saturated fat and sodium and high in vitamins A, D and C, dietary fiber, manganese and copper (Kazi *et al.,* 2013).

In Nigeria, fruit salad is classified as a street food and so are sometimes prepared in public places and sold by vendors on streets and in other similar places in small transparent covered plastic bowls. They may be consumed where it is purchased or can be taken away and eaten elsewhere (Nwachukwu *et al*., 2008).

Fruit salad, due to its nature is of great public health concern. This is because it is prone to contamination from its preparation to the selling point due to either improper handling, contamination/ cross contamination by insects and preparation utensils, packaging materials, handling and marketing (Edward *et al.,* 2012; Uzeh *et al.,* 2009; Osamwonyi *et al.,* 2015). Contamination and cross contamination of street foods especially fruit salads are increased by unsanitary processing and preservation methods. Pathogens may invade the interior surfaces of the produce during peeling, cutting and other processes like packaging, handling and marketing (Barro *et al*., 2007). The use of dirty utensils as well as the open display of street produce encourages sporadic visits by flies, cockroaches, rodents and dusts. Another major source of contamination of fresh fruits and vegetables sold by street vendors is the washing water (Muinde and Kuria, 2005). In addition to this, it is difficult for one to attest to the

hygiene of the processors or the sanitary conditions at the point of preparation. Holding of sliced fruits that requires no further processing before consumption at ambient temperature during retail, maintains the produce at optimum temperature for proliferation/ invasion by pathogenic mesophiles (Barro *et al*., 2007).The use of simple facilities like wheelbarrow, trays, mats tables and make shift stalls by the street vendors further increase the risk of food contamination (Ray and Bhunia, 2007).

Difference in microbial profiles of various fruits and organisms result largely from unrelated factors, such as resident microflora on the soil, application of non-resident microflora via animal manures, sewage or irrigation water, transportation and handling by individual retailers (Ofor *et al*., 2009). In developing countries such as Nigeria, continued use of untreated wastewater and manure as fertilizers for the production of fruits and vegetables is a major contributing factor to contamination (Amoah *et al*., 2009).

1.2 Aim and Objectives of the Study

This study had the aim to investigate the fungal profile/load of ready-to-eat mixed fruit salad sold in Benin City.

The specific objectives were;

Deleted[test]: include

(i) isolate, characterize and identify fungi associated with mixed fruit salads over a keeping/storage duration of 10h,

(ii) determine the effect of room temperature, RT, (27±2^{o}C) and refrigeration or cool temperature, CT, (4^{o}C) storage conditions on the fungal load of ready-to-eat mixed fruit salad,

(iii) determine the rate of increase of fungal load,

(iv) determine the change in pH with increase in fungal load over storage duration of 10h .

CHAPTER TWO

LITERATURE REVIEW

2.1 Fruits and Fruit Salad

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 mixed fruits, popularly known as fruit salad, has increased over the years in many parts of the world (Oranusi and Olorunfemi, 2011). In Nigeria, fruit salad is classified as ready-to-eat street food because they are often bought directly from street vendors or hawkers or at road side kiosks and eaten form they are bought without further processing like washing, peeling or slicing. Many consumers patronize the street vended fruit salad due partly to the fact that they are cheaper than whole fruits, convenient to eat and partly because they are easily available (Edward *et al.,* 2012).

Fruit salad is usually a combination of various fresh fruits such as apples, water melon, pineapples, cucumber, pawpaw and orange (Rashed *et al.,* 2013). They are usually sliced into small immediately in the pieces and packaged in small transparent plastic bowls. The salad can be eaten using fork or tooth pick, with or without milk added to it. Since fruit salad consists of different fruits, the overall nutritional status of the salad reflects the nutritional quality of the individual fruits used in its preparation. According Edward *et al.* (2012), fruit salad is low in cholesterol, sodium and saturated fat but high in vitamins A, C and D, manganese, copper and dietary fiber.

Fruit salad is prepared and sold in road side kiosks, recreational areas and busy streets around Benin City. The different fruits used in preparing the salad are usually kept on the ground near the slicing tables without any form of protection. Hence the microbiological quality of the prepared fruit salad remains doubtful (Nichols *et al.,* 2000).

2.2 Health Benefits of Fruit Salads

The fruits and vegetables used for salad preparations are rich sources of human dietary requirements which include vitamins, carbohydrates, proteins, fats, minerals and phytochemicals that serve as antioxidants, phytoestrogens, and antiinflammatory agents. They also contain dietary fibres, flavonoids, carotenoids and other phyto nutrients that reduce the risk of heart related disorder and cancer (Pollack, 2001).

2.3 Microbial Contamination of Fresh fruit Produce

Fresh fruit and vegetables are an essential component of a healthy and balanced diet. Their consumption has increased worldwide in recent years (Betts, 2014) as a result of the promotion of healthier lifestyles. In many cases, these commodities are ready-to-eat (RTE). Consumers often do not subject these foodstuffs to any processing step prior to consumption to ensure the effective removal or inactivation of contaminants such as chemical residues or pathogenic microorganisms. Their increased consumption, allied with the globalization and large scale of production of RTE foodstuffs (Olaimat and Holley, 2012), has resulted in longer distribution times and greater distribution distances, which increases the complexity and importance of food safety management. Notably, the number of produce related disease outbreaks has risen in recent years (Kirezieva *et al.,* 2015)

Ready-to-eat foodstuffs (specifically fruits and nuts, leafy vegetables, root vegetables, sprouts, and vine-stalk vegetables) were responsible for 6.7% of total foodborne disease outbreaks reported by the Foodborne Disease Outbreak Surveillance System between 1998 and 2008 (Gould et al., 2013). The contribution of these foodstuffs to reported foodborne disease outbreaks had increased to 9.7% in the time period between 2011 and 2015 (CDC, 2017).

Microbial contamination of fresh produce has been widely reviewed in the literature (Miceli and Settanni, 2019). When considering the farm-to-fork chain, microbial contamination of

fresh produce can occur at multiple steps (Matthews, 2013). Contamination can take place during the cultivation of fresh produce, at harvest, during preparation/washing, within distribution chains and transport to shops, and even at the final step in the consumers' kitchen (Gutierrez-Rodriguez and Adhikari, 2018).

2.4 Sources of Microbial Contamination of Fruits

Fruits and vegetable possess normal or natural microbial flora, as also found in other living organisms. There could also be contamination from soil, environment, and inoculation via water irrigation. But, this may be altered in the course of harvesting, transportation and processing for consumption (Ofor *et al.,* 2009). According to WHO, level of microbial contamination in production systems can occur because of many variables including water, post-harvest practices, local environment, workers' health and hygiene and fertilizers (WHO, 2008). Some pathogenic microorganisms could have access to fruits and vegetables via damaged surfaces, and such organisms adapt, survive and reproduce in them and subsequently pose health hazard to consumers (Brooks, 2014). Although, microbial contamination of vended fruits and vegetables can change at every stage of the food chain, from cultivation to processing and point of consumption, poor hygienic conditions and environmental pollution during cultivation could also increase the risk of contamination (Wadamori *et al.,* 2017).

Contamination during cultivation can be caused by contaminated soil. Soil intended for crop production is often amended with treated or untreated animal manure/human biosolids applied as fertilizers, which serve as a cost effective nutrient source, although able to harbor pathogenic microorganisms (Brooks, 2014). Transport of microorganisms from contaminated soil to produce can be mediated by splashing originating from water droplets. Both rain and irrigation water droplets have been shown to carry over soil particles to the surface of plants,

leading to produce contamination (Allende *et al.,* 2017). Direct contact of plant surfaces with manure is also a source of contamination (Alegbeleye *et al.,* 2018).

Water used for irrigation of produce, as well as pesticide application, cooling, or protection of crops from frost, can be sourced from municipal water supplies, groundwater, recovered rainwater, surface water, or reutilized wastewater. Water sources of poorer microbiological quality (surface water and reutilized wastewater) can be a source of contamination of fresh crops. Furthermore, the type of irrigation utilized in a given crop also influences the potential for microbial contamination of fresh produce (Jongman and Korsten, 2018).

Meteorological conditions are also important factors to consider regarding microbial contamination of produce. The influence of extreme weather events is well documented and reviewed in literature. Extreme rain can lead to flooding of terrains or run off events that can result in contamination of crops. On the other hand, extreme drought conditions can lead to the utilization of water of lower microbiological quality, due to the lack of potable water, increasing the chances of contamination (Yeni and Alpas, 2017). Microbial contamination of produce has also been shown to be dependent on growth season (Marine *et al*., 2015). The survival of microorganisms in produce may be dependent on factors such as air temperature, solar radiation, or humidity. Survival of *Escherichia coli* and *Listeria innocua* in lettuce plants has been demonstrated to be greater in colder weather conditions (fall-winter) than in warmer conditions (spring-summer), in similar growth practices (Oliveira *et al.,* 2012). However, it is relevant to mention that the influence of weather conditions on plant contamination may be specific for each microorganism/plant/weather condition combination, meaning that a definite trend cannot be established (Ward et al., 2015).

2.5 Microorganisms associated with Fruit contamination

A study conducted on safety profile of fresh tomatoes presented in major markets of South-Eastern Nigeria revealed the presence of the following bacteria: *Staphylococcus aureus, Bacillus cereus* and *Lactobacillus* spp. The fungal isolates consist of *Aspergillus oryzae, Penicillum spp.* and *Aspergillus niger*. The authors showed that the isolated microbes were traced in water that was used in washing vegetable and therefore recommended the adoption of good agricultural practices (Ofor *et al.,* 2009).

In another study, 200 samples of ready to eat pineapple, pawpaw, and watermelon fruits packaged in polyethylene were provided from various street vendors in Abeokuta Ogun State and were analyzed for microbial contamination. The mean aerobic plate counts ranged from 6.34 log10 cfu/g to 8.99 log10 cfu/g, the total fungal counts ranged from 6.18 log10 cfu/g to 8.40 log10 cfu/g, while total coliform counts ranged from 6.18 log10 cfu/g to 8.43 log10 cfu/g. Fungi isolated included *Saccharomyces cerevisiae, Penicillium spp., Aspergillus flavus, Aspergillus niger, Fusarium spp., Rhizopus spp.,* and *Neurospora* spp., while bacteria isolated were *Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Proteus vulgaricus, Enterobacter aerogenes, Salmonella typhii, Bacillus spp.* and *Shigella dysenteriae.* Afolabi *et al.* (2015) concluded that fruits from these locations were below acceptable standard due to the presence of foodborne pathogens and thus recommended the need for adequate pre-treatment before consumption.

Various pathogenic microbes can contaminate fresh fruits at any point in the chain. *Escherichia coli* O157:H7, *Salmonella* spp., *Listeria monocytogenes*, *Staphylococcus aureus* and *Campylobacter* spp. are the most important pathogenic organisms that can contaminate the fresh fruits (Wadamori *et al.,* 2017). The pathogens of main concern are *Escherichia coli* O157:H7 and *Salmonella* spp. (Keith *et al.,* 2009).

Ehimemen *et al*. (2019) isolated Gram positive and Gram negative bacteria consisted of *Enterobacter* spp*., Staphylococcus aureus, Escherichia coli, Streptococcus* spp*., Citrobacter* spp. and *Klebsiella* spp. and from vegetables and fruits sold in North Western Nigeria. Among the isolated organisms, *Staphylococcus aureus* was the most prevalent one with 80% occurrence, while Streptococcus spp. was the least prevalent with 2% occurrence. Applying clean water and good handling for washing of vegetables and fruits by retailers and consumers was suggested by authors (Ehimemen *et al*., 2019).

Brook conducted a research on street vended fruit salad in Calabar, Cross River State. Different fruits combinations were used for the preparation of the fruit salad. The experimental results on 20 samples used revealed high microbial load up to 90%. Yeasts (50%), molds (70%), fecal coliform (60%), nonfecal coliform (80%), and Heterotrophic bacteria (90%). Faecal and total coliform counts were in the range of 3.2×10^5 to 5.8×10^5 cfu/g, and from 3.7×10^5 to 6.8×10^5 cfu/g respectively; while total fungal and staphylococcal counts ranged from 3.4 to 6.5 cfu/g in both cases, exceeding the recommended microbiological standards. The authors searched for microbial analysis of fruit salad for human consumption (Brooks, 2014).

Adesetan *et al.* (2013) in the street vended fruits of Ijebu area in Ogun state evaluated the antimicrobial sensitivity of bacterial isolates. A total of 75 samples of sliced fruits including coconut (15), watermelons (15), pineapples (20) and pawpaw (25) were screened. The microbial load for these fruits ranged as follows: Coconut ($0.5–5.6\times10^5$ cfu/ mL), Pineapple ($1.2–2.3\times10^5$ cfu/mL), pawpaw ($2.6- 8.0\times10^5$ cfu/mL), and watermelon ($3.0–9.3\times10^5$ cfu/ mL). The bacteria isolated included *Enterococcus faecalis, Staphylococcus aureus, Bacillus subtilis, Micrococcus* spp*., Streptococcus* spp*., Lactobacillus* spp*., Bacillus cereus, Escherichia coli, Serratia plymuthica, Serratia ficaria, Klebsiella pneumoniae* and *Proteus mirabilis*. Majority of isolates were sensitive to antibiotics. They recommended the need for a

health education in other to eliminate bacteria mediated food poisoning (Adesetan *et al.*, 2013).

Eni *et al.* (2010) investigated the microbial quality of 15 samples of fruits and vegetables procured from three different areas, a cafeteria, road side vendor, and a local market in Sanga-Ota, Ogun State. The prevalence of bacterial found in the samples include *Escherichia coli* (4.2%), *Actinomycetes* (4.2%), *Salmonella* spp. (12.5%), *Klebsiella* spp. (12.5%), *Staphylococcus* spp. (12.5%), and *Staphylococcus aureus* (29.2%). Increasing vinegar concentration from 0.5-2.5% declined microbial contamination by 15-82%. Authors suggested that consumers should be educated on the risk associated with consumption of contaminated vegetables and fruits (Eni *et al.*, 2010).

Imafidor *et al.* (2018) assessed the microbial content of lettuce sold in Benin metropolis, Edo State. Whole and soft rot samples of the vegetables part were bought, processed and analyzed. Nutrient agar plated for lettuce samples showed bacterial contamination ranging from 2.0×10^3 to 4.7×10^7 cfu/mL. Bacteria isolated were *Bacillus* spp., and *Pseudomonas* spp., where *Pseudomonas* spp. was the most prevalent. For MacConkey agar, *Klebsiella* spp., *Enterobacter* spp. and *Escherichia coli* were isolated, with bacterial counts ranging from 2.3×10^3 to 5.7×10^7 cfu/ml. The authors concluded that the consumption of rotten fruits samples could be injurious to consumers' health (Imafidor *et al.*, 2018).

2.6 Health Significance of Fruit Contamination

Fruits are prone to microbial contamination because they are constantly in contact with soil, dust and water, and by handling at harvest or during postharvest processing. Pathogenic microorganisms may also enter the fruits through damaged surfaces, such as punctures, wounds, cuts and splits. Such pathogens may become internalized, survive and grow within the fruit and consequently become health hazard to consumers (FDA, 1999). *Salmonella* sp

has been reported to survive and grow rapidly on water melon held at room temperature and the level of contamination did not change when the melon was stored at refrigeration temperature (FDA, 1999). Outbreaks of listeriosis and salmonellosis have also been associated with the consumption of ready-to-eat fruit salad (Brooks, 2014). In Nigeria where street food vending is very common, there is paucity of information on the incidence of food borne diseases related to the street vended foods. However, microbial studies on such foods in American, Asian, European and some African countries have revealed increased bacterial pathogens in fruit salad (Mahale *et al*., 2008). 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 in Benin City.

2.7 Means to Minimizing Contamination of Fruits

Various approaches of curtailing microbial contamination of vegetables and fruits were recommended by authors. They include adoption of good sanitary condition, while handling, soaking vegetables and fruits in appropriate amount of vinegar for at least 10 minutes, so as to minimize the level of microbial contamination (Nwachukwu and Chukwu, 2013). Mahapatra *et al.* (2015) recommended that appropriate and feasible disinfection system should be developed by the government. Kibitok and Nduko (2016) recommended that the government should establish safety control measure as well as analysis of the hazards and the critical control point control principles.

In some earlier studies, *Staphylococcus aureus,* a flora of the human skin and nasal cavity could have occurred through inappropriate use of unwashed hands by buyers or end users during the time of selecting the choice of fruits to buy (Iyoha and Agoreyo, 2015). It was also reported that most venders of fruits and vegetables are neither educated on proper hygiene

practice. Oluwatoyin *et al.* (2015) recommended the use of high concentration of salt or chlorinated water in washing sliced fruits packaged in polyethylene to ensure that pathogens are removed before consumption. Although, modified atmospheric packaging (MAP) may reduce spoilage by aerobic microorganisms, it can also enhance the virulence of microbes, e.g. *Escherichia coli* O157:H7 (Chua *et al.,* 2008).

Formatted[test]: Font: Italic

Polluted water used in washing processing and packaging materials after slicing or cutting, which were also listed as major source of contamination should be avoided (Daniyan and Ajibo, 2011). Chemical disinfection can also produce minimal reduction in the initial bacterial contamination, but irradiation may produce a more efficient result. Organic fertilizer, manure and municipal sludge should be well decontaminated before application for plantation purposes. Also, pathogens arising from humans or animals reservoirs as well as the environment due to unhygienic production practice are not left out of place (Kinitok and Nduko, 2016).

In attempt to maximize profit, most venders combine contaminated fruits with healthy ones during storage leading to the spread of bacteria from contaminated fruits to the healthy ones (Din *et al.,* 2011). Being that there might not be visible signs of spoilage at the initial stage of bacteria infestation, consumers are unlikely to detect the damage and thus go ahead to purchase and consume these contaminated fruits. Sensitizing venders on the need of disposing or segregating contaminated fruits from healthy ones will go a long way in reducing consumption of contaminated fruits by the populace (Din *et al.,* 2011).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Sample collection

Samples were purchased from New Benin, Ring road, Uselu, Ugbowo areas of Benin City, Edo State. Samples were then transferred to the laboratory for analysis within 1 hour.

3.2 Sample Processing

Cultural analysis was done immediately samples got to the laboratory viz: one part sample was weighed and transferred aseptically into 9 parts sterile distilled water (25 g : 225g) in a secure, sterilized container. The container was agitated after which 1ml of aliquot was pipetted into petri dishes in preparation for culturing using pour plate technique. After the plates were poured they were left to cool down and then incubated for 48-72hrs. This first culture was recorded for 0h. Test was repeated subsequently for cultural analysis every 2hrs for 7 more hours.

3.3 Sample Categories

The samples were grouped into 2 categories and analysed accordingly viz: Samples stored under room temperature, RT, (27 ± 2^{o}C) and refrigeration/cool temperature, CT, (4^{o}C).

3.4 Control Samples

Control samples were prepared in the laboratory complying with HACCP Standards.

Analysis was done every 2 hours for RT and CT samples.

3.5 Cultural Analysis

3.6 Preparation of Culture Media

All media were prepared according to manufacturer's instruction. The medium used in this study was Potato dextrose agar. Streptomycin was added to the potato dextrose agar to inhibit bacterial growth.

Potato Dextrose Agar

The medium was prepared from commercially available dehydrated powder, available from most suppliers of culture media. 39 g of Potato dextrose agar powder was dissolved in one (1) litre of distilled water in a sterile conical flask covered with cotton wool and aluminum foil paper. It was mixed thoroughly and autoclaved at 121^{o}C for 15 min. The medium was cooled after autoclaving to 45-50^{o}C and then dispensed aseptically into Petri dishes already containing inoculum (pour plate) and then rocked gently. The plates were labeled and incubated aerobically at 37^{o}C for 24 h (nutrient agar) and 72 h (potato dextrose agar). The plates were then examined macroscopically and microscopically for bacterial growth, and fungal growth (after 72 h), and then sub-cultured to obtain pure cultures according to Cheesbrough (2000).

3.7 Characterization and Identification of Isolated Fungi

Cultural and Microscopic examination

The growth pattern, pigmentation and size of colonies were recorded during the incubation period.

A drop of lactophenol blue stain was dropped on a clean, grease-free sterilized glass slide and after this, a sterile inoculating wire loop was used to pick the mycelium unto the glass slide from the mold culture .The mycelium was spread evenly on the slide. Teasing was carried out to separate the mycelium in order to get a homogenous mixture and the mixture was then

covered with cover slip gently and then allowed to stay for some seconds before observing under X40 under the microscope. The microscope examination of actively growing mold was on the basis of structures bearing spores and presence or absence of septate hyphae.

pH

pH was recorded using a pH meter (H12210 Hanna Instruments).

3.8 Data Analysis

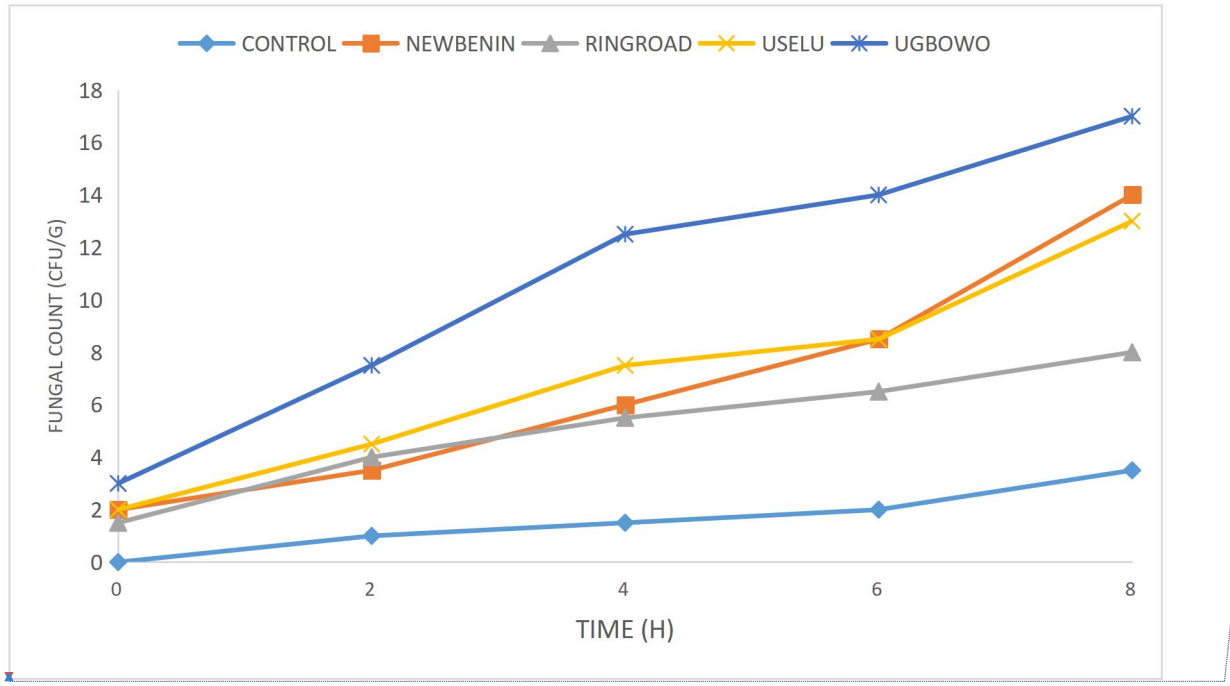
All data was analysed using SPSS package version 16.00. Results are expressed as mean ± standard deviation (S.D). All test were performed in duplicates.

CHAPTER FOUR

RESULTS

Figure 1A shows the total heterotrophic Fungal Counts of ready to eat mixed fruit salad stored at room temperature, RT, over a duration of 10h. There was a consistent increase in count as the hours progressed with UGBOWO having the highest count (17.00 ± 1.00 cfu/g) and the least count recorded by CONTROL (3.50 ± 1.50 cfu/g).

For samples stored at refrigeration/cool temperature, CT, (Fig. 1B), there was also a consistent increase in count as the hours progressed with RINGROAD having the highest count (8.50 ± 0.50 cfu/g) while the least count was recorded by CONTROL (0.50 ± 0.50 cfu/g) all after 9 h.



Deleted[test]: <sp><sp>

Formatted[Unknown]: Font: (Asian) Calibri

Figure 1A: Total Heterotrophic Fungal Counts of Ready-to-eat Mixed Fruit Salad Stored at RT

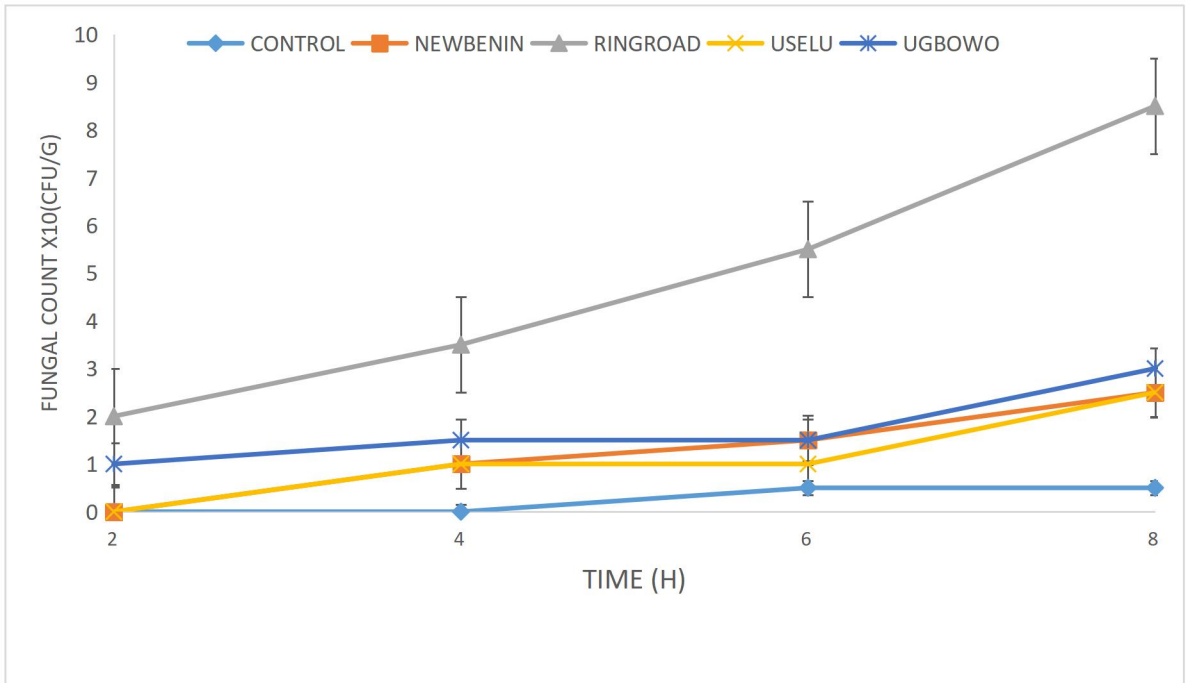


Figure 1B: Total Heterotrophic Fungal Counts of Ready-to-eat Mixed Fruit Salad Stored at CT

Table 1 shows the isolated fungi from sampled ready-to-eat mixed fruit salads. The fungi isolated where *Rhizopus* spp, *Penicillium* spp*, Aspergillus niger* and *Fusanum* spp. Table 2 shows the cultural and morphological characteristics of these isolated fungi.

The distribution of the fungal isolates is shown in Table 3 while Table 4 shows the frequency of occurrence of the isolated fungi. The highest frequency of occurrence was recorded by *Rhizopus* spp (40%) while the lowest was recorded by *Fusarium* spp (10%).

Table 1: Fungi isolated from Ready-to-eat Mixed Fruit Salads

No	Isolate
1	*Rhizopus* spp.
2	*Penicillium* spp.
3	*Aspergillus niger*
4	*Fusarium* spp.

Formatted[test]: Font: Bold

Formatted[test]: Line spacing: single

Formatted[test]: Font: Not Italic, Font color: Text1

Formatted[test]: Font: Not Italic, Font color: Text1

Table 2: Cultural, Morphological and Microscopic Characteristics of Isolated Fungi

Isolate	Morphological/Cultural characteristics	Microscopic characteristics
***Rhizopus* spp.**	White fluffy mycelium with creamy reverse side.	Non septate hyphae with branches, bat or cloud shaped sporangium, and no visibly attached sporangiospore. Long clear sporangiospore.
***Penicillium* spp.**	Flat, green colony, abundant, with reverse side off white	Brush-like condidia, septate branching conidiphore was smooth/rough walled
Aspergillus niger	Black fluffy colonies with reverse side yellow.	Long, non-septate clear hyphae, smooth conidiophore.-
***Fusarium* spp.**	White cottony mycelium, with reverse side pink	Multi-segmented canoe-like spores with branched and segmented conidiophores

Formatted[test]: Space After: 0 pt

Formatted Table[test]

Deleted[test]: *y*

Formatted[test]: Font: Not Italic

Table 3: Distribution of Isolated Fungi from ready to eat mixed fruit salads

Isolate	CONTROL	NEW BENIN	RINGROAD	USELU	UGBOWO
Rhizopus spp.	+	+	+	_	+
Penicillium spp.	_	+	_	_	+
Aspergillus spp.	+	_	+	+	_
Fusarium spp.	_	_	_	_	+

Deleted[test]: ***y***

Deleted[test]: <sp>

Formatted[Unknown]: Font: (Asian) Calibri

Deleted[test]: <sp>

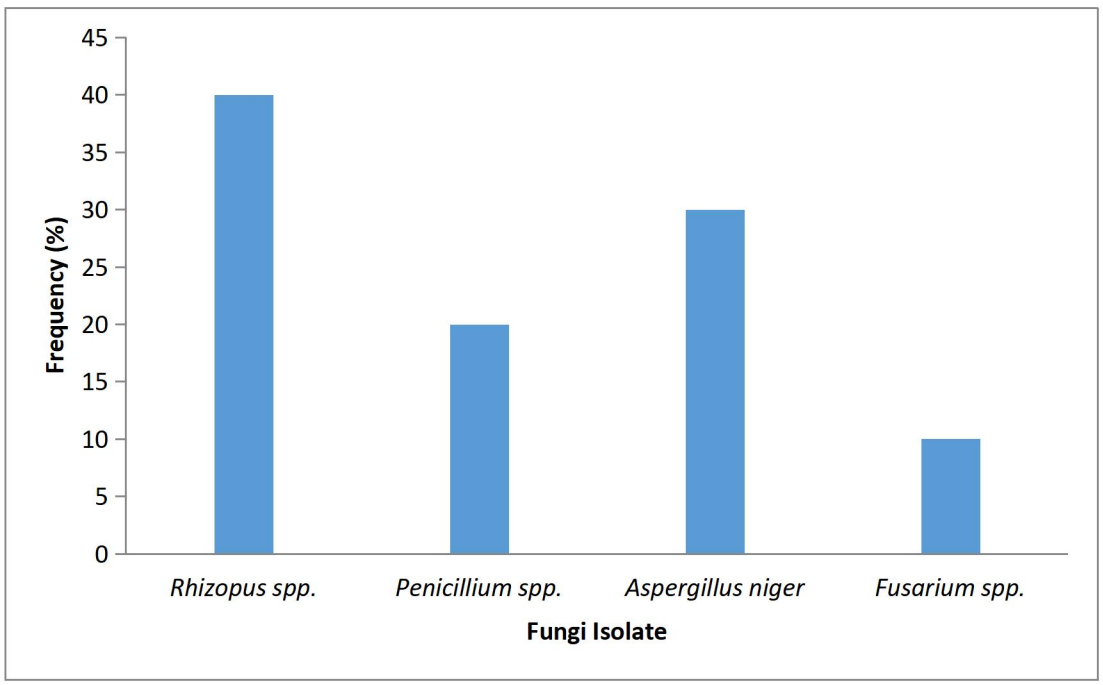


Figure 2: Frequency of Occurrence of Isolated Fungi from Ready-to-eat Mixed Fruit Salads

Deleted[test]:

Table 4: Changes in pH of ready to eat mixed fruit salads during storage

Time (h)	Room Temperature					Refrigeration temperature				
	Control	New Benin	Ring Road	Uselu	Ugbowo	Control	NewBenin	RingRoad	Uselu	Ugbowo
0	4.95±0.005	5.02±0.010	5.02±0.000	5.00±0.010	5.00±0.010	4.97±0.020	5.04±0.030	5.00±0.010	5.00±0.010	5.00±0.010
2	5.00±0.005	5.10±0.030	5.04±0.010	5.01±0.010	5.01±0.010	5.00±0.000	5.10±0.030	5.01±0.010	5.00±0.000	5.01±0.020
4	5.10±0.035	5.30±0.020	5.20±0.020	5.03±0.010	5.04±0.010	5.00±0.000	5.10±0.010	5.10±0.010	5.01±0.010	5.02±0.010
6	5.22±0.010	5.73±0.270	5.23±0.020	5.10±0.010	5.11±0.010	5.02±0.005	5.20±0.010	5.10±0.000	5.03±0.010	5.10±0.010
8	5.32±0.015	5.51±0.180	5.34±0.040	5.13±0.020	5.20±0.010	5.04±0.010	5.12±0.010	5.11±0.010	5.04±0.010	5.13±0.010

RT = Room temperature (27 ± 2°C), CT = Cool temperature (4°C)

Deleted[test]:

Formatted[test]: Line spacing: single

Formatted Table[test]

Formatted[test]: Space Before: 12 pt, Line spacing: Double

Formatted Table[test]

Formatted[test]: Space Before: 12 pt, Line spacing: Double

Formatted[test]: Space Before: 12 pt, Line spacing: Double

Formatted[test]: Space Before: 12 pt, Line spacing: Double

Formatted[test]: Space Before: 12 pt, Line spacing: Double

CHAPTER FIVE

DISCUSSION

Microbes such as fungi are associated with every single matter that exists except sterilized bodies or places. This means that fungi are inevitable life forms on earth because they are almost everywhere (Gadafi *et al.,* 2020). Their variation in different forms gives them the ability to survive in almost all kinds of environments. Since they are found in all unsterilized environments, then it is not surprising that they are found on fresh fruits and vegetables (Septembre-Malaterre *et al.,* 2018).

This study investigated the fungal load of ready to eat mixed fruit salad sold in Benin City metropolis. The samples where purchased from New Benin, Ring Road, Uselu and Ugbowo areas of Benin City. The samples were processed in duplicate, each for every sampling hour. There was a consistent increase in fungal count from 0 to 10h for samples stored at both room temperature, RT, (27±2^{o}C) and refrigeration/cool temperature, CT, (4^{o}C). For the room temperature samples, the highest count was recorded by sample UGBOWO (17.00±1.00cfu/ml) at 10h. As there is ready availability of nutrients, the fungal load will continue to increase rapidly as time progresses. The implication of this is that fruit salad contaminated in this manner become highly unsafe for consumption in a short period of time. This highlights the importance of refrigerating food that is not meant for immediate consumption, as shown in the contrast in results of the RT and CT sample counts. The samples stored at RT comparatively recorded higher counts than CT stored samples. This result is similar to the work carried out by Alyexandra *et al.* (2020) who recorded refrigerated samples having lower microbial contamination than samples stored at room temperature.

The fungi isolated in this study from ready to eat fruit salads purchased in Benin City metropolitan areas include *Rhizopus* spp., *Penicillium* spp., *Aspergilllus niger and Fusanum*

Deleted[test]:

Formatted[test]: Line spacing: Multiple 1.15 li

Deleted[test]: 0

Deleted[test]: ,

Deleted[test]: y

oxysporium. The presence of these fungi in fruit salad samples could be due to poor handling of fruits with contaminated hands during processing. Fungi can also contaminate fruits from farm during harvest as they adhere to the outer layers of most fruit. The atmosphere, soil and water are also source of contamination through which these fungi can transfer and adhere to fruits. Results for fungi presence in fruit salad samples is similar to findings obtained by Edward *et al*. (2011) who isolated same fungi from ready to eat fruit salads purchased in Port Harcourt fruit salads can easily be contaminated with microorganisms due to handling, preparation and packing processes. The way in which these foods are sold also influences the rate of microbial contamination (Oluwasanmi *et al*., 2019). If the load is high, and above WHO acceptable limits (10^2 cfu/g), then there is the possibility of the consumer being exposed to illness due to poisoning or intoxication (Oluwasanmi *et al*., 2019).

Rhizopus spp. showed the highest occurrence (40%), followed by *Aspergillus niger* (30%) while the least occurrence was recorded by *Fusarium oxysporium* (10%). *Rhizopus* spp. and *Aspergillus niger* are ready colonizers of food substances, and are found readily in a host of environments. In the work conducted by (Brooks, 2014), the fungi also showed similar occurrence rates; *Aspergillus niger* (25%) and *Rhizopus* sp (30%).

Rhizopus spp have been implicated as spoilage fungi of fruits, but pose less risk to humans. *Fusarium* species are also relatively pathogenic to plants than animals while *Penicillium* species can be harmful and cause infections called penicilliosis. Species of this genus have been mentioned in association with infections such as keratitis, endophtalmitis, otomycosis, pneumonia, endocarditis and urinary tract infections (Egbuta *et al.,* 2017). On the other hand, *Aspergillus* species are known to be pathogenic to man due to production of aflatoxins (Okonko *et al.,* 2009*,* Eni *et al.,* 2010). Filamentous fungi, especially those belonging to the genus *Aspergillus* such as *Aspergillus niger* and the genus *Penicillium* (such as *Penicillium*

citrinum) are being utilized in the food and pharmaceutical industries as a result of some metabolites they produce (Laich *et al.,* 2002). These filamentous fungi have also been reported in association with infections and disease such as aspergillosis, keratitis and otomycosis (Walsh *et al.,* 2004). Some filamentous fungi have been reported to cause both superficial infections in the case of skin and nail infections, as well as invasive infections particularly in immune-compromised individuals (Person *et al.,* 2010).

The pH recorded for all the samples over the research duration (10h) showed a trend of an increase for room temperature samples. After 10h, USELU had the lowest pH (5.13±0.020) while NEW BENIN recorded the highest pH (5.51±0.180). These pH values increase possibly due to the activities of the contaminating organisms, their metabolism, secretions and multiplication. Similar case study by Martins *et al.* (2016) conducted on fruit salad recorded lower acidic pH of 3.19 compared to higher pH values of 5.13 and 5.51 obtained for fruit salad samples from Uselu and New Benin.

Recommendations

Fruit handlers, farmers, professionals and market women involved in the food production industry ranging from production to the market should be made aware of the potential risk associated with various practices and possible chances of food contamination. They should be educated to gain sufficient knowledge on the source of etiological agents responsible for the contamination and their resultant diseases.

Conclusion

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. Thus, urgent steps including awareness are needed to enhance personal and environmental hygiene of the vendor and processing. This will improve the quality of the vended fruits as well as lifespan and wellbeing of its consumers.

REFERENCES

Adesetan, T.O., Egberongbe, H.O. and Ilusanya, O.A. (2013). Antimicrobial sensitivity of bacterial isolates from street vended fruits in Ijebu area of Ogun State, Nigeria. *International Research Journal of Microbiology* **4**: 220-225.

Afolabi, O., Oloyede, A. and Abibu, W. (2015). Microbial safety of polyethylene packaged sliced fruits sold in Abeokuta, South-West Nigeria. *Journal of Natural Scientific Research* **5**:16-21.

Alan, D. (1999). *Oxford companion to food*. Oxford University Press: Oxford. p.323

Alegbeleye, O.O., Singleton, I. and Sant'Ana, A.S. (2018). Sources and contamination routes of microbial pathogens to fresh produce during field cultivation: A review. *Food Microbiology,* **73**: 177–208.

Allende, A., Castro-Ib´ a˜ nez, I., Lindqvist, R., Gil, M. I., Uyttendaele, M. and Jacxsens, L. (2017). Quantitative contamination assessment of *Escherichia coli* in baby spinach primary production in Spain: Effects of weather conditions and agricultural practices. *International Journal of Food Microbiology* **257**: 238–246.

Alyexandra, A., Lorenza, M., Ilaria, F., Valentina, G., Riccardo, A. and Giovanni A(2020). Microbiological quality of Ready-to-Eat leafy green salads during shelf-life and home-refrigeration. *Foods* **9**: 1-11.

Amoah, P., Drechse, P., Abaidoo, R.C. and Abraham, E.M. (2009). *Improving food hygiene in Africa where vegetables are irrigated with polluted water.* Regional Sanitation and Hygiene Symposium, 3-5 Nov. 2009, Accra, Ghana.

Barro, N., Bello, A.R., Itsiembou, Y., Savadogo, A., Quattara, C.A.T., Nikiema, A.P., De Souza C. and Traore, A.S. (2007). Street vended foods improvement: contamination mechanism and application of food safety objective strategy: Critical review. *Pakistan Journal of Nutrition* **6**: 1-10.

Betts, R. (2014). Microbial update: Fruit and salad. *International Food Hygiene* **25**(3): 9–12.

Brooks, A.A. (2014). Evaluation of microbial contamination of street-vended fruit salad in Calabar, Nigeria. *International Journal of Current Microbiology and Applied Science.* **3**:1040-1046.

Cheesbrough, M. (2000). *District laboratory practical in tropical countries*. Part 2, Cambridge University Press, United Kingdom. Pp. 63-70.

Chua, D., Goh, K. and Saftner, RA. (2008). Fresh cut lettuce in modified atmosphere packages stored at improper temperatures supports enteroemorrhagic *E. coli* isolates to survive gastric acid challenge. *Journal Food Sci.* **73**: 12-17.

Daniyan, S.Y. and Ajibo, C.Q. (2011). Microbiological examination of selected fruit sold in Minna metropolis. *International Research Journal of Pharmacy* **2**:124 -129.

Din, A., Parveen, S. and Ali, A.M. (2011). Safety issues in fresh fruits and vegetables-a review. *Pakistan Journal of Nutrition* **21**:1- 6.

Deleted[test]: s

Deleted[test]:

Formatted[test]: Font: Italic

Deleted[test]: *h*

Deleted[test]:

Deleted[test]: ,

Deleted[test]: ,

Formatted[test]: Font: Italic

Edward, K.C., Umoh, E.E. and Eze, V.C. (2012). Microbial Quality of already prepared fruit salad sold in Port Harcourt, Nigeria. *Journal of Biology Agriculture and Healthcare* **2**(1): 74-79.

Egbuta, M.A., Mulunda, M. and Olubukola, O.B. (2017). Health risks associated with exposure to filamentous fungi. *International Journal of Environmental Research and Public Health.* **14**(7): 719.

Ehimemen, N.E., Mukhtar, M.F. and Salisu, N. (2019). Prevalence of bacterial loads on some fruits and vegetables sold in Kaduna central market, Northwestern Nigeria. *Journal of Applied Science* **19**(1):20- 24.

Eni, A.O., Oluwawemitan, I.A. and Oranusi, U.S. (2010). Microbial quality of fruits and vegetables sold in Sango Ota, Nigeria. *African Journal of Food Science* **4**:291-296.

FDA (1999). Food and Drug Administration: Internalization of Microorganisms into fruits and vegetables. 23p.

Gadafi, I.B., Denis, D.Y., Vera, G.A. and Priscilla, A.K. (2020). Microbial contamination, an increasing threat to the consumption of fresh fruits and vegetables in today's world. *International Journal of Microbiology* **12**: 1-13.

Gould, L. H., Walsh, K. A., Vieira, A. R., Herman, K., Williams, I. T., Hall, A. J. and Cole, D. (2013). Surveillance for foodborne disease outbreaks—United States, 1998–2008.*Morbidity and Mortality Weekly Report: Surveillance Summaries* **62**(2): 1–34.

Gutierrez-Rodriguez, E. and Adhikari, A. (2018). Pre-harvest farming practices impacting fresh produce safety. *Microbiology Spectrum* **6**(2): 12-15.

Imafidor, H.O., Obemeata, O. and Iris, O. (2018). Bacteriological assessment of lettuce vended in Benin City Edo State. *Journal of Applied Science* **4**:7-13.

Iyoha, O. and Agoreyo, F. (2015). Bacterial contamination of ready to eat fruits sold in and around ugbowo campus of University of Benin (Uniben), Edo State, Nigeria. *Brazilian Journal of Medical Research* **7**:155-60.

Jones, D. (1990). Foodborne Listeriosis. *The Lancet* **336**(8724): 1171-1174.

Jongman, M., and Korsten, L. (2018). Irrigation water quality and microbial safety of leafy greens in different vegetable production systems: A review. *Food Reviews International* **34**(4): 308–328.

Kazi, N.A., Yadav, J.P. and Agale, M.G. (2013). Nutritional value of fruits. *Scholarly Research journal for Interdisciplinary Studies* **3**: 2937-2943.

Keith, W., Ann, H. and Azadeh, N. (2009). Recent advances in the microbial safety of fresh fruits and vegetables. *Advanced Food Nutritional Research* **57**:155-208.

Kibitok, S.K. and Nduko, J.M. (2016). Evaluation of microbial contamination of consumed fruits and vegetables salad (Kachumbari) around Egerton University, Kenya. *Journal of Food Safety and Hygiene* **2**: 26-29.

Kirezieva, K., Luning, P. A., Jacxsens, L., Allende, A., Johannessen, G. S., Tondo, E. C. and van Boekel, M. (2015). Factors affecting the status of food safety management systems in the global fresh produce chain. *Food Control* **52**: 85–97.

Laich, F., Fierro, F. and Martin, J.F. (2002). Production of *Penicillin* by fungi growing on food products: Identification of a complete *Penicillin* gene cluster in *Penicillium griseofulvum* and a truncated cluster in *Penicillium verrucosum*. *Applied Environmental Microbiology* **68**:1211–1219.

Mahale, D.P., Khade, R.G. and Vaidya, V.K. (2008). Microbiological analysis of street vended fruit juices from Mumbai city, India. *Internet Journal of Food Safety* **10**: 31-34.

Mahapatra, S., Chaly, P.E. and Girija, A.S. (2015). Effectiveness of various disinfection techniques on vegetables and fruits: an *in-vitro* study. *Journal of Food Safety and Hygiene* **1**:1-8.

Marine, S.C., Pagadala, S., Wang, F., Pahl, D.M., Melendez, M.V., Kline,W. L. and Micallef, S.A. (2015). Growing season, but not farming system,a food safety risk determinant for leafy greens in the mid-Atlantic region. *Applied and Environmental Microbiology* **81**(7): 2395–2407

Martins, E.M., Afonso Mota, R., Maurilio, L.M. and Bruno, R. (2016). Fruit salad as a new vehicle for probiotic bacteria. *Food Science and Technology* **10**: 1-9

Matthews, K.R. (2013). Sources of enteric pathogen contamination of fruitsand vegetables: Future directions of research. *Stewart Postharvest Review* **9**(1):1–5.

Miceli, A. and Settanni, L. (2019). Influence of agronomic practices and pre-harvest conditions on the attachment and development of *Listeria monocytogenes* in vegetables. *Annals of Microbiology* **69**(3): 185–199

Muinde, O.K. and Kuria, E. (2005). Hygienic and sanitary practices of vendors of street foods in Nairobi, Kenya. *Journal of Food Safety and Hygiene* **5**:1-3.

Nichols, G., Gillespie, I, and deLouvois, J. (2000). The Microbiological quality of ice used to cool drinks and ready to eat fruits from retail and catering premises in the United Kingdom. *Journal of Food Protection* **63**:78- 82.

Nwachukwu, E, Ezeama, C. F. and Ezeanya, B. N. (2008) Microbiology of po yethylene-packaged sliced watermelon (*Citrullus lanatus*) sold by street vendors in Nigeria. *African Journal of Microbiology* **2**: 192-195.

Nwachukwu, E. and Chukwu, C.M. (2013). Effect of chemical treatments on the microbial load of fruits and vegetables. *Journal of Food Safety and Hygiene* **1**:16 -19.

Ofor, M.O., Okorie, V.C. and Ibeawuchi, I.I. (2009). Microbial contaminants in fresh tomato wash water and food safety considerations in South-Eastern Nigeria. *Life Science Journal* **6**:80-82.

Okonko, I.O., Adejoye, O.D., Ogun, A.A., Ogunjobi, A.A., Nkang, A.O. and Adebayo, B.C. (2009) Hazard analysis critical control points (HACCP) and Microbial qualities of sea foods as affected by handler's hygiene in Ibadan and Lagos, Nigeria. *African Journal of Food Science* **3**(2): 35-50.

Olaimat, A.N. and Holley, R.A. (2012). Factors influencing the microbial safety of fresh produce: A review. *Food Microbiology*, **32**(1): 1–19.

Oliveira, M., Vinas, I., Usall, J., Anguera, M. and Abadias, M. (2012). Presence and survival ofEscherichia coliO157:H7 on lettuce leaves and in soil treated with contaminated compost and irrigation water. *International Journal of Food Microbiology*, **156**(2): 133–140.

Oluwasanmi, A.A., Boluwatife, M.F. and Oluwapelumi, O.A. (2019). Microbial contamination of fresh vegetable salads from food vendors in Oyo Metropolis. *Nigerian Journal Pure and Applied Science* **32**(1): 3374-3379.

Oluwatoyin, A., Adejare, O. and Wasiu, A. (2015). Microbial safety of polyethylene packaged sliced fruits sold in Abeokuta, South-West Nigeria. *Natural Science Research* **5**:16-21.

Oranusi, S. and Olorunfemi, O.J. (2011). Microbiological safety evaluation of street vended ready-to-eat fruits sold in Ota, Ogun state, Nigeria. *International Journal of Research in Biological Sciences* **1**(3): 27-32.

Osamwonyi, O.U., Obayagbona, O.N., Aborisade, T.W., Olisaka, N.F., Uwadiae, E. and Igiehon, O.N. (2013). Bacteriological Quality of Vegetable Salads Sold at Restaurants Within Okada Town, Edo State, Nigeria. *Journal of Pharmacy and Biological Sciences* **5**(2):87-90.

Person, A.K., Chudgar, S.M., Norton, B.L., Tong, B.C. and Stout, J.E. (2010). *Aspergillus niger*: An unusual cause of invasive pulmonary aspergillosis. *Journal of Medical Microbiology* **59**:834–838.

Pollack, S. (2001). *Consumer demand for fruit and vegetables: Changing Structure of Global Food Consumption and Trade. Washington, DC: Economic Research Service/United States Department of Agriculture.*; Pp. 49-54.

Rashed, N., Md Aftab, U., Md Asizul, H., Saurab, K. M., Mrityunjoy, A. and Majibur, R. (2013). Microbiological study of vendor and packed fruit juices locally available in Dhaka City, Bangladesh. *International Food Research Journal* **20**(2): 1011-1015.

Ray, B. and Bhunia, A.K. (2007). *Fundamental Food Microbiology*, 4th ed. CRC Press, USA. p. 492.

Septembre-Malaterre, A., Remize, F. and Poucheret, P. (2018). Fruits and vegetables, as a source of nutritional compounds and phytochemicals: changes in bioactive compounds during lactic fermentation. *Food Research International* **104**: 86-99.

Uzeh, R.E., Alade, F.A. and Bankole, M. (2009). The microbial quality of pre packed mixed vegetable salad in some retail outlets in Lagos Nigeria. *African Journal of Food Science* **41**: 270-272.

Wadamori, Y., Gooneratne, R. and Hussain, MA. (2017). Outbreaks and factors influencing microbiological contamination of fresh produce. *Journal of Science, Food and Agriculture* **97**:1396-1403.

Walsh, T.J., Groll, A., Hiemenz, J., Fleming, R., Roilides, E. and Anaissie, E. (2004). Infections due to emerging and uncommon medically important fungal pathogens. *Clinical Microbiology and Infections* **10**:48–66.

Ward, M., Dhingra, R., Remais, J. V., Chang, H. H., Johnston, L. M., Jaykus, L. A. and Leon, J. (2015). Associations between weather and microbial load on fresh produce prior to harvest. *Journal of Food Protection* **78**(4): 849–854.

WHO (2008). *Microbiological hazards in fresh leafy vegetables and herbs, in MRA Series , World Health Organization,* 85p.

Yeni, F. and Alpas, H. (2017). Vulnerability of global food production to extreme climatic events. *Food Research International* **96**: 27–39.

APPENDIX

APPENDIX I

Potato dextrose agar

Typical composition

Ingredients & conditions	**Value**
potatoes (sliced washed unpeeled)	4g (from 200g infused potato)
dextrose	20g
agar powder	20g
final pH	5.6±0.2

Formatted Table[test]

APPENDIX II

Deleted[test]:

Total Heterotrophic count of Ready-to-eat Mixed Fruit Salads

TIME (h)	CONTROL	NEWBENIN	RINGROAD	USELU	UGBOWO
RT					
0	0.00±0.00	2.00±1.00	1.50±0.50	2.50±0.50	3.00±2.00
2	1.00±0.00	3.50±1.50	4.00±1.00	4.50±0.50	7.50±1.50
4	1.50±0.50	6.00±1.00	5.50±2.50	7.50±0.50	12.50±0.50
6	2.00±1.00	8.50±1.50	6.50±1.50	8.50±1.00	14.00±1.00
8	3.50±1.50	14.00±3.00	8.00±2.00	13.00±1.00	17.00±1.00
CT					
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.50±0.50
2	0.00±0.00	0.00±0.00	2.00±1.00	0.00±0.00	1.00±1.00
4	0.00±0.00	1.00±1.00	3.50±0.50	1.00±0.00	1.50±0.50
6	0.50±0.50	1.50±0.50	5.50±0.50	1.00±0.00	1.50±0.50
8	0.50±0.50	2.50±1.50	8.50±0.50	2.50±0.50	3.00±0.00

Key: RT = Room temperature (27 ± 2ºC), CT = Cool temperature (4ºC)

Frequency of occurrence of the isolated fungi

Isolate	Occurrence	Frequency (%)
***Rhizopus* spp.**	4	40
***Penicillium* spp.**	2	20
Aspergillus niger	3	30
***Fusarium* spp.**	1	10
Total	10	100