# MICROBIAL ANALYSIS OF A MIXED FRUIT JUICE SAMPLE USING BRINE AS THE PRESERVATIVE

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## CERTIFICATION

This is to certify that this project work was carried out by **Martha JUMBO** (Miss) with Matriculation Number **LSC2103962** in the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City under my supervision.

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**DR.(MRS) C.G. DIMOWO** **Date**

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Prof. F.I. AKINNIBOSUN Date

**(Head of Department)**

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External Examiner Date

## APPROVAL

I certify that this work has been accepted in partial fulfillment of the requirement for the award of Bachelor of Science (B.Sc.) in the Department of Microbiology, University of Benin, Benin City.

_____ _____

**PROF. F. I. AKINNIBOSUN** **Date**
**(Head of Department)**

## DEDICATION

I dedicate this project to God, whose guidance and blessings have been my strength and inspiration. I also extend my heartfelt gratitude to my parents, whose unwavering support, love, and encouragement have been instrumental in my journey. Thank you for believing in me and being my constant pillars of strength.

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I would like to appreciate my friends for their encouragement and support throughout my academic journey.

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## ABSTRACT

The study microbial analysis of a mixed fruit juice sample using brine as the preservative was carried with the aim of producing mixed fruit juice from the extracted juices of watermelon and pineapple, and also to determine the effect of different rations of brine solution in preserving the produced mixed fruit juice. The fruit juice from the watermelon and orange was extracted using the pressing and filtration technique while the mixed fruit juice was prepared by mixing different rations of orange and watermelon (80:20), orange and watermelon (30:70) and orange and watermelon (60:40). The brine solution was prepared by using different rations (10:90, 20:80, 40:60 and 60:40) of sodium chloride and calcium chlorides respectively. The effect of the different rations of the brine solutions on the microbiological and physiological properties of the mixed fruit juices was done using standard laboratory procedures. The overall acceptability of the different mixed fruit juices after day 7 of the experiment was found to be 45%, 35% and 70% for the orange and watermelon (80:20), orange and watermelon (30:70) and orange and watermelon (60:40) respectively. The results of the effect of the different concentration of the brine solutions for the preservation of the different rations of the extracted juice showed that mixtures ration orange and watermelon (60:40) was more stable in terms of microbial growth on the juice while the pH of the different juice rations was found to be slightly neutral at day 0 of the experiment and then acidic at day 7 of the experiment. The results of the effect of the different brine solutions on the microbial count of the juice mixture recorded no microbial growth in any of the treatments at day 7 of the experiment. This was attributed to the increased acidity of the juice at day 7 of the experiment. Findings from this study has shown that the juice mixture orange and watermelon (60:40) was found to be more stable in terms of the sensory, physiological and microbiological evaluation.

# CHAPTER ONE

# INTRODUCTION

## 1.1 Background of the Study

Foods and beverages often contain different types of food additives, among which preservatives play an important role (PetanovskaIlievska *et al*., 2017); these are included in one of the 26 major additive categories that are utilized in foods (Kregiel, 2015). Before the advent of preservatives, food was placed in containers such as clay jars to preserve them from spoiling.

Food storage can be traced back to every ancient civilization such as Egyptian, Greek, Roman, Sumerian, and Chinese (Anand and Sati, 2013). Fruit juices, particularly those derived from tropical fruits like pineapple and watermelon, are popular for their refreshing taste and nutritional benefits. However, they are highly susceptible to microbial contamination due to their high moisture content and natural sugars, which provide an ideal environment for spoilage organisms. Pineapple juice is a low pH fruit juice with sweet taste and beneficial health compounds. These compounds include ascorbic acid, bromelain, carotenoids, phenolic compounds and flavonoids among others (Lagnika *et al.,* 2017).

The preservation of food has been in existence for a long time now. Preservatives are defined as substances that are added to products such as food or beverages to prevent, stop, and/or delay any food deterioration due to microbial growth. An ideal food preservative remains effective until the product is consumed (Adeshinavand, 2012). Brine preservatives are included in biological preparations to kill or inhibit the growth of microorganisms inadvertently introduced during manufacture or use which may cause microbial deterioration. The use of preservatives in products is to reduce the likelihood of microbial growth in aqueous products and also to minimize the chances of microbial survival in anhydrous products that may be contaminated

(Baird, 2004). They are also added to industrial products which may, by their nature, support the growth of bacteria and moulds causing spoilage of the product and possibly infection of the user  Juices are aqueous liquids expressed or otherwise extracted usually from one or more fruits (Bello *et al*., 2014).

Juices are prepared mechanically by squeezing or macerating the pulp of fresh fruits or vegetables without application of heat or solvent to give an unfermented clouded, unclarified and untreated juice ready for consumption. Diluting or blending is a common practice as many fresh juices are either too acidic or too strongly flavored to be pleasant for consumption (Noah, 2020). Fruits are parts of plants that houses seeds or flesh covering of nuts or succulent, they are pulpy in character, often juicy and since the develop from flowers of plant. Fresh fruit and vegetable juices are an important part of modern day diet in many parts of the world as they are rich source of nutrients such as vitamins, minerals and other naturally occurring phytochemicals which are of health and therapeutic benefits (Ukwo *et al.*, 2011).

Fruit juices are important commodities in the global market, providing vast possibilities for new value-added products to meet consumer demand for convenience, nutrition, and health.  Vending of fresh unpasteurized juices is widely practiced in many developing countries, particularly in urban areas (Tambekar *et al*., 2009 ). These juices are in great demand for their perceived health, nutritional benefits and availability at reasonable prices (Noah, 2020). Juices served in permanent structures are offered in better hygienic conditions than those in make-shift structures (Abdalla *et al*., 2008) . The microbiological quality of street-vended juices, just like most street foods, is largely questionable because of the poor hygienic environment associated with their processing (Alamgir *et al*., 2015) . Pineapple and watermelon are not just summer fruits; they are packed with nutrients and beloved for their juicy, refreshing taste. When mixed, they create a

delightful and refreshing beverage. But like all natural products, they come with the challenge of spoilage. Both fresh pineapple and watermelon juices is a popular product due to its pleasant aroma, flavor and numerous functional properties (Rattanathanalerk *et al*., 2005). Its content in vitamin C is particularly emphasizes and reported to largely vary from 9.2 to 93.8 mg/100mL (Hounhouigan *et al*., 2014) . Fresh unpasteurized juices clearly are products worthy of concern in causing potential food safety risk (Torres-Vitela *et al.,*2013) .

The high moisture content of fruit juice predisposes them to microbial spoilage by bacteria, molds, and yeasts, since most are acid tolerant (Aderinola and Adeniran, 2015). Fruit juices are spoiled primarily due to proliferation of acid tolerant and osmophilic microbiota. There is also risk of food-borne microbial infections or intoxications, which may be associated with the consumption of fruit juices. In order to reduce the incidence of outbreaks, fruit juices are preserved by various techniques (Aneja *et al*., 2014b).

Traditionally, the stability of fruit drinks and beverage has been achieved by thermal processing. However, thermal processing tends to reduce the product quality and freshness; therefore, preservatives are a good option because these products display several advantages such as retention of sensory qualities and nutritional values over traditional thermal processing (Rupasinghe and Yu, 2012). Food preservatives may be classified as natural and conventional, which play a very important role in the beverage industry, citric acid is a good example of a naturally occurring preservative, sodium benzoate and potassium sorbate are representatives of the second type.

The choice of appropriate preservatives for fruit drinks and beverages should take into consideration specific product requirements, the type of spoilage organisms associated with it, the product pH, the intended shelf life, and the mode of application. The pH and nutritional

parameters are among the most decisive factors in choosing a preservative. In general, preservatives are only effective when the initial microbial contamination level is low. Brine is essentially a solution of salt in water. It's been used to preserve food because of its ability to inhibit the growth of bacteria and other microorganisms (Oliveira *et al*.,2012).

In delving deeper into this study, it's crucial to recognize the inherent complexities of fruit juices and microbial activities. Pineapple and watermelon, despite their robust nutrient profiles, are susceptible to microbial spoilage due to their high water content and natural sugars. This vulnerability necessitates efficient preservation techniques to maintain their freshness and safety for consumption (Shahnawaz *et al*., 2013).

Most microbiological problems arise because of poor quality raw materials and poor process hygiene which lead to overcoming of the preservation system applied during manufacture by the spoilage organisms (Riikka *et al*., 2011). Good hygienic practices are essential to guarantee the quality of the products (Sospedra *et al*., 2012).

Consumption of fresh juices has resulted in foodborne illnesses such as salmonellosis , staphylococcal food poisoning and shigellosis from among other pathogenic microorganisms *Escherichia coli*, *Salmonella, Shigella* and *Staphylococcus aureus* (Addisu, *et al*., 2016). Illnesses caused by fresh juices are reported to arise from contamination from raw materials, equipment, processing conditions, improper handling, unpotable water as well as increased length of storage at room temperature (Imathiu, 2017). Furthermore, the exposure of these juices to airborne dust and unhygienic conditions such as dirty waste water and garbage near the food establishments allows vectors like houseflies to transfer microorganisms to the finished products (Tambekar *et al*., 2009, Addisu, *et al*., 2016).

Preservation techniques play a vital role in extending the shelf life of fruit juices while retaining their sensory and nutritional qualities. Brine, a solution of salt in water, has been traditionally used as a preservative for various foods, including fruits and vegetables. Its effectiveness lies in its ability to create an osmotic environment that inhibits microbial growth. This study aims to explore the potential of brine as a preservative for mixed fruit juice from pineapple and watermelon, assessing its impact on microbial stability and overall juice quality.

## 1.2 AIM AND OBJECTIVES

The aim of this study was to produce mixed fruit juice from both watermelon and pineapple, subjecting to brine solution as a method of preservation and to determine the microbial load of the mixed fruit juice.

**The specific objectives were to**:

i. analyze the microbial content in mixed fruit juice samples of pineapple and watermelon.

ii. evaluate the effectiveness of brine as a preservative in inhibiting spoilage organisms.

iii. assess changes in physicochemical properties (such as pH, color, and titratable acidity) during storage with brine.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1. MIXED FRUIT JUICES

Fruit juices are highly favored worldwide, not only for their refreshing qualities but also for their nutritional benefits. Mixed fruit juices, in particular, have gained popularity due to the combination of flavors and nutrients derived from a variety of fruits. These beverages are rich in essential vitamins, minerals, antioxidants, and other bioactive compounds, making them an excellent source of nutrients that support overall health and well-being (Saeed *et al*., 2015). For instance, many mixed fruit juices provide high concentrations of Vitamin C, potassium, fiber, and phytonutrients, which have been linked to reduced risks of chronic diseases such as heart disease, cancer, and diabetes (Slavin and Lloyd, 2012). The diversity of fruits in a single blend enhances the nutrient profile and taste, appealing to a wide range of consumers looking for both flavor and health-promoting properties in their beverages (Liu, 2013).

Despite their many benefits, mixed fruit juices are particularly vulnerable to microbial contamination, which poses serious public health concerns. Microorganisms such as bacteria, yeasts, and molds can thrive in fruit juices due to the high moisture content, nutrient-rich environment, and acidity, although most fruit juices are acidic (with pH values between 3.0 and 4.0), which tends to inhibit the growth of certain pathogens (Tournas *et al*., 2006). However, the low pH is not a guarantee of safety, as certain acid-tolerant microorganisms, including spoilage organisms and pathogens such as *Escherichia coli* O157, *Salmonella* spp., and *Listeria monocytogenes*, can survive and even proliferate in fruit juices under favorable conditions (Tauxe *et al*., 1997).

The production process of mixed fruit juices often involves several stages, including washing, peeling, juicing, blending, pasteurization (in some cases), and packaging. Each of these stages presents a potential risk for microbial contamination if proper hygiene and quality control measures are not strictly adhered to. Raw fruits, which serve as the main ingredient in juices, can harbor various microorganisms on their surfaces due to contamination from soil, water, insects, or handling (Tournas and Katsoudas, 2005).

Poorly sanitized processing equipment, inadequate cleaning of the fruit before juicing, and contamination during transportation and storage are other factors that contribute to the microbial load in fruit juices (Ryu *et al*., 1998). The presence of these microorganisms in fruit juices can lead to spoilage, off-flavors, and even foodborne illnesses, making it crucial to understand and mitigate these risks.

Moreover, natural microbial flora, including yeasts and lactic acid bacteria, are commonly found in fruit juices and are responsible for fermentation and spoilage. Yeasts, such as *Saccharomyces cerevisiae*, and molds *like Aspergillus niger* and *Penicillium* spp., are frequently associated with juice spoilage, leading to gas production, off-odors, and cloudiness in the juice (Fleet, 1992; Moss, 2008). These spoilage organisms, although not typically pathogenic, can significantly reduce the shelf life of mixed fruit juices and impact their sensory attributes, leading to economic losses for producers and dissatisfaction among consumers.

On the other hand, pathogenic bacteria such as *E. coli, Salmonella*, and *Listeria monocytogenes* are of greater concern due to their potential to cause serious foodborne diseases. For instance, outbreaks of *Salmonella* spp. and *E. coli* O157 associated with unpasteurized fruit juices have been reported in both developed and developing countries, underscoring the importance of

effective processing and preservation techniques (Parish, 1997; Scallan *et al*., 2011). These pathogens can contaminate fruit juices at various stages of production, especially if proper hygiene practices are not followed or if contaminated water is used for washing the fruits.

## 2.2. COMPOSITION OF MIXED FRUIT JUICES

Mixed fruit juices are formulated by blending the juices of two or more fruits, such as oranges, apples, pineapples, mangoes, and berries, resulting in a product that boasts diverse flavors and nutritional profiles. The composition of these juices is influenced by the types of fruits used, their ripeness, and the processing methods employed. This section explores the key components of mixed fruit juices, highlighting their nutritional benefits and the factors affecting their composition.

### 2.2.1. Vitamins and Minerals

Mixed fruit juices are particularly noted for their high vitamin content, with vitamin C (ascorbic acid) being one of the most abundant. Vitamin C is a powerful antioxidant that plays a vital role in protecting cells from oxidative damage, enhancing immune function, and aiding in the absorption of iron (Carr and Frei, 1999). In addition to vitamin C, mixed fruit juices provide significant amounts of essential minerals, including potassium, magnesium, calcium, and folate. Potassium is crucial for maintaining healthy blood pressure levels, while magnesium plays a role in numerous biochemical reactions in the body (He *et al*., 2006).

### 2.2.2 Natural Sugars

The sweetness of mixed fruit juices primarily comes from natural sugars, mainly glucose, fructose, and sucrose. These sugars contribute to the overall energy content of the juice and provide a quick source of energy for the body. While the natural sugars in fruit juices are

healthier than added sugars, it is important to consume these beverages in moderation, as excessive sugar intake can lead to health issues such as obesity and type 2 diabetes (Malik *et al*., 2006).

### 2.2.3 Organic Acids

Mixed fruit juices contain various organic acids, including citric, malic, and tartaric acids, which impart a tart flavor and contribute to the overall taste profile of the juice. These organic acids also play a significant role in regulating the pH levels of the juice. The typical pH range for fruit juices falls between 2.5 and 4.5, creating an acidic environment that is unfavorable for the growth of certain pathogenic bacteria (Vanderlinde *et al*., 2010). While this acidic environment can inhibit some microbial activity, it is insufficient to completely prevent spoilage or contamination.

### 2.2.4. Phytonutrients and Antioxidants

In addition to vitamins and minerals, mixed fruit juices are rich in phytonutrients and antioxidants, such as flavonoids and carotenoids. These compounds have been associated with numerous health benefits, including anti-inflammatory properties, improved cardiovascular health, and a reduced risk of certain cancers (Anjum *et al*., 2012). The presence of these bioactive compounds enhances the overall health-promoting potential of mixed fruit juices, making them a desirable choice for consumers seeking nutritious beverages.

#### 2.2.1.1 Factors Affecting Composition

The composition of mixed fruit juices can be influenced by several factors, including, The choice of fruits used in the juice blend directly affects its nutritional profile. For example, juices containing citrus fruits tend to have higher vitamin C content, while those made from berries

may be richer in antioxidants (Rohloff, 2008). The ripeness of the fruits at the time of juicing can significantly impact their nutrient content. Ripe fruits generally contain higher concentrations of vitamins and sugars compared to unripe ones (Hernandez *et al*., 2011). The methods employed during the processing and storage of mixed fruit juices can also influence their composition. Pasteurization, for instance, can reduce microbial loads but may also lead to a loss of some heat-sensitive vitamins (Klein and Smith, 2018). Additionally, the use of preservatives or additives can alter the nutritional composition and overall quality of the juice.

## 2.3. USE OF PROCESSED FRUIT JUICES

In the developed countries the trend is shifting to the use of processed juices rather than fresh juices. Processed juices have more nutritional contents and are available and consumed easily. Orange juice is rich in vitamin C, so it is most appreciated and consumed among orange juices. The parametres like colour, flavour and the evolution of vitamin C during storage determine the quality and shelf life of the orange juice (Zerdin *et al*., 2003). Vitamin C take part in prevention of many diseases because it has high antioxidant power (Tannenbaum *et al*., 1985). The storage conditions play a vital role in the stability of vitamin C, it is lost during poor storage conditions due to its oxidation (Kabasakalis *et al*., 2000). The factors that are responsible for loss of vitamin C are temperature, dissolved oxygen and oxygen barrier (Tannenbaum *et al*., 1985). The quality of the product during shelf life and its cost are also contributing factors.

## 2.4. SHELF LIFE OF FRUIT JUICES

The main objective of processing is to preserve the fruit or fruit juice in a stable form to supply to local and distant markets. The shelf life of the fruit juice depends upon the packaging material used and a lot of research work has been done on this topic (Zerdin *et al*., 2003). Imported juice

products are packed using good quality packing material unlike that local products have poor quality packing materials and lack proper labeling. Juices should have labels indicating the shelf life of the products. Most of the imported juices have labels indicating their shelf life. Shelf life of the juice products also depend upon volume of the pack. Normally juices in 250ml packs have shelf life less than 6 months while the juices that are in larger packs have shelf life from 6 months to 1 year.

The main factors that contribute shelf life are quality and quantity of preservative and conditions of the production process. Several factors encourage, prevent or limit the growth of microorganisms in juices; the most important are pH, hygienic practice and storage temperature and concentration of the preservative. Storage of products at refrigerator temperature or below is not always best for the maintenance of desirable quality of some fruits. The growth of the pathogens can be favoured by changing pH of the juice products (FDA, 2001).

## 2.5. MICROBIAL CONTAMINATION IN FRUIT JUICES

Fruit juices, especially mixed fruit juices, are susceptible to microbial contamination at various stages, including processing, packaging, transportation, and storage. This contamination is a significant concern as it can lead to spoilage, reduce the shelf life of the products, and pose potential health risks to consumers. Understanding the sources and types of microbial contaminants is essential for developing effective food safety measures.

Microbial contamination in fruit juices can originate from multiple sources.The primary source of microorganisms in fruit juices is the raw fruits themselves. Fruits can harbor bacteria, yeasts, and molds on their surfaces, stemming from soil, water, or contact with contaminated surfaces during harvesting, processing, or handling. For example, fruits like apples and peaches can carry

*E. coli* or *Salmonella* if contaminated during agricultural practices (Deng *et al*., 2014). Water used during the juicing process can also introduce pathogens. If the water is not potable or contains contaminants, it can lead to microbial contamination of the final juice product (Moyne *et al*., 2016).

Therefore, using safe, treated water is critical in juice processing. Equipment used in juice production can serve as reservoirs for microbial growth if not properly sanitized. Residual organic matter and biofilms can develop on surfaces, providing a suitable environment for the proliferation of microorganisms (Banerjee *et al*., 2018). The hands of processing personnel can also be a source of contamination. Inadequate personal hygiene practices, such as not washing hands or handling food without proper gloves, can transfer pathogens to the juice (Tessema *et al*., 2020).

Several studies have reported various microorganisms in fruit juices, highlighting the need for stringent quality control measures, Bacterial contaminants such as *Escherichia coli*, *Salmonella spp.*, and *Lactobacillus spp.* are commonly found in unpasteurized fruit juices (Campos *et al*., 2009). *E. coli*, in particular, is a well-known pathogen that can cause severe gastrointestinal illnesses. Its presence in fruit juices indicates potential contamination with fecal matter, raising serious health concerns.Yeast species, such as *Saccharomyces cerevisiae* and *Candida spp.*, are prevalent in fruit juices. They can initiate fermentation processes, leading to spoilage and off-flavors in juices due to their ability to thrive in acidic environments (Fleet, 1992). The growth of yeast can also contribute to the production of carbon dioxide and alcohol, altering the sensory attributes of the juice.

Molds, including species from the genera Aspergillus and Penicillium, have been identified in fruit juices, especially in products with high sugar content or stored under unsanitary conditions (Tournas *et al*., 2006). These molds can produce mycotoxins, which pose significant health risks if ingested. Viral contamination is also a concern, particularly from noroviruses and hepatitis A virus, which can be transmitted through contaminated water or food. These viruses can survive in acidic conditions and pose a significant health threat (López *et al*., 2012).

The presence of microorganisms in fruit juices can lead to several adverse effects. Microbial activity can cause changes in flavor, aroma, and color, rendering the juice unpalatable and unsuitable for consumption (Khan *et al*., 2015). Consumption of contaminated fruit juices can lead to foodborne illnesses, resulting in symptoms such as nausea, vomiting, diarrhea, and, in severe cases, hospitalization (Bennett *et al*., 2018). Microbial contamination can significantly decrease the shelf life of fruit juices, leading to economic losses for producers and retailers.

### 2.5.1. Bacterial Contaminants

Mixed fruit juices can harbor various pathogenic bacteria that pose significant health risks to consumers. The presence of these bacteria often indicates poor hygiene practices during handling, processing, or storage. Various bacteria such as *Bacillus alvei, Bacillus polymyxa, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia, Enterobecter* and *Escherichia coli* are found in fruit juices (Iqbal *et al*., 2015). Micro-organisms use human foods as source of their nutrients. They are causing food deterioration by increasing their numbers, produce enzymatic changes and contribute to flavor. So these microbes must be eliminated from the contaminated food to preserve it (Ahmed, 1991). Although fruit juices are acidic in nature, high moisture content favors the growth of bacteria and yeast. Normally at room

temperature, alcoholic fermentation and oxidation of alcohol and fruit acid by yeast and mould take place (Macrae *et al*., 1997).

*Escherichia coli* (*E. coli*) is a gram-negative bacterium that serves as an indicator of fecal contamination. The detection of *E. coli* in fruit juices is alarming, as it is primarily associated with human and animal waste. Several studies have highlighted the prevalence of pathogenic strains, particularly *E. coli* O157, which can cause severe gastrointestinal illness, including diarrhea, abdominal cramps, and hemolytic uremic syndrome (HUS) (Jay *et al*., 2005). In a study conducted by Sandeep *et al*. (2004), high levels of *E. coli* were detected in street-vended mixed fruit juices, demonstrating a significant public health risk. This finding underscores the need for stringent hygiene practices in juice preparation and serving, particularly in street-vending settings. Additionally, the presence of *E. coli* in unpasteurized juices has been linked to outbreaks of foodborne illness, emphasizing the importance of proper sanitation and food handling protocols (Liu *et al*., 2016).

*Salmonella* spp. are facultative anaerobic bacteria that can contaminate fruit juices, particularly when hygiene during handling and packaging is compromised. The ingestion of contaminated juice can lead to salmonellosis, characterized by symptoms such as diarrhea, fever, and abdominal cramps (Tauxe *et al*., 1997). In a study investigating the microbiological safety of fruit juices, *Salmonella* was isolated from several samples, indicating a significant risk for consumers (Norrung *et al*., 2015). Moreover, the occurrence of *Salmonella* in fruit juices is often associated with cross-contamination from raw fruits, improper washing of fruits before juicing, or contamination from processing equipment.

Many outbreaks of Salmonella spp. have been linked with the consumption of unpasteurized juices (Harris *et al*., 2003). Federal Analysis Critical Control Point Program has been implemented by the U.S. Food and Drug Administration to reduce microbial population from fruit juices by 5 log 10 cycles (Goodrich *et al*., 2005). One of the best methods for removing microbes from fruit juices is Pasteurization (Silva and Gibbs, 2001). Being most popular thermal pasteurization may damage the nutritional ingredients. To mitigate the risk of salmonellosis, it is essential to adopt proper sanitation practices and consider pasteurization as a means of ensuring the microbiological safety of fruit juices.

*Shigella* spp. are another group of pathogenic bacteria associated with fruit juice contamination, particularly in areas with poor sanitation. They can cause bacillary dysentery, leading to severe diarrhea, fever, and abdominal pain (Cohen *et al*., 2012). *Shigella* contamination in fruit juices can occur through direct contact with contaminated water or fruits. To minimize the risk, it is crucial to ensure that all water used in juice preparation is potable and that fruits are thoroughly washed before processing.

While *Listeria monocytogenes* is more commonly associated with dairy products and ready-to-eat meats, it has been isolated from fruit juices as well. This pathogen is particularly dangerous for vulnerable populations, including pregnant women, newborns, and immunocompromised individuals, as it can cause listeriosis, a severe illness with a high mortality rate (Farber and Peterkin, 1991). Listeriosis can lead to symptoms such as fever, muscle aches, and gastrointestinal distress, and in severe cases, it can cause meningitis or sepsis (Choi *et al*., 2020). The presence of *Listeria* in fruit juices highlights the importance of maintaining proper hygiene and sanitation practices throughout the entire juice production process, including stringent cleaning of equipment and monitoring of processing conditions.

### 2.5.1.1 Survival of bacteria in fruit juices

The growth and survival of bacteria is limited due to low pH of fruit juices. pH range for citrus juices is 2.2 to 2.6 which do not support the growth of spoilage bacteria. Orange juice has pH between 3.4 to 4.0 which supports the growth and survival of *Lactobacillus* spp. and *Leuconostoc* spp. Pathogenic bacteria can survive at pH as low as 2.5 (De Jonge *et al*., 2003). Abnormal flavor and color of juices have been reported by these bacteria. These bacteria cannot grow in concentrates because they have high sugar concentrations.

The temperature which favors the growth of bacteria is also an important factor. When fruit juices are kept at normal temperatures it enhances the growth of acetic acid bacteria. Normally fruit juices are kept at moderate temperatures before refrigeration so it can increase the microbial population in the juices. During the processing, the use of high temperature evaporator killed most of the microbes, if some survive they are killed during freezing, the freezing process will preserve the ultimate survivors. Improper processing methods and unheigenic conditions are indicated by higher counts of microbes in fruit juices. So there should be few microbes in the frozen juice concentrates.

## 2.5.2. Yeast Contaminants

In addition to bacteria, yeasts are common spoilage organisms in fruit juices. They can ferment the sugars present in the juice, leading to off-flavors, gas production, and cloudiness. Common yeast contaminants in mixed fruit juices include *Saccharomyces cerevisiae*, *Candida tropicalis*, and *Rhodotorula mucilaginosa* (Fleet, 1992).

*Saccharomyces cerevisiae* is a yeast widely used in fermentation processes, such as bread-making and brewing. However, its presence in fruit juices is considered a spoilage indicator.

Under certain conditions, *S. cerevisiae* can ferment the sugars in fruit juice, producing alcohol and carbon dioxide, which can lead to spoilage (Kurtzman *et al*., 2011). The fermentation process not only alters the flavor profile of the juice but also reduces its overall quality and marketability.

Yeast species such as *Candida tropicalis* and *Candida parapsilosis* are frequently associated with fruit juice spoilage. These yeasts can grow rapidly in refrigerated conditions, fermenting sugars and leading to spoilage (Fleet, 1992). The presence of *Candida* spp. can result in the production of undesirable by-products, causing changes in taste and aroma that make the juice unappealing to consumers.

### 2.5.3. Mold Contaminants

Molds, although less prevalent than bacteria and yeasts, can pose a significant risk in fruit juices due to their ability to produce mycotoxins. Common molds found in fruit juices include *A. niger*, *Penicillium* sp., and *Fusarium* sp. Mycotoxins, such as ochratoxin A and patulin, can have serious health implications, including carcinogenic, immunosuppressive, and teratogenic effects (Tournas *et al*., 2006). *A. niger* is frequently found in fruit products and can produce mycotoxins like ochratoxin A, which is harmful to human health (Pitt and Hocking, 2009). The presence of *A. niger* in fruit juices can compromise product safety, and its spores can lead to respiratory issues if inhaled.

*Penicillium* species are known for producing patulin, a mycotoxin that can contaminate apple-based products and, consequently, mixed fruit juices containing apples. The presence of this mold is of particular concern, as patulin is associated with various health risks, including

gastrointestinal issues and immune system suppression (Moss, 2008). Proper handling and storage conditions are essential to minimize the risk of mold contamination in fruit juices.

## 2.6. SPOILAGE OF FRUITS AND FRUIT JUICES

Fruit and fruit juices are generally contaminated with bacteria, often from insect damage. Fallen fruit thus be avoided were possible for all of the risks. Flavoring, water and other chemical are all potential sources of microbial contamination. Bacterial that have been associated with spoilage in fruit juice industries include *Acetobacter, Bacillus* spp, *Clostridium* spp, *Gluconobacter, Lactobacillus*, *Leccuonostoc*, *Saccharobacter*, *Zymobacter* and *Zymomonas* . *Gluconobacter* is common spoilage agent of fruit juices. The types of spoilage associated with fruit drink includes flat sour by *Bacillus thermoacidurans*, butyric anaerobes by *Clostridium butyricum* and nonspore formers (mostly lactic acid types of bacteria) by yeast and moulds. Common food poisoning bacteria *Salmonella breandercup*, *Salmonella enteritidis*, *Staphylococcus pyogenes, Clostridium welchii, Clostridium botulinum, Vibrio parahaemolyticus* and *Bacillus cereus.* Acid forming microaerophilic bacteria such as the lactobacilli tolerate lower pH values down to 3.5 in the case of some strains (De Jonge *et al*., 2003).

## 2.7. FRUIT JUICE INTAKE AND HUMAN ILLNESS

The consumption of raw and unpasteurized fruit juices has been associated with human illness in United States (Deroever, 1998). Salmonellosis, *E. coli* O157:H7 infections and enterotoxigenic *E. coli* were found to be associated with various fruits and vegetables. Various types of microorganisms are found on the surface of fruits before harvest and it depends upon the conditions (Brackett, 1999). Microbes associated with most vegetables are Gram negative bacteria whereas yeast are found dominating the surface of fruits due the low acidic pH of fruit

tissue (Splittstoesser, 1987). Various outbreaks of illness in humans has been reported to be associated with consumption of unpasteurized fresh fruit juices. In 1995, more than 60 visitors were affected due to the consumption of unpasteurized fresh orange juice contaminated with salmonella in Florida Theme Park, USA (Schmidt *et al*., 1997).

In recent years, there are also reports of *E. coli* 0157:H7 and Salmonella infections (Centers for Disease Control, 1996) which have developed awareness about the pathogens present in fruit juices which were previously thought to be pathogen free due to their acidic nature. U.S. Food and Drug Administration have altered regulations labeling laws, to include warning labels on unpasteurized fruit juices. The interest about the development of effective methods to inactivate or kill microbes is also the part of these regulations.

## 2.8. PRESERVATION OF FRUIT JUICES

In Nigeria, chemical preservation is predominantly employed due to its cost-effectiveness compared to other methods. This approach involves the use of various chemical preservatives to inhibit microbial growth, thus preventing spoilage of fruit juices. It is commonly noted that preservatives are often used in combination, as no single preservative is sufficiently effective when applied alone (Chipley, 1983). Among the most frequently used preservatives are sodium benzoate and potassium metabisulfite, known for their significant antimicrobial properties (Lueck, 1990). Sodium benzoate, for instance, has been reported to be effective against certain yeast strains and spoilage-causing organisms (Sofos *et al*., 1986). According to Codex Standard (2005), the maximum allowable levels for these chemicals are 1000 mg/kg for sodium benzoate and 500 mg/kg for potassium metabisulfite.

Emerging foodborne diseases have raised concerns regarding the illegal use of these preservatives, as microbial resistance can develop over time due to their improper application (Akinpelu, 2001). In addition to chemical methods, non-thermal treatments have been explored to mitigate microbial populations in fruit juices. One notable method is the germicidal effect of ultraviolet (UV) light, which has been evaluated for its ability to combat various microorganisms (Bintsis *et al*., 2000).

For instance, the disinfection of surfaces in fruit juice processing plants, as well as water and liquid foods, has been achieved using UV light at a wavelength of 254 nm (Guerrero and Barbosa, 2005). This method offers the advantage of not generating toxic waste products during processing, while also ensuring that certain organic contaminants can be effectively removed. Furthermore, it does not alter the flavor or color of the juice products, and the energy expenditure is minimal (Keyser *et al*., 2008).

Conversely, thermal processing can result in undesirable changes to the color and nutritional content of fruit juices (Choi and Nielson, 2005). In contrast, UV light treatment preserves the physical attributes of juices, including their color and aroma (Tran and Farid, 2004). The modern consumer increasingly demands safe, fresh foods (Mittal and Griffiths, 2005), necessitating the development of preservation technologies that retain the nutritional integrity of products. High-intensity pulsed electric field treatment is one such method, effectively inactivating pathogenic and spoilage microbes without compromising flavor, color, or nutritional value (Cserhalmi *et al*., 2006). The combination of this technology with other preservation methods has been shown to yield even better results (Sobrino and Martin-Belloso, 2006). In practice, fluids are positioned between two electrodes, where high-intensity electric pulses are applied to reduce or inactivate microbial populations.

This pulsed electric field method has demonstrated efficacy against *Salmonella* spp. and *E. coli* in orange juices (Mosqueda *et al*., 2007). It is crucial that microbial inactivation data using this technology meet commercial standards, as stipulated by regulatory bodies (Liang *et al*., 2002). According to the U.S. Food and Drug Administration (USFDA, 2002), novel preservation technologies should achieve a reduction of 5 $\log_{10}$ in microorganisms to ensure safety. With the development of advanced preservation methods, it has become feasible to supply high-quality fruit juices to large populations, thereby improving food safety and public health.

### 2.8.1. Role of Brine in Preservation

In addition to the aforementioned preservatives, sodium chloride (brine) plays a significant role in the preservation of fruit juices. The addition of sodium chloride not only enhances flavor but also helps in controlling microbial growth by creating a hypertonic environment. High concentrations of sodium chloride can lead to osmotic stress in microbial cells, inhibiting their growth and metabolic activities (Friedman, 1996). This property is particularly beneficial in the preservation of fruit juices, as it helps extend shelf life while maintaining product quality.

Research has shown that sodium chloride can synergistically work with other preservatives, enhancing their antimicrobial effects (Bennett *et al*., 1998). For instance, the combination of sodium chloride with other chemical preservatives can lead to a more significant reduction in microbial populations, thereby improving the overall safety and quality of fruit juices (Beuchat, 1996). However, it is crucial to maintain an optimal concentration of sodium chloride, as excessively high levels may alter the sensory attributes of the juice and deter consumer acceptance.

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 SAMPLE COLLECTION

A total of 8 samples of mixed fruit juice, comprising a blend of Orange, Watermelon and Apple, were prepared for analysis. The juice samples were divided equally into two groups: one group was treated with brine (5 % sodium chloride solution) as a preservative, and the other group was left untreated to serve as a control. For post-preservation analysis, samples were collected at four different time intervals: immediately after preparation (Day 0), and on Days 7, 14, and 21 of storage. Each sample consisted of 100 mL of juice, which was collected using sterile pipettes and transferred into sterile, screw-capped bottles. All samples were transported to the laboratory in ice-packed coolers and were analyzed within 1 hour after collection to ensure the integrity of the microbiological data.

## 3.2 STERILIZATION OF MATERIALS

Materials such as Petri-dishes, pipette, glass containers (conical flask, round bottom flask) and bottles were washed, drained and dried. They were wrapped with aluminum foil and sterilized in a hot-air oven at 160 $^{0}$C for 1 hr. They were allowed to cool after sterilization before usage. An aseptic working environment was achieved with the use of Bunsen burner flame and disinfection of work surfaces with alcohol (Dubey and Maheshwari, 2002).

## 3.3. PREPARATION AND STERILIZATION OF MEDIA

The media used were Nutrient agar, Eosin Methylene Blue Agar, MacConkey Agar, Citrate agar, Triple Sugar Iron agar and Potato Dextrose Sugar (PDA). All media were prepared according to manufacturer's instructions and sterilized using the autoclave at 121 °C for 15minutes.

## 3.4 Isolation of Microorganisms

About 10 mL of each mixed fruit juice sample, both brine-treated and untreated, was measured and homogenized in 90 mL of sterile peptone water using a vortex mixer. The homogenized samples were then subjected to ten-fold serial dilutions in sterile peptone water. Following the serial dilutions, 1 mL of the $10^{-4}$ dilution was dispensed into sterile Petri dishes. The Petri dishes were pre-labeled according to the type of agar being used.

After gelling, the petri-dishes that contained nutrient agar, macConkey agar and potato dextrose agar was incubated at 37 °C for 24hrs while the petri-dishes that contained potato dextrose agar was incubated at 25 °C for 3days. The nutrient agar, macConkey agar and potato dextrose agar was used to check for total bacterial count, total coliform count respectively. At the end of the incubation period, the plates was brought out of the incubators and the colonies was counted using a colony counter device and each count was expressed in colony forming unit per ml (CFU $ml^{-}$).

$$Colony\ forming\ unit\ per\ millilitre\ \frac{cfu}{ml} = \frac{number\ of\ colonies}{amount\ plated\ x\ dilution\ factor}$$

## 3.5 Identification of Isolates

The distinct colonies on nutrient agar and potato dextrose agar was carefully examined using microscope for their morphological characteristics like colour. Then these colonies was subcultured using streaking method , using a sterile inoculating loop, a small portion of the colony was streaked across the surface of an agar plate in four quadrants. Each streak dilutes the sample further, allowing individual colonies to grow in isolated sections by the fourth quadrant.

The isolated colonies with uniform morphology are selected and re-streaked onto fresh plates, repeating this process until no mixed colonies remain. This iterative streaking confirms that a pure, single-species culture is achieved. After successful isolation and purification, the pure microbial isolates are transferred to agar slants for storage and was incubated at 37 °C for 24hrs for the bacterial isolates and 25 $^{0}$C for 72hrs for the fungi isolates.

Gram staining and other biochemical tests was carried out based on the method of Cheesbrough (2006). The biochemical tests performed here include catalase, oxidase, indole, coagulase, Methyl red, Voges proskauer, Citrate, Sugar fermentation and Lactophenol cotton blue test for the fungi isolates.

## 3.6. Antibiotic susceptibility test

The identified colonies of bacteria were used to determine the susceptibility and resistance of bacterial isolates, which were subjected to standard antibacterial susceptibility testing (AST) to decipher their resistance or susceptibility to common antibiotics used for treatment within the locality. The standard discs were produced by Oxoid, UK, which was used to execute the disc diffusion method employed in this study. For this assay, a fully grown bacterial culture (from 18-24 hours) was cultured on MHA.

The inoculum corresponding to 1.5 x $10^8$ cells/ml McFarland standard was streaked using a sterile loop onto the MHA plates before the introduction of antibiotic discs and were added with extreme care to the plates with the aid of sterile forceps. The susceptibility results were recorded after incubation for 24 hours at 37 ºC. Following the standard or rules of AST established in 2017 by CLSI (Clinical Laboratory Standards Institute). The inhibition zone around each disc (measured using a meter rule in diameter) was assessed and interpreted based on the 2020 CLSI standard as Resistant (R), Intermediate resistant (I) and Sensitive (S).

### 3.6.1 Measuring of pH of fruit juices

The pH of fruit juices was measured on day 0,day 3 and day 7 duration. pH of fruit juice was measured by pH meter

### 3.6.2 Determination of titratable acidity

***Preparation of 0.1M sodium hydroxide***

Forty grams of sodium hydroxide pellets was weighed in a weighing balance. The pellets were dissolved in distilled water and then made to 1000mls.

The titratable acidity was determined as described by Friedrich (2001) using colorimetric acidity titration as follows: 10mls of the different samples was transferred into a clean Erlenmeyer flask and 2 drops of 1% phenolphthalein indicator solution and a magnetic stir bar before stirring on a magnetic stir plate. Then 0.1N NaOH was carefully titrated against the sample solution to the end point of pH 8.2 until a faint but definite pink colour, which was stable for 5 to 10 seconds was obtained. The titrable acidity was calculated using the equation:

$$\% \text{ acid} = \frac{[\text{mls NaOH used}] \times [0.1 \text{ N NaOH}] \times [\text{milliequivalent factor}] \times [100]}{\text{grams of sample}}$$

Where V is volume (ml) of NaoH solution used for titration

N = Normality of NaoH solution, meq.

wt is milliequivalent weights of acid: lactic acid (0.1941)

Vs= sample volume

The analysis was performed in triplicate to find the mean titrable acidity of each sample.

### 3.6.3 Acceptability of the different rations of the juice mixtures

The results of the mixture of different rations of the extracted watermelon juice and orange juice shows that the ratios orange and watermelon (Plate 1), orange and watermelon (Plate 2) and orange and watermelon (Plate 3) was found to be acceptable in terms of the taste, aroma and colour. The overall acceptability of the orange and watermelon (80:20), orange and watermelon (30:70) and orange and watermelon (60:40) were 75%, 65% and 85% respectively at day 0 of the experiment. While the overall acceptability after 7 of the experiment was 45%, 35% and 70% for the orange and watermelon (80:20), orange and watermelon (30:70) and orange and watermelon (60:40) respectively.

# CHAPTER FOUR

# RESULT

## 4.1 Preservation of the juice mixtures using different concentration of the brine solution

The results of the effect of the different concentration of the brine solutions for the preservation of the different rations of the extracted juice showed that mixtures ration orange and watermelon (60:40) was more stable in terms of microbial growth on the juice

**Effect of the different brine solutions on the pH values of the juice mixture**

The results of the effect of the different brine solutions on the pH values of the juice mixture (80:20) treated with brine solution A (10:90) was highly acidic throughout the experimental period with pH value of 3.4 in day 1 and 1,8 at day 7. The pH values of the juice mixture (80:20) treated with brine solution B (**20:80**) was found to be more stable with pH values of 8.2 at day 0 and 4.2 at day 7

The results of the effect of the different brine solutions on the pH values of the juice mixture (70:30) treated with brine solution A (70:30) was slightly basic throughout the experimental period with pH values ranging from of 6.5 at day 1 to 3.4 at day 7.

The results of the effect of the different brine solutions on the pH values of the juice mixture (60:40) shows that the juice mixture (60:40) was slightly basic throughout the experimental period with pH values ranging from of 6.5 at day 1 to 3.4 at day 7.

**Effect of the different brine solutions on the titratable acidity values of the juice mixture**

The results of the effect of the different brine solutions on the titratable acidity values of the juice mixture shows that the juice mixture (80:20) treated with brine solution B has the highest titratable acidity value of 15.1% while the juice mixture (80:20) treated with brine solution C had the lowest titratble acidity value of 3.1% at day 7 of the preservation.

The results of the effect of the different brine solutions on the titratable acidity values of the juice mixture shows that the juice mixture (70:30) treated with brine solution C has the highest titratable acidity value of 8.1% while the juice mixture (70:30) treated with brine solution D had the lowest titratble acidity value of 7.1% at day 7 of the preservation.

The results of the effect of the different brine solutions on the titratable acidity values of the juice mixture shows that the juice mixture (60:40) treated with brine solution D has the highest titratable acidity value of 11.1% while the juice mixture (60:40) treated with brine solution A had the lowest titratble acidity value of 7.1% at day 7 of the preservation.

**Table 4.1.1: Sensory Properties of the Juice Mixtures at Day 0 and Day 7**

| **Day 0** | | | |
|---|---|---|---|
| **Sensory Properties (Day 0)** | **Orange and Watermelon (30:70)** | **Orange and Watermelon (30:70)** | **Orange and Watermelon (60:40)** |
| Taste | 6 | 5 | 7 |
| Aroma | 4 | 4 | 5 |
| Colour | 5 | 4 | 5 |
| **Overall acceptability** | **75%** | **65%** | **85%** |
| **Day 7** | | | |
| Taste | 4 | 3 | 6 |
| Aroma | 2 | 1 | 4 |
| Colour | 3 | 3 | 4 |
| **Overall acceptability** | **45%** | **35%** | **70%** |

**Table 4.2.1** pH of the Juice mixture (80:20) across the different preservatives concentration

| pH of the Juice mixture (80:20) | | | |
|---|---|---|---|
| S/N | Day 0 | Day 3 | Day 7 |
| A (10:90) | 3.10 | 3.30 | 6.3 |
| B (20:80) | 3.30 | 4.50 | 15.1 |
| C (40:60) | 3.10 | 3.80 | 3.1 |
| D (60:40) | 3.15 | 4.40 | 4.40 |

**Keys**:

A=10:90
B=20:80
C=40:60
D= 60:40

**Figure 4.1: pH values of the juice mixture (80:20) across the different brine concentration**

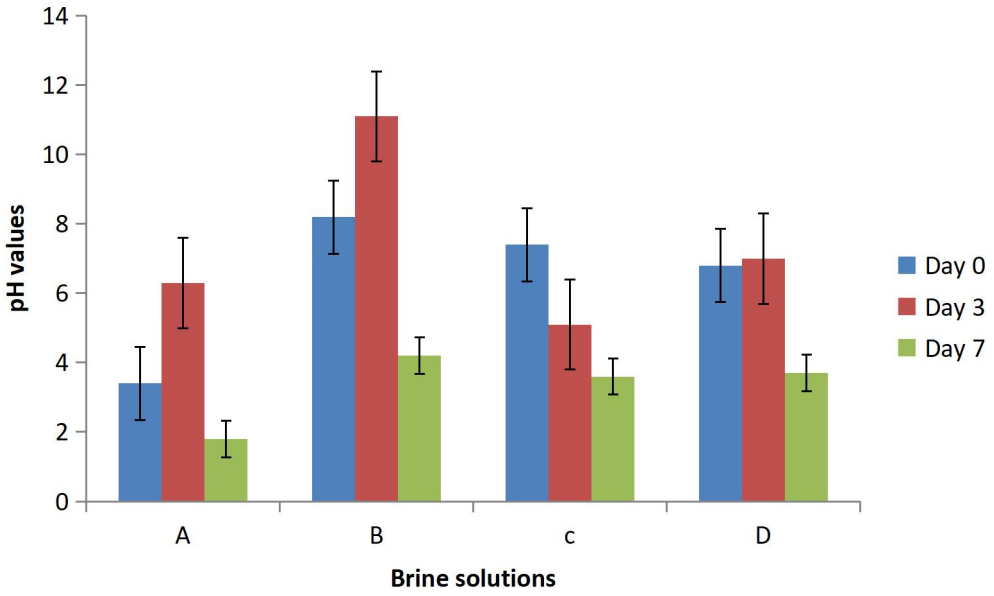


**Table 4.2.2 pH of the Juice mixture (70:30) across the different preservatives concentration**

| pH of the Juice mixture (70:30) | | | |
|---|---|---|---|
| S/N | Day 0 | Day 3 | Day 7 |
| A (10:90) | 6.6 | 6.6 | 3.6 |
| B (20:80) | 6.7 | 6.7 | 3.6 |
| C (40:60) | 6.5 | 6.5 | 3.4 |
| D (60:40) | 6.9 | 6.9 | 3.4 |

**Keys**:

A=10:90
B=20:80
C=40:60
D= 60:40

**Figure 4.1.2: pH values of the juice mixture 70:30) across the different brine concentration**

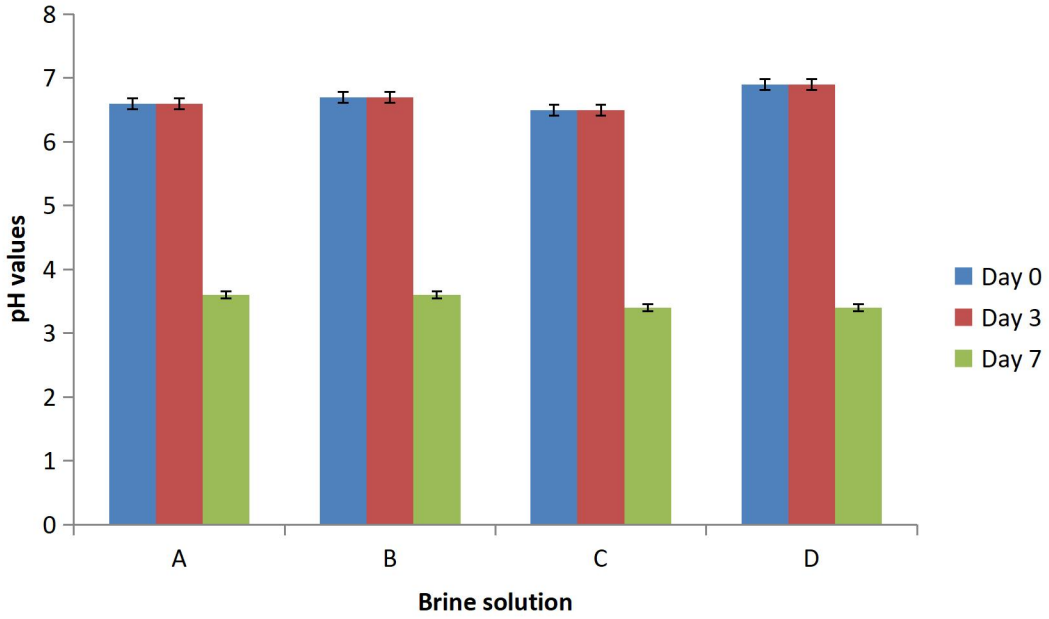


**Table 4.2.3: pH of the Juice mixture (60:40) across the different preservatives concentration**

| pH of the Juice mixture (60:40) | | | |
|---|---|---|---|
| S/N | Day 0 | Day 3 | Day 7 |
| A (10:90) | 6.6 | 6.6 | 3.3 |
| B (20:80) | 6.7 | 6.7 | 3.5 |
| C (40:60) | 6.5 | 6.5 | 3.5 |
| D (60:40) | 6.9 | 6.9 | 3.4 |

**Keys**:

A=10:90
B=20:80
C=40:60
D= 60:40

**Figure 4.1.3: pH values of the juice mixture 60:40) across the different brine concentration.**

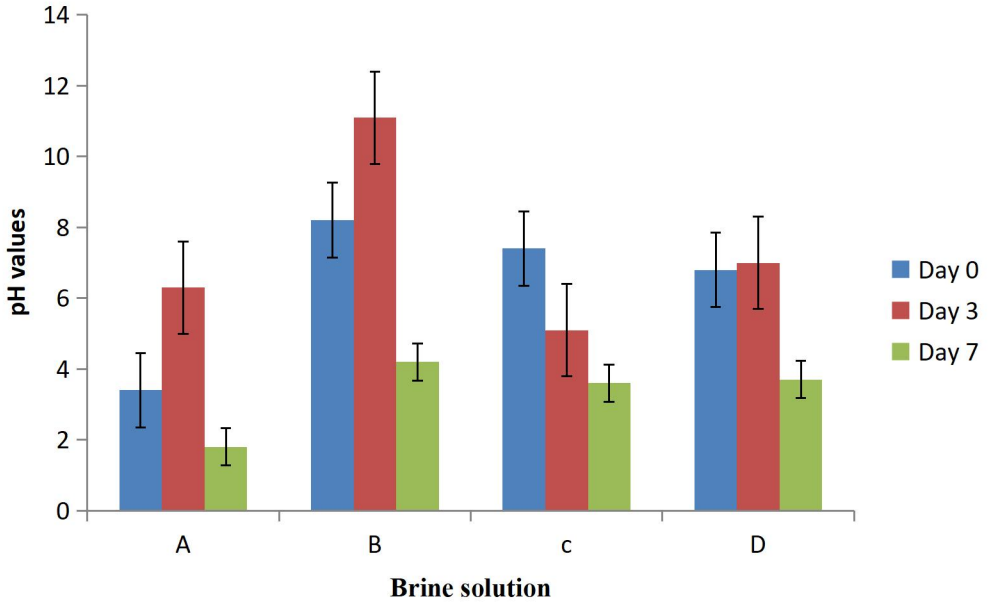


**Table 4.3.1: Titratable acidity of the Juice mixture (80:20) across the different preservatives concentration**

| Titratable acidity of the Juice mixture (80:20) | | | |
|---|---|---|---|
| S/N | Day 0 | Day 3 | Day 7 |
| A (10:90) | 3.10 | 3.80 | 6.3 |
| B (20:80) | 3.30 | 4.50 | 15.1 |
| C (40:60) | 3.10 | 3.80 | 3.1 |
| D (60:40) | 3.15 | 4.40 | 4.40 |

**Keys:**

A = 10.90

B = 20:80

C = 40:60

D = 60”40

**Figure 4.3.1**: **Titratable acidity values of the juice mixture (80:20) across the different brine concentration**

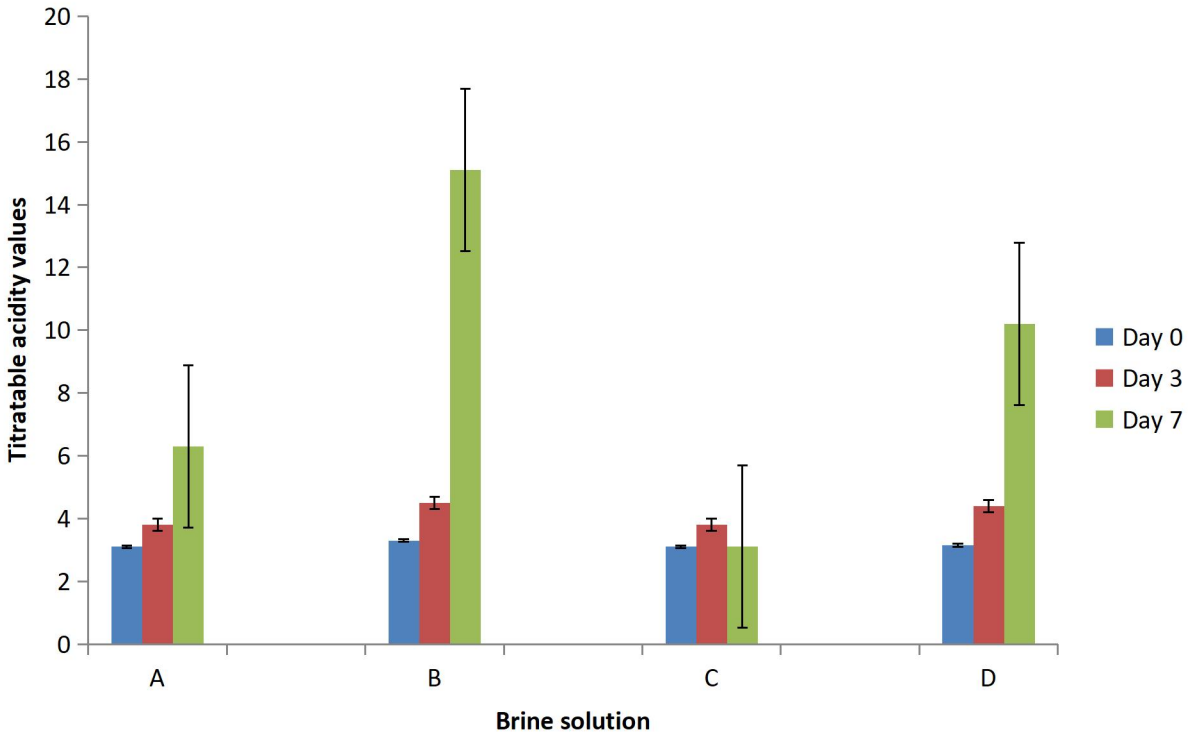


**Table 4.3.2.** Titratable acidity of the Juice mixture (70:30) across the different preservatives concentration

| Titratable acidity of the Juice mixture (70:30) | | | |
|---|---|---|---|
| S/N | Day 0 | Day 3 | Day 7 |
| A (10:90) | 2.40 | 4.2 | 8.0 |
| B (20:80) | 2.45 | 4.31 | 8.1 |
| C (40:60) | 2.40 | 4.10 | 8.9 |
| D (60:40) | 2.40 | 4.2 | 7.1 |

**Keys:**

A = 10:90

B = 20:80

C = 40:60

D = 60”40

**Figure 4.3.2. Titratable acidity values of the juice mixture (70:30) across the different brine concentration**

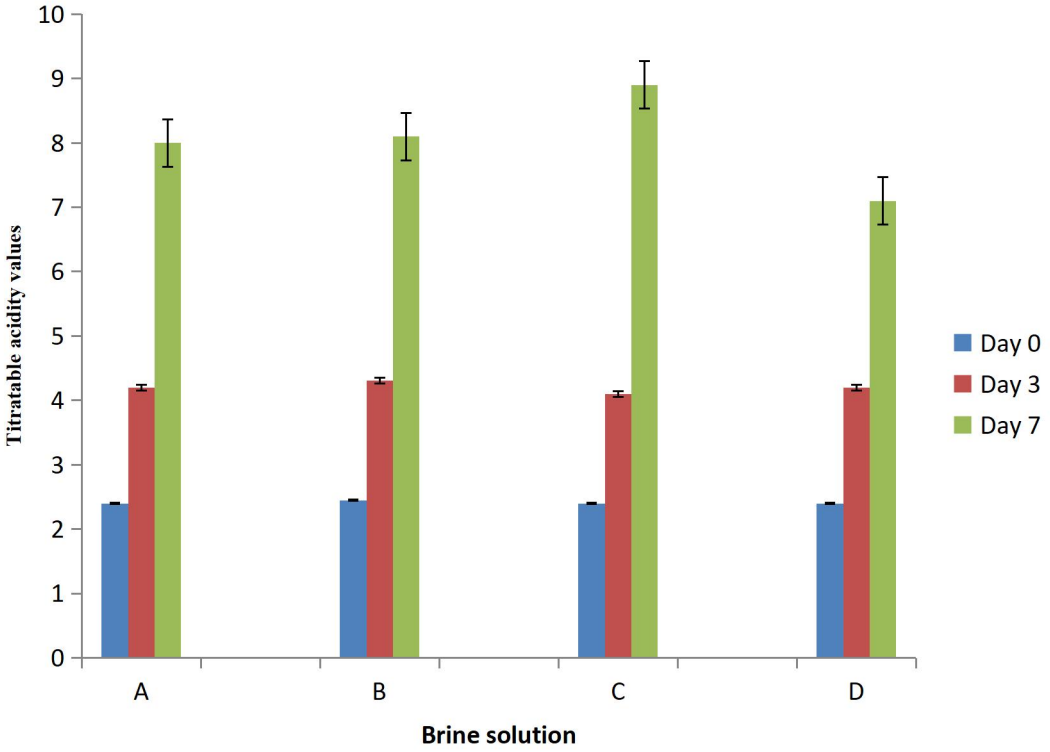


**Table 4.3.2.** Titratable acidity of the Juice mixture (60:40) across the different preservatives concentration

| Titratable acidity of the Juice mixture (60:40) | | | |
|---|---|---|---|
| S/N | Day 0 | Day 3 | Day 7 |
| A (10:90) | 1.20 | 3.4 | 7.1 |
| B (20:80) | 1.20 | 3.51 | 10.9 |
| C (40:60) | 1.25 | 3.33 | 10.3 |
| D (60:40) | 1.25 | 3.43 | 11.1 |

**Keys:**

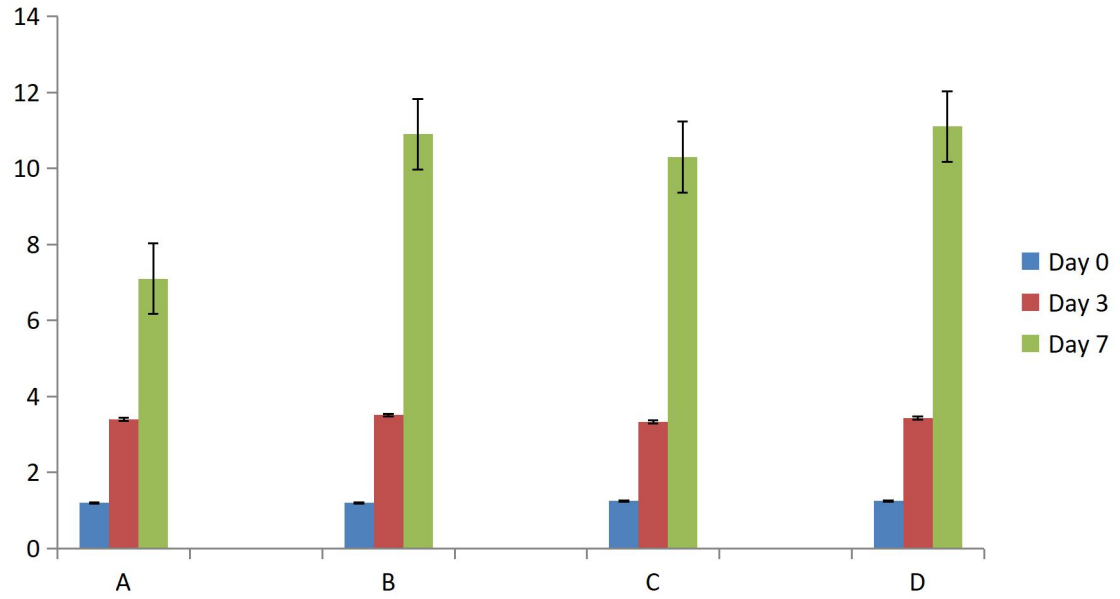
A = 10.90

B = 20:80

C = 40:60

D = 60:40

**Figure 4.3.3. Titratable acidity values of the juice mixture (60:40) across the different brine concentration.**



**4.4 Effect of the different brine solutions on the bacterial count of the juice mixture**

The results of the effect of the different brine solutions on the microbial count of the juice mixture shows that the juice mixture (80:20) treated with brine solution A has the highest microbial count of 7cfu/ml while the juice mixture (80:20) treated with brine solution D had the lowest microbial count of 3cfu/ml at day 3 of the preservation. There was no microbial growth in any of the treatment at day 7 of the preservation.

At (70:30), the results of the effect of the different brine solutions on the microbial count of the juice mixture shows that the juice mixture (80:20) treated with brine solution A has the highest microbial count of 7cfu/ml while the juice mixture (80:20) treated with brine solution D had the lowest microbial count of 3cfu/ml at day 3 of the preservation. There was no microbial growth in any of the treatment at day 7 of the preservation.

The juice mixture (60:40) had the highest bacterial growth of 3 cfu/ml at day 1 of the experiment while no bacterial growth was observed in day 3 and 7 of the experiment apart the solution treated with brine solution A which had a bacterial growth of 2 cfu/ml at day 3 of the experiment.

**Table 4.4.1** Bacterial count of the Juice mixture (80:20), (70:30), (60:40) across the different

| Bacterial count of the Juice mixture (80:20) | | | |
|---|---|---|---|
| S/N | Day 0 | Day 3 | Day 7 |
| A (10:90) | 2 | 7 | - |
| B (20:80) | - | 5 | - |
| C (40:60) | 3 | 3 | - |
| D (60:40) | 2 | 3 | - |

preservatives concentration.

**Keys:**

A = 10.90

B = 20:80

C = 40:60

D = 60:40

**Figure 4.3.1 Bacterial count of the Juice mixture (80:20) across the different preservatives concentration**

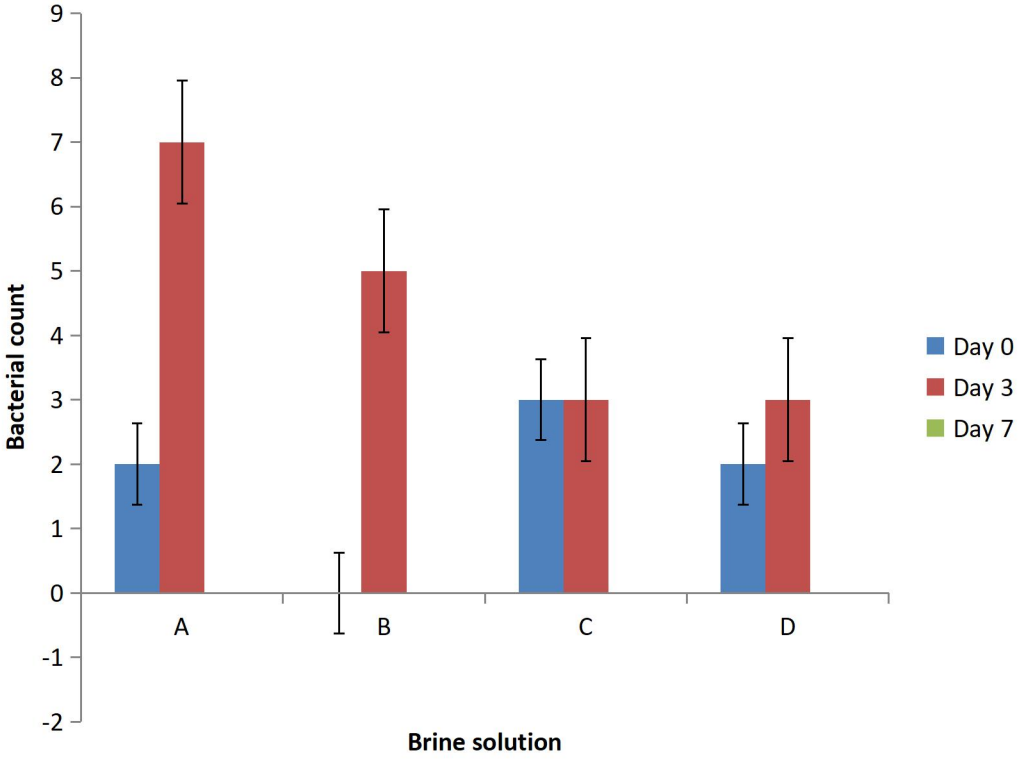


**Table 4.4.3: Bacterial count of the Juice mixture (60:40), across the different preservatives**

| | Bacterial count of the Juice mixture (60:40) | | |
|---|---|---|---|
| S/N | Day 0 | Day 3 | Day 7 |
| A (10:90) | 2 | 7 | - |
| B (20:80) | - | 5 | - |
| C (40:60) | 3 | 3 | - |
| D (60:40) | 2 | 3 | - |

**concentration**

**Keys:**

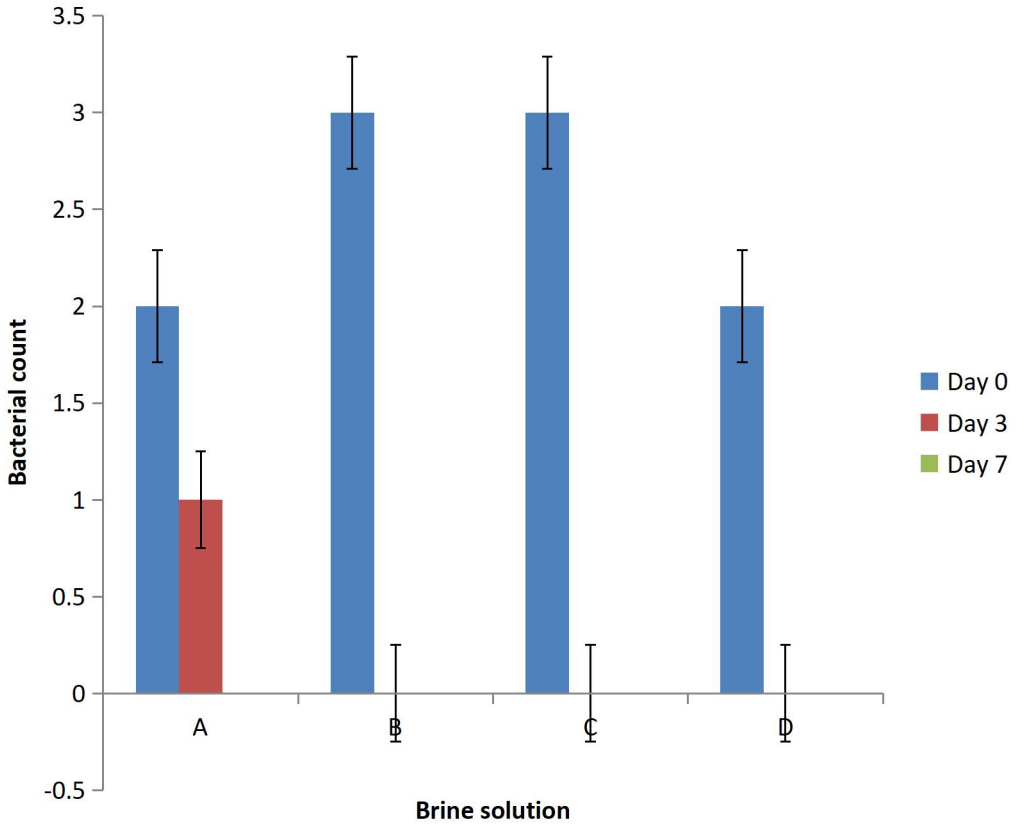
A = 10.90

B = 20:80

C = 40:60

D = 60:40

**Figure4.3.3: Bacterial count of the Juice mixture (60:40) across the different preservatives concentration.**



# CHAPTER FIVE

## 5.1 DISCUSSION

Fruit juices are important trade commodities. This is because fruits juices consist of high nutritive values and pose considerably less harmful effects on health and they are fat-free, nutrient-dense beverages rich in vitamins, minerals and naturally occurring phytonutrients that contribute to good health. Most fruits contain carbohydrates, proteins and minor nutrients such as minerals and vitamins (Vasavada, 2003; Franke *et al*. 2005). Consumption of natural juices can provide health benefits due to the antioxidants and high content of vitamins and minerals (Edwards *et al*., 2003). In this study orange and watermelon was used in the preparation of the fruit juice at varying rations.

Storage factors such as pH and acidity can affect the shelve life of fruits juice. According to Bates *et al*., (2001) increased acidity helps to prevent the growth of microorganisms in the product, thereby increasing their shelf life. The excellent keeping quality of fruits and soft drinks is influenced by low pH. In this study, the pH of the different juice rations was found to be slightly neutral at day 0 of the experiment and then acidic at day 7 of the experiment.

The acidity can be increased by the addition of citric acid and be should be less than 1%. In this study, orange (citric acid) was used to increase the acidity of the juice mixtures (Yadav *et al*., 2013). However, the acidity of the juice mixtures was found to be above 1% at the end of the experiment.

The extension of shelf life can be resulted primarily from the inhibition of spoilage microorganisms. The acid percentage of the mixed fruit juice drink can be increased slightly than the normally recommended value. This should be done without having any undesirable effects on

the sensory properties and nutritive values of the product. It has been found that destruction of vitamin C occurs at higher acid levels. At low pH vitamin C is a more powerful antioxidant (Gramlich *et al*., 2002), hence degrades more quickly at low pH. To have increased acid percentage without altering the taste the sugar level also has to be increased.

According to Yadav *et al*. (2013) the number of micro-organisms in fruit juices can affect the microbial quality of fresh fruit juices. In this study, the no of bacteria count in the juice mixture was found to increase from day 0 to day 3 and afterward no growth was observed. This might be due to decrease in the pH values of the juice mixtures as the experiment progress.

The acceptable limit of microorganisms in fruit juice varies by regulatory authority and depends on the specific microorganism being tested. According to the World Health Organization (WHO) and Codex Alimentarius standards, microbiological criteria for fruit juices typically focus on total plate count, coliforms, yeast, mold, and pathogens such as Salmonella and Escherichia coli (*E. coli*).

Total Plate Count (TPC): Generally should not exceed 10$ to $10^a$ CFU/mL, depending on whether the juice is pasteurized or not. Coliforms (including *E. coli*): Should be absent in 1 mL of juice, as coliform presence indicates possible fecal contamination. Yeast and Mold: Typically limited to ≤100 CFU/mL in pasteurized juices. Pathogens (e.g., Salmonella, Listeria monocytogenes: Should be completely absent in 25 mL of juice.

## 5.2 CONCLUSION

Fruit juices, particularly those made from oranges and watermelons, are valuable commodities due to their high nutritional value. They are rich in vitamins, minerals, antioxidants, and phytonutrients that support good health, making them a preferred beverage for maintaining a healthy lifestyle. Natural fruit juices also offer significant health benefits by providing essential nutrients and antioxidants.

The pH and acidity levels of fruit juices play a crucial role in determining their shelf life. Lower pH and higher acidity can inhibit the growth of microorganisms, thereby extending the shelf life of the juice. In this study, the juice's pH became slightly acidic by day 7, which aligns with the role of acidity in preserving the product.

The addition of citric acid, particularly from oranges, is important for increasing the acidity of fruit juices, which helps prevent microbial growth. However, maintaining acidity levels below 1% is critical, as higher acidity can degrade vitamin C, a powerful antioxidant. While increasing acidity is beneficial for preservation, it must be balanced to avoid compromising the taste and nutritional value of the juice.

The number of microorganisms in the fruit juice mixtures was observed to increase initially but stabilized after day 3, likely due to the increasing acidity. Microbial control is essential for maintaining juice quality and safety. Regulatory standards, such as those set by the WHO and Codex Alimentarius, provide clear guidelines for acceptable microbial limits, particularly concerning total plate counts and the presence of harmful pathogens like E. coli and Salmonella.

The shelf life of fruit juices can be extended by adjusting acidity levels without negatively affecting the taste or nutritional quality. However, careful monitoring is necessary to avoid the degradation of sensitive nutrients like vitamin C at low pH levels. Additionally, to balance acidity, sugar levels may need to be adjusted to maintain the flavor profile of the juice.

Thus, while fruit juices offer excellent health benefits, careful attention to acidity and pH levels is essential to prolong shelf life, maintain quality, and ensure microbial safety.

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## APPENDIX II

## PREPARATION OF MEDIA

### Nutrient Agar

Twenty-eight grams (28 g) of nutrient agar was dissolved in 1000 ml of distilled water in a conical flask corked with cotton wool and foil paper and allowed to dissolve in 1000 ml of distilled water in a conical flask. The medium was placed in an autoclave to sterilize it for 15 minutes at 121 °C. After sterilization, the flask was allowed to cool and then dispensed into sterile petri dishes.

### Eosin Methylene Blue Agar

Eosin methylene blue agar was prepared by dissolving 36 g of agar powder in 1000 ml of distilled water under heat and continuous stirring. The mixture was then immersed in an autoclave and sterilized at $121^{oC}$ for 15 minutes. The agar was then poured into Petri dishes after cooling to $50^{oC}$ and the allowed to solidify.

MacConkey Agar

MacConkey agar was prepared by dissolving 49.53 grams of dehydrated medium in 1000 ml of distilled water and boiled to completely dissolve the agar. The medium was sterilized by autoclaving at 15 lb pressure, 121 °C for 15 mins. The medium was cool to 45-50 °C and then dispensed aseptically into sterile Petri dishes.

### Citrate agar

24.28grams of agar was suspended in 1000 ml distilled water and heated to boiling, to dissolve the medium completely. It was then mixed properly and distributed in conical flasks. The

medium was sterilized by autoclaving at 15 lbs pressure (121 °C) for 15 minutes and then left to cool before dispension on sterile petri dishes.

**Triple Sugar Iron agar**

64.6 g of powder was dissolved in 1L of distilled water and then heated to properly dissolve the mixture. The mixture was autoclaved to sterilize the agar before it is dispensed into tubes and sterilized again at 121 °C for 15 minutes. The agar was then left to solidify with short slant and good butts.

**Preparation of Potato Dextrose Agar**

Thirty nine (39 g) grams of potato dextrose agar powder was dissolved in 1 liter of distilled water in a conical flask covered with cotton wool and aluminum foil paper. It was mixed thoroughly and sterilized by autoclaving at 121°C for 15 minutes. The medium was cooled to 45-50°C and before dispensing aseptically into Petri dishes. Plates were then incubated for 72 hrs at 25 °C (room temperature).

**Gram Staining**

A thin smear of the isolates was carried out on different slides with the aid of a wire loop and left to dry and after they will be heat fixed and allowed to cool. Then the different smears were covered with crystal violet stain for 30-60seconds and rapidly washed off with clean water. Then the smears were covered with Lugol's iodine for 30-60seconds and rapidly washed off with clean water. The smears were decolourised rapidly with alcohol and washed out immediately with clean water.

Then the smears were covered with safaranine for 30-60sec and washed immediately with clean water. The stained smears were then allowed to air-dry. After drying, a few drops of oil immersion was dropped on the stained smears and viewed with the aid of a microscope (×100 oil objective lens) to check for the microscopic properties of the organisms like the Gram reaction, morphology (Cheesbrough, 2006). For the fungal isolate, a drop of lactophenol cotton blue stain was dropped in the centre of a clean slide. And then a fragment of the fungus were collected with the aid of a wireloop and placed in the drop of the stain and teased gently and covered with a coverslip. The coverslip was not pushed down or tapped to avoid the dislodging of the conidia from the conidiophores. Then the stained isolate was viewed under the microscope with ×10 and ×40 objective lens for its morphological characteristics (Cheesbrough, 2006).

Biochemical test

**Oxidase test**

This is mainly used to differentiate between *pseudomonas* from other gram-negative rod bacteria. Oxidase test was carried out to identify bacteria species that will produce cytochtome oxidase enzyme. *Staphylococcus aureus* and *Escherichia coli* which are gram positive and gram negative respectively were employed as control. A piece of filter paper using sterilizes wire loop 2-3 drops of freshly prepared oxidase reagent (1% aqueous tetramethyl-3-phenyl nediamine dichloride) was added. A positive oxidase test is indicated by purple colouration within 10 sec (Cheesbrough, 2006).

Urease test.

This is used to test organisms that have the abilities to produce the enzyme urease which catalyzes the breakdown of urea to produce ammonia. The test is usually used to differentiate organisms like *Proteus mirabilis* from other non-urease positive organism. A sterilized medium

was dispensed into test tubes aseptically and the test bacteria isolated were inoculated into the medium and incubated at 37 degree centigrade for 24 hrs. A change in colour from yellow to red-

**Indole production test**

An indole test was carried out to demonstrate the ability of certain bacteria that can decompose amino acid **tryptophane** to **indole**. The indole production test is essential in identifying the *Enterobacteriaceae* family that breaks down the amino acid tryptophan by releasing indole in the presence of intracellular enzymes called "tryptophanase." Several drops of Kovac's indole reagent were placed on a filter paper. A portion of a pure isolated colony picked from the TSA pure culture with an inoculating loop was smeared onto the reagent-saturated area of the filter paper. It was allowed and examined to observe for colour development within 2 - 3 minutes. **In this spot test**, indole combined with the reagents in the filter paper matrix to produce a **blue-to-blue-green colour change** on the bacterial smear **and adverse** reactions remained colourless or light pink (Cheesbrough, 2006).

Citrate utilization test

The citrate utilization test is a part of the test used to differentiate organisms on their ability to utilize citrate as the primary energy source. A citrate test was performed to differentiate members of Enterobacteriaceae capable of fermenting citrate in the presence of the enzyme citrate. Simon's citrate agar contained citrate as significant energy and was prepared for inoculation on Petri dishes. Well-prepared and sterilized citrate agar plates were inoculated from the pure isolated culture by streaking the surface with a sterilized loop. The plates were then incubated at 37°C for 24 hours. There were changes in colour due to bacterial growth of the organisms on the medium due to citrate metabolism, which gave a positive citrate test. The shift in pH turns the bromothymol blue indicator in the medium from green to blue (positive result). A negative test

was demonstrated with no growth, no colour change, or the colour of the medium remains green (Cheesbrough, 2006).

**Catalase test**

This is a test to detect the presence or absence of catalase enzyme. The catalase enzyme catalyses the breakdowns of hydrogen peroxide to release free oxygen gas and the formation of water. A few drops of freshly prepared 3% hydrogen peroxide were added onto the bacterial isolates smeared on a slide. The production of gas bubble indicated catalase enzyme positive (Cheesbrough, 2006).

**Triple sugar iron (TSI) agar test**

The Triple Sugar Iron (TSI) test is an ability to test an organism's capability to ferment sugars and to produce hydrogen sulphide ($H_2S$) or gas ($O_2$), or both. The test was used primarily to differentiate members of the *Enterobacteriaceae* family based on their sugar fermentation patterns and from other Gram-negative rods. An agar slant prepared of a TSI agar was used in carrying out this test in a sterile test tube at a slanted angle. The slanted medium was inoculated with TSA pure culture using a straight inoculation needle by stabbing first through the center to the bottom of the tube and streaking the agar slant's surface. After inoculations, the test tubes were covered with foil paper and left at an ambient temperature of 36°C to incubate for 24 hrs. Reactions on test tubes were examined, and sugar fermentations were indicated by the production of $H_2S$, gas and a change in colours from red (alkaline) to yellow (acid). When an alkaline/acid (red top/yellow bottom) slant reaction appeared, it only indicated dextrose (glucose) fermentation. When an acid/acid (yellow top/yellow bottom) slant reaction appeared, it showed the fermentation of dextrose, lactose and/or sucrose. The appearance of an alkaline/alkaline (red top/red bottom) slant reaction represented the absence of sugar fermentation. The blackening of

the medium in the slant indicated $H_2S$ production. Bubbles, cracks, or bottom-raised space in the slanted agar indicated gas production (formation of $CO_2$ and $H_2$) (Fawole and Oso, 2007).

**Voges Proskauer Test**

This test is used to differentiate *Bacillus* sp. and enteric bacteria which ferment glucose with the production of acetoin which can be detected by oxidation reaction. 2 ml of sterile Methyl red-Voges Proskauer broth was inoculated with test organism and incubated at 37 $^0$ C for 24 hrs. A small amount of 10 % alpha-naphthol was added and then mixed. About 3 ml KOH was added and shaken. The set up was then left for an hour at room temperature. A pink to red colour indicated a positive result (Omomowo *et al.,* 2015).

**Methyl Red Test**

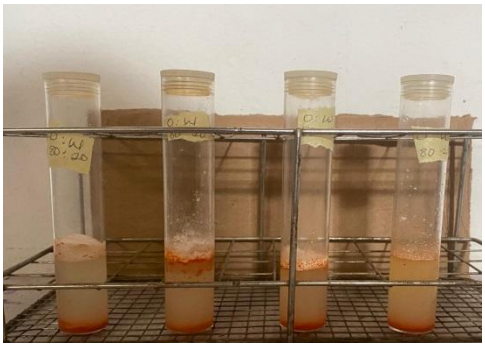
The test is used to check acid production in the medium usually for coliform organisms which ferment dextrose rapidly causing a fall in the pH. Methyl red-Voges Proskauer broth was prepared. 10 ml of the broth was dispensed into test tubes and sterilized. Inoculation was subsequently done and incubated at 30 $^0$ C for 24 hrs. After incubation a few drop of methyl red indicator was added to the culture and a resultant red colouration indicated a positive reaction (Omomowo *et al*., 2015).

# APPENDIX I

**Plate 1: Orange and Watermelon (80:20) mixture**

**Plate 2: Orange and Watermelon (30:70) mixture**

**Plate 3: Orange and Watermelon (60:40) mixture**



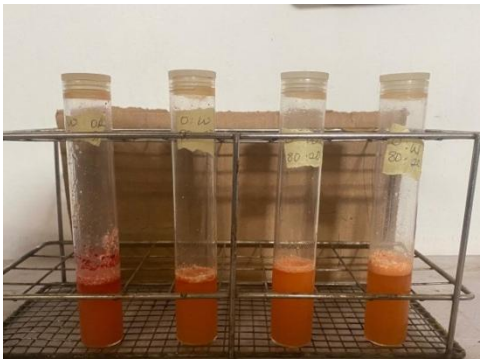
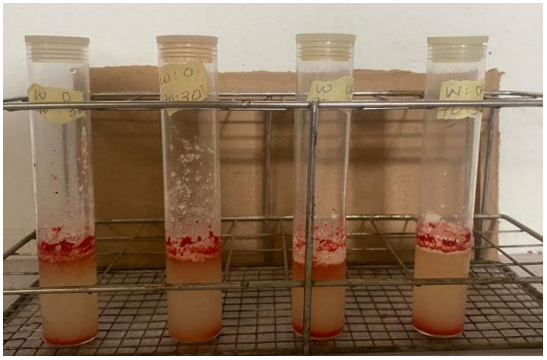


Plate 4 (Stable) Plate 5 (Shaken)

**Plates 4 and 5: Orange and Watermelon (80:20) mixture after 7 days of preservation with brine solution**



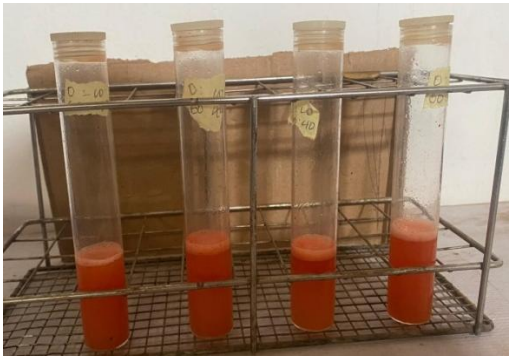


Plate 6 (Stable) Plate 7 (Shaken)

**Plates 6 and 7: Orange and Watermelon (30:70) mixture after 7 days of preservation brine solution**

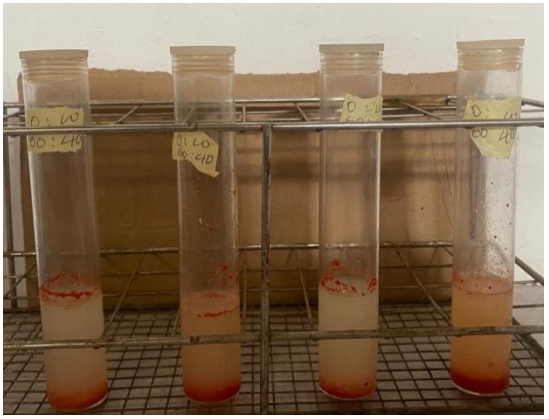


Plate 8 (Stable) Plate 9 (Shaken)

**Plates 8 and 9: Orange and Watermelon (60:40) mixture after 7 days of preservation with bine solution**.