International Journal of Bacteriology and Mycology ISSN 2756-3669 Vol. 13 (1), pp. 001-007, February, 2025.
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*Full Length Research Paper*

# Evaluation of the Microbiological Quality of Commercial and Lab-Prepared Orange Juice

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Accepted 19 February, 2025

**Because of its energizing flavors, natural sugars that provide immediate energy, and nutrients that promote the growth of bacteria, yeasts, and molds that can withstand acid, orange juice has become one of the most popular natural beverages. The goal of the current study was to examine the microbiological analysis of freshly and commercially made orange juices. After being pasteurized, carbonated, condensed, and chemically treated with sodium metabisulphite as a preservative, freshly made orange juices were kept for 90 days at room temperature, in a freezer, and in a refrigerator. The commercialized, fresh, and stored samples had pH values ranging from 3.0 to 4.5, respectively. The commercially, newly, and stored samples had total bacterial loads ranging from 1.6x105 to 3.6x106 and total yeast cell counts ranging from 1.7x104 to 4.8x106 cfu/ml. From the orange juices, twenty-two microbial species were identified, including five bacterial isolates, six yeast isolates, and eleven mold isolates. Bacillus megaterium, Bacillus cereus, Bacillus pantothenticus, Bacillus aeruginosa, and Escherichia coli were the bacterial isolates that were found in the samples. Trichoderma sp., Aspergillus sp., Mycelia sp., Chrysosporium sp., Blastomyces sp., Saccharomyces cerevisiae, Saccharomyces rouxii, and Saccharomyces telluris were the fungal isolates. Bacillus species dominated the bacterial isolates, whereas Saccharomyces and Mycelia species dominated the fungal isolates, accounting for five of the eleven isolates. The bacterial, yeast, and mold isolates seem to have remained consistent over the course of the investigation and may serve as markers of the quality of the microbiota. Combining processing techniques like chemical preservatives with pasteurization, concentration with carbonation, and controlled microbiological environments like freezer and refrigeration temperatures can result in a safe microbial load and a decrease in contamination of orange juice and other fruit juices.**

**Key words:** Orange juices; Processing; Microbial load; Microbial quality; Microbial contamination.

## INTRODUCTION

Due to its freshness, high vitamin content, and low calorie content, fresh juice consumption has significantly increased [1]. It may be made at home with a range of manual and electric juicers [3] and is made by mechanically squeezing or macerating fresh fruits and vegetables without the use of heat or solvent [2]. The comparatively short shelf-life duration brought on by pathogen attacks is one of the limiting variables that affect the fruits' economic value [4]. Even in affluent nations, it is estimated that infections damage 20–25% of produced fruits during post-harvest processing [4-6]. Because of insufficient facilities for storage and transportation, post-harvest losses are frequently more severe in poor nations [4]. When fruits and vegetables are grown or harvested, they may sustain wounds, splits, cuts, or punctures that allow pathogenic organisms to enter [7].

Because of the physical and chemical characteristics of fruit juices, molds and yeasts are preferred over bacteria as fruit juice spoilers [8, 9]. A few of these characteristics are the fruit juices' low pH, their water activity, their positive oxidation reduction potential, and their rich nutritional makeup [8, 9]. Microorganisms in juices can induce cloud loss, the development of off flavors, the creation of carbon (IV) oxide, and changes in color, texture, and appearance,

all of which can lead to product degradation [10, 11]. Acetobacter, Alicyclobacillus, Bacillus gluconobacter, Lactobacillus, Leuconostoc, Zymomonas, and Zymobacter are the bacterial genera that are most frequently reported. Among the yeasts that cause juice spoiling, Pichia, Candida, Saccharomyces, and Rhodotorula are frequently found genera [12]. Numerous fruit juices have been known to degrade due to common molds such Penicillium sp., Aspergillus sp., Eurotium sp., Alternaria, Cladosporium, Paecilomyces, Botrytis, Colletotricum, and Curvularia [10, 13].

When it comes to fruit juices (orange juice), quality is the culmination of all the characteristics that contribute to their acceptability, desirability, and nutritional value as food for humans [14–16]. Juices and beverages are tested for microbiological quality to protect customer safety. Indicator organisms, as opposed to diseases, have historically been identified and counted. The primary determinant of a drink's acceptability for eating is its coliform level [17]. Food indicator organisms are used to assess the product's microbiological quality. Additionally, the aerobic plate count is a valuable indicator of product quality, enables shelf-life prediction, and identifies possible hazards through the use of appropriate markers or pathogen direct detection [14].

Fruit juices make up to 90% of fruit production in underdeveloped countries like Nigeria [3]. Fruit has long been recognized as an essential dietary item that is both commercially and nutritionally significant [4]. Fruits are fundamental to human nutrition because they include growth elements like vitamins and minerals that are needed for a healthy diet and can help maintain good health [4].

Therefore, the current study looks on the microbiological safety of orange juices that are processed in labs and sold commercially. This would give background microbiological information for the creation of techniques that would successfully lower the microbial load and contamination of fruit juices, especially those that are thought to pose a risk to consumer health and spoilage.

## 2. Materials and Methods

### 2.1. Gathering Samples and Making Orange Juices

As shown in Table 1, a total of 14 orange juice preparations—12 treated samples and one commercially processed juice—were employed in the investigation. We bought commercially processed orange juice and mature, ripe delicious oranges (Citrus sinensis) from the Kaduna State market in Nigeria.With minor adjustments, six hundred medium-sized, intact sweet oranges (Citrus sinensis) were chosen, cleaned, peeled, and cut in half using the AKpapunam et al. [18] processing procedure. A hand juicer was used to remove the juice from the chopped oranges (mesocarp). A sterile hand Monilex blender was used to homogenize the extracted juice and pulp. To get a whole juice, or clear juice, the homogenate was filtered through sterile cheesecloth (Figure 1).As indicated in Table 1, this was split up into five batches, each containing 400 milliliters, and subjected to various procedures.The carbonated juice (CAB) was treated with carbon (iv) oxide gas at a concentration of 1.0 kg/100 ml of orange juice at a pressure of around three atmospheres at 10 °C. Each batch of 400 ml of the extracted juice was pasteurized (PASD) at 90 °C for five minutes in a water bath.The concentrated juice (COND) was chemically treated with a preservative (sodium metabisulphite 0.035gm/100ml of juice) and heated to boiling using a distillation apparatus.Orange juices that were commercially processed (COS) and freshly made in the lab (FRH) served as control samples.The concentrated and pasteuerized samples were left to cool to between 30 and 400 degrees Celsius.For ninety days, the CHM, CAB, COND, and PASD treated samples were kept at room temperature, in a freezer, and in a refrigerator (Figure 1).

**Measurement of pH: The pH of juice samples were measured using a pH meter.**

Microbiological analysis: The Lateef et al. [19] method for counting microorganisms in fresh, pasteurized, carbonated, chemically treated, and commercially processed orange juices was used to make this determination. The serial dilution agar plate method was used to analyze each of the different orange juice samples. 90 milliliters (90 ml) of sterile peptone water were combined with an aliquot of 10 milliliters (10 ml) of each orange juice sample, and the mixture was shaken by hand to homogenize it. In sterile peptone water, each sample was serially diluted ten times (10-1, 10-2, 10-3, and 10-10 dilutions). To count the bacteria and fungus, 0.1 ml of each diluent was plated in duplicate on the different agar substrates. To count the total viable counts of bacteria, PCA plates were incubated at 37°C for 24 to 48 hours. Three to four days later, yeasts and molds were counted. Every count was given in colony forming units per milliliter, or cfu/ml.

Identification of Bacteria: The bacterial isolates were identified following standard microbiological procedures as described by Cheesbrough [20] and Kampfer, et al. [21]. The purified bacterial isolates was observed under microscope by Gram stain method and further various biochemical tests were performed for identification of bacteria such as catalase test, oxidase test, starch hydrolysis test, IMViC test and sugar fermentation test [21].

Identification of Yeasts and Moulds: According to "Fungi and food spoilage" [22], morphological traits, sugar fermentation, and urea hydrolysis are among the techniques used to identify yeast. Morphological and cultural traits, including colony color, surface, appearance, presence or lack of cell walls, and asexual and sexual reproductive structures, were used to identify the molds. Additional mold identification was done using the techniques outlined in "fungi and food spoilage" [22]. At 250C, molds were grown on Czapek Yeast Extract agar (pH6.7).

## 3. Results

Table 2 displays the counts of bacteria and yeast cells from various juices kept in freezers, refrigerators, and spaces with varying temperatures. It demonstrates that the bacterial total aerobic plate counts (TAPC) per milliliter for each sample varied between 1.50x105 and 4.1x106 cfu/ml. The sample that was pasteurized and carbonated and kept in the freezer exhibited the highest bacterial counts, ranging from 8.1x105 to 9x105 cfu/ml. The counts of the pasteurized, concentrated, carbonated, and chemically treated samples kept in the refrigerator were 3.75x106cfu/ml, respectively. The counts for the chemically treated, carbonated, concentrated, and pasteurized samples were 1.50x106, 1.73x106, 2.7x106, and 3.4x106 cfu/ml when they were kept at room temperature. The CFU/ml of the commercial and fresh samples were 4.40x105 and 1.6x105, respectively. The range of the yeast cell total plate count was 1.7x106 to 4.75x106cfu/ml. The counts for the pasteurized, carbonated, concentrated, and chemically treated samples in the freezer were 5.95x105, 1.99x106, 6.4x105, and 5.75x105 cfu/ml, respectively. For concentrated, carbonated, chemically treated, and pasteurized samples, the counts were 1.7x104, 1.7x104, and 4.75x106 cfu/ml in the refrigerator, whereas the counts for samples kept at room temperature were 8.0x105, 9.2x104, 2.5x106, and 3.4x106 cfu/ml in the ambient temperature samples. The counts of 8.5x104 and 5.5x104 cfu/ml were found in the commercial and fresh samples, respectively.

Tables 3, 4, and 5 display the findings of the bacterial and fungal isolates according to gram reaction, cultural, morphological, and biochemical assays. The fluids were categorized into two bacterial species, four yeast species, and seven mold species, yielding a total of 22 microbial species, including five bacterial isolates, six yeast isolates, and eleven mold isolates. Bacillus megaterium, Bacillus cereus, and Bacillus pantothenticus were the most common genera among the bacterial species isolates. Escherichia coli and Pseudomonas aeruginosa are two more (Table 3).

Rhodotorula mucilaginosa, Brettanomyces anomalus, Candida mesenterica, Saccharomyces cerevisiae, Saccharomyces telluris, and Saccharomyces rouxii were among the yeasts that were isolated (Table 4).

Mycelia sp., Helminthosporium sp., Blastomyces sp., Chrysosporium sp., Aspergillus sp., and Trichoderma sp. were among the mold species that were isolated (Table 5). Five of the eleven isolates were from the genus Mycelia sp., making it the most common.

## 4. Discussion

As environmental or raw material contaminants, a variety of microorganisms can be identified in fruit juices and soft drinks throughout their cultivation in fields, orchards, vineyards, or greenhouses, as well as during harvest, post-harvest handling, and distribution [2]. However, only a small number of microorganisms can thrive in an acidic, low-oxygen environment; yeast is the most important group linked to fruit juice and soft drink deterioration [2]. Bacterial levels on the surface of most fruits are 1x105cfu/ml [7, 23–25]. When fruits are not properly cleaned, these bacteria are added to the juices, causing contamination [7]. The study's findings demonstrated that, under various processing and storage settings, the microbial counts of all the orange juices examined were comparatively high, ranging from 1.5x106 to 4.1x106 for bacteria and 1.7x106 to 4.75x106cfu/ml for yeast. High numbers of 1.6x105 and 4.4x105 cfu/ml were also observed in the freshly generated and commercially processed control samples.

The samples' microbial counts of bacteria, yeasts, and molds were higher than the highest levels advised by the Food and Agricultural Organization (FAO) [27], the National Agency for Food and Drug Administration and Control News (NAFDAC) [28], the International Commission on Microbiological Specifications of Foods (ICMSF) [26], and the SON-Standard Organization of Nigeria [29].

These organizations state that the maximum amount of mesophilic aerobic bacteria and fungus that can be found in fruit juices, dried foods, and soft beverages is 103 cfu/ml and 2x104 cfu/ml, respectively. However, as no microorganisms should be found in any meal intended for human consumption, the microbial counts found in this study are significantly high [27, 30, 31]. As a result, the samples can pose a health risk to customers. Fruits are mostly contaminated with different microorganisms from dirt and dust during harvest, which may be the reason for the high microbial counts of bacteria, yeasts, and molds [7]. Furthermore, a high viable count frequently denotes inadequate sanitation, improper production or storage circumstances, or an inappropriate time or temperature [32, 33]. The study's typically high microbial counts may be explained by the impact of environmental conditions on the microbial population, which has been demonstrated to have a major impact on food product quality [30, 34–38].

The most common bacterial and fungal isolates in the microbial population were Bacillus species, Saccharomyces species, and Mycelia species. Given that the majority of these organisms are known to flourish in environments rich in fermentable substrates, including sugars, which frequently result in the generation of acids during fermentation, the existence of some of these species is not surprising [39]. These organisms' growth and metabolic processes played a part in the orange juice's deterioration while it was being stored. It is well known that fruit concentrates and juices provide sufficient nutrients to promote the growth of microorganisms [40, 41]. The microbes' use of these nutrients may lead to the synthesis of compounds such acetyl and diacetyl methyl carbinol [14, 40, 42]. These chemicals will affect the juice's pH and lead to spoiling. According to certain workers' reports, fruit juice deterioration may be indicated by pH [10, 11, 13, 43]. Furthermore, research has demonstrated that molds and yeasts can cause spoiling in processed foods because they can withstand high osmotic pressure and low pH levels and thrive at refrigerated temperatures [44]. Orange juice, apple juice, and pineapple squash can harbor Pseudomonas and E. coli bacteria, according to reports by Parish et al. [45], Ghenghesh et al. [46], and Raybaudi-Massilia et al. [1]. The

survival of Samonella species and Enterohaemorrhagic E. coli (EHEC) in fruit juices (pasteurized orange juice) was covered in Food Safety and Hygiene. They came to the conclusion that Salmonella and EHEC can endure for several days, particularly at refrigerator temperature, in fruit juices and other acidic foods with a pH below 4.5 [47]. These data support the findings of the current study, which found that Pseudomonas and E. coli were among the bacteria identified from orange juice samples that were commercialized, pasteurized, chemically treated, and kept at room temperature and in a refrigerator, respectively. In one investigation by Essien et al. [39], Abdalla et al. [48], Gabriel and Abdul [49], Bacillus spores were recovered from a variety of Egyptian canned juices and veggie fruit juices and bottled drinks. Spores from Bacillus species, which are spore formers, can withstand high processing temperatures [39]. These bacteria' spores' thermoduric nature guarantees their survival at pasteurization temperatures [39]. Accordingly, these studies validate the potential isolation of the Bacillus species from the orange juice sample under analysis, particularly the pasteurized and those kept in the freezer, refrigerator, and room temperature. According to reports by Beech and Davenport [50], Bacillus and pseudomonas species are able to withstand extremely cold temperatures. Fruits' high sugar and acid content frequently allows molds and yeasts to dominate fruit juices [8, 51]. The most common mold pollutants found in the examined samples were Mycelia species. Fungi, particularly yeasts, are more likely to thrive during delayed thawing, as evidenced by the fact that the mold and yeast species isolated from the test samples were primarily from those kept in the freezer. This is due to their inability to be destroyed by freezing [7]. This outcome is consistent with the findings of earlier research [7, 52]. They found that even while the surviving microorganisms may sustain damage when the ice thaws, they usually regain their viability, which may allow them to survive when the ice melts into the fluids ([7, 52]). According to reports by Covadonga et al. [44], the International Commission on Microbiological Specifications of Food (ICMSF) [26], Renard et al. [53], and Obire et al. [8], Candida sp., Saccharomyces sp., and Brettanomyces sp. were found to be yeast contaminants of fruit juices, particularly orange juice. According to reports by Bevilacqua et al. [12] and Kamal et al. [13], representatives of the genera Brettanomyces and Candida have been known to cause delayed spoiling in fruit juice and soft beverages, whereas Saccharomyces sp. has been known to cause large-scale spoiling. The quantity of bacteria and yeasts found in the test samples and the control did not differ statistically significantly ($p \leq 0.05$).

Yeasts and molds are frequently found in fruit and fruit juice sources due to insect damage. Therefore, it is best to avoid falling fruits [2]. Sources of contamination for mold growth could include environmental mold spores or used additives, as it is known that some of these compounds decay over time if improperly sealed, which leads to the growth of mold. This is clearly demonstrated in the current investigation, where yeast and mold growth in orange juices stored at pH values of 3.0-3.5 and even 4.5 was not inhibited by a level of sodium metabisulphate of 350 ppm (0.035%) source of sulfur dioxide. Despite this, Beuchat [54] discovered that sulfur (IV) oxide was an inhibitory agent against Byssochlamys niver ascospores among the antimycotic medicines he examined. Given that numerous species of yeasts and molds were isolated from the orange juice samples evaluated in storage, it would not be a viable preservative for fruit juices, particularly orange juice, and soft beverages in Nigerian markets.

**Conclusion and Recommendations**

Techniques comparable to those used in this study are used by fruit juice processors in Nigeria. Pasteurization, carbonation, concentration, and use of chemical preservatives are some of these processes. Their goods are preserved using freezer, refrigeration, and room temperature, all of which are similar to the methods used in this study. Among other things, the procedures were created to prolong the products' shelf life by limiting microbial development and contamination.

In orange juice samples that were examined, the average counts of bacterial and fungal isolates were significantly higher than the upper limit permitted for items that are intended for consumer consumption. These elevated levels point to a public health issue and are suggestive of significant contamination.

The investigation also revealed that there were many different types of bacteria and fungi, with Bacillus, Mycelia, and Saccharomyces being the most common. It is possible to screen the Saccharomyces sp. isolated from these orange juice samples for leavening capacity. Therefore, the creation of a high-quality product with a low microbiological count and production of high-quality raw fruits is crucial.

It may be inferred from the quantity of isolated bacteria and fungus found in the orange juice samples under test that various bacterial and fungal species are present in fruits and juice-making materials. The findings indicate that fresh fruit juices, such as sweet oranges, may include germs that could pose a health risk to the general public.

Based on the study's processing techniques and storage circumstances, it can be said that combining different processing techniques can result in a safe microbiological load and less contamination of orange juice and other fruit juices:Pasteurization, carbonation, and chemical preservations should be used in regulated microbiological conditions, such as freezer and refrigerator temperatures.

The present study's findings have led to the following recommendations:Orange juice manufacturing in Nigerian markets should utilize a combination of food preservatives, sodium metabisulphite (100 ppm) and sodium benzoate (350 ppm), as an adjuvant, flavoring agent, and antibacterial.To prevent and lessen microbiological contamination of fruit products, orange fruit surfaces should be cleaned with hot water and chemical sanitizers such chlorine dioxide, ozone, and peracetic acid before juice is extracted following good agricultural manufacturing standards.For locally produced commercial orange juice and

fruit juices, the Standards Organization of Nigeria (SON) and the National Agency for Food and Drug Administration and Control (NAFADAC) should make sure that the manufacturers strictly follow the guidelines for the maximum allowable limit of microorganisms for commercial fruit juices.They ought to conduct routine testing of products on the shelf and inspections of production facilities to make sure they meet food safety regulations.

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