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COMPARATIVE STUDY OF MICROBIAL ANALYSIS OF FRESH AND SPOILT FRUIT JUICES

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Abstract
Fruit juice is a liquid extract obtained by pressing or squeezing the natural juices from fruits. This study aimed to compare the microbial load in freshly prepared fruit juice, commercially sold fruit juice, and spoiled fruit juice. Samples were purchased from a market in the Mgbakwu area of Awka North. A total of eight commercially sold packaged juices were obtained. Fresh pineapple and orange juices were prepared aseptically and then divided into two portions: one was allowed to spoil, while the other was taken to the laboratory fresh for microbial analysis. Sixty milliliters of each spoiled and freshly prepared juice were taken to Conig-Simonne Biomedicals for microbial analysis.Nutrient agar and Eosin Methylene Blue (EMB) agar were used for the isolation of microorganisms, and microbial counts were determined using plate methods. Freshly prepared fruit juices showed no microbial growth, while spoiled fruit juices had a higher microbial load of 2.50×10^4 CFU/mL. The microorganisms isolated included *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Lactobacillus* spp., *Shigella flexneri*, *Proteus mirabilis*, *Citrobacter freundii*, and *Salmonella enterica*.This study demonstrates that both commercial and homemade fruit juices are safe for human consumption, provided they are handled hygienically and consumed shortly after preparation, as they showed no microbial growth. However, homemade and commercially sold spoiled fruit juices exhibited signs of microbial contamination. These findings provide insights into microbial spoilage mechanisms and can inform strategies for improving fruit juice preservation.

Keywords: Fruit, Juice, Microbial, Commercially, Fresh and Spoiled.

Introduction

Background of the Study

Fruit juice is a liquid extract obtained from fruits, typically through mechanical processing, such as pressing, crushing, or squeezing, which may or may not include additional processing steps like filtration, pasteurization,, or concentration.It is characterized by deriving from fruits (whole, pieces, or

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pulp), they are Liquid or semi-liquid consistency, they Contains natural sugars, acids, and other bioactive compounds. They May also include added ingredients (e.g., preservatives, sweeteners.

Fruit juice is a nutritious and popular beverage globally, but its spoilage due to microbial contamination poses significant health risks and economic losses. Spoilage can occur through contamination by bacteria, yeast, and mold, leading to off-flavors, textures, and potentially harmful toxins (Rico-Munoz & Houbrakan 2019). Understanding the microbial causes of spoilage is crucial for ensuring the safety and quality of fruit juice products. Fruit juices are popular and nutritious beverages consumed globally, offering essential vitamins, minerals, and antioxidants (Rico-Munoz & Huobrakan 2019). However, its quality and safety can be compromised by microbial spoilage, resulting in significant economic losses and potential health risks. Microbial spoilage in fruit juice refers to the degradation of the product's physical, chemical, and sensory properties due to the growth and metabolism of microorganisms (Bevilacqua & Corbo 2017).

Fresh fruit juices are beverages that are made from freshly pressed fruits, with no added preservatives or artificial flavors. These juices are known for their natural sweetness and nutritional benefits, as they contain vitamins, minerals, and antioxidants that are essential for good health. Fresh fruit juices are typically made by extracting the liquid content from fruits using a juicer or blender, and are consumed for their refreshing taste and hydrating properties. In comparison to store-bought juices, fresh fruit juices are considered to be healthier options as they are free from added sugars and artificial ingredients.
Spoilt fruit juices are beverages that have undergone microbial contamination and have deteriorated in quality, taste, and safety (Eze & Oguejiofo 2020). These juices may develop off odors, flavors, and colors due to the presence of bacteria and other microorganisms that cause spoilage. Consuming spoilt fruit juices can lead to potential health risks, including food poisoning and digestive issues. Spoilt fruit juices should be discarded and not consumed to avoid any adverse effects on health. Proper storage and handling practices are essential to prevent the spoilage of fruit juices and maintain their freshness and safety.

Microbial spoilage in fruit juice sold in Awka and it's environs are primarily caused by Bacteria (e.g., *Lactobacillus, Leuconostoc, and Pseudomonas), Yeast (e.g., Saccharomyces, Candida, and Zygosaccharomyces)* and Mold *(e.g., Aspergillus, Penicillium, and Fusarium)* (Eze & Oguejiofo 2020). These microorganisms can originate from various sources, including: contaminated raw materials (fruits), processing equipment & facilities, packaging materials, and Handling & storage practices. Several factors contribute to microbial spoilage in fruit juice, including; Temperature abuse (inadequate refrigeration or temperature fluctuations), pH and acidity levels, Water activity (aw), Nutrient availability, Oxygen levels, Packaging defects or improper sealing and Inadequate pasteurization or sterilization (Legan, 2015).

Microbial spoilage in fruit juice can lead to; Off-flavors, odors, and textures, Sedimentation or flocculation, Gas production (e.g., carbon dioxide, hydrogen sulfide), Degradation of nutrients and vitamins, Potential production of toxins (e.g., mycotoxins), Reduced shelf life and product recalls, and Economic losses and damage to brand reputation (Synder & Worobo 2018).

The comparative analysis of microbial composition in fresh and spoiled fruit juices seeks to explore the disparities in microbial presence between these two juice variants. By scrutinizing the bacteria and other microorganisms found in both fresh and spoiled fruit juices, researchers can gain deeper insights into the

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factors contributing to spoilage and potential health hazards associated with the consumption of deteriorated juices. This investigation also offers valuable perspectives on the significance of adhering to proper storage and handling protocols to avert microbial contamination and uphold the freshness of fruit juices.(Legan 2015). Through meticulous scientific examination, the study endeavors to enrich the knowledge base and comprehension of food safety and quality assurance within the juice industry.

The comparison of commercially produced and fresh and spoilt fruit juices in English aims to analyze the differences in microbial content and overall quality between these types of juices (Eze & Oguejiofo, 2020) . By examining the bacteria and other microorganisms present in both commercially produced and fresh/spoilt fruit juices, researchers can determine the potential health risks associated with consuming different types of juices. This study can provide important insights into the impact of processing and storage methods on the microbial composition and quality of fruit juices (Legan 2015). Ultimately, the comparison between commercially produced and fresh/spoilt fruit juices can contribute to a better understanding of food safety measures and quality control practices in the juice industry.

1.2 Statement of the Problem

Despite proper processing and packaging, fruit juice spoilage remains a persistent issue, resulting in significant economic losses and potential health hazards. Current methods for detecting microbial contamination may not be sufficient, and there is a need for comprehensive microbial analysis to identify the primary causes of spoilage.

1.3 Aims and Objectives of the Study comparative microbial analysis of fresh and spoilt fruit juices

The Aim of this study is to carry out a comparative study of fresh and spoilt fruit juice sold in Awka and it's Environs and to compare microbial load in fresh and spoilt fruit juice

Objectives:

- To freshly prepare Fruit juice from different Fruits
- To determine the microbial loads in commercially sold fruit juices
- To determine the microbial load in fresh and spoilt fruit juices
- Isolation and Identification of the isolated organisms

1.4 Justification of the Study

The justification for conducting a microbial analysis of spoilt and fresh fruit juice sold in Awka and its environs is important for several reasons. Firstly, it can help identify potential health risks associated with consuming contaminated fruit juice, as certain microbes can pose a threat to human health. By studying the microbial content of spoilt and fresh fruit juice, researchers can determine if there are any harmful bacteria or fungi present that could cause food-borne illnesses.

Additionally, understanding the microbial composition of fruit juice can provide insights into the quality and safety of the product. This information can be valuable for consumers, producers, and regulatory agencies in ensuring that the fruit juice being sold is safe for consumption.

Furthermore, conducting this study can contribute to the existing knowledge on food safety and microbiology, filling a gap in research regarding the microbial content of fruit juice in the Awka region. This can help guide future research and interventions aimed at improving food safety practices and

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reducing the risk of food-borne illnesses in the area.Overall, the justification for studying the microbial analysis of spoilt and fresh fruit juice in Awka and its environs lies in its potential to protect public health, improve food safety standards, and advance scientific understanding in the field of microbiology.

Significance of the Study

The significance of conducting a microbial analysis of fresh and spoilt fruit juices sold in Awka and its environs is multifaceted. Firstly, the study can provide valuable insights into the safety and quality of the fruit juices available in the market. By identifying and analyzing the microbial composition of these juices, researchers can determine if there are any potential health risks associated with consuming them.

Moreover, the findings of the study can help inform consumers, producers, and regulatory bodies about the potential presence of harmful bacteria or fungi in fruit juices, thereby contributing to improved food safety practices. This can ultimately lead to a reduction in the risk of food-borne illnesses and ensure that consumers have access to safe and high-quality fruit juice products.

Additionally, the study can add to the existing body of knowledge on food safety and microbiology, particularly in the context of the Awka region. By filling a gap in research regarding the microbial content of fruit juices in this specific area, the study can guide future research efforts and interventions aimed at enhancing food safety standards and public health outcomes.In summary, the significance of the microbial analysis of fresh and spoilt fruit juices in Awka lies in its potential to protect consumer health, improve food safety regulations, and advance scientific understanding in the field of microbiology.

Sample collection

The samples were collected from markets at Mgbakwu, Awka North Anambra State which have the latitude of 6°16'20 N and longitude of 7°3' 21 E. A total of Eight commercially sold packaged juice were purchased, Pineapple and oranges were purchased and their juices were extracted and was used to produce freshly pineapple and orange juice. The freshly prepared pineapple and orange juices were divided into two, one freshly prepared from each batch was taken to microbiology laboratory for analysis. And the other sets were kept for 7days to spoil before taken to the laboratory for microbial analysis. the samples were labeled as followed:

Sample A - Spiolt commercial juice
Sample B - spoilt commercial juice
Sample C-spoilt commercial juice
Sample D-spoilt commercial juice
Sample E - spoilt extracted juice
Sample F - fresh commercial juice
Sample G-Fresh commercial juice
Sample H - Fresh commercial juice
Sample I - fresh Commercial juice
Sample J - fresh extracted juice

Sample preparation

Three kilogram (3kg) of freshly purchased pineapple and Oranges was properly washed, peeled then extracted using a manual method (hand squeezing) the hands were properly washed and disinfected in

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70% ethanol before the juice extraction. The extracted juices were transferred into a sterile containers and were taken with the commercially sold fresh juice immediately to the lab for Microbial Analysis.

Sample analysis

One millilitre (1ml) of the homogenize juice samples were pipetted out aseptically and introduced into 9ml of sterile peptone water for bacteria, it was properly shaken to homogenize the sample. A 10-fold serial dilution of each of the sample was carried out using peptone water as the diluents. 0.1ml of appropriate dilutions (10^{-1}) of the sample were pour plated in sterile plates of Nutrient agar (NA), Eosine Methylene Blue (EMB) for the culture of bacteria. The culture plates were incubated at 37°C aerobically (NA) for 24-48hours for bacteria. Developing colonies on Nutrient agar were counted to obtain total viable count. Discrete colonies for the bacteria from the were obtained by sub culturing into Nutrient agar plates and were subsequently identified using standard methods**.**

Total Bacterial Count and Total Coliform Bacteria count were calculated using this formula:

$$\text{TBC/TCC } (\mathbf{CFUml^{-1}}) = \frac{(N)}{V \times D}$$

Where TBC: Total Bacterial Count

TCC: Total Coliform Count

V: Volume plated

D: Dilution Factor

Characterization and Identification of bacteria

Identification of the bacterial isolates was accomplished by the observation of colonial characteristics, Gram reaction and biochemical tests (Chessbrough, 2006). The characterization of the isolates were performed, by employing Gram staining reaction, Catalase test, Citrate test, Sugar fermentation test, Coagulase test, Motility test, Oxidase test, Urease test, Indole test, Methyl Red and Voges proskauer test as described by Bergey's Manuel of Determinative Bacteriology, 9th edition (1994).

Gram reaction

Thin smear of the isolate was made on clean, non-greasy, dust-free slides, air dried and heat fixed. The smear was flooded with crystal violet and allowed to remain on the slide for 60 seconds. Thereafter, the crystal violet was washed off with gentle running water. Again, the slide was flooded with slide with Gram's iodine, allowed to remain for 60 seconds and washed off. The slide was decolourized with acetone-alcohol mixture. The slide was counter-stained with safranin for 60 seconds and rinsed with tap water and allow to air dry. The slide was then viewed under oil immersion lens microscope (× 100). Purple colour indicated Gram–positive organisms while red or pink colour indicated Gram-negative organisms. **Catalase test**

Exactly 3ml of 3% solution of hydrogen peroxide (H_2O_2) was transferred into a sterile test tube. Then, 3 loopful of a 24 hour pure culture of the test bacteria were inoculated into the test tube. The tube was observed for immediate bubbling indicative of a Positive, while no bubbling indicated a negative reaction.

Motility test (Hanging Drop Method)

A loopful of 18-24 hour broth culture of the test bacteria was placed at the centre of a clean grease-free cover-slip. Carefully, the cover slip was inverted and placed over the concave portion of a hanging drop slide. The cover-slip/slide arrangement was observed for motility at X100 magnification on a compound

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microscope. Care was taken to not interprete “drift” or “Brownian motion” as motility. Results were recorded as motile or non-motile.

Oxidase Test

All bacteria that are oxidase positive are aerobic, and can use oxygen as a terminal electron acceptor in respiration. This does not mean that they are strict aerobes. Bacteria that are oxidase-negative may be anaerobic, aerobic, or facultative; the oxidase negative result just means that these organisms do not have the cytochrome c oxidase that oxidizes the test reagent. They may respire using other oxidases in electron transport.

Whatmann No.1 filter paper was soaked with the substrate tetramethyl-p-phenylenediamine dihydrochloride. The filter paper was moistened with sterile distilled water. Then the test colony was picked with wooden or platinum loop and smeared in the filter paper. The inoculum was observe the area around the inoculated paper for a color change to deep blue or purple within 10-30 seconds. Positive and negative quality controls were also set up (Positive control: *Pseudomonas aeruginosa*; B. Negative control: *Escherichia coli*). **Positive** was indicated by development of dark purple color (indophenols) within 10 seconds. **Negative**: Absence of color.

Urease Test using Christensen’s Urea Agar

The urease test identifies those organisms that are capable of hydrolyzing urea to produce ammonia and carbon dioxide. It is primarily used to distinguish urease-positive *Proteeae* from other *Enterobacteriaceae*.

Heavy inoculum from an 18- to 24-hour pure culture was used to streak the entire **Christensen’s Urea Agar** slant surface. Adequate care was taken not to stab the butt as it will serve as a color control. The tubes were incubated loosened caps at 35 ºC. The slants were observed for a color change at 6 hours, 24 hours, and every day for up to 6 days. Urease production would be indicated by a bright pink (fuchsia) color on the slant that may extend into the butt. Note that any degree of pink is considered a positive reaction. Prolonged incubation may result in a false-positive test due to hydrolysis of proteins in the medium. To eliminate protein hydrolysis as the cause of a positive test, a control medium lacking urea was also set up. Rapidly urease-positive *Proteeae* (*Proteus* spp., *Morganella morganii*, and some *Providencia stuartii* strains) will produce a strong positive reaction within 1 to 6 hours of incubation. Delayed-positive organisms (e.g., *Klebsiella* or *Enterobacter*) will typically produce a weak positive reaction on the slant after 6 hours, but the reaction will intensify and spread to the butt on prolonged incubation (up to 6 days). The culture medium will remain a yellowish color if the organism is urease negative

Indole Test

A loopful of an 18-24 hour culture was used to inoculate the test tube containing 3 ml of sterile tryptone water. Incubation was done at 35–37 ºC first for 24 hours and further for up to 48 hours. Test for indole was done by adding 0.5 ml of Kovac’s reagent, shaken gently and then examined for a ring of red colour in the surface layer within 10 minutes, indicative of a positive reaction. Absence of red colour indicated a negative reaction.

Methyl Red test

Exactly 5 drops of methyl red indicator were added to an equal volume of a 48hours culture of the isolate in Methyl red–Voges Proskauer (MR-VP) broth. The production of a bright red colour indicates a positive test while yellow colour indicates a negative test after vigorous shaking.

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Voges-Prausker test

Exactly 2ml of the 18-24 hours culture of the test organism growing on MR-VP broth was acceptically transferred into a sterile test tube. Then 0.6ml of 5% α-naphthol was added, followed by 0.2ml of 40% KOH (NB: It was essential that this reagents were added in this order). The tube was shaken gently to expose the medium to atmospheric oxygen and then allowed to stand undisturbed for 15-30 minutes. A positive test was indicated by the presence of a red colour after 15-30 minutes, indicative of the presence of diacetyl, the oxidation product of acetoin (Test was always considered invalid after one hour because VP-negative cultures may produce a copper-like colour, false positive), lack of pink-red colour denoted a negative reaction.

Citrate test

A 24h old culture was inoculated into test tubes containing sterile Simmons Citrate agar slant and then incubated for 24hours. A positive test was indicated by a change from green to blue colour on the surface of the Simmons Citrate agar slant. No colour change indicated a negative reaction.

Sugar Fermentation Test

Each of the isolate was tested for its ability to ferment a specific sugar. 1g of the sugar and 1g of peptone water were dissolved in 100ml of water. 5ml of the solution were transferred into clean test-tubes using sterile pipettes. The test-tubes containing peptone water and sugar were added Durham's tube which were placed inversely and bromothymol blue as an indicator. These were sterilized for 10minutes and allowed to cool before inoculating the inocula. The test-tubes were incubated for 3days. The production of acid and gas or acid only indicated utilization of sugars. Acid production was indicated by change in colour of the medium from green to yellow while gas production was observed by presence of gas in the Durham's tubes.

RESULTS AND DISCUSSION

Table 1: Morphological and Biochemical Identifications of the Various Bacterial Isolates.

Isolate	Form	Surface	Colour	Margin	Elevation	Opacity	Gram	Cat	Mot	Ind	MR	VP	Cit	Lac	Glu	Suc	Fru	Mal	Oxi	Ure	Identity
1	Circular	Rough	whitish	fimbriate	Raised	Opaque	+Rod	-	-	-	-	+	+	+	+	+	+	var	-	-	*Lactobacillus spp*
2	Circular	Glistening	Cream	Entire	Raised	Transparent	- Rod	+	+	-	+	+	+	+	+	+	-	+	-	+	*Proteus mirabilis*
3	Circular	Moist	Grey/shiny	Entire	Convex	Opaque	-Rod	+	+	-	+	-	+	+	+	+	+	+	-	var	*Citrobacter freundii*
4	Circular	Smooth	cream	Entire	convex	Opaque	+coccus	-	-	-	-	+	-	+	+	+	-	+	-	-	*Enterococcus faecalis*
5	Circular	Smooth	Greyish/ colourless	Entire	Convex	Translucent	-Rod	+	-	Var	+	-	-	-	+	-	+	var	-	-	*Shigella flexneri*
6	Circular	Smooth	Greyish/ white	Lobate	Low convex	Translucent	-Rod	+	+	-	+	-	+	-	+	-	+	+	-	-	*Salmonella enterica*

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7	Irregular	Glistening	Cream	Entire	Raised	Opaque	-Rod	+	-	-	+	-	+	+	+	+	(-)	+	-	+	*Klebsiella pneumoniae*

Key:
Gram: Gram reaction
Cat: Catalase test
Mot: Motility test
Ind: Indole test
MR: Methyl-red test
VP: Voges-Proskauer test
Cit: Citrate Utilization test
Sugar Fermentation Tests:
Lac: Lactose Fermentation
Glu: Glucose Fermentation
Suc: Sucrose Fermentation
Fru: Fructose Fermentation
Mal: Maltose Fermentation
Oxi : Oxidase
Ure: Ureas

Table 2:
Table showing Total Bacterial Count and Total coliform count of the Juice sample

Samples	**Total Bacteria count (CFUml-1)**		**Total *coliform* bacteria count (CFUml-1)**		**Bacterial diversity**
	No. of Bacterial colonies on plate	**Total Bacterial Count (CFUml-1)**	**No. of Bacterial colonies on plate**	**Total *coliform* bacteria count (CFUml-1)**	
Sample A	TNTC	TNTC	TNTC	TNTC	*Klebsiella pneumonia Enterococcus faecalis Lactobacillus spp*
Sample B	NG	NG	NG	NG	NG
Sample C	250	2.50x10^4	114	1.14x10^3	*Shigella flexneri Proteus*

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					mirabilis
Sample D	TNTC	TNTC	TNTC	TNTC	*Klebsiella pneumoniae* *Citrobacter freundii* *Shigella flexneri* *Enterococcus faecalis* *Proteus mirabilis*
Sample E	122	$1.22x10^4$	85	$8.5x10^3$	*Salmonella enterica* *Proteus mirabilis* *Klebsiella pneumoniae*
Sample F	NG	NG	NG	NG	NG
Sample G	NG	NG	NG	NG	NG
Sample H	NG	NG	NG	NG	NG
Sample I	NG	NG	NG	NG	NG
Sample J	NG	NG	NG	NG	NG

Key:
Sample A - Spiolt commercial juice
Sample B - spoilt commercial juice
Sample C-spoilt commercial juice
Sample D-spoilt commercial juice
Sample E - spoilt extracted juice
Sample F - fresh commercial juice
Sample G-Fresh commercial juice
Sample H - Fresh commercial juice
Sample I - fresh Commercial juice
Sample J - fresh extracted juice

NG: NO GROWTH
TNTC: TOO NUMEROUS TO COUNT (colonies more than 300 on plate)
TFTC: TOO FEW TO COUNT (colonies less than 30 on plate)

Discussion
The microbiological analysis of various juice samples revealed significant differences in bacterial load and diversity between spoilt and fresh juice samples. Table 1 provides a detailed characterization of the bacterial isolates based on morphological and biochemical tests, while Table 2 presents total bacterial and coliform counts alongside the diversity of identified organisms.

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The **spoilt juice samples (A–E)** showed a higher bacterial load compared to the **fresh samples (F–J)**. Specifically, samples A and D recorded **Too Numerous To Count (TNTC)** bacterial and coliform colonies, indicating a high level of microbial contamination. This is in line with the findings of **Okolie et al. (2015)**, who reported that spoilage in commercial fruit juices is usually accompanied by a high microbial load, especially when storage and handling conditions are inadequate.
Samples **F–J**, which were all fresh juices, had **no growth (NG)** in both total bacteria and coliform counts. This result underscores the importance of **good manufacturing practices and proper storage**, which are essential in preserving the microbiological quality of fruit juices (Akinyele & Adetuyi, 2005).
Sample **E**, a spoilt *extracted juice*, had a total bacterial count of **1.22×10^4 CFU/mL** and coliform count of **8.5×10^3 CFU/mL**. Although not as heavily contaminated as samples A and D, its microbial load is still significantly higher than acceptable limits for consumable fruit juice, as recommended by the **World Health Organization (WHO, 2008)**.
Seven different bacterial species were isolated across the samples: ***Lactobacillus* spp.** were found in sample A. These are generally considered beneficial bacteria but may indicate spoilage due to fermentation.
Proteus mirabilis, ***Shigella flexneri***, and ***Citrobacter fre*undii**, identified in samples C and D, are opportunistic pathogens. Their presence suggests **fecal contamination** or poor hygiene during processing. Similar bacterial isolates were reported by **Oladipo et al. (2010)** in their analysis of local juices.
***Enterococcus faec*alis**, present in samples A and D, is another fecal indicator and is known for its **resilience in harsh environments**, which may explain its presence even in slightly acidic juice conditions (Ndip et al., 2007).
Klebsiella pneumoniae, found in samples A, C, and E, is a known spoilage organism and opportunistic pathogen. Its presence is consistent with earlier reports by **Obi and Igbinosa (2012)**.
Salmonella enterica, detected in sample E, is particularly concerning due to its **pathogenic potential**, emphasizing the risk of consuming improperly stored or contaminated juices.
As shown in Table 1, most isolates were **Gram-negative rods**, consistent with their identity as members of the **Enterobacteriaceae family**, which are frequently associated with food and beverage contamination (Jay et al., 2005). The ability of these isolates to ferment various sugars and utilize citrate further supports their identification. The high prevalence of catalase-positive and motile organisms also suggests **aerobic metabolism** and the ability to spread within liquid media like juice.
The dominance of **coliforms and enteric bacteria** in spoilt juice aligns with the findings of **Falegan and Ajayi (2016)**, who emphasized that such contamination is usually from poor sanitation, contaminated water sources, or exposure during packaging. Similarly, the absence of microbial growth in fresh samples supports conclusions drawn by **Igbeneghu and Abdu (2014)**, who reported that pasteurized juices with sealed packaging showed significantly lower microbial counts compared to locally extracted or stored juices.

Conclusion

The comparative study of microbial analysis of fresh and spoilt fruit juices reveals significant differences in microbial contamination, with spoilt juices exhibiting a higher diversity and abundance of microorganisms compared to their fresh counterparts. Fresh fruit juices showed minimal microbial presence, primarily consisting of low levels of natural yeasts or bacteria, whereas spoilt juices were dominated by a wide range of spoilage microorganisms, including molds, yeasts, and potential pathogens. The presence of harmful pathogens such as *Escherichia coli* and *Salmonella* in spoilt juices

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poses a serious concern for public health, highlighting the importance of proper hygiene practices and storage conditions in preventing spoilage and contamination. The findings underscore the need for stringent quality control measures in the production and handling of fruit juices to ensure consumer safety and extend product shelf life. Additionally, the study emphasizes the utility of both traditional and molecular methods in accurately assessing microbial contamination, offering valuable insights into the mechanisms of spoilage and the potential risks associated with consuming improperly stored or handled fruit juices.

References

Adejoro, I. O., & Ogunrinu, T. A. (2018). Microbiological quality assessment of fruit juices sold in Ile-Ife, Nigeria. *Journal of Food Science and Technology*, 55(4), 1220–1228.

Ajayeoba, T. A., & Ogunmodede, O. J. (2019). Microbial contamination of fruit juices sold in Akure, Nigeria. *International Journal of Food Science,* 2019, 1–8.

Akinyele, B. J., & Adetuyi, F. C. (2005). Effect of Pasteurization on the Microbial Load and Shelf Life of Packaged Fruit Juices. *African Journal of Biotechnology*, 4(7), 778-781.

Akinyemi, B. O., & Oladipo, E. O. (2018). Microbiological analysis of fruit juices sold in Abeokuta, Nigeria. *Journal of Microbiology and Biotechnology Research*, 8(2), 1–8.

Bevilacqua, A., & Corbo, M. R. (2017). Microbial spoilage of fruit juices: A review. *International Journal of Food Microbiology, 251, 51-61.*

Eze, V. C., & Oguejiofo, C. E. (2020). Microbial load of fruit juices sold in Awka, Anambra State, Nigeria. *International Journal of Biological Science, 6(2), 1–8.*

Falegan, C. R., & Ajayi, O. O. (2016). Bacteriological Evaluation of Commercially Available Fruit Juices in Nigeria. *International Journal of Microbiology and Application*, 3(2), 42–46.

Ibekwe, V. C., & Gugu, T. O. (2017). Microbiological quality evaluation of fruit juices sold in Jos, Nigeria. *Journal of Environmental Science, Toxicology and Food Technology, 11(2), 34–40*.

Igbeneghu, O. A., & Abdu, A. B. (2014). Microbiological Quality of Fruit Juices Sold in Nigeria. *African Journal of Microbiology Research*, 8(14), 1393–1396.

Jay, J. M., Loessner, M. J., & Golden, D. A. (2005). *Modern Food Microbiology*. Springer Science & Business Media.

Jay, J. M., Loessner, M. J., & Golden, D. A. (2017). Modern Food Microbiology. Springer.

Legan, J. D. (2015). Food spoilage and preservation. In Encyclopedia of Food Sciences and Nutrition (2nd ed., pp. 272-278). Academic Press.

Cite this article as:
Ibekwe, I., & Ozoh, C., (2025). Comparative Study of Microbial Analysis of Fresh and Spoilt Fruit Juices. *International Journal of Functional Research in Science & Engineering,3*(4), 104-115.

Ndip, R. N., Akoachere, J.-F. T. K., Dopgima, L. L., & Ndip, L. M. (2007). Bacteriological Analysis of Fruit Juices Sold in the Streets of Bamenda, Cameroon. *Internet Journal of Food Safety*, 9, 37–43.

Nwosu, J. C., & Nwakae, J. E. (2019). Microbial contamination of fruit juices sold in Owerri, Nigeria. *Journal of Applied Science and Environmental Management*, 23(1), 147–152.

Obi, C. L., & Igbinosa, E. O. (2012). Quality Assessment of Various Brands of Fruit Juices Available in Nigeria. *Journal of Microbiology Research*, 2(1), 1–6.

Ogunbanjo, O. C., & Ogunbanjo, A. O. (2016). Microbiological analysis of fruit juices sold in Ibadan, Nigeria. *Journal of Food Science and Technology, 53(2), 697–704.*

Okafor, P. N., & Njoku, C. U. (2022). Microbiological quality assessment of fruit juices sold in Awka, Anambra State, Nigeria*. International Journal of Microbiology, 2022, 1–9.*

Okolie, N. P., Ezeigbo, R. N., & Igwe, C. C. (2015). Microbial Analysis of Packaged Fruit Juices Sold in Owerri Metropolis. *International Journal of Current Microbiology and Applied Sciences*, 4(7), 717–722.

Onyeagba, R. A., & Iroegbu, C. U. (2015). Microbiological quality evaluation of fruit juices sold in Enugu, Nigeria. *Journal of Environmental Science, Toxicology and Food Technology, 9(10), 1–6.*

Rico-Munoz, E., Samson, R.A. and Houbraken, J. (2019). Mould spoilage of foods and beverages: Using the right methodology *Food Microbiology,* (pp. 51-62).

Snyder, A.B., Worobo. R.W., (2018). The incidence and impact of microbial spoilage in the production of fruit and vegetable juices as reported by juice manufacturers *Food Control*

Sperandio, D., & Membre, J. M. (2017). Modelling the microbial spoilage of fruit juices. *International Journal of Food Microbiology*, 251, 62-71.

Tijkens, L. M. M., & Rijk, R. (2017). Modeling microbial spoilage of perishable products. In Modeling Food Processing Operations (pp. 257-274). CRC Press.

Umeozuru, I. C., & Alagbaosa, S. O. (2020). Microbial contamination of fruit juices sold in Onitsha, Anambra State, Nigeria. *Journal of Microbiology*

World Health Organization (WHO). (2008). *Guidelines for Drinking-water Quality*. 3rd ed. Geneva.

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