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Sugar Concentration and Microbial Growth: A Comparative Study between Orange Juices and Coca-Cola Canned Beverages

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ABSTRACT

Sugary beverages specifically Coca-Cola and orange juice are popularly consumed all around the world, often consumed or stored within proper refrigeration. This practice raises health concerns as microorganisms can thrive in these high-sugar environments which leads to potential spoilage and contamination. This study aims to investigate the effect of sugar concentration on microbial growth in commonly consumed beverages. Potato agar was prepared as the growth medium and poured into Petri dishes. Samples of Coca-Cola and orange juices were inoculated onto the agar and incubated for five days at room temperature to simulate typical storage conditions. After incubation, visible microbial colonies with varying colours and textures were observed, indicating active growth of bacteria and fungi. The results expectedly showed that the beverage with high sugar concentration, orange juice exhibited denser microbial growth compared to Coca-Cola. These findings suggest that sugary drinks provide favourable conditions for microorganisms, especially when left unrefrigerated. Therefore, proper storage practices are essential to ensure beverage safety and reduce the risk of microbial contamination.

1. Introduction

1.1 Research Background

Microorganisms are small living organisms that exist almost everywhere, including food, water, soil, and inside the human body [9]. Although they cannot be seen with the naked eye, their presence is highly significant because they influence processes such as food spoilage, fermentation, and human health [12]. The growth of microorganisms is affected by several factors, including temperature, pH, oxygen availability, nutrients, and moisture. Among these factors, nutrients are particularly important and correlate closely with the presence of sugar, which serves as a key energy source for many microbes [7].

Sugar plays a unique role in microbial growth because its effect varies depending on concentration. At low to moderate levels, sugar promotes microbial proliferation by providing a readily available substrate for respiration and fermentation [4]. This is why many microbes, including

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yeasts and bacteria, thrive in sugar-containing environments. However, when sugar is present at extremely high concentrations, it can inhibit microbial growth due to osmotic stress, which causes microbial cells to lose water and eventually become dehydrated [6, 23]. This dual effect explains why products such as jams and syrups with high sugar content are more resistant to spoilage compared to fresh juices [23].

Microorganisms such as bacteria, yeasts, and molds are naturally present in the environment and play a significant role in food spoilage [23]. Beverages such as fruit juices and soft drinks often contain sugar as their primary ingredient, serving as an energy source for microbial growth. The concentration of sugar in these beverages influences microbial activity in two opposing ways. Firstly, at low to moderate sugar levels, microorganisms thrive because sugar serves as a readily available substrate for respiration and fermentation [6]. Secondly, at extremely high sugar concentrations, microbial growth is suppressed due to osmotic pressure that dehydrates cells and restricts cell division [5, 6]. This principle explains why high-sugar foods are generally more shelf-stable, whereas low-sugar beverages spoil more quickly [23].

However, the relationship between sugar concentration, microbial growth, and human health remains complex. At low to moderate sugar levels, beverages can support microbial contamination if not stored properly or consumed beyond their expiry date, posing risks such as foodborne illness and spoilage [22]. Conversely, beverages with extremely high sugar concentrations can contribute to long-term metabolic and cardiovascular problems when consumed excessively [13]. These include obesity, Type 2 diabetes, cardiovascular diseases, and dental caries—all of which are major global health conditions linked to excessive intake of sugar-sweetened beverages [13].

Previous studies on sugar and health have predominantly focused on metabolic outcomes such as lipid accumulation, dyslipidaemia, and insulin resistance [13], while fewer have explored the microbiological aspects of sugar concentration in everyday beverages. Therefore, there is a need for small-scale experimental studies that clearly demonstrate how sugar concentration affects microbial growth in common drinks. Such research enhances understanding of food safety and preservation while also highlighting sugar's dual role as both a microbial nutrient and a health risk.

This study aims to investigate how different sugar concentrations in fruit-flavoured drinks and carbonated beverages affect microbial growth. The findings will illustrate the connection between food safety and health, demonstrating how sugar can act simultaneously as a preservative and a potential health hazard depending on its level and consumption pattern.

1.2 Problem Statement

It is generally known that orange juices and Coca-Cola canned drinks are consumed globally. Orange juice is often preferred for their perceived nutritional value, while canned drinks are chosen for their taste, which provides a sense of satisfaction to consumers. According to statistics, orange juices are the most famously purchased juice in Malaysia while Coca-Cola is already one of the biggest carbonated drink franchises, which explains its popularity even in Malaysia. However, both beverages undeniably contain high levels of sugar content. While fruit juices may be perceived as natural, nowadays manufacturers increase the sugar concentration of the drinks because of many reasons during the processing.

Sugar is one of the most important components in beverages to enhance taste and consumer appeal. However, sugars such as glucose and sucrose are the main catalysts for microorganisms to grow. They act as the main energy source for microorganisms to grow when the conditions are favourable. This will lead to spoilage, economic losses to manufacturers, and potential health risks to

the consumers of the products. Poor storage and handling of these beverages can easily affect the spoilage rate of both fruit juices and canned drinks.

Limited research has directly compared the impact of varying sugar concentrations on microbial growth in natural beverages. Most existing studies focus either on general microbial contamination or on single factors such as pH or preservatives, without isolating the specific role of sugar content across different beverage types. The gap in knowledge makes it difficult to fully understand the actual causes of spoilage of these drinks. A few factors that can be listed as the main catalysts of microbial growth include pH levels, carbonation, surrounding temperature, and preservatives. Hence, this complexity makes it challenging for manufacturers to predict spoilage rates accurately and for consumers to recognize early signs of deterioration. The rising trend in sugary beverages be it orange-flavoured drinks or Coca-Cola canned drinks among the younger generations further highlight the vitality of this study. The findings are expected to support the development of better safety guidelines, storage practices, and quality control measures for both the industry and consumers.

Despite the widespread consumption of sugar-sweetened beverages, limited research has directly compared the effect of sugar concentration on microbial growth across different beverage types under controlled conditions. Most existing studies focus on general microbial contamination or isolate single factors such as pH or preservatives, without examining how sugar concentration interacts with these variables in real storage environments. This gap in knowledge makes it difficult to fully understand the actual causes of beverage spoilage and the relative contribution of sugar content. Besides that, limited studies have provided clear information on the storage of canned beverages and partly consumed fruit juices to preserve their quality and shelf-life.

Therefore, this study aims to address this gap by experimentally comparing microbial growth in fruit-based and carbonated beverages with different sugar concentrations under ambient storage conditions. The findings are expected to provide clearer insights into the role of sugar in microbial proliferation and contribute to improved food safety practices, storage guidelines and consumer awareness.

1.3 Research Questions

1. What is the sugar concentration in orange juices compared to Coca-Cola carbonated canned drinks?
2. What is the correlation between sugar concentration and microbial growth under ambient storage conditions?
3. What storage conditions minimise microbial growth in sugar-sweetened beverages?

1.4 Research Objectives

1. To determine and compare the sugar concentration in selected orange juices and Coca-Cola beverages.
2. To examine the relationship between sugar concentration and the rate of microbial growth in both beverage types under ambient storage conditions over a 5-day period by observing the microbial growth rate between both beverages.
3. To develop evidence-based recommendations for the appropriate storage and handling of sugar-sweetened beverages to reduce microbial spoilage and mitigate the risk of foodborne illnesses.

1.5 Hypotheses

- Null Hypothesis (H_0):

There is no significant difference in microbial growth rate between orange juices and Coca-Cola canned beverages under ambient storage conditions.

- Alternative Hypothesis (H_1):

Orange juices exhibit a significantly higher microbial growth rate than Coca-Cola canned beverages due to differences in sugar concentration and preservation characteristics.

1.6 Significance of Study

This study enhances scientific understanding of how sugar concentration influences microbial growth in orange juices and Coca-Cola carbonated canned drinks. By investigating the relationship between sugar levels and microbial activity under normal storage conditions, the research provides empirical data that enriches current knowledge in food microbiology and beverage safety. These findings also serve as a reference for future studies exploring the chemical and biological factors influencing beverage spoilage.

From a practical perspective, this research offers useful insight for consumers and policymakers. By identifying how sugar content influence microbial growth, it helps these stakeholders make informed decisions regarding the handling and storage of canned beverages. Thus, this study can help to maintain the quality of drinks.

On a broader scale, the study holds strong public health value as it highlights the potential risks of microbial contamination in commonly consumed beverages. By promoting awareness of safe beverages storage and consumption habits, the research supports healthier dietary practices in the community. Hence, the findings contribute to efforts in strengthening public health awareness, fostering a safer and more informed community.

1.7 Literature Review

Microorganisms play a crucial role in determining the quality, safety, and shelf life of beverages. Their growth in sugary environments is influenced by factors such as nutrient composition, pH, temperature, and osmotic pressure [20]. Factors affecting the fermentation process are the substrate sugar concentration and fermentation time [3]. Among these, sugar concentration is particularly significant because it acts as both a nutrient source and an osmotic regulator for microbial cells [14]. Previous studies have shown that microorganisms such as *Saccharomyces cerevisiae*, *Lactobacillus*, and *Streptococcus* species can thrive in high-sugar beverages when storage conditions are favourable [2, 16]. The tolerance of potential spoilage microorganisms to high sugar levels and their ability to grow under chilled conditions represent a challenge for the microbial stability of high sugar fruit products [15]. Sugar concentration primarily affects microbial growth by reducing water activity and imposing osmotic stress.

Several researchers have compared microbial activity in different beverage types. Kaddumukasa *et al.* [8] observed that fruit juices, especially freshly prepared and unpasteurized varieties, often contain higher microbial loads due to limited processing and the absence of preservatives. In contrast, carbonated canned drinks typically exhibit lower microbial growth because of their high acidity, carbonation, and use of antimicrobial additives [1]. Kregiel, D. [10] further noted that while carbonated beverages

may contain high sugar levels, their shelf stability and microbial safety are significantly enhanced through controlled manufacturing parameters, carbonation, and sealed packaging.

However, the debate persists regarding whether "healthier" fruit-flavoured drinks truly pose lower risks. Studies by Ray and Bhunia [20] suggested that variable pH levels and natural sugars in fruit-based drinks create optimal conditions for spoilage microorganisms, including yeasts and lactic acid bacteria. Research on fruit juices demonstrates that their natural sugars significantly promote *Streptococcus mutans* proliferation and biofilm formation, showing that even natural fruit-flavoured beverages carry dental health risks [18]. This indicates that even "healthier" fruit-flavoured beverages can enhance microbial proliferation and contribute to dental health risks.

Another important factor considered in this study is the role of pH in influencing microbial growth alongside sugar concentration. Acidity in food acts as a barrier against the growth of spoilage and pathogenic microorganisms, particularly when combined with other factors such as low water activity and low temperature, thereby improving food shelf life and safety [11].

However, certain spoilage bacteria, yeasts, and molds have developed resistance to acidic conditions. One example is Alicyclobacillus acidoterrestris, a major spoilage bacterium commonly associated with fruit juices [17]. The pH values of Coca-Cola Original and Coca-Cola Zero Sugar are 2.37 and 2.96 respectively, while Tropicana 100% Orange Juice has a pH of 3.80 [21]. The acidity of Coca-Cola is mainly attributed to phosphoric acid and dissolved carbon dioxide. Additionally, a study conducted by Ravindra *et al.,* [19] on the shelf life of carbonated beverages reported that uncarbonated control samples remained sensorily acceptable for only 14 days, while carbonated beverages remained acceptable for up to 30 days. These findings suggest that acidity and carbonation contribute significantly to the inhibition of microbial growth and the extension of beverage shelf life.

Despite these findings, limited research has directly compared microbial growth between commercial fruit-flavoured and carbonated beverages under controlled conditions. This study addresses this gap by examining orange juices and Coca-Cola, focusing on how differences in sugar concentration influence microbial survival. Understanding these dynamics is vital for improving beverage safety and raising awareness of the potential health risks associated with excessive sugar consumption.

2. Methodology

2.1 Research Design

This study employed a quantitative research design to investigate the relationship between sugar concentration and microbial growth in commercially available beverages. Quantitative analysis was applied to the measurement of sugar concentration and microbial colony growth in the beverage samples. Experiments were conducted using orange juice and Coca-Cola variants under controlled laboratory conditions, where samples were examined for microbial colony development to generate measurable numerical data. This approach enabled systematic comparison and analysis of how varying sugar levels influence microbial survival, spoilage rate, and beverage stability. In addition, descriptive analysis was used to observe the presence and progression of microbial growth in the PDA agar medium.

2.2 Population and Sample

The target population of this study comprises commercially available orange juice and Coca-Cola canned beverages, which are widely distributed across local supermarkets and convenience stores.

Four beverage types were selected as samples, representing two principal categories: Marigold Orange Juice and Tropicana Twister Orange Drink as the orange juice samples, and Coca-Cola Original together with Coca-Cola Zero Sugar as the carbonated canned beverage samples. These specific brands were chosen based on their widespread accessibility, high consumer prevalence, and notable differences in sugar formulation, rendering them particularly suitable for comparative analysis.

Microbial colony development on each plate was observed over a five-day period, with readings recorded every 24 hours to monitor the progression of microbial growth continuously. Sugar concentration was measured using a digital refractometer, with three readings obtained per sample to minimise instrumental error and enhance measurement reliability. Each individual reading required approximately one to two minutes to collect.

The experiment was conducted using a total of five independent bottles or cans per beverage type (n=5), yielding 20 sample units in total. Sourcing five samples from five distinct containers of the same brand and formulation ensured consistency and accuracy in microbial growth results. All selected beverages were within their stated expiry dates, sealed prior to use, and obtained from few different locations to reduce sampling bias. The sampling bias specifically refers to the effect of uniform storage conditions that may arise when samples are bought in bulk from a single outlet. This sample size was considered sufficient to show reliable comparative results and manageable within a pre-university laboratory setting. Observations were conducted at consistent time intervals, and the appearance of colonies was systematically recorded using grading system to evaluate the rate of microbial growth in each sample. This observation frequency was selected to enable continuous tracking of microbial growth development without the risk of overgrowth affecting results.

2.3 Sampling Method

The sampling method employed in this study was cluster sampling, a probability sampling technique in which the population is divided into naturally occurring groups or clusters. In this research, retail outlets including Jaya Grocer, Hero Market, Eco Shop, and 99 Speed Mart were treated as clusters. Beverage samples were then obtained from each cluster to ensure representation from different storage environments and retail conditions.

All samples were purchased within a one-week period to minimise variation in expiry dates, and handling conditions. This approach improved the reliability of the comparison between sugar concentration and microbial growth by reducing potential bias associated with obtaining all samples from a single retail outlet Furthermore, the use of multiple retail clusters increased the relevance of the findings to real-world consumer purchasing conditions.

2.4 Research Instrument

<u>Sugar concentration measurement</u>

- Sugar (beverages samples)

Sugar serves as the primary variable in the experiment. Sugar concentration directly influences microbial growth, as many microorganisms utilise sugars as an energy source through fermentation or respiration.

- Distilled water

Provides a pure solvent free from minerals, ions or contaminants that could interfere with microbial growth or alter sugar concentration measurements.

- Measuring cups

Ensures precise measurement of liquids and solid substances, maintaining accuracy and reproducibility in experimental design.

- Spoon

A simple essential tool for transferring, mixing, and dissolving solid substances such as sugar or agar powder.

- Clean glass beaker

Provides a sterile, transparent container for mixing, heating, and holding solutions. Glass resists chemical reactions and allows easy observation of contents.

Microbial growth measurement

- Agar powder

Agar is a polysaccharide derived from seaweed and used as a solidifying agent in microbiological media. It provides a stable surface for microbial growth without being metabolised by most organisms.

- Potatoes

Potatoes are used to prepare potato dextrose agar (PDA), a nutrient-rich medium that supports fungal and bacterial growth. The starch and other nutrients in potatoes enhance microbial growth.

- Petri dishes

Petri dishes are flat, sterile containers used to culture microorganisms on solid media. Their shallow design allows even distribution of agar and easy observation of microbial colonies.

- Aluminium foil

Aluminium foil provides a protective covering to maintain sterility and prevent contamination during preparation and storage.

- Marker

Marker ensures clear labelling of samples, preventing mix-ups and ensuring traceability throughout the experiment.

Safety and sterilisation equipments

- Gloves

Gloves protect the researchers from direct contact with potentially harmful microorganisms and prevent contamination of samples by human skin.

- Disinfectant

Disinfectant eliminates unwanted microorganisms from surfaces, instruments, and hands to maintain a sterile working environment.

3. Results

3.1 Observation

The observations of the microbial growth are recorded in Figure 1 below.

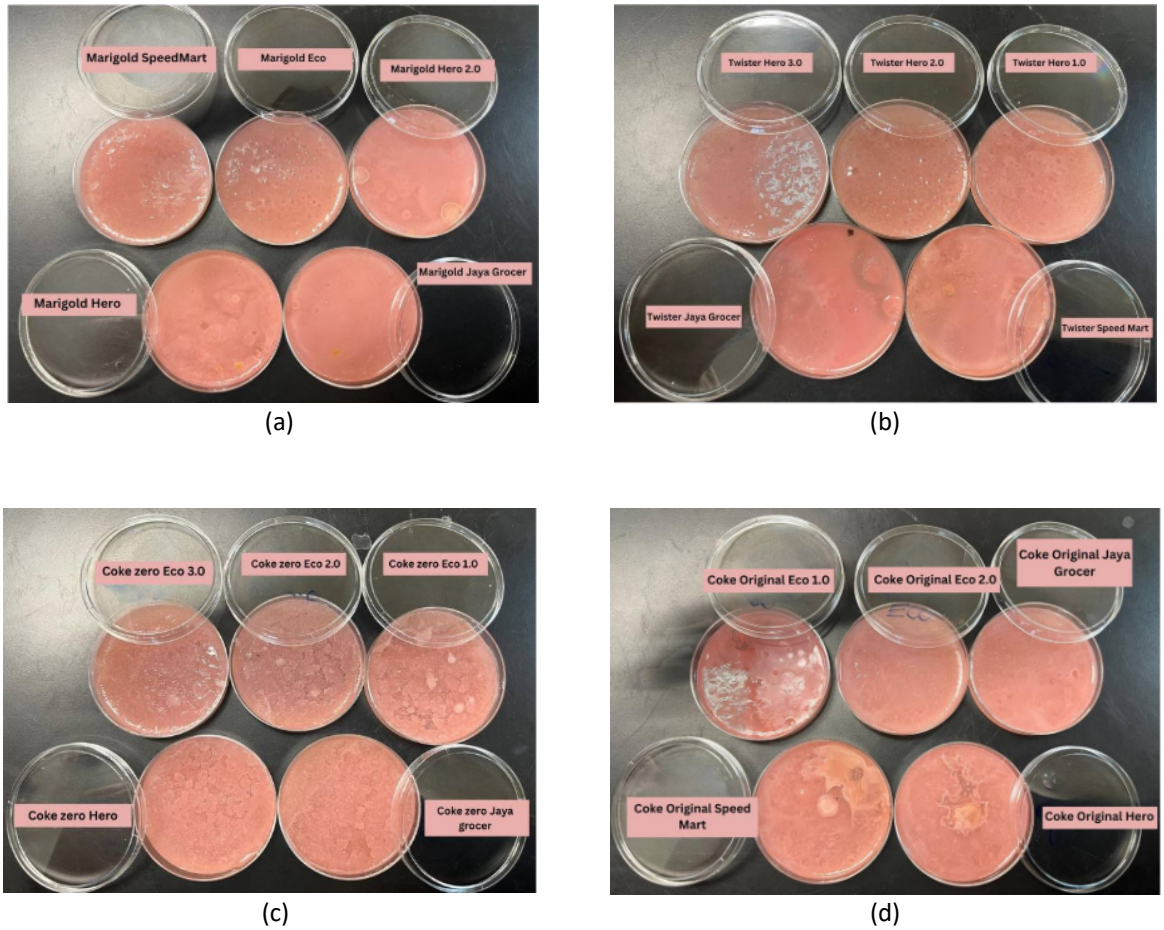

Fig. 1. Observations of microbe colonies recorded on PDA plates containing each beverage samples after 5 days. (a) Marigold (b) Tropicana Twister (c) Coca-Cola Zero Sugar (d) Coca-Cola Original

A grading system was used to categorise the microbial growth. Grades ranging from 0 to 3 each represents level of growth as explained below:

*Grade 0 (No growth) - Agar was completely clear
*Grade 1 (Slight growth) - A few isolated, distinct colonies were visible
*Grade 2 (Moderate growth) - Many colonies were present
*Grade 3 (Heavy growth) - The plate was almost completely covered in a merged sheet of microbes

Table (a) Marigold
Average grade: 2.6

Store Name	Sugar Concentration (°Brix)	Grade
Jaya Grocer	6.2	3
99 Speed Mart	6.0	3
Hero Market 1.0	6.2	2
Hero Market 2.0	6.2	2
Eco Shop	6.2	3

Table (b) Tropicana Twister
Average grade: 2.6

Store Name	Sugar Concentration (°Brix)	Grade
Jaya Grocer	5.0	2
99 Speed Mart	5.0	2
Hero Market	5.0	3
Hero Market 2.0	5.0	3
Hero Market 3.0	5.0	3

Table (c) Coca-Cola Zero Sugar
Average grade: 2.4

Store Name	Sugar Concentration (°Brix)	Grade
Jaya Grocer	0.6	3
Hero Market	0.8	3
Eco Shop 1.0	0.8	2
Eco Shop 2.0	0.6	2
Eco Shop 3.0	0.6	2

Table (d) Coca-Cola Original
Average grade: 1.8

Store Name	Sugar Concentration (°Brix)	Grade
Jaya Grocer	5.0	2
99 Speed Mart	5.0	2
Hero Market	5.0	2
Eco Shop 1.0	5.0	1
Eco Shop 2.0	5.0	2

Fig. 2. Average grade of microbial growth observed on the PDA plates for the 5 samples of each brand

The orange-flavoured drinks showed highest susceptibility towards microbial growth in ambient conditions with both brands yielding a high average growth of Grade 2.6. For Marigold recorded in Table (a), heavy growth was observed on samples from Jaya Grocer, 99 Speed Mart and Eco Shop with each showing Grade 3 growth, while both Hero Market samples showed moderate growth. Tropicana Twister orange juice in Table (b) showed identical growth with an average Grade 2.6. Three out of five samples displayed Grade 3 growth, completely covering the agar surface. The remaining samples from Jaya Grocer and 99 Speed Mart maintained moderate growth (Grade 2).

The carbonated drinks displayed lower average growth grades compared to the fruit juices. Coca-Cola Zero Sugar in Table (c) finished with an average grade of 2.4. Two out of the five samples reached heavy growth at Grade 3, while all three Eco Shop samples maintained a moderate level of growth at Grade 2. Coca-Cola Original based on Table (d) proved to be the most resistant to spoilage across the entire experiment, yielding the lowest average growth grade of 1.8. Only one of the samples which is Eco Shop 1.0 showed the lowest growth of Grade 1 while the others displayed growth of Grade 2.

3.2 Discussion

Based on the results obtained, different beverages showed varying levels of microbial growth. Both Marigold and Tropicana recorded the highest average growth (2.6), followed by Coca-Cola Zero Sugar (2.4). In contrast, Coca-Cola Original showed the lowest microbial growth with an average grade of 1.8.

These findings suggest that the relationship between sugar concentration and microbial growth is not entirely straightforward. While sugar can serve as a nutrient source for microorganisms, other factors such as acidity, preservatives, and beverage composition may also influence microbial growth. The low microbial growth observed in Coca-Cola Original may be due to its high acidity and carbonation, which creates conditions that are less favourable for microorganisms to survive and multiply.

Therefore, the results indicate that microbial growth in beverages is influenced not only by sugar concentration but also by other environmental and chemical factors present in the drinks.

Sugar generally acts as a source of energy and carbon for microorganisms, enabling them to grow and reproduce. Microorganisms can metabolize sugars through cellular respiration or fermentation, producing energy needed for cell growth and colony formation.

However, extremely high sugar concentrations can also inhibit microbial growth. High levels of sugar create a hypertonic environment, causing water to leave microbial cells through osmosis, which may lead to plasmolysis and reduced microbial activity. Additionally, many soft drinks contain preservatives and have a low pH, which further suppress microbial growth.

There are also several limitations in this experiment. The grading system used only provides an estimate of colony growth rather than an exact colony count, which may reduce accuracy. Furthermore, differences in handling, storage conditions, and possible contamination during sampling may have influenced the results.

Future studies could improve accuracy by using controlled sugar solutions, precise colony counting methods, and standardized experimental conditions to better understand the relationship between sugar concentration and microbial growth.

4. Conclusions

The findings demonstrate that microbial growth in beverages is determined not only by sugar concentration but also by beverage composition, particularly acidity, carbonation, and preservatives. Orange-flavoured drinks showed rapid and dense microbial growth, whereas Coca-Cola Original and Coca-Cola Zero Sugar showed minimal and significantly slower microbial growth.

In conclusion, this study provides a clear, empirical demonstration of the relationship between sugar content, beverage composition, and microbial growth. The comparative analysis of orange-flavoured drinks (Marigold and Tropicana Twister) and carbonated canned beverages (Coca-Cola Original and Coca-Cola Zero Sugar) revealed that beverages with moderate sugar levels and minimal preservatives support rapid and highly dense microbial growth under ambient storage. Visual observations on Day 5 confirmed widespread, confluent microbial colonies across the fruit-flavoured samples.

To ensure the scientific validity of these results and eliminate storage condition bias, the beverage samples were intentionally sourced from multiple distinct retail locations, this includes Hero Supermarket, Jaya Grocer, Eco Shop, and 99 Speed Mart. Consistent growth patterns were observed across all store variations in orange drinks and minimal growth in all Coca-Cola samples. By this observation, it can be confidently concluded that these results are driven by the

chemical properties of the beverages themselves, rather than localized environmental storage at a single location.

In contrast to the fruit drinks, the carbonated beverages, despite their high sugar content, exhibited significantly slower and heavily inhibited microbial growth regardless of where they were purchased. This demonstrates that the suppression of microbial activity in Coca-Cola is caused not by sugar alone, but by a combination of factors including its low pH carbonation, and the presence of antimicrobial additives.

Based on these findings, the null hypothesis is rejected, and the alternative hypothesis is accepted. The results affirm that while sugar serves as a primary nutrient for microbes, sugar concentration is just one factor in a beverage's susceptibility to spoilage. Variables like acidity, carbonation, and preservatives play a much more critical role in restricting microbial metabolic activity.

This study successfully met its objectives by quantifying sugar levels in selected beverages and observing microbial growth patterns over a five-day period. Ultimately, this research underscores the vital importance of proper refrigeration and hygiene practices, especially for non-carbonated fruit beverages commonly consumed by children. For future research, it is recommended that studies explore a wider array of beverage types, utilize longer observation periods, and employ molecular techniques to identify specific microbial species to deepen the understanding of spoilage mechanisms and their associated health implications.

This study provides important implications for both food safety practices and consumer awareness, particularly in reducing risks associated with improper storage of fruit-based beverages. Fruit-flavoured drinks, which are widely consumed especially by children, should be stored under proper refrigeration to inhibit rapid microbial growth. Public awareness about beverage storage conditions is therefore essential in preventing foodborne illness and ensuring consumer safety.

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