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Overview of the Effect of Storage Duration of Sweet Orange Juice (*Citrus sinensis* L.) on Bacterial Count Using the MPN (Most Probable Number) Method

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Sweet oranges (*Citrus sinensis* L.) are rich in nutrients that support overall health, particularly brain function. They contain essential components such as vitamin C, folic acid, phytochemical fibers, and various minerals including calcium, potassium, and magnesium. These nutrients may contribute to the prevention of conditions such as cancer, kidney stones, digestive disorders, high cholesterol, and hypertension. This study aimed to determine the bacterial count in sweet orange juice using the Indonesian National Standard (SNI) No. 3719-20 method at room temperature. The research was conducted as a descriptive observational study. Sweet oranges were purchased from the Amifruits Shop in Pontianak. The juice was extracted and diluted with water at a 1:1 ratio, then divided into five samples and stored at room temperature for 2, 3, 4, 5, and 6 hours, respectively. Samples were cultured on LB media and incubated at 37°C for 24 hours to observe bacterial growth, indicated by changes in the medium. Based on the Most Probable Number (MPN) method, two samples tested positive for coliform bacteria, with an estimated bacterial count of approximately 25. It can be concluded that there was some Coliform bacterial contamination at specific times.

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1. INTRODUCTION

Eating and drinking are essential activities for human survival—not only to meet nutritional needs but also to ensure safety, meaning food and beverages must be free from harmful microorganisms. Contamination by microbes from ingredients in the food and drinks we consume can pose serious health risks. Moreover, food and beverages should contain compounds necessary for tissue repair, development, and energy production to support various life activities (Sukamto et al., 2023).

Fruits complement human dietary needs with their variety of flavors, colors, and fibers beneficial to health. In addition to being consumed fresh, fruits are also commonly processed into products such as fruit juice. According to SNI 3719-2014, fruit juice is a beverage made from fruit extract and water without added sugar or other permitted food additives (Badan Standarisasi Nasional, 2014). The definition of fruit juice as stated in the Head of the Food and Drug Supervisory Agency Decree No. HK.00.05.52.4040 Year 2006 on food categories outlines the basic characteristics of fruit juice, including raw materials, processing, and final products. It defines juice as the liquid obtained from the edible parts of fruit, which is crushed, clarified (if necessary), unpasteurized, and packaged for direct consumption (Badan Pengawas Obat dan Makanan Republik Indonesia, 2006).

Sweet orange (*Citrus sinensis* L.), commonly consumed as freshly squeezed juice, has a sweet and slightly tangy flavor, making it refreshing whether eaten or drunk (Surbakti, 2019). This fruit grows well in tropical regions at altitudes of 900–1200 meters above sea level with high humidity and sufficient water (Adelina, Adelina, & Hasriyanty, 2017). Sweet oranges can be eaten directly, juiced, or used as a food and beverage ingredient.

Sweet oranges are rich in nutrients beneficial for both physical and mental health. Their primary contents include vitamin C, folic acid, phytochemical fibers, and carotenoids. Nutrients found in sweet oranges include water, energy, carbohydrates, sugars, fiber, vitamin B6, vitamin C, calcium, iron, and zinc. Sweet oranges are proven to have high vitamin C content, making them a good natural antioxidant source. Key metabolites in the fruit include narirutin (17.33 mg/g), limonoid (398.30 mg/g), synephrine (2.81 mg/g), and hesperidin (191.30 mg/g) (Sun et al., 2013).

They also contain polyphenols, pectin, folacin, calcium, potassium, thiamine, niacin, and magnesium, which biologically help prevent cancer, kidney stones, gastric ulcers, and reduce cholesterol and high blood pressure (Etebu & Nwauzoma, 2014). Flavonoids and bioflavonoids in sweet oranges act as anti-inflammatory, anti-allergic, antiviral, anticancer, and antioxidant agents. Bioflavonoids help regulate body fat and improve fat metabolism. The high vitamin C content can also help treat mouth ulcers.

Sweet oranges can fulfill 75% to 88% of the daily vitamin C requirement. Consuming 10 grams of fiber per day can reduce the risk of heart disease by 14% and coronary heart disease by 30%. The fiber in sweet oranges helps bind bile acids, which are the end products of cholesterol metabolism. Eating one orange daily can also meet the body's vitamin A needs, which is vital for eye health. To maintain sufficient vitamin C levels, it is recommended to consume 30–100 mg/day (Pacier, & Martirosyan, 2015)

Coliform bacteria are a group of bacteria used as indicators of water contamination. Their presence in water indicates the possible presence of pathogenic or toxin-producing microorganisms that can harm human health (Wardani, & Setiyaningrum, 2019). The most commonly used indicator bacteria for food contamination is *Escherichia coli* (*E. coli*), which is a sanitation indicator bacterium typically found in the human gut. Coliforms are divided into two groups: fecal coliforms (e.g., *E. coli*) and non-fecal coliforms (e.g., *Enterobacter aerogenes*). *E. coli* can cause health issues such as diarrhea if it enters the digestive system through contaminated food or drink (Badan Standarisasi Nasional, 2014).

The Most Probable Number (MPN) test is a microbiological method based on qualitative data from microbial growth in a series of specific liquid media tubes to estimate the microbial count (MPN/mL or MPN/g). While fruit juices are generally consumed immediately, they are sometimes left out and not drunk right away. According to SNI No. 3719-2014, the MPN limits for fruit juices are: coliform ≤ 20 MPN/mL and *E. coli* ≤ 3 MPN/mL. The purpose of this study was to determine the bacterial count in sweet orange juice (*Citrus sinensis* L.) stored at room temperature using the MPN method as per the SNI No. 3719-20 standard.

2. METHOD

This study is a descriptive observational research that aims to measure the Most Probable Number (MPN) value in sweet orange (*Citrus sinensis* L.) juice. The primary objective is to assess the level of Coliform bacterial contamination in freshly squeezed sweet orange juice, based on established microbiological safety standards. The testing evaluates the microbiological quality of the juice in relation to storage time at room temperature.

The population of this study consists of sweet oranges originating from Sambas Regency, purchased from the AMI Fruit Store. A total of 3 kilograms of fresh oranges were used as samples, selected through purposive sampling, which involves the intentional selection of samples based on specific characteristics relevant to the research objectives. The criteria included freshness and suitability for microbiological examination.

This study utilized primary data, which refers to data obtained directly from the source through field observation and laboratory analysis. The primary data in this study consisted of the results of Coliform bacterial identification in the sweet orange juice samples. Primary data is defined as information directly collected from research subjects or phenomena, in this case, the juice samples tested for microbial contamination.

The data collection technique employed was observation, considered appropriate for research involving natural phenomena such as microbial growth. Observation is especially suitable when the sample size is relatively small and when direct monitoring of the research object is required. Once data collection was completed, the data were analyzed descriptively to provide an overview of the MPN values in sweet orange juice, which serve as an indicator of the juice's microbiological safety and quality.

3. RESULTS AND DISCUSSION

This study aims to determine the storage duration of orange juice (*Citrus sinensis* L.) and its effect on bacterial count using the MPN (Most Probable Number) method. The sample used was sweet oranges purchased from AMI fruit store on Jl. Merdeka, Pontianak City. Sampling was conducted on January 28, 2024, and the research was carried out from January 29 to February 7, 2024, at the Microbiology Laboratory of Poltekkes Kemenkes Pontianak with a total of 25 treatment samples. The data from this study is intended to observe the level of Coliform bacterial contamination incubated at 37°C. The research results are presented in tables as follows:

Table 1. Most Probable Number Index of Sweet Orange Juice (*Citrus sinensis* L.) at 37°C

Group	Presumptive			Coliform
	10 ml	1 ml	0.1 ml	
R1 2 hours	0/5	0/1	0/1	0
R2 2 hours	0/5	0/1	0/1	0
R3 2 hours	0/5	0/1	0/1	0
R4 2 hours	0/5	0/1	0/1	0
R5 2 hours	0/5	0/1	0/1	0

Based on Table 1, no bacterial growth was observed in the presumptive test, so the confirmed test was not carried out. All samples (5 samples) 100% meet the requirements of SNI No. 3719-2014: coliform \leq 20 MPN/ml.

Table 2. Most Probable Number Index of Sweet Orange Juice (*Citrus sinensis* L) at 37°C

Group	Presumptive			Coliform
	10 ml	1 ml	0.1 ml	
R1 3 hours	0/5	0/1	0/1	0
R2 3 hours	0/5	0/1	0/1	0
R3 4 hours	0/5	0/1	0/1	0
R4 5 hours	0/5	0/1	0/1	0
R5 5 hours	0/5	0/1	0/1	0

Based on Table 2, no bacterial growth was observed in the presumptive test, so the confirmed test was not carried out. All samples (5 samples) 100% meet the requirements of SNI No. 3719-2014: coliform \leq 20 MPN/ml.

Table 3. Most Probable Number Index of Sweet Orange Juice (*Citrus sinensis* L) at 37°C

Group	Presumptive			Coliform
	10 ml	1 ml	0.1 ml	
R1 4 hours	0/5	0/1	0/1	0
R2 4 hours	0/5	0/1	0/1	0
R3 4 hours	0/5	0/1	0/1	0
R4 4 hours	2/5	0/1	0/1	0
R5 4 hours	1/5	0/1	0/1	0

Group	Presumptive			Confirmed at 37°C			Coliform
	10 ml	1 ml	0.1 ml	10 ml	1 ml	0.1 ml	
R4 4 hours	2/5	0/1	0/1	0	0	0	0
R5 4 hours	1/5	0/1	0/1	0	0	0	0

Based on Table 3, the presumptive test showed positive results as indicated by gas production in Durham tubes. Positive results were found in the 4th and 5th samples. The 4th sample had 2 positive results and the 5th sample had 1 positive result.

Table 3 also shows that positive samples from the presumptive test were followed up with a confirmed test and all showed negative results. All samples (5 samples) 100% meet the requirements of SNI No. 3719-2014: coliform \leq 20 MPN/ml. In R4 and R5 at 4 hours, gas formation was observed, possibly due to the growth of coliform bacteria, which is highly dependent on environmental factors such as temperature, pH, and available nutrients.

Table 4. Most Probable Number Index of Sweet Orange Juice (*Citrus sinensis* L) at 37°C

Group	Presumptive			Confirmed at 37°C			Coliform
	10 ml	1 ml	0.1 ml	10 ml	1 ml	0.1 ml	
R1 5 hours	0/5	0/1	0/1	0	0	0	0
R2 5 hours	0/5	0/1	0/1	0	0	0	0
R3 5 hours	0/5	0/1	1/1	0	0	1	1.8
R4 5 hours	0/5	0/1	0/1	0	0	0	0
R5 5 hours	1/5	1/1	1/1	1	0	0	2

Group	Presumptive			Confirmed at 37°C			Coliform
	10 ml	1 ml	0.1 ml	10 ml	1 ml	0.1 ml	
R3 5 hours	0/5	0/1	1/1	0	0	1	1.8
R5 5 hours	1/5	1/1	1/1	1	0	0	2

Based on Table 4, the presumptive test showed positive results indicated by gas formation in the Durham tubes. Positive results were found in the 3rd and 5th samples. R3 had 1 positive sample, and R5 had 3 positive samples. Confirmed test showed 1 positive sample in R3 and 1 positive sample in R5. Positive results were marked by gas bubbles in the Durham tubes and turbidity. All samples (5 samples) still met the requirements of SNI No. 3719-2014: coliform \leq 20 MPN/ml. In R3 and R5 at 5 hours, gas production was observed due to coliform growth, which depends on environmental factors such as temperature, pH, and available nutrients.

Table 5. Most Probable Number Index of Sweet Orange Juice (*Citrus sinensis* L) at 37°C.

Group	Presumptive			Coliform
	10 ml	1 ml	0.1 ml	
R1 6 hours	0/5	0/1	0/1	0
R2 6 hours	0/5	0/1	0/1	0
R3 6 hours	0/5	0/1	0/1	0
R4 6 hours	0/5	0/1	0/1	0
R5 6 hours	0/5	0/1	0/1	0

Based on Table 5, no bacterial growth was observed in the presumptive test, so the confirmed test was not carried out. All samples (5 samples) 100% meet the requirements of SNI No. 3719-2014: coliform \leq 20 MPN/ml. This may be due to the depletion of nutrients needed by bacteria in the orange juice, thus hindering bacterial growth and leading to a decrease in coliform count.

DISCUSSION

A visual inspection was carried out by the researcher with additional time intervals of 2, 3, 4, 5, 6, 7, 8, and 9 hours, using both closed and open containers. The results showed that between 2 and 7 hours, the sweet orange juice remained in good condition in the closed container, while in the open container, it began to spoil or foam starting from the 5th hour. Between the 5th and 9th hours, the quality of the juice was found to depend on the storage method, temperature, sterility, and other factors. Thus, orange juice can still be consumed as long as it is stored properly and kept at the correct temperature to avoid contamination by Coliform bacteria.

Research by Rahayu (2015) showed similar findings regarding the quality of drinking water, where the contamination levels of bacteria were almost the same across samples. Three depots showed a decrease in the number of Coliform bacteria from day 0 to day 7, while 20 other depots showed an increase. This happens because Coliform bacteria growth is heavily dependent on environmental conditions such as temperature, pH, and nutrient availability. Bacteria require nutrients to grow and reproduce. According to Rahayu (2015), bacteria need carbon, nitrogen, non-metal elements like sulfur and phosphorus, metal elements such as calcium, zinc, sodium, potassium, copper, manganese, magnesium, and iron, as well as vitamins and water to support their growth.

Coliform determination is used as an indicator because the number of colonies correlates positively with the presence of pathogenic bacteria (Akita et al., 2021). The fewer Coliform bacteria, the better the water quality. Gas bubbles formed in Durham tubes are due to microbial respiration. A Durham tube is considered positive if there is turbidity and gas bubbles inside.

In the study, 3 positive samples were found after 24 hours of incubation at 37°C, which corresponds to a 12% rate, using Lactose Broth medium. Lactose Broth is used to detect Coliform bacteria through acid and gas formation as a result of lactose fermentation. These bacteria come from the genera *Escherichia*, *Enterobacter*, and *Klebsiella*.

Lactose Broth is made from beef extract, peptone, and lactose, which provide nutrients for Coliform bacterial metabolism. Each component in Lactose Broth serves a specific purpose: peptone and beef extract are essential for bacterial metabolism, while lactose serves as a carbohydrate source for fermentation (Naufal et al., 2022). Coliform bacteria can ferment lactose, producing gas and causing turbidity in the water. Negative bacteria also grow optimally and ferment lactose, producing gas (Alihar, 2018).

The second stage of testing involved a confirmation test by inoculating samples from Lactose Broth onto Brilliant Green Lactose Broth (BGLB) medium using a sterilized inoculating loop. After 24 hours of incubation at 37°C, two positive samples were obtained, corresponding to an 8% rate. BGLB medium inhibits gram-positive bacterial growth and promotes Coliform bacteria growth. It contains peptone, oxgall, lactose, and brilliant green. Each component has a specific function: peptone provides essential nutrients for bacterial metabolism, lactose is the carbohydrate source for fermentation, and brilliant green and oxgall inhibit gram-positive bacteria while promoting Coliform growth. Gas produced during fermentation is trapped in the Durham tube, and its presence confirms the presence of Coliform bacteria. A positive result is indicated by color changes and gas bubbles (Naufal et al., 2022).

In the confirmed test at 37°C, two positive samples were obtained at the 5th hour. This was due to sample contamination from the air, resulting in a positive result, but without causing further contamination or bacterial growth. According to the research data, at the 6th hour, the sweet orange juice was still fit for consumption because the tightly closed container prevented bacteria from the air from coming into contact with the juice. Only the bacteria present in the juice were able to grow. This may occur because the nutrients needed by the bacteria in the juice had been depleted, thus hindering bacterial growth and reducing the number of Coliform bacteria in the juice.

4. CONCLUSION

Based on the results of the study on the effect of storage duration of sweet orange juice (*Citrus sinensis* L) on bacterial count using the MPN (Most Probable Number) method, it can be concluded that there was some Coliform bacterial contamination at specific times. Firstly, in the Confirmed Test for Coliform at 37°C, 2 positive samples were found from 25 samples, and the positive results still met the requirements of SNI No. 3719-2014, which is Coliform at 20 MPN/ml. Secondly, in the Confirmed Test for Coliform at 37°C, 2 positive samples were found at the 5th hour, and the results still met the SNI No. 3719-2014 standard of 20 MPN/ml. It is suggested that future research could focus on testing the quality of orange juice sold at street stalls using the TPC (Total Plate Count) method.

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