

**Survey Of Total Microbes And Molds In Avocado Juice  
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**ABSTRACT**

*Fresh avocado juice is a very popular drink, but it carries the risk of microbial contamination if it is handled with little attention to hygiene and sanitation. The high total number of microbes and yeasts in food that exceeds SNI provisions indicates that the food product is rotten and is not suitable for consumption. High total microbial contamination can be a sign of the presence of harmful microorganisms. The purpose of this study is to ascertain the overall microbiological, yeast and mold contamination content found in avocado juice in Sukolilo District, Surabaya. This research uses a descriptive method by taking samples from all avocado juice traders in Sukolilo District, Surabaya. The parameters tested were total microbes, total yeast mold and hygiene and sanitation observations of juice traders (ingredients, processes, workers) as supporting parameters. The study's findings demonstrated that all microorganisms in avocado juice in Sukolilo District ranged from  $3.2 \times 10^2$  CFU/ml to  $9.9 \times 10^4$  CFU/ml, there were 65.22% of samples exceeding the limit determined by SNI ( $1.0 \times 10^4$  CFU/ml). The total mold-yeast ranged between  $8.20 \times 10^3$  CFU/ml and the greatest was  $1.07 \times 10^5$  CFU/ml, there were 100% of samples exceeding the limit determined by SNI ( $1.0 \times 10^2$  CFU/ml). Seller's hygiene is not paid attention to, such as not wearing aprons, gloves, head coverings, and not washing hands when making juice. Sanitation is also not paid attention to, including: the fruit used is of poor quality and is kept at room temperature during serving, the blender is not washed with detergent, the refilled water is used to make juice*

*Keywords: juice; mold; yeast; total microbes; hygiene and sanitation*

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**INTRODUCTION**

Many people need fruit juice as a thirst-quenching drink, a source of nutrition, or as a therapy or treatment for an illness. Content of the high polyphenols and antioxidants in fruit juice cause consumers to like drinking fruit juice (Abountiolas & do Nascimento Nunes, 2018). Food or drink which are high in antioxidants, if consumed can reduce the risk of disease cardiovascular (Zhou et al., 2021). Avocado juice is a highly favored beverage due to its abundance of beneficial vitamins and nutrients and has a soft fruit texture. Most of the avocado juice consumed is fresh, not processed and has gone through a pasteurization process (Sariningsih & Srimati, 2018).

Avocado juice is a product that spoils quickly and cannot be stored at room temperature for long periods of time (Anova & Kamsina, 2013). The damage to avocado juice is caused by the high water and nutrient content in it, which makes microbes immediately use it as the main ingredient for their life.

Food products, such as fruit juice, become unfit to be marketed and consumed, due to microbiological quality deviations. Consuming food products whose microbiological values exceed the specified standard limits (BSN, 2014) can result in fever, vomiting, disorientation, diarrhea, and nausea. Some germs may even result in health problems ranging from fainting, nerve cell damage to death. Products whose microbiological quality exceeds the specified SNI standards are more easily damaged resulting in a short shelf life and are not suitable for consumption. food microbiological quality is used as an indicator of the cleanliness and hygiene of the production process. In food products with a total microbial content that exceeds SNI, certain products even indicate the presence of pathogenic bacteria.

Processed fresh foods such as fruit juice are widely reported to contain microbial contamination as follows: (Atma, 2016) reported that the total microbial content in the pineapple carrot juice drink sold at the Mawar Setu Cipayung Posdaya, East Jakarta was quite high, namely  $1.36 \times 10^3$  CFU/ml. And the mold and yeast content is  $1.25 \times 10^3$  CFU/ml. (Astuti et al., 2020) reported that the total aerobic bacterial contamination in avocado juice in snacks on Jalan Surakarta ranged from  $6.07 \pm 0.37$  to  $8.95 \pm 0.04$  log CFU/ml) also reported on avocado and mango juice drinks sold around the Muhammadiyah University Surakarta campus, showing that the total microbial count was  $8 \times 10^7$  CFU/ml for avocado juice and  $3.7 \times 10^8$  CFU/ml for mango juice. The making and presentation of beverages with the presence of bacteria contamination in beverages may will be influence by hygiene factors are not qualified. From this study it could be concluded that the quality of boiled water and washing water are risk factors Escherichia coli bacteria contamination on fruit juice in Tembalang area (Lestari & , Nurjazuli, 2015). Twenty-four avocado juices samples have been collected from different juice houses, cafés, and restaurants of Debre Birhan town in 2019 and 2020 Results show that the mean of E. coli and yeast and mould count of avocado juice samples were  $1.31 \times 10^6$  and  $1.25 \times 10^6$  cfu/ml respectively (Worku & Hailu, 2023)

Therefore, this research was conducted to determine the microbiological quality and to analyze relation between hygiene sanitation with presence of microbe on avocado juice in Sukolilo District, Surabaya. This research is important to assess the cleanliness and microbiological quality of avocado juice available on the market. By knowing the level of presence and number of microbes present, appropriate preventive measures can be taken to maintain the safety and quality of avocado juice consumed by the public. Once the survey results have been carried out, they will be used to support research on the food safety of fresh juice drinks in Surabaya in terms of their microbiological properties.

The survey on the total number of microbes and total mold in avocado juice is aimed at providing information to the general public to be selective in choosing the juice they buy, specifically this research is aimed at traders to be able to improve their sales practices in terms of sanitation and hygiene so that the juice sold has microbiological quality. better, and does not pose a danger to human health

## **METHOD**

### ***Material***

The main ingredient used is avocado juice obtained from fruit juice traders in the Sukolilo District Surabaya, area. Additional materials used include Aquadest which is used to dissolve the agar, 0.85% NaCl which is used to make a diluent solution which is mixed with the sample, potato dextrose agar as a mold-yeast growth medium, plate count agar as a total microbial growth medium.

### ***Tool***

The tools used in this research were petri dishes, 1 ml pipettes, micro pipettes, baker's glasses, laminar air flow (LAF), water bath, autoclave, colony counter and test tubes. All tools and materials are sterile.

### **Research Design**

This research was conducted using descriptive methods. This sampling was determined using non-probability sampling using a saturated sampling method. Nonprobability sampling is a sampling method wherein not every element or member of the population has an equal chance of being chosen as a sample (Rizki et al., 2022). When every member of the population is used as a sample, the process is known as saturated sampling. This is frequently used in research that aims to generate generalizations with extremely small mistakes or in populations that are relatively small—less than 30 people (Mahmud & Astuti, 2019).

This research procedure began by conducting a survey in Sukolilo District, Surabaya, how many juice traders there were. Determining the number of samples tested, followed by taking samples of avocado juice from traders. Then the purchased avocado juice samples are put into a cool box and taken to the testing laboratory. Microbial analysis includes total microbes and total yeast mold, followed by a pH test and observation of hygiene and sanitation conditions at avocado juice traders. The data obtained was made into a bar chart and then compared with the SNI standard for fruit juice drinks to conclude whether the avocado juice sample was safe for consumption or not.

### **Microbiological test**

To carry out a total count of microbes, molds and yeasts in avocado juice, the sample must undergo dilution which is carried out in the following way: 25 grams of sample is blended with 225 ml of sterile 0.85% NaCl solution, at normal speed to obtain a homogenate. Next, successive dilutions were made from  $10^{-1}$  to  $10^{-7}$ .

#### **Determination of Total Microbes**

Take 1 ml of sample from the intended series of dilutions and transfer it into a sterile petri dish, then pour in sterile liquid Plate Count Agar media with a temperature of  $\pm 45^{\circ}\text{C}$ , amounting to  $\pm 10$  ml. then the petri dish is shaken gently so that it is evenly distributed and allowed to solidify. Incubate at  $37^{\circ}\text{C}$  for 1 to 2 days, count the number of all colonies that grow (Tlay et al., 2024).

#### **Determination of Total Yeast Mold**

Take 1 ml of sample from the series of dilutions you want and add it to a sterile petri dish, then pour in sterile liquid Potato Dextrose Agar media with a temperature of  $\pm 45^{\circ}\text{C}$ , amounting to  $\pm 10$  ml. then the petri dish is shaken gently so that it is evenly distributed and allowed to solidify. Incubate at  $37^{\circ}\text{C}$  for 1 to 5 days, count the number of all growing colonies (Suhartatik, N., Mustofa, A Wijaya, D., ES, Yuliasuti., Astuti, 2024).

### **Hygiene and sanitation observations of Avocado Juice Traders**

Hygiene observations Avocado juice sanitation observed the personal hygiene conditions of traders and the sanitation facilities of avocado juice traders in Sukolilo District by using an inspection form in the form of an observation sheet (Mengistu et al., 2021). The data results were analyzed to determine the state of sanitation hygiene of avocado juice traders in Sukolilo District. The hygiene and sanitation observation sheet for avocado juice traders can be seen in the attachment.

## RESULTS AND DISCUSSION

This research was carried out on avocado juice in terms of total microbes, mold and yeast as well as the hygiene and sanitation of traders. Of the 30 fruit juice traders there, it is known that only 23 traders sell avocado juice, therefore the entire avocado juice sample was used in this study

### ***Total Microbial Content of Avocado Juice in Sukolilo District***

The goal of the Total Plate Count (TPC) test is to count all of the microorganisms in avocado juice. TPC is usually used to determine whether the sample has experienced rot/damage or not. In several studies, even total microbes that exceed the SNI can indicate the presence of pathogenic microbes that endanger human health. Based on the research results, the total microbes in avocado juice sold in Sukolilo District ranged from  $3.2 \times 10^2$  CFU/ml to  $9.9 \times 10^4$  CFU/ml, with an average value of  $2.61 \times 10^4$  CFU/ml. The lowest TPC result was the AJ sample, namely with a value of  $3.2 \times 10^2$  CFU/ml, the highest value was the AA sample, namely with a value of  $9.9 \times 10^4$  CFU/ml. It is evident from the image that of the 23 samples there are 15 samples (65,22% ) that exceed the SNI regulatory threshold ( $1.47 \times 10^4$  CFU/ml) which is marked with a dotted line in the histogram and 8 (34,78%) samples that do not exceed SNI. The average TPC value in avocado juice sold in Sukolilo District is  $2.61 \times 10^4$  CFU/m, this value is lower than research reported by (Shiferaw & Kibret, 2018), which stated that the average number of aerobic mesophilic microbes from the surface of the fruit avocados surveyed in Northwest Ethiopia were 5.24 Log<sub>10</sub> CFU/g. Likewise, reports stated that twenty-four fruit juice samples were collected from different juice shops, cafes and restaurants in the city of Debre Birhan, Ethiopia, in 2019 and 2020, shows that the average total number of aerobic bacteria is  $1.89 \times 10^6$  CFU/ml (Worku & Hailu, 2023).

This shows that if we buy avocado juice in Sukolilo District, there is a 65% chance of buying juice that is indicated to be rotten and possibly contains pathogenic microbes that are harmful to health. This is in accordance with research by Anwar (2017) that avocado and mango juice sold around the Surakarta Muhammadiyah University campus also exceeds SNI provisions with a total microbial count of  $8 \times 10^7$  CFU/ml for avocado juice and  $3.7 \times 10^8$  CFU/ml for mango juice. Likewise, the report by (Iqbal et al., 2015) the total plate number of fresh fruit juice was  $6.80 + 1.91$  log CFU/ml compared to pasteurized juice which reached  $2.39 + 0.43$  log CFU/ml.

Based on observations made by researchers, all avocado juice sellers in Sukolilo District do not wash their hands when making juice, do not use masks, aprons, gloves or head coverings. Of the 23 avocado juice sellers, only 1 uses plastic gloves, regarding seller hygiene and sanitation. This shows that the seller's lack of awareness of hygiene has resulted in avocado juice being contaminated by microbes. It can be seen that the total number of microbes in avocado juice sold in Sukolilo District is quite high.

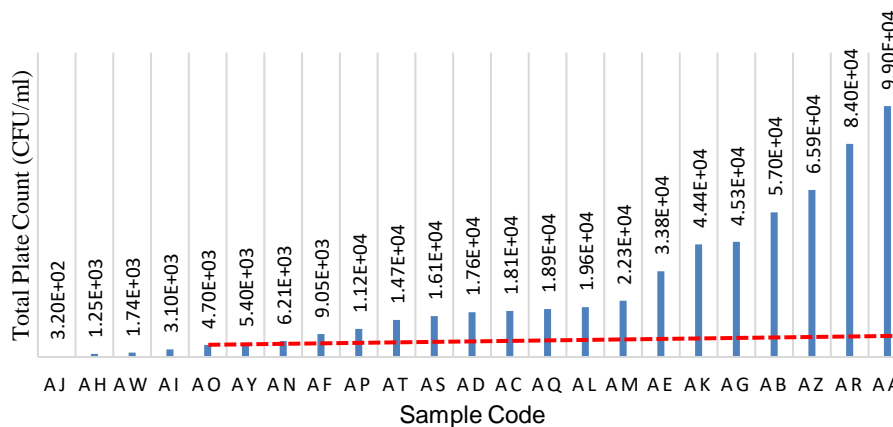


Figure 1. Bar Diagram of Total Microbes in Avocado Juice at Sukolilo District, Surabaya

The water used as the main ingredient in making juice can also influence the total number of microbes in avocado juice. (Khuswataningrum & Pawenang, 2015) said that water plays a very important role in the juice making process as an additional ingredient. Most of the water used by avocado juice sellers in Sukolilo District is refillable drinking water, which is also easily contaminated with microorganisms. Research by (Telan et al., 2015) reported that 40% of refillable drinking water was contaminated with microorganisms. The total microbes contained in avocado juice sold in Sukolilo District are quite high, this indicates that there is a possible source of the high total microbes coming from contamination of the refill water used.

**Total Yeast Mold Content of Avocado Juice in Sukolilo District**

The total yeast mold testing that was carried out in the research aims to determine the number of molds and yeasts in avocado juice. This total test was carried out because avocado juice contains many nutritious components high in carbohydrates in the form of glucose, as reported by (Kusyawati, 2020) that the fruit contains high levels of carbohydrates.

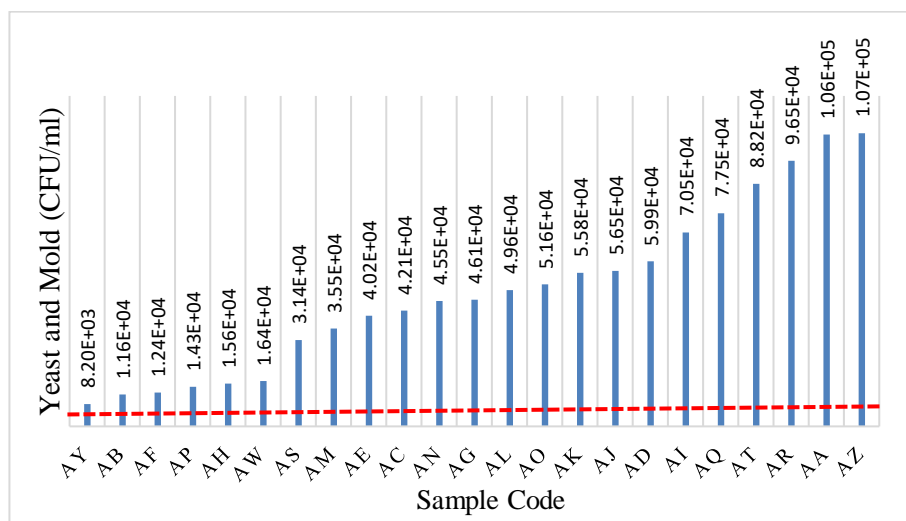


Figure 2. Bar Diagram of Total Yeast and Mold in Avocado Juice at Sukolilo District, Surabaya

The picture showed the total number of yeast molds in avocado juice sold in Sukolilo District. These results are then compared with the maximum standards set by SNI, which are marked with

a dotted line on the graph. Based on the results of this research (Figure 2), total yeast and molds in avocado juice had a number between  $8.20 \times 10^3$  CFU/ml to  $1.07 \times 10^5$  CFU/ml, with an average value of  $4.95 \times 10^4$  CFU/ml. Based on the picture, it can be seen that of the 23 samples) that exceeded the SNI regulatory threshold ( $1.0 \times 10^2$  CFU/ml) which is marked with a dotted line in the histogram. This shows that if we buy avocado juice in Sukolilo District, there is a chance of buying juice that is indicated to contain yeast mold that exceeds the SNI limits which can be harmful to health. This is caused by very poor hygiene and sanitation conditions carried out by traders. Apart from that, there are important things that also need attention, namely the quality of the fruit purchased is not fresh enough, the storage is not refrigerated, thus supporting the growth of mold and yeast in avocado juice. The water used to make juice using refillable water, tools such as blenders that are not washed using detergent, as well as the unhygienic habits of traders in making juice are the causes of high levels of yeast mold in avocado juice.

### Sanitation and Hygiene Research Results

Based on the results of the researcher's observations using a questionnaire sheet, it shows that the hygiene and sanitation of avocado juice sellers in Sukolilo District, Surabaya need to be improved. Because seller hygiene is one of the factors causing contamination by pathogenic bacteria. Therefore, it is very important to pay attention to the hygiene of food sellers. The following are the results of a hygiene and sanitation survey among avocado juice traders in Sukolilo District. This research is in accordance with (Sofia et al., 2021) which states environmental sanitation, fruit juice sellers' sales places in Banda Sakti District It's not good to see from the rubbish that's scattered around, it's open, the condition of the washing place is not good to see from the traders who use the washing placehands, tools and materials in one container simultaneously; and the condition of the drainage channels is not good.

### Seller Hygiene

Seller hygiene is one of the rules that every handler needs to pay attention to to maintain food safety so that it does not become cross-contaminated which can come from serving utensils and from the juice seller's behavior when serving. Regulation of the Minister of Health of the Republic of Indonesia No.1096/Menkes/Per/VI/2011, that one of the factors that influences food contamination is the behavior of food handlers.

Based on observations, almost all traders serve buyers while chatting, which could result in the juice being contaminated during manufacture and serving. This observation was made when the seller made avocado juice for the buyer. Personal hygiene carried out by juice traders can be seen in the following table.

Table 1. Hygiene of avocado juice sellers

No	Seller hygiene		N	%
1.	Use a mouth covering or mask	Yes	0	0
		No	23	100
2.	Keep hair, nails and clothes clean	Yes	6	26
		No	17	7.4
3.	Using an apron	Yes	3	1.3
		No	20	87
4.	Use a plastic mat or gloves	Yes	1	4.3
		No	22	95.6
5.	Do not smoke or scratch your body parts	Yes	23	100
		No	0	0
6.	Don't sneeze or cough in front of fruit juice	Yes	23	100
		No	0	0

Based on the table 1 above, it is generally known that the hygiene of fruit juice sellers in Sukolilo sub-district still does not meet the seller's hygiene rules well enough. This can be seen from 100% of sellers not using mouth coverings or masks during the juice making process, and 7.4% of respondents found that juice sellers do not keep their nails, hair and clothes clean when selling. These findings are consistent with studies carried out by (Mengistu et al., 2021) that 65 (83.3%) food vendors do not wear aprons or uniforms, and 41 (52.6%) food vendors always wash their hands with water and soap. In addition, the study discovered a statistically significant link between juice contamination with *Staphylococcus* and unhygienic conditions that put food handlers in danger.

In this result can be seen when the seller makes juice and has direct contact with the fruit to be processed. A total of 22 people (95.6%) and 20 people (87%) did not use plastic gloves and aprons to cut and peel fruit. Regulation of the Minister of Health of the Republic of Indonesia No.1096/Menkes/Per/VI/2011, that one of the factors that influences food contamination is the behavior of food handlers. Based on observations, almost all traders serve buyers while chatting, which could result in the juice being contaminated during manufacture and serving. This observation was made when the seller made avocado juice for the buyer. According to (Astuti et al., 2020), the TPC in avocado juice is relatively high, namely  $U\ 6.07 \pm 0.37$  to  $8.95 \pm 0.04$  log CFU/ml. This TPC value is high exceeds permitted standards, this is related to poor hygiene and sanitation implementation. Influencing factors high levels of microbial contamination in juice, it can be said to be the initial quality fruit, water, devices, and the environment around, especially those who can contribute the most a lot of it is water

### Equipment Sanitation

Equipment sanitation is an effort to control factors such as materials, food handlers, places and equipment used that may cause disease or other health problems. Food or other drinks can be contaminated by microorganisms through equipment used for the juice production process. The following is equipment sanitation which can be seen in the table.

Table 2. Sanitation of equipment used by juice traders

No	Equipment Sanitation		N	%
1.	Is the equipment used such as a blender washed with clean water?	Yes	23	100
		No	0	0
2.	Do you always wash the blender after every use?	Yes	0	0
		No	23	100
3.	After cutting the fruit, store it in the refrigerator	Yes	0	0
		No	23	100
4.	Wash the fruit with running water	Yes	23	100
		No	0	0
5.	The water used to wash equipment uses running water	Yes	50	50
		No	50	50
6.	Wash the fruit before processing/blending	Yes	0	0
		No	23	100

Based on the table 2 above, it can be seen that the sanitation of the equipment used by fruit juice traders is still not good. This can be seen from the results of observations made, as many as 100% of respondents did not properly wash equipment such as blenders. Blenders that have been used are only rinsed using running water and do not use cleaning soap, this causes the possibility of remaining juice remaining on the blender. As many as 100% of respondents did not put cut fruit in the refrigerator or cooler. This can cause contamination from equipment that is not washed properly. This result is in accordance with the results of (Khuswataningrum & Pawenang,

2015) research that there are 80% of respondents who are easy to understand cleaning equipment, 20% do not. As many as 33.3% of respondents rinsed equipment use running water and 66.7% do not. A total of 8 respondents (53.3%) dried the equipment for making fruit juice with a dry cloth and 46 did not. In line with this research (Mengistu et al., 2022) found a significant relationship between bacterial contamination of fruit juice and several hygiene and safety conditions, such as the place used to store fruit juice after processing, the actions taken to cause the juice to go bad, the frequency of cleaning agents used to store fruit juice such as cupboards, ice and materials used to store fruit juice, type of cooking, place for hand washing, availability of hand washing facilities and type of waste container

## CONCLUSION

Total Plate Count and Total Yeast and Mold in avocado juice in Sukolilo District, Surabaya District, there were 65.22% of samples exceeding the limits determined by SNI for TPC and 100% of samples exceeding SNI limits for total yeast and Mold. Seller's hygiene is not paid enough attention, such as not wearing aprons, gloves, head coverings, and not washing hands when making juice. Sanitation is also not paid attention to, including: the fruit used is of poor quality and stored at room temperature when served, the blender is not washed with detergent, the refilled water is used to make juice

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