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The Effect of Adding Avocados (*Persea americana*) on Microbiological and Chemical Qualities in Yoghurt Drinks

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Abstract: Yoghurt is one of the fermented products from cow's milk and skim milk as a source of protein to cover nutritional needs. With the development of innovation and the creation of food products, yogurt has many flavors from various fruits and vegetables. Yogurt food product innovations can also be made from skim milk combined with the addition of avocados. Avocado is a fruit that contains fiber, riboflavin (B2), niacin (B3), vitamins A, C, E, K, vitamin B6, magnesium, glutathione, and unsaturated fatty acids. The purpose of this study was to describe the effect of the concentration of avocados (*Persea americana*) on the quality of microbiology (total LAB) and chemistry (pH, fat content, and protein content) in yogurt drinks. The research used laboratory experimental methods, and data analysis was done descriptively. The treatment of adding avocados is 0%, 10%, 15%, and 20%. The results of the study obtained the effect of the concentration value of adding avocados (Persea americana) with the results of the study: the total value of LAB increased from 4.1x109±0.20 CFU/mL (10%) to 4.4x109±0.07 CFU/mL (15%), the pH value decreased from 4.30±0.02 (0%) to 3.89±0.01 (20%), The fat content value increased from 0.05±0.007 % w/b (0%) to 0.57±0.005 % w/b (20%), the protein content value decreased from 3.46±0.02% w/b (0%) to 2.94b±0.00 % w/b (20%). The conclusion of this study is the effect of adding avocados (*Persea americana*) on the quality of microbiology (total LAB) and chemistry (pH, fat content, and protein content) in yogurt drinks.

Keywords: Avocado; Fat Content; Protein Content; Total LAB; pH; Yohgurt.

Introduction

Yogurt is a probiotic drink. Yogurt is a processed fermented milk product made from lactic acid bacteria (LAB) starter [1]. Yogurt made from skim milk can increase the nutritional value of yoghurt and provide test results with better consistency and shape [2]. Avocados (*Persea americana*) tend to quickly deteriorate and rot if not handled carefully after peeling. Combining it with a yoghurt drink is the best solution to prolong its freshness. Avocado (*Persea americana*) is a fruit that contains fiber, riboflavin (B2), niacin (B3), vitamins A, C, E, and K, vitamin B6, magnesium, glutathione, and unsaturated fatty acids. One of the preparations is to make avocado (*Persea americana*) in the form of yogurt [3].

The most important factor that makes avocado (*Persea americana*) suitable as an additional ingredient in yogurt is that it can reduce the risk of heart disease. This can be supported by clinical research published in the Journal of the American Heart Association; consuming avocado (*Persea americana*) (≥ 2x servings per week) is associated with a reduced risk of cardiovascular disease and the incidence of coronary heart disease [4]. Based on the Global Burden of Disease and Institute for Health Metrics and Evaluation (IHME) 2014 – 2019, heart disease is the highest cause of death in Indonesia. Basic Health Research (Riskesdas) data for 2013 and 2018 shows an increasing trend in heart disease from 0.5% in 2013 to 1.5% in 2018 [5].

Several factors cause an increase in heart disease, including cholesterol. Cholesterol is also the main cause of the development of various diseases in the body [6]. Avocado (*Persea americana*) is a food that contains unsaturated fat, which can increase *high-density lipoprotein* (HDL) [7]. Avocado (*Persea americana*) contains several active ingredients that are thought to reduce blood cholesterol levels, namely niacin (vitamin B3), β-*sitosterol*, vitamin C, pantothenic acid, oleic acid, *Monounsaturated Fatty Acid* (MUFA), folic acid, selenium, amino acids, and fiber [8]. One type of MUFA often found in nature is omega-9 (Oleate). Omega-9 fatty acids are found in many nuts, avocados, and olive oil [9].

Avocado (*Persea americana*) is suitable as an addition to yogurt drinks because it contains Omega-9. Yogurt drinks contain factors that inhibit cholesterol synthesis, so they can reduce cholesterol levels and prevent blockage of blood vessels (*atherosclerosis*), which causes coronary heart disease [10]. To meet your fat needs, especially essential fats, you can use skim milk when making yogurt drinks and add avocado (*Persea americana*) to it, which contains oleic-rich oil with a low saturated fat content and makes avocado oil suitable for direct consumption and has nutritional support, which is very good and overcomes cardiovascular disease [11].

This study aimed to describe the effect of increasing the concentration of avocado (*Persea americana*) on microbiological quality (total LAB) using total plate count (TPC) and chemistry (pH using a digital pH meter, fat content using Roese-Gottlieb and protein content using Kjeldahl) yogurt drink. Based on this description, this research was carried out by making yogurt with adding avocado (*Persea americana*) with varying concentrations of 0%, 10%; 15%; and 20%. According to [12], the results of

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his research showed that the combination of adding soursop fruit with treatment concentrations of 10%, 15%, and 20% had the best effect on yogurt, the best impact on adding soursop fruit juice at a concentration of 10% and a fermentation time of 12 hours.

Research Methods

Tools and Materials

The tools to be used are glassware, pH meter (Krisbow-10206579), Infrared Moisture Determination Balance (FD-610), laminar airflow (Thermo Scientific 1300 Series A2), analytical balance (Denver S1-234), micropipette (Eppendorf Research Plus), incubator (Temperature Controller XH-W3001), autoclave (Hirayama HVE-50), stirrer (DLAB MS7-H550-Pro), centrifugator (Hrttich EBA 20), vortex (Tab-Net S0200), vortex mixer (Faithful Xh-D), electric stove (Maspion), fume hood, pumpkin kjeldahl, measuring flask, condenser, three-neck flask, stative, clamps, biuret, stopcock, drip pipette, erlenmeyer, spirtus burner, lighter, spray bottle, knife, cutting board, blender, strainer, tube rack, petri dish dish, centrifuge tube, spatula, sterile glass bottle, watch glass, stirrer magnet.

The materials needed are skim milk (*Greenfield*), avocado fruit (*Persia americana*), Sugar, Starter (*Biokul plain*), water, MRSA (Merck), NaCl for analysis (Merck), Alcohol 70%, $CaCO_3$, pH indicator.

Research Procedure

Avocado Fruit Intake

Taking avocados 3 avocados then cleaned with running water. Next, separate the avocado from the skin and seeds, weigh it, and then mash it. Avocado mashed with a ratio of avocado to water 1:2 using a blender ±3 minutes. After blending, it is then filtered and placed into a container.

Yoghurt Making

Making yogurt begins with skim milk and is measured at as much as 400 ml. Skimmed milk is heated at a pasteurization temperature of ±80ºC. After that, turn off the stove and wait for the nails to warm. Pasteurized milk is added with 15% *Biokul Plain* starter, 8% sugar, and 0%, 10%, 15%, and 20% avocado variations. Then, put it into a sealed bottle that has been labeled. After that, the fermentation process is started and put into the incubator at 37°C for 12 hours.

Total Lactic Acid Bacteria (LAB)

Total lactic acid bacteria (LAB) were analyzed using the Total Plate Count (TPC) method—preparation of 100 ml NaCl solution, as much as 0.85 grams of NaCl. Then, the manufacturer of 200 ml MRS A media, as much as 13.4 grams of MRS A, added 1% $CaCO_3$ as much as 1 gram. A petridish dish is prepared as much as needed, then sprayed with 96% alcohol. All preparations are put in plastic and an autoclave for ±3 hours until the temperature drops to 50ºC. Then, it was incubated in the reverse position for 48 hours at 37ºC with the position of the petridish dish turned upside down. Growing colonies can be calculated in units of CFU (Colony Forming Unit)/mL.

Acidity (pH)

Acidity (pH) is analyzed using the digital pH meter method. The pH meter is calibrated first with pH 4 and pH 7 buffers according to the pH range of yogurt. The measurement is done by dipping the pH meter electrode indicator into the sample.

Fat Content

Fat levels were analyzed using the Roese-Gottlieb method. Weighing 5 grams of sample, wetted with NH_4OH, then shaken with Polyethylene solvent. The fatty extract in Polyethylene is collected in a saucer, and the solvent Polyethylene is evaporated. Then, the extracted fat is weighed. The calculation of crude fat content can use the following formula:

$$\text{Fat Content (\% b/b)} = \frac{w2-(w3+w4)}{w1} x100$$

Description:
W1: Sample weight
W2: Tube weight + fat
W3: Tube weight after fat removal
W4 : Weight of residual fat in blanks

Protein Levels

Protein levels were analyzed using the Kjeldahl method. The destruction stage is carried out by weighing 5 grams and then inserted into a 100 ml Kjeldahl tube, which is then added with concentrated H_2SO_4 without samples. Then, it is destroyed at a temperature of 200 – 250ºC for 2 – 3 hours (until the color is clear green). The destruction tube is cooled, and the volume is adjusted with aquades to 50 ml. A total of 20 ml is taken and put into a distillation flask, and then 20 ml of NaOH is added. PP indicator (Phenolphthalein) is added drop by drop. Samples and blanks were distilled using 0.2 M HCl solution. The titration process is ended until the color of the solution in the Erlenmeyer changes from clear green to clear purple, indicating that all N elements react with Cl. The calculation of crude protein content can use the following formula:

$$\text{Protein Levels (\% b/b)} = \frac{(Va - Vb)HCl\ x\ N\ HCl\ x\ 14{,}007\ x\ 6{,}25\ x\ 100\%}{W\ x\ 1000}$$

Description :
Va: ml HCl sample
Vb: ml HCl titration stamp
N: Normality of standard HCl used
14.007: Atomic weight of nitrogen
6.25: Fruit protein conversion factor
W: Sample weight

Results and Discussion

The Effect of Adding Avocados To Lactic Acid Bacteria (LAB)

Total LAB testing was conducted to determine the effect of adding avocados on the growth of lactic acid bacteria in yogurt drinks. This test uses the Total Plate Count (TPC) method to see the total amount of BAL that can grow. The media used is de Man Rogose and Sharpe (MRS) Agar with the addition of $CaCO_3$ and NaCl solvent. The principle of the Total Plate Count (TPC) method is to grow microbial

cells that are still alive on a medium so that the microbial cells multiply to form colonies that can be seen directly by the eye. Colonies that create a clear zone in the media react between lactic acid produced by LAB and CaCO3 during incubation and produce Ca-lactate. The reaction occurs as follows:

$$C_3H_6O_3 + CaCO_3 \rightarrow Ca(C_3H_6O_3) + H_2O + CO_2$$

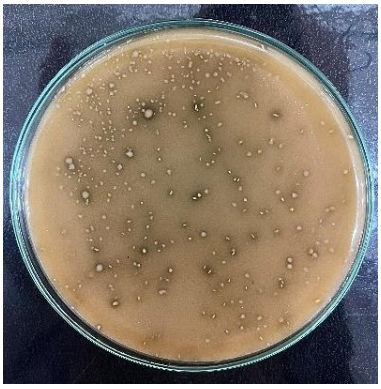

Figure 1. Growing LAB Colonies

Figure 1. indicates that white spherical colonies are formed on MRS Agar and $CACO_3$ media. In addition, a clear zone formed around the colony caused by the reaction between the acid produced by LAB with $CaCO_3$ and the solvent NaCl. The total LAB calculation results are shown in Table 1.

Table 1. The Effect Of Adding Avocados on Lactic Acid Bacteria (LAB) Yoghurt Drinks

No	Addition of Avocados (concentration)	Total LAB (log CFU/mL)
1	0%	$9.3x10^{8a}$ ±0.04
2	10%	$4.1x10^{9a}$±0.20
3	15%	$4.4x10^{9b}$±0.07
4	20%	$10.53x10^{8a}$±0.05

Description: values followed by different letters show a significant difference ($p<0.05$).

The statistical analysis results using One Way Anova showed a significant value of $p<0.05$, indicating the effect of adding avocados on LAB growth. Furthermore, Duncan's Post Hoc analysis was carried out to identify significant differences in each treatment. Statistical results showed a significant difference at concentrations of 10 – 15%, but at concentrations of 0%, 10%, and 20%, there was no noticeable difference. The addition of avocados affects the growth of LAB yogurt drinks, with optimal growth of LAB found at a concentration of 15% from $4.1x10^{9a}$ CFU/mL to $4.4x10^{9b}$ CFU/mL.

According to the Indonesian National Standard (INS), the total LAB in yogurt is at least 107 CFU/mL. An increase in LAB at a concentration of 15% indicates the peak of the exponential phase of LAB growth. LAB can grow rapidly in the exponential phase because it can utilize nutrients in the growing medium [13].

This follows [14], which states that lactic acid bacterial cells can grow and divide exponentially to the maximum number affected by environmental conditions and nutrients in the media. LAB growth in food is closely related to the growing medium available. The development of LAB through the fermentation process can improve the quality of fermented materials because LAB can produce enzymes that can degrade fiber [15].

The process of making yogurt is done by fermentation. Fermentation is a chemical change in food caused by enzymes. Enzymes that play a role can be produced by microorganisms or enzymes that already exist in food [16]. Therefore, adding avocados to yogurt drinks can increase LAB and lower pH. According to research [17], the lower the pH and the higher the LAB, the more LAB utilizes fiber for cell metabolism, hydrolyzes it into simpler compounds, and ferments it through glycolysis to acids. In addition, the fermentation process that breaks down milk lactose into lactic acid can increase acidity, decreasing pH.

The Effect of Adding Avocados on pH

pH testing is carried out to determine the degree of acidity in the sample using the digital pH meter method. The data obtained was normal, so a statistical analysis of One Way ANOVA was carried out. The results showed a significance value of $p<0.05$, indicating the effect of adding avocados to the pH value. Next, Duncan's Post Hoc test was carried out to see the difference between treatments. The pH test results are shown in Table 2.

Table 2. The Effect of Adding Avocados on The pH of Yoghurt Drinks

No	Addition of Avocados (concentration)	pH
1	0%	4.30^{a} ±0.02
2	10%	4.23^{b}±0.01
3	15%	4.19^{c}±0.01
4	20%	3.89^{d}±0.01

Description: values followed by different letters show a significant difference ($p<0.05$).

The results showed a significant difference in the 0 - 20% concentration when adding avocados. The addition of avocados affects the lowering of the pH value in yogurt drinks, as seen in table 2. The pH continued to decrease with each treatment, reaching 3.89 at a concentration of 20% compared to the initial pH at a 0% concentration of 4.31. This is due to the age of the starter and the bacteria in it reaching the logarithmic phase, thus lowering the pH of avocado yogurt. According to INS, yogurt quality requirements range from 3.8 – 4.4 pH yoghurt. The decrease in pH is one of the consequences of the fermentation process due to the production of lactic acid derived from LAB. This is in accordance with [18] that the acidity and pH values are closely related to the increase in metabolism so that lactic acid production increases while the pH value decreases.

The Effect of Adding Avocados on Fat Content

Fat content testing is carried out to determine the crude fat content in the sample using the Roese-Gottlieb method. Fat content is a very important percentage of nutrients because it is a source of energy to improve texture and taste and a source of vitamins A, D, E, and K. The principle of the Roese-Gottlieb method is to extract samples with diethyl ether and petroleum ether. The fat content test results are shown in Table 3.

Table 3. The Effect of Adding Avocados on The Fat Content of Yoghurt Drinks

No	Addition of Avocados (concentration)	Fat Content (% b/b)
1	0%	0.05^{a} ±0.007
2	10%	0.33^{b}±0.003
3	15%	0.48^{c}±0.004
4	20%	0.57^{d}±0.005

Description: values followed by different letters show a significant difference ($p<0.05$).

The data obtained was normal, so a statistical analysis of One Way ANOVA was carried out. The results showed a significance value of $p<0.05$, indicating the effect of adding avocados on protein levels. Furthermore, Duncan's Post Hoc test was carried out to see the significant difference in each treatment. Statistical results show a significant difference in concentrations of 0 – 20%. Adding avocados is known to influence the fat content of yogurt drinks, characterized by increased concentration. The concentration at the beginning of adding avocados is 0.05%, which gradually increases to 0.57% after 20%.

Increased fat levels occurred in each treatment with an average value of 0.05 – 0.57%, according to SNI 01-2981-1992 [19]. The fat content in yogurt that meets the quality requirements must have a maximum fat content of 3.8%. The results showed that the fat content met the INS yoghurt quality requirements. According to [20], Yogurt fat can be distinguished from full-fat yogurt (>3%), half-fat yoghurt (0.5% - 3%), and low-fat yoghurt (<0.5%). Avocado yogurt is low-fat because avocados have a relatively low-fat content of 14.66 grams. According to [21], Lactic acid bacteria have secondary lipolytic activity, meaning lipolytic activity occurs after other microorganisms break down milk fat into simpler compounds. During fermentation, the lipase enzyme hydrolyses fats into simpler compounds, producing fatty acids and glycerol. The activity of the lipase enzyme reflects the amount of fat broken down into simpler, more digestible compounds. Low-fat content goes hand in hand with [22]. Lipotic activity is controlled by lipase enzymes produced by lactic acid bacteria, and decreasing pH and lipase will free fatty acids from fat molecules. In addition, fermentation bacteria also have strong reducing properties. Under active fermentation conditions, media containing sugar and other compounds will reduce aldehydes to alcohol, affecting the increase in fat [23].

The Effect of Adding Avocados on Protein Levels

Protein content testing is done to determine the crude protein levels in the sample using the Kjeldahl method. Protein content is the percentage of protein content in a product, and nasal protein content is the remaining protein not used by starter bacteria during storage. The principle of the Kjeldahl method is that nitrogen in proteins is released as ammonia through a digestion process using concentrated H_2SO_4 by heating. Next, ammonia is bound by concentrated H_2SO_4 to ammonium sulfate. The results of the protein content test are shown in Table 4.

The data obtained was normal, so One Way Anova statistical analysis was conducted. The study's results showed a significant value of $p<0.05$, which indicates the influence of adding avocado on the protein content value. According to [24], the more concentration of klenceng honey (*Trigona sp*) is added, the more yogurt protein levels will increase. Furthermore, Duncan's Post Hoc test was carried out to see the significant difference in each treatment. Statistical results showed a significant difference at concentrations of 0 – 10%, but there was no significant difference at concentrations of 10 – 20%. The addition of avocados has an effect on reducing the protein levels of yogurt drinks, where protein levels at an initial concentration of 3.46% decreased to 2.94% at a concentration of 20%.

Table 4. The Effect of Adding Avocados on The Protein Content of Yoghurt Drinks

No	Addition of Avocados (concentration)	Protein Levels (% b/b)
1	0%	3.46^{a} ±0.02
2	10%	3.03^{b}±0.08
3	15%	2.98^{b}±0.02
4	20%	2.94^{b}±0.00

Description: values followed by different letters show a significant difference ($p<0.05$).

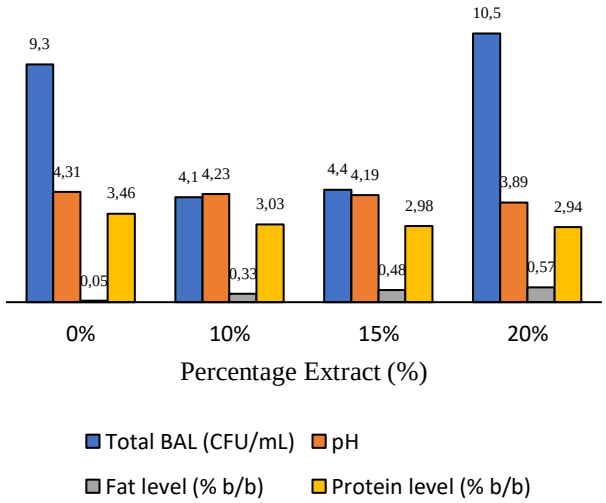

Figure 2. Bale Growth, pH Decrease, and Fat Levels Increase in Yogurt Based on the Addition of Avocados.

Increased fat levels are also correlated with decreased protein levels. The decrease in protein levels occurred at a concentration of 0% from 3.46% to 3.03%; according to the Indonesian National Standard (INS), good yoghurt has a minimum protein content of 2.7%. Adding avocados causes a decrease in protein levels due to increased bacterial division activity, requiring more amino acids as an energy source. This follows [25] that optimal BAL formulation and fermentation time will produce the highest levels of latured protein. The process of making yogurt using the same lactic acid bacteria (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*). *Lactobacillus bulgaricus* bacteria are better because they ferment glucose and lactose and can grow at 45 - 50 °C temperatures. *Lactobacillus bulgaricus* is more tolerant of high acidity [26]. *Streptococcus thermophilus* is a spherical type of bacteria [27] with an optimal growth temperature of 40 - 45 ° C and an acidity tolerance of 0.85 - 0.89%.

According to [28], fat content decreases after fermentation because *Lactobacillus bulgaricus* can reduce fat content by being absorbed as a source for growth. The

optimum temperature for the development of *Lactobacillus bulgaricus* is 44ºC. These lactic acid bacteria will produce lipase enzymes, decomposing fat into fatty acids. Then, these fatty acids will be broken down into compounds with a distinctive yogurt aroma.

According to [29], the number of living bacterial cells influences protein levels, and an increase in the number of living bacterial cells will increase the number of enzymes, including protein-breaking enzymes (proteases), which are used to break down proteins (proteolytic activity) and increase protein synthesis. Proteins will be broken down into peptides and further hydrolyzed into amino acids. These breakdown results serve as precursors for enzymatic and chemical reactions to form flavors.

Conclusion

Based on data obtained in research regarding the effect of adding avocado (*Persea americana*) on the microbiological and chemical quality of yoghurt drinks with the addition of avocado (*Persea americana*) concentrations of 0%, 10%, 15%, 20%. The addition of avocado (*Persea americana*) affected the microbiological quality in that the total BAL value increased from 4.1x109±0.20 CFU/mL (10%) to 4.4x109±0.07 CFU/mL (15%) so that Avocado yoghurt drink (*Persea americana*) is one of the products that influences LAB growth and reaches optimal levels due to enzyme activity. The addition of avocado (*Persea americana*) affects the chemical quality as the pH value decreases from 4.30 ± 0.02 (0%) to 3.89 ± 0.01 (20%) so that the avocado (Persea americana) yoghurt drink. It meets the quality requirements for the acidity level of yoghurt. The addition of avocado (*Persea americana*) affects chemical quality in that the fat content value increases from 0.05±0.007 % w/w (0%) to 0.57±0.005 % w/w (20%), so it meets the requirements quality low-fat yoghurt. The addition of avocado (*Persea americana*) affects chemical quality. The protein content value decreases from 3.46 ± 0.02% w/w (0%) to 2.94 ± 0.00 % w/w (20%), so it is not recommended as high in protein.

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