Jurnal Riset Rumpun Ilmu Kesehatan (JURRIKES)
Vol.2, No.1 April 2023
e-ISSN: 2828-9374; p-ISSN: 2828-9366, Hal 01-17

SANCACO COFFEE SYINBIOTIC (PROBIOTIC AND PREBIOTIC) AND ANTIBACTERIAL ASSAY AGAINST *Escherichia coli*

Miksusanti[1*], Adik Ahmadi[2], Syndi N.K[2,] Dasril Basir[1], Heni Yohandini K[1]

[1]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Sriwijaya University, Indralaya, Ogan Ilir, South Sumatra, Indonesia

[2,]Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Sriwijaya University, Indralaya, Ogan Ilir, South Sumatra, Indonesia
Corresponding authors: miksusanti@gmail.com

ABSTRACT

Robusta coffee is one of the plants used as a drink and has an efficacious content that can be modified to be used as a cure for diarrhea. This research was conducted To determine the effect of synbiotic coffee with a combination of coffee, prebiotic inulin chicory, probiotic B. bifidum, and glucose on the growth and antibacterial activity of the probiotic Bifidobacterium bifidum BRL-130. Organoleptic testing of synbiotic coffee was performed on 30 panelists, then the growth test used the total plate count method, and the antibacterial test used the paper diffusion method. The organoleptic test showed that the selected formula 1 had the highest average taste of 3.76. In the symbiotic coffee growth test, the best results were obtained in week 1 of plain water with a temperature of 40°C with the number of colonies 5.95 x10^{8} CFU/mL. Antibacterial activity test using paper disc diffusion method with a variation of the selected formula 1 treatment control, 2 treatment control pure robusta coffee powder, positive control of ciprofloxacin antibiotic, and negative control of aquadest solvent. The antibacterial activity of synbiotic coffee against E. coli showed that there was an inhibitory response in formula 1 against Escherichia coli bacteria in the first week of storage, 12.3±1.1 mm with strong criteria.
***Keywords**: Sancaco robusta coffee, synbiotics, Bifidobacterium bifidum BRL-130, inulin chicory, E. coli.*

ABSTRAK

Kopi jenis robusta merupakan salah satu tumbuhan yang digunakan sebagai minuman dan memiliki kandungan yang berkhasiat dan dapat diramu dengan tepat untuk membantu mengobati diare. Penelitian ini bertujuan melihat pengaruh kopi Sancaco sinbiotik dengan kombinasi kopi sancaco, prebiotik inulin cikori, probiotik *B. bifidum* dan glukosa terhadap pertumbuhan dan aktivitas antibakteri probiotik *Bifidumbacterium bifidum* BRL-130. Dilakukan pengujian organoleptik terhadap kopi Sancaco sinbiotik pada 30 panelis, kemudian pada uji pertumbuhan menggunakan metode total plate count, dan pada pengujian antibakteri menggunakan metode difusi kertas. Hasil uji organoleptik menujukan formula 1 yang terpilih memiliki rata-rata rasa tertinggi 3,76. Pada uji pertumbuhan kopi Sancaco sinbiotik diperoleh hasil terbaik pada minggu 1 air biasa dengan suhu 40°C dengan jumlah koloni 5,95 x10^{8} CFU/mL. Uji aktivitas antibakteri menggunakan metode difusi cakram kertas dengan variasi kontrol perlakuan formula 1 terpilih, kontrol perlakuan 2 bubuk kopi Sancaco robusta murni, kontrol positif antibiotik ciprofloxacin dan kontrol negatif pelarut aquades. Hasil uji aktivitas antibakteri kopi

Received November 02, 2022; Revised November 20, 2022; Accepted Desember 22, 2022

Sancaco sinbiotik terhadap *E. coli* menunjukkan bahwa penyimpanan tiap minggunya terdapat respon hambatan pada formula 1 terhadap bakteri *Escherichia coli* dengan hambatan paling kuat pada minggu pertama sebesar 12,3±1,1 mm dengan kriteria kuat.

Kata kunci: Kopi Sancaco robusta, sinbiotik, *Bifidobacterium bifidum* BRL-130 ,inulin cikori, *E. coli*.

INTRODUCTION

In this era of globalization, the increasing needs and desires of humans influence lifestyle changes, especially the daily diet. The wrong diet triggers various diseases, especially digestive tract disorders such as diarrhea. According to the (Depkes RI, 2011; WHO, 2016), from 30 provinces in Indonesia, data on diarrhea estimates in health facilities reached 5,097,247 people in 2015. This data is related to the 2007 *Basic Health Research Report*, which shows that most of the Indonesian population still consumes less fiber from vegetables and fruits. Fruit, lack of exercise, and consuming foods containing preservatives and synthetic dyes (Rautiainen et al., 2015). Low whole fruit intake represents a potentially more serious global population health threat than previously recognized, especially in light of the emerging research on whole fruit and fruit fiber health benefits (Dreher, 2018). This can be overcome by changing and adjusting the diet and consuming food products that are good for digestion (Dreher, 2018).

At this time food technology has changed the function of food, apart from being used as a hunger tool. The potential mechanisms by which nutraceuticals/functional foods/food supplements may alter a host's health are also highlighted in this paper (Cencic & Chingwaru, 2010). It also produces functional food products, which can protect the human body and even treat several diseases (Martirosyan et al., 2021). One of the functional food products that can be developed is synbiotic drinks. (Senditya et al., 2014) states that synbiotic is a term used in naming food products in which there is a mixture of probiotics and prebiotics (Cencic & Chingwaru, 2010). This food uses a mixture of prebiotics and probiotics because it has a good working mechanism for increasing digestive endurance (González-Herrera et al., 2021). This synbiotic food can also inhibit the growth of pathogenic bacteria (Pandey et al., 2015). Probiotics compete in using nutrients (Dahiya & Nigam, 2022). Synbiotics are a combination of the concepts of probiotics and prebiotics. So synbiotics contain live microbes that are stimulated by the presence of prebiotics (Verma et al., 2012). The advantage, in addition to the health effects of commercial probiotics, is also the presence of prebiotics that encourage the growth of probiotic organisms in the colonic complex (Miksusanti et al, 2016; Miksusanti et al., 2020; Miksusanti et al, 2022).

Probiotics are generally called healthy drinks which contain lactic acid bacteria. These bacteria can survive in the acidity of the stomach and can occupy the intestines in large enough quantities. This serves to balance the microbes in the digestive tract so that digestion is protected from pathogenic bacteria (Tanaka et al., 2017). Lactic acid bacteria need good food for growth in the digestive system, namely prebiotic compounds that contain lots of fiber and carbohydrates such as inulin, FOS (Fructo-oligosaccharides), and GOS (gluco-oligosaccharides) (Miksusanti et al, 2016), where the food content is not digested and can have

a positive impact. For digestion because it can stimulate the growth and activity of normal microflora in the intestine.

Coffee is one of the most popular drinks worldwide and covers every circle, from the lower to the upper class (Yeretzian et al., 2017). Coffee has a unique taste and interesting aroma that gives the effect of a fresher body (Seninde & Chambers, 2020). The content of coffee besides caffeine is chlorogenic acid, trigonelline, volatile compounds, amino acids, and carbohydrates (N.Farhaty &Muchtaridi, 2016; Handayani & Muchlis, 2021). The most abundant polyphenolic compound in coffee is chlorogenic acid, which reaches 90% of the total phenol in coffee (Yusmarini, 2011), Chlorogenic acid has strong antioxidant activity because it is antifungal, antiviral, hepatoprotective, antioxidant, anti-inflammatory, and antibacterial (Jung et al., 2017; Segheto et al., 2018).

METHODOLOGY

The materials used consisted of the probiotic microbial culture *B. bifidum BRL*-130, and pathogenic bacteria *Escherichia coli*, Sancaco robusta coffee, chicory inulin powder (crafty), skim milk, sodium alginate, sterile distilled water, ciprofloxacin antibiotic tablets, NaCl 0, 9%, MRSA media (de Mann Rogose and Sharpe Agar), MRSB media (de Mann Rogose and Sharpe Broth), NA media, NB media.

Bacterial Preparation

The test bacteria used were *B. bifidum* obtained at the Center for Food and Nutrition Studies, Gadjah Mada University. Mixture Preparation of *Bifidobacterium bifidum* BRL-130, Sodium Alginate, and Freeze Drying Skin Milk (NuaireNU9483GC) (J. Y. Kim et al., 2010). Preparation of the material to be freeze-dried is carried out by dissolving 1 dose of *B. bifidum* suspension into 10 grams of skim milk in 100 ml of distilled water, diluted, and then incubating at 37°C for 8 hours, then adding 4 grams of sodium alginate in 100 ml. mL of distilled water, then freeze-dried and then frozen at -23°C for 24 hours.

Composition of Synbiotic Sancaco Coffee

The formulation of synbiotic coffee beverage preparations can be seen in table 1. The process of making probiotic coffee refers to the modified (Fawzan et al., 2019). For the organoleptic test samples, three variants of the synbiotic coffee formulation were made. Pure robusta coffee powder was added with probiotics (a mixture of *B. bifidum* and sodium alginate, skim milk from (freeze drying) then added inulin powder from chicory was. Adding a 0.5% inulin concentration to the preparation was the most effective modification in increasing the growth probiotic (M. H. Marhamatizadeh et al., 2014). Acidophilus when compared with other treatments. In this study, the concentration was combined again for 3 formulas, added glucose according to the combination of formula table 1 below, and stirred evenly using a mortar until homogeneous, after mixing 25 grams were taken for each formula, and then added water with a temperature of 40°C as much as 300 ml and then stirred until smooth using a glass spatula for 15 seconds and distributed to each panelist as much as 20 ml for organoleptic. One of the best synbiotic coffee formulas that the panelists liked the most and then stored for 1 week, 2 weeks, and 3 weeks was used for further analysis.

Table 1. Formulation of Synbiotic Sancaco Coffee

Formula	Coffee	Glucose	Chicory Powder	*B. bifidum powder*
F1	20. 62%	3. 75%	0. 15%	0. 50%
F2	20. 22%	3. 75%	0. 02%	1. 00%
F3	19. 25%	3. 75%	0. 50%	1. 50%

Organoleptic Test (SNI-01-2346, 2006)

Organoleptic tests were carried out on the three variants of the coffee formula. Then organoleptic tests were carried out to assess the differences in the composition of the three variants to find the best and most preferred formula composition. Organoleptic includes taste, aroma, color, and texture (Sabam Malaua et al., 2018). Then the sample was assessed by 30 untrained panelists. Samples were placed in a container and coded according to treatment and given 20 ml to each panelist. Panelists were asked to rate each sample on the questionnaire sheet that had been presented. The scale used in this study consisted of five numerical scales, namely strongly dislike (1), dislike (2), neutral (3), and like. (4), very like (5).

Table 2. Effect of Inulin Chicory on the Growth of *B. bifidum* In Sancaco Coffee

No	Treatment	Description
1	Positive (+)	Control Suspension *B. bifidum* +MRSB
2	Negative (-)	Control Aquades sterile+ *B. bifidum* +MRSB
3	Treatment Control	Selected Coffee (Robusta coffee + *B. bifidum* powder + inulin + glucose + aquades + MRSB) with storage time 7.14, and 21 days and brewing at 40°C and 70°C

Selected synbiotic coffee, which was stored for 7 days, 14 days, and 21 days, was then used as two control treatments, namely treatment control 1 synbiotic coffee brewed with 40°C plain water. Treatment control 2, brewed with 70°C hot water and tested every week. his. Bacterial growth was tested using the total plate count method with MRS agar media. 1 ml sample of selected synbiotic coffee was taken into a test tube with a micropipette and then added physiological NaCl was so that the volume was exactly 10 mL. The test solution groups of each concentration were diluted 10^{-1}-10^{-8} and were carried out in duplicate 10^{-6}-10^{-8} dilutions into MRS agar media. Petri dishes were incubated at 37ºC for 48 hours in an inverted position. Then the number of probiotic bacteria was calculated by the calculation formula based on (SNI-01-2346, 2006)

Antibacterial Activity Assay

The antibacterial activity test refers to (Miksusanti et al., 2016) which has been modified using the paper disk diffusion method. Disk paper with a diameter of 6 mm was taken aseptically using sterilized tweezers. Disk paper was immersed in the treatment control of 1 selected synbiotic copy. Ordinary water treatment was 40°C as a control in treatment 1. The positive control was ciprofloxacin antibiotic, and the negative control was distilled water. And control in treatment 2 was pure robusta coffee. The media were incubated for 24 hours at 37°C. The antibacterial activity of synbiotic coffee was seen from the inhibition zone obtained (Akhlaghi et al., 2019). The inhibition zone looks clearer than the surrounding area and is not overgrown with bacteria. The inhibition zone was measured by placing a caliper on the outer boundary of the disk paper up to the longest limit and the shortest limit of the inhibition zone formed so that the radius of the longest inhibition zone and the radius of the shortest inhibition zone were obtained.

Data analysis

The results of organoleptic tests, data on the growth of probiotic bacteria, and data on antibacterial activity were analyzed using the SPSS for Windows v.24.0 programs. Data analysis includes testing of normally or abnormally distributed data. The normality test used was Shapiro-Wilk, with a significance level of 0.05. If the results obtained are normal, then they are analyzed using the Independent t-test. If the results are not normal, then continue with the Kruskal Wallis test (Vargha et al., 1998). Then proceed with testing one way ANOVA data with a 95% confidence level to see if there is a significant difference or not. If it shows significant results, namely there is a significant difference, then it is continued with the LSD Post Hoc test analysis to determine the difference (the significance between test groups) with > 0.05.

RUSULT AND DISCUSSION

Sancaco Synbiotic Coffee

This research was conducted using robusta coffee beans (*coffee canephora*) originating from the Agung Lawangan village, North Dempo District, Pagaralam City, South Sumatra. The choice of robusta coffee is because the coffee contains caffeine, phenolic compounds, trigonelline, and chlorogenic acid, which have antibacterial activity. Synbiotic coffee is made by adding several components such as glucose, chicory inulin, and probiotics (*B. bifidum*).

The addition of glucose affects the growth of *B. bifidum* bacteria because glucose is an important nutrient for the growth of LAB as an energy source, where the glucose is broken down into pyruvic acid through the Embden Meyrhrof-Parnas (EMP) pathway into lactic acid so that it can trigger the growth of LAB colonies quickly in large quantities. (Mbae et al., 2022). Meanwhile, the addition of chicory inulin plays a role in increasing the growth of *B. bifidum* bacteria, where inulin will work as a carbon source in LAB cells, which will produce energy that is used for growth, cell reproduction, and activity of probiotic bacteria (Kurnia, 2021). In addition to producing energy for metabolism and cell division, this fermentation also produces a by-product in the form of lactic acid which causes the pH in the large intestine to decrease so

that the growth of pathogenic bacteria is inhibited. Freeze drying was carried out at the LIPI Research Center for Biomaterials, Cibinong, Bogor, West Java.

Synbiotic Coffee Analysis

Organoleptic Test

Organoleptic tests were carried out visually to determine the physical appearance of the synbiotic coffee preparation. It was conducted using 30 untrained panelists to provide assessing the three coffee compositions given (Seninde & Chambers, 2020). Panelists were given a sample of formula 1.

Formula 2 and Formula 3 were treated with water at a temperature of 40°C and plain water and observed organoleptically. This organoleptic test was carried out by 30 panelists. The organoleptic results of synbiotic coffee can be seen in Figure 1

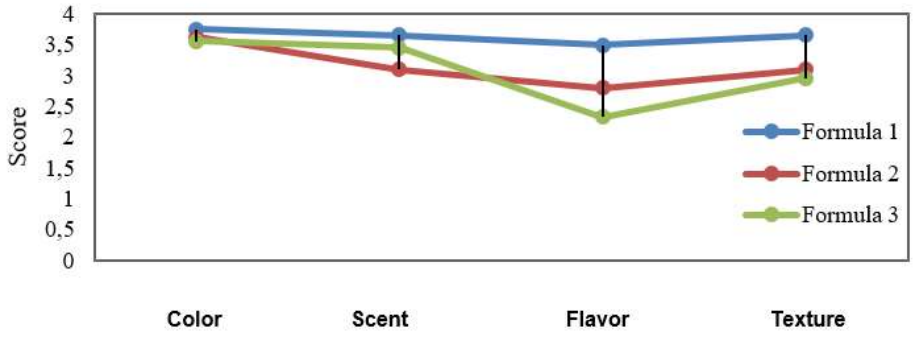

Figure 1. The graph above is the average value of the organoleptic response

From 30 panelists assessing the taste and aroma produced by each formula. From the analysis results, it can be seen that formula 1 has the most preferred level, namely 14 panelists with an overall average of 2.91. Formula 2 is 9 panelists with an average of 2.52, and Formula 3 has only 7 panelists with an average of 2. ,45. A significant difference is found in the taste factor in coffee drinks because the taste is a factor that greatly determines the consumer's final decision to accept or reject a drink, even though it is known that other formulas are better if the taste is not good or not liked, then the drink is rejected (McCain-Keefer et al., 2020). The composition of robusta coffee in Formula 1 is the highest compared to the others, which is 20.62% and from the three formulas, it only differs in the amount of coffee composition and other additives, especially the number of probiotics, resulting in a sharper taste and smell so that panelists are interested in it. The results of the three formulas show a neutral category.

Furthermore, the SPSS®24 test analysis was carried out, based on organoleptic value data, the difference was tested using Kruskal Wallis (Vargha et al., 1998) because the data analyzed were more than two formulas which were ranking tests of three unpaired data. The test results showed that there was no significant effect on the odor and taste parameters ($p>0.05$). Meanwhile, the color and texture parameters have a significant effect ($p<0.05$). This is because the results of the color and texture organoleptic test values in formulas 2 and 3 tend to be lower than in Formula 1. After all, formulas 2 and 3 have a dark brown color and the

texture is a bit thick, then in formulas 2 and 3 also the aroma and The taste is less sharp so it is not liked by many panelists because the two problems are in the lack of coffee composition in formulas 2 and 3. affect the level of preference of the panelists.

Effect of Sancaco Synbiotic Coffee on Growth of *Bifidobacterium bifidum* BRL-130

Testing the effect of synbiotic coffee drinks on the growth of *B. bifidum* using the best control formula treatment that had been selected from 30 panelists during the organoleptic test that had been stored for 1-3 weeks at room temperature in plastic clips, then made two treatments namely control, treatment 1 was brewed with plain water at a temperature of 40°C, and control treatment 2 with hot water at a temperature of 70°C aimed to compare the 2 temperatures on the growth of synbiotic coffee bacteria (Majeed et al., 2019). The positive control was in the form of a suspension of *B. bifidum* to compare the number of *B. bifidum* probiotic bacteria that had not been mixed with other additives. The negative control used was sterile distilled water and *B. bifidum* probiotic bacteria (Chaturvedi & Chakraborty, 2021). The aim is to find out whether aquadest solvent can affect the growth of *B. bifidum* bacteria.

Measurement of total lactic acid bacteria was carried out using the cup count method or commonly known as the total plate count method. The purpose of this method is to analyze the total number of microbes contained in a sample by culturing on MRSA growth media. The suitable medium for growing lactic acid bacteria is Man Rogosa Sharpe Agar (MRSA) which is a selective medium for the growth of lactic acid bacteria (Rahmawati et al., 2021). Lactic acid bacteria were inoculated on MRS media to grow lactic acid bacteria colonies, as indicated by a clear zone around the growing colonies (Husain et al., 2020). The clear zone formed indicates the presence of lactic acid production by these bacteria.

Calculation of total LAB begins with homogenization of the sample and then diluting in NaCl at a ratio of 1:9. The dilution was carried out from 10-1 -10-8. The dilution solution used is in the form of a physiological salt containing NaCl (0.9%) which functions to maintain the ion balance of microbial cells so that bacteria can survive.

Table 3. Effect of storage time on the Growth of *Bifidobacterium bifidum* BRL-130 in Synbiotic Coffee

treatment		Probiotic Bacteria (CFU/mL)
Week 1	Temperature 40°C	5.95 x10^8 CFU/ mL
(F1)	Temperature 70° C	4.08 x 10^8 CFU/ mL
Week 2	Temperature 40° C	5.46 x 10^8 CFU/ mL
(F1)	Temperature 70° C	2.90 x 10^8 CFU/mL
Week 3	Temperature 40° C	4.99 x 10^8 CFU/ mL
(F1)	Temperature 70° C	1.15 x 10^8 CFU/ mL
Positive control (suspense *B. bifidum*)		6 x 10^8 CFU/ mL
Negative Control (aquadest *sterile* + *B. bifidum*)		8.03 x 10^7 CFU/ mL

Based on the results of testing the growth of *B. bifidum* bacteria after being stored for 3 weeks at room temperature with plastic clips, and being tested for growth every 1 week, the positive control results were obtained in the form of a suspension of *B. bifidum*. The negative control in the form of *B. bifidum* bacteria and sterile distilled water as solvents was used as a solvent to see whether distilled water would affect or not the activity of bacterial growth, it means that distilled water affected the growth of *B. bifidum* probiotic bacteria even though it was only slightly 8.03 x10^7CFU/mL.

The highest number of colonies produced was in the control of ordinary water treatment at 40°C with a storage time of 7 days with the number of colonies of 5.95 x 10^8CFU/mL. This high total value of lactic acid was due to the incubation time the glucose reshuffle process occurred by LAB is longer and optimal so that the lactic acid produced is high, and the increase in LAB activity in breaking down lactose will become lactic acid which is the end product of LAB metabolism (Bintsis, 2018). It was followed by week 2 with the number of colonies of 5.46 x 10^8 CFU/mL, and the last week the number of colonies was 4.99 x 10^8 CFU/mL. The number of colonies in the 70°C hot water treatment in week 1 with the number of colonies was 4.08 x10^8 CFU/mL, followed by the second week of 2.90 x 10^8 CFU/mL, and the last week 3 was 1.15x 10^8 CFU/mL. The number of *B. bifidum* microbes at a temperature of 40°C and 70°C with a storage period of 3 weeks during the test period decreased due to the accumulation of lactic acid, acetic acid, and other acid products that caused a decrease in pH. The difference in the total amount of LAB from each beverage treatment with various additions of LAB was influenced by the availability of substrate in the media and storage time. Meanwhile, each lactic acid bacteria (LAB) has a different generation time, causing the ability of the log phase (adaptation) of the bacteria to varying. The growth of LAB colonies in this study was also influenced by the sugar content in synbiotic coffee as a carbon source (Wang et al., 2021).

The decreased viability of probiotic powder is influenced by the temperature factor in storage (Trisnawita et al., 2018; Østlie et al., 2005). From the results above, the control of hot water treatment with a temperature of 40°C and 70°C decreased every week due to cell dehydration, which resulted in cells suffering from osmotic shock and cell leakage. Heat has been reported to damage various cell structures including damage to cell membranes, ribosomes, DNA, RNA, and enzymes. DNA is still defined as a lethal target molecule because using hot water temperatures exceeding 45-50°C can damage the survival of *B. Bifidum* bacteria. However, in this study, the temperature of ordinary hot water at 70°C for the 3rd week still met the requirements of the Indonesian National Standard (SNI-7552, 2009), which states that the minimum requirement for a good total LAB value is 10^6 CFU/mL. The requirement for a product to be said to be probiotic is the product contains a total LAB that is still alive at the time of consumption of 10^6 CFU/mL.

The acids produced during storage cause a decrease in viability in several ways, namely lactic acids (CH_3CHOOH) dissociate or split into CH_3CHOO^- and H^+. Acid molecules that are not dissociated into the cytoplasm will then dissociate into H^+ resulting in a decrease in cytoplasmic pH which causes proteins or enzymes to be denatured. Additionally, it is suspected that the organic acids produced by LAB cause disruption of the host cell wall, resulting in a

decrease in cell viability. In general, cell viability depends on the strain used, the culture conditions, the oxygen content, the acidity of the product, and the concentration of lactic acid (Bedani et al., 2013). The main factors that cause a decrease in the viability of *Bifidobacterium* strains are a decrease in the pH of the medium and the accumulation of organic acids (Parhi et al., 2022).

The addition of 10% skim milk in the freeze-drying process and 3.75% glucose also affected the growth of *B. bifidum* bacteria because both are important nutrients for LAB growth as an energy source, where glucose is broken down into pyruvic acid via the Embden Meyrhrof-Parnas (EMP) pathway(Jojima et al., 2021). into lactic acid so that it can trigger the growth of LAB colonies quickly in large quantities (Samsul Rizal et al., 2016) then the addition of sodium alginate can protect lactic acid bacterial cells against low pH to provide high lactic acid growth when applied to products that have low pH (Mahmoud et al., 2020; Coelho-Rocha et al., 2018).

The growth of probiotic bacteria is influenced by growing conditions such as pH and nutritional and nutritional supplements. The better the media used contains the nutrients needed by probiotic bacteria, the higher the growth of probiotic bacteria (Widowati et al., 2011) MRS Agar media has nutrients that are suitable for the growing needs of lactic acid bacteria. The increase in the number of bacteria that grow in the media is due to the presence of sugar substrates consumed in the media used by bacteria for cell growth, and maintenance, as well as the formation of organic acids, especially lactic acid.

Statistical Analysis on the growth of Probiotic

The results obtained were then analyzed using SPSS 24 (Daniel Arkkelin, 2014). Based on the normality test data for the growth of symbiotic coffee with plain water at 40°C with a sig value of more than 0.05 (p-value> 0.05). The results of the normality test indicate that the data is normally distributed. Then the homogeneity test was carried out, where the sig value obtained was homogeneous. Then we continued with the One Way ANOVA test (T. K. Kim, 2017) where the sig value obtained was more than 0.05 (p-value < 0.05). This shows that there is a significant difference. However, the post-hoc output data can be seen at week 1 against week 3 for positive control and negative control with sig values obtained below 0.05. Additionally, from week 2 to week 3, the sig value obtained was > 0.05, which indicated that there was a significant difference in the length of storage time.

Symbiotic coffee growth test data with hot water at 70°C, sig value more than 0.05 (p-value > 0.05). Then the results of the normality test indicate that the data is normally distributed. Then the homogeneity test was continued, where the sig value obtained was homogeneous. Then proceed with the Way ANOVA test (T. K. Kim, 2017) where the sig value obtained is <0.05 (p-value> 0.05). This shows that there is a significant effect. Based on the post-hoc output data, it can be seen that at week 1 to week 3 and negative control and also at week 3 to the positive and negative control, the sig value obtained is below 0.05, which indicates that there is a significant difference between test control.

Furthermore, for the comparison between the control treatment with plain water at 40°C and hot water at 70°C, a normality test was carried out which showed that the data were normally distributed (sig> 0.05). Next, a paired sample test was conducted to determine

whether there were differences in the growth of probiotic bacteria in synbiotic coffee due to temperature differences (Majeed et al., 2019). The results obtained, a value of sig 0.00, which indicates that there is a significant effect of temperature on the control treatment between ordinary water at 40°C and hot water at 70°C. It can be concluded that normal water at 40°C can increase the growth of *B. bifidum* bacteria, which is more effective than hot water with a temperature of 70°C.

Testing the antibacterial activity of synbiotic coffee using the Kirby-Bauer method (diffusion agar) using disk paper (Sathyabama et al., 2012). The working principle of this method is to see the clear zone around the paper disk. The presence of an inhibitory response to bacterial growth by an antibacterial compound is indicated by the presence of a clear zone around the paper disk (Sari et al., 2017)The diameter of the disk paper used in this test is 6 mm. The basis for choosing this method is that it is the simplest and fastest way to see the sensitivity and effectiveness of an antibacterial substance and see the response to its growth inhibition.

Antibacterial testing used samples from the viability test results, namely the control treatment of formula 1 plain water at 40°C which had been stored from 7 days to 21 days, the positive control used was ci ciprofloxacin antibiotic tablets, the negative control was sterile distilled water, and the control treatment 2 was Robusta coffee. Pure temperature 40°C. The purpose of this antibacterial test is to see the effect of synbiotic coffee bacteria inhibition on *E. coli* diarrhea bacteria with a storage period of 21 days or 3 weeks.

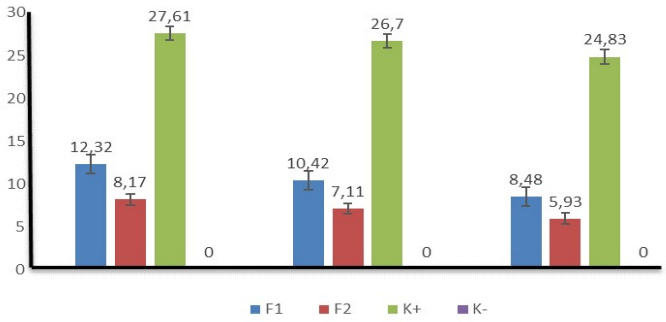

Figure 2. Antibacterial of Synbiotic Coffee against *E. coli*
F1: Sancaco robusta coffee, chicory inulin, *B. bifidum* powder, and glucose.
F2: Pure Sancaco robusta coffee (without mixture)
K+: Ciprofloxacin antibiotic tablet solution
K-: Aquades sterile + *B. bifidum*

Based on the results of testing the antibacterial activity of synbiotic coffee against *E. coli* bacteria, it showed that the most optimal inhibitory response in the positive control group was the ciprofloxacin antibiotic, an average of 27.6 ± 0.2 mm from week 1 which was a very zone criterion, while in the control group the treatment was 1 plain water with a temperature of 40°C with a storage time of 7 days or the first week obtained an inhibition zone of 12.3 ± 1.1 mm where the inhibition response criteria were strong. Followed by a temperature of 40°C and a storage time of 14 days or the second week of 10.42 ± 0.43 mm is the criteria for a strong inhibition zone and the last one is storage for 21 days or the third week, which is 8.48 ± 0.17 mm, which is a criterion of moderate inhibition zone. Determination of these

criteria is based on (Davis & Stout, 1971) who reported that the provisions of antibacterial power were as follows: the inhibition area of 20 mm was very strong, the inhibition area was 10 - 20 mm in the strong category, the inhibition area was 5-10 mm in the medium category, and the inhibition area was in the moderate category. 5 mm or less is in the weak category.

Based on the results obtained, there is a decrease in the inhibition zone that is not too significant for weekly storage. A decrease in the diameter of the inhibition zone each week due to the activity of lactic acid bacteria is influenced by the reduced nutrients available in the media along with storage. Nutrients that continue to decrease make lactic acid bacteria that continue to breed unable to survive, then are also influenced by growth temperature, so that it affects the organic compounds produced during the long storage of synthetic coffee so that the activity of the antibacterial compounds produced is different (Nuhu, 2014), Although it is known that lactic acid and acetic acid produced have high activity in inhibiting pathogenic bacteria, the inhibition of bacterial growth is influenced by several factors, namely, temperature, decrease in pH, storage time, the number of *B. bifidum* bacteria in the presence of oxygen and the presence of bacteriocins (Md Sidek et al., 2018).

In the control treatment 2 (F2), namely Robusta coffee, there are inhibition zones each week of 8.17 ± 0.63 mm, 7.1 ± 0.1 mm, and 5.93 ± 0.07 mm. This is indicated by (Sari et al., 2017) who state that robusta coffee beans have been shown to have antimicrobial activity. The difference in the diameter of the inhibition zone between F1 and F2 was because in treatment group 1 (F1) there was an important additive composition, namely the content of inulin, where the mechanism of inulin is very helpful for probiotics as prevention of diarrhea by increasing the colonization of probiotic bacteria (*B. bifidum*) in the intestine. The lumen of the gastrointestinal tract, so that the entire intestinal mucosal epithelium has been occupied by probiotic bacteria through receptors in intestinal epithelial cells so that there is no place for pathogenic bacteria to attach themselves to intestinal epithelial cells and eventually colonize. (Roberfroid, 2007). While the control treatment was only pure robusta coffee, and there were no other additives so the inhibitory power was not as big as treatment control 1.

The negative control used in the form of sterile distilled water showed no inhibitory response (MIC=0) (Rodríguez-Tudela et al., 2003). This shows that the aquadest solvent used does not have antibacterial activity (not bactericidal or bacteriostatic), and aquadest is a neutral and universal solvent, so it does not change the pH of the solution. Therefore, aquadest is not able to inhibit *E. coli*. While the inhibitory activity of the positive control, namely the antibiotic Ciprofloxacin, was included in the very strong category, both from the positive control group and the best growth control group each week, namely 27.6 ± 0.2 mm, 26.7 ± 0.1 mm, and 24.8 ± 0, 08mm.

Statistical Analysis of Antibacterial Activity Assay

The results that had been obtained were then analyzed using SPSS 24. Based on the output data of the normality test, a sig value> 0.05 is obtained which indicates that the data is normally distributed. Furthermore, the homogeneity test is continued where the sig value obtained is > 0.05, which indicates that the data is homogeneous. Then proceed with one way

ANOVA testing (T. K. Kim, 2017) where the sig value obtained is >. 0.05. This indicates that there is a significant effect between storage times.

Based on the output data on the positive control for the 1st, 2nd, and 3rd-week formulas as well as the negative control and pure robusta coffee, the value of sig <0.05, indicates that there is a significant difference between the test groups. This is because the positive control produces antibacterial activity with the largest zone of inhibition in inhibiting *E. coli*. (D. S. Handayani et al., 2019) Meanwhile, the negative control showed a sig value <0.05 for the 1st, 2nd, and 3rd-week formulas because there were no obstacles in distilled water. This is because the negative control was in the form of an aquadest solvent, where distilled water did not show any antibacterial activity with no inhibition zone around the paper disk. Additionally, aquadest is a solvent that is not bactericidal. In the control treatment of pure robusta coffee to the formula 1 week 1, 2, and 3, as well as positive and negative controls with a value of sig <0.05, which indicates that there is a significant difference between the test groups. In the control treatment of pure robusta coffee at weeks 1, 2, and 3, as well as positive and negative controls with a sig value < 0.05, which indicates that there is a difference.

CONCLUSION

Based on the results of the research that has been carried out, it can be concluded that the organoleptic test showed the best synbiotic Robusta Sancaco coffee, namely formula 1 with the most composition of Robusta Sancaco coffee at 20.62%, followed by 0.15% chicory inulin, 0.50% *B. bifidum* powder. , and 3.75% glucose. Synbiotic Robusta Sancaco coffee at week 1 had the highest growth yield of *B. bifidum* in the control treatment with ordinary water at 40°C, which was 5.95 x10^8 CFU/mL. At a temperature of 70°C, the 3rd week still met the requirements for good growth of *B. bifidum* of 1.15 x 10^8 CFU/mL. In formula 1, the largest inhibition zone was found in the control of ordinary water treatment with a temperature of 40°C at week 1 of 12.3 ± 1.1 mm in the strong category. Storage of formula 1 in Robusta Sancaco synbiotic coffee for 21 days showed a decrease in the number of colonies and inhibition which was not too significant (p<0.05) for coffee brewing at 40°C and 70°C each week. Sancaco synbiotic robusta coffee has antibacterial properties with the greatest inhibition at week 1 storage of 8.17 ± 0.63 mm with a moderate inhibition zone category.

ACKNOWLEDGMENTS

This publication of this article was funded by DIPA of Public Service Agency of Universitas Sriwijaya 2022, SP DIPA -023.17.2.677515/2022, On December 13, 2021. In accordance with the Rector's Decree Number :0109/UN9.3.1/SK/2022, On April 28, 2022".

REFERENCES

Akhlaghi, N., Sadeghi, M., Fazeli, F., Akhlaghi, S., Mehnati, M., & Sadeghi, M. (2019). The antibacterial effects of coffee extract, chlorhexidine, and fluoride against *Streptococcus mutans* and *Lactobacillus Plantarum*: An in vitro study. *Dental Research Journal*, *16*(5), 346–353. https://doi.org/10.4103/1735-3327.266093

Bedani, R., Rossi, E. A., & Saad, S. M. I. (2013). Impact of inulin and okara on *Lactobacillus acidophilus La-5* and *Bifidobacterium animalis* Bb-12 viability in a fermented soy product

and probiotic survival under in vitro simulated gastrointestinal conditions. *Food Microbiology*, *34*(2), 382–389. https://doi.org/10.1016/j.fm.2013.01.012

Bintsis, T. (2018). Lactic acid bacteria as starter cultures: An update in their metabolism and genetics. *AIMS Microbiology*, *4*(4), 665–684. https://doi.org/10.3934/microbiol.2018.4.665

Cencic, A., & Chingwaru, W. (2010). The role of functional foods, nutraceuticals, and food supplements in intestinal health. *Nutrients*, *2*(6), 611–625. https://doi.org/ 10.3390/nu2060611

Chaturvedi, S., & Chakraborty, S. (2021). Review on potential non-dairy synbiotic beverages: a preliminary approach using legumes. *International Journal of Food Science and Technology*, *56*(5), 2068–2077. https://doi.org/10.1111/ijfs.14779

Coelho-Rocha, N. D., De Castro, C. P., De Jesus, L. C. L., Leclercq, S. Y., De Cicco Sandes, S. H., Nunes, A. C., Azevedo, V., Drumond, M. M., & Mancha-Agresti, P. (2018). Microencapsulation of lactic acid bacteria improves the gastrointestinal delivery and in situ expression of a recombinant fluorescent protein. *Frontiers in Microbiology*, *9*(OCT), 1–11. https://doi.org/10.3389/fmicb.2018.02398

Dahiya, D., & Nigam, P. S. (2022). Probiotics, Prebiotics, Synbiotics, and Fermented Foods as Potential Biotics in Nutrition Improving Health via Microbiome-Gut-Brain Axis. *Fermentation*, *8*(7). https://doi.org/10.3390/fermentation8070303

Daniel Arkkelin. (2014). Using SPSS to Understand Research and Data Analysis. *Psychology Curricular Materials*, *1*, 194. http://scholar.valpo.edu/psych_oerhttp://scholar.valpo.edu/psych_oer/1%0Ahttp://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.139.2050&rep=rep1&type=pdf

Davis, W. W., & Stout, T. R. (1971). Disc plate method of microbiological antibiotic assay. II. Novel procedure offering improved accuracy. *Applied Microbiology*, *22*(4), 666–670. https://doi.org/10.1128 /aem.22.4.666-670.1971

Depkes RI. (2011). Buku Saku Petugas Kesehatan Lintas Diare. *Departemen Kesehatan RI, Direktorat Jendral Pengendalian Penyakit Dan Penyehatan Lingkungan*, 1–40.

Dreher, M. L. (2018). Whole fruits and fruit fiber emerging health effects. *Nutrients*, *10*(12). https://doi.org/10.3390/nu10121833

Fawzan, A. Al, Cahya, G., Darma, E., & Soewondo, B. P. (2019). Formulasi Minuman Kopi Probiotik dengan Kultur Starter Lactobacillus acidhopillus sebagai Minuman Fungsional. *Prosiding Farmasi*, *5*(2), 536.

González-Herrera, S. M., Bermúdez-Quiñones, G., Ochoa-Martínez, L. A., Rutiaga-Quiñones, O. M., & Gallegos-Infante, J. A. (2021). Synbiotics: a technological approach in food applications. *Journal of Food Science and Technology*, *58*(3), 811–824. https://doi.org/10.1007/s13197-020-04532-0

Handayani, D. S., Pranoto, Saputra, D. A., & Marliyana, S. D. (2019). Antibacterial activity of polyeugenol against *staphylococcus aureus* and *escherichia coli*. *IOP Conference Series:*

Materials Science and Engineering, *578*(1). https://doi.org/10.1088/1757-899X/578/1/012061

Handayani, R., & Muchlis, F. (2021). Review: Manfaat Asam Klorogenat Dari Biji Kopi (Coffea) Sebagai Bahan Baku Kosmetik. *FITOFARMAKA: Jurnal Ilmiah Farmasi*, *11*(1), 43–50. https://doi.org/10.33751 /jf.v11i1.2357

Husain, D. R., Gunawan, S., & Sulfahri, S. (2020). Antimicrobial potential of lactic acid bacteria from domestic chickens (Gallus domesticus) from south Celebes, Indonesia, in different growth phases: In vitro experiments supported by computational docking. *Iranian Journal of Microbiology*, *12*(1), 62–69. https://doi.org /10.18502/ijm.v12i1.2519

Jojima, T., Igari, T., Noburyu, R., Watanabe, A., Suda, M., & Inui, M. (2021). Coexistence of the Entner–Doudoroff, and Embden–Meyerhof–Parnas pathways enhances glucose consumption of ethanol-producing Corynebacterium glutamicum. *Biotechnology for Biofuels*, *14*(1), 1–9. https://doi.org/10.1186/s13068-021-01876-3

Jung, S., Kim, M. H., Park, J. H., Jeong, Y., & Ko, K. S. (2017). Cellular Antioxidant and Anti-Inflammatory Effects of Coffee Extracts with Different Roasting Levels. *Journal of Medicinal Food*, *20*(6), 626–635. https://doi.org/10.1089/jmf.2017.3935

Kim, J. Y., Kwon, J. H., Ahn, S. H., Lee, S. Il, Han, Y. S., Choi, Y. O., Lee, S. Y., Ahn, K. M., & Ji, G. E. (2010). Effect of probiotic mix (*Bifidobacterium bifidum, Bifidobacterium lactis, Lactobacillus acidophilus*) in the primary prevention of eczema: A double-blind, randomized, placebo-controlled trial. *Pediatric Allergy and Immunology*, *21*(2 PART 2). https://doi.org/10.1111/j.1399-3038.2009.00958.x

Kim, T. K. (2017). Understanding one-way ANOVA using conceptual figures. *Korean Journal of Anesthesiology*, *70*(1), 22–26.

Kurnia, S. N. (2021). Viabilitas Probiotik Dalam Kopi Yang Mengandung PRebiotik Dan Uji Antibakteri Terhadap *Escherichia coli*. *Skripsi Fak. Farmasi UNSRI*, *3*(2), 6.

M. H. Marhamatizadeh, B, E. E., & Gholami, P. (2014). *The Effect of Coffee Extract on the Growth and Viability of Lactobacillus acidophilus and Bifidobacterium bifidum in Probiotic Milk and Yoghurt*. *4*(1), 37–48.

Mahmoud, M., Abdallah, N. A., El-Shafei, K., Tawfik, N. F., & El-Sayed, H. S. (2020). Survivability of alginate-micro en-capsulated *Lactobacillus Plantarum* during storage, simulated food processing, and gastrointestinal conditions. *Heliyon*, *6*(3). https://doi.org /10.1016/j.heliyon.2020.e03541

Majeed, M., Majeed, S., Nagabhushanam, K., Arumugam, S., Beede, K., & Ali, F. (2019). Evaluation of probiotic *Bacillus coagulans* MTCC 5856 viability after tea and coffee brewing and its growth in GIT hostile environment. *Food Research International*, *121*(December), 497–505. https://doi.org/ 10.1016/j.foodres.2018.12.003

Martirosyan, D., Kanya, H., & Nadalet, C. (2021). Can functional foods reduce the risk of disease? Advancement of functional food definition and steps to create functional food products. *Functional Foods in Health and Disease*, *11*(5), 213–221.

https://doi.org/10.31989 /ffhd.v11i5.788

Mbae, J., Koskei, R., & Mugendi, B. (2022). Effect of Addition of Coffee Extract on Microbial Growth and Functional Properties of Yoghurt. *European Journal of Agriculture and Food Sciences*, *4*(1), 76–80. https://doi.org/10.24018/ejfood.2022.4.1.445

McCain-Keefer, H. R., Meals, S., & Drake, M. A. (2020). The sensory properties and consumer acceptance of cold brew coffee. *Journal of Sensory Studies*, *35*(6). https:// doi.org/10.1111/joss.12604

Md Sidek, N. L., Halim, M., Tan, J. S., Abbasiliasi, S., Mustafa, S., & Ariff, A. B. (2018). Stability of bacteriocin-like inhibitory substance (BLIS) produced by pediococcus acidilactici kp10 at different extreme conditions. *BioMed Research International*, *2018*. https://doi.org/ 10.1155/2018/5973484

Miksusanti, Harian Saputra, Sofia Sandi, H. (2016). The Effect of *Lactobacillus acidophilus* and Chito−Oligosaccharide on Antibacterial Activity and Organic Acid Production. *Indonesian Journal of Fundamental and Applied Chemistry*, *1*(2), 29–34. https://doi.org/10.24845/ijfac.v1.i2.29

Miksusanti, Indah Solihah, Dina Permata Wijaya, N. A. (2022). *Antibacterial Edible Coating of Ipomea batatas Incorporated with Lactobacillus acidophilus*. *2*(2), 135–146.

Miksusanti, M., Herlina, H., Annuria, F. N., & Zulhijjah, Z. (2020). Patch film from cellulose derivative incorporated with virgin coconut oil and its physical and antibacterial properties. *Key Engineering Materials*, *840 KEM*, 351–359. https://doi.org/10.4028/www.scientific.net/kem.840.351

Naeli Farhaty, M. (2016). Tinjauan Kimia dan Aspek Farmakologi Senyawa Asam Klorogenat pada Biji Kopi: Review. *Farmaka*, *14*(1), 214–227.

Nuhu, A. A. (2014). Bioactive Micronutrients in Coffee: Recent Analytical Approaches for Characterization and Quantification. *ISRN Nutrition*, *2014*, 1–13. https://doi.org/10.1155/2014/384230

Østlie, H. M., Treimo, J., & Narvhus, J. A. (2005). Effect of temperature on growth and metabolism of probiotic bacteria in milk. *International Dairy Journal*, *15*(10), 989–997. https://doi.org/10.1016/j.idairyj.2004.08.015

Pandey, K. R., Naik, S. R., & Vakil, B. V. (2015). Probiotics, prebiotics, and synbiotics- a review. *Journal of Food Science and Technology*, *52*(12), 7577–7587. https://doi.org/10.1007/s13197-015-1921-1

Parhi, P., Song, K. P., & Choo, W. S. (2022). Growth and survival of *Bifidobacterium breve* and *Bifidobacterium longum* in various sugar systems with fructooligosaccharide supplementation. *Journal of Food Science and Technology*, *59*(10), 3775–3786. https://doi.org/10.1007/ s13197-022-05361-z

Rahmawati, N., Syukri, M., Darmawi, D., Zachreini, I., Yusuf, M., & Idroes, R. (2021). Identification of lactic acid bacteria from etawa goat milk kopelma Darussalam Village, Banda Aceh. *IOP Conference Series: Earth and Environmental Science*, *667*(1).

https://doi.org/10.1088 /1755-1315/667/1/012022

Rautiainen, S., Wang, L., Lee, I. M., Manson, J. A. E., Buring, J. E., & Sesso, H. D. (2015). A higher intake of fruit, but not vegetables or fiber, at baseline is associated with a lower risk of becoming overweight or obese in middle-aged and older women of normal BMI at baseline. *Journal of Nutrition*, *145*(5), 960–968. https://doi.org /10.3945/jn.114.199158

Roberfroid, M. (2007). Prebiotics: The concept revisited. *Journal of Nutrition*, *137*(3). https://doi.org/10.1093/jn/137.3.830s

Rodríguez-Tudela, J. L., Barchiesi, F., Bille, J., Chryssanthou, E., Cuenca-Estrella, M., Denning, D., Donnelly, J. P., Dupont, B., Fegeler, W., Moore, C., Richardson, M., & Verweij, P. E. (2003). Method for the determination of minimum inhibitory concentration (MIC) by broth dilution of fermentative yeasts. *Clinical Microbiology and Infection*, *9*(8), i–viii. https://doi.org /10.1046/j.1469-0691.2003.00789.x

Sabam Malaua, Siagianb, A., Siraitc, B., Ambaritab, H., Pandiangana, S., Sihotanga, M. R., Naibahoa, B., & Simanjuntak, R. (2018). Variability of the organoleptic quality of Arabica coffee. *Anadolu Journal of Agricultural Sciences*, *33*(October), 241–245. https://doi.org /10.7161/omuanajas.405418

Samsul Rizal, Maria Erna, Fibra Nurainy, dan A. R. T. (2016). Karakteristik Probiotic Probiotic Characteristic of Lactic Fermentation Beverage of Pineapple Juice with Variation of Lactic Acid Bacteria (LAB) Types. *Terap.Indones*, *18*(1), 63–71. http://kimia.lipi.go.id/inajac/index.php

Sari, P. P., Nurliana, N., Hasan, M., Sayuti, A., Sugito, S., & Amiruddin, A. (2017). 3. *Lactobacillus casei* Fermented Milk as a Treatment for Diabetes in Mice (Mus musculus). *Jurnal Medika Veterinaria*, *11*(1), 15–19. https://doi.org/10.21157/j.med.vet..v11i1.4088

Sathyabama, S., Vijayabharathi, R., Bruntha devi, P., Ranjith kumar, M., & Priyadarisini, V. B. (2012). Screening for probiotic properties of strains isolated from feces of various human groups. *Journal of Microbiology*, *50*(4), 603–612. https://doi.org/10.1007/s12275-012-2045-1

Segheto, L., Santos, B. C. S., Werneck, A. F. L., Vilela, F. M. P., Sousa, O. V. de, & Rodarte, M. P. (2018). Antioxidant extracts of coffee leaves and its active ingredient 5-caffeoylquinic acid reduce chemically-induced inflammation in mice. *Industrial Crops and Products*, *126*(June), 48–57. https://doi.org/10.1016/j.indcrop.2018.09.027

Senditya, M., Hadi, M. S., Estiasih, T., & Saparianti, E. (2014). *Efek Prebiotik dan Sinbiotik Simplisia Daun Cincau Hitam (Mesona palustris BL) Secara In Vivo : KKajian Pustaka In Vivo Prebiotic and Synbiotic Effect of Black Grass Jelly (Mesona palustris BL) Leaf Simplicia : A Review*. *2*(3), 141–151.

Seninde, D. R., & Chambers, E. (2020). Coffee flavor: A review. *Beverages*, *6*(3), 1–25. https://doi.org/10.3390/beverages6030044

SNI-01-2346, S. N. I. (2006). Petunjuk Pengujian Organoleptik dan atau Sensori. BSN (Badan Standarisasi Nasional), 2–14.

SNI. (2009). Minuman Susu Fermentasi. *BSN (Badan Standarisasi Nasional)*.

Tanaka, K., Tsukahara, T., Yanagi, T., Nakahara, S., Furukawa, O., Tsutsui, H., & Koshida, S. (2017). *Bifidobacterium bifidum* OLB6378 simultaneously enhances systemic and mucosal humoral immunity in low birth weight infants: A non-randomized study. *Nutrients*, *9*(3). https://doi.org/10.3390/nu9030195

Trisnawita, Y., Silalahi, J., & Sinaga, S. M. (2018). The effect of storage condition on the viability of lactic acid bacteria in the probiotic product. *Asian Journal of Pharmaceutical and Clinical Research*, *11*(Special Issue 1), 85–86. https://doi.org /10.22159/ajpcr.2018.v11s1.26574

Vargha, A., Delaney, H. D., & Vargha, A. (1998). The Kruskal-Wallis Test and Stochastic Homogeneity. *Journal of Educational and Behavioral Statistics*, *23*(2), 170. https://doi.org/10.2307/1165320

Verma, S. K., David, J., & Chandra, R. (2012). Synbiotics: Potential dietary supplement in functional foods. *Indian Dairyman*, *May*, 58–62.

Wang, Y., Wu, J., Lv, M., Shao, Z., Hungwe, M., Wang, J., Bai, X., Xie, J., Wang, Y., & Geng, W. (2021). Metabolism Characteristics of Lactic Acid Bacteria and the Expanding Applications in Food Industry. *Frontiers in Bioengineering and Biotechnology*, *9*(May), 1–19. https://doi.org/10.3389/fbioe.2021.612285

WHO. (2016). Preventing diarrhea through better water, sanitation, and hygiene. *World Health Organization*, 1–48. http://apps.who.int/iris/bitstream/10665/150112/1/9789241564823-eng.pdf?

Widowati, E., Si, S., Andriani, I. M., Kusumaningrum, A. P., Pengajar, S., Teknologi, J., Pertanian, H., Pertanian, F., Surakarta, U., Program, A., & Teknologi, S. (2011). Kajian Total Bakteri Probiotik Dan Aktivitas Antioksidan Yoghurt Tempe Dengan Variasi Substrat. *Jurnal Teknologi Hasil Pertanian*, *IV*(1), 18–31.

Yeretzian, C., Blank, I., & Wyser, Y. (2017). Protecting the Flavors-Freshness as a Key to Quality. *The Craft and Science of Coffee*, *December*, 329–353. https://doi.org/10.1016 /B978-0-12-803520-7.00014-1

Yusmarini. (2011). Senyawa Polifenol pada Kopi : Pengaruh Pengolahan, Metabolisme dan Hubungannya dengan Kesehatan. In *Sagu* (Vol. 10, Issue 2, pp. 22–30).