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Viability and Antibacterial Activity of *Bifidobacterium bifidum* in Fermented Robusta Coffee for Diarrhea Treatment

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Abstract

***Background**: Diarrhea can be treated with probiotic bacteria such as Bifidobacterium bifidum, which decreases the intestinal environment's pH to become acidic so that pathogenic bacteria cannot thrive. Objective: To make fermented coffee that can increase the number of probiotic bacteria Bifidobacterium bifidum and has antidiarrheal activity against pathogenic bacteria Escherichia coli. **Methods**: Robusta coffee (20.25% and 19.75%) was fermented using Saccharomyces cerevisiae, and then the probiotic bacteria Bifidobacterium bifidum was added. Unfermented coffee was compared with the same concentration (20.25% and 19.75%) to obtain four formulas. Organoleptic panelists tested all formulas to determine the best formula for fermented and non-fermented coffee. The number of Bifidobacterium bifidum and antibacterial activity was calculated on the optimum formulation using the Total Plate Count and Disc Diffusion Method. **Result**: The optimum formula obtained at fermented and unfermented coffee concentration was 20,25%. The number of probiotic bacteria Bifidobacterium bifidum growing in fermented and non-fermented coffee was 7.3 x 108 ± 32.4 and 3.1 x 108 ± 30.7 ($p < 0.05$). The diameter of the inhibition zone of the best fermented and non-fermented coffee was 11.5 ± 0.5 mm and 8.5 ± 0.5 mm, respectively ($p < 0.05$). **Conclusion**: Fermented coffee can increase the growth of the probiotic bacteria Bifidobacterium bifidum and has strong antibacterial activity against Escherichia coli bacteria.*

***Keywords**: Bifidobacterium bifidum, disc diffusion, Escherichia coli, fermented coffee, total plate count*

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INTRODUCTION

Diarrhea is a disease associated with environmental hygiene. Diarrhea is mainly caused by food contamination of pathogenic bacteria such as *Escherichia coli*, *Salmonella*, and *Shigella* (Wang *et al*., 2015). According to research by Zhou *et al*. (2018), *E. coli* is the most common bacteria that causes diarrhea. Diarrheal disease management can be treated with antibiotics such as metronidazole, vancomycin, ciprofloxacin, tetracycline, and doxycycline (Giannelli, 2017). In addition, there are other antidiarrheal agents, such as probiotics (Guarino *et al*., 2015).

Probiotics are live microorganisms that provide the host health benefits when administered appropriately. Microorganisms that can act as probiotics are non-pathogenic microorganisms tolerant of acids and bile, modulate the immune system, and can produce lactic acid (Riddle *et al*., 2016). One of the most widely used microorganisms as probiotics is *Bifidobacterium bifidum* (*B. bifidum*) (Pandey *et al*., 2015). Probiotics can prevent the attachment of pathogenic bacteria to the intestinal mucosa, increase the immune response, and increase the microflora in the intestine (Plaza-Diaz *et al*., 2019). The growth of probiotic bacteria can be assisted with a prebiotic agent.

Prebiotics are a nutrient source rich in simple carbohydrates (Arslanoglu *et al*., 2008). The fermentation process can obtain simple carbohydrates (Pokuesava *et al*., 2011). In this study, robusta coffee was used as a source of carbohydrates. Based on the research of Mindarti *et al*. (2020), Robusta coffee contains carbohydrates up to 62.78% *w/w*. The main carbohydrate in robusta coffee is sucrose (Wulandari *et al*., 2021). Complex carbohydrates in robusta coffee will be fermented using *Saccharomyces cerevisiae*, which can break down complex carbohydrates into simpler components to be used as a source of nutrition for *B. bifidum* (Rizal *et al*., 2020). *S. cerevisiae* can break down sucrose into glucose and fructose (Marques *et al*., 2016).

The fermented coffee will be added with the probiotic bacteria *B. bifidum* to become a symbiotic product. In fermented products, the probiotic bacteria *B. bifidum* will degrade carbohydrates into organic acids such as lactic acid, succinic acid, and acetic acid (Chichlowski *et al*., 2011; Stiverson *et al*., 2014; Wang *et al*., 2021). These organic acids have a strong antimicrobial effect against bacterial pathogens (Makras *et al*., 2006). *Bifidobacterium* can kill pathogenic bacteria such as *Clostridioides difficile* (Yang & Yang, 2019), *Salmonella enterica* (Symonds *et al*., 2012), and *E. coli* (Abdelhamid et al., 2018).

Based on the description above, researchers are interested in making a fermented robusta coffee as a symbiotic product containing the probiotic bacteria *B. bifidum* and testing the growth of the probiotic bacteria using the total plate count method. Then, the antibacterial activity was tested against pathogenic bacteria *E. coli* using the Disc Diffusion Method. Non-fermented robusta coffee was used as a comparison.

MATERIALS AND METHODS

Materials

The materials used consisted of robusta coffee (South Sumatra, Indonesia), *S. cerevisiae* (buy at Sriwijaya University, Indonesia), distilled water (Bratachem, Indonesia), Man Rogosa and Sharpe media (Merck, Indonesia), *B. bifidum* BRL-130 (buy at Gadjah Mada University, Indonesia), *E. coli* ATCC-25922 (buy at Sriwijaya University, Indonesia), ciprofloxacin, sodium alginate (Merck, United States), barium chloride (Sigma-Aldrich, United States), sodium chloride (Sigma-Aldrich, United States), hydrochloric acid (Bratachem, Indonesia), and concentrated sulfuric acid (Bratachem, Indonesia).

Equipment

The equipment used consisted of Freeze Dryer (Nuaire® NU9483GC), glassware (Pyrex® and Iwaki®), magnetic stirrer (IKA® C-MAG HS 4), micro pipette (DragonLab®), analytical balance (Ohaus®), autoclave (Lequitron®), incubator (Biosan®), Oven (Memmert®), Furnace (Thermolyne®), butyrometer.

Methods

Production of *Bifidobacterium bifidum* probiotic powder

The suspension of the probiotic bacteria *B. bifidum* in de Man, Rogosa & Sharpe (MRS) broth media was dried using the freeze-drying method. The probiotic powder was made by mixing *Bifidobacterium* suspension into 10% skim milk solution and 4% sodium alginate solution, then incubated at 37°C for 8 hours. The mixed suspension was then dried at -23°C for 24 hours (Holkem *et al*., 2016).

Production of fermented robusta coffee

Robusta coffee was fermented using 3% *S. cerevisiae* yeast for 5 hours. After that, it is washed thoroughly and dried in the sun. After drying, the coffee beans are cleaned and then roasted at a temperature of 120°C. Then the coffee beans are ground to obtain fermented robusta coffee powder (Pereira *et al*., 2014).

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Table 1. The formula of probiotic coffee

Samples	Formula Concentration (%)			
	F1	F2	F3	F4
Fermented coffee	20.25	-	19.75	-
Non-fermented coffee	-	20.25	-	19.75
Probiotic powder	1.00	1.00	1.00	1.00
Glucose	3.75	3.75	3.75	3.75

Production of robusta coffee symbiotic

Robusta coffee symbiotic products are made from fermented and non-fermented coffee with added probiotic powder containing the *B. bifidum*. Fermented coffee powder or non-fermented coffee is added with probiotic powder. Then add glucose and mix until smooth. The formula for probiotic coffee can be seen in Table 1.

Organoleptic test

Organoleptic tests were conducted to determine the best formula with assessments including color, smell, taste, and texture from samples shown by 30 untrained panelists with a target age of 15 to 50 years. Samples are placed in containers and coded according to the formula. Panelists were asked to rate each sample on the questionnaire sheet. The scale used in this study consisted of five numerical scales, namely strongly dislike (1), dislike (2), neutral (3), like (4), and like very much (5).

Proximate test of robusta coffee beans and fermented coffee

The proximate test refers to the rules of SNI 01-2891-1992, including water, ash, protein, fat, and carbohydrate content.

Water content test

Moisture content was measured using the Thermogravimetric Method. The best probiotic coffee preparations were dried in an oven at 105°C for 5 hours and then weighed until a constant weight was obtained.

Total ash content test

Total ash content was measured by calculating the constant weight of the sample, which had been heated at 600°C for 2 hours using a furnace.

Protein content test

A number of samples were mixed with 1.9 g K_2SO_4, 40 mg HgO, and 2 mL concentrated H_2SO_4, then boiled until the solution became clear. The solution was then distilled with 10 mL of 60% NaOH, 5 mL of boric acid, and 5 mL of MB:MM indicator for 15 minutes. The solution was then titrated with 0.02 N HCl.

Fat content test

A total of 10 mL of H_2SO_4 and a number of samples were put into the butyrometer. Add amyl alcohol and stir until homogeneous. The mixture was heated at a temperature of 65 - 70°C for 5 minutes and then mixed. Put the mixture upside down and reheat for 2 - 3 minutes. Calculate the percent fat on the butyrometer line.

Carbohydrate content test

The carbohydrate content of the sample was calculated by subtracting 100% of the nutritional content of the sample from the moisture content, total ash content, protein content, and fat content.

Probiotic bacteria growth test

The growth test of the probiotic bacteria *B. bifidum* was carried out using the Total Plate Count Method. In this test, a suspension of *B. bifidum* was used as a positive control, and distilled water as a negative control. Selected probiotic coffee formula from fermented and non-fermented coffee was brewed with warm water. Then the dilution was performed from 10^{-1} to 10^{-8} using 0.9% NaCl solution. Then the solution was poured into MRS Agar media and incubated at 37°C for 48 hours (Rosburg *et al*., 2010). The amount of growth of probiotic bacteria was calculated using equation 1.

$$Number\ of\ Colony\ (N) = \frac{\sum C}{(1xn1)+(0.1xn2)+(0.01xn3)xdf} \quad [1]$$

Information:
N = Number of product colonies (cfu/mL or cfu/g)
ΣC = Number of colonies in all counted plates
n1 = Number of cups in the first dilution is calculated
n2 = Number of cups in the second dilution is calculated
n3 = Number of cups in the third dilution is calculated
Fp = First dilution calculated

Antibacterial activity test against *E. coli*

The antibacterial activity test was carried out using the Disc Diffusion Method (Mohammed *et al*., 2020). The antibiotic ciprofloxacin was used as a positive control, and distilled water was used as a negative control. Filter paper with a diameter of 6 mm was dipped in each sample (positive control, negative control, and coffee samples) for 5 minutes and then planted on MRS Agar media which already contained the pathogenic bacterium *E. coli*. The Petri dish was then incubated at 37°C for 24 hours. Antibacterial activity was measured from the diameter of the resulting inhibition zone.

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Data analysis

Data analysis was carried out statistically using SPSS version 23. The normality test was carried out using the Shapiro-Wilk method. If the data were normally distributed ($p > 0.05$), then it was continued with the one-way ANOVA test to see the differences between the test groups.

RESULTS AND DISCUSSION

Probiotic powder containing *B. bifidum* bacteria has a smooth and dry texture. Drying was carried out using the freeze-drying method with the help of coating materials such as sodium alginate and skim milk. According to research by Chandramouli *et al.* (2004), sodium alginate coating has several advantages, such as being non-toxic, easy to form a gel matrix around bacterial cells, and easy release of active substances when contact with the intestine fluid. In addition, skim milk, which contains high protein, can also prevent damage to bacterial cell membranes (Amine *et al.*, 2014).

The growth and metabolic activity of the probiotic bacteria *B. bifidum* can be selectively increased by the presence of carbohydrates in the environment. Robusta coffee is used as a carbohydrate source for the growth of the probiotic bacteria *B. bifidum*. Robusta coffee is known to contain mucilage consisting of pectin and carbohydrates (Haile & Kang, 2019). Based on the research of Mindarti *et al.* (2020), Robusta coffee contains carbohydrates up to 62.78% *w/w*. In this study, the robusta coffee beans used had a water content of 6.63 ± 0.14%, ash content of 3.68 ± 0.18%, protein content of 7.84 ± 0.11%, fat content of 0.86 ± 0.03%, and carbohydrate content of 75.98 ± 0.07% (Table 2). In addition, S. cerevisiae also contains β-Galactosidases which can convert lactose into galactooligosaccharides (GOS) (Macfarlane *et al.*, 2008; Osman *et al.*, 2012). Carbohydrates that can be used as nutrients for probiotic bacteria B. bifidum are simple carbohydrates such as inulin, fructooligosaccharides (FOS), galactooligosaccharides (GOS), and lactulose (Gibson *et al.*, 2010). In this study, robusta coffee will be fermented using the yeast *Saccharomyces cerevisiae* to help the degradation process of complex carbohydrates into prebiotic compounds. Fermentation is a chemical process that can convert complex compounds into simpler compounds. S. cerevisiae is known to have the enzyme Fructosyl-transferase (FTase), which is an enzyme capable of converting fructose into inulin and fructooligosaccharides (FOS) (Louis *et al.*, 2016; Mohkam *et al.*, 2016). S. cerevisiae can also hydrolyze sucrose into glucose and fructose (Marques *et al.*, 2016).

Organoleptic is an essential parameter in functional food products to increase consumer attractiveness. Fermented and non-fermented robusta coffee was carried out by organoleptic tests using 30 panelists to determine the best formula. Based on this test, fermented coffee formula one and non-fermented formula two were chosen as the best formula according to Figure 1. The results of statistical analysis showed that there were no significant differences in taste and odor parameters ($p > 0.05$) while color and texture had significant differences ($p < 0.05$). Formula 1 generally has the best results from all parameters. It indicates that the fermentation process in coffee can improve the quality of the coffee produced. Robusta coffee fermented with *S. cerevisiae* has been proven to make coffee that has good taste qualities such as the presence of a caramel aroma in the coffee, a sweet taste at the beginning and bitter at the end, and a fresh aroma (Bressani *et al.*, 2020; Evangelista *et al.*, 2014; Silva *et al.*, 2013). This change is because the coffee fermentation process will produce new byproducts such as acetic acid, citric acid, malic acid, lactic acid, and succinic acid (Da Mota *et al.*, 2020).

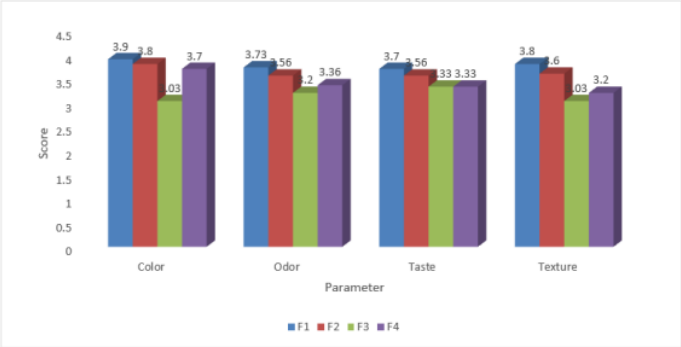

Figure 1. Organoleptic results of symbiotic coffee (n = 30, assessed using questionnaire)

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Table 2. Test results of coffee beans and fermented coffee the best formula (n = 5)

Parameter (%)	Robusta coffee beans	Fermented coffee	SNI 6685, 2009
Water content	6.63 ± 0.14	8.76 ± 0.13	Max 7
Ash content	3.68 ± 0.18	4.48 ± 0.21	Max 1
Protein content	7.84 ± 0.11	38.11 ± 0.49	Min 1
Fat content	0.86 ± 0.03	1.77 ± 0.02	Min 0.6
Carbohydrate content	75.98 ± 0.07	46.87 ± 0.61	-

Table 4. Inhibition zone diameter in samples (n = 5)

Treatment Group	Inhibition zone diameter (mm)	Category
Positive control	26.5 ± 0.5	Very strong
Negative control	0.0 ± 0.0	None
F1- Fermented coffee	11.5 ± 0.5	Strong
F2- Non-fermented coffee	8.5 ± 0.5	Moderate

The proximate results of the best-fermented robusta coffee have met the standards of SNI 6685, 2009, namely water content of 8.76 ± 0.13%, ash content of 4.48 ± 0.21%, the protein content of 38.11 ± 0.49%, fat content was 1.77 ± 0.02%, and carbohydrate content was 46.87 ± 0.61 (Table 2).

Formula 1 and 2 were continued by testing the viability of probiotic bacteria. The results of the viability test of the probiotic bacteria *B. bifidum* can be seen in Table 3.

Table 3. Viability of the probiotic bacteria *B. bifidum* in samples (n = 5)

Treatment Group	Number of colonies (cfu/mL)
Positive control	7.4×10^8
Negative control	1×10^8
F1-Fermented coffee	7.3×10^8
F2-Non-fermented coffee	3.1×10^8

Based on the results of statistical analysis for the viability of probiotic bacteria, there was no significant difference between the positive control and the fermented coffee formula 1 ($p > 0.05$). In contrast, the non-fermented coffee formula 2 differed significantly from the positive control and formula 1 ($p < 0.05$). It proves that the fermentation process increases the growth of the probiotic bacteria *B. bifidum*. According to the description above, *S. cerevisiae* can convert complex carbohydrates such as sucrose and lactose into FOS and GOS. FOS and GOS have been shown to increase the growth of the bacterium *B. bifidum* (Saulnier *et al*., 2008).

Furthermore, Formula 1 and 2 were continued with antibacterial activity tests against *E. coli* pathogenic bacteria. The results of the antibacterial activity test can be seen in Table 4.

Statistical analysis showed significant differences between groups ($p < 0.05$). It indicates that fermented coffee and non-fermented coffee produce different antibacterial activities. Fermented coffee makes a larger diameter of inhibition zone than non-fermented coffee and is included in the category of antibacterial solid compounds, namely 11.5 ± 0.5 mm. It proves that the fermentation process can positively affect antibacterial activity. As described above, the robusta coffee fermentation process will produce prebiotic compounds such as FOS and GOS. FOS and GOS will then be further fermented by the bacterium *B. bifidum*. FOS will be fermented into organic acids such as lactic acid and succinic acid, while GOS will be fermented into acetic acid, which can inhibit the growth of *E. coli* bacteria (Bondue & Delcenserie, 2015; Stiveson *et al*., 2014). Organic acids will make the environment acidic so *E. coli* bacteria cannot grow. In addition, the undissociated form of organic acids will enter the *E. coli* bacterial cell and dissociate in the cytoplasm, which causes the bacterial cell to lysis (Bermudez-Brito *et al*., 2012). *B. bifidum* probiotics can also produce bacteriocins in the form of Bifidocin A. Bifidocin A enters the cells of *E. coli* bacteria to form pores that cause leakage of intracellular compounds such as proteins, nucleic acids, and ions (Liu *et al*., 2015). *B. bifidum* can stimulate mucus secretion, strengthening intestinal epithelial defenses (Denkova *et al*., 2017). Pathogenic bacteria attach to the intestinal epithelium due to the interaction between Microbe-Associated Molecular Pattern (MAMP) and Pattern Recognition Receptor (PRR) (Madsen, 2012). *B. bifidum* can recognize these receptors to replace pathogens attached to the intestinal epithelium (Sarkar & Mandal, 2016).

In addition to the probiotic effect, the antibacterial activity of fermented and non-fermented robusta coffee can also be caused by the content of robusta coffee.

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Robusta coffee contains flavonoid compounds, alkaloids, tannins, and terpenoids (Less & Kamengon, 2021). Flavonoids can act as antibacterial by inhibiting nucleic acid synthesis, inhibiting porin formation, disrupting membrane structure, and changing membrane permeability (Xie *et al*., 2015). Alkaloids can act antibacterial by inhibiting ATP-dependent transport of compounds across the cell membrane (Mabhiza *et al*., 2016). Tannins work as antibacterial by interfering with the metabolism of bacterial cells (Kaczmarek, 2020). Meanwhile, terpenoids act as antibacterial by disrupting the integrity of cell membranes (Guimarães *et al*., 2019). However, the antibacterial activity of fermented and non-fermented coffee was not as strong as the antibiotic ciprofloxacin. But for fermented coffee, the antibacterial activity is in a strong category.

CONCLUSION

The fermented robusta coffee produced in this study meet with SNI requirements in characteristics parameter namely water content, ash content, the protein content, the fat content, and carbohydrate content. Fermented robusta coffee also has a strong antibacterial activity against *E. coli* while the non-fermented coffee has moderate antibacterial activity. Fermented robusta coffee can also increase the growth of *B. bifidum* bacteria due to the presence of prebiotic compounds such as FOS and GOS. So, fermented robusta coffee can be used as an alternative symbiotic product for diarrhea management.

AUTHOR CONTRIBUTIONS

Conceptualization, M., E. F. A.; Methodology, M., E. F. A.; Sotfware, M., E. F. A.; Validation, M., E. F. A., D. N. A.; Formal Analysis, M., E. F. A., D. N. A.; Resources, M., E. F. A., D. N. A.; Investigation, M., E. F. A., D. N. A.; Data Curation, M., E. F. A., D. N. A.; Writing - Original Draft, M., E. F. A.; Writing - Review & Editing, M., E. F. A.; Visualization, M., E. F. A; Supervision, M., E. F. A.; Project Administration, M., E. F. A.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

Abdelhamid, A. G., Esaam, A. & Hazaa, M. M. (2018). Cell Free Preparations of Probiotics Exerted Antibacterial and antibiofilm Activities Against Multidrug Resistant *E. coli*. *Saudi Pharmaceutical Journal; 26*; 603–607. doi: 10.1016/j.jsps.2018.03.004.

Amine, K. M., Champagne, C. P., Salmieri, S., Britten, M., St-Gelais, D., Fustier, P. & Lacroix, M. (2014). Effect of Palmitoylated Alginate Microencapsulation on Viability of *Bifidobacterium longum* during Freeze-drying. *Food Science and Technology*; *56*; 111-117. doi: 10.1016/j.lwt.2013.11.003.

Arslanoglu, S., Moro, G. E., Schmitt, J., Tandoi, L., Rizzardi, S. & Boehm, G. (2008). Early Dietary Intervention with a Mixture of Prebiotic Oligosaccharides Reduces the Incidence of Allergic Manifestations and Infections during the First Two Years of Life. *Journal of Nutrition*; *138*; 1091–1095. doi: 10.1093/jn/138.6.1091.

Bermudez-Brito, M., Plaza-Diaz, J., Muñoz-Quezada, S., Gómez-Llorente, C. & Gil, A. (2012). Probiotic Mechanisms of Action. *Annals of Nutrition and Metabolism*; *61*; 160–174.

Bondue, P. & Delcenserie, V. (2015). Genome of Bifidobacteria and Carbohydrate Metabolism. *Korean Journal for Food Sciences of Animal Resources*; *35*; 1-9. doi: 10.5851/kosfa.2015.35.1.1.

Bressani, A. P. P., Martinez, S. J., Sarmento, A. B. I., Borém, F. M. & Schwan, R. F. (2020). Organic Acids Produced during Fermentation and Sensory Perception in Specialty Coffee using Yeast Starter Culture. *Food Research International*; *128*; 108773. doi: 10.1016/j.foodres.2019.108773.

Chandramouli, Y., Gandhimati, R., Yasmeen, B. R., Vikram, A., Mahitha, B. & Imroz, S.M. (2004). Review On Cocrystal as An Approach with Newer Implication in Pharmaceutical Field. *International Journal of Medicinal Chemistry and Analysis*; *2*; 91-100.

Chichlowski, M., German, J. B., Lebrilla, C. B. & Mills, D. A. (2011). The Influence of Milk Oligosaccharides on Microbiota of Infants: Opportunities for Formulas. *Annual Review of Food Science and Technology*; *2*; 331-351. doi: 10.1146/annurev-food-022510-133743.

Da Mota, M. C. B., Batista, N. N., Rabelo, M. H. S., Ribeiro, D. E., Borém, F. M. & Schwan, R. F. (2020). Influence of Fermentation Conditions on the Sensorial Quality of Coffee Inoculated with Yeast. *Food Research International*; *136*; 109482. doi: 10.1016/j.foodres.2020.109482.

Denkova, R., Goranov, B., Teneva, D., Denkova, Z. & Kostov, G. (2017). Antimicrobial Activity of

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E-ISSN: 2580-8303

Probiotic Microorganisms: Mechanisms of Interaction and Methods of Examination. *Antimicrobial Research: Novel Bioknowledge and Educational Programs*; *34*; 201-212.

Evangelista, S. R., Miguel, M. G., de Souza Cordeiro, C., Silva, C. F., Pinheiro, A. C. & Schwan, R. F. (2014). Inoculation of Starter Cultures in a Semi-Dry Coffee (*Coffea arabica*) Fermentation Process. *Food Microbiology*; *44*; 87–95.

Giannelli, F. R. (2017). PA-C Antibiotic-associated Diarrhea. *Journal of the American Academy of Physician Assistants*; *30*; 46-47. doi: 10.1097/01.JAA.0000524721.01579.c9.

Gibson, G. R., Scott, K. P., Rastall, R. A., Tuohy, K. M., Hotchkiss, A., Dubert-Ferrandon, A., Gareau, M., Murphy, E. F., Saulnier, D. & Loh, G. (2010). Dietary Prebiotics: Current Status and New Definition. *Food Science & Technology Bulletin Functional Foods*; *7*; 1–19.

Guarino, A., Guandalini, S. & Vecchio, A. (2015). Probiotics for Prevention and Treatment of Diarrhea. *Journal of Clinical Gastroenterology*; *49*; 37-45. doi: 10.1097/MCG.0000000000000349.

Guimarães, A. C., Meireles, L. M., Lemos, M. F., Guimarães, M., Endringer, D. C., Fronza, M. & Scherer, R. (2019). Antibacterial Activity of Terpenes and Terpenoids Present in Essential Oils. *Molecules; 24*; 2471. doi: 10.3390/molecules24132471.

Haile, M. & Kang, W. H. (2019). The Role of Microbes in Coffee Fermentation and Their Impact on Coffee Quality. *Journal of Food Quality*; *2019*; 1-16. doi: 10.1155/2019/4836709.

Holkem, A., Raddatz, G., Nunes, G., Cichoski, A., Jacob-Lopes, E., Grosso, C. & Menezes, C. (2016). Development and Characterization of Alginate Microcapsules Containing Bifidobacterium BB-12 Produced by Emulsification/Internal Gelation Followed by Freeze Drying. *Food Science and Technology*; *71*; 302-308. doi: 10.1016/j.lwt.2016.04.012.

Kaczmarek, B. (2020). Tannic Acid with Antiviral and Antibacterial Activity as A Promising Component of Biomaterials-A Minireview. *Materials; 13*; 3224.

Liu, G., Song, Z., Yang, X., Gao, Y., Wang, C. & Sun, B. (2015). Antibacterial Mechanism of Bifidocin A, a Novel Broad-Spectrum Bacteriocin Produced by *Bifidobacterium animalis* BB04. *Food Control*; *62*; 309-316. doi: 10.1016/j.foodcont.2015.10.033.

Louis, P., Flint, H. J. & Michel, C. (2016). How to Manipulate the Microbiota: Prebiotics. *Advances in Experimental Medicine and Biology*; *902*; 119-142. doi: 10.1007/978-3-319-31248-4_9.

Macfarlane, G., Steed, H. & Macfarlane, S. (2008). Bacterial Metabolism and Health-Related Effects of Galacto-Oligosaccharides and Other Prebiotics. *Journal of Applied Microbiology*; *104*; 305–344.

Mabhiza, D., Chitemerere, T. & Mukanganyama, S. (2016). Antibacterial Properties of Alkaloid Extracts from *Callistemon citrinus* and *Vernonia adoensis* against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *International Journal of Medicinal Chemistry*; *2016*; 1-7. doi: 10.1155/2016/6304163.

Madsen, K. L. (2012). Enhancement of Epithelial Barrier Function by Probiotics. *Journal of Epithelial Biology & Pharmacology*; *5*; 55–59.

Makras, L., Triantafyllou, V., Fayol-Messaoudi, D., Adriany, T., Zoumpopoulou, G., Tsakalidou, E., Servin, A. & DeVuyst, L. (2006). Kinetic Analysis of the Antibacterial Activity of Probiotic Lactobacilli Towards *Salmonella enterica* Serovar *typhimurium* Reveals a Role for Lactic Acid and Other Inhibitory Compounds. *Research in Microbiology*; *157*; 241–247. doi: 10.1016/j.resmic.2005.09.002.

Marques, W., Raghavendran, V., Stambuk, B. U. & Gombert, A. K. (2016). Sucrose and *Saccharomyces cerevisiae*: A Relationship Most Sweet. *Federation of European Microbiological Societies Yeast Research*; *16*; 1-16. doi: 10.1093/femsyr/fov107.

Mindarti, S., Zalizar, L., Damat, Saati, E. A. & Fajriani, S. (2020). Characterization of Fiber Fraction, Physical and Chemical Properties of Coffee Flour (*Coffea* sp.) as Functional Foodstuff for Diabetes Mellitus Patient. *IOP Conferences Series: Earth and Environmental Science*; *462*; 1-10.

Mohammed, M., Raman, N., Alhoot, M. & Alwan, R. (2020). Antibacterial Activities of *Allium sativum* (Garlic) Extracts Against *Staphylococcus aureus* and *Escherichia coli*. *European Journal of Molecular & Clinical Medicine*; *7*; 526-534.

Mohkam, M., Nezafat, N., Berenjian, A., Negahdaripour, M., Behfar, A. & Ghasemi, Y. (2016). Role of *Bacillus genus* in the Production of Value-Added Compounds. Basel: Springer.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Osman, A., Tzortzis, G., Rastall, R. A. & Charalampopoulos, D. (2012). BbgIV is an Important *Bifidobacterium* β-galactosidase for the Synthesis of Prebiotic Galactooligosaccharides at High Temperatures. *Journal of Agriculture and Food Chemistry*; *60*; 740–748.

Pandey, K. R., Naik, S. R. & Vakil, B. V. (2015). Probiotics, Prebiotics and Symbiotics - a Review. *Journal of Food Science and Technology*; *52*; 7577–7587.

Pereira, G., Thomaz-Soccol, V., Pandey, A., Madeiros, A., Lara, J., Gollo, A. & Soccol, C. (2014). Isolation, Selection and Evaluation of Yeasts for Use in Fermentation of Coffee Beans by the Wet Process. *International Journal of Food Microbiology*; *188*; 60-66. doi: 10.1016/j.ijfoodmicro.2014.07.008.

Plaza-Diaz, J., Ruiz-Ojeda, F. J., Gil-Campos, M. & Gil, A. (2019). Mechanisms of Action of Probiotics. *Advances in nutrition*; *10*; 49–66. doi: 10.1093/advances/nmy063.

Pokusaeva, K., Fitzgerald, G. F. & van Sinderen, D. (2011). Carbohydrate metabolism in Bifidobacteria. *Genes & Nutrition*; *6*; 285–306. doi: 10.1007/s12263-010-0206-6.

Riddle, M. S., DuPont, H. L. & Connor, B. A. (2016). ACG Clinical Guideline: Diagnosis, Treatment, and Prevention of Acute Diarrheal Infections in Adults. *American Journal of Gastroenterology*; *111*; 602-622. doi: 10.1038/ajg.2016.126.

Rizal, S., Murhadi, Kustyawati, M. A. & Hasanudin, U. (2020). Growth Optimization of *Saccharomyces cerevisiae* and *Rhizopus oligosporus* during Fermentation to Produce Tempeh with High β-glucan Content. *Biodiversitas*; *21*; 2667-2673. doi: 10.13057/biodiv/d210639.

Rosburg, V., Boylston, T. & White, P. (2010). Viability of Bifidobacteria Strains in Yogurt with Added Oat Beta-Glucan and Corn Starch During Cold Storage. *Journal of Food Science*; *75*; 439–444. doi: 10.1111/j.1750-3841.2010.01620.x.

Sarkar, A. & Mandal, S. (2016). Bifidobacteria - Insight into Clinical Outcomes and Mechanisms of Its Probiotic Action. *Microbiological Research*; *192*; 159-171. doi: 10.1016/j.micres.2016.07.001.

Saulnier, D. M., Gibson, G. R. & Kolida, S. (2008). In Vitro Effects of Selected Symbiotics on the Human Faecal Microbiota Composition. *Federation of European Microbiological Societies Microbiology & Ecology*; *66*; 516–527.

Silva, C. F., Vilela, D. M., de SouzaCordeiro, C., Duarte, W. F., Dias, D. R. & Schwan, R. F. (2013). Evaluation of a Potential Starter Culture for Enhances Quality of Coffee Fermentation. *World Journal of Microbiology & Biotechnology*; *29*; 235–247. doi: 10.1007/s11274-012-1175-2.

Stiverson, J., Williams, T., Chen, J., Adams, S., Hustead, D., Price, P., Guerrieri, J., Deacon, J. & Yu Z. (2014). A Comparative Evaluation of Prebiotic Oligosaccharides using In Vitro Cultures of Infant Fecal Microbiome. *Applied and Environmental Microbiology*; *80*; 7388-7397. doi: 10.1128/AEM.02200-14.

Symonds, E. L., O'Mahony, C., Lapthorne, S., O'Mahony, D., Sharry, J. M., O'Mahony, L. & Shanahan, F. (2012). *Bifidobacterium infantis* 35624 Protects Against Salmonella-Induced Reductions in Digestive Enzyme Activity in Mice by Attenuation of the Host Inflammatory Response. *Clinical and Translational Gastroenterology*; *3*; 15.

Wang, X., Wang, J., Sun, H., Xia, S., Duan, R. & Liang, J. (2015). Etiology of Childhood Infectious Diarrhea in a Developed Region of China: Compared to Childhood Diarrhea in a Developing Region and Adult Diarrhea in a Developed Region. *PLoS ONE*; *10*; 1-14. doi: 10.1371/journal.pone.0142136.

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*; 1-19. doi: 10.3389/fbioe.2021.612285.

Wulandari, S., Ainuri, M. & Sukartiko, A. C. (2021). Biochemical Content of Robusta Coffees Under Fully-Wash, Honey, and Natural Processing Methods. *IOP Conferences Series: Earth and Environmental Science*; *819*; 1-9.

Xie, Y., Yang, W., Tang, F., Chen, X. & Ren, L. (2015). Antibacterial Activities of Flavonoids: Structure-Activity Relationship and Mechanism. *Current Medicinal Chemistry*; *22*; 132–149. doi: 10.2174/0929867321666140916113443.

Yang, J. & Yang, H. (2019). Antibacterial Activity of *Bifidobacterium breve* Against *Clostridioides difficile*. *Frontiers in Cellular and Infection Microbiology*; *9*; 1-12. doi: 10.3389/fcimb.2019.00288.

P-ISSN: 2406-9388
E-ISSN: 2580-8303

Zhou, Y., Zhu, X., Hou, H. et al. (2018). Characteristics of Diarrheagenic *Escherichia coli* among Children Under 5 Years of Age with Acute Diarrhea: A Hospital Based Study. *BMC Infectious Disease*; *18*; 1-10. doi: 10.1186/s12879-017-2936-1.

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