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IMPLEMENTATION OF CONSORTIUM BACTERIES OF *Bacillus cereus* ISOLATED FROM SEA AS PROTEIN SOURCE FOR PRO COFFEE BEVERAGE

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ABSTRACT

Pro coffee is a fermented beverage using *Bacillus cereus* bacterial consortium with single-cell protein. This study aims to determine the best composition of the *B. cereus* consortium bacteria mixture in organoleptic aspects that panelists most prefer and the effect of adding B. cereus consortium bacteria on proximate analysis and pro coffee flavor. Organoleptic testing of probiotic coffee was conducted on 20 panelists, then continued with proximate analysis of pro coffee. The composition with the best organoleptic flavor is obtained in code D, with the treatment of adding 5% bacterial consortium *B. cereus* for 15 days. The analysis of taste obtained has a value of 0.000 <0.05, and the study results show that the significance value is smaller than the significance level. Hence, the treatment results give the effect of adding bacterial consortium to pro-coffee. The addition of bacterial consortium B. cereus to probiotic coffee, in the form of moisture, ash, protein, fat, and carbohydrate content, is 0.000 <0.05. The analysis results show that the significance value is greater than the significance level, so the treatment results do not give the effect of adding bacterial consortium to pro coffee.

Keywords: Coffee, Probiotics, *Bacillus cereus*, Protein

1. INTRODUCTION

Food technology has changed the function of food as a functional product, which can maintain the human body and even treat various diseases. One of the functional food products that can be developed is probiotic drinks. Probiotic bacteria have been shown in multiple studies to help the process of food digestion in animals and humans. Probiotic bacteria are growing significantly, as seen in various studies that prove that probiotic bacteria have benefits for human health[1].

Bacteria have high potential as probiotics. One of the bacteria that has the potential to be developed as a single-cell protein agent is *B. cereus,* which is classified as a heterotrophic bacterium[2]. Studies state that *B. cereus* is a probiotic that can inhibit several pathogenic bacteria and degrade toxic organic matter in the environment, especially in water. Its implementation has been done by making probiotic capsules[3].

Single Cell Protein (PST) is used for crude or purified proteins derived from simple one- or multi-celled microorganisms, such as bacteria, fungi, algae, and protozoa. Microbes that are often used in single-cell protein research are yeasts, fungi, algae, and bacteria. The crude protein contained in several types of mycorrhizae, such as yeast, ranges from 45-55%, the crude protein content is 30-45%, and algae crude protein content is 40- 60%, and in bacteria, the crude protein ranges from 50- 65%[4].

Coffee has its appeal from various circles, so increasing the added value of coffee commodities is necessary. At the processing stage, the fermentation process utilizing microorganisms has now become a study by various researchers. The improvement of coffee quality during the

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fermentation process is due to the microbial activity in coffee, which produces alcohol and organic acids that provide aroma and flavor for quality coffee production[5]. The addition of probiotic bacteria in coffee has yet to be widely done, and this supports the quality improvement of protein drinks using probiotic bacteria *B. cereus* under the name pro coffee.

This study aimed to determine the best composition of the mixture of *B. cereus* consortium bacteria in the organoleptic aspect that panelists and the effect of the addition of *B. cereus* consortium bacteria on proximate analysis and pro coffee flavor most prefer.

2. RESEARCH METHOD

Time and Place

This research was conducted from October to December 2023. Bacterial inoculation for making pro-coffee was performed at the Marine Microbiology Laboratory, Department of Marine Science, Faculty of Fisheries and Marine Sciences, Universitas Riau. The proximate test was conducted at the Nutrition and Chemistry Laboratory, Faculty of Agriculture and Forestry, Universitas Riau.

Method

The method used in this research is an experimental method using a completely randomized design (CRD), which uses four levels of treatment with three repetitions. The bacterial isolates used were the addition of *B. cereus* consortium bacteria from (*B. cereus* SN7, *B. cereus* SP4, *B. cereus* S5, *B. cereus* Xmb051, and *B. cereus* BF2) in the Marine Microbiology Laboratory. Then, organoleptic tests and proximate analyses were conducted.

Procedures

Sterilization of Tools and Materials

The first step in preparing the media is sterilizing tools and materials, such as glass tools, which are first washed and dried. Then, they are covered with paper and heated in an autoclave at 121^{o}C with a pressure of 2 atm for 15 minutes.

Starter Preparation

Culture of *B. cereus* Bacteria

The first step is to make NA media in a Petri dish aseptically. Then sterilized with an autoclave. After sterilization, NA media was transferred into petri dishes aseptically. Then, after the NA media is solid, bacteria from the old media are inoculated into the new media using an ose needle aseptically. Bacteria were incubated for 24 hours at 36^{o}C in an incubator.

Making Modified Media

The manufacturing procedure, by adding or homogenizing water, sugar, and milk with other micronutrients such as vitamin B12, is homogenized on a hotplate at 47°C±20 minutes.

Table 1. Composition of the growth media for the *B. cereus* bacterial consortium

Ingredients	Unit
UHT milk (Ultra Milk)	55 mL
Sugar (Gulaku)	20 g
Cleo Water	400 mL
Vitamin B12 (IPI)	25 g

Preparation of *B. cereus* Bacteria Suspension

Sterilized distilled water was added to the test tube using a 10 ml dropper. The bacterial strains used were *B. cereus* SN7, *B. cereus* SP4, *B. cereus* S5, *B. cereus* Xmb051 and *B. cereus* BF2. Then, the five bacterial strains incubated are suspended into each test tube according to their names aseptically using an ose needle. Homogenize using a vortex until the turbidity of the five suspensions is equivalent to the turbidity of the McFarland 10^5 solution that has been made.

Bacterial Cultures and Subcultures on Growth Media

The culture was carried out aseptically by inserting five strains of bacteria

suspended in a test tube into an Erlenmeyer bottle containing modified growth media. The five strains of bacteria incorporated in the media are named the *B. cereus* consortium bacteria. Then, the *B. cereus* consortium bacteria included in the modified media were allowed to stand for 6 hours of bacterial growth at room temperature.

Pro Coffee Making

Robusta coffee is made with 10 g of sugar and 10 g of coffee. Next, all ingredients dissolved in 100 ml of boiling water until homogeneous for 15 seconds. Then, let it rest at room temperature (± 25^0C). Then, the bacterial suspension was inoculated on media containing robusta coffee with a completely randomized design (CRD) based on the treatment;

A = No addition, Coffee Mix 100 mL
B = 1 mL Bacterial Consortium Suspension, 99 mL Coffee Mix
C = 3 ml suspension, 97 mL coffee
D = 5 mL Suspension, 95 mL coffee

Then, the culture of *B. cereus* bacteria was incubated at a temperature of ±25^0C. Observation of bacterial growth on days 0,7 days to 15 days and each probiotic coffee sample will be taken and stored for further analysis.

Organoleptic Tests

The organoleptic test involves trained panelists assessing texture, aroma, color, and taste. Hedonic tests, on the other hand, involve potential consumers evaluating their overall level of satisfaction with the product[6]. At this stage, testing was carried out by giving questionnaires and innovative products to 20 panelists. The panelists were assessed on several aspects, namely aroma and taste, on a scale of 1 to 5, ranging from very like to dislike.

Proximate Analysis Observations

Determination of the content of each sample was carried out through proximate tests at the Nutrition and Chemistry Laboratory, Faculty of Agriculture, Universitas Riau.

3. RESULT AND DISCUSSION

Organoleptic Test

Organoleptic testing of innovative products in the form of robusta coffee with a bacterial suspension of *B.cereus* consortium, which has a different composition, has been done on 20 panelists, assessing two types of aspects in the form of taste and aroma. Robusta coffee with a bacterial suspension of *B.cereus* consortium presented is a representative sample of drinks that are suitable for consumption, namely innovation products with codes A (Control), B (1%), C (3%), and D (5%) having a 15-day treatment. Based on the study's results, the highest value that many panelists liked was the innovation with code D (5%).

Table 2. Percentage of organoleptic test of product flavor

Treatment	Very dislike	Dislike	Neutral	Like	Like very much	Total %
A	0,00	10	80	10	00	100
B	0,00	15	35	50	00	100
C	0,00	00	40	50	10	100
D	0,00	05	30	50	15	100

Flavor

In product innovation, flavor is an essential factor that can determine the attractiveness for consumers, especially in a food product. Factors including temperature, liquid consistency, length of production, or cooking time can influence flavor in food products.

Based on Table 2, containing the results of the organoleptic test on the taste of robusta coffee with a bacterial suspension of the *B. cereus* consortium, the percentage of results obtained in the highest organoleptic

test in the very like category is found in coffee with treatment D, namely 15%.

The average test conducted on panelists regarding the taste of coffee with the addition of a bacterial suspension of *B. cereus* consortium showed the highest score in treatment D or the composition of the highest bacterial administration. This is because the addition of *B. cereus* bacterial consortium suspension affects coffee's original taste, so panelists prefer the addition of *B.cereus* bacterial consortium suspension. Factors that can affect the flavor of a product are chemical compounds, interactions with other components, and temperature. Flavor also dramatically affects panelists' level of acceptance of a product[7].

Aroma

Aroma is an indication of food eligibility. Besides indicating aroma, it can also detect whether or not a processed food is suitable for consumption.

Table 3. Percentage of product aroma organoleptic test

Treatment	Very dislike	Dislike	Neutral	Like	Like very much	Total %
A	00	00	50	50	00	100
B	00	25	20	55	00	100
C	00	10	50	35	05	100
D	00	00	25	65	10	100

Based on Table 3, containing the results of the organoleptic test on the aroma of robusta coffee with a suspension of *B. cereus* consortium bacteria, the percentage of results obtained in the highest organoleptic test in the category of very like the aroma of the treatment, is found in coffee with treatment D, which is 10%.

The average test conducted on panelists regarding the aroma of coffee with the addition of a bacterial suspension of *B. cereus* consortium showed the highest score in treatment D or the provision of the highest composition. The aroma can directly influence panelists' interest in choosing a food product[8]. The addition of *B. cereus* bacterial consortium suspension affects the original aroma of robusta coffee, so panelists prefer coffee with the addition of *B. cereus* bacterial consortium suspension. Aroma can be a direct factor that influences consumers' interest in choosing food products[9].

Proximate Analysis

Figure 1 shows that the results of the analysis of the total water content in robusta coffee with a bacterial suspension of *B. cereus* consortium show that the highest value of total water content is in treatment A, which is 98.61%, namely in the control treatment, while the lowest value for total water content is in treatment D with a percentage of 98.13%. The test in this study obtained the lowest moisture content of 98.13%, where the moisture content value is still too high and has not met the standard of ≤10%. This is related to the speed of water propagation (diffusion) in the cell tissue of coffee beans. The lower the water content in coffee beans, the lower the water evaporation speed because the water molecules' position is located further away from the surface of the beans[10].

Figure 2 shows the analysis results of the total ash content of robusta coffee with a suspension of *B. cereus* bacterial consortium. The highest value of total ash content was found in treatment D, which amounted to 3.21%, while the lowest value for total ash content was in treatment A, with a percentage of 3.21%, namely as a control treatment. These levels are still within the appropriate level for ash content that should be contained in food ingredients. Ash content in food ingredients is ≤ 10%.

In this study, the ash content obtained was by the standard. Ash content in food determines the amount of minerals in it[11]. Minerals are essential in deciding nutrition and observing parameters. Minerals will not

burn during the charring process, while organic matter will be consumed. Ash content is closely related to water content, as high water content will be followed by ash content. A high ash content indicates that a high amount of minerals are also present.

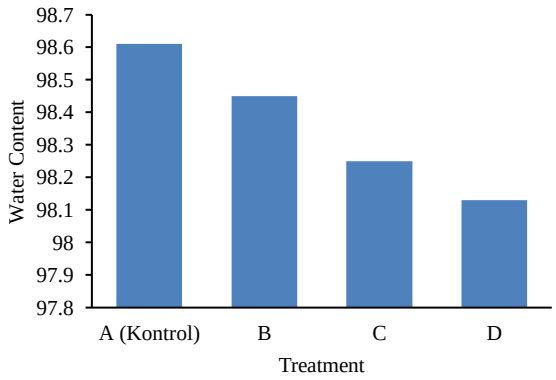

Figure 1. Total moisture content

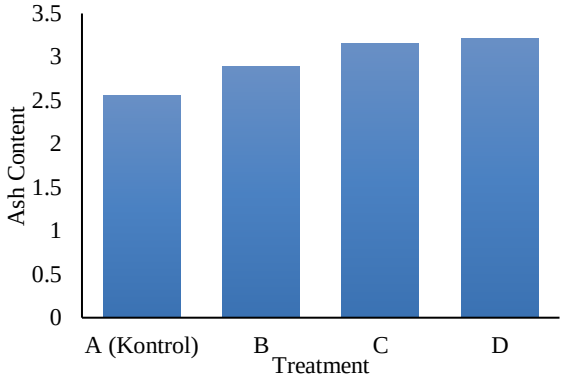

Figure 2. Total ash content

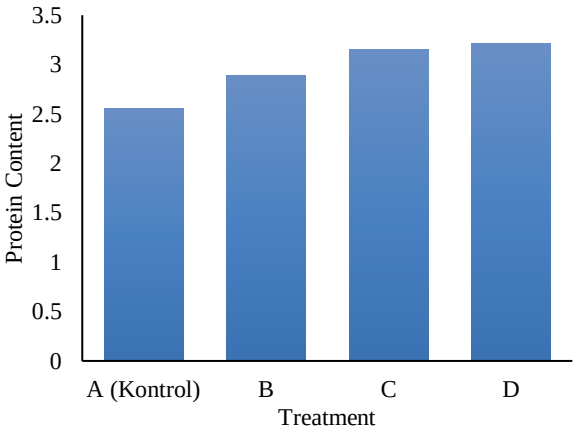

Figure 3. Total protein content

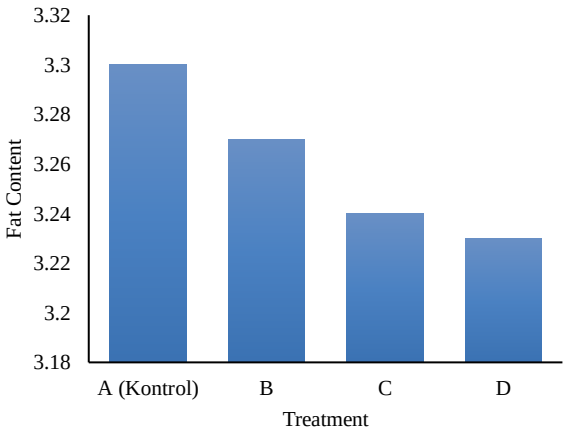

Figure 4. Total fat content

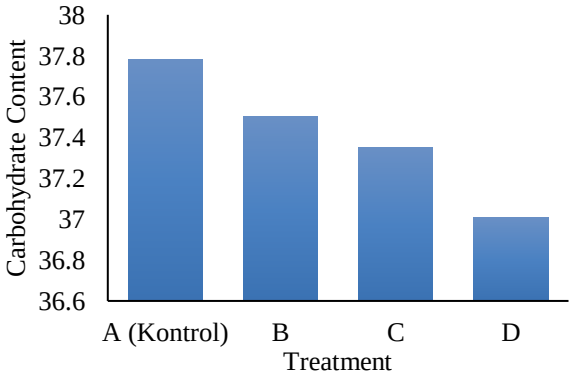

Figure 5. Total carbohydrate

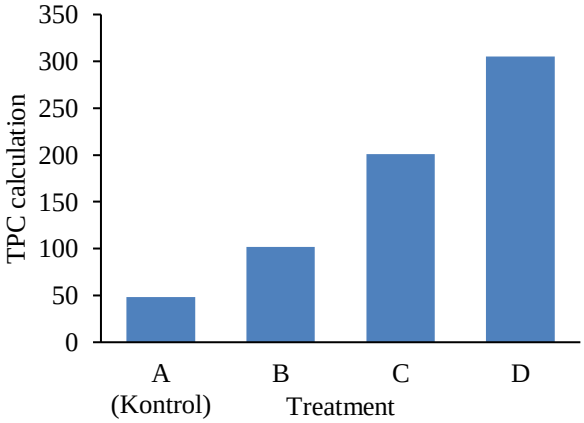

Figure 6. TPC count

Figure 3 shows the results of the analysis of the total protein content in robusta coffee with a suspension of the bacterial consortium *B. cereus*. It shows that the highest total protein content value is in treatment D, which is 13.11%, namely in the provision of the highest bacterial suspension, while the lowest value for total protein content is in treatment A, with a percentage of 12.25%, namely in the control treatment.

Based on the results of the proximate analysis of protein content in robusta coffee with a suspension of *B. cereus* consortium bacteria above, a discussion can be obtained in the form that adding a suspension of *B.*

cereus consortium bacteria in robusta coffee does not significantly affect protein content. However, the addition of a bacterial suspension of *B. cereus* consortium in robusta coffee can inhibit the appearance of bacteria or pathogens because *B. cereus* is one of the bacteria from the Bacillus genus that has the potential to produce protein. *B. cereus* is also often used as a probiotic material that functions as an inhibitor of heterotrophic bacteria, so toxic materials in the environment can be degraded. This is reinforced by bacteria that generally produce proteins, such as *Bacillus* sp[12].

Figure 4 shows the results of the analysis of total fat content in robusta coffee with a suspension of bacterial consortium *B. cereus. The* highest value of total fat content is in treatment A, which is 3.30%, namely in the control treatment, while the lowest value is in treatment D, with a percentage of 3.23%, namely in the provision of the highest dose of 5 mL of bacterial consortium *B. cereus* suspension.

Based on the results of the proximate analysis of fat content in robusta coffee with a suspension of *B. cereus* consortium bacteria above, a discussion can be obtained in the form of the addition of a suspension of *B. cereus* consortium bacteria in robusta coffee does not significantly affect fat content. Before adding a bacterial suspension of *B. cereus* consortium, Robusta coffee contains protein, alkaloid compounds, carbohydrates, and fat. Coffee comprises insoluble and water-soluble compounds like alkaloid compounds, fats, proteins, and carbohydrates[13].

Figure 5 shows that the results of the analysis of the total carbohydrate content of robusta coffee with a suspension of bacterial consortium *B. cereus* show that the highest value of total carbohydrate content is in treatment A, which is 37.78%, namely in the control treatment, while the lowest value for total water content is in treatment D with a percentage of 37.01%, namely in the provision of the highest dose of 5 mL of bacterial consortium *B. cereus* suspension.

Based on the results of proximate analysis of carbohydrate content in robusta coffee with a suspension of *B. cereus* consortium bacteria above, a discussion can be obtained in the form of the addition of a suspension of *B. cereus* consortium bacteria in robusta coffee does not have a significant effect on carbohydrate content. The carbohydrate content obtained based on the graph above has decreased carbohydrate value. The decrease in carbohydrate value is likely from adding a bacterial suspension of *B. cereus* consortium in robusta coffee. *B. cereus* consortium bacteria in robusta coffee use carbohydrates for bacterial growth as an energy source. In addition to glucose as an energy source, microorganisms also use other types of carbohydrates as materials to fulfill energy needs[13].

Figure 6 shows the results of the analysis of the TPC value in robusta coffee with a suspension of *B. cereus* bacterial consortium. This indicates that the highest total fat content value is in treatment D, which is 305 CFU/mL, in the control treatment. In comparison, the lowest value for total water content is in treatment A at 48 CFU/mL, namely in providing the highest dose of 5 mL of bacterial suspension of *B. cereus* consortium.

Based on the proximate analysis of TPC results, it is known that the colonies that grow in coffee indicate the number of all microorganisms, including the *B. cereus* consortium bacteria. The aim is to describe the microbiological quality of coffee added with *B. cereus* consortium bacteria suspension. The lowest TPC value obtained from the test was 48 CFU/mL, and the highest was 305 CFU/mL. As a sample of beverages with bacterial suspensions, this value is sufficient for the minimum requirement of the TPC value. The requirement for probiotic drinks is that they must contain more than 10^8 CFU/mL in a living state[14].

4. CONCLUSION

The composition with the best organoleptic flavour is obtained in code D,

with the treatment of adding 5% *B. cereus* consortium bacteria for 15 days, with a total score of 10% aroma and 15% flavour. The addition of bacterial consortium *B. cereus* to probiotic coffee, in the form of moisture, ash, protein, fat, and carbohydrate content, is 0.000 <0.05. The analysis results show that the significance value is greater than the significance level, so the treatment results do not give the effect of adding bacterial consortium to pro coffee. While the analysis of taste obtained has a value of 0.000 <0.05, the results show that the significance value is smaller than the significance level. Hence, the treatment results give the effect of adding consortium bacteria to pro coffee.

Further research needs to be done on variations in storage temperature and extended storage time of probiotic coffee drinks in order to obtain probiotic coffee drinks that are safe for consumption.

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