Production of pro coffee as healthy coffee with the addition of *bacillus cereus* bacteria

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Abstract. Pro coffee is one of the coffee drinks enriched with *Bacillus cereus* bacterial consortium. The bacterial consortium is a rich single-cell protein. This study aimed to determine the best formula from the organoleptic aspect includes smell, taste, colour, and texture as well as proximate analysis. The method used was experimental by using complete random design (CRD) with a series of tests: organoleptic to 20 respondents, and proximate analysis. With 5 levels, such as the addition of 0% (P0), 1% (P1), 3% (P2) and 5% (P3) with a fermentation period of 0, 7 and 15 days. The results showed that the formulation that had the best organoleptic was P3 (5% bacterial consortium of *B. cereus*) for 15 days. The highest and the lowest of total protein content was also in P3 (13.11%) and P0 (12.25%), respectively. The addition of *B. cereus* suspension increased the protein content of pro coffee.

1 Introduction

Microbes that are often used in single-cell protein research are yeast, function, algae, and bacteria. Bacteria have great potential to be developed in the field of biotechnology industry. Crude protein contained in several types of microbes such as yeast ranges from 45-55%, fungi the crude protein content is 30-45% and algae the crude protein content is 40-60% and in bacteria the crude protein ranges from 50-65% [1]. Probiotic bacteria are growing significantly and this is seen in various studies that prove that probiotic bacteria have benefits to human health. 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]. This is evident from several studies that state *B. cereus* to be a probiotic that can inhibit several pathogenic bacteria, and is also a heterotrophic bacterium that can degrade toxic organic matter in the environment, especially in waters. Its implementation has been carried out by making probiotic capsules.

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Coffee has its own appeal from various circles, so it is necessary to increase the added value of coffee commodities. The application of technology to improve coffee quality can be done through various processes such as the plantation, processing and storage stages. At the processing stage, the fermentation process by utilizing microorganisms has now become a study by various researchers. The improvement of coffee quality during the fermentation process is due to microbial activity in coffee during fermentation which produces alcohol and organic acids that provide aroma and flavour for quality coffee production [3]. The addition of probiotic bacteria in coffee has not been widely done, this supports the improvement of the quality of protein drinks using probiotic bacteria *B. cereus* under the name pro coffee. Based on this background, researchers want to develop probiotic coffee drinks, the *B. cereus* bacteria used consist of 5 isolates that are combined. According to the results of research [4] there are five species of *B. cereus* that have the potential to be developed as single. cell proteins, namely (*B. cereus SN7, B. cereus SP4, B. cereus S5, B. cereus Xmb051* and *B. cereus BF2*), otherwise known as the *B. cereus* consortium bacteria. So, it is necessary to conduct research on "Implementation of *Bacillus cereus* consortium bacteria isolated from the sea as a protein source for Pro Coffee Drinks".

Pro coffee is a coffee that contains protein, which is derived from probiotic microorganisms. The *B. cereus* species used have conducted a series of tests, including challenge tests against pathogenic bacteria, namely *Pseudomonas* sp*., Aeromonas* sp., and *Vibrio alginolyticus* [5]. With the ability of these bacteria can inhibit the growth of these pathogenic bacteria. The development of this pro coffee product has been carried out through the replacement of microorganism culture media, namely with sugar and milk so that probiotic microorganisms can still live and coffee is suitable for human consumption. The addition of probiotic bacteria in coffee has not been widely done, this supports the improvement of the quality of protein drinks using probiotic bacteria *B. cereus* under the name pro coffee. The purpose of this study was to determine the best composition of the mixture of *B. cereus* consortium bacteria in the organoleptic aspect that is most preferred by respondents and the effect of the addition of *B. cereus* consortium bacteria on proximate analysis and pro coffee flavour.

2 Material and Method

Bacterial inoculation for making pro coffee was carried out at the Marine Microbiology Laboratory, Department of Marine Science, Faculty of Fisheries and Marine Sciences, Riau University. 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, namely the addition of 0% (P0), 1% (P1), 3% (P2) and 5% (P3) with a storage fermentation period of 0, 7 and 15 days. 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 test and proximate analysis were conducted.

2.1 Culture of *B. cereus* bacteria on NA media

The first step is to make NA media in a petri dish aseptically. The procedure for making growth media, by adding or homogenizing mineral water (400 ml), sugar 20 gr (GULAKU), UHT milk 55 ml (Ultra Milk) with other micronutrients such as vitamin B12 (0.25 mg), which is homogenized on a hotplate at 47 °C ± 20 minutes. After the growth medium is in sterile conditions, it can then be used as a testing medium for B. cereus.

2.2 Preparation of *B. cereus* Bacteria Suspension

Sterilized distilled water was put into a test tube using a 10 ml dropper. Five bacterial strains were used, namely *B. cereus* SN7, *B. cereus* SP4, *B. cereus* S5, *B. cereus* Xmb051 and *B. cereus* BF2. Then 5 strains were incubated and then suspended into each test tube with the turbidity of Mc Farland 10^8 solution that had been made. About 5 strains of bacteria that have been suspended in a test tube are then placed into an Erlenmeyer bottle containing modified growth media. The 5 strains of bacteria incorporated in the media were named the *B. cereus* consortium bacteria. Then the *B. cereus* consortium bacteria that have been included in the modified media are incubated for 6 hours of bacterial growth period at room temperature.

2.3 Making Pro Coffee

Pro coffee is made with a robusta coffee (*Coffea canephora*) composition of 10 grams of sugar and 10 grams of coffee dissolved in 100 ml of boiling water. Then let stand at room temperature (± 27°C). Then the bacterial suspension was inoculated into the media and fermented for 0, 7 and 15 days.
P0 = 100 ml coffee
P1 = Coffee 99 ml +1 ml bacterial suspension of *B. cereus* consortium
P2 = 97 ml coffee + 3 ml *B. cereus* consortium bacteria suspension
P3 = 97 ml coffee + 5 ml *B. cereus* consortium bacteria suspension
Then the coffees were incubated in an incubator at a temperature of ± 27 °C

2.4 Organoleptic Test

Organoleptic tests involve trained respondents to assess organoleptic aspects such as aroma, colour, texture and taste. Hedonic tests, on the other hand, involve potential consumers to evaluate their overall level of satisfaction with the product [6]. At this stage, testing was carried out by providing questionnaires and giving innovative products to 20 respondents, with several assessments, namely aroma and taste with a scale rating of 1 to 5 ranging from very like to dislike. Determination of the content of each sample is carried out proximate test, namely. Moisture content, ash content, protein content, weak content and carbohydrate content. Observation of bacterial growth on day 0, 7 days to 15 days and will be taken each probiotic coffee sample and then stored for further analysis. the number of bacterial colonies is carried out by the total plate count method with the formula for calculating bacteria, namely:

$$\text{CFU/ml} = \frac{\text{Number of colonies}}{\text{Sample volume x Dilution factor}}$$

3 Result and Discussion

3.1 Proximate Analysis

3.1.1 Water Content

Most of the pro coffee content is water, Fig.1., it can be seen that the P0 treatment is the highest value of 98.61% as the control treatment, while the lowest value for total water content is in the P3 treatment with a percentage of 98.13%. The lowest water content result was 98.13% (P3). In food and beverage content, high water content will result in faster

spoilage due to microbial contamination [7]. The shelf life of food is certainly strongly influenced by the water content in it. If a food ingredient has a low water content, its shelf life will also be more durable. The effect of adding *B. cereus* suspension can reduce water content and slow down decay.

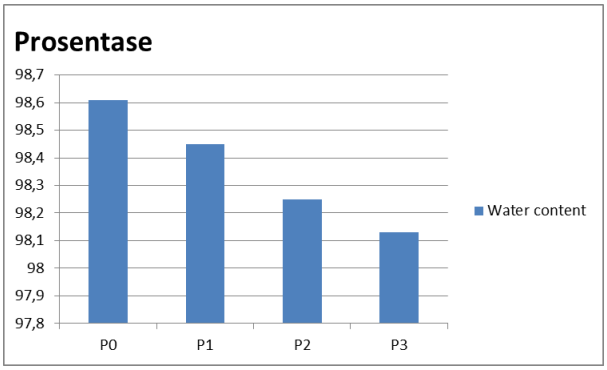

Fig. 1. Graph of total water content of pro coffee. P0 = No addition of bacteria, P1 = Addition of 1 ml *B. cereus* consortium suspension, P2 = Addition of 3 ml *B. cereus* consortium. P3 = Addition of 5 ml *B. cereus* consortium.

3.1.2 Ash content

Organic constituents that come from minerals are ash. The following are the results of the analysis of total ash content in robusta coffee with a suspension of *B. cereus* bacterial consortium.

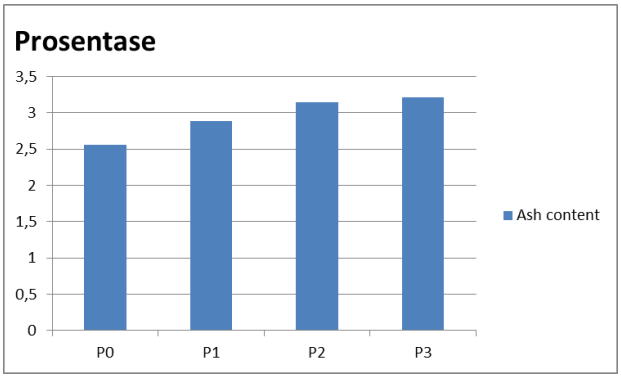

Fig. 2. Graph of total ash content of robusta coffee. P0 = No addition of bacteria, P1 = Addition of 1 ml *B. cereus* consortium suspension, P2 = Addition of 3 ml *B. cereus* consortium. P3 = Addition of 5 ml *B. cereus* consortium

Fig 2. shows the results of the analysis of the total ash content in robusta coffee with a suspension of *B. cereus* consortium, that the highest value of total ash content was found in treatment P3, which amounted to 3.21%, while the lowest value for total ash content was in treatment with a percentage of 2.56%, namely as a control treatment. where these levels are still within the appropriate level for ash levels that should be contained in food ingredients. Ash content in food ingredients is ≤ 10% (SNI 3753:2014).

In this study, the ash content obtained was in accordance with the standard. Ash content in food is used to determine the amount of minerals in it [8]. Minerals are very important and necessary in determining nutrition and observation parameters. During the process of

ignition, minerals will not burn, while organic matter will be consumed. Ash content is closely related to moisture content, the high value of moisture content will be followed by ash content. A high ash content indicates a high amount of minerals contained therein.

3.1.3 Protein Content

The nutritional content that has a function as a building and regulating substance for the body is protein, therefore protein levels in food are very necessary. The following are the results of the analysis of total protein content in robusta coffee with a suspension of *B. cereus* bacterial consortium.

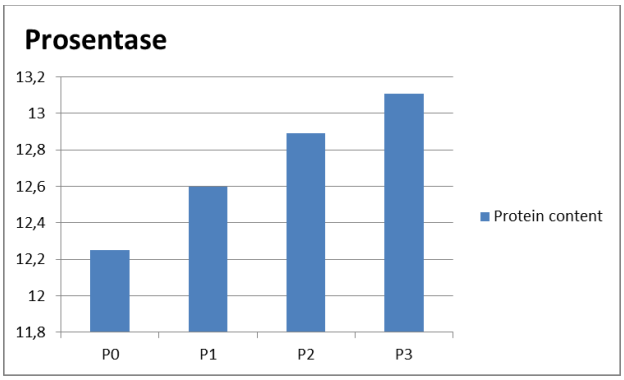

Fig. 3. Graph of total protein content of pro coffee. P0 = No addition of bacteria, P1 = Addition of 1 ml *B. cereus* consortium suspension, P2 = Addition of 3 ml *B. cereus* consortium. P3 = Addition of 5 ml *B. cereus* consortium

Fig. 3., it can be seen that the results of the analysis of the total protein content in robusta coffee with a suspension of bacterial consortium *B. cereus* show that the highest value of the highest total protein content is found in the P3 treatment which is 13.11%, namely in the provision of the highest bacterial suspension, while the lowest value for total protein content is in the P0 treatment with a percentage of 12.25%, namely in the control treatment.

Based on the results of 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 of the addition of a suspension of *B. cereus* consortium bacteria in robusta coffee does not have a significant effect on protein content. However, the addition of *B. cereus* bacterial consortium suspension in robusta coffee can inhibit the appearance of bacteria or pathogens because *B. cereus* is one of the bacteria sourced from the Bacillus genus which has the potential to inhibit the growth of pathogenic bacteria. *B. cereus* [9] is also often used as a probiotic material that functions as a heterotrophic bacterium so that toxic materials in the environment can be degraded.

3.1.4 Fat Content

Fat has an important role in the human body, namely as a source of energy for the body of living things.

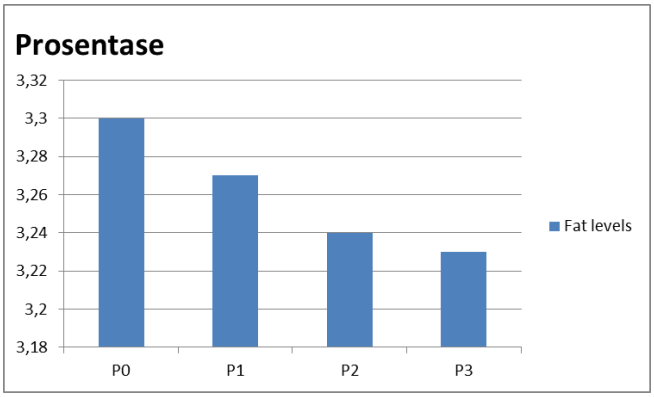

Fig. 4. Graph of total fat content of pro coffee. P0 = No addition of bacteria, P1 = Addition of 1 ml *B. cereus* consortium suspension, P2 = Addition of 3 ml *B. cereus* consortium. P3 = Addition of 5 ml *B. cereus* consortium

Based on fig 4. it can be seen that the results of the analysis of total fat content in robusta coffee with a suspension of *B. cereus* consortium bacteria show that the highest value of total fat content is found in the P0 treatment which is 3.30%, namely in the control treatment, while the lowest value for total fat content is in the P3 treatment with a percentage of 3.23%, namely in the provision of the highest dose of 5 ml of suspension of *B. cereus* consortium bacteria.

The results of 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 have a significant effect on fat content. Robusta coffee before the addition of bacterial suspension of *B. cereus* consortium contains protein, alkaloid compounds, carbohydrates, and fats. This is because coffee is composed of insoluble and water-soluble compounds in the form of alkaloid compounds, fats, proteins, and also carbohydrates [10].

3.1.5 Carbohydrate Content

Carbohydrates play an important role in the human body, plants, and animals. The main function of carbohydrates is to be the body's main source of energy

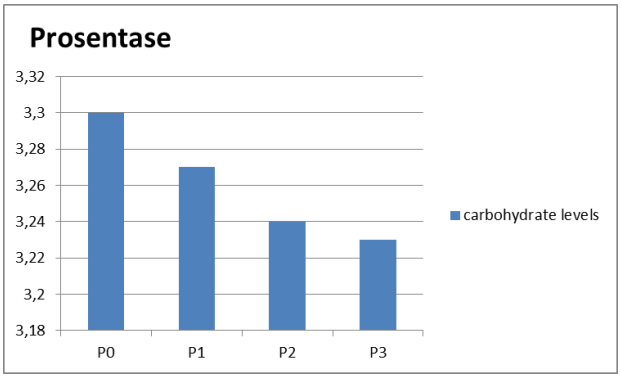

Fig. 5. Graph of total carbohydrate content of pro coffee. P0 = No addition of bacteria, P1 = Addition of 1 ml *B. cereus* consortium suspension, P2 = Addition of 3 ml *B. cereus* consortium. P3 = Addition of 5 ml *B. cereus* consortium.

3.2 Total Plate Count (TPC) of *B. cereus*

The total number of bacteria in pro coffee varied from each treatment.

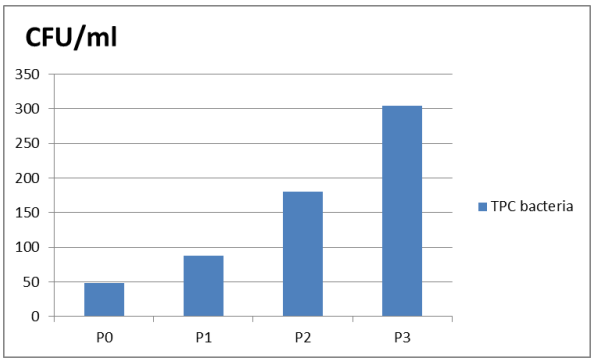

Fig. 6. Graph of TPC count of pro coffee products. P0 = No addition of bacteria, P1 = Addition of 1 ml *B. cereus* consortium suspension, P2 = Addition of 3 ml *B. cereus* consortium. P3 = Addition of 5 ml *B. cereus* consortium.

Based on Fig.6. it can be seen that the results of the analysis of the TPC value in robusta coffee with a suspension of *B. cereus* consortium bacteria. shows that the highest value of total fat content is highest in the P3 treatment, which is 305 CFU / ml, namely in the control treatment, while the lowest value for total water content is in the P0 treatment of 48 CFU / ml, namely in the provision of the highest dose of 5 ml of suspension of *B. cereus* consortium bacteria. The results of proximate analysis of TPC results, it is known that the colonies that grow in coffee show the number of all microorganisms including the *B. cereus* consortium bacteria. The aim is to describe the microbiological quality in coffee that is given the addition of a suspension of *B. cereus* consortium bacteria. The lowest TPC value obtained from the test was 48 CFU/ml to the highest 305 CFU/ml. As a beverage sample with a bacterial suspension, this value is sufficient for the minimum requirement of TPC value. The requirement for probiotic drinks is that they must contain more than 108 CFU/ml in a living state [10].

3.3 Organoleptic Test

A total of 20 respondents were used in organoleptic testing of innovative products in the form of robusta coffee with a suspension of *B. cereus* consortium bacteria. The assessment was carried out in two types of aspects in the form of aroma and taste. The average test conducted on respondents regarding the aroma of coffee with the addition of a suspension of *B. cereus* bacterial consortium showed the highest score in treatment D or the provision of the highest composition. Aroma can affect the direct interest of respondents in choosing a food product. The addition of *B. cereus* bacterial consortium suspension affects the original aroma of robusta coffee, so respondent 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 [11].

3.3.1 Flavour

In a type of product innovation, flavour is an important factor that can determine the attractiveness for consumers, especially in a food product. Flavour in food products can be

influenced by factors including temperature, liquid consistency, length of production or cooking time.

Table 1. Percentage of organoleptic test of product flavour

Treatment	Very dislike	Dislike	Neutral	Like	Very like	% Total
P0	0,0	10	80	10	0	100
P1	0,0	15	35	50	0	100
P2	0,0	0	40	50	10	100
P3	0,0	5	30	50	15	100

According to Table 1, contains the results of the organoleptic test on the taste of robusta coffee with a suspension of bacterial consortium *B. cereus* , the percentage of results obtained in the highest organoleptic test in the very like category is found in coffee with treatment P3, namely 15%. The average test conducted on respondents regarding the taste of coffee with the addition of a suspension of *B. cereus* consortium bacteria showed the highest score in treatment D or the composition of the highest bacterial administration. This is because the addition of *B. cereus* consortium bacteria suspension affects the original taste of coffee, so respondents prefer the addition of *B. cereus* consortium bacteria suspension. Factors that can affect the taste of a product are chemical compounds, interactions with other components, and temperature [12]. Flavour also greatly affects the level of acceptance of a product by respondents.

3.3.2 Aroma

Aroma is an indication of food feasibility, besides being an indication of aroma, it can also detect whether a processed food is suitable for consumption or not. The following Table 4 is the percentage of aroma organoleptic test on robusta coffee with a suspension of *B. cereus* consortium bacteria with different compositions.

Table 2. Percentage of product aroma organoleptic test

Treatment	Very dislike	Dislike	Neutral	Like	Very like	% Total
P0	0	0	50	50	0	100
P1	0	25	20	55	0	100
P2	0	10	50	35	5	100
P3	0	0	25	65	10	100

According to Table 2, 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, found in coffee with treatment P3 which is 10%.

4 Conclusion

The composition that has the best flavour is obtained in the P3 treatment, with the treatment of adding 5% *B. cereus* consortium bacteria for 15 days, having a total score of 10% aroma and 15% flavour. The addition of *B. cereus* bacterial consortium to probiotic coffee affects the moisture, ash, protein, fat and carbohydrate content of taste and aroma.

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