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Anthocyanin and Nutritional Contents of Fermented Lebui Bean (*Cajanus* sp.) through SSF Method and Induced by *Rhizopus* sp. and *Saccharomyces* sp.

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Abstract. Anthocyanins are bioactive compounds that were very important to support health and prevent oxidation. The existence of anthocyanin in plants, one of which is characterized by the presence of their striking colors in the plant. Lebui bean (*Cajanus* sp.) have a deep black color and identified to contain anthocyanins and phenolic compounds. However, these two important bioactive compounds are not obtained in free conditions, because they were still bound in cells and cell walls through the glycoside bond, also bound to other chemical bonds in the cell. Therefore, a method needed to break the bioactive compounds from their bonds. The easiest, cheapest, and most applicable method is fermentation. Fermentation is a method of processing food that utilizes microbial functions to degrade the compounds contained in the material to produce simpler components, while at the same time breaking various binding chains between components in the material, including solid state fermentation (SSF) method. Through fermentation, it is expected that bioactive compounds including anthocyanins will be cut off and released from the bonds of glycosides and form free compounds that have functional properties. Therefore, this study aims to obtain the type of microbes (*Rhizopus* sp. and *Saccharomyces* sp.) with the most optimal fermentation time to produce fermented bean powder which has the best anthocyanin content and nutritional component. A nested design with two factor was used. The main factor is the type of microbes (*Rhizopus* sp. and *Saccharomyces* sp.) and the second factor is the fermentation time (1, 2, and 3 day) nested on the main factor. Fermentation carried out for two days using *Rhizopus* sp. is the best treatment. Fermentation treatment by *Rhizopus* sp. for 1 to 3 days, produced an average level of anthocyanin in dry bases conditions is 129,81-153,55 ppm whether by *Saccharomyces* sp. was in the range of 107,12-128,11 ppm. Fermented lebui bean powder induced by *Rhizopus* sp. for 2 days fermentation has highest protein content and lowest fat content. The nutritional content of this best treatment is protein, fat, water, ash, and carbohydrate content i.e 22,39%, 0,41%, 6,73%, 3,18%, and 67,29%, respectively.

Keywords: SSF fermentation method, anthocyanin, protein, *Rhizopus* sp., *Saccharomyces* sp.

1. Introduction

Anthocyanins are colored water-soluble pigments which belong to widespread class of phenolic compounds i.e. flavonoid are naturally synthesized as secondary metabolism. Its common used to be colorant food that are responsible for the blue, purple, violet, magenta and red coloration [1,2], and would be replace the synthetic ones because of the negative effect when consumed by human body [3]. Due to derive from plant tissue, anthocyanins are non-toxic since it have been include in feed of animal or in food of the human in many years without generate symptoms of the diseases [4]. In



addition, anthocyanins also indicate a number of merit in biological systemic, i.e. as antioxidant, anti-carcinogen, hepato protection capacity and also could improve the memory system [5,6,7].

Leguminosae or common call Fabaceae is one of anthocyanin source which abundant in Indonesia. There are classified in flowering plants family known as the legume family, bean family, pulse family or pea family, which has a large growth area mainly in Indonesia. Lebui beans (*Cajanus* sp.) is one of the bean family that grown throughout the year and not much used in Lombok Island. In common, lebui beans were used as local dishes or just left on the field caused by limitations reference related processing or utilization of lebui beans. Anthocyanins attached in legume could generate mostly by complicated extraction method and expensive. However, recently research as done by Mushollaeni et al., [8] has shown that by applied SSF (Solid-state Fermentation) extraction method in lebui beans would derived anthocyanin consider from lebui beans powder that contain purple color.

SSF or solid-state fermentation has been used as a potential technology based on microbial utilization to result some product such as food, feed, fuel, pharmaceutical products and industrial chemicals [9]. The application include in bioleaching, biobeneficiation, bioremediation, biopulping, etc that offer some advantages. Technically, solid-state fermentation is defined as the microbial cultivation process neither in the absence nor near absence of free water in the substrate [10]. However, there must be enough moisture present to support cell growth. To support the fermentation, bacteria, yeast and fungi produce have different groups of enzymes. The selection of a suitable strain for the required purpose depends upon a number of factors, in particular upon the nature of the substrate and environmental conditions. In this research, *Rhizopus* sp. and *Saccharomyces cerevisiae* have been chosen to ferment the lebui beans to release the bioactive compounds in this case are anthocyanins. *Rhizopus* sp is the main microorganism plays important role in tempe fermentation process. Besides, other microorganism such as yeast, i.e. *Saccharomyces cerevisiae* may also contribute in fermentation process [11]. This process is easier because are not carried out under aseptic condition although starter culture is added. Moreover, the existence of those microorganism is quite abundant and cheaper. The aims of this research are to obtain the best type of microorganism both *Rhizopus* sp. and *Saccharomyces cerevisiae* to release bioactive compound such as anthocyanin through optimal fermentation time that show higher nutritional component.

2. Materials and Methods

2.1 Raw materials

Lebui beans (*Cajanus* sp.) were obtained from Lombok Island, West Nusa Tenggara Indonesia. Those beans were sorted to remove the dirt and mold, then dried in cabinet dryer for 20 min in 40°C until the water content is 12-13%. This beans then finely grounded and sieved 60 mesh to obtain the lebui bean powder (LBP).

2.2 SSF procedures

The LBP was heated in cabinet dryer at 70-80°C for 15 minutes, then fermented for 1 to 3 days using 2% dry culture of *Rhizopus* sp. (R) or *Saccharomyces cerevisiae* (S) at 27-28°C. After fermentation, LBP was dried by using cabinet dryer at 40°C for 5 h, then grounded and sieved 60 mesh into a fermented LBP and stored in a tightly closed glass container.

2.3 General experimental procedures

This research was statistically planned by a nested design with two factor was used. The main factor is the type of microbe (*Rhizopus* sp. and *Saccharomyces cerevisiae*) and the second factor is the fermentation time (1, 2, and 3 day) nested on the main factor. Observation parameters were total anthocyanin, protein, fat, moisture, ash, and carbohydrate content. The measurement of anthocyanin by UV-Visible Spectroscopy [12]. The proximate contents analysis were measured by AOAC [12] i.e. water content, protein content, ash content, and fat content. Total anthocyanin was determined by Juniarka et al. [13]. The research data were analyzed using nested ANOVA.

3. Result and Discussion

3.1 Total Anthocyanin

The aims of the Lebui Bean fermentation process is to release glycoside bonds attached inside the cells, so that the bioactive compounds including anthocyanins could be used. Fermenting process proven safety than applied high temperatures which could defect the compounds, moreover it may also improve the nutritional quality of beans. The anthocyanin content of fermented lebui beans with *Rhizopus* sp. was in the range 129,810-153,350 ppm, whereas fermented with *Saccharomyces cerevisiae* had lower level was 107,120-128,110 ppm. The anthocyanin levels in fermented lebui beans with *Rhizopus* sp.(R) or *Saccharomyces cerevisiae* (S) might tend to degenerate related to extension of fermentation time. In accordance to Balik [14], biochemical, metabolism, degradation, and destruction in fermentation process was conducted by microbes to produce more sugar components to support its growth, meanwhile for non-sugar components and secondary metabolite compounds would decreased including anthocyanin. Longer fermentation would increase microbial mass and positively correlated with decreased levels of anthocyanin in fermented products (Figure 1).

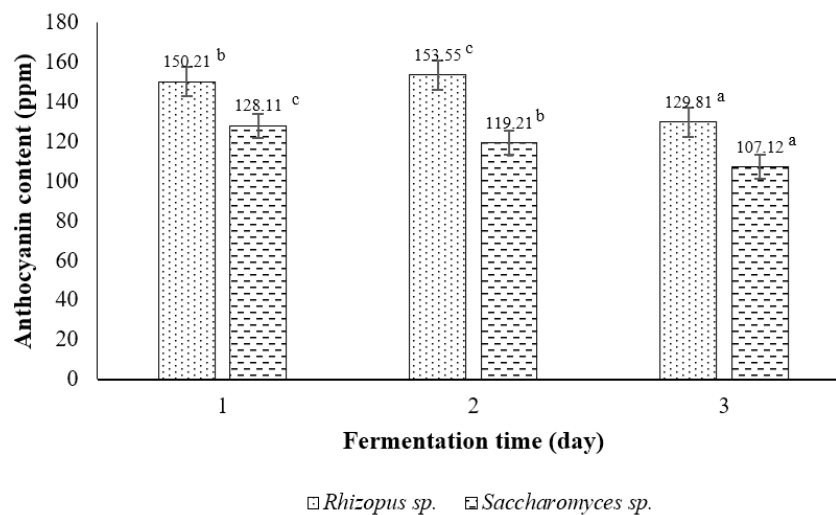


Figure 1. Comparison of nutrient content of LBP fermented by *Rhizopus* sp. and *Saccharomyces cerevisiae*

Reduction of anthocyanin levels during the fermentation period was also explained by Afoakwa et al. [15]. Anthocyanin levels may decrease with during fermentation period which is indicate by dark color reduced of the extract into lighter colors. As long as an anthocyanins are belong to polyphenol components, those were highly sensitive to changes in temperature and pH. Therefore, longer time of fermentation and environmental influences would decrease of anthocyanin levels [16].

In fermentation process, there also include hydrolysis that causes anthocyanin shift to be anthocyanidin, then gain a simple sugar group. It's underlies the reduction in anthocyanin content during fermentation period. The decline of these anthocyanin levels continues and reached the lower peak on the 3rd day of fermentation [15]. Based on the results of experiment, fermentation treatment up to 2 days gave better results than day 3. This relates to the occurrence of changes after more than 2 days of fermentation caused by the increase in temperature in the material. This increase in temperature will result in odor changes, discoloration, and anthocyanin levels, either fermented with R or S.

3.2 Physicochemical properties

3.2.1 Protein content. The average of protein content of LBP fermented with R is 22,39% higher than fermented with S is 19,09% (Table 1). Igbabul et al. [17] stated that the process of fermentation of the mahogany bean (*Afzelia africana*) up to 72 h could obtain the protein content from 21.88% to 22.43-26.8%. The fermentation process can lead to hydrolyze of protein molecules, resulting in amino acids and nitrogen. According to Kasprowicz-Potocka et al. [18], levels increased of this protein is influenced by biomass enhancement of microbes. Moreover, they were hydrolyze the protein substrate to produce free amino acids and nitrogen to support its growth. Handoyo and Morita [19], Engel et al. [20] and Meussen et al. [21] shown that the optimal growth of *Rhizopus* sp. in range pH 3,6-7.

3.2.2 Fat content. The average fat content of LBP fermented with R and S, respectively are 0,41% and 1.25% (Table 1).. Fermentation using culture R or S indicate not significant difference in fat content between fermentation time 1, 2, and 3 days. The highest average of fat content was indicate when the fermentation have been done by S within 3 days On the 3rd day, the data shown that the fat content in fermentation by S was higher is 1,30% which is signify that the number of simple fatty acid compounds have been produced by microbial hydrolysis and the breakdown of the lipoprotein complex of the material, even the microbes have not been used entirely by microbes to support its metabolism [22, 23]. In other hand, fermentation by R indicate a decrease of fat content. Khetarpaul and Chauhan [24] reported that during fermentation up to 72 h, they research also signify that fat content tend to reduced. The existence of lipolytic enzymes that produced by microbes during fermentation, which is part of hydrolysis process, caused reduction levels of the fat [25].

3.2.3 Water content. This research shown that throughout the fermentation by R or S, the water content alteration in LBP followed by an increase or decrease in the value of other nutrients. The average water content of LBP fermented with R is 6,60-6,81% whereas fermented with L shows a higher average value is 9.05-9,08% Figure 1. Orhevba [26] and Obadina et al. [25] explained that usually water content would decrease in the first period of fermentation, then it would increases until the end of fermentation. However, this condition is the opposite of the carbohydrate compounds that increase with reduction of water content of LBP when fermented by R with average 67,29%. This condition in line with research conducted by Morris et al. (2004), and they explained that the percentage of water content was caused by relatively high of CO₂ during the fermentation process and might include in biochemical process (Table 1).

Water content controlling at the time of LBP fermentation was done by aeration performing in top-down reversing in every 12 h. Nout and Kiers [27] suggest to provide sufficient distance between the products and regulate the thickness of the fermented product, thereby heating temperature from the mass of *Rhizopus* sp. will not reach a maximum temperature of 40-50°C so it's still suitable for its growth. SSF method used in this research has been able to avoid the rotten of fermented LBP in high water content.

Table 1. Comparison nutrient fermented by R and S in LBP.

Microbial	Average					
	Protein	Fat	Water	Ash	Carbohydrate	Fiber
<i>Rhizopus</i> sp.	22,39	0,41	6,73	3,18	67,29	32,71
<i>Saccharomyces cerevisiae</i>	19,09	1,25	9,08	3,31	67,27	32,73

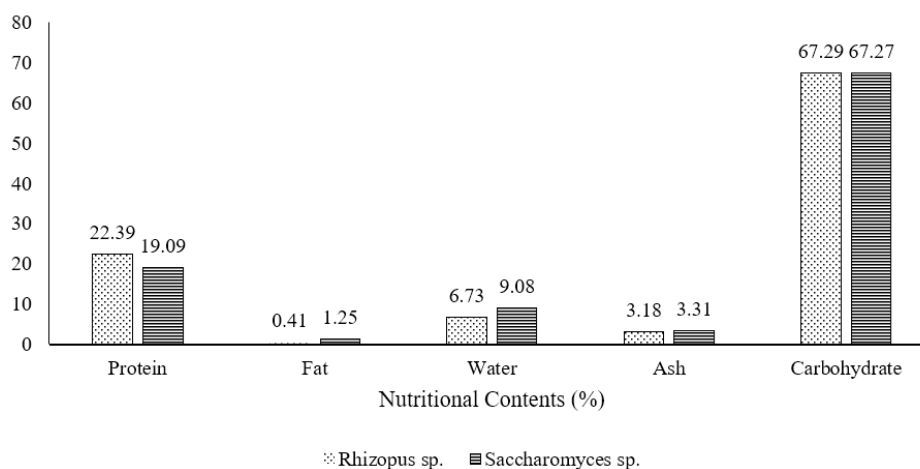


Figure 2. Comparison of nutrient content of LBP fermented by *Rhizopus* sp. and *Saccharomyces cerevisiae*

3.2.4 Ash content. Average ash content (%) LBP fermented with R and S respectively R3 <R1 <R2 is 3.21 <3.25 <3.08 <3.20 and S3 <S1 <S2 is 3.34 <3.34 <3.24 (Table 1). Mostly, the average of ash content of LBP fermented with S (3.31%) was higher than that fermented with R (3.21%). Although the overall ash content of the fermented LBP is lower than 3.5% and meets the standards for food preparations made from the seeds of a plant, it's could still be used for food needs [28]. This research was also in line in ash content of other beans in the Leguminosae group that ranges from 2.02-9,36% [29] and 3.53.9% [30]. Osman [31] reported that fermentation does not caused significant effect on the alteration in ash content of the final product. Conducted to Difo et al. [32], ash content was decreased when some mineral content in materials such as Fe, Na, Mg, Zn, and K were used by microbes to support its growth for fermentation.

3.2.5 Carbohydrate content. Carbohydrate levels of LBP fermented with R shown higher than by S, which is the average carbohydrate content respectively 67,29 and 67,27 % (Table 1). Carbohydrates levels was increased when R applied as microbial fermenter in LBP with value 66,83 to 67, 89. Whereas it will reduce from 67,33 to 67, 23 when S applied as fermenter during fermentation. The declining trend in carbohydrate levels is also indicated by the Osman [31]. Due to enzymatic activity by microbial fermenter may cause the reduction of carbohydrate levels during fermentation. Its influence by a low pH condition which is could inhibit the amylase release by microbes. Moreover, antimicrobial compounds including fermentation by microbe such as terpenoid groups was hampered.

3.2.6 Total fiber content. Total fibre content in fermented Lebui Bean crude fiber content tends to increase with length of fermentation time until the end of day 3 whether by R and S (Table 1). The height of total fiber to the end of fermentation indicate that cellulase enzymes from both types of microbes have been able to digest the cell walls in into simpler components including fiber components. In line with Mirnawati et al. [33] research, which is reveals the function of cellulase enzymes in crush the plant cell wall such as palm kernel so thereby could increase the fiber content. Mirnawati et al. [34] add that by applying fungus i.e. *Eupenicillium javanicum* as fermenter, with longer fermentation would might cause the total fiber content reduction.

4. Conclusion

SSF method have mostly effect to decrease the LBP nutrient, especially anthocyanin. Even there were found increase in first day fermentation, in longer fermentation were tend to degenerate the concentration. In fact, the reduction of anthocyanin was not quite significant from the initial nutrient of Lebui Beans. Fermentation carried out for two days with *Rhizopus* sp. shown the best treatment in this research.

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