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Determination and Characterization of Phenolics, Flavonoids, and Dietary Fiber in Fermented Lebui Bean (*Cajanus* sp.) Extracts by the SSF Method

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Lebui beans (*Cajanus* sp.) is a type of black bean originating from Lombok Island, Indonesia. Lebui beans are only used as food, though the potential is very high as a food source containing bioactive compounds. Bioactive compounds that are impo rtant for health include phenolics, flavonoids, and dietary fiber. However, these bioactive compounds are present in cells and are bound to the sugar component in the glycoside bond. These bioactive compounds are very heat-resistant, easy to oxidize, and easily damaged due to changes in pH. Therefore, in order to obtain bioactive compounds in beans, a process that can break down the glycoside bonds without damaging the properties of these compounds is needed. An effective method for breaking down glycoside bonds in a material and not using high temperatures is through a fermentation process. This study aims to obtain and characterize the components of bioactive compounds from phenolics, flavonoids, and dietary fiber groups in fermented lebui bean extracts using the solid-state fermentation (SSF) method. A nested design with two factor was used. The main factor is the type of microbe (*Rhizopus* sp. and *Saccharomyces* sp.) and the second factor is the fermentation time (1, 2, and 3 day) nested on the main factor. The results of this study obtained fermentation carried out for two days using *Rhizopus* sp. is the best treatment. Fermentation treatment using *Rhizopus* sp. for 1 to 3 days, produce an average level of dietary fiber in dry bases conditions which include levels of SDF, IDF, and TDF respectively are 10%; 27.59%; and 37.58%. In this treatment also produced the final product containing phenolic and flavonoids components in the range of 78.18-78.54 mg GAE/g (db) and 313.22-317.36 mg QE/g (db), respectively.

Keywords: fermentation, phenolics, flavonoids, dietary fiber, *Rhizopus* sp.

INTRODUCTION

Bioactive compounds according to the NCI Dictionary of Cancer Terms (2016) are compounds derived from fruit, vegetables, nuts, oil, plant seeds, animals or microbes (Guaadaoui et al., 2014) that can affect biological activity in the body (Cammack et al., 2006) and pharmacologically can support health (Bernhoft, 2010). Bioactive compounds are also interpreted as components of secondary metabolites produced by groups of plants or animals as a result of biochemical processes in cells (Tan and Zou, 2001; Bernhoft, 2010).

The types of bioactive compounds not only include phenolic components, flavonoids, isoflavones, saponins, anthocyanins, and quercetin in the *Leguminosae* group (Akashi et al., 2005; Lin and Lai, 2006; Atchibri et al., 2010; Hanganu et al., 2010) , polyphenols in plants and animals (Ferreira et al., 2008), dietary fiber in fruits, vegetables, cereals, and various beans (Liu, 2013; Guaadaoui et al., 2014; Mushollaeni et al.,

2018), as well as microbial alkaloids, terpenoids and steroids through a fermentation process (Tan and Zou, 2001), but also included in the bioactive group are other types of compounds produced by plants or animals that have biological activities as antioxidants (Elhassaneen et al., 2016; Kala et al., 2016), anti-microbial, anti-cancer, anti-malaria and anti-infection (Strobel and Daisy, 2003; Bernhoft, 2010). Therefore, bioactive compounds are also called bioactive food components (Swanson, 2003), biologically active substances (Dubtsova et al., 2012), or biocompounactive (Guaadaoui et al., 2014).

Lebui bean (*Cajanus* sp.) is a native bean plant of Lombok Island, Indonesia. This plant can grow throughout the year and is able to grow in dry soil conditions. Its abundant potential is not comparable to its utilization. Lombok people only use lebui bean for food during traditional events and in some places are used as traditional medicinal ingredients. The use of lebui beans as one of the ingredients of traditional medicine has been able to show that there are bioactive compounds in these beans. However, there have been no studies to reveal the types of bioactive compounds contained in lebui beans. The weakness in the study of using this lebui bean is the hardness of the outer shell of the bean and the presence of glycoside bonds that bind strongly to the bioactive compounds. Therefore, an appropriate method is needed to hydrolyze glycoside bonds that bind bioactive compounds without causing damage to the biological properties of bioactive compounds by fermentation. Research conducted by Mushollaeni et al. (2018) and Mushollaeni et al. (2019) is using a solid-state fermentation (SSF) method to hydrolyze glycoside bonds that bind bioactive compounds in black beans and reduce cyanide acid in rubber seeds. Research conducted by Mushollaeni et al. (2018) and Mushollaeni et al. (2019) is using a solid-state fermentation method to improve the quality of nutrient compounds in black beans and to hydrolyze chemical bonds in cells that bind cyanide acid in rubber seeds. The SSF method is able to improve the quality of protein content and hydrolyze glycoside bonds that bind anthocyanin compounds in black beans, resulting in free anthocyanin.

MATERIALS AND METHODS

Raw materials

The main raw material of this research is lebui beans obtained from Mataram, Lombok, Indonesia. Beans are sorted from damaged beans and non-uniform size, then dried in an oven at a drying temperature of 40°C for 20 minutes until the water content reaches 12-13%. Lebui beans are ground and sifted using a 60 mesh size stainless steel sieve. Lebui bean powder is stored in a glass jar and given silica gel to maintain its dry condition before treatment.

Fermentation process

Lebui bean powder is heated in an oven with a temperature of 70-80°C for 15 minutes, then mixed with 125 ml of distilled water for every 100 g of lebui bean powder. 2% of the dried culture of *Saccharomyces* sp. (S) or *Rhizopus* sp. (R) mixed into the first mixture containing lebui bean powder and distilled water. Fermentation is carried out at room temperature ± 27°C in dark conditions for 1, 2 and 3 days (according to treatment). Preparation of fermented bean powder begins by drying fermented bean powder at 40°C for 5 hours. Then proceed with milling and sifting using a 60 mesh size sieve. Storage of fermented lebui bean powder is carried out in a glass jar that is tightly closed and equipped with silica gel.

Research design and general experimental procedures

This research was carried out with a randomized nested design using types of microbes (*Saccharomyces* sp. and *Rhizopus* sp.) as the main factor and the duration of fermentation (1, 2, and 3 day) as the second factor. Total phenol, flavonoid, and soluble fiber levels were tested on dried lebui bean powder before and after fermentation. Soluble dietary fiber (SDF), insoluble dietary fiber (IDF), and total dietary fiber (TDF) were also tested as quality parameters. Tests for dietary fiber (DF) levels were carried out according to the method of Asp et al. (1983), whereas total phenolic and total flavonoids were based on the method of the Airlangga University Testing Laboratory (ULP) (2015).

RESULTS AND DISCUSSION

Phenolic content

The highest concentration of phenolic compounds contained in cotyledons of plant seeds is a product of plant metabolism and part of a group of secondary metabolites that are polar and easily soluble in water. Total phenolic of unfermented lebui bean powder is 30.501 mg GAE/g (db), while lebui bean powder which has

been fermented for one to three days using R is 32.458-78,363 mg GAE/g (db) and fermented using S is 31,403-50,160 mg GAE/g (db). Fermented lebui bean powder has a greater total phenolic than unfermented bean powder. This condition indicates that the fermentation process has been effective in breaking down the cell wall and outlining the glycoside bonds that bind bioactive compounds in beans including phenolic components. Affirmed by Rasouli et al. (2017), the bioactive component of the phenol group has one or more benzene rings that are generally found in nature as glycosides. The relationship between the degradation of cell walls that protect bioactive components and the increase in the concentration of bioactive compounds that are detached from their bound forms into free components. Total phenolic of unfermented and fermented lebui bean powder can be seen in Figure 1.

The results showed that the phenolic content of fermented bean powder increased from the first day to the third day. The increase in phenolic content ranged from 2-48% in fermented bean powder using R and an increase of 1-28% in lebui bean powder fermented using S. Lebui bean powder which is fermented using *Rhizopus* sp. for two days, produces a final product that has a greater average phenolic content (78,363 mg GAE/g db) than if fermented for one (32,458 mg GAE/g db) or three days (60,635 mg GAE/g db). The same results also occurred in lebui bean powder which was fermented using *Saccharomyces* sp. for two days, resulting in a final product which had an average phenolic level greater (58,075 mg GAE/g db) than if fermented for one (31,403 mg GAE/g db) or three days (50,160 mg GAE/g db).

The increase in the phenolic content of fermented bean powder compared to unfermented bean powder indicates that the fermentation process has been able to degrade the lebui bean cell and release glycoside bonds that bind phenolic bioactive compounds, so that the phenolic component becomes free form. During fermentation, microbes produce proteolytic enzymes to hydrolyze phenolic complexes, so that free phenolic compounds are formed. In addition, the increase in phenolic content in the bean powder after fermentation, shows that the process steps carried out starting from the processing of lebui bean powder, drying, and fermentation have been able to protect phenolic bioactive compounds that are highly heat-resistant. This is in line with the statement of Mushollaeni et al. (2018), the fermentation process can improve the quality of bioactive compounds and protect them from exposure to changes in temperature, pH and excessive light.

Flavonoid content

Flavonoids are the largest component of phenolic groups other than anthocyanin (Harborne, 1993; Wink, 2013). Flavonoids are a very important component of bioactive compounds, because of their high antioxidant properties. Flavonoids are found in high concentrations in several types of beans from the *Leguminosae* group.

Flavonoid content of lebui bean powder which was fermented for 2 days was higher than that fermented for 3 days and greater than that fermented for 1 day (2 days> 3 days> 1 day). This condition occurs in both types of microbes used for fermentation (Figure 2). The mold used as a starter in the fermentation process has a higher advantage when compared to other microbes. Fermentation using molds as a starter, will produce several types of enzymes that have higher effectiveness to decompose bonds in bioactive compounds, including to break down glycoside bonds in the phenolic group. The β-glucosidase enzyme produced by microbes during the fermentation process can hydrolyze glycoside bonds that bind bioactive compounds, including compounds from the flavonoid group in cells. Differences and decreases in flavonoid levels during fermentation are possible because of differences in bean species or raw materials used, as well as the length of fermentation time. T

here were differences in the levels of bioactive compounds after the treatment process, including those caused by differences in species and varieties. Differences in flavonoid levels can also be caused by geographical conditions and climatic conditions.

Dietary fiber content

Dietary fiber (DF) is a component of non-starch polysaccharides, included in the fiber group in plants, and also grouped in bioactive components (biologically active compound) that can affect body health (Liu, 2013; Mudgil and Barak, 2013; Guaadaoui et al. , 2014; Yang et al., 2017). Total Dietary Fiber (TDF), Soluble Dietary Fiber (SDF), and Insoluble Dietary Fiber (IDF), showed that lebui bean powder fermented at all fermentation times had a higher dietary fiber value than unfermented lebui bean powder. The results of the TDF, SDF and IDF analysis can be seen in Figure 3.

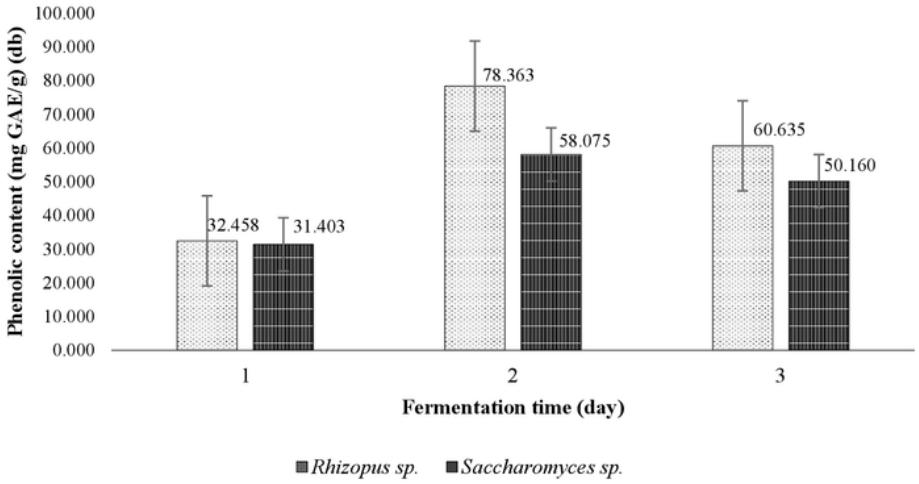

Figure 1. Phenolic content of fermented lebui bean powder

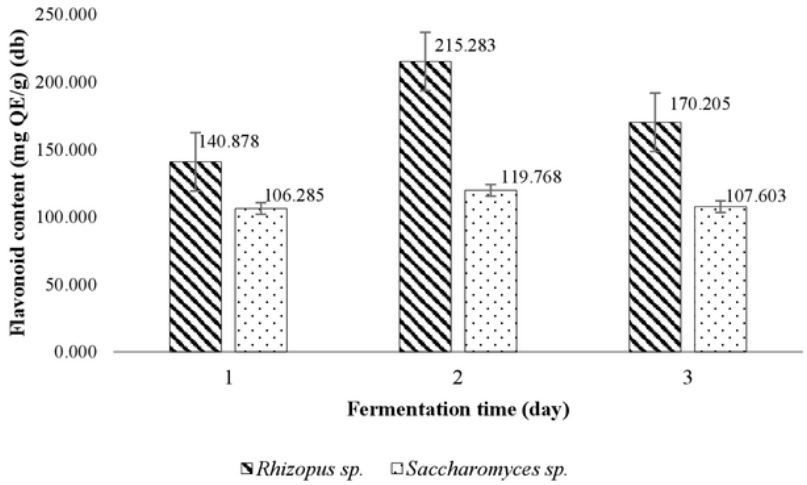

Figure 2. Flavonoid content of fermented lebui bean powder

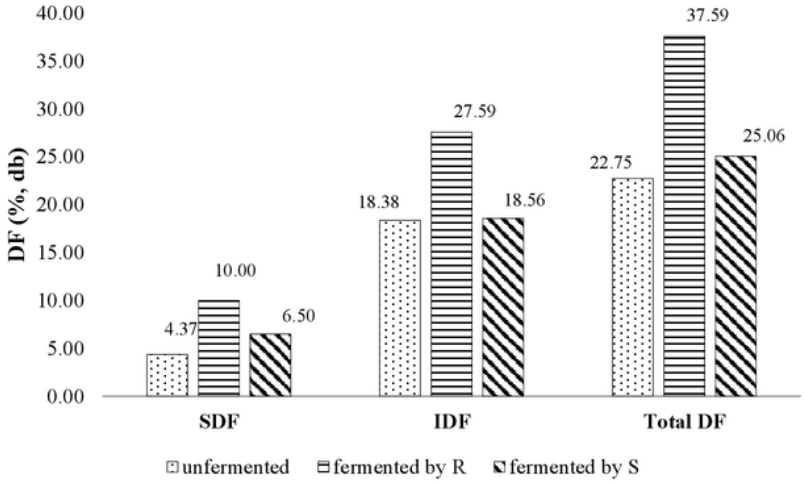

Figure 3. Dietary fiber content of fermented lebui bean powder (2 days fermentation)

However, the average levels of TDF, SDF, and IDF from fermented bean powder using R were higher than if fermented using S. The difference in the level of DF which is clearly seen from fermentation using R and S is in the treatment of fermentation for 2 days. The average levels of TDF from lebui bean powder fermented for 2 days using R (37.59%) were higher when compared with S (25.06%), and TDF levels from lebui bean powder fermented with S were higher than those of unfermented (22.75%). This condition indicates that enzymes produced by *Rhizopus* sp. are more effective in degrading cell walls and softening the hardened of lebui bean shells so that they can release more fiber components and DF.

Increased levels of total DF are caused by microbial activity during fermentation which secretes cellulase and hemicellulase enzymes that are able to degrade cell walls, resulting in the release of bonds between organic components in cells, including DF components (FAO, 1998). The activity of cellulase and esterase enzymes released by *Rhizopus* sp. can degrade cell walls, free the cellulose chain, and soften the hard outer shell of the seeds.

CONCLUSION

Fermentation was carried out for two days using *Rhizopus* sp. is the best treatment. the final product containing phenolic and flavonoids components in the range of 78.18-78.54 mg GAE / g (db) and 313.22-317.36 mg QE / g (db), respectively. Fermentation treatment using *Rhizopus* sp. for 1 to 3 days, produce an average level of dietary fiber in dry bases conditions which include levels of SDF, IDF, and TDF respectively are 10%; 27.59%; and 37.58%.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest".

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AUTHOR CONTRIBUTIONS

WM and LT has performed the experiments and also wrote the manuscript. All authors read and approved the final version.

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