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Differences in effect of Pre-Microencapsulation process on components of Bioactive compounds in local Lebui Bean extract from West Nusa Tenggara

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Microencapsulation is one of the technological engineering methods developed on a material to protect bioactive components by coating. However, the extract of lebui bean (*Cajanus* sp.) which contain bioactive compounds is still in a very susceptible condition to damage due to its unstable condition from the effects of oxidation. In addition, the development of local black beans from Lombok Island will be hampered because of its low utilization and only as local food ingredients. The aim of the research was to determine the pre-microencapsulation method and the type of solvent to produce lebui bean extract which has the best nutritional composition and bioactive compounds. Nested Random Design was used. The main factor was the type of pre-microencapsulation method (microwave, fermentation, and vacuum pressure) and the nesting factor was the type of solvent (ethanol and n-hexane). The result showed the lebui powder which was pre-microencapsulated using a microwave, which was then extracted using n-hexane as solvent was the best combination. The extract contains protein, lipid, moisture, ash, fiber, and carbohydrates respectively 26.22%; 13.60%; 14.24%; 3.28%; 15.77%; and 26.33%. The bioactive compounds identified in the extract were phenolic compounds, flavonoids, anthocyanins, and dietary fiber respectively 38.54 mg GAE/g (d.b); 49.36 mg QE/g (d.b); 107.67 ppm; and 40.05 %. The yield and antioxidant activity were 80.86% and 12.04 IC50,mg/mL. It has been found that more than 10 types of bioactive compounds from the phenolics group and more than 20 types of essential fatty acids in the extract play an important role in the health of the body, and there are also important compounds as a source of herbal medicinal ingredients, such as *Neophytadiene, a*nthocyanin, and *Cajanus lactone.*

Keywords: pre-microencapsulation; bioactive compounds; lebui bean (*Cajanus* sp.); nutritional composition; phenolics; flavonoids; anthocyanins; dietary fiber; antioxidant activity; black beans

INTRODUCTION

Process engineering technology that aims to protect components or compounds that are prone to damage in a natural material is microencapsulation. The components of the material are coated using certain materials, so that they are protected from the effects of extreme heat, oxygen, and pH, as well as for the development of industrial-scale functional food products. Bioactive compounds and potential secondary metabolites were also found in various amounts and types in the local black bean (*Cajanus* sp.) extract of Lombok Island known as lebui beans, including anthocyanins, polyphenols, flavonoids, phenolics, and terpenoids (Mushollaeni et al. 2017; 2018; 2019; 2020). Although found in abundance, lebui beans are only used as food and traditional medicine or are only left on the land during the main harvest. Scientific information regarding the advantages and further utilization of its bioactive compounds is also very low. Whereas based on the results of previous studies, Mushollaeni et al. (2017) stated the phenolic levels of lebui beans ranged from 30.501–78.363 mgGAE/g (d.b.) which were higher than *Phaseolus lunatus* and *Vigna angularis* beans, respectively 0.11–9.72 and 8.18 ± 0.12 mgGAE/g by Agostini-Costa *et al.* (2015). The phenolic content of lebui beans is in the medium-high group with a range of 3000- > 5000 mgGAE/100 g^6 and its anthocyanin content is in the range of 107,120–153,350 ppm (Mushollaeni et al. 2020; Chew et al., 2011). Given the importance of the potential of local ingredients as a source of bioactive compounds, as well as the need for natural sources of antioxidants in functional foods, it is very necessary to engineer microencapsulation technology as a protection for leukabean bioactive compounds that are better than using only extraction methods, and can later support product characterization for further development. on an industrial scale.

The bioactive compounds and secondary metabolites that were free from intercellular bonds and other components were extracted using a combination method of maceration-percolation-stirring and organic solvents of

ethanol and hexane. Based on the results of previous studies (Mushollaeni et al. 2017; 2018; 2020) phenolic compounds and flavonoids have better solubility in polar organic solvents and terpenoid compounds and non-polar fatty acids are extracted better in hexane solvents, so that the resulting yield will be high (Perinelli et al. 2020; Raddatz and de Menezes, 2021). However, there are still weaknesses, namely the extract is still very susceptible to damage if it is not enveloped and the three pre-microencapsulation methods combined with extraction have not been studied, so research is urgently needed to reveal their effectiveness against bioactive compounds in lebui bean extract.

The pre-microencapsulation and extraction processes are very important steps before entering the microencapsulation process. The basis for selecting the three pre-microencapsulation methods in this research, namely microwave, fermentation, and vacuum pressure, is to produce bioactive compounds in free form, improve the weakness of extract yields using only extraction methods as well as determine the amount, type, and antioxidant activity of bioactive compounds. and other secondary metabolites (Mushollaeni et al. 2017; 2018; 2019; 2020). Microwaves produce microwaves that can damage cells and release bioactive compounds[9], while the fermentation method is able to release glycoside bonds through hydrolysis of bonds between components without damaging cells to form free bioactive compounds with high antioxidant activity (Bintari and Elyani, 2017). In contrast to vacuum pressure, this process uses the principle of pressure and temperature (121 °C, 1 atm, 5 min) as cell lysis, so that bioactive compounds are easier to extract. The three processes were measured for their effectiveness through testing of bioactive compounds and their antioxidant activity.

MATERIALS AND METHODS

2.1. Materials

The research materials included lebui beans from Lombok Island (harvested at 3 months), n-hexane, 90% ethanol, 70% ethanol, Whatman paper no.41, filter paper, N_2, *Rhizopus* sp. culture, PDA media, physicochemical and phytochemical screening chemicals. Profilling, antioxidant activity, total anthocyanins, phenolic flavonoids chemical materials. Research equipment includes electric dryer, microwave, maceration-percolation apparatus, analytical balance, centrifuge, pH meter, autoclave, thermometer, incubator, microbiological analyzer, Barnstead SHKE2000 shaker app., KLT app., KLT Kiesel Gel GF254, UV rays (366–254 nm), Buchi rotary vaporizer, colorimeter Konica Minolta CR10, spectrophotometer UV-1700 Pharma Spec., spectrophotometer UV-Vis 1240, vortex, propipet, GC-MS.

2.2. Methods

2.2.1. Experimental Design

Nested design with pre-microencapsulation method (microwave, fermentation, and vacuum pressure) and the nesting factor was the type of solvent (ethanol and n-hexane). These experimental designs could determine the best result in different types of pre-microencapsulation and solvent extraction which produces lebui bean extract with the highest quality nutritional composition and bioactive compounds. This study applied ethanol and n-hexane (pro analyst) as solvents and the concentrations was 90%, and the experimental was done at room temperature which is the relative humidity was about 79%. A combination of each treatment was repeated three times. The data were analyzed by Analysis of Variance (ANOVA) for nested design (SPSS 26 for windows) and calculation of the best treatment using the effectiveness index method.

2.2.2. Preparation Process

Lebui beans with a diameter of 0.5–0.8 cm were collected and sun-dried for 2–3 days. Then sorted and oven dried until the water content reaches 12–13%. The beans were then finely grounded, sifted by 60 mesh size (BSS sieve) and stored in a tightly sealed glass container with silica gel.

2.2.3. Pre-Microencapsulation Methods

Pre-microencapsulation methods used three methods. These methods are the fermentation method (F), the vacuum pressure method (V), and the microwave method (M). Extraction using two types of solvents are ethanol (E) and n-hexane (H). Lebui powder used in the fermentation method was heated in an oven at 70–80 °C for 15 min. Then, mixed thoroughly with distilled water 125 mL/100 g. Rhizopus sp. culture with biomass in approximately 2% was added on lebui beans powder and incubated for 2 days at 27–28 °C in a dark fermentation cabinet. Lebui beans powder fermented result was taken and fore dried at 40 °C for 5 h using an oven (Memmerth Oven 10 L), henceforth terminated by grounded and sifted by a 60 mesh size (Hur et al. 2014; Kang et al. 2016).

In the treatment using vacuum pressure, lebui bean powder was put into an autoclave, then processed at 121 °C for 10 min. The lebui bean powder that has been vacuumed was then put in a desiccator for 10 min to lower the temperature and reduce the moisture content. It was grounded and sieved using a 60 mesh sieve. The lebui bean powder that has been processed, then put in a glass container that was tightly closed and lined with aluminum foil, added with silica gel, and stored in a dry and dark place.

Microwave oven has been used as a tool in the pre-microencapsulation treatment with the microwave method. The lebui bean powder was put in a microwave oven and processed at 800 W for 30 s. Then, it was ground and

sieved using a 60 mesh sieve. Similar to the other two pre-microencapsulation methods, all the processed lebui bean powder was stored in a glass container with a tight lid and lined with aluminum foil. Storage is done in a dry place, not damp, not exposed to direct sunlight, and in a dark place at a temperature of 27–30 °C.

RESULTS

Tests and data analysis were carried out using the average difference test method on the nested ANOVA test (for more than 2 groups). If the results of nested anova show a significant difference, then continue with the LSD test. If the notation of further test results shows a difference between 2 different groups, then the two groups are significantly different, but if the notation between the 2 groups is the same, then the two different groups are declared insignificant. The researchers where also used control sample treatment s to differentiate the effect pf microencapsulation on the physicochemical profile of the product. Hypothesis testing and analysis using the following methods that:

H_0: There is no significant average difference between groups based on the variables measured;

H_1: There is a significant average difference between groups based on the variables measured.

The test criteria are as follows:

If the calculated F value > F table, and p-value < 0.05, then H_0 is rejected;

If the calculated F value < F table, and p-value > 0.05, then H_0 is accepted.

3.1. Protein Content

Tests on the effect of the pre-microencapsulation method (F, M, and V) with nesting solvents (E and H) in each of these methods on protein content were carried out using nested ANOVA analysis. Based on the results of the analysis, it was founded that the calculated F value of the two factors were greater than the F Table 5% and 1%, and the p-value were smaller than (0.05 and 0.01), so it concluded that there was a very significant average difference between treatments on the measured protein content. To determine the difference in the average protein content of the pre-microencapsulated factor and the type of solvent nested in the pre-microencapsulated factor, a follow-up test was carried out using the 5% LSD test (**Table 1**).

Table 1: The average protein content of the extract generated by pre-microencapsulated factor and the type of solvent nested in the pre-microencapsulated factor.

F		M		V	
Method	**Average**	**Method**	**Average**	**Method**	**Average**
Extract-F	23.315^{a}	**Extract-M**	25.963^{c}	**Extract-V**	23.463^{b}
Ethanol	23.355^{a}	Ethanol	25.600^{a}	Ethanol	23.600^{b}
n-hexane	23.275^{a}	n-hexane	26.325^{b}	n-hexane	23.325^{a}

Insignificant differences between treatments are indicated by the same notation (5% LSD test).

The results of the 5% LSD test showed that the pre-microencapsulation method factors showed a significant difference between the methods with the highest average in lebui bean powder processed using the M method. However, based on the table above, it was also shown that there was an insignificant difference between the types of solvents E and H. The extract of lebui powder using the M and V methods showed a significant difference with the use of solvents E and H (indicated by different notations).

3.2. Lipid Content

Based on the results of the analysis using Nested ANOVA on lipid content, it was found that there was a very significant average difference between treatments on lipid content (F count was greater than F Table 5% and 1%, and p-value was smaller than 0.05 and 0.01).

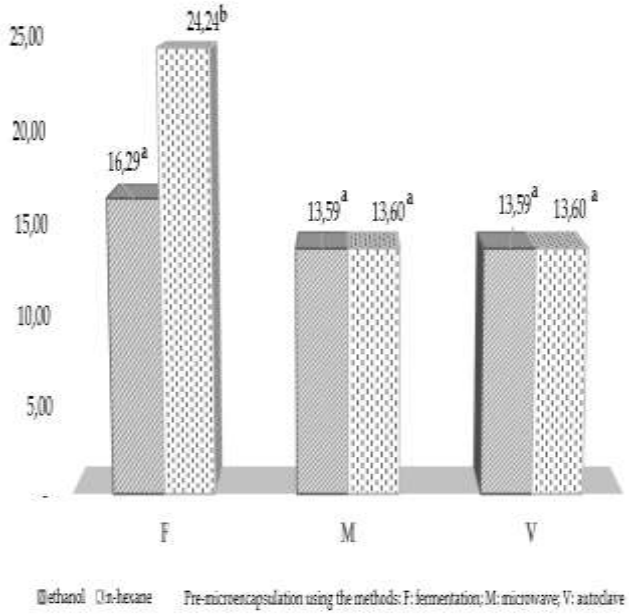


Figure 1: The average of lipid content of lebui powder treated by pre-microencapsulation method (F, M, and V) and extracted using ethanol or n-hexane as solvent. Insignificant differences between treatments are indicated by the same notation (5% LSD test).

Table 2: The average moisture content of the extract generated by the pre-microencapsulated factor and the type of solvent nested in the pre-microencapsulated factor.

F		M		V	
Method	**Average**	**Method**	**Average**	**Method**	**Average**
Extract-F	12.995 [a]	Extract-M	14.845 [b]	Extract-V	14.845 [b]
Ethanol	15.280[b]	Ethanol	15.455[b]	Ethanol	15.455[b]
n-hexane	10.710[a]	n-hexane	14.235[a]	n-hexane	14.235[a]

Insignificant differences between treatments are indicated by the same notation (5% LSD test).

3.3. Moisture Content

The results of the 5% BNT test showed a significant difference between the pre-microencapsulated method with the highest average in the lebui bean extract extracted using M and V solvents. Each extract showed a significant difference between those extracted using solvents E and H indicated by different notations.

3.4. Ash Content

The source of the diversity of factors in the pre-microencapsulation method with an F value greater than the F table value 5% and 1%, and a p-value smaller than 0.05 and 0.01, so it can be concluded that there is a very significant average difference between the treatments used. to ash content. However, the source of the diversity factor of the type of solvent contained in the pre-microencapsulation method factor with an F value smaller than F Table 5% and 1%, and a p-value greater than 0.05 and 0.01, so it can be concluded that there is a difference in the mean insignificant mean between the treatments used on the ash content.

Table 3: The average ash content of the extract generated by the pre-microencapsulated factor and the type of solvent nested in the pre-microencapsulated factor.

F		M		V	
Method	**Average**	**Method**	**Average**	**Method**	**Average**
Extract-F	3.530[b]	Extract-M	3.270[a]	Extract-V	3.270[a]
Ethanol	3.535[a]	Ethanol	3.265[a]	Ethanol	3.265[a]
n-hexane	3.525[a]	n-hexane	3.275[a]	n-hexane	3.275[a]

Insignificant differences between treatments are indicated by the same notation (5% LSD test).

3.5. Fiber Content

Table 4: The average fiber content of of the extract generated by pre-microencapsulated factor and the type of solvent nested in the pre-microencapsulated factor.

Treatments	Average	Dev. Std.	Average of the extract (E)
F - ethanol	15.2700[b]	0.02828	E1 (F) = 13.123[a]
F - hexane	10.9750[a]	0.00707	
M - ethanol	15.7650[a]	0.00707	E2 (M) = 15.898[c]
M - hexane	16.0300[b]	0.04243	
V - ethanol	15.7650[a]	0.00707	E3 (V) = 15.898[b]
V - hexane	**16.0300[b]**	**0.04243**	

Insignificant differences between treatments are indicated by the same notation (5% LSD test).

3.6. Carbohydrate Content

The results of the LSD test of 5%, the factor of the pre-microencapsulation method showed a significant difference between the types of methods with the highest average of lebui bean extract processed using the vacuum pressure method. Based on Table 5, the extracts of white beans that have been processed using the F, M, and V pre-microencapsulation methods, all showed significant differences between the types of solvents used. This difference is indicated by a different notation

Table 5: The average of carbohydrate content of the extract generated by the pre-microencapsulated factor and the type of solvent nested in the pre-microencapsulated factor.

Treatments	Average	Dev. Std.	Average of the extract (E)
F - ethanol	26.275[a]	0.03536	E1 (F) = 26.778[b]
F - hexane	27.280[b]	0.02828	
M - ethanol	26.330[a]	0.02828	E2 (M) = 26.433[a]
M - hexane	26.535[b]	0.02121	
V - ethanol	28.330[a]	0.02828	E3 (V) = 28.933[c]
V - hexane	29.535[b]	0.02121	

Insignificant differences between treatments are indicated by the same notation (5% LSD test).

3.7. Anthocyanin, Phenolics, and Flavonoids Levels

The two factors showed a very significant average difference between treatments on anthocyanin, phenolics, and flavonoids levels.

Table 6: The average of anthocyanin generated by the pre-microencapsulated factor and the type of solvent nested in the pre-microencapsulated factor.

Treatments	Average	Dev. Std.	Average of the extract (E)
F - ethanol	117.225[a]	0.02121	E1 (F) = 23.315[c]
F - hexane	117.665[a]	0.07778	
M - ethanol	107.225[a]	0.02121	E2 (M) = 25.963[b]
M - hexane	107.665[b]	0.07778	
V - ethanol	101.115[a]	0.02121	E3 (V) = 23.463[a]
V - hexane	101.135[a]	0.02121	

Insignificant differences between treatments are indicated by the same notation (5% LSD test)

Table 7: The average of phenolics generated by the pre-microencapsulated factor and the type of solvent nested in the pre-microencapsulated factor.

Treatments	Average	Dev. Std.	Average of the extract (E)
F - ethanol	117.225[a]	0.02121	E1 (F) = 23.315[c]
F - hexane	117.665[a]	0.07778	
M - ethanol	107.225[a]	0.02121	E2 (M) = 25.963[b]
M - hexane	107.665[b]	0.07778	
V - ethanol	101.115[a]	0.02121	E3 (V) = 23.463[a]
V - hexane	101.135[a]	0.02121	

Insignificant differences between treatments are indicated by the same notation (5% LSD test)

Table 8: The average of flavonoids generated by the pre-microencapsulated factor and the type of solvent nested in the pre-microencapsulated factor.

Treatments	Average	Dev. Std.	Average of the extract (E)
F - ethanol	84.515[b]	0.29889	E1 (F) = 44.546[b]
F - hexane	4.577[a]	0.01032	
M - ethanol	48.217[a]	0.00651	E2 (M) = 48.788[c]
M - hexane	49.358[b]	0.05876	
V - ethanol	40.520[a]	0.42426	E3 (V) = 40.728[a]
V - hexane	40.935[a]	0.00707	

Insignificant differences between treatments are indicated by the same notation (5% LSD test).

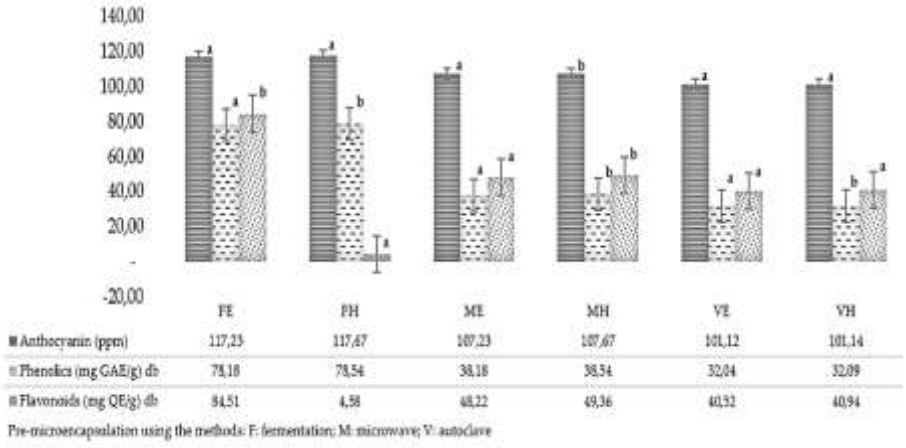


Figure 2: The average of anthocyanin, phenolics, and flavonoids of the extracts of lebui bean powder.

3.8. Dietary Fiber Levels

The 5% LSD test gave the result that the pre-microencapsulation method showed a significant difference between the extract produced and the highest average of DF was found in the lebui bean extract which was processed using the microwave method. The lebui bean powder given the F process showed no significant difference between the extracts produced from the use of ethanol and n-hexane as solvents. Lebui bean extract, which had previously been processed using the M and V methods, showed significant differences based on the extraction results using ethanol and n-hexane as solvents.

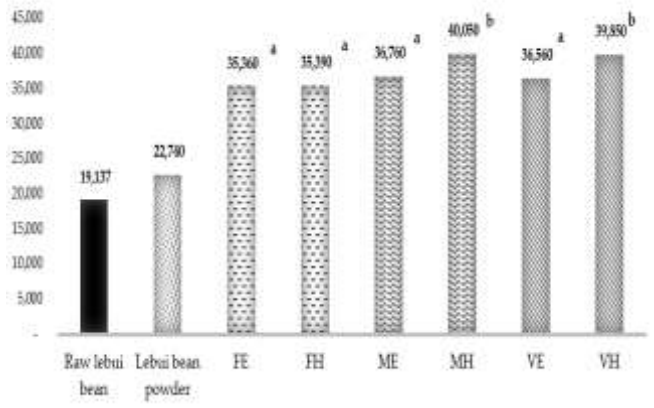


Figure 3: The average of dietary fiber of raw lebui bean, lebui bean powder, and the extracts of FE, FH, ME, MH, VE, and VH.

DISCUSSION

4.1. Protein Content (%, d.b.)

Tests of the effect of the pre-microencapsulation method (F, M, and V) within the type of solvent (E and H) nested in each of these methods on protein content, was carried out by nested ANOVA analysis. Based on the analysis, it is known that F value of the two factors is greater than F Table 5% and 1% was calculated, and the p-value is smaller than (0.05 and 0.01), so it can be concluded that there is a very significant average difference. between treatments on the measured protein content. Differentiation in the average protein content of the pre-microencapsulated factor and the type of solvent nested in the pre-microencapsulated factor was determined by follow-up test which was carried out using the 5% LSD test.

The results of the 5% LSD test were in Table 1, the type factor of the pre-microencapsulation method had been showen significant differences between treatments. The highest average protein content was obtained from the M method. Based on Table 1, it can also be seen that the type of solvent contained in the fermentation method was showed an insignificant difference in the protein content of the extract produced. It be differ when pre-microencapsulation method was carried out with microwave and vacuum pressure , it was seen that there were differences in the protein content of each extract produced. Overall, the protein content of the extracts produced from leucopean powder which was processed using the microwave method and extracted with ethanol or n-hexane, showed a higher value than the other 2 treatment combinations (M > V > F).

These results were conducted by previous research that extraction using the microwave method can speed up the extraction process and increase the speed of protein hydrolysis (Damm *et al.* 2010). Further strengthened by a similar research which states that extraction by applied microwaves could be streamline the extraction time, so as to improve the quality of extracts, including bioactive compounds and nutritional components (Piovesan *et al.* 2017). Between lebui bean powder (BKL) processed using the fermentation method and vacuum pressure, although there is a difference in notation, the difference between the two is not greater than 0.5 (0.148), so it can still be said that the results are almost comparable.

The protein content of whole lebui bean in this research is 18.49% and that of lebui powder which is processed into powder without going through the soaking process is 18.50% (Mushollaeni *et al.* 2021). The three methods, among processed using the F, M, and V methods produced extracts containing protein content of 23.315–25.963%. Based on these results, it can be stated that the three pre-microencapsulation methods used in this study have proven to be able to produce lebui bean extract with high protein content. It was also mentioned in previous studies that the protein content of lebui beans was based on the results of research conducted previously that the processing of lebui beans into powder form and which had undergone a fermentation process, more effective to help the extraction process of nutritional components and bioactive compounds in lebui beans (Mushollaeni *et al.* 2017; 2018; 2020; 2021). The processing of lebu beans into powder form has also resulted in the release of peptide bonds in proteins, resulting in the formation of smaller protein units and analyzed as protein content based on the Kjeldahl method. Applying the fermentation process for bean-commodities can increase the protein content as a result of the activity of protease enzymes by fermenting agents (Benjamin *et al.* 2021).

4.2. Lipid Content (%, d.b.)

Tests to determine the effect of the pre-microencapsulation method (F, M, and V) with multiple type of solvent at the extraction stage (ethanol, and n-hexane) nested in the extract on fat content were carried out using nested ANOVA analysis. Based on the analysis,

it was found that there were significant differences in the fat content of the extract from the lebui bean powder which had been fermented and then extracted with ethanol or n-hexane as solvent. However, different conditions, which were indicated by insignificant differences, occurred in the two types of extracts previously processed by means of microwave or vacuum pressure, both extracted using ethanol or n-hexane as a solvent. There is a significant difference or there is no difference, stated in the notation. Different notation shows that there is a significant difference and the same notation shows that there is no difference in the fat content results.

The fat content of whole lebui nuts is 0.97%, and alter to 0,88% while being processed into powder. Mushollaeni et al. (2017; 2018) state that after processing and extraction steps, the fat content showed a significant increase in the range of 13,593–24,235%. This considerable increase is very likely to occur due to the influence of processing processes that are able to extract the nutritional components in an ingredient, including the fat content. Previous studies shown that enhancement of fat content in the extract was caused by the large number of simple fatty acid compounds resulting from hydrolysis due to the processing carried out. The fat content of the extract in the pre-microencapsulation process using the fermentation method was higher than the other two methods. This can occur as a result of microbial activity or the breakdown of lipoprotein complexes by microbes that produce simple fatty acid components. This opinion is also strengthened by previous research which proved that the linoleic fatty acid content increased when the fermentation process was applied to raw peanuts up to 23.25% using *Lactobacillus* rhamnosus (Ziarno et al. 2020).

4.3. Carbohydrate Content (%, d.b.)

Tests to determine the effect of the pre-microencapsulation method and the type of solvent on the carbohydrate content of the extract used the nested ANOVA method, which obtained :

The source of the diversity of factors in the pre-microencapsulation method with a calculated F value of 9681,802 > F Table 5% and 1%, and a p-value of 0.000 < (0.05 and 0.01), so it can be concluded that there is a very significant average difference between treatments. used for the measured carbohydrate content.

The source of the diversity factor of the type of solvent nested in the pre-microencapsulation method factor with a calculated F value of 1100.692 > F Table 5% and 1%, and p-value < (0.05 and 0.01), so it can be concluded that there is an average difference very significant between the treatments used on the measured carbohydrate content.

The overall treatment indicate the degradation of carbohydrate content, namely from the initial content of lebui bean powder by 68.01% to 26.275–29.535%. In the pre-microencapsulation process with fermentation, the decrease in carbohydrate content was mostly caused by microbial activity, i.e *Rhizopus* sp. The decrease in carbohydrate content was caused by the enzymatic activity of microbes and began to appear in the first 24 h of fermentation. The application of microbial agents in the fermentation process had the effect of reducing carbohydrate levels and increasing lysine and arginine in peanut-based products. When the beans are processed into powder, the carbohydrate content will also decrease as the fermentation time increases (Adebo et al. 2020).

During fermentation at 12 to 24 h, microbes have begun to be active in hydrolyzing carbohydrate components into energy and other biological processes. Therefore, the carbohydrate content decreases. Carbohydrate levels decreased along with the increase in the number of microbes and an increase in the concentration of the amylase enzyme produced by microbes [1]. These enzymes would be hydrolyze the carbohydrates into modest sugar components which are utilized to support fermentor-agent growth (Hassan et al. 2006; Vincent et al. 2009; Osman, 2011; Assohoun et al. 2013; Kilonzo-Nthenge et al. 2013; Iqbabul et al. 2014).

Physical processing and high temperatures application would allows the alteration of nutritional content. Carbohydrates are the largest nutritional component in the *Legumes* group, which is between 24–61.2%. Processes that involve the use of heat, such as processing using vacuum and microwave pressure, can result in hydrolysis of starch granules. Starch granules expand and then break, which in turn facilitates a more random configuration of the structure and results in bond breaking (Uppal and Bains, 2012).

Total carbohydrates is calculated by applied the total carbohydrate by difference method, which is the result of reducing 100% of the total components of the ingredients with the percentage of water content, ash, fat and protein. When the percentage of non-carbohydrate components would decreases, the carbohydrate percentage will increase. The fat and protein content in the whole extract increased significantly, resulting in a decrease in the percentage of carbohydrates.

4.4. Moisture Content (%, d.b.)

Water content of the extract of lebui bean powder which has undergone pre-microencapsulation treatment by fermentation with treatment involving heat, namely the microwave and vacuum methods, showed different results, namely the fermentation process produced a lower water content of the extract than the other two methods. However, when compared with the moisture content of whole lentils (8.79%) and before processing (8.18–8.21%), the water content of the extract showed a higher value (10.71–15.45%).

Enhancement of water content during the fermentation process can be explained and is also related to previous studies. Generally in the fermentation process there will be a decrease in the water content at the beginning of the process and then the water content can increase until the end of the fermentation depending on

the type of microbe, the need for water, and the level of microbial life (Mushollaeni et al. 2017; 2018). This study has a similar phenomenon with the another research that for the basic ingredients of fermented beans, the water content will decrease until the 3rd day, then it will start to increase on the 4th day and so on. The decrease in water content can be caused by the increase in dry material as a result of microbial cell proliferation (Content et al. 2021).

Water content of the extract wasobtained by applied ethanol and or hexane in each pre-microencapsulated treatment using fermentation, microwave or vacuum pressure methods did not show a significant difference. This is possible due to the influence of the aquades content in the solvent and the process steps do not affect the water content much.

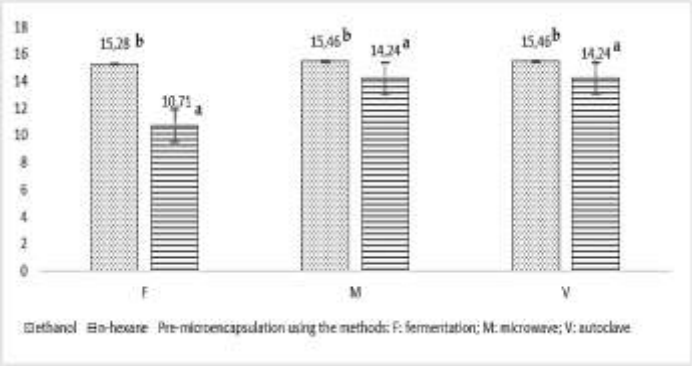


Figure 4: The average of moisture content.

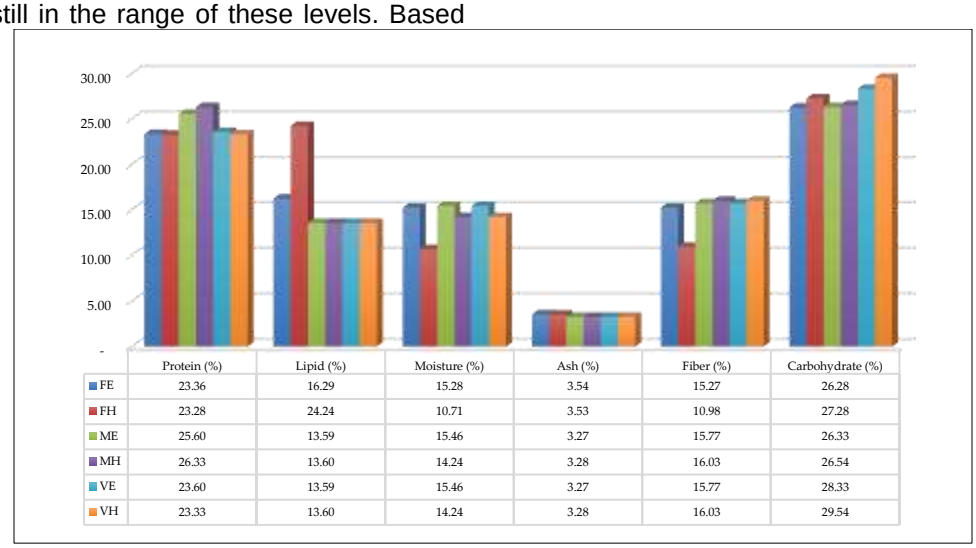
4.5. Ash Content (%, d.b.)

The ash content of beans in the Leguminosae group is in the range of 2.02–9.36% w/w and 3.5–3.9% w/w (Megat-Rusydi *et al.* 2011; Mushollaeni *et al.* 2015). The ash content in the extract was in ranged from 3.27–3.53%. When compared with the ash content of lebui powder before processing, the ash content of the extract was not much different or still in the range of these levels. Based on the results of the 5% LSD test on the tested factors, it showed that there was an insignificant average difference between the types of solvent treatments used on the ash content measured for all pre-microencapsulated methods. However, on the source of the diversity of factors of the pre-microencapsulation method, there is a very significant average difference between the treatments used for the measured ash content. The ash content of the extract from the highest to the lowest based on the type of pre-microencapsulation method was F > M>V.

4.6. Fiber Content (%, d.b.)

Results of the 5% LSD test showed that the type factor of the pre-microencapsulation method showed a significant difference in the total fiber content between the extracts produced, with the highest average being in the leukabean extract with the M and V methods, while the F method produced the extract with the lowest ash content. Based on nested ANOVA analysis, it was also found that all extracts from the pre-microencapsulated method showed significant differences between ethanol and n-hexane as indicated by different notations. The use of hexane solvent in the palm family extraction process showed higher levels of total fiber compared to ethanol up to 15% (Alvarenga et al. 2020).

Processes with involve high heat or pressure would produced a high solubility of fiber, moreover for the insoluble could also be degraded during the process (Wani *et al.* 2020). During the cooking process, components or products resulting from the Maillard process from the condensation of proteins and tannins, as well as resistant starch, can also be part of the fiber which will also be counted as total fiber. However, it would not happen in the all types of *Legumes*, depend on the characteristic of the solvent and also for the method.



	Protein (%)	Lipid (%)	Moisture (%)	Ash (%)	Fiber (%)	Carbohydrate (%)
FE	23.36	16.29	15.28	3.54	15.27	26.28
FH	23.28	24.24	10.71	3.53	10.98	27.28
ME	25.60	13.59	15.46	3.27	15.77	26.33
MH	26.33	13.60	14.24	3.28	16.03	26.54
VE	23.60	13.59	15.46	3.27	15.77	28.33
VH	23.33	13.60	14.24	3.28	16.03	29.54

Figure 5; The average of protein, lipid, moisture, ash, fiber, and carbohydrate content.

4.7. Anthocyanin, Phenolics, and Flavonoids Levels

The levels of anthocyanin extracts of lebui bean powder which were subjected to pre-microencapsulation treatment through fermentation, microwave, and vacuum pressure had a total anthocyanin value with a not large difference, each ranging from 117.21–117.72 ppm; 107.21–107.72 ppm; and 101.10–101.15 ppm. To determine the difference in the average levels of anthocyanins in the pre-microencapsulated method type factor, and also the solvent factor embedded in the pre-microencapsulated method type factor, a follow-up test was carried out using the 5% BNT test (LSD).

The total anthocyanin contained in the extract obtained from the extraction of lebui bean powder by the fermentation process has a higher value than the other two types of methods. This shows that the fermentation process is more effective in glucosidae enzyme bonds in bioactive compounds so that they become free compounds. *Rhizopus* sp. also has the ability to release esterase enzymes that can soften plant seed coats and produce anthocyanins by breaking down chemical bonds in cell walls and in cells (Lee *et al.* 2008).

The fermentation process for up to 24 h at moderate temperatures, will form lactic acid and acetic acid. This acidic condition will prevent the growth of other unwanted microbes in the fermentation and can provide optimum conditions for the enzymatic degradation of nutritional components and bioactive compounds contained in the ingredients. However, based on previous research, it showed that the average total anthocyanin levels in BKLT fermented with *Rhizopus* sp. tends to decrease with the longer fermentation time. During fermentation, biochemical processes, metabolism, degradation, and destruction are carried out by microbes to produce more sugar components to support their growth, resulting in a decrease in non-sugar components and secondary metabolites including anthocyanins. The longer the fermentation time, there was an increase in microbial mass and was positively correlated with a decrease in anthocyanin levels in the fermented product.

The factor causing the decrease in anthocyanin levels is the hydrolysis of anthocyanins into anthocyanidins and continues to become a group of simple sugars that occur during the fermentation of plant seeds, so that anthocyanin levels will decrease in the final product. The decrease in anthocyanin levels was also seen in the decrease in the black color intensity of the fermented product. The color of the product will be lighter than the color before fermentation. Anthocyanin which is a component of polyphenols is very sensitive and prone to damage due to changes in temperature and pH along with the increase in fermentation time (Mushollaeni et al. 2018). In pre-microencapsulation with the microwave method, heat or temperature factors will interact with electromagnetic fields to hydrolyze cells and glycoside bonds or other chemical bonds (Berhoft, 2008). Likewise with the vacuum pressure method, pressure and heat will open glycoside bonds, especially those that bind bioactive compounds, so that free bioactive compounds will be formed which can be extracted with a certain treatment.

The bioactive compounds commonly found in *Leguminosae* plants are flavonoids, proanthocyanidins, polyphenols, cajanol, isoflavones, saponins, phytosterols, inositol, hexaphosphates, sphingolipids, phenolic acids, alkaloids, and terpenoids (Berhoft, 2008). *Leguminosae* plant group contains bioactive compounds from the phenolic group, namely phenylpropanoids, catechins, anthocyanins, tannins, lignans, coumarins and furanocoumarins, as well as terpenoids, especially triterpenoids, steroidal saponins, and tetraterpenes (Berhoft, 2008; Wink, 2013). The extract produced from the treatment in this study also contained phenolic compounds which were calculated based on the total phenolic mg GAE/g in dry weight. The average total phenolic in the extracts that were treated pre-microencapsulated by the fermentation method and which was extracted using either ethanol or n-hexane, gave the extract yields with a higher total phenolic (78.176–78,544 mg GAE/g) compared to those processed using the other method (32,088–38,544 mg GAE/g).

The results of the research that have been carried out are in line with the results of research that fermentation using mold culture including *Rhizopus* sp. can increase the levels of total phenolic and anthocyanin which also results in an increase in the antioxidant activity of the final product (*Lee et al. 2008; Chaiyasut et al.* 2013). The -glucosidase enzyme produced by *Rhizopus* sp. able to decompose glycoside bonds that bind anthocyanins and release free anthocyanin aglycones including cyanidin which has high antioxidant activity. This decomposition and release process is effective for the first 0 to 24 h of black rice fermentation and will generally decrease with increasing fermentation time. The highest concentration of phenolic compounds contained in the cotyledons of plant seeds is a component of metabolic products and part of a group of secondary metabolites which are polar and easily soluble in water (Shahidi *et al.* 2015). However, although the total phenolic content of the extracts produced from the pre-encapsulation method with microwave and vacuum pressure was lower than that of the fermentation method, both types of extracts produced.

Research showed that the processing of natural materials using heat and microwave treatment, especially in legumes, has comparative advantages when compared to traditional processing, especially in terms of product texture, palatability, and composition. nutritional compounds (Khatoon et al. 2004; Eke et al. 2017). In terms of nutritional value, heat treatment can cause the product to undergo starch gelatinization, denaturation, and increase some of its nutritional availability, as well as inactivate toxic components and enzyme inhibitors. In addition, the use of heat in the microwave cooking method and the use of vacuum pressure can save energy, provide

convenience in using the equipment and save more time.

Table 9: Profile of active compounds in extract by GC-MS

No	Compounds	Chemical name	%	Function	References
1	*Neophytadiene*	*1-Hexadecene, 7,11,15-trimethyl-3-methylene / Triterpenoid*	10,10	Analgesic, antipyretic, anti-inflammatory, anti-microbial, antioxidant	Raman et al. (2012); Swamy et al. (2017); U.S. National Library of Medicine (2017)
2	*4-Methyl imidazole-5-[1,1-dimethyl butyric acid amide]*	*4-methyl-4-(5-methyl-1H-imidazol-4-yl) pentanamide*	18,15	Material for making drugs for anti-cholesterol, anti-protozoa, antiseptic	NTP (2007); U.S. National Library of Medicine (2017)
3	Anthocyanin	*1-benzopyry lium,2-phenyl-; Anthocyanidins; 2-phenyl chromenylium /* Flavonoid	17,23	Neuroprotectant/neuroprotective, anti-cancer, anti-inflammatory, anti-enteric disorders	Maran et al. (2015); U.S. National Library of Medicine (2017)
4	*Cajanus lactone*	7-hydroxy-5-*O*-methyl-8-(3-methyl-2-butyl ene)-4-phenyl-9,10-dihydro-benzopyran-2-one	11,78	Anti-bacterial, antifungal, anti-parasitic, anti-diabetic	Kong et al. (2010); U.S. National Library of Medicine (2017)

Testing the effect of the pre-microencapsulation method of lebui powder (F, M, and V) with the type of solvent at the time of extraction (ethanol and n-hexane) nested in the pre-microencapsulation method on total flavonoid content was carried out using nested ANOVA analysis. From the analysis process, it was found that the calculated F value was greater than the F table value 5% and 1%, and the p-value was smaller than the value (0.05 and 0.01), so it can be concluded that there is a very significant average difference between treatments used on the measured total flavonoid levels. To find out the difference in the average total flavonoid levels in the main factor and the factors nested in the main factor, a follow-up test was carried out using the 5% LSD test.

Flavonoids are the largest component of the phenolic group besides anthocyanins and are very important bioactive compounds due to their high antioxidant properties (Wink, 2013; Stephanie et al. 2021). Flavonoids are found in high concentrations in several types of *Leguminosae* beans, including faba beans and lentils. The best results from physicochemical parameters, as well as levels of anthocyanins, phenolics, and flavonoids, were then analyzed for antioxidant activity.

CONCLUSION

Our results demonstrated that the lebui powder which was pre-microencapsulated using a microwave, which was then extracted using n-hexane as solvent was the best combination. The extract contains protein, lipid, moisture, ash, fiber, and carbohydrates respectively 26.22%; 13.60%; 14.24%; 3.28%; 15.77%; and 26.33%. The bioactive compounds identified in the extract were phenolic compounds, flavonoids, anthocyanins, and dietary fiber respectively 38.54 mg GAE/g (d.b); 49.36 mg QE/g (d.b); 107.67 ppm; and 40.05 %. The yield and antioxidant activity were 80.86% and 12.04 IC50,mg/mL. It has been found that more than 10 types of bioactive compounds from the phenolics group and more than 20 types of essential fatty acids in the extract play an important role in the health of the body, and there are also important compounds as a source of herbal medicinal ingredients.

CONFLICT OF INTEREST

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

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AUTHOR CONTRIBUTIONS

Conceptualization, Writing, Analysis, Methodology, W.M.S., Writing, Analysis, L.N.T. All authors have read and agreed to the published version of the manuscript..

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