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RESEARCH ARTICLE

Determination of the Combination of Microencapsulation Materials in the Foam-Mat Process on Indonesian Local Black Bean Extract Using an Optimization Model

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ABSTRACT

Technology to protect bioactive compounds and secondary metabolites that have been extracted from a plant seed is very important in optimizing their potential as a natural source of antioxidants and medicinal ingredients. One of the friendly technologies for bioactive compounds to protect them from damage due to temperature, pH, and exposure to oxidation is microencapsulation. Microencapsulation is a technological method developed for a material to protect material and/or the bioactive components by coating. The aim of this study was to obtain an optimal combination of maltodextrin, tween 80, and sucrose for coating lebui bean extract which contains nutritional and bioactive compounds obtained from the pre-microencapsulation stage. Response Surface Method (RSM) was used in this study with a centralized composite design, included concentrations of maltodextrin (5%, 10%, 15% w/w), tween 80 (0.5%; 1%; 1.5% w/w) and sucrose (10%, 12%, 14% w/w). Software Design Expert 7.1.5 was used to identify the op-timal combination of maltodextrin, tween 80, and sucrose to produce powder containing the best levels of anthocyanin. The results of optimizing the formulations of maltodextrin, tween 80, and sucrose in calcula-tions with RSM are the use of maltodextrin of 13,71%, tween 80 of 2,20%, and sucrose of 11,46%. Based on the formulation, the quality was then characterized which included total anthocyanin, phenolic, flavo-noid, dietary fiber, and measuring the highest content of other bioactive compounds besides anthocyanins based on the results of compound profiling. The powder contains anthocyanin of 147,52 ppm, cajanin of 4,26%, and cyanidin of 2,09%, phenolic group of 158,02 mg EAG/g, flavonoid group of 385,04 mg EK/g, and dietary fiber content of 40,78%.

INTRODUCTION

Natural product process engineering aims to extract and/or protect important components or compounds that are prone to damage in these natural materials, including through the microencapsulation method. Components are coated using certain materials, so they are protected from extreme effects of 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 quantities and types in the local black bean (*Cajanus* sp.) extract of Lombok Island, known as lebui beans, including anthocyanins, phenolic compounds, flavonoids, dietary fiber, and identified 20 types of essential fatty acids in extracts play an important role in the health of the body[1-5]. Scientific information regarding the advantages and further utilization of bioactive compounds is still low. Whereas based on the results of previous studies, the phenolic content of lebui beans ranged from 30,501-78,363 mgEAG/g (d.b.)[4,21,23] and anthocyanin levels ranging from 107,120-153,350 ppm[3]. Given the importance of the potential of local ingredients as a source of bioactive compounds, as well as the need for sources of natural antioxidants in functional foods, it is very necessary to have the right microencapsulation technology to protect bioactive compounds rather than just using extraction methods and for further development on an industrial scale.

Microencapsulation is part of the encapsulation technology that processes a material into particle form, one of which is micrometer (1-1000μm) and nanometer in size. The purpose of using microencapsulation in food processing is to protect important compounds or elements such as bioactive compounds, vitamins, essential oils, medicinal ingredients, enzymes, and colors which are very easily damaged due to environmental influences[6]. Microencapsulates that are formed in optimal sizes can be more easily absorbed in the body. However, the selection of the optimal microencapsulation technique depends on the type of material, material structure, and final product quality parameters[7,8,22].

Emulsifier ingredients to coat active ingredients, including maltodextrin, gum, starch, stearic acid, tween, glyceride, casein, and gelatin. Selection of the right coating material can provide a good emulsion between materials in a solid/oil/water medium, because it reduces the surface tension between the two different substance phases and stabilizes the dispersion of the substance using a steric effect[7,8,9]. Previous research still revealed extraction and fermentation methods to extract bioactive compounds, but had not applied microencapsulation. If this is not done, then the bioactive compounds in lebui beans that have been disclosed and their great potential will be useless and only utilized in local areas. Therefore, research is urgently needed to optimize microencapsulation technology as a carrier for bioactive compounds from lebui beans which are rich in antioxidants and to characterize functional foods formulated with encapsulates. The aim of this study was to obtain an optimal combination of maltodextrin, tween 80, and sucrose for coating lebui bean extract which contains nutritional and bioactive compounds obtained from the pre-microencapsulation stage.

MATERIALS AND METHODS

Materials

The lebui beans used as the main ingredient in the study were obtained from Mataram, West Nusa Tenggara. Lebui beans are harvested at the age of three months with the characteristics of the black bean seeds, the seed size is 0.5-0.8 cm, the seed shape is round, and the outer shell of the seed has hardened. Chemicals for the process, chemical analysis, and equipment needed, including n-hexane, 90% ethanol, 70% ethanol, Whatman paper no.41, filter paper, N_2, physicochemical and phytochemical screening chemicals for profilling, antioxidant activity, total anthocyanins, and phenolic flavonoids chemical materials. Coating and supporting materials used include: maltodextrin (5%, 10%, 15%); tween 80 (0,5%; 1%, 1,5%); sucrose (10%, 12%, 14%); sodium bicarbonate 17,2%; citric acid 4,4%; tartaric acid 12,2%; salt 0,1%; aspartame 0,85%; and 3,7% grape flavor extract. Research equipment includes electric dryer, microwave, maceration-percolation apparatus, analytical balance, centrifuge, pH meter, autoclave, thermometer, incubator, 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.

Methods

Experimental Design

Response Surface Method (RSM) was used in this study with a centralized composite design, included concentrations of maltodextrin (5%, 10%, 15% w/w), tween 80 (0.5%; 1%; 1.5% w/w)) and sucrose (10%, 12%, 14% w/w). Software Design Expert 7.1.5 was used to identify the optimal combination of maltodextrin, tween 80, and sucrose to produce powder containing the best levels of anthocyanin. This research was divided into 3 stages consisting of: (1) Pre-microencapsulation process of lebui bean powder, (2) Extraction of lebui bean powder produced from the pre-microencapsulation stage, and (3) Optimization of powder production containing bioactive compounds from lebui beans extract using the response surface method.

Pre-Microencapsulation Process

The pre-microencapsulation process of lebui beans powder begins with grinding the lebui into powder form, then pre-microencapsulation using a microwave. The lebui beans are sorted and taken to have a diameter of 0.5–0.8 cm, then dried in a cabinet oven until the moisture content reaches 11-12%. The beans were then finely grounded, sifted by 60 mesh size (BSS sieve) and stored in a tightly sealed glass container with silica gel. Lebui beans powder that has been heated for 5 min at 40^{o}C, then put in the microwave and processed at 800 W for 30 s. then store in a dry place, not damp, not exposed to direct sunlight, and in a dark place at a temperature of 27–30 °C[5)].

Extraction Process

Extraction was carried out by percolation maceration method and combined with stirring method. The solvents used were 90% n-hexane and 90% ethanol. Extraction was carried out in 4 stages for each type of treatment and solvent concentration, until extracts were obtained from each of these treatments. The solvent ratio used was 1:8 (w/v) and the extraction was carried out at 25^{o}C for 24 h[5)].

Optimization of Powder Production Containing Bioactive Compounds from Lebui Beans Extract Using the Response Surface Method

This process aims to obtain an optimal combination of maltodextrin, tween 80, and sucrose for coating lebui bean extract which contains nutritional and bioactive compounds obtained from the pre-microencapsulation stage. Coating materials and supporting materials are mixed with lebui bean extract, then homogenized for 5 minutes using a homogenizer. Next, it is dried in an oven at 40-45^{o}C for 4 hours. Coated powder was obtained by grinding and sifting on an 80 mesh sieve. The coated powder obtained was then put in a brown glass container and covered with aluminum foil, tightly closed, and stored in a dry condition at room temperature. The analysis for each treatment was the anthocyanin content, while the analysis for the best treatment included total anthocyanins, phenolics, flavonoids, dietary fiber, and measuring the highest content of other bioactive compounds besides anthocyanins based on the results of compound profiling.

RESULTS

RSM is a suitable method for identifying the effect of a single variable and looking for optimum conditions in an efficient multivariable system. In this study, RSM was used with Box Wilson's Central Composite Design (CCD) which is one of the RSM designs with a fractional factorial design and an enlarged center point with a group of star points that allows determining the curve point[10)].

Determining the optimum point in this study requires an experimental area that is around the midpoint of the experiment. The experimental area consists of two levels of each variable coded with -1 and +1 and expanded with the values of $-\alpha$ and $+\alpha$, where the value of α is $2^{k/4}$ where k is the number of variables tested[11)].

Total Anthocyanins

Determination of the highest anthocyanin levels was obtained through the stages of the RSM method in phase 0, phase 1, and phase 2. The test in the zero phase is to test the response of each treatment by plotting and looking at the treatment regression equation for the response. If the graphs and equations show parabolas and quadratic equations, the research can be continued using the optimal point and one point around the optimal (**Figure 1**). Based on the results of phase 0 analysis which is shown by the shape of a parabolic graph and the presence of a quadratic equation, the RSM analysis can be continued in phase 1 with optimal predictive points at 15% maltodextrin, 1.5% tweeen 80, and 12% sucrose.

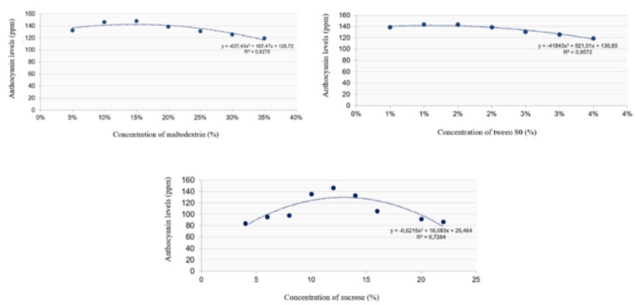


Figure 1. Determination of the highest anthocyanin levels through the stages of the RSM method

Phase 1 diagnosis can be seen through the lack of fit model test, if the lack of fit shows a significant value, then the linear model or interaction model cannot be used to model the response. Lack of fit is significant if the p-value < 0.05 (α = 5%). After seeing the significance of the lack of fit, then we can see the curvature which shows an indication that the response can be modeled in quadratic or cubic equations. This can be seen if the p-value curvature is <0.05 (α = 5%), because there is a curve or optimal point that can be predicted (**Figure 2**).

Response 1 Kadar Antosianin
ANOVA for selected factorial model
Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	261.13	6	43.52	59.03	< 0.0001	significant
A-Maltodekstrin	*0.024*	*1*	*0.024*	*0.033*	*0.8622*	
B-Tween	*63.17*	*1*	*63.17*	*85.68*	*< 0.0001*	
C-Sukrosa	*4.87*	*1*	*4.87*	*6.60*	*0.0424*	
AB	*134.64*	*1*	*134.64*	*182.62*	*< 0.0001*	
AC	*30.97*	*1*	*30.97*	*42.00*	*0.0006*	
BC	*27.45*	*1*	*27.45*	*37.24*	*0.0009*	
Curvature	575.20	1	575.20	780.16	< 0.0001	significant
Residual	4.42	6	0.74			
Lack of Fit	*4.20*	*1*	*4.20*	*96.11*	*0.0002*	*significant*
Pure Error	*0.22*	*5*	*0.044*			
Cor Total	840.75	13				

Figure 2. The results of the calculation of lack of fit and curvature with a design expert

The p-value curvature is 0.0001 which is less than 0.05 which indicates that the response model can be modeled quadratic or cubic, so that phase 1 of the RSM process can be continued to phase 2. This is also strengthened through contour maps. which shows the existence of a curve which indicates an optimal point that can be achieved by each treatment on anthocyanin levels. The 3D contour map also shows a curve indicating that the response and treatment relationship is quadratic and the optimal point of response can be found (**Figure 3 and 4**).

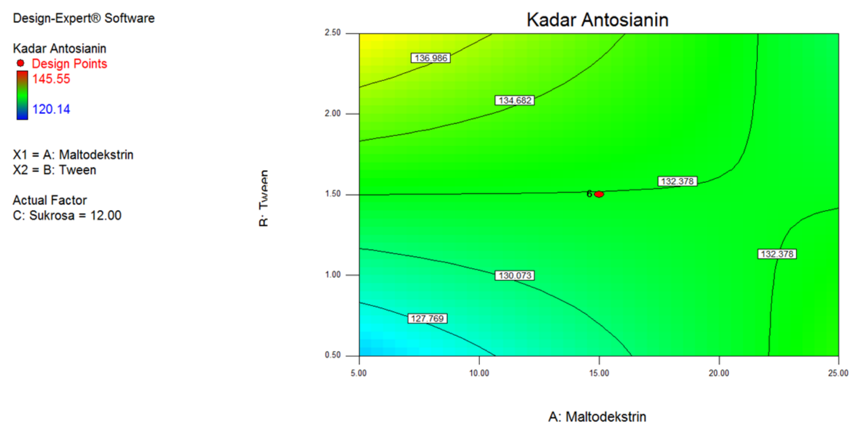


Figure 3. Contour map of maltodextrin, tween 80, and sucrose to anthocyanin levels

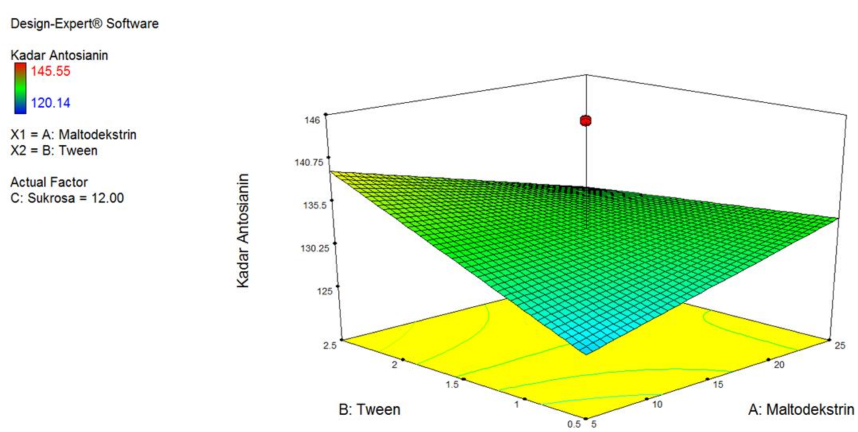


Figure 4. Contour map 3D of maltodextrin, tween 80, and sucrose to anthocyanin levels

RSM Methods and Quadratic Model

In order to get the optimum point with the RSM method, the treatment is extended to the level below and above the optimum point. The lower level (code -1) for the maltodextrin concentration variable is 5% while the upper level (code +1) is 25%. The lower level (code -1) for the tween concentration variable is 0.5% and the upper level (code +1) is 2.5%, while the lower level (code -1) for the sucrose concentration variable is 10% and the upper level (code +1) by 14%. Data on anthocyanin levels obtained from the treatment results and added to the expansion of the treatment, were then processed with the help of the Design-Expert DX 7.1.5 program for statistical data processing.

The quadratic model is used as a model to explain the relationship between variables X1 (concentration of maltodextrin), X2 (concentration of tween) and X3 (concentration of sucrose) to the response of Y (level of anthocyanin). After the quadratic model has been selected, an analysis of variance is performed on the model.

Analysis of variance (ANOVA) shows that the model has a significant effect on the response where the p value is less than 0.05 (5%). The results of the analysis of variance indicated that the concentration of maltodextrin (squared) had a significant effect on the response. Other factors, namely the concentration of maltodextrin (linear), the concentration of tween (linear), the concentration of sucrose (linear) and the interaction of two of the three factors had no significant (not significant) effect on the response. Likewise, for the other two factors, namely the tween concentration (squared) and the sucrose concentration (squared) which also did not affect the response. There was no significant effect on the concentration of tween and sucrose in this study, presumably because the combination of the two ingredients had been able to protect the anthocyanins contained in the powder, and therefore did not cause a significant reduction in anthocyanin levels. The results also showed that there was a significant effect on the concentration of maltodextrin (squared). This happened because the greater the concentration of maltodextrin, the anthocyanin levels also increased to a certain extent (15%) and tended to decrease if the concentration was increased.

DISCUSSION

The Effect of Maltodextrin, Tween 80, and Sucrose Concentrations on the Response of Anthocyanin Levels

Response graphs are used to simplify the description in knowing the effect of variables on the response of anthocyanin levels. Responses to anthocyanin levels are depicted in 3D curves and contour plots. Contour plots are 2D plots that are cross-sections of 3D curves. Contour plots are useful for analyzing the effect of interactions between factors on responses[12]. The concentration of maltodextrin and tween is a critical factor because it affects the level of anthocyanin produced. Therefore, it is necessary to conduct research on the proper concentration of maltodextrin and tween to produce lebui bean extract powder containing bioactive compounds that have optimal anthocyanin levels. The interaction between maltodextrin and tween concentrations, is shown in Figure 5.

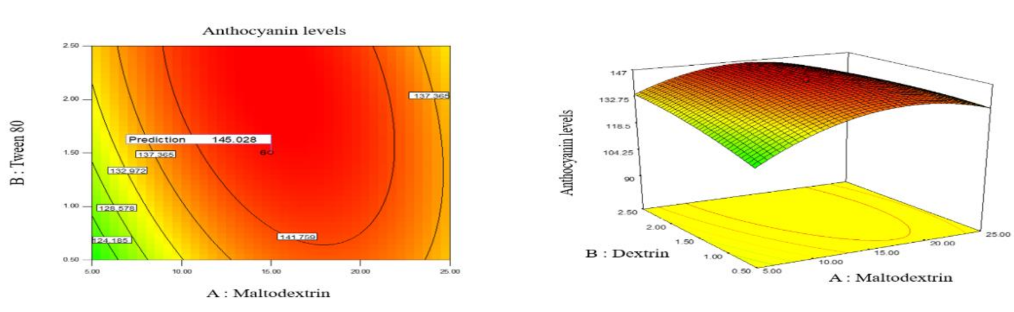


Figure 5. (a) Contour plot and (b) Surface curve 3D response of maltodextrin and tween concentrations to response of anthocyanin levels

The x-axis and y-axis in Figure 5 show the optimized variables. The x-axis shows the variable concentration of maltodextrin, while the y-axis shows the variable concentration of tween. The circular lines show the response. The optimal response is indicated by the presence of a flag in the middle of the contour indicating the optimal point information located at the point (node) indicated on the flag. On the contour plot, the optimum anthocyanin concentration is shown at the point (node) of 145,028.

Figure 5 (a) shows that the contour is in the form of a saddle. Maltodextrin and tween concentrations showed maximum and minimum points. Figure 5(b) shows the response to anthocyanin levels obtained from various concentrations of maltodextrin and tween under conditions of sucrose concentration at the optimum point, and Figure 5(b) also shows the existence of a curve which proves that the identification of a quadratic response can be found.

The Effect of Maltodextrin and Sucrose Concentrations on the Response of Anthocyanin Levels

The concentration of maltodextrin and sucrose is a factor that influences the anthocyanin content of the resulting powder. Therefore, it is necessary to study the interaction between the two to produce powders with optimal anthocyanin levels. The interaction between the concentrations of maltodextrin and sucrose, is shown in Figure 6. On the contour plot, the optimum anthocyanin concentration is shown at the point (node) of 145,028. Figure 6 shows the response to anthocyanin levels obtained from various concentrations of maltodextrin and sucrose. At the optimum point there is a curve which proves that a quadratic response is identified.

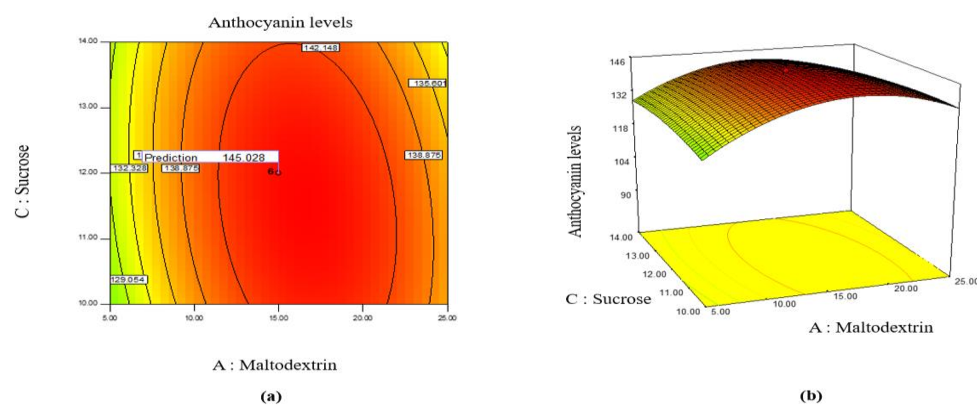
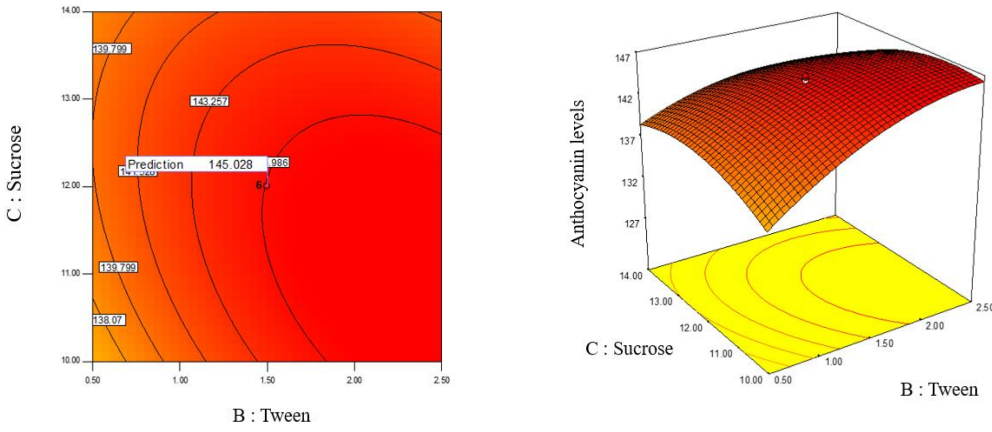


Figure 6. (a) Contour plot and (b) Surface curve 3D response of maltodextrin and sucrose concentrations to response of anthocyanin levels

The Effect of Tween and Sucrose Concentrations on the Response of Anthocyanin Levels

The interaction between tween and sucrose concentrations to produce powder with optimal anthocyanin content is shown in Figure 7. Figure 7 (a) shows that the contour is in the form of a saddle. The tween and sucrose concentrations show maximum points and minimum points. Figure 7 (b) shows the response to anthocyanin levels obtained from various concentrations of tween and sucrose, as well as the optimum point for using both concentrations. Figure 7 (b) also proves that quadratic response identification can be found. The circular lines show the response. The optimal response is indicated by a flag in the middle of the contour indicating the optimal point description located at the point (node) indicated on the flag. On the contour plot, the optimum anthocyanin concentration is shown at the point (node) of 145,028.



(a) (b)

Figure 7. (a) Contour plot and (b) Surface curve 3D response of sucrose and tween concentrations to response of anthocyanin levels

Determination of Optimum Conditions Response Anthocyanin Levels

Software Design Expert 7.1.5 is used to identify the optimal combination of maltodextrin, tween 80, and sucrose to produce coated powder which is formulated into an instant drink containing the best levels of anthocyanins. In this study, the optimal solution offered by the model is a maltodextrin concentration of 13,71%, a tween concentration of 2,20%, and a sucrose concentration of 11,46% with a predicted response of 146,176 with a desirability value of 1, where a desirability value of 1 indicates the perfect case. Data analysis using Design Expert 7.1.5 obtained a quadratic regression model equation formed from the variables X1, X2, and X3 which were constructed from the selected model in the form of a code equation.

$$Y = 145{,}03 + 3{,}65X_1 + 3{,}14X_2 - 0{,}74X_3 - 4{,}10X_1X_2 - 1{,}97X_1X_3 - 1{,}85X_2X_3 - 12{,}14X_1^2 - 2{,}21X_2^2 - 2{,}23X_3^2$$

Actual variables are also found in the Design Expert program. The form of the equation is called the actual equation, as shown below.

$$Y = -16{,}97547 + 5{,}80311X_1 + 27{,}02937X_2 + 15{,}89121X_3 - 0{,}41025X_1X_2 - 0{,}098375X_1X_3 - 0{,}92625X_2X_3 - 0{,}12141X_1^2 - 2{,}20816X_2^2 - 0{,}55823X_3^2$$

This equation is the best model to explain the relationship between responses and the independent variables given the concentrations of maltodextrin, tween, and sucrose. The results of optimizing the formulations of maltodextrin, tween 80, and sucrose in calculations with RSM are the use of maltodextrin of 13,71%, tween 80 of 2,20%, and sucrose of 11,46%. Based on these formulations, quantitative and qualitative characterization was then carried out.

The results of the analysis of powder anthocyanin levels using the optimum formulation, were obtained at 147,52 ppm. This result is higher than the predicted response of 146,176 with a desirability value of 1 in the optimization test. The combination of the ratio of maltodextrin: tween:

sucrose with maltodextrin concentration less than 15% to 10% with tween and sucrose each 1,5%, can still produce powder having anthocyanin content above 144 ppm. An increase in maltodextrin concentration of up to 5% and not followed by an increase in tween concentration of up to 2,5% at a sucrose concentration of ≥12%, will result in a sharp decrease in anthocyanin levels ranging from less than 130,48 ppm to 135,24 ppm and also resulted in an increase in the intensity of the dark color when compared to the levels of anthocyanin and the color of the extract. These conditions indicated the proper interaction between maltodextrin and tween 80 at a concentration of 13,71% and 2,20% which was able to form optimum protection for powders containing bioactive compounds. The decrease in anthocyanin levels in the powder is caused by the nature of anthocyanins which are easily damaged due to increased temperature, changes in pH, increased light intensity and UV rays, and also at temperatures higher than 50°C, resulting in a decrease in color degree[13,14]. Then, based on the formulation and anthocyanin level, the quality was then characterized which included phenolic, flavonoid, dietary fiber, and measuring the highest content of other bioactive compounds besides anthocyanins based on the results of compound profiling.

Characterization of The levels of Phenolics, Flavonoids, Dietary Fiber (DF), and Other Bioactive Compounds Besides Anthocyanins

The optimum formulation of total phenolic powder was 155,37 mg EAG/g. This level is quite high considering that the drying process is carried out in the manufacture of powder. This indicates the level of bioactive compounds present in the extract until it is processed into powder form is still in a stable condition or has low damage. Reported by previous experiment[15], that the use of drying temperatures of up to 50°C in processed wine waste is still safe to protect the bioactive compounds of the phenolic and anthocyanin groups from damage. The total flavonoid powder based on the optimum formulation was higher thanthat of the early lebui bean powder extract. Hydrolysis and degradation of the bonds between components in the material occur during the pre-microencapsulation process, so that the bioactive components become free components. These conditions resulted in increased concentrations of free polyphenols, flavonoids, and antioxidant activity[16,17].

The average value of total DF in powdered products was higher than lebui bean powder and higher than whole lebui bean, respectively, which were 40,78% > 22,74% > 19,14%. The main cause that resulted in an increase in Total Dietary Fiber (TDF) from the powder was the pre-microencapsulation process which turned out to be effective in breaking the glycosidic bonds in the polysaccharide chain and finally forming the components that make up the DF fiber. This is supported by research which explains that increased levels of Soluble Dietary Fiber (SDF) in cereal processing can occur at high temperatures of 100°C[18,19,20]. Analysis by GC-MS of the powder produced with the best formulation showed the presence of bioactive compounds from the cajanin and cyanidin groups, which have potential as antioxidant compounds, with respective levels of 4.26% and 2.09%.

CONCLUSIONS

Based on the results of the study it was concluded that the use of maltodextrin 13.71%, tween 80 2.20%; and 11.46% sucrose is the most optimal combination to produce powder from lebui bean extract which contains bioactive compounds in the anthocyanin group of 147.52 ppm, cajanin of 4.26%, and cyanidin of 2.09%, phenolic group of 158 .02 mg EAG/g, and the flavonoid group of 385.04 mg EK/g.

AUTHOR CONTRIBUTIONS

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

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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