

Optimization of Microencapsulation Process of Green Coffee Extract with Spray Drying Method as A Dietary Supplement

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Received : 31 October 2019 / Accepted : 5 February 2020

Abstract

Green coffee has high content of chlorogenic acid which is potential to be developed into slimming diet supplement. Microencapsulation was used to make herbal supplement. Spray drying process with the addition of maltodextrin as a coating material will protect the extract from high temperatures. The purpose of this study was to determine the percentage of maltodextrin and optimal skim milk to produce dietary supplement preparations and find out the quality of dietary supplement preparations obtained. Robusta coffee beans from Argopuro, Jember, East Java, was dry processed. This research optimized the microencapsulation process of green coffee extract using central composite design method. The method was response surface with two factors namely percentage of maltodextrin and percentage of skim milk. The response used was total phenolic content and antioxidant activity. The results showed the optimal percentage of maltodextrin and combined skim milk were 8.61% and 3.22% respectively with total phenol obtained at 58.75 mg GAE/g with an accuracy of 93.10% and IC_{50} 65.10 ppm with an accuracy of 95.76%. Dietary supplement products on the market contain total phenol of 57.52 mg GAE/g and IC_{50} 87.65 ppm. Comparison with other green coffee supplement products showed this microencapsulation have higher total phenol content and antioxidant activity.

Keywords: Green coffee bean, maltodextrin, spray drying, dietary supplement, skimmed milk, polyphenol, antioxidant

INTRODUCTION

Coffee is widely consumed as refreshing drink after mineral water and tea. There are two types of coffee that are popular in Indonesia namely Robusta and Arabica. According to Ministry of Agriculture data in 2017, Robusta coffee dominates 72.59% of coffee production in Indonesia. Green coffee is dried coffee beans that have been peeled off but not yet roasting. According to Isnindar *et al.* (2017), coffee contains several important substances

such as caffeine, phenolic compounds, chlorogenic acid, and antioxidant compounds that are able to protect body from free radicals. Polyphenols are chemical compounds as antioxidants those can be found in coffee. In green coffee, the main polyphenol compound is chlorogenic acid. Polyphenol compounds are used to reduce body weight so that currently green coffee can be consumed as supplement diets for obese people (Nagao *et al.*, 2009). According to data of Depkes (2009), the prevalence of obesity in Indonesia reaches

19.1% calculated from population aged of 15 years and above. The prevalence of obesity is expected to increase according to age, increase in population, diet, and lifestyle. Obesity will cause several diseases such as high blood pressure, coronary heart disease, diabetes, stroke, and cancer (Husnah, 2012). Therefore, it is necessary to do research on dietary supplements that produce high total polyphenols and antioxidants with the addition of optimal fillers. Microencapsulation is one of important process to make bioactive component more stable.

Method of microencapsulation can be used to make herbal supplement. Spray drying process with the addition of maltodextrin as a coating material that will protect the extract from high temperatures so that important components in it are not damaged and skim milk as a protein material. Spray drying is a method used to convert liquid feed into powder (Dewi & Loekman, 2015). According to Heldman *et al.* (1981), the advantages of the spray drying process are the relatively fast drying cycles, the retention of the product in a short drying chamber, and the finished product that has been dried ready to be packaged. Maltodextrine and skim milk are kind of carrier material that will protect bioactive component from high temperature on spray drying process.

The optimization method that can be used is the response surface method (RSM) with central composite design (CCD). According to Aritonang (2014), RSM is used for research that has complex processes and is widely used in food technology research. The RSM method is able to explore correlations between many factors to get the most optimal production conditions (Chang *et al.*, 2006). In RSM, CCD is used to build a polynomial model of a mathematical function of the independent

variables on the response formed (Montgomery, 2001). Therefore, this study aimed to determine the percentage of maltodextrin and optimal skim milk to produce dietary supplement preparations and to find out the quality of dietary supplement preparations obtained in form of microencapsulation.

MATERIALS AND METHODS

The material used in this research is Robusta coffee beans from Argopuro, Jember, East Java. Raw materials were obtained from Jember, harvested in July 2018 and processed by dry process and no pretreatment was carried out. The filling material used were maltodextrin and skim milk, and commercial green coffee supplement. The research was conducted at the Laboratory of Entrepreneurship and Bioindustry of Faculty of Agricultural Technology, Brawijaya University and the Laboratory of Pharmacy Biology, Faculty of Science and Mathematics, Indonesian Islamic University.

The research used response surface method (RSM) in two factors that are the percentage of maltodextrin and the percentage of skim milk so that 13 experimental design combinations were obtained. The response used in this study was total phenolic content and antioxidant activity. The results of optimization in the Design Expert 7.1.5 Portable software were validated by extracting in accordance with the optimal treatment results from the prediction of the response surface then testing the total phenolic content and antioxidant activity.

The level used for the percentage of maltodextrin was 8.50 as the lower limit and 9.50 as the upper limit, while for skim milk was 2.20 as the lower limit and 6.60 as the upper limit so that 13 experimental design combinations were obtained as in Table 1.

Tabel 1. Experimental design

Factor		Factor	
X ₁	X ₂	Percentage of maltodextrin (% of total extract)	Percentage of skim milk (% of total extract)
-1	-1	8.50	2.20
+1	-1	9.50	2.20
-1	+1	8.50	6.60
+1	+1	9.50	6.60
-1,414	0	8.29	4.40
+1,414	0	9.71	4.40
0	-1,414	9.00	1.29
0	+1,414	9.00	7.51
0	0	9.00	4.40
0	0	9.00	4.40
0	0	9.00	4.40
0	0	9.00	4.40
0	0	9.00	4.40

Coffee Powder Microencapsulation

Green coffee beans were aerated at room temperature for two hours to reduce water content. Furthermore, the coffee beans were grinded using a disk mill in stages and then sieved (40 mesh) to produce green coffee powder. The ground coffee obtained were stored in plastic containers at room temperature. The green coffee powder that did not pass the sieve was then grinded again using a blender.

Green coffee powder was taken as much as 10 g and then macerated in 100 mL of distilled water for 24 hours at room temperature ($\pm 25^\circ\text{C}$) without stirring. The extraction results were filtered with a paper filter and stored in glass bottles in cold temperatures.

Coffee Extract Microencapsulation

100 mL of green coffee extract was added with maltodextrin and skim milk using several proportions according to the experimental design. The three ingredients was then mixed for 15 minutes for homogenization. The spray drying process was then carried out with an inlet temperature of 120°C , an outlet temperature of $60\text{--}80^\circ\text{C}$ for 30 minutes. The microcapsules produced

were then analyzed for total phenolic content and antioxidant activity.

Total Phenolic Content (TPC)

A response to optimization process of microencapsulation of green coffee was total phenolic content. Every samples was analyzed total phenolic content. The optimum treatment was analyzed of TPC and compared with commercial green coffee supplement.

Standard curves of chlorogenic were set up by weighing chlorogenic acid powder as much as 0.01 g, then put into 100 mL volumetric flask and added distilled water to mark the limit and homogenized to form 100 ppm. The solvent was diluted with concentrations of 0 ppm, 20 ppm, 40 ppm, 60 ppm, 80 ppm and 100 ppm. Each concentration was taken 0.5 mL and added 2.5 mL of 10% folin reagent then homogenized and incubated 5 minutes in the dark. After that, 2 mL of 7.5% Na_2CO_3 was added and incubated 30 minutes in the dark. Absorbance measurement using 765 nm wavelength was then plotted into a curve where X is the concentration of gallic acid and Y is the absorbance so that the regression formula $Y = aX + b$ is obtained.

The solvent for analyzed was prepared by encapsulation powder samples weighed as much as 0.01 g and dissolved in 10 mL of distilled water. The solvent was taken 0.5 mL and put in a dark test tube. The solvent was mixed with 2.5 mL of Folin C reagent 10% distilled water and then homogenized and incubated 5 minutes in the dark and at room temperature. Then it was added with 2 mL of Na₂CO₃ solution, 7.5% distilled water, homogeneous, and incubated 30 minutes in the dark. The blank used was 0.5 mL distilled water added with 2.5 mL of 10% folin reagent and 2 mL of 7.5% sodium carbonate solution. Absorbance measurement used 765 nm wavelength. The amount of phenolic compounds was measured based on gallic acid standard curves and expressed as mg chlorogenic acid equivalent (CGAE/g extract). The levels of phenolic compounds in extracts were calculated by the equation:

$$C = \frac{c \times fk \times V}{g}$$

C = concentration of total phenolic content (mg CGAE/g)
 c = concentration of chlorogenic acid (μg CGAE/mL)
 V = volume of the extract solvent taken for analyzed (mL)
 g = extract weights used for analyzed (g)
 fk = conversion factor

Antioxidant Activity

The second response from optimization process was antioxidant activity. Analysis of antioxidant activity was carried out by the DPPH method by means of 0.01 g of microcapsule powder dissolved in 10 mL of methanol to obtain a 1000 ppm solvent. The solvent was diluted at concentrations of 60 ppm, 70 ppm, 80 ppm, 90 ppm and 100 ppm. Samples at each concentration were taken 2 mL and put in a dark test tube. Each test tube was added with 1 mL DPPH 0.2 mM, homogenized, and incubated in the dark for 30 minutes. The control solvent was made by 1 mL DPPH 0.2 mM put into 2 mL of methanol then homogenized and

incubated 30 minutes in the dark. Absorbance measurements used wavelength of 517 nm. DPPH uptake values before and after sample addition were calculated as percent inhibition (% inhibition) by the formula:

$$\% \text{ Inhibition} = \frac{A \text{ control} - A \text{ sample}}{A \text{ sample}} \times 100\%$$

Then the results are entered into the linear regression equation $Y = aX + b$ where Y is the percent inhibition and X is the concentration. The equation was used to determine the LC₅₀ value of each sample. The LC₅₀ value was the half maximal inhibitory concentration.

The chlorogenic acid sample test was carried out with LC-MS/MS equipment. The column used was the Hypersil Gold specification (50 mm x 2.1 mm x 1.9 μm). UHPLC brand ACCELLA type 1250 made by Thermo Scientific which consisted of a vacuum degasser, quartener pump, thermostatic auto-sampler controlled by a personal computer through the x-calibur 2.1 program. Solvent A consisted of 0.1% formic acid in aquabidest, solvent B consisted of 0.1% formic acid in acetonitrile. A linear gradient with a velocity of 300 μL/min with the following mobile phase adjustments: a) 0–0.6 minutes 95% A; 0.6–3.0 minutes 75% B; 3.0–3.5 minutes 75% B; 3.5–4.0 minutes 75% B and 4.0–5.5 minutes 95% A. The injection volume at LC is 2 μL. The column was controlled at 30°C, and the autosampler compartment was set to 16°C.

RESULTS AND DISCUSSION

Response data of total phenol was in the range of 17.03–61.00 mg GAE/gr while LC₅₀ value response data was in the range of 62.69–100.08 ppm. The results of a centralized composite design analysis can be seen in Table 2.

Table 2. Result of central composite design analysis

Factor		Response	
Percentage of maltodextrin (% of total extract)	Percentage of skim milk (% of total extract)	Total phenolic content (mg GAE/g)	IC ₅₀ value (ppm)
8.50	2.20	61.00	62.68
9.50	2.20	54.49	86.96
8.50	6.60	32.23	80.36
9.50	6.60	25.25	98.55
8.29	4.40	54.69	81.14
9.71	4.40	31.82	98.86
9.00	1.29	46.07	89.20
9.00	7.51	17.03	100.08
9.00	4.40	37.85	71.85
9.00	4.40	39.63	75.21
9.00	4.40	21.00	66.84
9.00	4.40	26.21	66.83
9.00	4.40	19.63	76.97

Total Phenolic Content

According to Table 2, it can be seen that the highest response of total phenol value was 61.00 mg GAE/g obtained from the composition with percentage of maltodextrin was 8.50 and percentage of skim milk is 2.20. The lowest total phenol is 17.03 mg GAE/gr obtained from the treatment with the percentage of maltodextrin 9.00 and the percentage of skim milk 7.51. The research data indicate that the total phenol extract tends to increase with the smaller percentage of maltodextrin and the small percentage of skim milk used. The results obtained were in accordance with Widarta & Hapsari (2014), the higher encapsulant's concentration, the lower total phenol of the microcapsule product. This was caused by the higher concentration of encapsulants resulting in a greater ratio of extracts to encapsulants.

Data processing using RSM shows that the chosen model was linear with both factors significant to the response. The polynomial equation obtained was

$$Y_1 = 35.92 - 5.73X_1 - 12.39X_2 \dots\dots (1)$$

$$Y_1 = 163.84 - 11.46X_1 - 5.630X_2 \dots (2)$$

In response surface optimization for total phenols, the most influential factor was the percentage of maltodextrin (X_1) with a coefficient value of 11.46 which shows that the percentage factor of maltodextrin gave an effect of 11.46 for every one point increased. Next was the percentage factor of skim milk (X_2) with a coefficient value of 5.630 which shows that the solvent ratio factor gives an effect of 5.630 for every one point increase.

The contour plot of the percentage factor of maltodextrin and skim milk to the total phenolic content response can be seen in Figure 1. The results of the response are shown through the contour lines in the picture. The largest total phenol was shown starting from the deepest line and the more out the total phenol value will be lower. The red contour indicates that the total phenol was higher, while the darker blue the total phenol content was lower. The surface curve of the percentage response of maltodextrin and skim milk to the total phenolic content response can be seen in Figure 2.

Based on the response surface curve it can be seen that the percentage factor of maltodextrin and skim milk had a significant

effect. The graph also shows a linear model, in which total phenols will increased along with low concentrations of maltodextrin and skim milk. Based on these results, maltodextrin will protect the material from nutrient released due to high temperatures, but the higher the percentage of maltodextrin and skim milk, the lower the total phenol content. This was due to the increasing number of total solids contained in the material so that it can reduce the intensity of the blue color in folin reagents

(Siska & Wahyono, 2013). According to Al Fakkar (2018), the combination of skim milk and green coffee extract will produced total phenol, antioxidants, and high chlorogenic acid content which was useful as a dietary supplement.

Antioxidant Activity

Antioxidant activity value was carried out to find out how strong the antioxidant

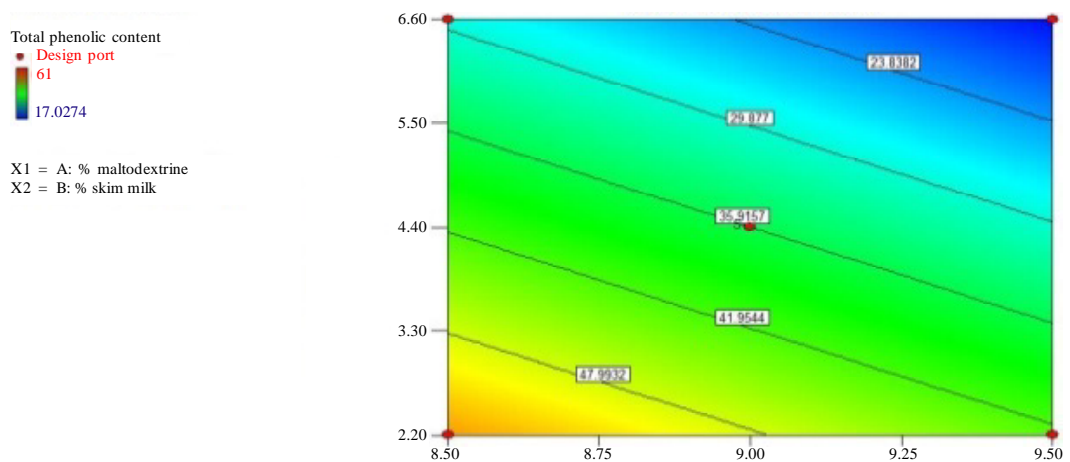


Figure 1. Contour plot response percentage of maltodextrin and percentage of skim milk to response total phenolic content microencapsulation of green coffee extract

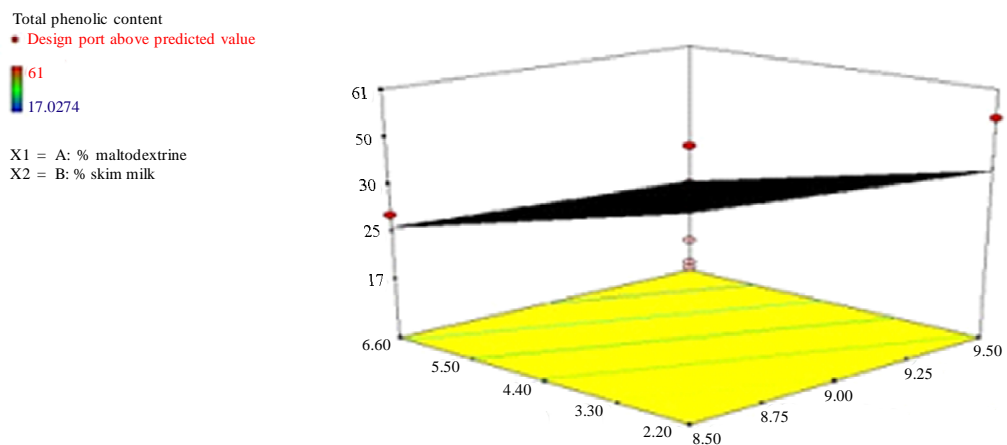


Figure 2. Response surface curve percentage of maltodextrin and percentage of skim milk to response total phenolic content microencapsulation of green coffee extract

activity was in the microencapsulation powder of green coffee extract produced. Antioxidant activity can be seen from the LC_{50} value where the smaller LC_{50} value, will make antioxidant activity higher. Based on the data obtained it can be seen that the response of antioxidant activity had the lowest LC_{50} value of 62.68 at 8.5% maltodextrin percentage and 2.2% skim milk percentage which means it had high antioxidant activity while the highest LC_{50} value was 100.08 at percentage of maltodextrin 9% and percentage of skim milk 7.51% which means it had low antioxidant activity. The research data obtained indicate that the antioxidant activity will increase along with the reduction in the composition of additional ingredients in green coffee extracts. The more composition of maltodextrin and skim milk were added, the lower the antioxidant activity as indicated by the higher LC_{50} value.

These results were linier with the research of Yuliawaty & Wahono (2015), the addition of higher concentrations of maltodextrin cause a decrease in levels of antioxidant activity. This was presumed by the increasing number of total solids contained in the material, namely maltodextrin as a filler so that the measured antioxidant activity was reduced, so that with increasing total solids in an ingredient, the measured antioxidant activity levels will be smaller. In addition, it was also thought to be caused by changes in antioxidant compounds due to the heating process, namely vitamin C and other oxidized phenol compounds. It was possible that warming causes the phenol compound to decompose so that its ability as an antioxidant had decreased.

The results of data processing using RSM show that the chosen model was quadratic. The percentage factor of maltodextrin was significant for the response but the percentage factor for skim milk was not significant for the response. The polynomial equation for the quadratic model on the response value of

LC_{50} (Y_2) which is influenced by the percentage factor of maltodextrin (X_1) and the percentage of skim milk (X_2) is as follows:

$$Y_1 = 71.55 + 8.44X_1 + 5.58X_2 - 1.52X_1X_2 + 6.68X_1^2 + 9X_2^2 \dots\dots\dots (1)$$

$$Y_1 = 2055.10 - 458.24X_1 - 1.3806X_2 - 1.3838X_1X_2 + 26.734X_1^2 + 1.8604X_2^2 \dots (2)$$

In response surface optimization for LC_{50} values, the most influential factor is the percentage of maltodextrin (X_1) with a coefficient value of -458.24, which indicates that the percentage factor of maltodextrin gives an effect of -458.24 for every one point increase. Next is the percentage factor of skim milk (X_2) with a coefficient value of -1.3806 which shows that the percentage factor of skim milk gives an effect of -1.3806 every increase of one point. The contour plot of the percentage factor of maltodextrin and percentage of skim milk to the LC_{50} value of microencapsulated green coffee extract can be seen in Figure 3.

Based on Figure 3, it shows that the higher the LC_{50} value means that antioxidant activity is lower. Conversely, the lower LC_{50} value means that antioxidant activity is higher. In addition, in the graph there are also different colors where the redder the antioxidant activity the smaller and the darker blue the color shown, the higher the antioxidant activity.

The contour plot of the total phenolic content response was different from the plot contour of the LC_{50} value response. The difference was seen from the contour color where the expected total phenolic content plot response contour was red, the highest total phenol content. In contrast, the contour color of the expected LC_{50} response was blue, which is the lowest LC_{50} value so that it has high antioxidant activity. The surface curve of the maltodextrin percentage response and the percentage of skim milk to the LC_{50} value response is presented in Figure 4.

Based on these curves it can be seen that both factors affect the LC_{50} value, but the percentage of maltodextrin was more dominant factor affecting the yield. The graph also shows a quadratic model, where it was shown through optimum conditions at the peak then decreased based on the two factors used.

The percentage of maltodextrin and the percentage of skim milk generally affect the antioxidant activity produced. Increasing the concentration of maltodextrin which was

higher causes a decrease in the level of antioxidant activity. More total solids are present in the material, namely maltodextrin as a filler and skim milk as a source of protein, which causes measurably less antioxidant activity. The antioxidant content was also thought to be affected by the heating process, namely vitamin C and other oxidized phenol compounds. It was possible that warming causes the phenol compound to decompose so that its ability as an antioxidant had decreased (Yuliawaty & Wahono, 2015).

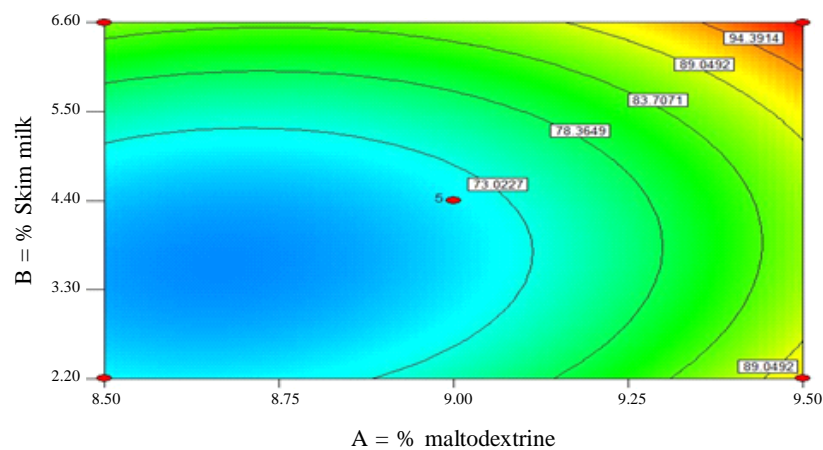


Figure 3. Contour plot response percentage of maltodextrin and percentage of skim milk to response LC_{50} value microencapsulation of green coffee extract

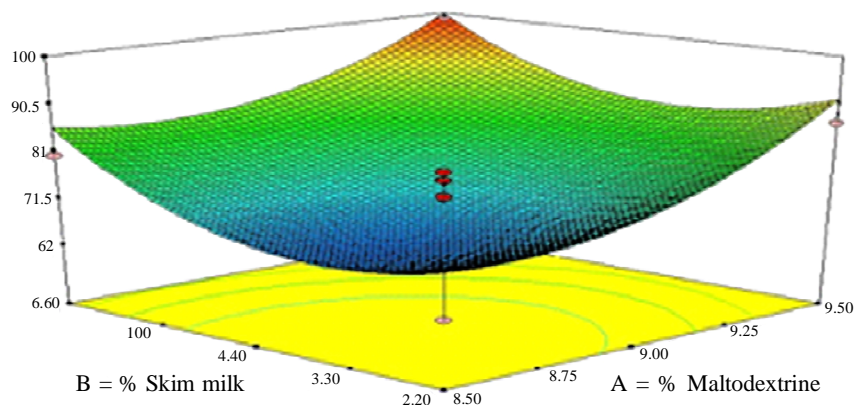


Figure 4. Response surface curve percentage of maltodextrin and percentage of skim milk to response LC_{50} value microencapsulation of green coffee extract

Optimum Solution for the Response

The study was conducted to determine the optimal solution results from the optimization of the percentage of maltodextrin and the percentage of skim milk in the microencapsulation process of green coffee extract by optimizing the total phenol and the antioxidant activity. Limitations of optimization for responses and factors can be seen in Table 3. Both responses were selected with maximum targets because the aim of the study was to obtain the highest total phenol yield and antioxidant activity from several treatment variations. Based on the limitations in Table 3, the optimum solution results obtained by expert design software 7.1.5 can be seen in Table 4. In addition to the optimal solution results predicted by the program, there is also an estimate of the lowest to highest value of the responses presented in Table 5. Through the table, the prediction value can be known for both responses.

The results of the optimization of total phenolic content response and antioxidant activity were also presented in the form of desirability which can be seen in Figure 5. According to Nurmiah *et al.* (2013), the

desirability value was the value of the objective optimization function that shows the ability of the program to fulfill desires based on the final predetermined criteria whose values range from 0 to 1. The closer to 1 the value of desirability shows the ability of the program to achieve the more perfect optimization goals.

Based on Figure 5 it was shown that the value of desirability was 0.926. The more outgoing the smaller the desirability value. The results of optimization carried out produce desirability values that are in the red contour, the highest contour. This shows that the program can achieve near-perfect optimization goals. Besides desirability, it was also shown by the response surface curve of the optimization results of total phenolic content response and antioxidant activity which can be seen in Figure 6.

The results of the calculation of green coffee microencapsulation powder samples can be seen in Table 6. The chlorogenic acid content of green coffee extracts was 1.19%. Chlorogenic acid percentage increase with increasing levels of caffeine. In the study of Urakova *et al.* (2008), determination of

Table 3. Optimization limits for responses and factors

Criteria	Name (Unit)	Target	Lower limit	Upper limit
Factor	Percentage of maltodextrin (% of total extract)	In range	8.50	9.50
Factor	Percentage of skim milk (% of total extract)	In range	2.20	6.60
Response	Total phenolic content (mg GAE/g)	Maximize	17.0274	61.0000
Response	Antioxidant activity(LC ₅₀ value in ppm)	LC ₅₀ -value Minimize	62.6899	100.0840

Table 4. Optimal solution results from design expert 7.1.5 software

Parameter	Prediction standard
Percentage of maltodextrin (%)	8.50
Percentage of skim milk (%)	2.32
Total phenolic content (mg GAE/g)	53.3343
LC ₅₀ value (ppm)	71.1029
Desirability	0.800
Information	Selected

Table 5. Prediction of the lowest to highest solution

Parameter	Prediction	SE Pred	Lowest prediction	Higher prediction
Total phenolic content (mg GAE/g)	53.3343	11.69	27.29	79.38
LC ₅₀ value (ppm)	71.1029	8.95	49.95	92.26

chlorogenic acid levels was carried out on unroasted coffee bean extracts (0.86%). Determination of chlorogenic acid levels in coffee beans is also done by Belay *et al.* (2008), obtained chlorogenic acid levels of 0.33% and 0.23%.

The appearance of microencapsulation of green coffee extract indicates a rift on the surface of the microcapsule caused by high temperature. This embezzlement can be caused by high temperature spray drying. This can lead to the loss of volatile components from within the microcapsules (Reineccius, 1988). Microcapsule particles in the control tend not to stick between the particles one with the others. This condition

is different from particles in microcapsules oleoresin ginger pulp that there is a lot of adhesion between particles one with the other particles.

Comparison with Other Products

Microencapsulation products that have been produced were then compared with similar products on the market. It aims to determine the quality and position of the products produced with products that were already known in the market. The parameters compared were total phenolic content, antioxidant activity, and solubility. The results of the comparison can be seen in Table 7.

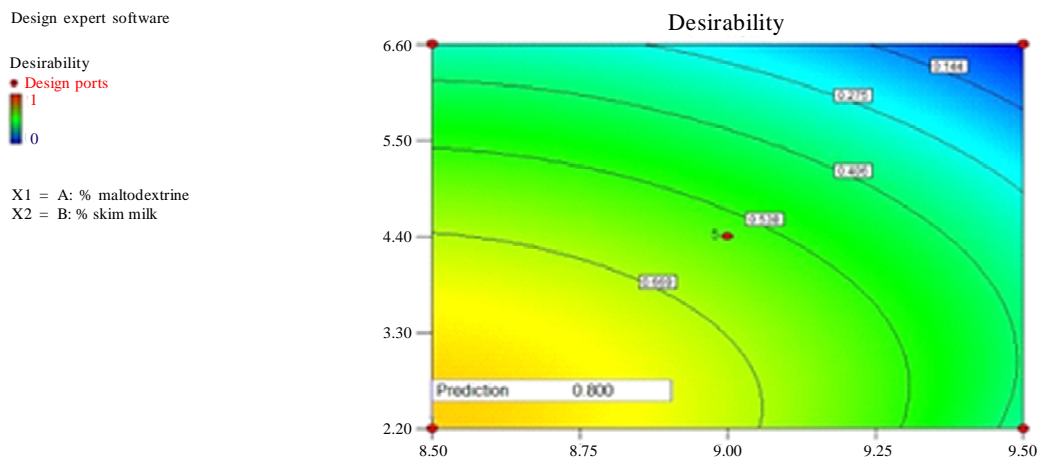


Figure 5. Desirability results of optimization of total phenolic content and antioxidant activity response

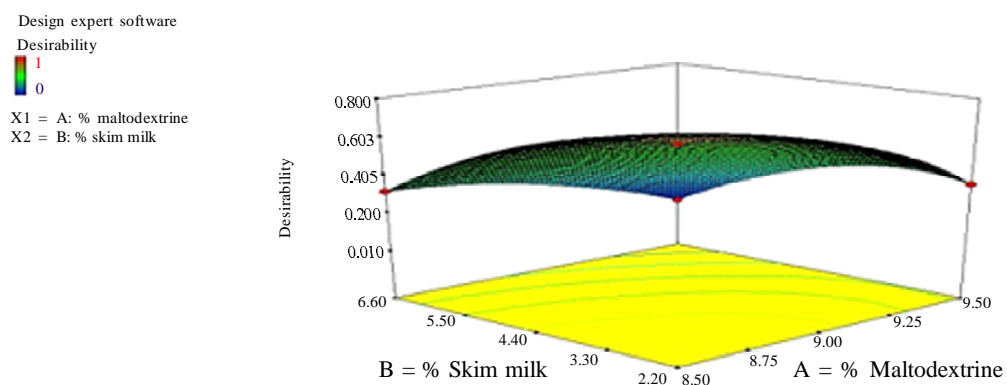


Figure 6. Response surface curve results optimization response of total phenolic content and antioxidant activity

Table 7. Product comparison

Parameter	Microencapsulation of green coffee extract	A comparative dietary supplement
Total phenol	53.33 mg GAE/g	46.32 mg GAE/g
LC ₅₀ value	71.10 ppm	87.65 ppm
Solubility	Soluble	Difficult to dissolve

Based on Table 7, it can be seen that the quality of the microencapsulated green coffee extract powder in this study has better quality than diet supplements that were already on the market. The lack of solubility in the comparative product is presumed to be due to the particle size of the capsule powder being less small or less smooth. The grinding process aims to reduce the size of the coffee making it easier for the coffee powder to dissolve in its solvent. Typically, water-soluble components were chlorogenic acid, caffeine, nicotinic acid, melanoidin compounds, and hydrophilic volatile compounds will be extracted more highly when using high temperatures and pressures. It was also thought to be due to other compositions in Exitox Greenco supplement namely *Garcinia cambogia*, *Garcinia mangostana* pericarpium, and *Guazuma ulmifolia*. The total phenol content and antioxidant activity which was lower than the microencapsulation product of green coffee extract by spray drying method was thought to be influenced by the type and polarity of the solvent at the time of extraction where the type and polarity of the solvent can affect the transfer of a single electron or transfer of hydrogen atoms which is a key aspect in testing antioxidant (Meitary, 2017).

CONCLUSIONS

The optimum conditions for green coffee extract microencapsulation process was predicted at 8.5% of maltodextrin and 2.32% of skim milk, producing a total phenol of 53.33 mg GAE/g and LC₅₀ value of 71.10 ppm. The comparative capsule had an LC₅₀ value

of 87.7 and a total phenol of 46.32 mg GAE/g. The quality of the green coffee extract microencapsulation product with spray drying method was better than diet supplements exist in the market.

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