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Optimization of coating ratio and homogenization conditions in the production of coconut shell liquid smoke nanoparticles

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Abstract

The liquid smoke in liquid form is considered less practical and susceptible to damage to phenolic compounds during storage. Liquid smoke encapsulation technology is expected to be able to overcome the problem of handling liquid smoke. This study aimed to determine the optimum conditions for the manufacture of liquid smoke nanoparticles in terms of the particle size distribution produced. The ratio of arabic gum and maltodextrin concentration, homogenization speed and homogenization time were optimized using the Box-Behnken Response Surface Methodology (RSM) experimental design. The response used in this study is the particle size distribution (z-average). Based on the results of the study, the optimum conditions for the manufacture of liquid smoke nanoparticles were obtained at a concentration ratio of gum arabic and maltodextrin 12:88, a homogenization speed of 4000 rpm and a homogenization time of 3 mins, with a particle size distribution (z-average) 78.53±1.48 nm.

1. Introduction

Liquid smoke is a mixture of solutions and colloidal dispersions of wood smoke vapour in water obtained from the pyrolysis of wood (Maga, 1987). According to Tranggono *et al*. (1996), liquid smoke originating from coconut shells has a chemical composition consisting of 5.13% phenol, 13.28% carbonyl and 11.39% acid. These compounds play a significant role in the formation of the desired food properties and have different functional properties. One of the compounds in liquid smoke that acts as an antioxidant and antimicrobial is a phenolic compound. However, handling liquid smoke in liquid form is considered less practical, especially with regard to distribution and transportation, because it requires a special container/place. In addition, the content of phenolic compounds in liquid smoke is easily damaged during storage. Therefore, it is necessary to develop technology to protect the active components and facilitate the handling of liquid smoke by making liquid smoke in the form of flour or powder through nanoencapsulation techniques.

In the nanoencapsulation process, it is necessary to pay attention to the selection of the right coating method and material. The type of coating material must be food grade and GRAS (Generally Recognized as Safe). One of the methods used to produce nanocapsules is spray drying. According to Young *et al*. (1993), the encapsulation process by spray drying must use a coating material with high solubility properties, forming a dry layer and low viscosity. One of the materials that have high solubility and low viscosity which is commonly used as an encapsulant is maltodextrin. However, the use of maltodextrin will produce an unfavourable wall structure because it can form agglomerations and cracks or ruptures in the walls of flour products which can trigger the release of active ingredients (Sansone *et al*., 2011). To increase the efficiency of encapsulation of phenolic compounds, in this study a combination of encapsulants using maltodextrin and gum arabic was used. According to Meer (1980) in Zuidam and Shimoni (2010), gum arabic is an excellent film-forming agent in absorbing encapsulated components. In addition, gum arabic contains arabinogalactan protein (AGP) and glycoprotein (GP) groups which act as effective emulsifiers and thickeners in protecting colloids, as well as preventing brittleness, wall cracking and leakage of active ingredients to protect the active components in liquid smoke.

Before drying with a spray dryer, liquid smoke was made in nanoparticles using a combination of gum arabic

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and maltodextrin coating. Although in general gum arabic can be used as a stabilizer in food matrices (Parnanto *et al*., 2018; Yudhistira *et al*., 2018) it can be used as an encapsulant. When making nanoparticles, it is essential to pay attention to the concentration of gum arabic and the process of adding gum arabic in liquid smoke. The process of adding gum arabic is carried out before maltodextrin to ensure there is an interaction between phenolic compounds in liquid smoke, and gum arabic can absorb phenolic compounds. Kanakdande *et al*. (2007) showed the results of observing the morphology profile of curcumin oleoresin microcapsules coated with a combination of maltodextrin encapsulants with a higher composition of gum arabic and modified starch, having a larger size than curcumin oleoresin microcapsules coated with a combination of arabic gum encapsulants with a higher ratio than maltodextrin. and modified starch. To ensure that the nanoparticles produced are nanometer in size, a homogenization process is carried out that considers the speed and length of time the homogenization takes place. Homogenization is a process that aims to uniform and reduce the size of the dispersed globules or particles. Based on this, the ratio of encapsulation concentration, speed and length of homogenization time are variables that need to be considered because they will affect the size distribution of the resulting particles. In this study, optimization was carried out with the Box-Behnken Response Surface Methodology (RSM) design.

2. Materials and methods

2.1 Materials

Research materials include coconut shell liquid smoke grade 1 obtained from PT Tropica Nucifera Industry, Depok, Sleman, Yogyakarta. The coating material used is maltodextrin combined with gum arabic as a co-encapsulant. Maltodextrin in small and fine white granules, having 10.8% DE and 6.67% moisture content, was obtained from Grain Processing Corp., Jowa USA. Gum arabic is the form of yellowish-white granules and has a water content of 13.45%.

2.2 Methods

This study consisted of five stages consisting of the characterization of the raw material (liquid smoke), determination of the zero points, determination of the optimum point, verification of optimum conditions and characterization of the optimized nanocapsules. The study consisted of three factors (variables), i.e. the ratio of arabic gum and maltodextrin concentrations, homogenization speed and homogenization time with the smallest particle size distribution response (z-average). Each factor consists of several levels, for the concentration ratio of arabic gum and maltodextrin consisting of 3 levels (8:92, 12:88, and 16:84), and the homogenization speed consists of 3 levels (4000 rpm, 5200 Rpm and 6400 rpm). In comparison, the homogenization time consists of 3 levels (1, 2, and 3 mins) (Table 1).

2.3 Liquid smoke characterization

The characterization of liquid smoke aims to determine the main components in liquid smoke, which used density, water content, total phenolic compounds, pH and total dissolved solids (Ali *et al*., 2014).

A pH-meter (Schott, Deutschland, Germany) was used to measure the pH of the nanoparticle solution at 26°C. Viscosity was determined by placing 250 mL of the nanoparticle solution in a cylindrical container with a volume of 300 mL, then measured using a Brookfield RVT Type (Middleboro, USA) viscometer at 25°C, spindle No. 1 at 100 rpm for 30 s. Total dissolved solids were measured using a hand-held refractometer N1 size (Atago, Tokyo, Japan) at 20°C, and expressed as degrees Brix (°Brix).

Total phenol analysis was carried out based on the method of Senter *et al*. (1989) with modifications. A total of 1 mL of liquid smoke was weighed or 1 g of liquid smoke nanocapsules were diluted to 25 mL, 1 mL was taken and diluted again to 10 mL (dilution factor = 250×), taken 2.5 mL and diluted again to 10 mL (dilution factor = 1000×). About 1 mL of the last dilution was put in a test tube, then 1 mL of saturated Na_2CO_3 (Merck, Germany) was added and left for 10 mins at room temperature. Then, 0.5 mL of Folinciocalteau reagent (Merck, Germany) and 7.5 mL of distilled water were added, homogenized with a vortex and then left for 30 mins at room temperature. The sample was measured at a wavelength of 770 nm. The phenol content of the sample was calculated based on the obtained standard curve equation.

Measurement of water content was done using the AOAC method (2005), Briefly, 1 g of sample (a) was put

Table 1. Codes and variables of experimental design

Treatment	Unit	Code	-1	0	1
Gum arabic and maltodextrin ratio (X_1)	%	A	8:92	12:88	16:84
Homogenization Speed (X_2)	rpm	B	4000	5200	6400
Homogenization time (X_3)	min	C	1	2	3

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into a weighing bottle which had been dried in an oven at a temperature of 105°C and the constant weight was known. The sample was dried at 105°C for 10 hrs and then weighed. The sample was dried again and then weighed every 2 hrs until the weight was constant (the difference in sample weight at each weighing was less than 0.2 mg). The water content is calculated using the formula:

$$Water\ content = \frac{(a-b)}{b} \times 100\%$$

The dispersion of nanocapsules in distilled water was measured using a laser particle size analyzer (Malvern Zetasizer Nanoseries Nano ZS ver 6.20, Malvern Instruments Ltd, Malvern, UK) using the Dynamic Light Scattering method. The particle size distribution is determined by the span value. Measurements were carried out three times.

2.4 Nanoparticle production

Determination of the zero point begins with the manufacture of liquid smoke nanoparticles at various concentration ratios of gum arabic and maltodextrin with a homogenization treatment of 4000 rpm for 2 mins. For each combination of gum arabic and maltodextrin concentrations, the total amount of solids in liquid smoke nanoparticles is 11% (w/v) of the total liquid smoke used or 25 g of encapsulation in 225 mL of liquid smoke. The analysis carried out is the particle size distribution (z-average) using a particle size analyzer. Furthermore, the manufacture of nanoparticles with a concentration ratio of gum arabic and maltodextrin used a homogenization speed of 4000 within 2 mins based on the method of referring to Saloko *et al*. (2012).

2.5 Process optimization

The analysis of liquid smoke nanoparticles with the Box-Behnken design included z-average, total dissolved solids and observations of phenolic compounds. Verification is an act of checking to cross-checking the results of the analysis of the concentration ratio of gum arabic and maltodextrin and the homogenization process (speed and time) which gives the optimum response in terms of the particle size distribution (z-average). However, to determine the optimum point, the response used is z-average only. By using RSM software, the ratio of arabic gum and maltodextrin concentrations will be obtained as well as the homogenization process (speed and time) which produces an optimal response (Table 2). After the optimization process, verification of the optimum point is carried out, which aims to prove the optimum point solution of each independent variable based on the design expert 7 (DX7) program. The actual result is compared with the predicted value based on the solution provided by the program. The optimization process and construction of predictive response curves are in 3 dimensions. The significant test of the response variable was carried out at $P<0.05$. Prediction is declared successful if the verification results are close to or right from the predicted value.

3. Results and discussion

3.1 Liquid smoke characteristics

In the encapsulation process, in addition to the required coating material, the main material is also needed to be encapsulated and protected from environmental influences. The main ingredient used in this research is liquid smoke grade 1, which contains the main components of total phenol, carbonyl and acid,

Table 2. The treatment design was based on the Central Composite Design (CCD) construction for the variables of gum arabic and maltodextrin ratio, Homogenization Speed, and Homogenization time

Run	Gum arabic and maltodextrin ratio (X_1)	Homogenization Speed (X_2)	Homogenization time (X_3)
1	1	0	1
2	0	1	1
3	1	-1	0
4	0	-1	1
5	-1	1	0
6	0	0	0
7	1	1	0
8	-1	-1	0
9	-1	0	-1
10	0	0	0
11	-1	0	1
12	0	0	0
13	0	-1	-1
14	0	1	-1
15	1	0	-1

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each of which has an important role in food product preservation, antioxidant, antibacterial, flavour enhancement, flavour and aroma of smoked products. The characteristics of liquid smoke can be seen in Table 3. The water content in the young coir coconut is free water content, so it will quickly evaporate when pyrolysis. In the pyrolysis process, the water content contained in the coconut fibre will follow evaporates at 100°C and experience condensation when water vapour passes through the condenser, thereby increasing the amount of smoke that condensate the resulting liquid (Pamori *et al*., 2015).

Table 3. Characteristics of coconut shell liquid smoke

Parameter	Value
Water content (%wb)	90.13±1.98
Total Soluble Solid (°Bx)	5.00±0.09
pH	2.54±0.02
Fenolic compound (%wb)	2.96±0.05
Density (g/m^3)	0.98±0.01

The pH values in liquid smoke are indicated to know the level of raw materials decomposition process by pyrolysis (Haji *et al*., 2012). According to Yuningsih (2014), the pH of liquid smoke is also influenced by the total phenol content, especially by the aromatic ring and because phenol is able to release H^+ ions from its OH group. Liquid smoke with a low pH has good quality in terms of preserving food products, especially durability and shelf life. In this study, the pH of liquid smoke was 2.54±0.02, which has almost the same value as the study by Amperawati *et al*. (2012) with a pH value ranging from 2.91. The analysis indicates that liquid smoke has acidic properties. This acidic nature comes from the components of liquid smoke, especially acetic acid and other types of acids Liquid smoke with a pH of 1.5-3.7 causes microbes to be unable to live and grow, it can then be used as a natural food biopreservative material. Phenol content and total acid titrated formed from the components of cellulose, hemicellulose and lignin contained in young coconut coir, and undergo decomposition to produce acid, phenol and carbonyl compounds. When the pyrolysis of cellulose takes place it will form acetic acid and its homologues together with water and a small amount of furans and phenols (Girard, 1992), as a result, the acid and phenolic compounds contained in liquid smoke from the coconut coir pyrolysis process young people are different, this is because the level of raw material for young coconut coir used varies (Pamori *et al*., 2015).

In this study, the main component analyzed was total phenol, which showed levels of 2.96±0.05% (wb). Phenolic compounds are components that act as antioxidants and antimicrobials. As antioxidants, phenolic compounds can stabilize free radicals by complementing the lack of electrons possessed by free radicals and inhibit chain reactions from the formation of free radicals. These phenols have -OH bonds that can function as hydrogen (H) donors. The amount of yield produced is influenced by the type of material and the level of water in the material. This shows that the higher the water content of young coconut coir material the value of the resulting specific gravity is getting lower (Pamori *et al*., 2015). Girard (1992), mentions that the composition of chemical substances that are contained in liquid smoke can depend on several factors, such as water content from the material, temperature and duration of burning, type of wood or raw materials that are used, condensation temperature and purification type that used. The quantity and quality of phenol compound that can be found in liquid smoke is related to lignin content and pyrolysis temperature. Phenol is the result of wood component disintegration, which is lignin, the more lignin content in the wood, then the greater of phenols content in liquid smoke. Furthermore, lignin degradation occurs at temperatures of 310 – 500°C, if the temperature has not been reached then it will influence lignin degradation and phenol content from liquid smoke that is produced (Demirbas, 2005).

3.2 Process optimization

Determination of the zero points begins with the manufacture of nanoparticles using the concentration ratio of the combination of gum arabic and maltodextrin with various variations in the homogenization treatment of 4000 Rpm for 2 mins. The use of a homogenization speed of 4000 Rpm is based on the research of Darmadji *et al*. (2012). Based on Figure 1, all the resulting particles are nanometers (10^{-9}m) in size. The smallest value is in the nanoparticles with the composition ratio of the concentration ratio of gum arabic and maltodextrin 12:88. Figure 1 shows that with the increase in the amount of gum arabic used in the manufacture of nanoparticles, the z-average also increases. It is assumed that the viscosity increased in proportion to the increase in the concentration of gum arabic. The minor particle size distribution is used as a parameter because the smaller the size of a particle, the larger its surface area. Thus, it is expected that the nanocapsules formed can have high solubility, increase bioavailability and control release. The zero point was obtained in the treatment of the concentration ratio of gum arabic and maltodextrin 12:88. The concentration ratio is used in determining the zero point with variations in the speed of homogenization (4000, 5200 and 6400 rpm) within 2 mins (Figure 2). The size of nano is the size of a component that is at intervals of 1-100 nm (Reis *et al*., 2006).

Based on Figure 2 the smallest z-average value is in

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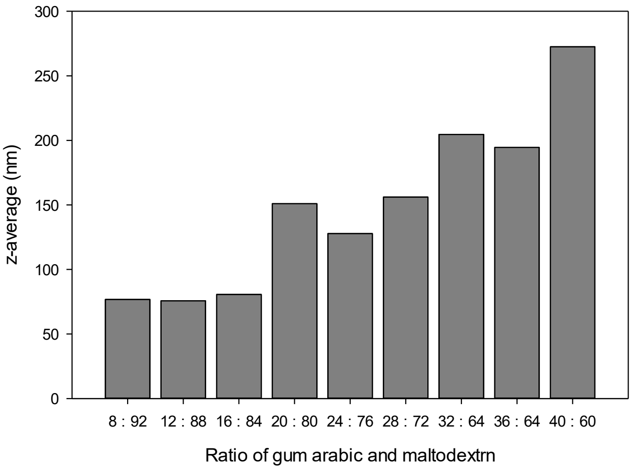

Figure 1. Effect of the ratio of the concentration of gum arabic and maltodextrin on z-average

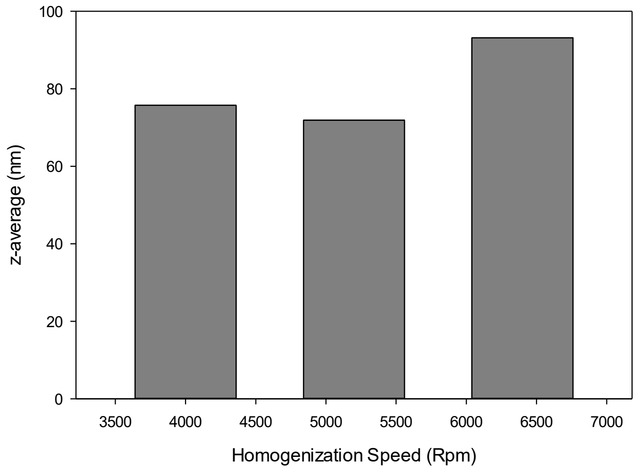

Figure 2. Effect of homogenization speed on z-average

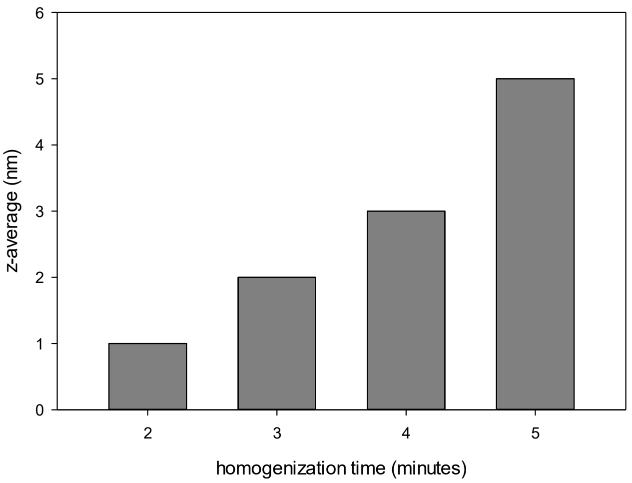

Figure 3. Effect of homogenization time on z-average

the 5200 rpm homogenization treatment. Homogenization is a process that aims to homogenize and reduce the size of the dispersed globules or particles, due to the homogenizer being able to break up intermolecular reactions. The zero point was obtained in the homogenization treatment of 5200 rpm. The speed of homogenization is used in determining the zero point with variations in homogenization time (1, 3, 4 and 5 mins). The research of Ningsih *et al*. (2017) showed that the increase in chitosan caused the particle size to increase as well. Based on Figure 3, the smallest particle size distribution value is obtained at 2 mins of homogenization time and it can be seen that when the homogenization time is longer, the particle size increases. It is suspected that the longer the homogenization, the more agglomeration will occur which causes large particles. Darmadji *et al*. (2012) conducted a study on the manufacture of coconut shell liquid smoke nanoparticles using chitosan and maltodextrin encapsulants with homogenization using an ultra-thurax homogenizer at a speed of 4000 Rpm for 2.5 mins capable of producing nanometer-sized particles. The average particle size formed from chitosan (0.5% w/v) and maltodextrin (9.5% w/v) in acetic acid was 16.21 nm. The same thing was done by Ali (2014) in the manufacture of liquid smoke nanoparticles with chitosan (0.12% w/v) and maltodextrin (0.88% w/v) encapsulation at a homogenization speed of 4000 rpm but with a different time of 2 mins. resulting in a particle size distribution of 29.16 nm. Meanwhile, Saloko (2014) encapsulated liquid smoke using chitosan and maltodextrin using different homogenization speeds and homogenization times. With a homogenization speed of 4000 rpm for 2.5 mins, smaller particle sizes were produced than at a homogenization speed of 5200 rpm for 5 mins. Therefore, in this study, various variations of the speed and time of homogenization were carried out to obtain encapsulated particles in nanometer-sized liquid smoke dispersions.

3.3 Optimum condition verification

The results showed that the optimum condition of z-average was the concentration ratio of gum arabic and maltodextrin 12:88, homogenization of 4000 rpm for 3 mins with a z-average value of 77.95±0.89 nm (Table 4). Meanwhile, based on RSM analysis, the optimum z-average condition was obtained at the concentration ratio of gum arabic and maltodextrin 12:88, homogenization of 4000 rpm for 3 mins which resulted in a value of 75.24 nm. The optimum response can be described visually through the response surface plot and the contour plot. Through the plot, it can be seen the form of the relationship between the response and the independent variable. The plot contour is a visual representation of the z-average surface shape as a response and change in the independent variables, in this case, the ratio of arabic gum and maltodextrin concentrations, homogenization speed and homogenization time. Suppose the contour plot of the interaction of the three variables on the response has the

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Table 4 Particle size distribution, PdI and TSS coconut shell liquid smoke nanoparticles

Sample code	Particle size (nm)	PdI	Total Soluble Solid (°Bx)
1	120.77±8.48	1.00±0.03	13.27±0.08
2	146.17±4.09	1.00±0.04	13.00±0.03
3	118.97±2.59	1.00±0.04	13.00±0.01
4	77.95±0.89	1.00±0.03	13.40±0.08
5	269.50±7.14	0.59±0.03	13.20±0.08
6	121.37±4.20	0.93±0.15	13.20±0.08
7	127.97±3.38	1.00±0.02	13.40±0.00
8	129.28±1.14	0.85±0.16	13.20±0.01
9	87.25±4.53	1.00±0.01	13.07±0.10
10	117.50±1.37	1.00 ±0. 02	13.20 ±0.03
11	116.72±9.17	1.00±0.04	13.20±0.08
12	120.10±3.47	0.92±0.16	13.40±0.04
13	161.92±9.73	0.89±0.49	13.00±0.08
14	102.72±5.55	1.00±0.01	13.13±0.10
15	138.07±13.09	1.00±0.01	13.20±0.08

optimum point in the middle. In that case, it means that the optimization result model can correctly describe the relationship between the variables and the response. Based on Figure 4, it can be seen that in the contour drawing, all variations that affect the z-average value have 1 central point. Based on ANOVA in the DX-7 program obtained a linear equation with the iteration of the z-average (Y) with the ratio of arabic gum and maltodextrin concentration (X_1), homogenization speed (X_2) and homogenization time (X_3) as follows:

$$Y = -132.36 + 153.42X_1 + 0.04X_2 - 106.50X_3 - 0.02X_1X_2 - 11.69X_1X_3 + 0.02X_2X_3$$

The value of the equation has a mixed sign, namely negative and positive. This indicates that the resulting graph is in the form of a saddle, as shown in Figure 4. Based on the study results, it was also found that all the results of the analysis of the particle size distribution (z-average) showed values on the nanometer scale. This means that the optimization process that has been carried out can produce nanometer-sized liquid smoke particles.

The last stage after the optimization process is to verify the optimum point, which aims to prove the optimum point solution for each independent variable based on the design expert 7 (DX7) program. The actual result is compared with the predicted value based on the solution provided by the program. Prediction is declared successful if the verification results are close to or right from the predicted value (optimization). As previously explained, based on the RSM program, the optimum particle size distribution results were treated with a concentration ratio of gum arabic and maltodextrin 12:88, homogenization speed of 4000 rpm within 3 mins which resulted in a z-average value of 75.24 nm. Meanwhile, based on the verification process, the z-average value is 76.9 nm, 79.8 nm and 78.9 nm, or if the average results in a z-average value of 78.53±1.48 nm. Thus, indicating that the verification results are close to the predicted value from RSM, the optimization results are declared successful.

Measurement of the particle size distribution (z-average) aims to determine the size distribution of liquid smoke nanoparticles using a particle size analyzer. In addition, to determine whether the optimized encapsulation process can produce nanometer-sized particles (10-9 m). Based on Figure 4 from one of the replicated analyses of the verification results that have been carried out, shows that the nanoparticle size is distributed starting from 789.1 nm (86%), 12.53 nm (7.4% intensity) to 46.53 nm (6.6% intensity) with a final z-average of 79.8 nm. The mechanism for the formation of nanometer-sized particles that can encapsulate the active compound (in this case, phenolic compounds) is the stirring of liquid smoke, which is added first with gum arabic and then maltodextrin, which can provide strength in absorbing phenol. It is suspected that the H group in gum arabic will bind to the O group in liquid smoke phenolic compounds through hydrogen bonds. As a result, phenolic compounds will be adsorbed in gum arabic and covered by maltodextrin on the outside. With the homogenization process, the liquid smoke particles that have bonded with gum arabic and maltodextrin become uniform and small (nanometers).

The use of core material in the encapsulation of active compounds can affect their protective properties and release rates (Yudhistira *et al*., 2022). In addition, polydipersity index (PdI) determines the level of uniformity or homogeneity of a particle. If the PdI value is below 0.5, it indicates a good level of homogeneity, but if the PdI value is above 0.5, it is said to be heterogeneous (Onwulata, 2005). The optimized nanoparticles (concentration ratio of gum arabic and maltodextrin 12:88, homogenization speed of 4000 Rpm for 3 mins) had a Pdl value of 0.906±0.16 so it can be said that the level of homogeneity or uniformity was uneven. The variety of particle sizes can be caused by agglomeration which results in large particles (Liu *et al*., 2011). The research of Ningsih *et al*. (2017) showed that the increase in chitosan caused the particle size to decrease as well.

4. Conclusion

The optimum condition that produces the smallest particle size distribution (z-average) of 78.53±1.48 nm is the ratio of the concentration of gum arabic and maltodextrin 12:88 with a homogenization speed of 4000 rpm for 3 mins.

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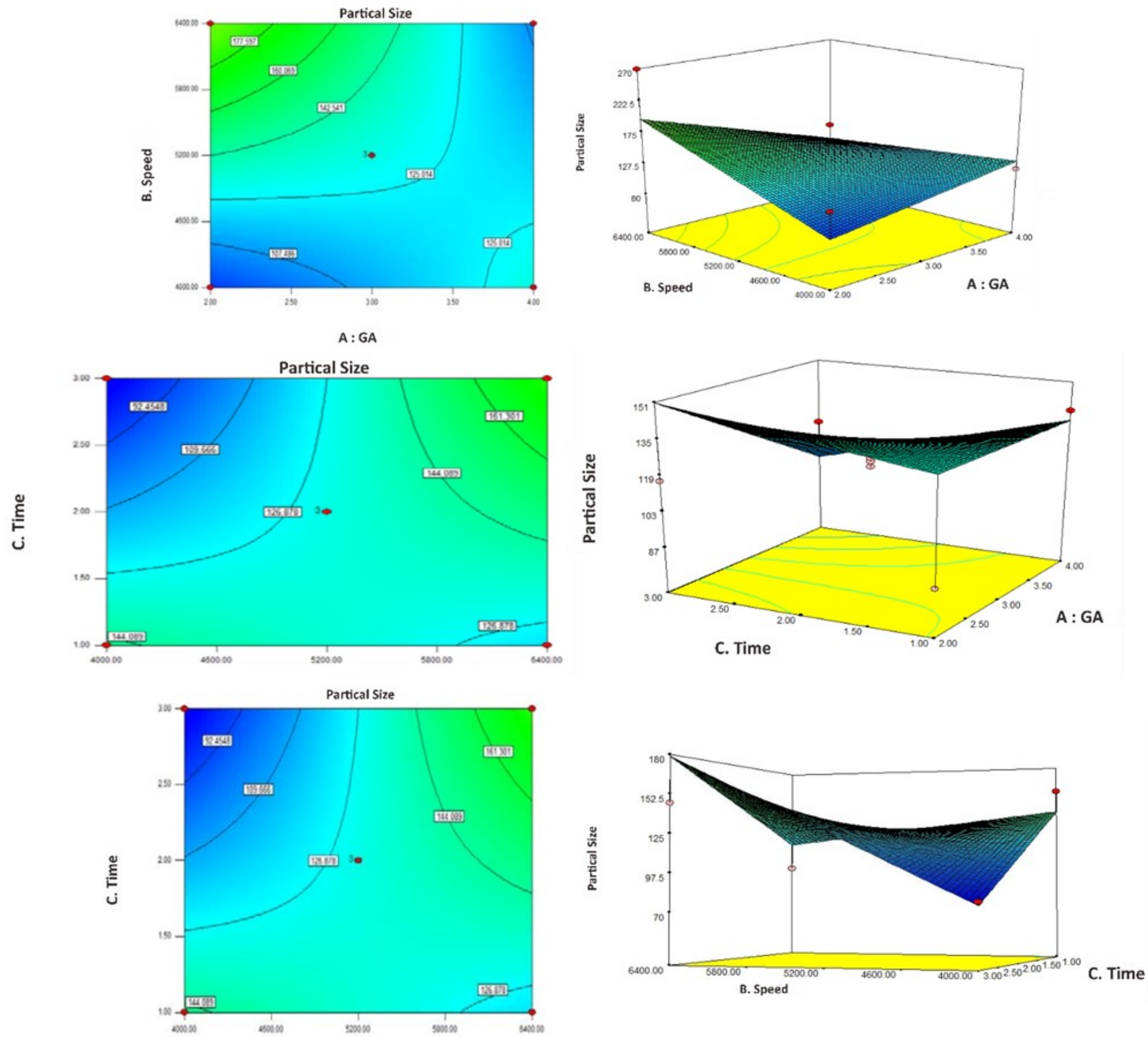

Figure 4. (a) The effect of gum arabic concentration ratio and homogenization speed, (b) The effect of concentration ratio of gum arabic and malto and homogenization time, (c) The effect of homogenization speed and homogenization time

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