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ORIGINAL ARTICLE

Optimization of encapsulated agents and stirring speed on the physicochemical characteristics of vacuum dried nutmeg seed oleoresin (*Myristica fragrans*)

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Abstract

Nutmeg seed oleoresin (*Myristica fragrans* Houtt) from nutmeg seed extraction contains active substances. However, oleoresins' active substances are commonly heat sensitive, so encapsulation is needed. Encapsulation is the process of wrapping particles containing active ingredients in a homogeneous or heterogeneous matrix that produces encapsulated powder. The objective of this study was to obtain the best combination of encapsulated agents' concentration (maltodextrin and whey protein isolate) and agitation speed on the physicochemical characteristics of nutmeg seed oleoresin encapsulated using a vacuum drying method. Encapsulation of nutmeg seed oleoresin was performed with comparative parameters, namely agitation speed (3000, 3500, 4000 rpm), maltodextrin (MD) concentrations (ratio of MD to nutmeg seed oleoresin = 2:4, 4:4, 6:4), and whey protein isolate (WPI) concentrations (ratio of WPI to nutmeg oleoresin = 6:4, 4:4, and 2:4). The physicochemical analysis consisted of trapped oil content, antioxidant activity, yields, water content, surface oil, water activity, and color testing. The physicochemical data were further analyzed by response surface methodology (RSM) to get an optimum formula. The best formula resulted from a process at an agitation speed of 3500 rpm and the addition of 4 g maltodextrin and 4 g WPI. That formula had a trapped oil content 10.23%, antioxidant activity 91.50%, yield 66.79%, water activity 0.55, moisture content 8.63, and color intensity L^* 65.47, a^* 7.90, and b^* 19.57. This formula could be applied to produce nutmeg seed oleoresin powder with good physicochemical properties.

Practical applications

Encapsulation of nutmeg seed oleoresin by vacuum drying produced a more stable powder with longer shelf life compared to those in the form of nutmeg seed oleoresin. The viscous liquid of nutmeg seed oleoresin is prone to oxidation and degradation during storage. This encapsulated nutmeg seed oleoresin powder can be used as a food ingredient for different applications, that is, beverage, confectionery, bakery products, and soup seasonings.

1 | INTRODUCTION

Nutmeg (*Myristica fragrans* Houtt) is one of the main crop commodities in Indonesia, originating from Banda Island, Maluku. Indonesian nutmeg production continuously increases every year in parallel with increasing nutmeg exports each year. Nutmeg exports by Indonesia can supply up to 60% of the world's nutmeg demands. Currently, nutmeg is exported in the form of seeds and mace nutmeg, either as "simplisia" or powder. The selling value of nutmeg seeds could be improved by processing the raw products into nutmeg oleoresin with higher added value.

Nutmeg oleoresin (*Myristica fragrans* Houtt), as the result of fresh nutmeg seed extraction with ethanol, contains active substances. Nowadays, the utilization of nutmeg oleoresin as a flavoring agent is preferable by the food industry compared to fresh herbs because of its stability and highly concentrated form. In addition, oleoresin also has a homogenous flavor, aroma, pungency, standardized quality, and longer shelf life (Khadka, 2018). However, oleoresin is prone to oxidation in the presence of air, light, and water. Therefore, the encapsulation process is carried out to create a barrier between the active substances and other external factors (Jafari, 2017).

The most common coating materials for encapsulation are maltodextrin and Arabic gum (Lantigua et al., 2011). Based on research by Zilberboim et al. (1986), the bell pepper oleoresin encapsulated with Arabic gum was considered an expensive and non-feasible coating ingredient. Research by Nurlaili and Darmadji (2014) reported that the microencapsulation of pulp ginger oleoresin with maltodextrin could reach an encapsulation efficiency of up to 22%. Therefore, maltodextrin is preferably used as a coating agent because of its affordable price, neutral taste and aroma, water-soluble and film-forming properties, low viscosity at high solids concentrations, and is less prone to oxidation (de Barros Fernandes et al., 2014). The disadvantage of using maltodextrin is unstable emulsion stability to trap oleoresin. In this case, emulsifier addition could help to obtain a better coating performance. Whey protein isolate (WPI) is considered a suitable emulsifier in the food system. Principally, WPI will be absorbed in the interface of oil-in-water (o/w) droplets and forms a layer that can protect droplets from coalescence (Assagaf et al., 2013). The agitation speed of the homogenizer could affect the droplet size. The higher the agitation speed, the smaller the size of the oil droplet. Research by Jayanudin et al. (2018) reported that the higher agitation speed would increase the Reynolds number (Re) and reduce the emulsion droplet size.

Based on the explanation above, it is necessary to optimize the encapsulation of nutmeg oleoresin with different agitation speeds and ratios of maltodextrin and WPI.

Processing data by applying response surfaces methodology (RSM) made this research important by analyzing the optimum points and illustrating them in a three-dimensional graph.

2 | MATERIALS AND METHODS

2.1 | Materials

The extraction materials were nutmeg (*Myristica fragrans*), ethanol 96% solvent, and Whatman filter paper number 1. The encapsulation materials were extracted nutmeg oleoresin, maltodextrin (DE 15–20), whey protein isolate (WPI) 90, and distilled water; while materials for analysis were diphenyl picryl hydrazyl (DPPH) solution 0.06 mM, methanol 99.98%, ethanol 96%, and filter paper.

2.2 | Nutmeg oleoresin extraction with ultrasound-assisted extraction (UAE)

First, fresh nutmeg seeds were dried in the oven at 45°C for 24 h. The dried nutmeg seeds were cut and ground before sieving with 36 mesh size. Nutmeg powder was dissolved with ethanol 96% by a ratio of 1:10. The extraction was carried out with an Erlenmeyer containing a sample, soaked in the ultrasonic cleaner UC-10SD at 50°C and 45 kHz frequency for 37.5 min. After that, the mixture was stored at chiller ±4°C for 30 min for fat phase separation (Assagaf et al., 2012), then filtered. The solvent in the filtrate was evaporated with a rotary vacuum evaporator (40°C, speed 52 rpm, and pressure 0.09 MPa) until all solvent evaporated and a thick nutmeg oleoresin was obtained (Trendafilova et al., 2010 modified). Oleoresin was kept in a glass bottle laminated with aluminum foil and stored in a chiller.

2.3 | Response surface methodology (RSM)

The formula determination was generated from the Statistica 6.0 Response Surface Methodology (RSM) software as presented in Table 1 and produced 17 treatments for oleoresin encapsulation. The range of agitation speed was set between 2700 and 4000 rpm, and 0.64 and 7.36 g for maltodextrin and WPI. RSM generated three levels of oleoresin and total coatings materials (MD and WPI) ratio. The ratio of 1:1 was applied on treatments 3, 7, 12 and 13; ratio of 1:2 was applied on treatments 1, 4, 5, 8, 9, 10, 15, 16, and 17; and ratio of 1:3 was applied on treatments 2, 6, 11, and 14. RSM with factorial design, namely the central composite design (CCD), could simplify the number of experiments and be useful for testing multiple process variables. The CCD design is a 2^k factorial design or called partial factorial. It is expanded by adding observation points at the center, so the predicted parameter coefficients will be on the quadratic surface (second order) (Montgomery, 2001 in Lubis, 2010). Generally, CCD consists of a factorial point (2^k), an axial point (2 k), and a center point (nc); where k is the variable number. The 2^k factorial design is used for experiments consisting of k factorial, where the low level is coded as (−1), the middle level as (0), the high level as (+1), and the minimum and maximum level at the axial point as (−α) and (+α). The calculation of the α value on the rotatable design CCD is as follows:

TABLE 1 Composition of materials in the process of nutmeg seed oleoresin encapsulate analyzed with RSM

Treatment	Agitation speed (rpm)	Oleoresin (g)	Coatings		Distilled water (ml)
			MD (g)	WPI (g)	
1 (F)	3000	4	6	2	16
2 (F)	3000	4	6	6	16
3 (F)	3000	4	2	2	16
4 (F)	3000	4	2	6	16
5 (F)	4000	4	6	2	16
6 (F)	4000	4	6	6	16
7 (F)	4000	4	2	2	16
8 (F)	4000	4	2	6	16
9 (A)	2700	4	4	4	16
10 (A)	4300	4	4	4	16
11 (A)	3500	4	7.36	4	16
12 (A)	3500	4	0.64	4	16
13 (A)	3500	4	4	0.64	16
14 (A)	3500	4	4	7.36	16
15 (C)	3500	4	4	4	16
16 (C)	3500	4	4	4	16
17 (C)	3500	4	4	4	16

α = [number of runs factorial point] $^{1/4} = (2^k)^{¼}$.

In this study, three variables were used, so $\alpha = (2^3)^{1/4} = 1.682$. The magnitude of the variable with codes $-\alpha$ and $+\alpha$ could be calculated using the equation below:

$$-\alpha = (0) - 1.682\left[(0) - (-1)\right] \parallel +\alpha = (0) + 1.682\left[(0) - (-1)\right]$$

RPM: $-\alpha = (3500) - 1.682\left[(3500) - (3000)\right] = 2659$

$+\alpha = (3500) + 1.682\left[(3500) - (3000)\right] = 4341$

MD: $-\alpha = (4) - 1.682\left[(4) - (2)\right] = 0.636$

$+\alpha = (4) + 1.682\left[(4) - (2)\right] = 7.364$

WPI: $-\alpha = (4) - 1.682\left[(4) - (2)\right] = 0.636$

$+\alpha = (4) + 1.682\left[(4) - (2)\right] = 7.364$

Therefore, each factor would have five levels of test points. The results of CCD analysis are presented in the form of graphs based on mathematical models and response surfaces. Those outputs are useful to predict the optimal value from the responses and to provide information on the interaction between the dependent and independent variables (Yousefi et al., 2016).

2.4 | Encapsulation of nutmeg oleoresin

Maltodextrin (MD) and whey protein isolate (WPI) were prepared and weighed. The suspension was made by adding distilled water to the MD and WPI mixture followed by agitation with a rotor-stator homogenizer at a particular speed for 15 min. Subsequently, 4 g of oleoresin was added to MD-WPI suspension. The mixture was homogenized at a particular speed for 10 min. Then, the mixture was poured into a glass pan to form a thin layer and dried using a vacuum oven at 50°C and 0.5 atm.

2.5 | Analysis of encapsulated nutmeg seed oleoresin

Physicochemical analyses of encapsulated oleoresins were trapped oil content, antioxidant activity, yield, water content, surface oil, water activity, and color intensity.

2.5.1 | Trapped oil content

One gram of encapsulated sample was placed in an Erlenmeyer, dissolved in 20 ml of ethanol 96%, and covered with aluminum foil. The sample was extracted by an ultrasonicator instrument at 50°C and 45 kHz frequency for 45 min. Filtration was carried out to separate the insoluble polymer fragments. The filtrate was transferred into an empty porcelain cup of known weight and then put in an oven at 45°C for 24 h. The measurement results were recorded as the final weight of the cup (Asyhari, 2013; Nugraheni et al., 2015). The total trapped oil yield was calculated by using the following formula:

$$\text{Total oil}\,(\%) = \frac{\text{Final weight of cup}\,(\text{g}) - \text{Empty cup weight}\,(\text{g})}{\text{Weight of sample}\,(1\,\text{g})} \times 100\,\%$$

$$\text{Trapped oil}\,(\%) = \text{Total oil}\,(\%) - \text{Surface oil}\,(\%)$$

2.5.2 | Antioxidant activity analysis

Approximately 0.5 g of encapsulated sample was weighed, then dissolved in 5 ml of ethanol 96%, and left for 2 h. After that, 0.1 ml of liquid was taken and dissolved with 3.9 ml of DPPH solution in a test tube and homogenized. The test tube with the sample was incubated in a dark room at 25°C for 30 min. After that, the sample absorbance was measured with a spectrophotometer at $\lambda = 517$ nm. The blank sample (control) was made by replacing the sample with 0.1 ml of ethanol (Amin et al., 2013; Hussein et al., 2017). Antioxidant activity was calculated as % inhibition using the formula below:

$$\text{Antioxidant activity}\,(\%) = \left[\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}}\right] \times 100\%$$

2.5.3 | Yield calculation

The yield was calculated based on the weight of the encapsulated powder produced from vacuum drying compared to the total solids of the emulsion material (encapsulating material and oleoresin) (Yuniarti et al., 2013). The yield content (dry basis) was determined by the following formula:

$$\%\,\text{Yield} = \frac{\text{Weight of microcapsule powder(g)}}{\text{Weight of emulsion solids (g)}} \times 100\,\%$$

2.5.4 | Moisture analysis

The moisture content of the sample was tested by using a moisture analyzer (Lindani, 2016). Half until One gram of sample was placed into the tool. The instrument will heat the sample until the value of the water content is shown constantly (approximately for 10 min).

2.5.5 | Surface oil analysis

One gram of encapsulated sample was put into a centrifuge tube and 5 ml of ethanol 96% was added. The mixture was centrifuged at 1700 rpm for 15 min. After that, the sample was filtered through filter paper and washed with 7.5 ml of ethanol twice. The filtrate was transferred in a cup of known weight, then dried in an oven for 24 h. After that, the sample was put in a desiccator for 15 min and weighed as the final weight (Hussein et al., 2017 and Yazicioglu et al., 2015 with modification). The amount of surface oil was calculated by using the formula below:

$$\text{Surface oil}\,(\%) = \frac{\text{Cup final weight (g)} - \text{Empty cup weight (g)}}{\text{Sample weight (1 g)}} \times 100\,\%$$

2.5.6 | Water activity (a_w) analysis

Water activity was measured by using an a_w meter. First, a homogenized sample was put into a clean and dry container cup, completely covering the bottom of the cup. The container was filled with samples until half a cup. The sample was measured with an a_w meter for 15 min and the result appeared on display (reader) (AquaLab, 2016).

2.5.7 | Color measurement

Color testing on encapsulating was carried out using Chroma Meter Minolta CR400. After instrument calibration, the encapsulated sample was placed in transparent plastic and a chroma meter beam was released. The measurement showed the values of L^*, a^*, and b^*. The value of L^* (lightness) of 100 indicates a light colored sample. The value of a^* indicates the tendency of red (+) and green (−). The b^* value indicates yellow (+) and blue (−) (Nguyen et al., 2018 with modification).

3 | RESULTS AND DISCUSSION

3.1 | Nutmeg seed oleoresin

Nutmeg seed oleoresin was processed by extraction using ethanol to dissolve the polar substances in nutmeg powder. The ethanol solvent was chosen because of its polarity by the presence of the -OH group to dissolve polar molecules. Oleoresin is a polar substance, while nutmeg butter is a non-polar substance. In addition, ethanol has a low boiling point at 78.5°C and 1 atm to easily remove the solvent from the extract (Joshi & Adhikari, 2019).

3.2 | Encapsulation of nutmeg seed oleoresin

The encapsulation process aimed to protect the active substance from oxidation by air and light, thereby increasing the shelf life of the product. The emulsification applied various levels of agitation speed by using a homogenizer. Homogenizer could reduce the oil globules' size and stabilize the emulsion by preventing coalescence. The agitation speed during homogenization could affect the droplet size, where increasing the agitation speed would result in a smaller emulsion droplet size.

In this study, a thin-layer drying technique with a vacuum dryer was used. Principally, this technique will create a thin layer on the glass pan surface, followed by drying in the oven. The thickness of the layer should be uniform by assuming a uniform temperature distribution (Onwude et al., 2016). This technique is very efficient with low-temperature (<60°C) application, so it will not damage the heat-sensitive substances. In addition, the utilization of a vacuum oven at a low-pressure setting (0.5 atm) would evaporate the water below the normal boiling point. It could preserve the texture and appearance of the material, minimize the loss of active substances such as aroma and volatile compounds, reduce nutrition

degradation, reduce browning due to oxidation, and save energy (Prasetyaningrum, 2010).

The measurement results of trapped oil, antioxidant activity, yield, moisture content, surface oil, and water activity could be seen in Table 2. To evaluate the significance of each factor, an analysis of variance (ANOVA) was performed. ANOVA results showed that the polynomial quadratic model was a suitable model to represent the experimental data at a 95% confidence level. The correlation coefficient from ANOVA statistical analysis is shown in Table 3. Based on the statistical analysis, agitation speed (RPM), MD, and WPI addition had a significant effect on trapped oil, antioxidants, and yield ($p < 0.05$). Coefficient regression tables to predict results through polynomial equations are presented in Table 4.

3.3 | Trapped oil of nutmeg seed oleoresin encapsulate

Trapped oil content is a parameter representing how much oleoresin (core material) is encapsulated by the coating material. By encapsulation, the core material or active substances could be protected from degradation reactions, aroma, and volatile compound loss, thus maintaining flavor stability during storage (Kanakdande et al., 2007). The result of the trapped oil measurement is presented in the experimental response table (Table 2) and illustrated as a three-dimensional graph (Figure 1).

Based on Figure 1 and Table 3, all three variables (RPM, MD, and WPI) had a significant effect on trapped oil content. From the graphs (Figure 1), there is a rising ridge chart where the critical point

TABLE 2 Experimental responses based on agitation speed, MD, and WPI concentrations

Treatment	Pattern	RPM	MD	WPI	Trapped oil (%)	Antioxidant activity (%)	Yield (%)	Moisture content (%)	Surface oil (%)	a_w (%)
1	−+−	3000	6.00	2.00	8.9 ± 0.71	53.79 ± 38.89	62.83 ± 18.38	7.57 ± 0.10	4.1 ± 2.97	0.57 ± 0.04
2	−++	3000	6.00	6.00	6.7 ± 2.62	53.25 ± 32.12	71.88 ± 0.00	8.43 ± 0.63	3.2 ± 0.57	0.55 ± 0.05
3	−	3000	2.00	2.00	8.1 ± 2.40	73.26 ± 24.53	68.75 ± 17.68	7.39 ± 0.65	7.6 ± 5.02	0.57 ± 0.06
4	−−+	3000	2.00	6.00	4.7 ± 2.26	55.45 ± 30.72	62.92 ± 5.30	8.08 ± 0.16	4.0 ± 1.27	0.55 ± 0.00
5	++−	4000	6.00	2.00	10.9 ± 3.39	13.31 ± 2.67	60.83 ± 8.25	7.93 ± 0.93	2.3 ± 0.28	0.57 ± 0.02
6	+++	4000	6.00	6.00	6.1 ± 2.05	51.89 ± 1.74	67.81 ± 5.75	8.87 ± 0.69	4.2 ± 0.85	0.55 ± 0.04
7	+−−	4000	2.00	2.00	8.8 ± 0.21	60.61 ± 4.59	51.25 ± 1.77	7.78 ± 0.25	7.5 ± 0.57	0.56 ± 0.04
8	+−+	4000	2.00	6.00	4.5 ± 4.31	46.48 ± 3.85	60.00 ± 9.43	9.12 ± 0.04	3.4 ± 1.41	0.57 ± 0.00
9	a00	2700	4.00	4.00	7.4 ± 1.20	67.25 ± 25.78	70.83 ± 0.00	8.17 ± 0.34	3.4 ± 0.49	0.56 ± 0.08
10	A00	4300	4.00	4.00	7.2 ± 3.39	73.47 ± 19.28	72.92 ± 2.95	8.42 ± 0.60	5.4 ± 1.13	0.55 ± 0.04
11	0A0	3500	7.36	4.00	8.6 ± 3.39	17.25 ± 7.32	71.60 ± 4.60	8.77 ± 0.18	3.6 ± 0.57	0.58 ± 0.04
12	0a0	3500	0.64	4.00	2.2 ± 1.91	91.33 ± 0.69	58.47 ± 7.37	8.64 ± 0.61	9.8 ± 3.04	0.56 ± 0.09
13	00a	3500	4.00	0.64	13.7 ± 0.14	61.68 ± 42.81	54.42 ± 4.91	8.00 ± 0.75	5.4 ± 1.20	0.57 ± 0.04
14	00A	3500	4.00	7.36	7.0 ± 4.67	61.07 ± 25.47	63.46 ± 11.41	9.30 ± 0.04	2.9 ± 1.98	0.58 ± 0.06
15 (C)	000	3500	4.00	4.00	10.3 ± 2.62	91.21 ± 2.43	66.67 ± 5.89	8.61 ± 0.44	3.1 ± 1.56	0.54 ± 0.07
16 (C)	000	3500	4.00	4.00	10.8 ± 3.82	89.80 ± 1.01	66.67 ± 5.89	8.96 ± 0.06	2.6 ± 1.41	0.55 ± 0.07
17 (C)	000	3500	4.00	4.00	9.6 ± 1.98	91.71 ± 1.11	66.67 ± 5.89	8.23 ± 0.21	7.7 ± 9.40	0.56 ± 0.06

TABLE 3 Significant levels of ANOVA polynomial quadratic models

	p-value					
Factor	Trapped oil	Antioxidant	Yield	Moisture content	Surface oil	a_w
RPM	0.652814	0.345729	0.150548	0.100713	0.768573	0.711615
RPM × RPM	0.004669[a]	0.063227	0.256910	0.120237	0.781478	0.997576
MD	0.001167[a]	0.008870[a]	0.020329[a]	0.657418	0.022711[a]	0.617207
MD × MD	0.000301[a]	0.009345[a]	0.411353	0.654599	0.249141	0.272313
WPI	0.000098[a]	0.925792	0.047565[a]	0.003462[a]	0.143077	0.449984
WPI × WPI	0.991724	0.021194[a]	0.028434[a]	0.542120	0.662044	0.103064
RPM × MD	0.703507	0.629725	0.229139	0.575373	0.976896	0.907489
RPM × WPI	0.207726	0.321592	0.288194	0.511938	0.666199	0.381533
MD × WPI	0.818747	0.124712	0.268150	0.831498	0.127129	0.510786

[a]Significant.

TABLE 4 Coefficient regression value for polynomial quadratic models

				Coefficient regression value					
Factor	Trapped oil	Anti-oxidant	Yield	Moisture content	Surface oil	a_w	Lightness	a^* value	b^* value
Mean	−53.5064[a]	−346.601	173.6736	−3.82726	10.07204	0.654362[a]	−12.7101	−12.7101	12.53253
RPM	0.0318[a]	0.242	−0.0565	0.00588	0.00349	−0.000020	0.0105	0.0105	−0.00311
RPM×RPM	−0.0000[a]	−0.000	0.0000	−0.00000	−0.00000	0.000000	−0.0000	−0.0000	0.00000
MD	3.6348[a]	23.211	−4.3574	0.43105	−3.03389	−0.006375	0.6135	0.6135	2.07388
MD×MD	−0.4390[a]	−3.750[a]	−0.2505	−0.01308	0.16536	0.001041	−0.1027	−0.1027	−0.19311
WPI	0.4961	−2.295	0.4477	0.07146	−1.97972	−0.025062	1.1285	1.1285	3.65184
WPI×WPI	0.0007	−3.124[a]	−0.7892[a]	−0.01795	−0.06003	0.001638	−0.0600	−0.0600	−0.11548
RPM×MD	0.0001	−0.003	0.0018	−0.00008	−0.00002	0.000000	0.0001	0.0001	0.00008
RPM×WPI	−0.0004	0.005	0.0016	0.00009	0.00028	0.000004	−0.0002	−0.0002	−0.00053
MD×WPI	0.0187	2.187	0.4095	−0.00734	0.27031	−0.000719	−0.0894	−0.0894	−0.10865
R^2	0.95	0.84	0.83	0.79	0.71	0.50	0.84	0.84	0.88

[a]Significant.

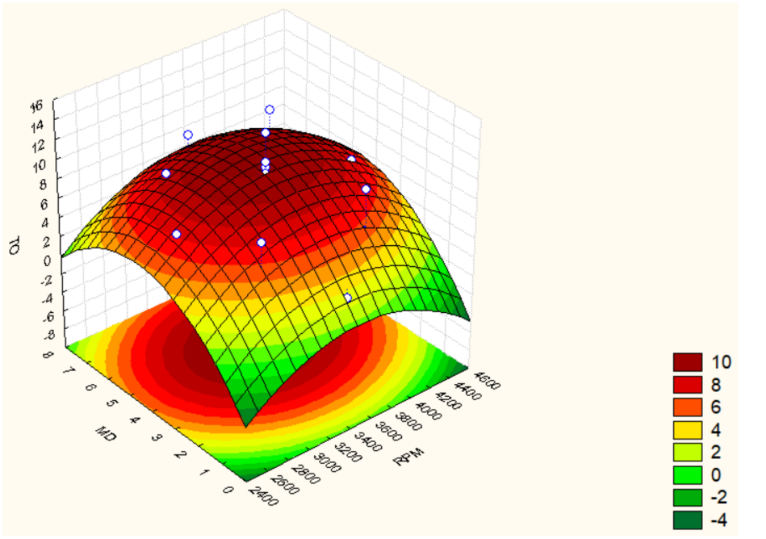
or stationary point is not in the experimental area but occupies the maximum point. Formula with the ratio of oleoresin and coating 1:1 had the highest trapped oil yield of 8.1%–13.7%, followed by the formula with the ratio of 1:2 (4.5%–10.9%) and 1:3 (6.1%–8.6%), respectively. Maltodextrin as a coating material plays an important role in the effectiveness of oil trapping. If the coating material amount is insufficient to wrap core materials, there will be a lot of core material on the outer surface of the encapsulate (Jayanudin et al., 2017). WPI contains up to 90% of free proteins which makes those proteins easily dissolve in the emulsion system and interact with oil (oleoresin). Thus, the more WPI added, the more stable the emulsion system will be. Whey protein isolate could function as a suitable encapsulating agent (Young et al., 1993 in Nasrullah, 2010). In addition, emulsion stability is affected by the higher agitation speed, which reduces the size of the oil globule (oleoresin) where oil globules can be completely covered by the coating material. The homogenization process can also reduce the tendency of fat globules to clump or coalescence due to smaller droplet sizes (Tetra Pak, 2015).

3.4 | Surface oil of nutmeg seed oleoresin encapsulate

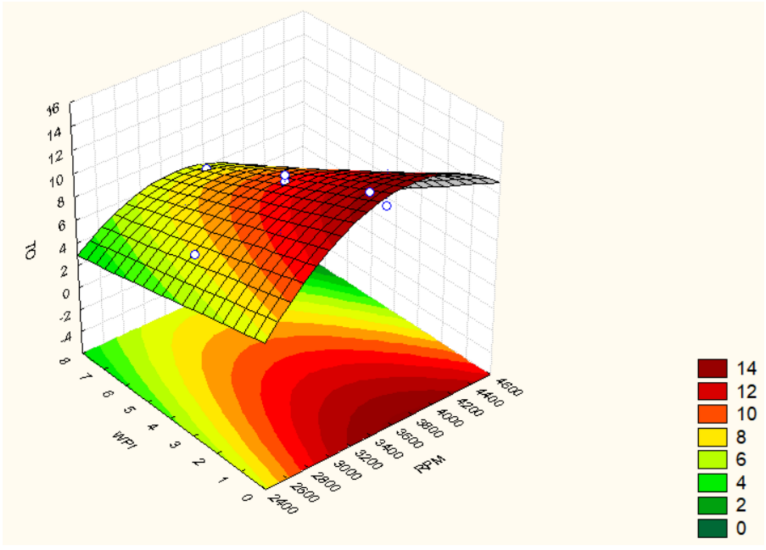
As one of the encapsulation process parameters, surface oil indicates the amount of oil present on the surface and not encapsulated well. This parameter could analyze how much oleoresin can be encapsulated completely (Nasrullah, 2010). Non-encapsulated oleoresin or free oleoresin on the surface will be easily damaged due to evaporation and oxidation (Shaidi & Han, 1993 in Nasrullah, 2010).

Figure 2 shows a saddle system graph where the elliptical contour extends significantly along with one of its main axes. The amount of coating material influenced the surface oil of the encapsulate. Formula with core and coating material ratio of 1:1 showed the highest surface oil yield (5%–9%), followed by the ratio of 1:2 (2.3%–7.7%) and 1:3 (2.9%–4.2%), respectively. As presented in Table 2, it can be seen that increasing the amount of coating material will reduce the amount of surface oil. It might be due to the thicker encapsulated wall formed, so the amount of oleoresin that comes out will be less (Jayanudin et al., 2017).

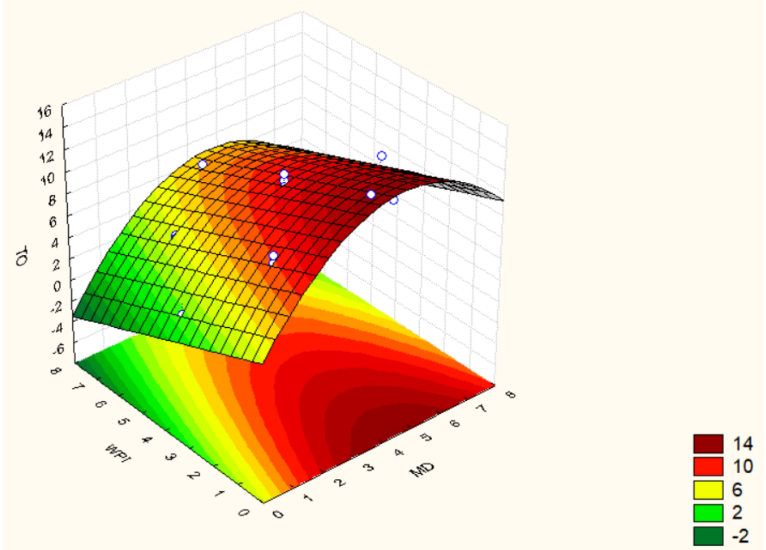
Table 3 indicates that MD has a substantial effect on the surface oil amount. The low amount of maltodextrin to core material will result in insufficient coating material to cover the whole surface of the oleoresin droplets to strengthen the capsule wall (Laohasongkrama et al., 2011). The addition of maltodextrin which is not balanced with whey protein will increase the level of surface oil. Maltodextrin is a lipophobic compound, so it cannot bind to the oil molecule. Therefore, it is not enough to emulsify the oil to be encapsulated and result in a lot of oil that is not encapsulated. Therefore, the addition of WPI is used in the formula since WPI is considered a suitable emulsifier in the food system. Principally, WPI will be absorbed in the interface of oil-in-water (o/w) droplets and forms a layer that can protect droplets from coalescence (Assagaf et al., 2013).



(a)

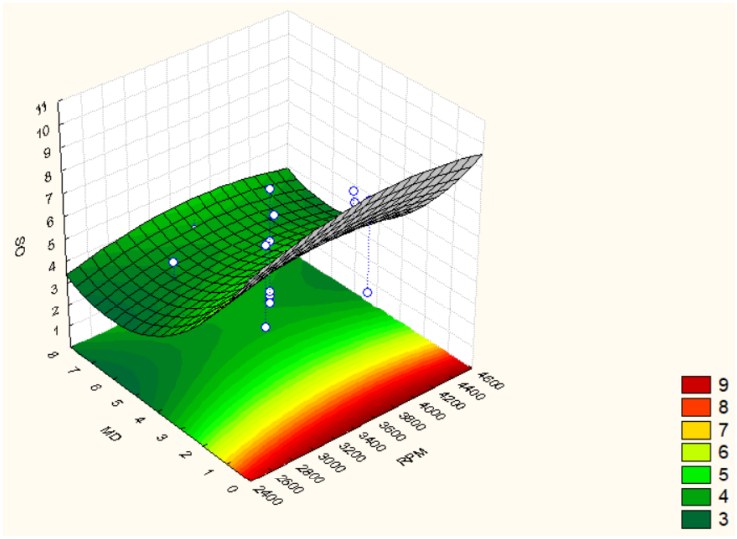


(b)



(c)

FIGURE 1 Fitted surface of trapped oil. (a) effect of agitation speed (rpm) and maltodextrin concentration on trapped oil encapsulate (b) effect of agitation speed (rpm) and whey protein isolate concentration on trapped oil encapsulate (c) effect of concentration of maltodextrin and whey protein isolate on trapped oil encapsulate.



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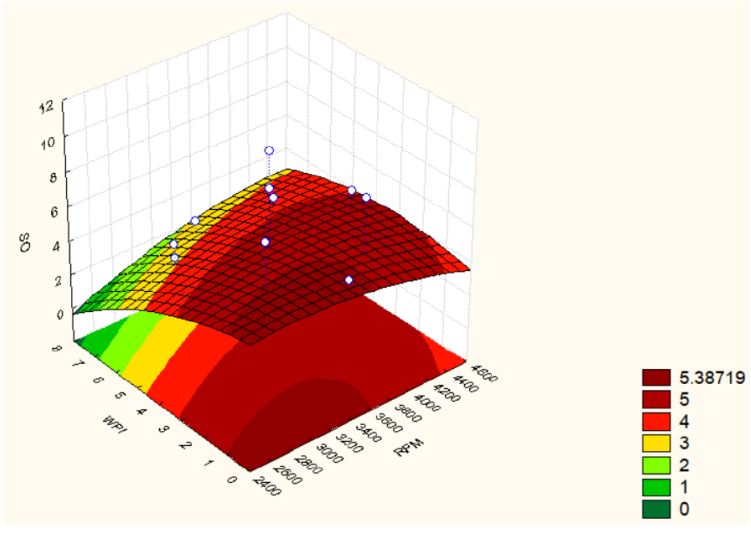

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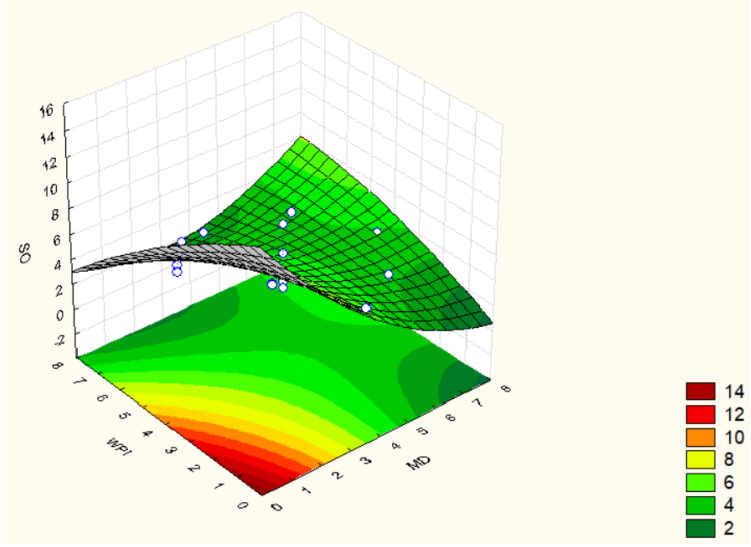


(c)

FIGURE 2 Fitted surface of surface oil. (a) the effect of agitation speed (rpm) and the concentration of maltodextrin on encapsulate surface oil (b) the effect of agitation speed (rpm) and the concentration of whey protein isolate on surface oil encapsulate (c) the effect of concentration of maltodextrin and whey protein isolate on surface oil encapsulate.

3.5 | Antioxidant activity of nutmeg seed oleoresin encapsulate

An antioxidant is a substance to inhibit or prevent oxidation in the substrate. Free radicals are unstable and highly reactive molecules with one or more unpaired electrons present in their outermost orbitals. To be more stable, free radicals tend to react with the other molecules to obtain electron pairs (Karim et al., 2015).

In this research, 1,1-diphenyl2-picrylhydrazyl (DPPH) becomes the free radical reacted with active substances from the encapsulated oleoresin. Flavonoids nutmeg oleoresin will donate hydrogen radicals (H+) or oxidized by the DPPH and result in more stability and low reactivity of free radicals (Amic et al., 2003 in Karim et al., 2015). A study by Sharma et al. (2015) reported that total flavonoids in onions would decrease after heating at a high temperature. It indicates that some flavonoids might be destroyed at high-temperature treatment. Nutmeg oleoresin contains phytochemical compounds with antioxidant activity such as myristicin, isoeugenol, and eugenol compounds (Ginting et al., 2017).

Based on DPPH in vitro testing, the antioxidant activity of fresh nutmeg oleoresin was 94.23%, while the antioxidant activity of nutmeg oleoresin encapsulate was ranging from 13.31% to 91.33% (see Table 2). The lowest antioxidant activity value was obtained from treatment 5 with an agitation speed of 4000 rpm, 6 g of maltodextrin, and 2 g of whey protein isolate. The highest antioxidant activity values were obtained from treatment 12 with an agitation speed of 3500 rpm, 0.64 g of maltodextrin, and 4 g of whey protein isolate. Based on research by Ginting et al. (2017) about the antioxidant activity of n-hexane extract of nutmeg plants, the antioxidant activity of nutmeg seeds was in the range 60.86%–87.85%.

From the result in Table 2, there was an interaction between surface oil and antioxidants, where encapsulates with low surface oil will have low antioxidants and vice versa. In other words, the encapsulation with a higher amount of surface oil will be more susceptible to damage (oxidation) compared to encapsulates that have low surface oil or not too high antioxidant activity.

In Figure 3, there are three graphs illustrating a maximum surface visual where the critical point is in the experimental region and the stationary point is at the maximum point. The antioxidant activity values of encapsulating at three different core and coating material ratios (1:1, 1:2, and 1:3) were 53.79%–91.33%, 13.31%–91.71%, and 17.25%–61.07%, respectively. The high amount of maltodextrin as encapsulating material will produce low antioxidant activity if the ratio of MD:WPI is not proportional. This is due to the wall being formed getting thicker. Maltodextrin has good stability against oil oxidation but has low oil retention, thus it is usually combined with an emulsifier (Kenyon, 1995 in Nasrullah, 2010). If the composition of maltodextrin is high and not balanced with whey protein, some oleoresin compounds might be damaged during the drying process because of their presence on the surface. At treatment 17 (91.71%) (ratio of core material: coating = 1:2), antioxidants were produced higher than at treatment 12 (91.33%) (ratio of core material: coating = 1:1), this can be caused by the number of solids that are too high which results in puffing (swelling) and cracking of particles so that the encapsulate ruptured because of high temperatures, and the core material comes out of the capsule (Li et al., 2015).

3.6 | Yield of nutmeg oleoresin encapsulate

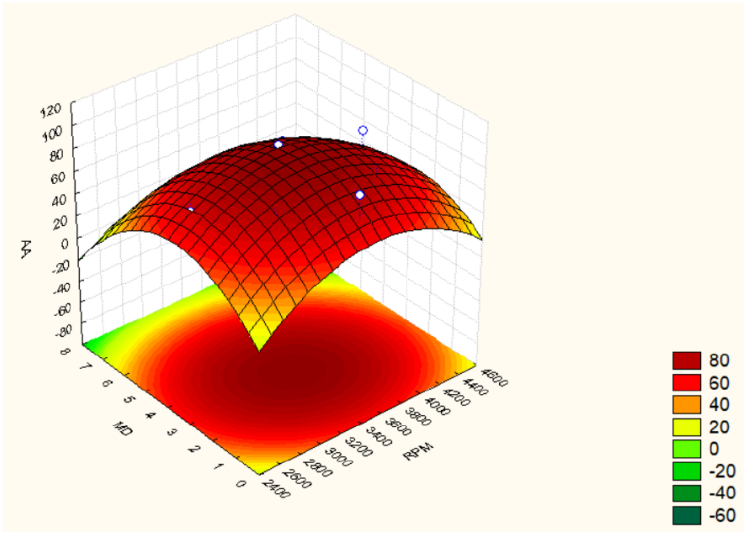
The yield (in %) of encapsulation could indicate how optimal the powder produced from each formula and how much loss in each formula is. Based on Table 2, the yield value of the encapsulate is not too high and ranges from 51.25% to 72.92%. It might be due to many product losses during the processing. Based on Table 3, the MD and WPI variables have a significant effect on the encapsulate yield ($p < 0.05$). The addition of maltodextrin and whey protein isolate as a coating material has a higher total solid, thus giving a higher yield.

Figure 4 is forming a saddle system graph where the elliptical contour extends significantly along one of its main axes and the rising ridge graph where the critical point or stationary point is not in the experimental area and the stationer point is at the maximum point. Formula with the ratio of core and coating material 1:1 has the lowest yield of encapsulating (51.25%–68.75%), followed by the ratio of 1:2 (60.00%–72.92%) and 1:3 (63.46%–71.88%), respectively.

3.7 | Moisture content of nutmeg oleoresin encapsulate

Moisture content is one of the encapsulated quality aspects. The higher moisture content in encapsulates will trigger the oxidation and hydrolysis reaction resulting in quality degradation and biological damage (Bakry et al., 2015). According to SNI 01–3709-1995, the maximum moisture content of spice powder is 12% (National Standardization Agency 1995). The moisture content of encapsulated powder in this study was in the range 7.39%–9.30%, so they met the SNI water content specification. Based on ANOVA results in Table 3, the WPI variable had a significant effect on water content ($p < 0.05$). Whey protein isolate is very hygroscopic or sensitive to moisture and stickiness (Hogan & O'callaghan, 2013). Hence, the addition of whey protein isolate could increase the water content of the encapsulated powder.

Figure 5 shows the rising ridge graph where the critical point or stationary point is not in the experimental region and the stationer point is at the maximum point. The addition of coating material affected the water content of encapsulates powder. The lowest water content was obtained from the formula with the ratio of core and coating material 1:1, while the highest water content was from the formula with a ratio of 1:3. The addition of whey protein isolate has a significant effect in increasing the water content of the encapsulated powder due to the hygroscopic properties of whey protein. Based on Prasetyo in (Ramadhani, 2016), too much addition of coating material as a filler will cause clotting and case hardening. As a result, the moisture inside the droplet cannot come out and contact with the drying air. The droplet surface is covered by solid substances and will minimize the water–hot air contact area. Therefore, adding coating material could increase the water content.



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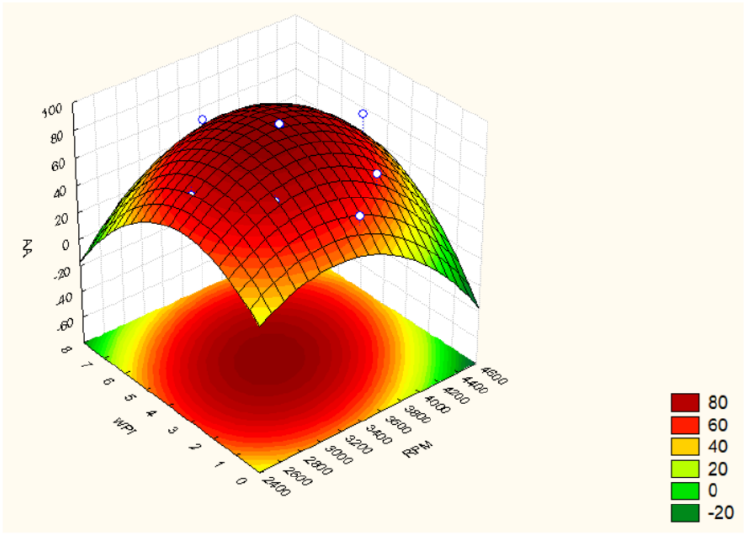

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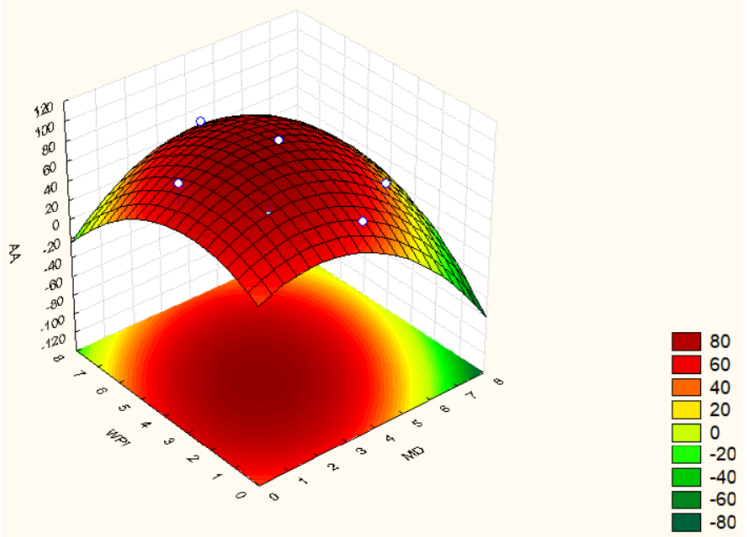

(c)

FIGURE 3 Fitted surface of antioxidant activity. (a) the effect of agitation speed (rpm) and the concentration of maltodextrin on the antioxidant activity of the encapsulate (b) the effect of the agitation speed (rpm) and the concentration of whey protein isolate on the antioxidant activity of the encapsulate (c) the effect of the concentration of maltodextrin and whey protein isolate on the antioxidant activity of the encapsulate.

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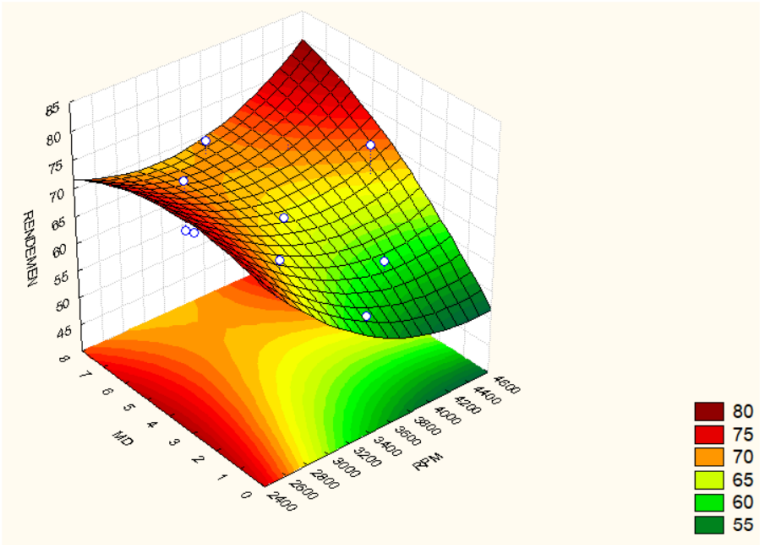

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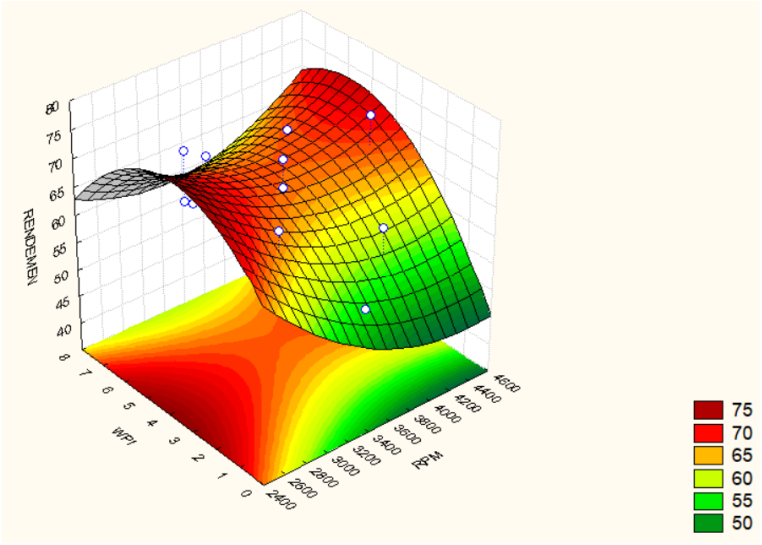

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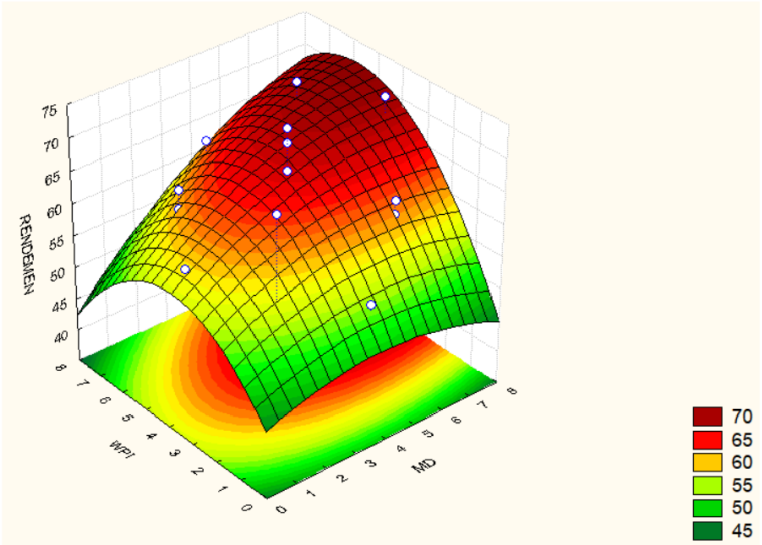
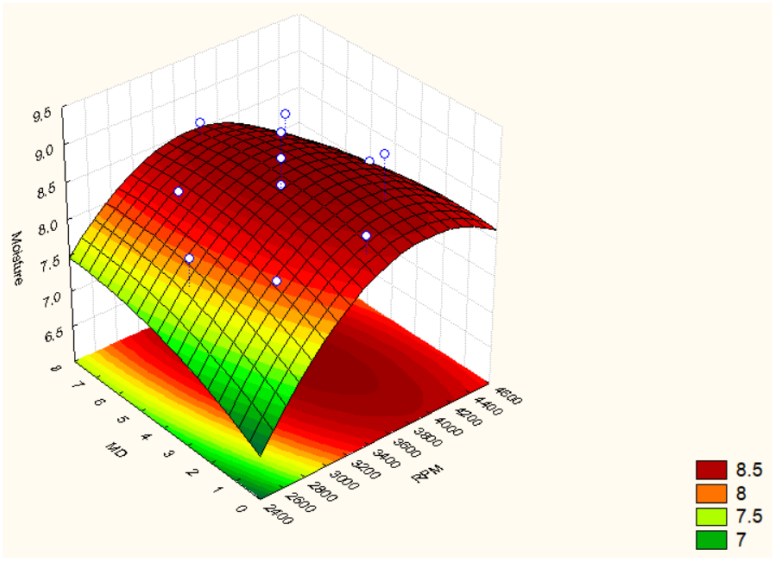


(c)

FIGURE 4 Fitted surface of yield. (a) the effect of agitation speed (rpm) and maltodextrin concentration on the percentage of encapsulate yield (b) the effect of agitation speed (rpm) and the concentration of whey protein isolate on the percentage of encapsulate yield (c) effect of the concentration of maltodextrin and whey protein isolate on the percentage of encapsulate yield.



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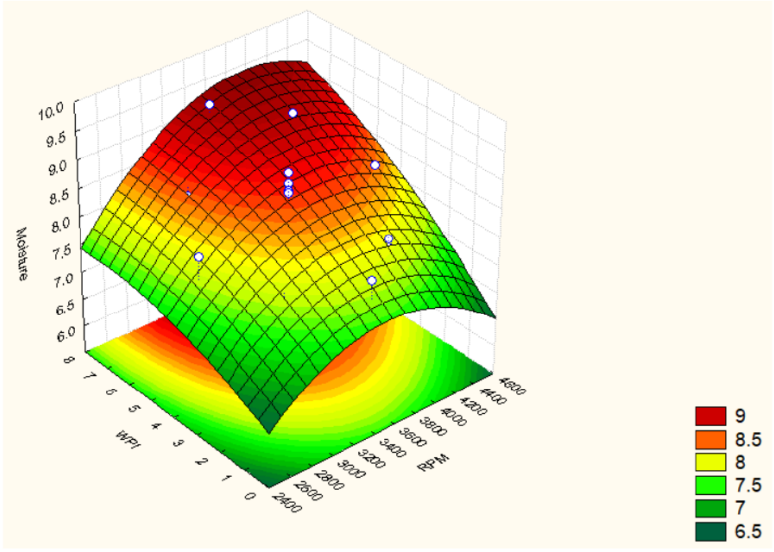

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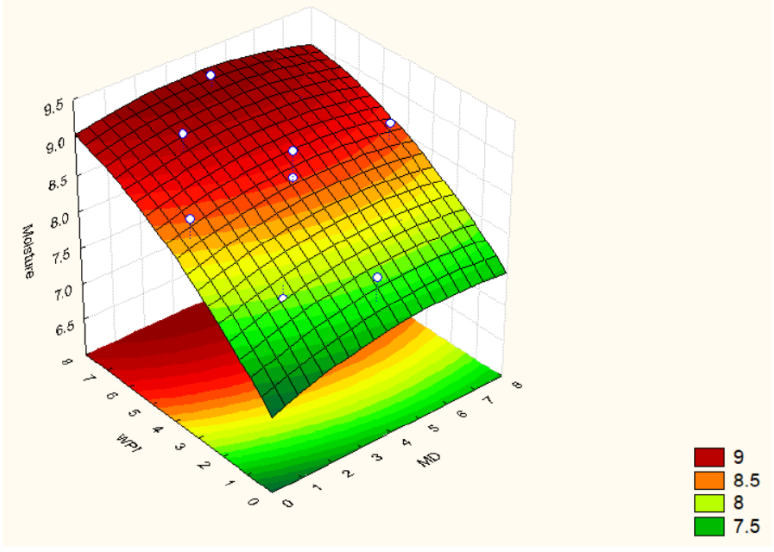

(c)

FIGURE 5 Fitted surface of moisture. (a) the effect of agitation speed (rpm) and maltodextrin concentration on encapsulate water content (b) effect of agitation speed (rpm) and whey protein isolate concentration on encapsulate water content (c) the effect of concentration of maltodextrin and whey protein isolate on encapsulate water content.

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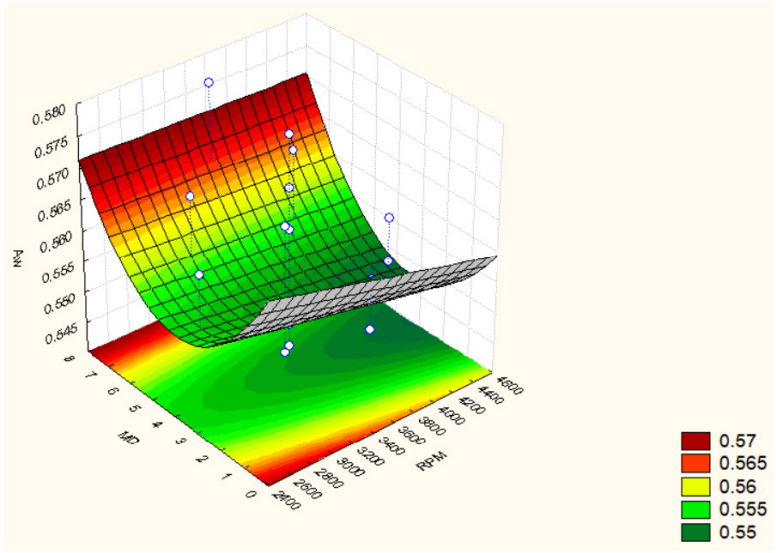

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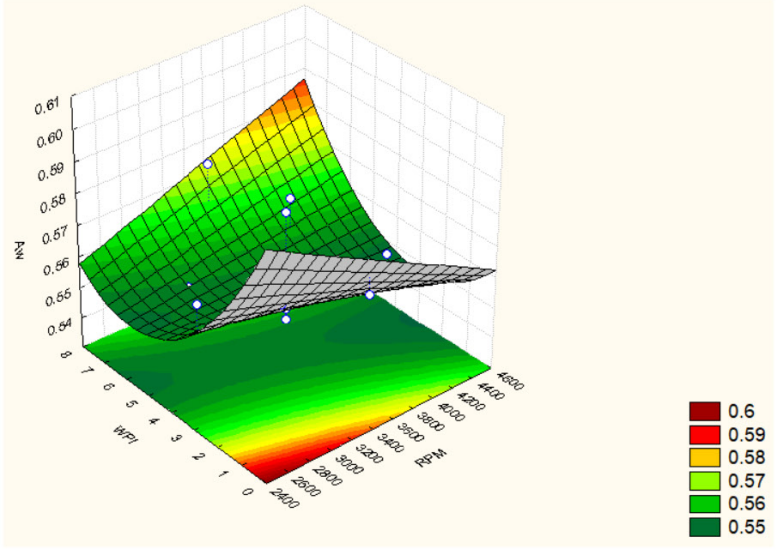

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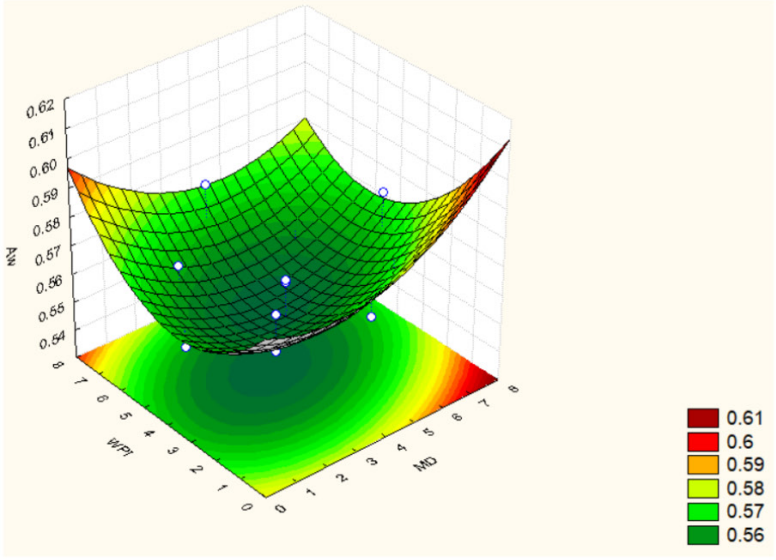

(c)

FIGURE 6 Fitted surface of water activities. (a) the effect of agitation speed (rpm) and maltodextrin concentration on encapsulate water activity (b) effect of agitation speed (rpm) and whey protein isolate concentration on encapsulate water activity (c) effect of concentration of maltodextrin and whey protein isolate on encapsulate water activity.

3.8 | Water activity of nutmeg oleoresin encapsulate

Water activity (a_w) indicates the amount of free water used by microorganisms to grow. Therefore, this parameter is important to define the microbiology risk in encapsulated powder and the stability during storage. The water activity values of oleoresin encapsulate in this study were in the range 0.54–0.58. Tapia et al. (2020) stated that the food product must have water activity below 0.6 to prevent mold growth. Based on ANOVA analysis in Table 3, no variable affects water activity in the powder. It might be due to the vacuum oven's ability to produce water vapor during off conditions. In addition, wehey protein isolate could function as a suitable encapsulating agent, so that the water vapor in the vacuum oven will be easily absorbed this increase the water activity.

Figure 6 shows the graphs that form various models. The interaction graph of agitation speed with maltodextrin shows the falling ridge graph where the critical point or stationary point is not in the experimental area and the stationary point is at the minimum point. The interaction graph of mixing speed with whey protein shows the saddle graph system where elliptical contours extend significantly along one of its main axes (Taylor & Francis, 2008).

3.9 | Color analysis of nutmeg oleoresin encapsulate

The color of the encapsulated powder indicated the physical properties based on the constituent materials. Principally, the chroma meter worked through the interaction of energy diffuse light and atoms or molecules of an object being analyzed. The light source of a Xenon lamp was beamed onto the sample surface and was reflected by the spectral sensor. Six high-sensitivity silicon photocells with a dual-back beam system measured the reflected light of the sample (Candra et al., 2014). The L^* indicator was indicated by a value of 0 (black/dark) to 100 (light/white). The reflected light of the L^* indicator showed the achromatic colors of white, gray, and black. An a^* indicator showed a chromatic color of red if positive and green if negative. A positive b^* indicator indicated a yellow chromatic color and a negative b^* value indicated a blue color intensity.

Based on Table 5, the difference in agitation speed showed no effect on color of encapsulated powder, while the addition of a coating material increased the values of L and b^*, and decreased the value of a^*. The addition of coating material could reduce the density of the brown color of oleoresin. However, differences in coating formulations did not produce significant differences in values of L, a^*, and b^*. The brown color of encapsulated powder decreased as the amount of coating material increased.

3.10 | Optimization of process parameter combinations

The optimum point was predicted by response surface methodology from the combination of optimal conditions and interactions between independent variables (Ratnawati et al., 2018). In the optimization step, the independent variables for optimization were trapped oil, antioxidant activity, and yield. Those variables (parameters) could reflect the effectiveness and efficiency of encapsulation. The Statistica 6.0 RSM program generated five optimum formula

TABLE 5 Results of measurement parameters of encapsulated color

					Colors		
Treatment	Pattern	RPM	MD	WPI	*L*	*a*	*b*
1	−+−	3000	6,00	2,00	66.15 ± 2.33	7.72 ± 0.29	18.05 ± 0.21
2	−++	3000	6,00	6,00	70.27 ± 5.19	6.08 ± 0.93	20.35 ± 0.10
3	−	3000	2,00	2,00	59.68 ± 5.03	7.49 ± 1.33	16.55 ± 0.03
4	−−+	3000	2,00	6,00	66.07 ± 0.64	7.55 ± 0.31	20.24 ± 1.15
5	++−	4000	6,00	2,00	70.84 ± 2.36	7.60 ± 1.32	18.69 ± 0.41
6	+++	4000	6,00	6,00	69.47 ± 0.12	5.54 ± 1.14	18.51 ± 2.76
7	+−−	4000	2,00	2,00	60.09 ± 4.66	7.12 ± 1.77	16.53 ± 5.43
8	+−+	4000	2,00	6,00	64.49 ± 1.00	6.21 ± 1.59	18.45 ± 5.53
9	a00	2700	4,00	4,00	72.26 ± 0.00	6.48 ± 0.15	19.90 ± 0.05
10	A00	4300	4,00	4,00	70.95 ± 0.79	7.11 ± 0.14	20.39 ± 0.08
11	0A0	3500	7,36	4,00	74.06 ± 3.53	6.20 ± 0.46	19.28 ± 0.78
12	0a0	3500	0,64	4,00	58.67 ± 11.87	7.19 ± 0.52	15.71 ± 3.86
13	00a	3500	4,00	0,64	61.42 ± 3.58	8.40 ± 1.56	17.14 ± 0.74
14	00A	3500	4,00	7,36	72.18 ± 2.13	5.96 ± 0.48	19.61 ± 0.64
15 (C)	000	3500	4,00	4,00	65.61 ± 7.25	7.91 ± 2.15	18.76 ± 1.88
16 (C)	000	3500	4,00	4,00	62.71 ± 9.83	8.62 ± 2.97	19.28 ± 1.75
17 (C)	000	3500	4,00	4,00	67.61 ± 1.68	7.21 ± 0.99	20.63 ± 0.31

TABLE 6 Formulas generated in optimization stages

Factor	Level factor	Predicted total oil (%)	Predicted antioxidant activity (%)	Predicted yield (%)	Desirability value
RPM (rpm)	2659.104	6.97	71.67	73.62	0.68
	3079.552	9.37	88.18	69.21	0.79
	3500.000	10.23	91.50	66.79 17.00	0.79
	3920.448	9.56	81.65	66.38	0.73
	4340.896	7.35	58.62	67.97	0.59
MD (g)	0.636	3.13	72.25	58.72	0.28
	2.318	7.92	92.48	63.46	0.66
	4.000	10.23	91.50	66.79	0.79
	5.682	10.06	69.31	68.70	0.73
	7.364	7.40	25.91	69.19	0.39
WPI (g)	0.636	13.45	55.54	53.66	0.39
	2.318	11.84	82.36	62.46	0.73
	4.000	10.23	91.50	66.79	0.79
	5.682	8.63	82.98	66.66	0.71
	7.364	7.03	56.79	62.07	0.49

Note: The best formula resulted from a process at an agitation speed of 3500 rpm and the addition of 4 g maltodextrin and 4 g WPI. That formula had a trapped oil content of 10.23%, antioxidant activity of 91.50%, yield of 66.79%, water activity of 0.55, moisture content of 8.63, and color intensity L^* 65.47, a^* 7.90, and b^* 19.57. This formula could be applied to produce nutmeg seed oleoresin powder with good physicochemical properties.

solutions as presented in Table 6. Process conditions with an agitation speed of 3500 rpm, 4 g of maltodextrin, and 4 g of whey protein isolate would produce an encapsulated powder with characteristics for an optimization target of 79.39%. Then, the optimum formula could be achieved by using polynomial quadratic models shown in Table 4.

4 | CONCLUSION

The nutmeg oleoresin encapsulation process was optimized by the response surface methodology (desirability value of 0.794) and resulted in the following setting variable: 3500 rpm of agitation speed, 4 g of maltodextrin, and 4 g of whey protein isolate addition. It means that those setting variables could produce nutmeg oleoresin encapsulates as desired (optimum) at 79.39%. The optimum formula had a trapped oil content of 10.23%, antioxidant activity of 91.50%, yield of 66.79%, water activity of 0.551, moisture content of 8.63%, and color properties $L = 65.47$, $a^* = 7.90$, and $b^* = 19.570$. As a suggestion, further research on the stability and safety (in vivo testing) of nutmeg oleoresin encapsulation needs to be done.

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CONFLICT OF INTEREST

There are none to declare.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

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