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Title:

Optimization of Encapsulated Agents and Stirring Speed on the Physicochemical Characteristics of Vacuum Dried Nutmeg Seed Oleoresin (*Myristica fragrans*)

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1. Manuscript Submission



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13-Jun-2022

Dear Dr. Ananingsih:

Your manuscript entitled "Optimization of Encapsulated Agents and Agitation Speed on the Physicochemical Characteristics of Vacuum Dried Nutmeg Seed Oleoresin (*Myristica fragrans*)" by Ananingsih, Victoria; Soedarini, Bernadeta; Andriani, Cynthia; Konstantia, Bernadine Adi; Santoso, Birgitta, has been successfully submitted online and is presently being given full consideration for publication in the Journal of Food Processing and Preservation.

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17-Jul-2022

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1 **Optimization of Encapsulated Agents and Stirring Speed on the Physicochemical**
2 **Characteristics of Vacuum Dried Nutmeg Seed Oleoresin (*Myristica fragrans*)**

3

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13 **6,489 words**

14

15 **Vacuum Dried Nutmeg Seed Oleoresin**

16

17

18 **ABSTRACT:** Nutmeg seed oleoresin (*Myristica fragrans* Houtt) from nutmeg seed
19 extraction contains active substances. However, oleoresins' active substances are
20 commonly heat-sensitive, so encapsulation is needed. Encapsulation is the process
21 of wrapping particles containing active ingredients in a homogeneous or
22 heterogeneous matrix that produces encapsulated powder. The objective of this
23 study was to obtain the best combination of encapsulated agents concentration
24 (maltodextrin and whey protein isolate) and agitation speed on the physicochemical
25 characteristics of nutmeg seed oleoresin encapsulated using a vacuum drying
26 method. Encapsulation of nutmeg seed oleoresin was performed with comparative
27 parameters namely agitation speed (3000, 3500, 4000 rpm), maltodextrin (MD)
28 concentrations (ratio of MD to nutmeg seed oleoresin= 2:4, 4:4, 6:4), and Whey
29 Protein Isolate (WPI) concentrations (ratio of WPI to nutmeg oleoresin= 6:4, 4:4,
30 2:4). The physicochemical analysis consisted of trapped oil content, antioxidant
31 activity, yields, water content, surface oil, water activity, and colour testing. The
32 physicochemical data were further analysed by Response Surface Methodology
33 (RSM) to get an optimum formula. The best formula ~~was~~ resulted from a process at ~~a~~
34 an agitation speed of 3500 rpm and the addition of 4 grams of maltodextrin and 4
35 grams of WPI. That formula had a trapped oil content 10.23%, antioxidant activity
36 91.50%, yield 66.79%, water activity 0.55, moisture content 8.63, colour intensity L*
37 65.47, a* 7.90, and b* 19.57. This formula could be applied to produce nutmeg seed
38 oleoresin powder with good physicochemical properties.

39

40 Keywords: Nutmeg oleoresin, encapsulation, vacuum drying, Response Surface
41 Methodology

εϚ

εϛ **Practical Application:**

εε Encapsulation of nutmeg seed oleoresin by vacuum drying produced a more stable

εο powder with a longer shelf life compared to those in the form of nutmeg seed

εϛ oleoresin. The viscous liquid of nutmeg seed oleoresin is prone to oxidation and

εϜ degradation during storage. This encapsulated nutmeg seed oleoresin powder can be

εϝ used as a food ingredient for different applications i.e. beverages, confectionery,

εϞ bakery products, and soup seasonings.

02 1. Introduction

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

10

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

20

21 The most common coating materials for encapsulation are maltodextrin and arabic
22 gum (Lantigua et al., 2011 and Elsebaie & Essa, 2018). Based on research by
23 Zilberboim et al. (1986), the bell pepper oleoresin encapsulated with arabic gum was
24 considered an expensive and non-feasible coating ingredient. Research by Nurlaili et
25 al. (2014) reported that the microencapsulation of pulp ginger oleoresin with

86 maltodextrin could reach an encapsulation efficiency of up to 22%. Therefore,
87 maltodextrin is preferably used as a coating agent because of ~~the-its~~ affordable price,
88 neutral taste and aroma, water-soluble and film-forming properties, low viscosity at
89 high solids concentrations, and is less prone to oxidation (Fernandes et al., 2014).
90 The disadvantage of using maltodextrin is unstable emulsion stability to trap
91 oleoresin. In this case, emulsifier addition could ~~aid in improving~~~~help to obtain a~~
92 ~~better~~ coating performance. Whey protein isolate (WPI) is considered a suitable
93 emulsifier in the food system. Principally, WPI will be absorbed ~~in-at~~ the interface of
94 oil-in-water (o/w) droplets ~~and-where it~~ forms a layer that ~~can-protects~~ droplets
95 from coalescent (McCrae et al., 1999 in Assagaf et al., 2013). The agitation speed of
96 the homogenizer could affect the droplet size. The higher the agitation speed, the
97 smaller size of the oil droplet. Research by Jayanudin et al. (2018) reported that the
98 higher agitation speed would increase the Reynolds number (Re) and reduce the
99 emulsion droplet size.

100

101 Based on the explanation above, it is necessary to optimize the encapsulation of
102 nutmeg oleoresin with different agitation speeds and ratios of maltodextrin and WPI.
103 Processing data by applying Response Surfaces Methodology (RSM) made this
104 research important by analysing the optimum points and illustrating them in a three-
105 dimensional graph.

106

107 **2. Materials and Methods**

108 **2.1. Materials**

1.9 The extraction materials were nutmeg (*Myristica fragrans*), ethanol 96% solvent, and
1.10 Whatman filter paper number 1. The encapsulation materials were extracted
1.11 nutmeg oleoresin, maltodextrin (DE 15-20) and whey protein isolate (WPI) 90, and
1.12 distilled water; while materials for analysis were DPPH (Diphenyl Picryl Hydrazyl)
1.13 solution 0.06 mM, methanol 99.98%, ethanol 96%, and filter paper.

1.14

1.15 **2.2. Nutmeg Oleoresin Extraction with Ultrasound-assisted Extraction (UAE)**

1.16 First, fresh nutmeg seeds were dried in the oven at 45°C for 24 hours. The dried
1.17 nutmeg seeds were cut and ground before sieving with 36 mesh-size. Nutmeg
1.18 powder was dissolved with-in ethanol 96% by-at a ratio of 1:10. The extraction was
1.19 carried out with an Erlenmeyer containing a sample, soaked in the ultrasonic cleaner
1.20 UC-10SD at 50°C and 45 kHz frequency for 37.5 minutes. After that, the mixture was
1.21 stored at chiller $\pm 4^{\circ}\text{C}$ for 30 minutes for fat phase separation (Rodianawati, 2010
1.22 and Assagaf et al., 2012), then filtered. The solvent in the filtrate was evaporated
1.23 with a rotary vacuum evaporator (40°C, speed 52 rpm, and pressure 0.09MPa) until
1.24 all solvent evaporated, and a thick nutmeg oleoresin was obtained (Trendafilova et
1.25 al., 2010 modified). Oleoresin was kept in a glass bottle laminated by-with aluminium
1.26 foil and stored in a chiller.

1.27

1.28 **2.3. Response Surface Methodology (RSM)**

1.29 The formula determination was generated from the Statistica 6.0 Response Surface
1.30 Methodology (RSM) software as presented in Table 1 and produced 17 treatments
1.31 for oleoresin encapsulation. The range of agitation speed was set between 2700 and
1.32 4000 rpm, and 0.64 and 7.36 gram for maltodextrin and WPI. RSM generated three

123 levels of oleoresin and total coatings materials (MD and WPI) ratio. Ratio of 1:1
 124 applied on treatment 3,7,12 and 13; Ratio of 1:2 applied on treatment 1, 4, 5, 8, 9,
 125 10, 15, 16 and 17; Ratio of 1:3 applied on treatment 2, 6, 11 and 14. RSM with
 126 factorial design, namely the Central Composite Design (CCD), could simplify the
 127 number of experiments and useful for testing multiple process variables. The CCD
 128 design is a 2^k factorial design or called as partial factorial. It is expanded by adding
 129 observation points at the centre, so the predicted parameter coefficients will be on
 130 the quadratic surface (second order) (Montgomery, 2001 in Lubis, 2010). Generally,
 131 CCD consists of a factorial point (2^k), axial point ($2k$), and a centre point (n_c); where k
 132 is the variable number. The 2^k factorial design is used for experiments consisting of k
 133 factorial, where at the low level is coded as (-1), the middle level as (0), the high level
 134 as (+1), and the minimum and maximum level at the axial point as $(-\alpha)$ and $(+\alpha)$. The
 135 calculation of the α value on the rotate able design CCD is as follows:

136 $\alpha = [\text{number of runs factorial point}]^{1/4} = (2^k)^{1/4}$.

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

139 $-\alpha = (0) - 1.682 [(0) - (-1)]$ $+\alpha = (0) + 1.682 [(0) - (-1)]$

140 **RPM:** $-\alpha = (3500) - 1.682 [(3500) - (3000)] = 2659$

141 $+\alpha = (3500) + 1.682 [(3500) - (3000)] = 4341$

142 **MD:** $-\alpha = (4) - 1.682 [(4) - (2)] = 0.636$

143 $+\alpha = (4) + 1.682 [(4) - (2)] = 7.364$

144 **WPI:** $-\alpha = (4) - 1.682 [(4) - (2)] = 0.636$

145 $+\alpha = (4) + 1.682 [(4) - (2)] = 7.364$

146 Therefore, each factor would have 5 levels of test points. The results of CCD analysis
147 are presented in the form of graphs based on mathematical models and respond
148 surfaces. Those outputs are useful to predict the optimal value from the responses
149 and to provide information on the interaction between the dependent and
150 independent variables (Yousefi et al., 2016)

151

152 **2.4. Encapsulation of Nutmeg Oleoresin**

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

160

161 **2.5. Analysis of Encapsulated Nutmeg Seed Oleoresin**

162 Physicochemical analyses of encapsulated oleoresins were trapped oil content,
163 antioxidant activity, yield, water content, surface oil, water activity, and colour
164 intensity.

165

166 **2.5.1. Trapped Oil Content** (~~Asyhari, 2013 and Nugraheni et al., 2015~~)

167 Trapped oil content was estimated as outlined by Asyhari (2013) and Nugraheni et al.
168 (2015). One gram of encapsulated sample was placed in an Erlenmeyer, dissolved in
169 20 ml of ethanol 96% and covered with aluminium foil. The sample was extracted by

170 an ultrasonicator instrument at 50°C and 45 kHz frequency for 45 minutes. Filtration
171 was carried out to separate the insoluble polymer fragments. The filtrate was
172 transferred into an empty porcelain cup of known weight and then put in an oven at
173 45°C for 24 hours. The measurement results were recorded as the final weight of the
174 cup. The total trapped oil yield was calculated by using the following formula:

$$175 \quad \text{Total Oil (\%)} = \frac{\text{Final Weight of Cup (g)} - \text{Empty Cup Weight (g)}}{\text{Weight of Sample (1 g)}} \times 100\%$$

$$176 \quad \text{Trapped Oil (\%)} = \text{Total Oil (\%)} - \text{Surface Oil (\%)}$$

177

178 | **2.5.2. Antioxidant Activity Analysis** (Hussein et al., 2017 and Amin et al., 2013)

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

$$187 \quad \text{Antioxidant activity (\%)} = \left[\frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \right] \times 100\%$$

188

189 | **2.5.3. Yield Calculation** (Yuniarti et al., 2013)

190 The yield was calculated based on the weight of the encapsulated powder produced
191 from vacuum drying compared to the total solids of the emulsion material

192 | (encapsulating material and oleoresin) [\(Yuniarti et al., 2013\)](#). The yield content (dry
193 | basis) was determined by the following formula:

$$194 \quad \% \text{ Yield} = \frac{\text{weight of microcapsule powder (g)}}{\text{weight of emulsion solids (g)}} \times 100\%$$

195

196 | **2.5.4. Moisture Analysis** [\(Lindani, 2016\)](#)

197 | Moisture content of the sample was tested by using a moisture analyser [\(Lindani,](#)
198 | [2016\)](#). Half until 1 gram of sample was placed into the tool. The instrument will heat
199 | the sample until the value of the water content is shown constantly (approximately
200 | for 10 minutes).

201

202 | **2.5.5. Surface Oil Analysis** (Hussein at al., 2017 and Yazicioglu et al., 2015 with 203 | modification)

204 | One gram of encapsulated sample was put into a centrifuge tube and 5 ml of ethanol
205 | [\(96%\)](#) was added. The mixture was centrifuged at 1700 rpm for 15 minutes. After
206 | that, the sample was filtered through filter paper and washed with 7.5 ~~ml~~ mL of
207 | ethanol twice. The filtrate was transferred ~~in-to~~ to a cup of known weight, then dried in
208 | an oven for 24 hours. After that, the sample was put in a desiccator for 15 minutes
209 | and weighed as the final weight. The amount of surface oil was calculated by using
210 | the formula below:

$$211 \quad \text{Surface Oil (\%)} = \frac{\text{Cup final weight (g)} - \text{Empty cup weight (g)}}{\text{Sample weight (1 g)}} \times 100\%$$

212

213 | **2.5.6. Water Activity (a_w) Analysis** [\(AquaLab, 2016\)](#)

214 | Water activity was measured by using an a_w meter [\(AquaLab, 2016\)](#). First, a
215 | homogenized sample was put into a clean and dry containers cup, completely
216 | covering the bottom of the cup. The container was filled with samples until half a
217 | cup. The sample was measured with a_w meter for 15 minutes and the result
218 | appeared on display (reader).

219

220 | **2.5.7. Colour Measurement** [\(Nguyen et al., 2018 with modification\)](#)

221 | Colour testing on encapsulate was carried out using Chroma meter Minolta CR400.
222 | After instrument calibration, the encapsulated sample was placed in a transparent
223 | plastic and a Chroma meter beam was released [\(Nguyen et al., 2018\)](#). The
224 | measurement showed the values of L^* , a^* and b^* . The value of L^* (lightness) of 100
225 | indicates a light coloured sample. The value of a^* indicates the tendency of red (+)
226 | and green (-). The b^* value indicates yellow (+) and blue (-).

227

228 | **3. Results and Discussion**

229 | **3.1. Nutmeg Seed Oleoresin**

230 | Nutmeg seed oleoresin was processed by extraction using ethanol to dissolve the
231 | polar substances in nutmeg powder. The ethanol solvent was chosen because of its
232 | polarity by the presence of the -OH group to dissolve polar molecules. Oleoresin is a
233 | polar substance, while nutmeg butter is a non-polar substance. In addition, ethanol
234 | has a low boiling point at 78.4°C and 1 atm to easily remove the solvent from the
235 | extract (Susanti et al., 2012 and Yulianti, 2010).

236

237 | **3.2. Encapsulation of Nutmeg Seed Oleoresin**

238 | The encapsulation process ~~aimed~~aims to protect the active substance from
239 | oxidation by air and light, thereby increasing the shelf life of the product. The
240 | emulsification is applied at various levels of agitation speed by using a homogenizer.
241 | Homogenizer could reduce the oil globules' size and stabilize the emulsion by
242 | preventing coalescent. The agitation speed during homogenization could affect the
243 | droplet size, where increasing the agitation speed would result in a smaller emulsion
244 | droplet size.

245

246 | In this study, a thin layer drying technique with a vacuum dryer was used. Principally,
247 | this technique will create a thin layer on the glass pan surface, followed by drying in
248 | the oven. The thickness of the layer should be uniform by assuming a uniform
249 | temperature distribution (Onwude et al., 2016). This technique is very efficient with
250 | low temperature (<60°C) application, so it will not damage the heat-sensitive
251 | substances. In addition, the utilization of a vacuum oven at a low-pressure setting
252 | (0.5 atm) would evaporate the water below the normal boiling point. It could
253 | preserve the texture and appearance of the material, minimize the loss of active
254 | substances such as aroma and volatile compounds, reduce nutrition degradation,
255 | reduce browning due to oxidation, and save energy (Prasetyaningrum, 2010).

256

257 | The measurement results of trapped oil, antioxidant activity, yield, moisture content,
258 | surface oil, and water activity could be seen in Table 2. ~~To evaluate the significance~~
259 | ~~of each factor, an~~An analysis of variance (ANOVA) was performed. ~~to evaluate the~~
260 | significance of each factor. ANOVA results showed that the polynomial quadratic
261 | model was a suitable model to represent the experimental data at a 95% confidence

262 level. The correlation coefficient from ANOVA statistical analysis is shown in Table 3.
263 Based on the statistical analysis, agitation speed (RPM), MD, and WPI addition had a
264 significant effect on trapped oil, antioxidants, and yield ($p < 0.05$). Coefficient
265 | Regression Tables to predict results through polynomial equations ~~is~~are presented
266 | in Table 4.

267

268 **3.3. Trapped Oil of Nutmeg Seed Oleoresin Encapsulate**

269 Trapped oil content is a parameter representing how much oleoresin (core material)
270 is encapsulated by the coating material. By encapsulation, the core material or active
271 substances could be protected from degradation reactions, aroma and volatile
272 compound loss, thus maintaining flavour stability during storage (Kanakdande et al.,
273 2007). The result of trapped oil measurement is presented in the Experimental
274 Response Table (Table 2) and illustrated as a three-dimensional graph (Figure 1).

275

276 Based on Figure 1 and Table 3, all three variables (RPM, MD, and WPI) had a
277 significant effect on trapped oil content. From the graphs (Figure 1), there is a rising
278 ridge chart where the critical point or stationary point is not in the experimental area
279 but occupies the maximum point. Formula with the ratio of oleoresin and coating
280 1:1 had the highest trapped oil yield of 8.1-13.7%, followed by the formula with the
281 ratio of 1:2 (4.5-10.9%) and 1:3 (6.1-8.6%), respectively. Maltodextrin as a coating
282 material plays an important role in the effectiveness of oil trapping. If the coating
283 material amount is insufficient to wrap core materials, there will be a lot of core
284 material on the outer surface of the encapsulate (Jayanudin et al., 2017). WPI
285 contains up to 90% of free proteins which makes those proteins easily dissolve in the

286 emulsion system and interact with oil (oleoresin). Thus, the more WPI added, the
287 more stable the emulsion system will be. In addition, whey protein could function as
288 a suitable encapsulating agent when used in isolate form (Young et al., 1993 ~~in~~and
289 Nasrullah, 2010). In addition, emulsion stability is affected by the higher agitation
290 speed, which reduces the size of the oil globule (oleoresin) where oil globules can be
291 completely covered by the coating material. The homogenization process can also
292 reduce the tendency of fat globules to clump or coalesce due to smaller droplet
293 sizes (Tetra Pak, 2015).

294

295 **3.4. Surface Oil of Nutmeg Seed Oleoresin Encapsulate**

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

301

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

31.

311 Table 3 indicates that MD has a substantial effect on the surface oil amount. The low
312 amount of maltodextrin to core material will result in insufficient coating material to
313 cover the whole surface of the oleoresin droplets to strengthen the capsule wall
314 (Laohasongkrama et al., 2011). The addition of maltodextrin which is not balanced
315 with whey protein will increase the level of surface oil. Maltodextrin is a lipophobic
316 compound, so it cannot bind to the oil molecule. So, it is not enough to emulsify the
317 oil to be encapsulated, and result in a lot of oil that is not encapsulated. Therefore,
318 the addition of WPI is used in the formula since WPI is considered a suitable
319 emulsifier in the food system. ~~WPI is primarily absorbed at the interface of oil-in-water (o/w)~~
320 ~~droplets, where it forms a layer that protects the droplets from coalescence (McCrae et al., 1999;~~
321 ~~Assagaf et al., 2013). Principally, WPI will be absorbed in the interface of oil-in-water~~
322 ~~(o/w) droplets and forms a layer that can protect droplets from coalescent (McCrae~~
323 ~~et al., 1999 in Assagaf et al., 2013).~~

324

325 3.5. Antioxidant Activity of Nutmeg Seed Oleoresin Encapsulate

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

33.

331 In this research, 1,1-diphenyl-2-picrylhydrazyl (DPPH) ~~becomes~~ became the free
332 radical that reacted with active substances from the encapsulated oleoresin.
333 Flavonoids in nutmeg oleoresin will donate hydrogen radicals (H+) or oxidized by the

334 DPPH and result in more stability and low reactivity of free radicals (Amic et al., 2003
335 ~~in-and~~ Karim et al., 2015). A study by Sharma et al. (2015) reported that total
336 flavonoids in onions would decrease after heating at a high temperature. It indicates
337 that some flavonoids might be destroyed at high-temperature treatment. Nutmeg
338 oleoresin contains phytochemical compounds with antioxidant activity such as
339 myristicin, isoeugenol, and eugenol compounds (Ginting et al., 2017).

340

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

351

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352 From the results in Table 2, there was an interaction between surface oil and
353 antioxidants, where ~~in~~ encapsulates with low surface oil will have low antioxidants
354 and vice versa. In other words, the encapsulate with ~~a~~ higher amount of surface oil
355 will be more susceptible to damage (oxidation) ~~compared-to~~ ~~than an~~ encapsulates
356 that have low surface oil or ~~not-too-high~~ ~~moderate~~ antioxidant activity.

357

358 | In Figure 3., there are three graphs ~~illustrated~~ illustrating a maximum surface visual
359 | where the critical point is in the experimental region and the stationary point is at
360 | the maximum point. The antioxidant activity values of encapsulate at three different
361 | core and coating material ratios (1:1, 1:2, 1:3) were 53.79-91.33%, 13.31-91.71%,
362 | and 17.25-61.07%, respectively. The high amount of maltodextrin as encapsulating
363 | material will produce low antioxidant activity if the ratio of MD:WPI is not
364 | proportional. This is due to the wall being formed is getting thicker. Maltodextrin has
365 | good stability against oil oxidation but has low oil retention, thus it is usually
366 | combined with an emulsifier (Kenyon, 1995 in Nasrullah, 2010). If the composition of
367 | maltodextrin is high and not balanced with whey protein, some oleoresin
368 | compounds might be damage during drying process because their presence on the
369 | surface. At treatment 17 (91.71%) (ratio of core material: coating = 1:2) antioxidants
370 | produced at a higher rate than treatment 12 (91.33%) (ratio of core material: coating
371 | = 1:1), this can be caused by the number of solids that are too high which results in
372 | puffing (swelling) and cracking of particles so that the encapsulate ~~ruptured~~ ruptures
373 | because of high temperatures, and the core material comes out of the capsule (Li et
374 | al., 2015).

375

376 | **3.6. Yield of Nutmeg Oleoresin Encapsulate**

377 | The yield (in percentage) of encapsulation could indicate how optimal the powder
378 | produced from each formula and how much loss in each formula is. Based on Table
379 | 2, the yield value of the encapsulate is not too high and ranges from 51.25% to
380 | 72.92%. It might be due to many product losses during the processing. Based on
381 | Table 3, the MD and WPI variables have a significant effect on the encapsulate yield

382 (p<0.05). The addition of maltodextrin and whey protein isolate as a coating material
383 has a higher total solid, thus giving a higher yield.

384

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

391

392 **3.7. Moisture Content of Nutmeg Oleoresin Encapsulate**

393 Moisture content is one of the encapsulate quality aspects. The higher moisture
394 content in encapsulates will trigger the oxidation and hydrolysis reaction resulting in
395 quality degradation and biological damage (Bakry et al., 2015). According to SNI 01-
396 3709-1995, the maximum moisture content of spice powder is 12% (National
397 Standardization Agency 1995). The moisture content of encapsulated powder in this
398 | study was in the range of 7.39% to 9.30%, so ~~they-it~~ met the SNI water content
399 | specification. Based on ANOVA results in Table 3, the WPI variable had a significant
400 | effect on water content (p<0.05). Whey protein isolate is very hygroscopic or
401 | sensitive to moisture and stickiness (Hogan & Callaghan, 2013). Hence, the addition
402 | of whey protein isolate could increase the water content of the encapsulated
403 | powder.

404

4.0 Figure 5 shows the rising ridge graph where the critical point or stationary point is
4.6 | not in the experimental region and the ~~stationer~~stationary point is at the maximum
4.7 | point. The addition of coating material affected on the water content of
4.8 | encapsulates powder. The lowest water content was obtained from formula with the
4.9 | ratio of core and coating material 1:1, while the highest water content was from the
4.10 | formula with ratio of 1:3. The addition of whey protein isolate has a significant effect
4.11 | in increasing the water content of the encapsulated powder due to the hygroscopic
4.12 | properties of whey protein. Based on Prasetyo in Ramadhani (2006), too much
4.13 | addition of coating material as a filler will cause clotting and case hardening. As a
4.14 | result, the moisture inside the droplet cannot come out and contact with the drying
4.15 | air. The droplet surface is covered by solid substances and will minimize the water-
4.16 | hot air contact area. Therefore, adding coating material could increase the water
4.17 | content.

4.18

4.19 **3.8. Water Activity of Nutmeg Oleoresin Encapsulate**

4.20 Water activity (a_w) indicates the amount of free water used by microorganisms to
4.21 | grow. Therefore, this parameter is important to define the microbiology
4.22 | microbiological risk in encapsulate powder and the stability during storage. The
4.23 | water ~~activities~~activity values of oleoresin encapsulate in this study were in the
4.24 | range 0.54 - 0.58. Tapia et al. (2020) stated that the food product must have water
4.25 | activity below 0.6, to prevent the mold growth. Based on ANOVA analysis in Table 3,
4.26 | no variable affect water activity in the powder. It might be due to the vacuum oven
4.27 | ability to produce water vapor during off condition. In addition, whey protein isolate

428 properties is hygroscopic, so that the water vapor in the vacuum oven will be easily
429 absorbed and this will increase the water activity.

430

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

437

438 3.9. Colour Analysis of Nutmeg Oleoresin Encapsulate

439 The colour of the encapsulated powder indicated the physical properties based on
440 the constituent materials. Principally, the Chroma meter worked-works through the
441 interaction of energy- diffuse diffuse-light and atoms or molecules of an object being
442 analyzed. The light source of a xenon lamp was beamed onto the sample surface and
443 was reflected back to the spectral sensor. Six high-sensitivity silicon photocells with a
444 dual-back beam system measured the reflected light of the sample (Candra et al.,
445 2014). The L* indicator was indicated by a value of 0 (black/dark) to 100
446 (light/white). The reflected light of the L* indicator showed the achromatic colors of
447 white, gray and black. The a* indicator showed a chromatic color of red if positive
448 and green if negative. A positive b* indicator indicated a yellow chromatic colour and
449 a negative b* value indicated a blue colour intensity.

450

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

4.08

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

4.09

4. Conclusion

The nutmeg oleoresin encapsulation process was optimized by the Response Surface Methodology (desirability value of 0.794) and resulted in following setting variable:

3500 rpm of agitation speed, 4 grams of maltodextrin, and 4 grams of whey protein isolate addition. It means that those setting variables could produce nutmeg oleoresin encapsulates as desired (optimum) is 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 8.63% , and colour 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 encapsulate needs to be done.

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Conflicts of Interest

There are none to declare.

Data Availability

The data is not publicly available.

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JFPP-06-22-1489 - Optimization of Encapsulated Agents and Agitation Speed on the Physicochemical Characteristics of Vacuum Dried Nutmeg Seed Oleoresin (*Myristica fragrans*)

Dear Editorial Office,

I have revised the manuscript and sent it to Prof. Anet Rezek Jambrak, Should I submit it to the link of ScholarOne Manuscript as well, however I could not find the button for submission of revised manuscript.
I am looking forward to hearing from you.

Thank you.

Sincerely,
Dr. Victoria Ananingsih
Journal of Food Processing and Preservation

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4. Submission of Revised Manuscript



Kristina Ananingsih <kristina@unika.ac.id>

Journal of Food Processing and Preservation - Decision on Manuscript ID JFPP-06-22-1489

Kristina Ananingsih <kristina@unika.ac.id>
To: anet.rezek.jambrak@pbf.hr

Thu, Aug 4, 2022 at 6:47 PM

Dear Prof. Anet Rezek Jambrak,
Associate Editor Comments to Author

We have revised the manuscript based on the comments.
Could you please find attached files of our revised manuscript.
We are looking forward to your feedback.

Thank you.

Kind regards,
Victoria Kristina Ananingsih

[Quoted text hidden]

--
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Reviewer(s)' Comments to Author:

Reviewer #1

Comments to the Author

all comments are attached in PDF file

Revision #1

We have already revised it (attached)

Reviewer: 2

Comments to the Author

Dear Authors;

The article deals with the production of oleoresin and its encapsulation. However, I could not find any information about the composition of this oleoresin, both in the introduction and the results of the article. In the introduction, it is mentioned that the stability of oleoresin is good and the concentration is intense. The antioxidant effect has been examined and found to be effective, why does this effect arise?

Revision #2

We have added literature and results

Line 77-78

Nutmeg oleoresin extracted by maceration method obtained $20.07 \pm 0.23\%$ oleoresin in dry weight.

Line 249-250

The yield of oleoresin produced in this research was 30.4%.

Line 294-295

The higher trapped oil showed the effectiveness of encapsulation in maintaining oleoresin of nutmeg.

Line 360-361

Application of this process conditions could maintain the antioxidant activity of nutmeg seed oleoresin.

1 **Optimization of Encapsulated Agents and Stirring Speed on the Physicochemical**
2 **Characteristics of Vacuum Dried Nutmeg Seed Oleoresin (*Myristica fragrans*)**

3

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12

13 **6,489 words**

14

15 **Vacuum Dried Nutmeg Seed Oleoresin**

16

17

18 **ABSTRACT:** Nutmeg seed oleoresin (*Myristica fragrans* Houtt) from nutmeg seed
19 extraction contains active substances. However, oleoresins' active substances are
20 commonly heat-sensitive, so encapsulation is needed. Encapsulation is the process of
21 wrapping particles containing active ingredients in a homogeneous or heterogeneous
22 matrix that produces encapsulated powder. The objective of this study was to obtain
23 the best combination of encapsulated agents concentration (maltodextrin and whey
24 protein isolate) and agitation speed on the physicochemical characteristics of nutmeg
25 seed oleoresin encapsulated using a vacuum drying method. Encapsulation of nutmeg
26 seed oleoresin was performed with comparative parameters namely agitation speed
27 (3000, 3500, 4000 rpm), maltodextrin (MD) concentrations (ratio of MD to nutmeg
28 seed oleoresin= 2:4, 4:4, 6:4), and Whey Protein Isolate (WPI) concentrations (ratio of
29 WPI to nutmeg oleoresin= 6:4, 4:4, 2:4). The physicochemical analysis consisted of
30 trapped oil content, antioxidant activity, yields, water content, surface oil, water
31 activity, and colour testing. The physicochemical data were further analysed by
32 Response Surface Methodology (RSM) to get an optimum formula. The best formula
33 resulted from a process at an agitation speed of 3500 rpm and the addition of 4 grams
34 of maltodextrin and 4 grams of WPI. That formula had a trapped oil content 10.23%,
35 antioxidant activity 91.50%, yield 66.79%, water activity 0.55, moisture content 8.63,
36 colour intensity L* 65.47, a* 7.90, and b* 19.57. This formula could be applied to
37 produce nutmeg seed oleoresin powder with good physicochemical properties.

38

39 Keywords: Nutmeg oleoresin, encapsulation, vacuum drying, Response Surface
40 Methodology

41

42

43 **Practical Application:**

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

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67 **Introduction**

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

75

76 Nutmeg oleoresin (*Myristica fragrans* Houtt), as the result of fresh nutmeg seed
77 extraction with ethanol, contains active substances. Nutmeg oleoresin extracted by
78 maceration method obtained $20.07 \pm 0.23\%$ oleoresin in dry weight (Assagaf, et al.,
79 2012). Nowadays, the utilization of nutmeg oleoresin as a flavouring agent is preferred
80 by the food industry compared to fresh herbs, because of its stability and highly
81 concentrated form. In addition, oleoresin also has a homogenous flavour, aroma,
82 pungency, standardized quality, and longer shelf life (Khadka, 2018). However,
83 oleoresin is prone to oxidation by the presence of air, light and water. Therefore, the
84 encapsulation process is carried out to create a barrier between the active substances
85 and other external factors (Jafari, 2017).

86

87 The most common coating materials for encapsulation are maltodextrin and arabic
88 gum (Lantigua et al., 2011 and Elsebaie & Essa, 2018). Based on research by Zilberboim
89 et al. (1986), the bell pepper oleoresin encapsulated with arabic gum was considered
90 an expensive and non-feasible coating ingredient. Research by Nurlaili et al. (2014)

91 reported that the microencapsulation of pulp ginger oleoresin with maltodextrin could
92 reach an encapsulation efficiency of up to 22%. Therefore, maltodextrin is preferably
93 used as a coating agent because of its affordable price, neutral taste and aroma,
94 water-soluble and film-forming properties, low viscosity at high solids concentrations,
95 and is less prone to oxidation (Fernandes et al., 2014). The disadvantage of using
96 maltodextrin is unstable emulsion stability to trap oleoresin. In this case, emulsifier
97 addition could aid in improving coating performance. Whey protein isolate (WPI) is
98 considered a suitable emulsifier in the food system. Principally, WPI will be absorbed
99 at the interface of oil-in-water (o/w) droplets where it forms a layer that protects
100 droplets from coalescent (McCrae et al., 1999 in Assagaf et al., 2013). The agitation
101 speed of the homogenizer could affect the droplet size. The higher the agitation speed,
102 the smaller size of the oil droplet. Research by Jayanudin et al. (2018) reported that
103 the higher agitation speed would increase the Reynolds number (Re) and reduce the
104 emulsion droplet size.

105

106 Based on the explanation above, it is necessary to optimize the encapsulation of
107 nutmeg oleoresin with different agitation speeds and ratios of maltodextrin and WPI.
108 Processing data by applying Response Surfaces Methodology (RSM) made this
109 research important by analysing the optimum points and illustrating them in a three-
110 dimensional graph.

111

112 **1. Materials and Methods**

113 **2.1. Materials**

114 The extraction materials were nutmeg (*Myristica fragrans*), ethanol 96% solvent, and
115 Whatman filter paper number 1. The encapsulation materials were extracted nutmeg
116 oleoresin, maltodextrin (DE 15-20) and whey protein isolate (WPI) 90, and distilled
117 water; while materials for analysis were DPPH (Diphenyl Picryl Hydrazyl) solution 0.06
118 mM, methanol 99.98%, ethanol 96%, and filter paper.

119

120 **2.2. Nutmeg Oleoresin Extraction with Ultrasound-assisted Extraction (UAE)**

121 First, fresh nutmeg seeds were dried in the oven at 45°C for 24 hours. The dried
122 nutmeg seeds were cut and ground before sieving with 36 mesh-size. Nutmeg powder
123 was dissolved in ethanol 96% at a ratio of 1:10. The extraction was carried out with an
124 Erlenmeyer containing a sample, soaked in the ultrasonic cleaner UC-10SD at 50°C and
125 45 kHz frequency for 37.5 minutes. After that, the mixture was stored at chiller \pm 4°C
126 for 30 minutes for fat phase separation (Rodianawati, 2010 and Assagaf et al., 2012),
127 then filtered. The solvent in the filtrate was evaporated with a rotary vacuum
128 evaporator (40°C, speed 52 rpm, and pressure 0.09MPa) until all solvent evaporated,
129 and a thick nutmeg oleoresin was obtained (Trendafilova et al., 2010 modified).
130 Oleoresin was kept in a glass bottle laminated with aluminium foil and stored in a
131 chiller.

132

133 **2.3. Response Surface Methodology (RSM)**

134 The formula determination was generated from the Statistica 6.0 Response Surface
135 Methodology (RSM) software as presented in Table 1 and produced 17 treatments for
136 oleoresin encapsulation. The range of agitation speed was set between 2700 and 4000
137 rpm, and 0.64 and 7.36 gram for maltodextrin and WPI. RSM generated three levels

138 of oleoresin and total coatings materials (MD and WPI) ratio. Ratio of 1:1 applied on
 139 treatment 3,7,12 and 13; Ratio of 1:2 applied on treatment 1, 4, 5, 8, 9, 10, 15, 16 and
 140 17; Ratio of 1:3 applied on treatment 2, 6, 11 and 14. RSM with factorial design,
 141 namely the Central Composite Design (CCD), could simplify the number of
 142 experiments and useful for testing multiple process variables. The CCD design is a 2^k
 143 factorial design or called as partial factorial. It is expanded by adding observation
 144 points at the centre, so the predicted parameter coefficients will be on the quadratic
 145 surface (second order) (Montgomery, 2001 in Lubis, 2010). Generally, CCD consists of
 146 a factorial point (2^k), axial point ($2k$), and a centre point (n_c); where k is the variable
 147 number. The 2^k factorial design is used for experiments consisting of k factorial, where
 148 at the low level is coded as (-1), the middle level as (0), the high level as (+1), and the
 149 minimum and maximum level at the axial point as $(-\alpha)$ and $(+\alpha)$. The calculation of
 150 the α value on the rotate able design CCD is as follows:

151 $\alpha = [\text{number of runs factorial point}]^{1/4} = (2^k)^{1/4}$.

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

154 $-\alpha = (0) - 1.682 [(0) - (-1)]$ || $+\alpha = (0) + 1.682 [(0) - (-1)]$

155 **RPM:** $-\alpha = (3500) - 1.682 [(3500) - (3000)] = 2659$

156 $+\alpha = (3500) + 1.682 [(3500) - (3000)] = 4341$

157 **MD:** $-\alpha = (4) - 1.682 [(4) - (2)] = 0.636$

158 $+\alpha = (4) + 1.682 [(4) - (2)] = 7.364$

159 **WPI:** $-\alpha = (4) - 1.682 [(4) - (2)] = 0.636$

160 $+\alpha = (4) + 1.682 [(4) - (2)] = 7.364$

161 Therefore, each factor would have 5 levels of test points. The results of CCD analysis
162 are presented in the form of graphs based on mathematical models and respond
163 surfaces. Those outputs are useful to predict the optimal value from the responses
164 and to provide information on the interaction between the dependent and
165 independent variables (Yousefi et al., 2016)

166

167 **2.4. Encapsulation of Nutmeg Oleoresin**

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

175

176 **2.5. Analysis of Encapsulated Nutmeg Seed Oleoresin**

177 Physicochemical analyses of encapsulated oleoresins were trapped oil content,
178 antioxidant activity, yield, water content, surface oil, water activity, and colour
179 intensity.

180

181 **2.5.1. Trapped Oil Content**

182 Trapped oil content was estimated as outlined by Asyhari (2013) and Nugraheni et al.
183 (2015). One gram of encapsulated sample was placed in an Erlenmeyer, dissolved in
184 20 ml of ethanol 96% and covered with aluminium foil. The sample was extracted by

185 an ultrasonicator instrument at 50°C and 45 kHz frequency for 45 minutes. Filtration
186 was carried out to separate the insoluble polymer fragments. The filtrate was
187 transferred into an empty porcelain cup of known weight and then put in an oven at
188 45°C for 24 hours. The measurement results were recorded as the final weight of the
189 cup. The total trapped oil yield was calculated by using the following formula:

$$190 \quad Total\ Oil\ (\%) = \frac{Final\ Weight\ of\ Cup(g) - Empty\ Cup\ Weight(g)}{Weight\ of\ Sample\ (1\ g)} \times 100\%$$

$$191 \quad Trapped\ Oil\ (\%) = Total\ Oil\ (\%) - Surface\ Oil\ (\%)$$

192

193 **2.5.2. Antioxidant Activity Analysis**

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

$$202 \quad Antioxidant\ activity\ (\%) = \left[\frac{absorbance\ of\ control - absorbance\ of\ sample}{absorbance\ of\ control} \right] \times 100\%$$

203

204 **2.5.3. Yield Calculation**

205 The yield was calculated based on the weight of the encapsulated powder produced
206 from vacuum drying compared to the total solids of the emulsion material

207 (encapsulating material and oleoresin) (Yuniarti et al., 2013). The yield content (dry
208 basis) was determined by the following formula:

$$209 \quad \% \text{ Yield} = \frac{\text{weight of microcapsule powder (g)}}{\text{weight of emulsion solids (g)}} \times 100\%$$

210

211 **2.5.4. Moisture Analysis**

212 Moisture content of the sample was tested by using a moisture analyser (Lindani,
213 2016). Half until 1 gram of sample was placed into the tool. The instrument will heat
214 the sample until the value of the water content is shown constantly (approximately
215 for 10 minutes).

216

217 **2.5.5. Surface Oil Analysis**

218 One gram of encapsulated sample was put into a centrifuge tube and 5 ml of ethanol
219 96% was added. The mixture was centrifuged at 1700 rpm for 15 minutes. After that,
220 the sample was filtered through filter paper and washed with 7.5 mL of ethanol twice.
221 The filtrate was transferred to a cup of known weight, then dried in an oven for 24
222 hours. After that, the sample was put in a desiccator for 15 minutes and weighed as
223 the final weight (Hussein at al., 2017 and Yazicioglu et al., 2015). The amount of
224 surface oil was calculated by using the formula below:

$$225 \quad \text{Surface Oil (\%)} = \frac{\text{Cup final weight (g)} - \text{Empty cup weight (g)}}{\text{Sample weight (1 g)}} \times 100\%$$

226

227 **2.5.6. Water Activity (a_w) Analysis**

228 Water activity was measured by using an a_w meter (AquaLab, 2016). First, a
229 homogenized sample was put into a clean and dry container cup, completely covering

230 the bottom of the cup. The container was filled with samples until half a cup. The
231 sample was measured with a_w meter for 15 minutes and the result appeared on
232 display (reader).

233

234 **2.5.7. Colour Measurement**

235 Colour testing on encapsulate was carried out using Chroma meter Minolta CR400.
236 After instrument calibration, the encapsulated sample was placed in a transparent
237 plastic and a Chroma meter beam was released (Nguyen et al., 2018). The
238 measurement showed the values of L^* , a^* and b^* . The value of L^* (lightness) of 100
239 indicates a light coloured sample. The value of a^* indicates the tendency of red (+) and
240 green (-). The b^* value indicates yellow (+) and blue (-).

241

242 **3. Results and Discussion**

243 **3.1. Nutmeg Seed Oleoresin**

244 Nutmeg seed oleoresin was processed by extraction using ethanol to dissolve the
245 polar substances in nutmeg powder. The ethanol solvent was chosen because of its
246 polarity by the presence of the -OH group to dissolve polar molecules. Oleoresin is a
247 polar substance, while nutmeg butter is a non-polar substance. In addition, ethanol
248 has a low boiling point at 78.4°C and 1 atm to easily remove the solvent from the
249 extract (Susanti et al., 2012 and Yulianti, 2010). The yield of oleoresin produced in this
250 research was 30.4%.

251

252 **3.2. Encapsulation of Nutmeg Seed Oleoresin**

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

259

260 In this study, a thin layer drying technique with a vacuum dryer was used. Principally,
261 this technique will create a thin layer on the glass pan surface, followed by drying in
262 the oven. The thickness of the layer should be uniform by assuming a uniform
263 temperature distribution (Onwude et al., 2016). This technique is very efficient with
264 low temperature (<60°C) application, so it will not damage the heat-sensitive
265 substances. In addition, the utilization of a vacuum oven at a low-pressure setting (0.5
266 atm) would evaporate the water below the normal boiling point. It could preserve the
267 texture and appearance of the material, minimize the loss of active substances such
268 as aroma and volatile compounds, reduce nutrition degradation, reduce browning due
269 to oxidation, and save energy (Prasetyaningrum, 2010).

270

271 The measurement results of trapped oil, antioxidant activity, yield, moisture content,
272 surface oil, and water activity could be seen in Table 2. An analysis of variance
273 (ANOVA) was performed to evaluate the significance of each factor. ANOVA results
274 showed that the polynomial quadratic model was a suitable model to represent the
275 experimental data at a 95% confidence level. The correlation coefficient from ANOVA
276 statistical analysis is shown in Table 3. Based on the statistical analysis, agitation speed

277 (RPM), MD, and WPI addition had a significant effect on trapped oil, antioxidants, and
278 yield ($p < 0.05$). Coefficient Regression Tables to predict results through polynomial
279 equations are presented in Table 4.

280

281 **3.3. Trapped Oil of Nutmeg Seed Oleoresin Encapsulate**

282 Trapped oil content is a parameter representing how much oleoresin (core material)
283 is encapsulated by the coating material. By encapsulation, the core material or active
284 substances could be protected from degradation reactions, aroma and volatile
285 compound loss, thus maintaining flavour stability during storage (Kanakdande et al.,
286 2007). The result of trapped oil measurement is presented in the Experimental
287 Response Table (Table 2) and illustrated as a three-dimensional graph (Figure 1).

288

289 Based on Figure 1 and Table 3, all three variables (RPM, MD, and WPI) had a significant
290 effect on trapped oil content. From the graphs (Figure 1), there is a rising ridge chart
291 where the critical point or stationary point is not in the experimental area but occupies
292 the maximum point. Formula with the ratio of oleoresin and coating 1:1 had the
293 highest trapped oil yield of 8.1-13.7%, followed by the formula with the ratio of 1:2
294 (4.5-10.9%) and 1:3 (6.1-8.6%), respectively. The higher trapped oil showed the
295 effectiveness of encapsulation in maintaining oleoresin of nutmeg. Maltodextrin as a
296 coating material plays an important role in the effectiveness of oil trapping. If the
297 coating material amount is insufficient to wrap core materials, there will be a lot of
298 core material on the outer surface of the encapsulate (Jayanudin et al., 2017). WPI
299 contains up to 90% of free proteins which makes those proteins easily dissolve in the
300 emulsion system and interact with oil (oleoresin). Thus, the more WPI added, the more

301 stable the emulsion system will be. In addition, whey protein could function as a
302 suitable encapsulating agent when used in isolate form (Young et al., 1993 and
303 Nasrullah, 2010). In addition, emulsion stability is affected by the higher agitation
304 speed, which reduces the size of the oil globule (oleoresin) where oil globules can be
305 completely covered by the coating material. The homogenization process can also
306 reduce the tendency of fat globules to clump or coalesce due to smaller droplet
307 sizes (Tetra Pak, 2015).

308

309 **3.4. Surface Oil of Nutmeg Seed Oleoresin Encapsulate**

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

315

316 Figure 2 shows a saddle system graph where the elliptical contour extends significantly
317 along with one of its main axes. The amount of coating material influenced the surface
318 oil of the encapsulate. Formula with a core and coating material ratios of 1:1 showed
319 the highest surface oil yield (5-9%), followed by the ratio of 1:2 (2.3-7.7%) and 1:3 (2.9-
320 4.2%), respectively. As presented in Table 2, it can be seen that increasing the amount
321 of coating material will reduce the amount of surface oil. It might be due to the thicker
322 encapsulated wall formed, so the amount of oleoresin that comes out will be less
323 (Jayanudin et al., 2017). The lower surface oil was connected to the higher trapped oil,
324 which showed the effectiveness of encapsulation process.

325

326 Table 3 indicates that MD has a substantial effect on the surface oil amount. The low
327 amount of maltodextrin to core material will result in insufficient coating material to
328 cover the whole surface of the oleoresin droplets to strengthen the capsule wall
329 (Laohasongkrama et al., 2011). The addition of maltodextrin which is not balanced
330 with whey protein will increase the level of surface oil. Maltodextrin is a lipophobic
331 compound, so it cannot bind to the oil molecule. So, it is not enough to emulsify the
332 oil to be encapsulated, and result in a lot of oil that is not encapsulated. Therefore,
333 the addition of WPI is used in the formula since WPI is considered a suitable emulsifier
334 in the food system. WPI is primarily absorbed at the interface of oil-in-water (o/w)
335 droplets, where it forms a layer that protects the droplets from coalescent (McCrae et
336 al., 1999; Assagaf et al., 2013).

337

338 **3.5. Antioxidant Activity of Nutmeg Seed Oleoresin Encapsulate**

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

343

344 In this research, 1,1-diphenyl2-picrylhydrazyl (DPPH) became the free radical that
345 reacted with active substances from the encapsulated oleoresin. Flavonoids in nutmeg
346 oleoresin will donate hydrogen radicals (H⁺) or oxidized by the DPPH and result in
347 more stability and low reactivity of free radicals (Amic et al., 2003 and Karim et al.,
348 2015). A study by Sharma et al. (2015) reported that total flavonoids in onions would

349 decrease after heating at a high temperature. It indicates that some flavonoids might
350 be destroyed at high-temperature treatment. Nutmeg oleoresin contains
351 phytochemical compounds with antioxidant activity such as myristicin, isoeugenol,
352 and eugenol compounds (Ginting et al., 2017).

353

354 Based on DPPH in vitro testing, the antioxidant activity of fresh nutmeg oleoresin was
355 94.23%, while the antioxidant activity of nutmeg oleoresin encapsulate was ranged
356 from 13.31% to 91.71% (see Table 2). The lowest antioxidant activity value was
357 obtained from treatment 5 with an agitation speed of 4000 rpm, 6 grams of
358 maltodextrin, and 2 grams of whey protein isolate. The highest antioxidant activity
359 values were obtained from treatment 17 with an agitation speed of 3500 rpm, 4 grams
360 of maltodextrin and 4 grams of whey protein isolate. Application of this process
361 condition could maintain the antioxidant activity of nutmeg seed oleoresin. Based on
362 research by Ginting et al. (2017) about the antioxidant activity of n-hexane extract of
363 nutmeg plants, the antioxidant activity of nutmeg seeds was in the range of 60.86%
364 and 87.85%.

365

366 From the results in Table 2, there was an interaction between surface oil and
367 antioxidants, where in encapsulates with low surface oil will have low antioxidants and
368 vice versa. In other words, the encapsulate with a higher amount of surface oil will be
369 more susceptible to damage (oxidation) than an encapsulate that have low surface oil
370 or moderate antioxidant activity.

371

372 In Figure 3., there are three graphs illustrating a maximum surface visual where the
373 critical point is in the experimental region and the stationary point is at the maximum
374 point. The antioxidant activity values of encapsulate at three different core and
375 coating material ratios (1:1, 1:2, 1:3) were 53.79-91.33%, 13.31-91.71%, and 17.25-
376 61.07%, respectively. The high amount of maltodextrin as encapsulating material will
377 produce low antioxidant activity if the ratio of MD:WPI is not proportional. This is due
378 to the wall being formed is getting thicker. Maltodextrin has good stability against oil
379 oxidation but has low oil retention, thus it is usually combined with an emulsifier
380 (Kenyon, 1995 in Nasrullah, 2010). If the composition of maltodextrin is high and not
381 balanced with whey protein, some oleoresin compounds might be damage during
382 drying process because their presence on the surface. At treatment 17 (91.71%) (ratio
383 of core material: coating = 1:2) antioxidants produced at a higher rate than treatment
384 12 (91.33%) (ratio of core material: coating = 1:1), this can be caused by the number
385 of solids that are too high which results in puffing (swelling) and cracking of particles
386 so that the encapsulate ruptures because of high temperatures, and the core material
387 comes out of the capsule (Li et al., 2015).

388

389 **3.6. Yield of Nutmeg Oleoresin Encapsulate**

390 The yield (in percentage) of encapsulation could indicate how optimal the powder
391 produced from each formula and how much loss in each formula is. Based on Table 2,
392 the yield value of the encapsulate is not too high and ranges from 51.25% to 72.92%.
393 It might be due to many product losses during the processing. Based on Table 3, the
394 MD and WPI variables have a significant effect on the encapsulate yield ($p < 0.05$). The

395 addition of maltodextrin and whey protein isolate as a coating material has a higher
396 total solid, thus giving a higher yield.

397

398 Figure 4 shows the formation of a saddle system graph where the elliptical contour
399 extends significantly along one of its main axes and the rising ridge graph where the
400 critical point or stationary point is not in the experimental area and the stationary
401 point is at the maximum point. Formula with the ratio of core and coating material 1:1
402 has the lowest yield of encapsulate (51.25-68.75%), followed by the ration of 1:2
403 (60.00-72.92%), and 1:3 (63.46-71.88%), respectively.

404

405 **3.7. Moisture Content of Nutmeg Oleoresin Encapsulate**

406 Moisture content is one of the encapsulate quality aspects. The higher moisture
407 content in encapsulates will trigger the oxidation and hydrolysis reaction resulting in
408 quality degradation and biological damage (Bakry et al., 2015). According to SNI 01-
409 3709-1995, the maximum moisture content of spice powder is 12% (National
410 Standardization Agency 1995). The moisture content of encapsulated powder in this
411 study was in the range of 7.39% to 9.30%, so it met the SNI water content specification.
412 Based on ANOVA results in Table 3, the WPI variable had a significant effect on water
413 content ($p < 0.05$). Whey protein isolate is very hygroscopic or sensitive to moisture
414 and stickiness (Hogan & Callaghan, 2013). Hence, the addition of whey protein isolate
415 could increase the water content of the encapsulated powder.

416

417 Figure 5 shows the rising ridge graph where the critical point or stationary point is not
418 in the experimental region and the stationary point is at the maximum point. The

419 addition of coating material affected on the water content of encapsulates powder.
420 The lowest water content was obtained from formula with the ratio of core and
421 coating material 1:1, while the highest water content was from the formula with ratio
422 of 1:3. The addition of whey protein isolate has a significant effect in increasing the
423 water content of the encapsulated powder due to the hygroscopic properties of whey
424 protein. Based on Prasetyo in Ramadhani (2006), too much addition of coating
425 material as a filler will cause clotting and case hardening. As a result, the moisture
426 inside the droplet cannot come out and contact with the drying air. The droplet
427 surface is covered by solid substances and will minimize the water-hot air contact
428 area. Therefore, adding coating material could increase the water content.

429

430 **3.8. Water Activity of Nutmeg Oleoresin Encapsulate**

431 Water activity (a_w) indicates the amount of free water used by microorganisms to
432 grow. Therefore, this parameter is important to define the microbiological risk in
433 encapsulate powder and the stability during storage. The water activity values of
434 oleoresin encapsulate in this study were in the range 0.54 - 0.58. Tapia et al. (2020)
435 stated that the food product must have water activity below 0.6, to prevent the mold
436 growth. Based on ANOVA analysis in Table 3, no variable affect water activity in the
437 powder. It might be due to the vacuum oven ability to produce water vapor during off
438 condition. In addition, whey protein isolate properties is hygroscopic, so that the
439 water vapor in the vacuum oven will be easily absorbed and this will increase the water
440 activity.

441

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

448

449 **3.9. Colour Analysis of Nutmeg Oleoresin Encapsulate**

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

461

462 Based on Table 5, the difference in agitation speed showed no effect on colour of
463 encapsulated powder, while the addition of a coating material increased the values of
464 L and b*, and decreased the value of a*. The addition of coating material could reduce
465 the density of brown colour of oleoresin. However, differences in coating formulations

466 did not produce significant differences in values of L, a* and b*. The brown colour of
467 encapsulated powder decreased as amount of the coating material increased.

468

469 **3.10. Optimization of Process Parameter Combinations**

470 The optimum point was predicted by Response Surface Methodology from the
471 combination of optimal conditions and interactions between independent variables
472 (Ratnawati et al., 2018). In the optimization step, the independent variables for
473 optimization were trapped oil, antioxidant activity, and yield. Those variables
474 (parameters) could reflect the effectiveness and efficiency of encapsulation. The
475 Statistica 6.0 RSM program generated five optimum formula solutions as presented in
476 Table 6. Process conditions with an agitation speed of 3500 rpm, 4 grams of
477 maltodextrin and 4 grams of whey protein isolate would produce an encapsulate
478 powder with characteristics for an optimization target of 79.39%. Then, the optimum
479 formula could be achieved by using the polynomial quadratic models shown in Table
480 4.

481

482 **4. Conclusion**

483 The nutmeg oleoresin encapsulation process was optimized by the Response Surface
484 Methodology (desirability value of 0.794) and resulted in following setting variable:
485 3500 rpm of agitation speed, 4 grams of maltodextrin, and 4 grams of whey protein
486 isolate addition. It means that those setting variables could produce nutmeg oleoresin
487 encapsulates as desired (optimum) is 79.39%. The optimum formula had a trapped oil
488 content of 10.23%, antioxidant activity of 91.50%, yield of 66.79%, water activity of
489 0.551, moisture content 8.63%, and colour properties L=65.47, a*= 7.90, and

490 $b^*=19.570$. As a suggestion, further research on the stability and safety (in vivo
491 testing) of nutmeg oleoresin encapsulate needs to be done.

492

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501

502 **Conflicts of Interest**

503 There are none to declare.

504

505 **Data Availability**

506 The data is not publicly available.

507 **References**

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7. Manuscript Accepted



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Manuscript ID JFPP-06-22-1489.R1 - Journal of Food Processing and Preservation

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17-Aug-2022

Dear Dr. Ananingsih:

Your manuscript entitled "Optimization of Encapsulated Agents and Agitation Speed on the Physicochemical Characteristics of Vacuum Dried Nutmeg Seed Oleoresin (*Myristica fragrans*)" by Ananingsih, Victoria; Soedarini, Bernadeta; Andriani, Cynthia; Konstantia, Bernadine Adi; Santoso, Birgitta, has been successfully submitted online and is presently being given full consideration for publication in the Journal of Food Processing and Preservation.

Co-authors: Please contact the Editorial Office as soon as possible if you disagree with being listed as a co-author for this manuscript.

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Decision Letter (JFPP-06-22-1489.R1)

From: Charles.brennan@rmit.edu.au

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CC: kristina@unika.ac.id, bernadeta@unika.ac.id, cynthiaandriani@unika.ac.id, bernardine.agatha99@gmail.com, birgittadevina@gmail.com

Subject: Journal of Food Processing and Preservation - Decision on Manuscript ID JFPP-06-22-1489.R1

Body: 06-Sep-2022

Dear Dr. Ananingsih:

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Sincerely,


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Kristina Ananingsih <kristina@unika.ac.id>
To: help@hindawi.com

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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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Funding information

Ministry of Research and Higher
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AK/SP2H.1/RESEARCH/2019

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 "simplicia" 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 $\pm 4^\circ\text{C}$ 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 ($2k$), 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 $(-\alpha)$ and $(+\alpha)$. 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

$$\alpha = [\text{number of runs factorial point}]^{1/4} = (2^k)^{1/4}$$

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:

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

$$\begin{aligned} \text{RPM: } -\alpha &= (3500) - 1.682[(3500) - (3000)] = 2659 \\ +\alpha &= (3500) + 1.682[(3500) - (3000)] = 4341 \end{aligned}$$

$$\begin{aligned} \text{MD: } -\alpha &= (4) - 1.682[(4) - (2)] = 0.636 \\ +\alpha &= (4) + 1.682[(4) - (2)] = 7.364 \end{aligned}$$

$$\begin{aligned} \text{WPI: } -\alpha &= (4) - 1.682[(4) - (2)] = 0.636 \\ +\alpha &= (4) + 1.682[(4) - (2)] = 7.364 \end{aligned}$$

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:

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

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

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

Factor	p-value					
	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

^aSignificant.

TABLE 4 Coefficient regression value for polynomial quadratic models

Factor	Coefficient regression value									
	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	

^aSignificant.

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).

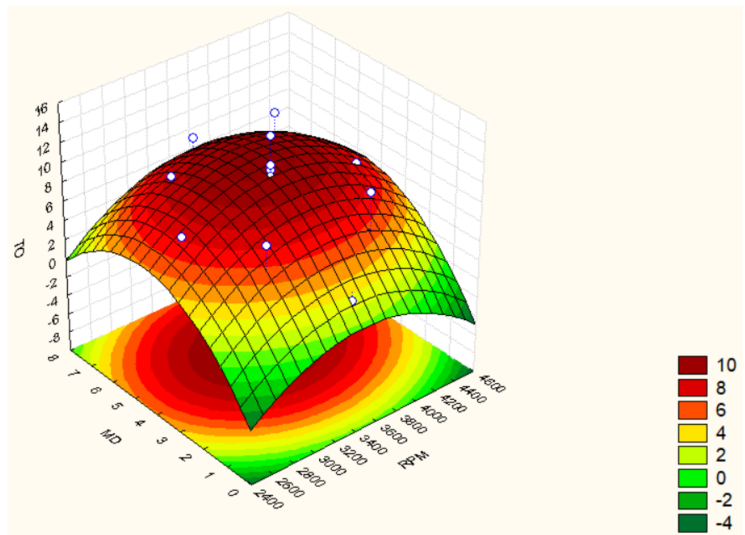
3.4 | Surface oil of nutmeg seed oleoresin encapsulate

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).

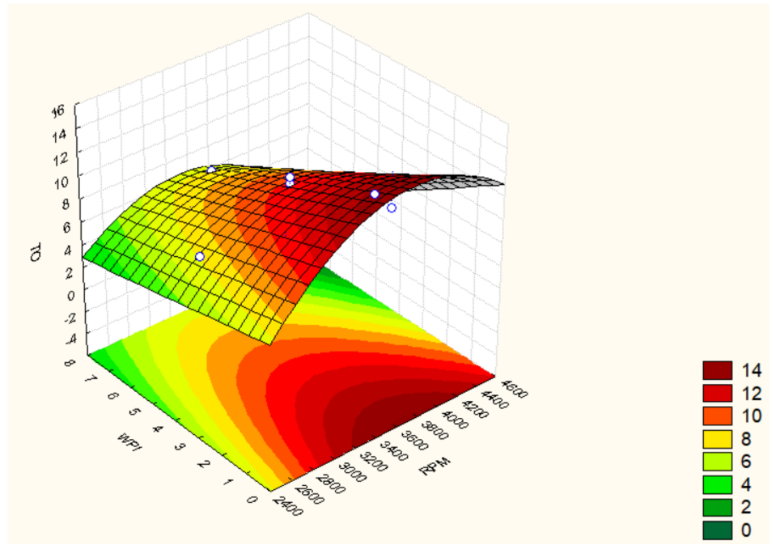
Figure 2 shows a saddle system graph where the elliptical contour extends significantly along with one of its main axes. The amount of coating material influenced the surface oil of the encapsulate. Formula with core and coating material ratio of 1:1 showed the highest surface oil yield (5%–9%), followed 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).

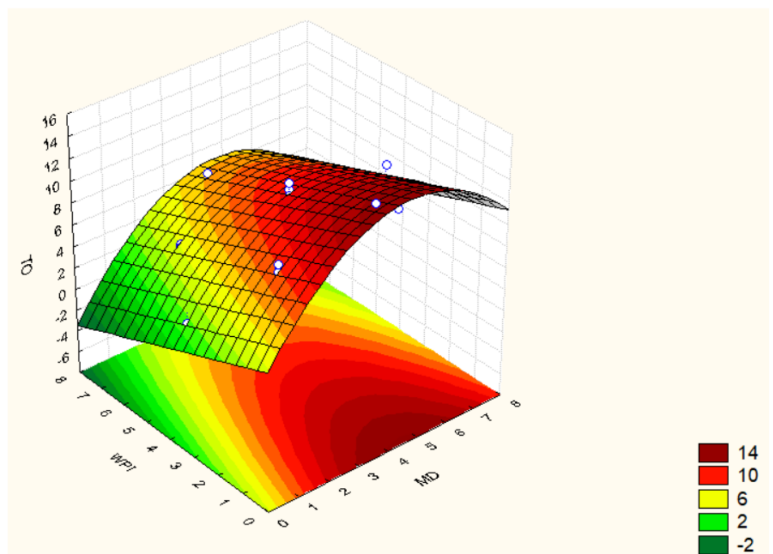
FIGURE 1 Fitted surface of trapped oil. (a) effect of agitation speed (rpm) and maltodextrin concentration on trapped oil encapsulate (b) effect of agitation speed (rpm) and whey protein isolate concentration on trapped oil encapsulate (c) effect of concentration of maltodextrin and whey protein isolate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



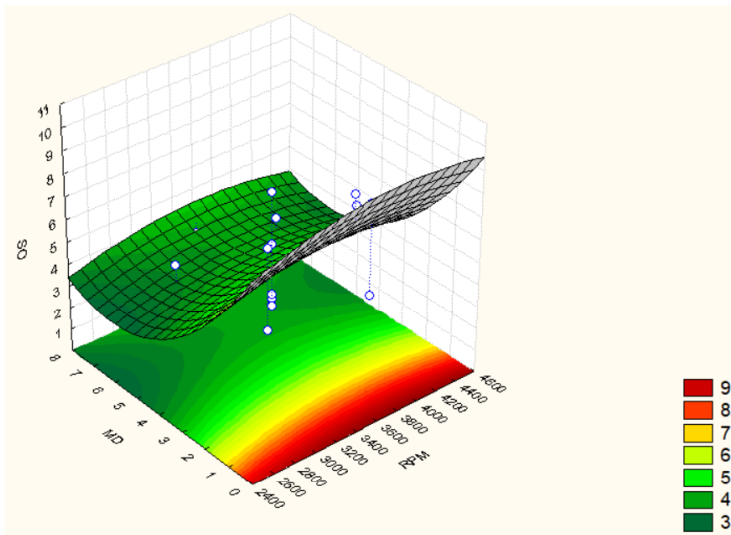
(a)



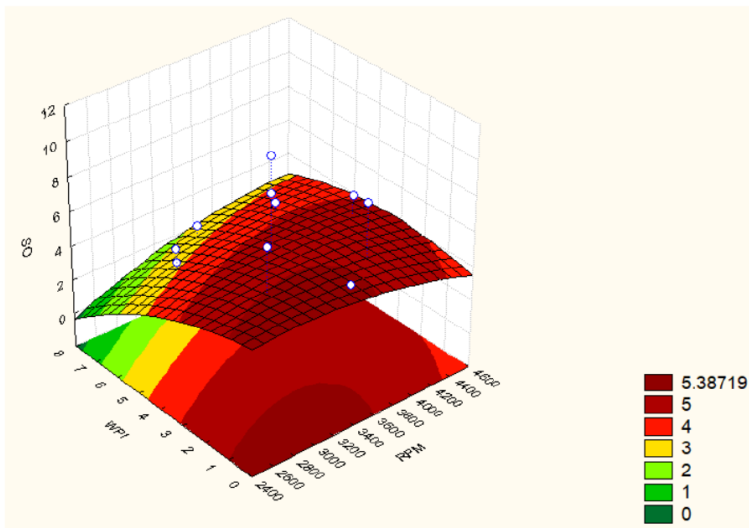
(b)



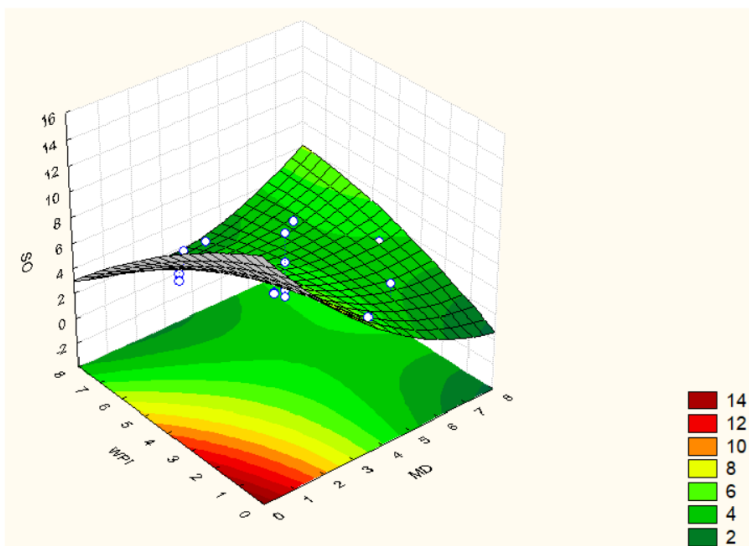
(c)



(a)



(b)



(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-diphenyl-2-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 4000rpm, 6g of maltodextrin, and 2g of whey protein isolate. The highest antioxidant activity values were obtained from treatment 12 with an agitation speed of 3500rpm, 0.64g of maltodextrin, and 4g 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

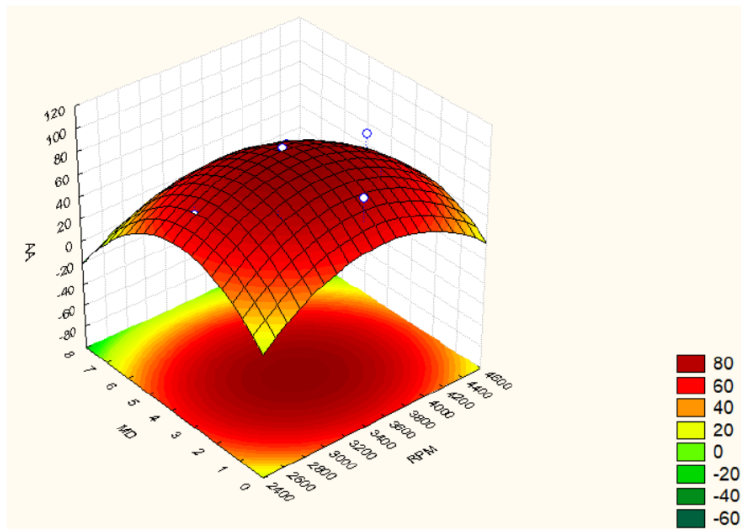
The yield (in %) of encapsulation could indicate how optimal the powder produced from each formula and how much loss in each formula is. Based on Table 2, the yield value of the encapsulate is not too high and ranges from 51.25% to 72.92%. It might be due to many product losses during the processing. 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.

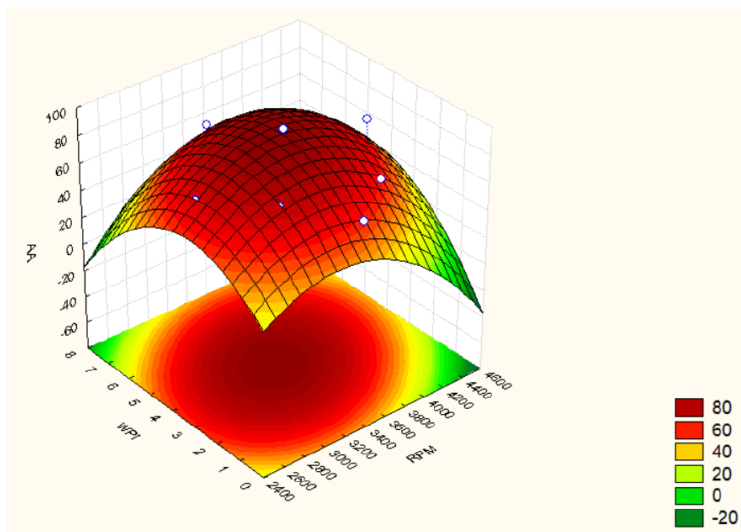
3.7 | Moisture content of nutmeg oleoresin encapsulate

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.

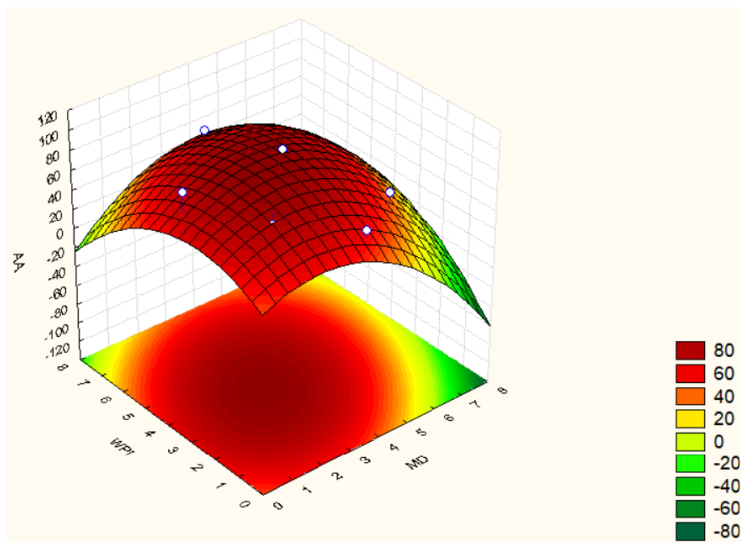
Figure 5 shows the rising ridge graph where the critical point or stationary point is not in the experimental region and the stationer point is at the maximum point. The addition of coating material affected the water content of encapsulates powder. The lowest water content was obtained from the formula 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.



(a)



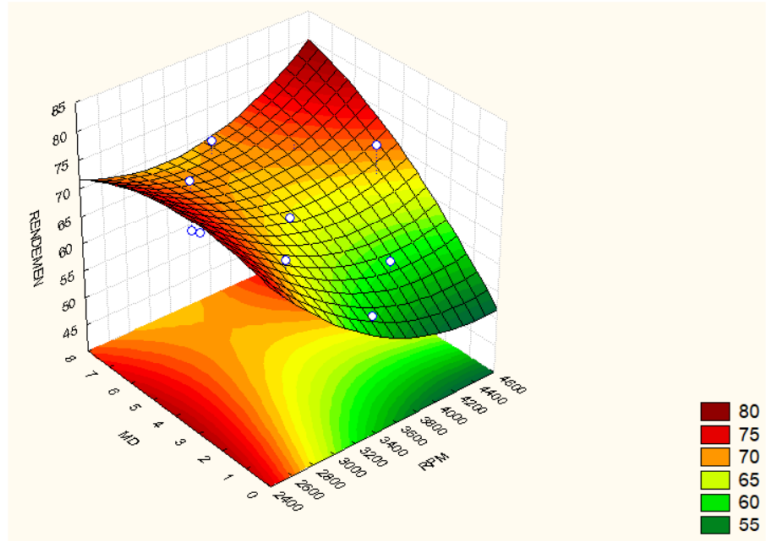
(b)



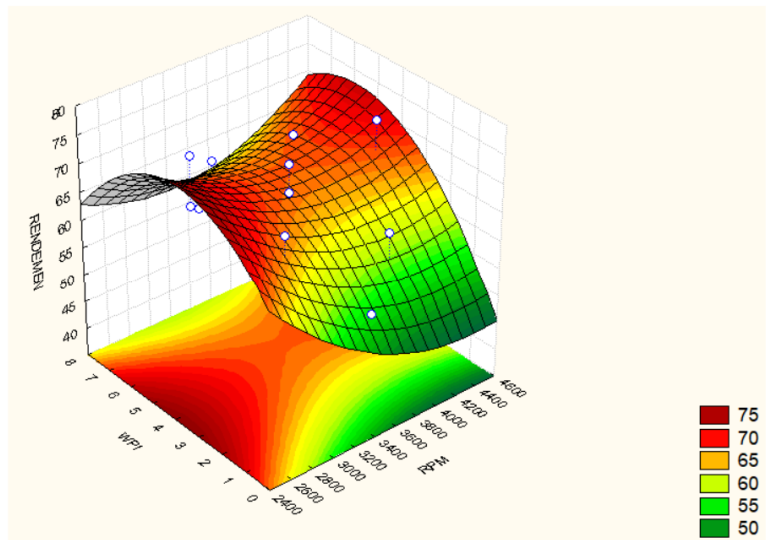
(c)

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.

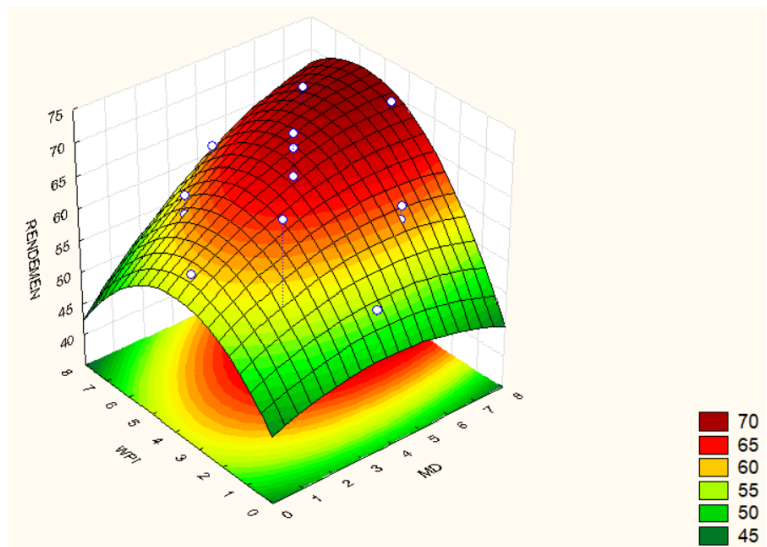
FIGURE 4 Fitted surface of yield. (a) the effect of agitation speed (rpm) and maltodextrin concentration on the percentage of encapsulate yield (b) the effect of agitation speed (rpm) and the concentration of whey protein isolate on the percentage of encapsulate yield (c) effect of the concentration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



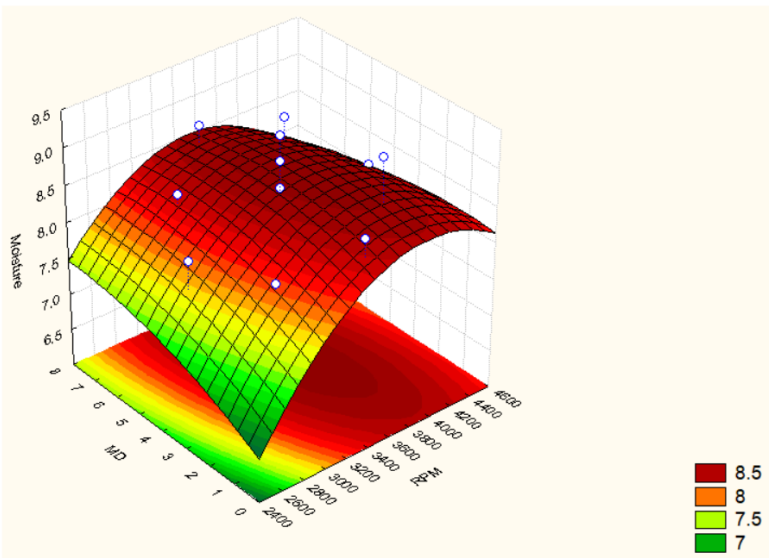
(a)



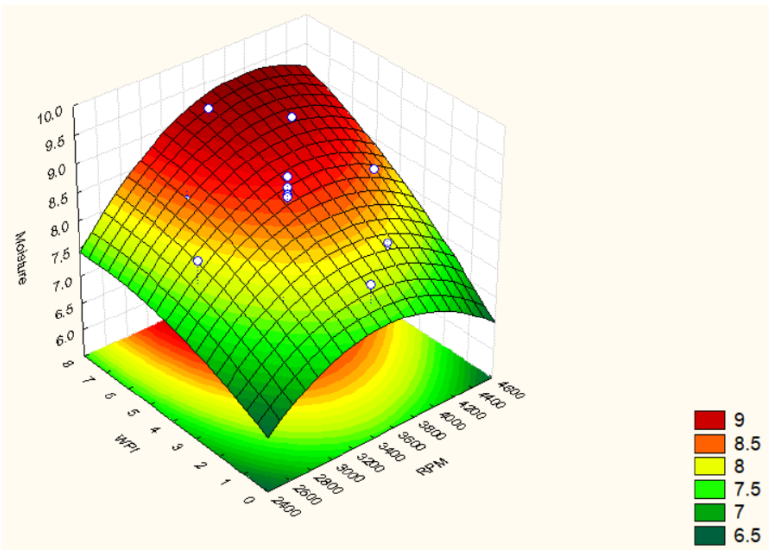
(b)



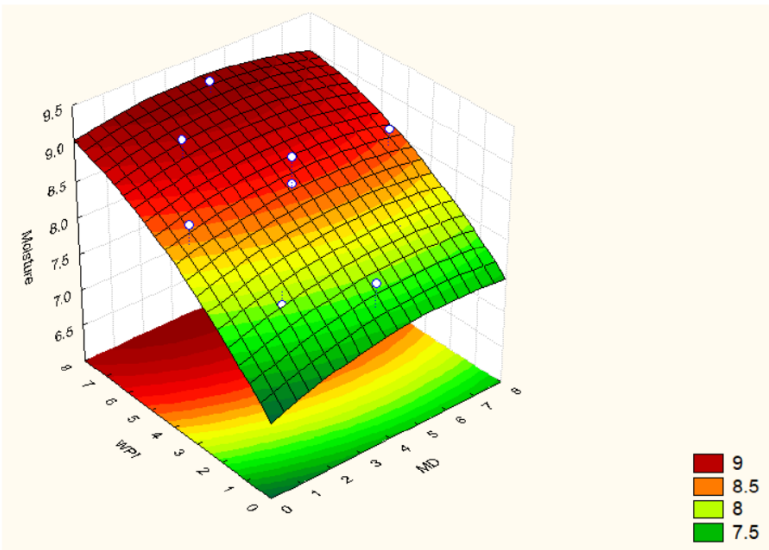
(c)



(a)



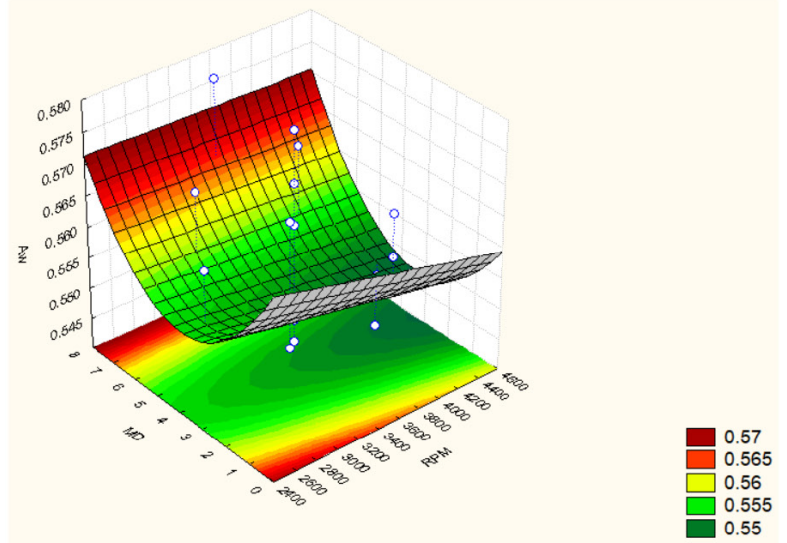
(b)



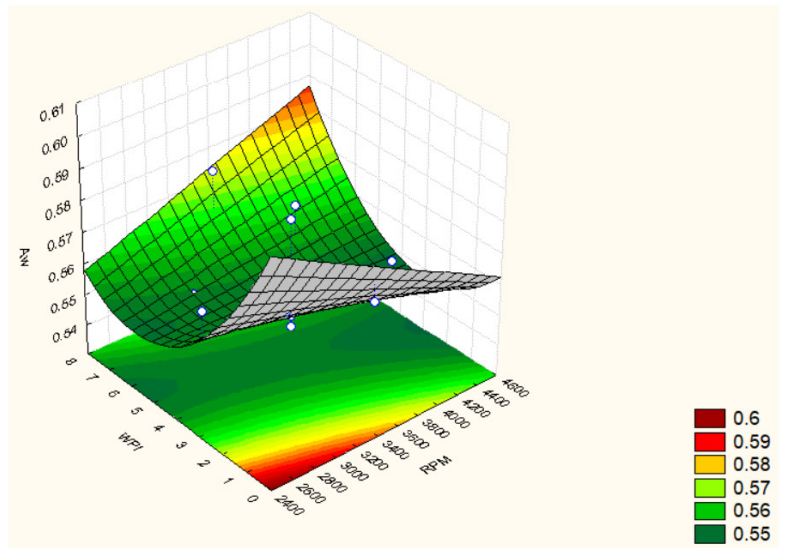
(c)

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.

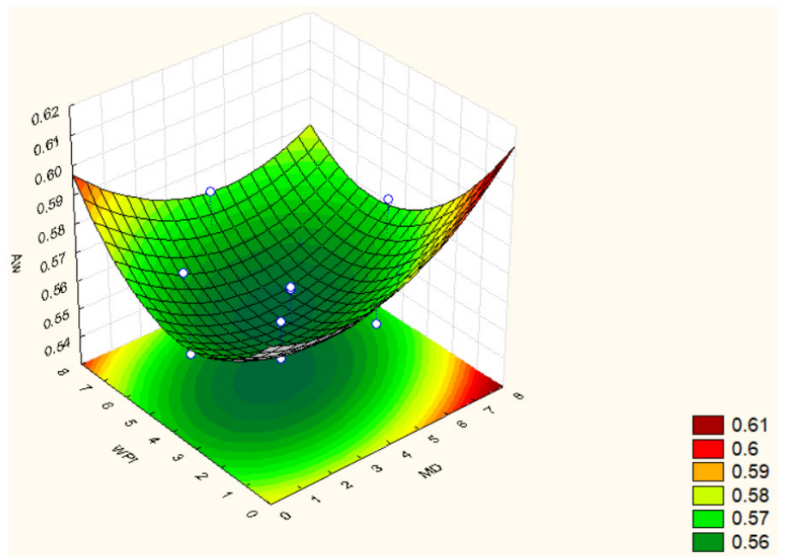
FIGURE 6 Fitted surface of water activities. (a) the effect of agitation speed (rpm) and maltodextrin concentration on encapsulate water activity (b) effect of agitation speed (rpm) and whey protein isolate concentration on encapsulate water activity (c) effect of concentration of maltodextrin and whey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



(a)



(b)



(c)

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

Treatment	Pattern	RPM	MD	WPI	Colors		
					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	0.79
				17.00	
	3920.448	9.56	81.65	66.38	0.73
MD (g)	4340.896	7.35	58.62	67.97	0.59
	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
WPI (g)	7.364	7.40	25.91	69.19	0.39
	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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