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Formulation Design and Optimization of Rice Bran Oil Microencapsulation with Ionic Gelation Method

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Abstract : Background: Rice bran oil (RBO) from *Oryza sativa* is the oil that has unique content and rich in bioactive compounds, γ-oryzanol. However, it is a problem in the use of RBO due to a rich in free fatty acid, causing limitations regarding handling and uses. Microencapsulation is one of the methods to simplify handling and to protect the oil from environmental influences. The aim of this study was to optimize a microencapsulation condition of RBO with ionic gelation method (cross-linking) of sodium alginate with calcium chloride using encapsulator. **Methods**: RBO was microencapsulated using Buchi microencapsulator using ionic gelation method. **Results**: three different formulations were prepared with ratio 1:1 by encapsulator. The method modifies electrode, flow rate, pressure, and frequency. The microcapsules were evaluated for optical graph analysis. Efficiency process was evaluated by microcapsules dry weight, efficiency proses of formula 1 is 34.36%, formula 2 is 35.75% and formula 3 is 35.55%. **Conclusion**: The results showed the formula 2 has the good characteristics, such as the highest process efficiency and the percentage efficiency.
Key words: Oryzanol, rice bran oil (RBO), microencapsulation, ionic gelation.

Introduction

Rice bran oil is the oil extracted from hard outer layer of rice, that has unique content and rich in bioactive compounds. One of the main components of RBO is γ-oryzanol [1]. Gamma oryzanol is a mixture of ferulic acid esters and alcohols triterpene; there are about 1-2% in the RBO. This compound has four major components, which is cycloartenil ferulic, 2,4-methylene cycloartenil ferulic, β-sitosterylferrulat and campesteril ferulic [2]. Resources of trans-ferulic acid and γ-oryzanol are potentially enormous because of their ubiquitous distribution in primary plant cell walls and crop bran [3]. Gamma oryzanol has been reported to demonstrate some biological activity such as: antihyperlipidemic, antioxidant, and hepatoprotective. Gamma Oryzanol is a fat-soluble compound having sterols and fatty acid held by ester bond, and this bond breakage requires esterase or lipase enzymes. These enzymes are secreted in pancreas and travel with food to the intestine and mix with bile juice secreted by gall bladder. It creates favorable conditions for the enzyme to act on dietary lipids as well as other ester molecules including nutritional oryzanol [4]. Gamma Oryzanol was also known to be a powerful inhibitor of iron-driven hydroxyl radical formation, and it was also reported to posses antioxidant

activity in stabilizing lipids [5]. It is a problem in the utilization of RBO because RBO is in oil form, that causing limitations in handling and uses[6]. Microencapsulation technology is an attractive option to overcome the problem through transformation from the liquid into a solid form, so as to facilitate the handling and use [7].

One method to prepared microcapsules is to use an ionic gelation method (cross-linking) between sodium alginate and calcium chloride [8]. Alginate is an anionic polysaccharide polymer extracted from seaweed. One of the biggest advantages of the alginate is in the form of a solution will be easy to form a hydrogel with divalent cations, especially calcium. When added to a solution of calcium alginate, calcium will close the position of the side poly glucuronic chain and the bounding will be put calcium ions in the middle of helix structure that resembles the egg box. This characteristic of sodium alginate is a natural function in forming hydrogels [9].

Methods

Materials:

Rice bran oil (RBO) was purchased from local market, Jakarta. Sodium alginate was obtained from Buchi, Switzerland. Calcium chloride, n-hexane and Tween 80 were purchased from Merck, Germany. All of the solvent and the material used was analytical grade.

Optimization condition of RBO microcapsules.

Preparation of rice bran oil microcapsules

Microencapsulation rice bran oil made using ionic gelation method with the following formula in Table 1 and was conducted using emulsion extraction technique [10].

Table 1. Formulation of Rice Bran Oil Microcapsules

Formula	Sodium Alginate Solution	Amount Sodium (g)	Rice Bran Oil (g)	Tween 80 (g)
1	10 g 1%	10	10	1
2	1.5%	10	10	1
3	2%	10	10	1

Sodium alginate solution (1%, 1.5%, 2%) and calcium chloride solution (2%, 100 ml) were prepared respectively with magnetic stirrer. Sodium alginate solution (1%, 1.5%, and 2%) and rice bran oil were mixed in ratio 1: 1 for 15 min at 5000 rpm by magnetic stirrer (IKA C-MAG HS 7). The oil was gradually added into the alginate solution until homogeneous, and tween 80 (1g) was added as emulgator into the mixture and emulsified for 30 min. The alginate-RBO emulsion was sprayed into a beaker containing calcium chloride solution using encapsulator (Buchi B-395 Pro, Switzerland) with nozzle diameter 700, 450 and 300µm. Set the frequency, pressure, and flow rate in certain condition to obtain stable droplets. The droplets were collected in 2% calcium chloride solution then left standing for 20 min for hardening and rinse twice with 50 ml distilled water and dried at room temperature. The microcapsules could be dried within 24 h.

Characterization of microcapsules

Organoleptic evaluation, including: shape, color, smell and texture of the surface

Particle size distribution:

The diameter and surface characteristics of microcapsules were determined by the optical microscope (Nikon Eclipse E200) with a magnification of 100x. In all measurements at least 30 particles were examined. The optical microscope was validated first before observation. Ocular micrometer scale was aligning with micrometer slide then measure the space. The measurement result obtained scale 7 adjacent with 0 whereas the scale 10 adjacent with 12. The space between 7-10 is 300 µm.

one part scale in microscope : $\frac{300}{120} = 2.5\mu m$

The efficiency of the process:

The efficiency process performed by comparing the total weight of the resulting dry microcapsules to the total material use on the current preparation of the microcapsules. efficiency process is calculated using the formula:

$$\text{Efficiency process } (\%) = \frac{(\text{The resulting weight of microcapsule})}{\text{total weight of starting material}} \text{ x } 100\%$$

Results and Discussion

Optimazion condition of RBO microcapsules

RBO microcapsules were made by encapsulator (Buchi B-395 Pro, Swizerland) using nozzle diameter 700, 450, 300µm and 4 parameters whereas electrode, flow rate, pressure and frequency. the result presented in table 1 Which indicate alginate 1,5% with nozzle 450 µm whereas electrode 1000 V, flow rate 7ml/min, pressure of 170 mbar, frequency 90 Hz lead to the optimal form microcapsules. With notes that optimal condition in this study not necessary right for other research.

Table 1: Optimization of RBO microencapsulation

Trial	Electrode (V)	Flow rate (ml/min)	Pressure (mbar)	Frequency (Hz)	Result
Alginate 1%(nozzle diameter 700µm)					
1	1000	19	320	40	Sticking (+++)
2	1000	10	152	80	Good
3	1000	10	362	80	Nonspherical (+)
4	1000	5,5	360	400	Sticking (+++)
5	1000	7	347	700	Sticking (++++)
6	1000	10	330	180	Caudate (++)
7	1000	7	169	100	Caudate (+)
8	1000	7	186	200	Caudate (+)
9	1000	6	362	540	Sticking (+++)
10	1000	6	225	300	Sticking (++)
11	1000	10	336	400	Sticking (++)
12	1000	7	380	80	Sticking (+++)
13	1000	7	327	300	Sticking (+++)
14	1000	7	172	140	Nonspherical (+)
15	1000	7	175	160	Caudate (+)
16	1000	7	220	232	Sticking (++)
17	1000	7	180	150	Nonspherical (+)
18	1000	9	215	150	Sticking (++)
19	1000	9	182	150	Nonspherical (+)
20	1000	9	150	150	Nonspherical (+)
21	1000	9	176	1500	Sticking (+++)
22	1000	8	178	130	Sticking (++)
23	1000	6	181	150	Nonspherical (+)
24	1000	7	172	140	Nonspherical (+)
Alginate 1.5% (nozzle diameter 450µm)					
1	1000	7	353	180	Caudate (+), sediment
2	1000	7	163	180	Good

3	1000	7	152	180	Caudate (+)
4	500	7	153	150	Sticking (++)
5	500	3	184	90	Sediment
6	500	7	170	90	Nonspherical (+)
7	1200	10	354	210	Sticking (+++)
8	1000	13.5	214	890	Sticking (++)
9	1000	7	349	780	Sticking (+++), sediment
10	1000	7	172	480	Caudate (+), sediment
11	1000	7	184	280	Caudate (+)
12	1000	7	164	480	Nonspherical (+)
13	1000	7	171	250	Sticking (+)
14	1500	7	165	250	Caudate (+)
15	1500	7	176	300	Sticking (+)
16	1000	7	170	90	Good
17	1000	7	160	100	Good
18	1000	7	174	90	Good
19	1000	7	197	90	Good
Alginate 2% (nozzle diameter 300µm)					
1	1000	4,5	384	1200	Good
2	1000	4.5	409	500	Sediment
3	1000	4.5	341	500	Sediment
4	800	4.5	289	400	Nonspherical (+)
5	1000	4.5	296	400	Good
6	1000	4.5	163	80	Good
7	1000	3	200	400	Sediment
8	1000	5	216	400	Sticking (+++), sediment

+= Low, ++ = Moderate, +++= High

Characteristics of microcapsules

Microcapsules design:

Observations of microcapsules include shape, color, smell and texture of the surface. Dried microencapsulated forms observed using an optical microscope with a magnification of 4x. The third formula has a spherical shape, brownish white and has a pungent odor. The result is in agreement with the findings of Ravindra et al[11].

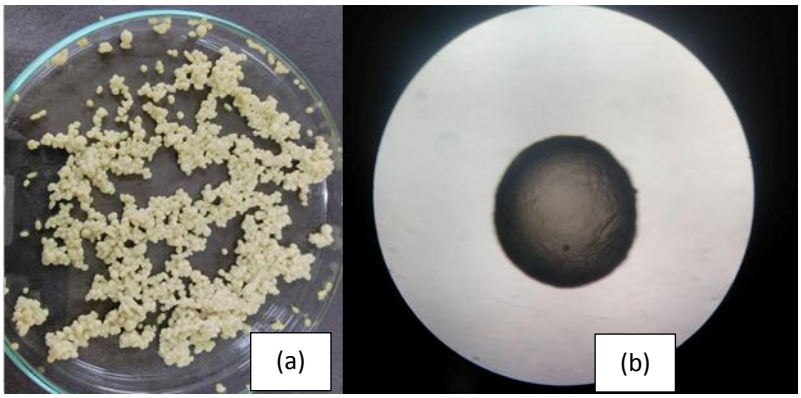

Figure 1: Results of RBO microcapsule containing 1% alginate (a) Microcapsule before drying, (b) Optical graph of RBO microcapsule

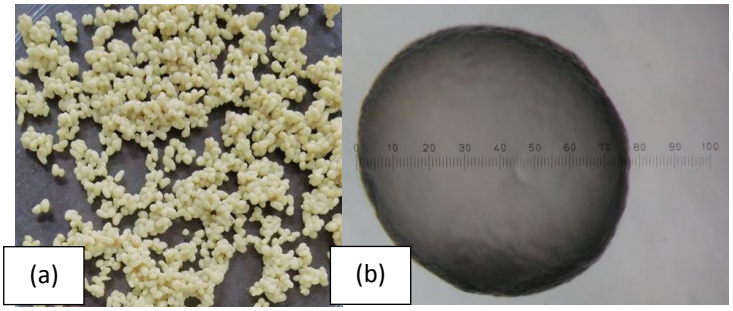

Figure 2: Results of RBO microcapsule containing 1.5% alginate. (a) Microcapsule before drying, (b) Optical graph of RBO microcapsule

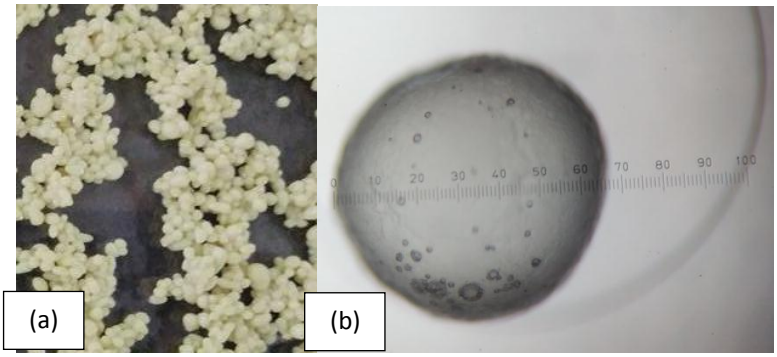

Figure 3: Results of RBO microcapsule containing 2% alginate (a) Microcapsule before drying, (b) Optical graph of RBO microcapsule

Particle size distribution

The shape and morphological microparticles homogeneously distributed without collapsed spheres. Measurement of 30 microcapsules using an optical microscope can be seen in Table 2

Table 2: Average of RBO microcapsules

No	Formula 1(scale)	Scale conversion (scale x 2.5 µm)	Formula 2(scale)	Scale conversion (scale x 2.5 µm)	Formula 3(scale)	Scale conversion (scale x 2.5 µm)
1	75	187.5	65	162.5	80	200
2	72	180	85	212.5	70	175
3	76	190	69	172.5	71	177.5
4	67	167.5	68	170	80	200
5	72	180	67	167.5	82	205
6	60	150	70	175	65	162.5
7	64	160	76	190	80	200
8	72	180	72	180	75	187.5
9	83	207.5	63	157.5	75	187.5
10	70	175	65	162.5	71	177.5
11	75	187.5	76	190	70	175
12	86	215	70	175	82	205
13	85	212.5	73	182.5	78	195
14	70	175	75	187.5	68	170
15	75	187.5	80	200	83	207.5

16	73	182.5	75	187.5	80	200
17	78	195	67	167.5	69	172.5
18	70	175	63	157.5	73	182.5
19	68	170	76	190	73	182.5
20	70	175	81	202.5	70	175
21	71	177.5	70	175	74	185
22	62	155	79	197.5	75	187.5
23	65	162.5	61	152.5	66	165
24	75	187.5	80	200	74	185
25	76	190	70	175	75	187.5
26	72	180	83	207.5	66	165
27	70	175	80	200	74	185
28	60	150	83	207.5	73	182.5
29	61	152.5	76	190	69	172.5
30	67	167.5	73	182.5	67	167.5
Total	2140	5350	2193	5477.5	2211	5520
Average	71.333	178.333	73.1	182.583	73.7	184

The averages of diameter microcapsules in this study were in the range of 178.33-184 µm. The diameter of F1 was slightly smaller than F2 and F3. The microcapsules were rough with some visible cracks. A good shape indicated that microcapsule forms a reticulated structure when contact with calcium ions [12].

Efficiency process

Results of efficiency process can be shown in Table 3. Regarding efficiency process, no significant differences in any of this studied were found. The performance was set with values between 34,6% and 35,55%. Low yield can be explained by the lost of alginate-RBO emulsion in syringe. For all formulation owning the high weigh loss during drying of microcapsules. The result is in agreement with the findings of Tous et al [13].

Table 3: Efficiency process of RBO microcapsules

Formula	Sodium alginate (g)	Rice bran oil	Tween 80	theoretical total weight	Dry weight of microcapsule	efficiency process (%)
1. Alginate 1%	10	10	1	21	7.216	34.36
2. Alginate 1.5%	10	10	1	21	7.509	35.75
3. Alginate 2%	10	10	1	21	7.463	35.55

Conclusion

Our results demonstrate that RBO microcapsules can be successfully prepared by encapsulator though the ionic gelation method. The best encapsulation formula is formula 2. No significant different between third formula.

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