Microencapsulation of Rice Bran Oil by Complex Coacervation Using Chitosan - k-carrageenan: Influence of Glutaraldehyde and Tween 20

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Microencapsulation of Rice Bran Oil by Complex Coacervation Using Chitosan – k-carrageenan: Influence of Glutaraldehyde and Tween 20

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Abstract: Rice bran oil (RBO) is obtained by extracting rice bran, a byproduct of the rice milling process. It contains vitamins, antioxidants, and nutrients that are very beneficial for the human body. In order to maintain its biological and functional characteristics, the microencapsulation technology is chosen. In this research the coacervation technique was applied using chitosan and k-carrageenan. The influence of the amount of glutaraldehyde as crosslinking agent and Tween 20 as emulsifying agent was studied. Research was conducting by dropping of solution of k-carrageenan containing RBO on the chitosan solution followed by adding glutaraldehyde. After washing and drying, the microcapsules were characterized by FTIR and SEM, and were analyzed for its yield and encapsulation efficiency. The FTIR shows that RBO was successfully loaded on the microcapsules. Analysis using SEM shows that micro-sized particles is not perfectly spherical, have irregular shape, rough surface and also agglomerated. The process has a yield of up 99.90 ± 1.29%. The highest encapsulation efficiency 66.49 ±4.8% was obtained from 0.5 mL Tween 20 and 0.5 mL glutaraldehyde. Increasing amount of glutaraldehyde tend to reduces the particle size since the wall became compact as degree of crosslinking increased. The amount of Tween 20 has little effect on encapsulation size.

Keywords: k-carrageenan; rice bran oil; microencapsulation; coacervation

1. Introduction

Rice is an important product consumed by the people of Indonesia. The rice needs to be processed further by milling. The rice milling process produces waste that can potentially be used as a source of oil[1)]. The rice milling process produces rice (57 - 60%), husks (18 - 20%), and bran (8 - 10%)[2)]. At this time, rice bran is mostly for animal feed with low economic value. According to data from the Ministry of Agriculture, in 2004 rice production reached 31.8 million tons, that give rice bran about 3.18 million tons. Rice Bran Oil (RBO) were produced by extraction process. Rice bran oil contains vitamins, antioxidants as well as nutrients needed by the human body. Rice bran oil contains several types of fats, including 47% monounsaturated, 33% polyunsaturated, and 20% saturated, as well as fatty acids; oleic acid 38.4%, linoleic 34.4%, linolenic 2.2%, palmitic 21.5%, and stearate 2.9%[3)].

The great potential in rice bran oil could be reduced because they are chemically unstable and susceptible to oxidative deterioration and loss of volatile compounds, especially when exposed to oxygen, light, moisture, and heat. To maintain their biological and functional characteristics the microencapsulation technology could be a better option[4)].

Microencapsulation is a physical process in which active material (core material), such as solid particles, water droplets, or gases, are packaged in secondary materials (walls) in the form of thin-film layers. Microencapsulation is a material packaging technology in the form of micron-sized closed capsules that can release their contents at a controlled speed under certain conditions[5)].

The microencapsulation method has wide applications. Materials that can be microencapsulated include drugs, oils, bioactive compounds, micronutrient such as vitamins[6)] and probiotics.

In the encapsulation process, what needs to be known is the material for encapsulation wall and the method of encapsulation. This wall material includes polymer, both synthetic polymers and natural polymers. Various natural polymers are used, including carbohydrates, gums, and proteins. New and innovative microcapsules also

developed, including nanostructures such as nanocellulose and chitosan nanoparticles[7]. Nanostructures provide advantages in use[8], however, many use ordinary structures in cellulose applications[9].

The microencapsulation technique used can be in the form of physical processes such as spray drying, freeze-drying, chemical processes such as molecular inclusions and physicochemical techniques such as complex coacervation and liposome entrapment.

In the coacervation technique, two polymers with opposite charges are used. Chitosan is a positively charged linear polysaccharide with a random arrangement of (1–4)-linked d-glucosamine and N-acetyl-d-glucosamine. Chitosan is a derivative of chitin that results from the deamination of chitin. Chitin itself is a biopolymer synthesized by living things such as shrimp and crabs. Chitin is obtained by extraction using acid or biologically by fermentation[10]. Chitosan is considered an environmentally friendly polymer because it has non-toxic, biodegradable and biocompatibility, high selectivity and antimicrobial activity properties[11]. With its beneficial properties chitosan can be used in many applications[12].

On the other hand, k-carrageenan is a natural polymer with a negative charge. K-carrageenan is sulfated galactans extracted from red seaweeds. K-carrageenan molecules are linear chains of alternating 3-O-substituted -d-galactopyranosyl units and 4-O-substituted -d-galactopyranosyl units. Because the sulfate ester groups are esters of a very strong acid, they are always ionized and the k-carrageenan molecules are always anionic [13].

The coacervation technique was chosen because of its easy way and has encapsulation efficiency of up to 90%. The polymers used are chitosan and k-carrageenan. These two opposite charge biopolymers can react to form polyelectrolyte[14,15]. Formation complex coacervation is start with adding the core material to polymer solutions, emulsification of this aqueous, adding the other polysaccharides along with dilution water, and following with adjusting the pH below the isoelectric point of protein to start electrostatic interactions and form agglomerates. In the emulsification step, surfactant is needed to improve the emulsifying properties and to maintain the core in dispersed state. Surfactants such as LSS, span and Tween are widely used. The presence of the non-ionic surfactant Tween 20 allows the formation of reduced size microcapsules[5].

Crosslinking agents are often used in encapsulation process[5] in order to stabilize the microcapsule structure. Crosslinking agent was added after the coacervation step. Among the crosslinking agents, glutaraldehyde is the most frequently used material. In this study, the effect of the amount of Tween 20 and glutaraldehyde on the properties of microencapsulation products was studied.

2. Materials and Method

2.1 Materials.

The materials used in this research were rice bran oil (local), k-carrageenan (Sigma), chitosan (local), tween 20 (Merck), glutaraldehyde (Sigma), glacial acetic acid (Merck), sodium acetate (Merck), ethanol (local), and n-hexane (local).

2.2 Encapsulation Procedure

Preparing Emulsion. Emulsion was prepared by dissolving 0.3 g of k-carrageenan in a 100 mL of acetic acid/sodium acetate buffer solution (pH = 4.6) and stirred at 250 rpm. Stirring was continued for 15 minutes while adding 0.5 mL of rice bran oil and 0.5 mL of Tween 20 . The volume of Tween 20 varied from 0.5 to 4 mL.

Microencapsulation process. Chitosan solution were made by dissolving 0.3 g of chitosan in 100 mL of acetic acid/sodium acetate buffer solution (pH = 4.6) accompanied by stirring at 250 rpm. Into the chitosan solution, emulsion is added by dropping to obtain the polyelectrolyte complexation. The system temperature was increased to 70˚C. and stirring was continued until it ran for 1.5 hours. The system was then cooled to 10-15 ^{o}C. Glutaraldehyde (0.25 , 0.5 0.75 or 1.25 mL) was added to the polyelectrolyte complex and the system temperature was raised to 50˚C. Stirring continues until it ran for 3 hours. The solid then was separated from the liquid by filtering. The solid was washed with water then with n-hexane and ethanol 96%. The particles were then oven dried for 12 hours at 50˚C.

2.3 Microcapsules characterization

The yield of encapsulation was calculated based on comparing the weight of the encapsulated powder produced and the weight of the initial raw material (polymer, oil and glutaraldehyde). The yield was calculated by using the following formula:

$$Yield\ (\%) = \frac{W}{W_1} \times 100 \quad (1)$$

Fourier Transformed Infrared Spectroscopy (Shimadzu Q-ATR spectrometer, Japan) was used for analyzed the functional groups. The morphology of the samples was analyzed using scanning electron microscope (SEM) (JEOL JSM-6510LA, Japan).

The encapsulation efficiency (EE%) is the percentage ratio between the amount of oil in loaded on the microcapsule and the initial amount used to make the formulation. The encapsulation efficiency (%) were calculated by following relationships.

$$EE(\%) = \frac{W_2}{W_3} \times 100\% \quad (2)$$

3. Results and Discussion

Chitosan is a natural polysaccharide with positively charged amino group, while k-carrageenan is a linear polysaccharide with negatively charged sulfate group.

Because chitosan and k-carrageenan have opposites charges, if the two polymers are united, they form microcapsules through polyelectrolyte complexation.

3.1 Yield of encapsulation.

Yield is used to determine the efficiency and effectiveness of a process. Higher yield means the more efficient the process. The yield of microencapsulation rice bran oil for various amount of glutaraldehyde is presented in Table 1. The yield of microcapsules ranged from 91.9 ± 6.55% to 99.90 ± 1.29%.

Both chitosan and k-carrageenan have hydroxyl group on their structure that make them easier to react with glutaraldehyde[16,17]. In the acidic condition, the reaction between glutaraldehyde and the hydroxyl group of chitosan and k-carrageenan were taken place[16]. The cross-linking that occurs between the polymer make the matrix strong. From the amounts of glutaraldehyde studied, it can be seen that increasing of glutaraldehyde tend to increase the yield. This result is accordance with from those found by Hariyadi[18] where the yield of microsphere albumin by alginate were increase by increasing the crosslinking agent. Jayanudin[19] also report the same phenomenon. The crosslinking reaction increases with increasing amount of glutaraldehyde, thus increasing the process solidification of the emulsion.

Table 1. Yield of encapsulation for various amount of glutaraldehyde (0.5 mL Tween).

glutaraldehyde (mL)	yield (%)
0.25	95.34±15.5
0.5	91.95±6.5
0.75	95.42±24.5
1.25	99.9±1.29

In this process, Tween 20 was added in the formulation of microcapsules. The microcapsules size and morphology and the oil encapsulation efficiency can be affected by the emulsification step. The role of Tween 20 was as emulsifying agent. The stability of emulsion is important in order to prevent the emulsion from layering out and separation into phases[20]. The effect of amount of Tween 20 can be seen in Table 2.

Table 2. The yield of encapsulation for varous amount of Tween 20 (0.5 mL glutaraldehyde).

Tween 20 (mL)	yield (%)
0.5	91.95±6.5
2	99.90±0.06
4	99.90±21.6

From Table 2 it can be seen that increasing the volume of Tween 20 would increase the yield. Adding surfactant increased the yield of encapsulation. Polyelectrolytes alone show low surface activity, making the droplets charged but they are insufficient to stabilize emulsion. The formation of stable microcapsules can be obtained from the presence of surfactants with rigid structures and can form hydrogen bonds[21].

3.2. FTIR spectroscopic analysis

The spectra of k-carrageenan, chitosan, RBO and RBO loaded crosslinked chitosan-k-carrageenan microcapsules are shown in Fig. 1. The spectra of k-carrageenan show absorption bands at 1261, 927 and 842 cm^{-1} indicate the presence of ester sulfate group, 3,6-anhydrogalactose and galactose-4-sulfate[22]. The chitosan FTIR spectra show four peaks in 1656, 1597, 1073 and 1030 cm^{-1}. The peaks of 1656 and 1597 cm^{-1} indicate the presence of amide I and amide II. The broad peak at 1073 cm^{-1} indicates an asymmetric stretch of C-O-C in glycosidic interrelationship, and its peak at 1030 cm^{-1} indicates the presence of C–O stretching vibrations[23]. FTIR spectra of rice bran oil show absorption bands in 2924, 1744 and 1162 cm^{-1}. Vibration modes are identified at 2923 and 1151 cm^{-1} for microencapsulation samples of 0.25 mL glutaraldehyde and 1155 cm^{-1} for microencapsulation samples of 0.5 mL glutaraldehyde. This point is also mentioned in the Rohman and Man experiment, which found rice bran oil specific peak points in 2922 and 1160 cm^{-1}[1, 24].

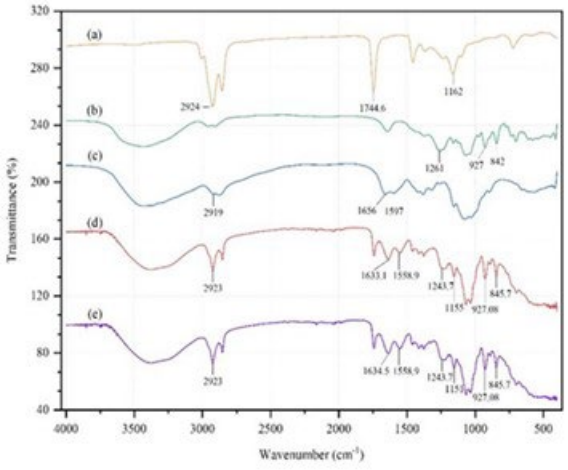

Fig. 1 FTIR spectra for of (a) RBO, (b) κ-K-carrageenan, (c) Chitosan, (d) Microencapsulation (0.5 mL glutaraldehyde), and (e) Microencapsulation (0.25 mL glutaraldehyde)

After the formation of polyelectrolyte complexes in microencapsulation rice bran oil, the crosslinking has been confirmed by new absorption bans. Peak at (d) 1633 and (e) 1634.5 cm^{-1} as the C=N bonding through crosslinking chitosan with glutaraldehyde , as well as in absorption band (d, e) 1558.9 cm^{-1} indicate the N-H bending . The spectra of microcapsules with 0.25 mL glutaraldehyde show the same pattern with that 0.5 glutaraldehyde. This confirm that the process

successfully loaded RBO to the complex chitosan carrageenan and crosslinked with glutaraldehyde.

The presence of the sulfate group, 3,6-anhydrogalactose and galactose-4-sulfate as the peak in microencapsulation rice bran oil showed interactions between the amine and sulfate groups chitosan and k-carrageenan success in forming complex polyelectrolytes. The microencapsulation rice bran oil FTIR spectra shows peak in 2919, 2923 and 2923 cm^{-1}. The peak indicates the presence of rice bran oil in the microcapsule.

3.3 Morphology of microcapsules

SEM analysis for microencapsulation of rice bran oil 0.5 mL with a k-carrageenan (0.3 grams), chitosan (0.3 grams), and Tween 20 emulsifiers (0.5 mL) with variations of glutaraldehyde are shown in Fig. 2. The SEM image shows that the morphology of microcapsules is not perfectly spherical, have irregular shape and a rough surface and also agglomerated. According to Piyakulawat[25] it was happened because the beads shrank during the drying process. This also reported by Lemos[26].

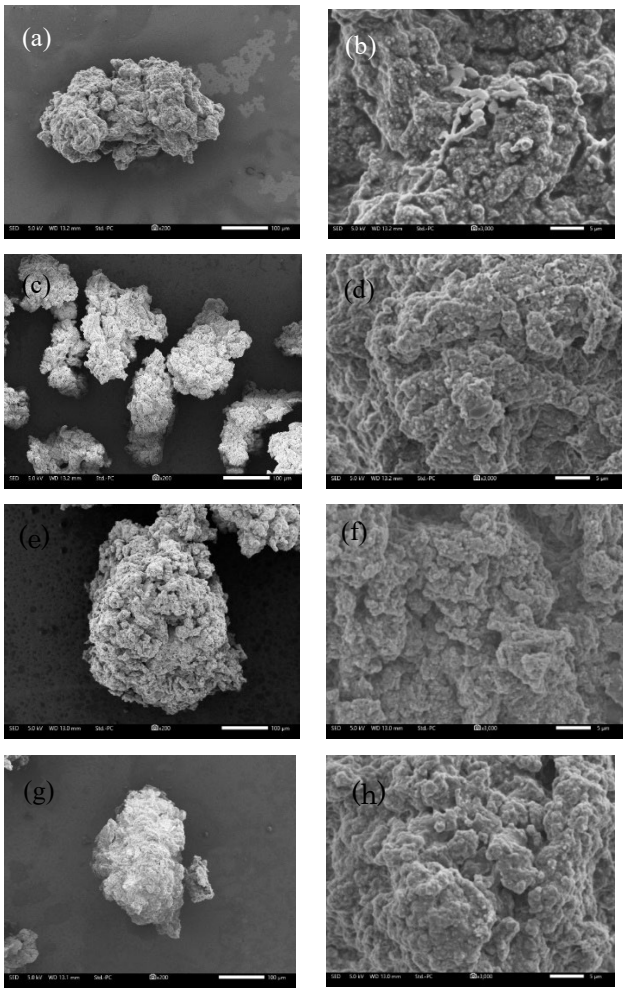

Fig 2. SEM image of microcapsules for various volume of glutaraldehyde: (a) 0.25 mL (magnification 200X) (b) 0.25 mL (magnification of 3000X) (c) 0.5 mL (magnification of 200X), (d) 0.5 mL (magnification of 3000X), (e) 0.75 mL (magnification of 200X), (f) 0.75 mL (magnification of 3000X), (g) 1.25 mL (magnification of 200X), and (h) 1.25 mL (magnification of 3000X).

Reduced in particle diameter is observed when the addition of the crosslinking agent increased from 0.25 mL to 0.5 mL and from 0.75 mL to 1.25 mL, but not from 0.5 mL to 0.75 mL. The microcapsule wall became compact as degree of crosslinking increased resulting in smaller particles[19,27]. A different phenomenon when adding 0.75 mL of glutaraldehyde may be due to the aggregation of the particles.

SEM analysis for microencapsulation of rice bran oil 0.5 mL with k-carrageenan (0.3 grams), chitosan (0.3 grams), and glutaraldehyde (0.5 mL) with variations of Tween 20 are shown in Fig. 3. From Fig. 3 it can be seen that particle have almost the same size. Theoretically the increasing of emulsifying agent would decrease the particle size[28]. From Fig. 2 and 3, the particle size of the microcapsules varies on the order of hundreds of microns (<500 μm). Lemos[26] reported that the Buriti oil microcapsules have particle size of 100 - 500 microns.

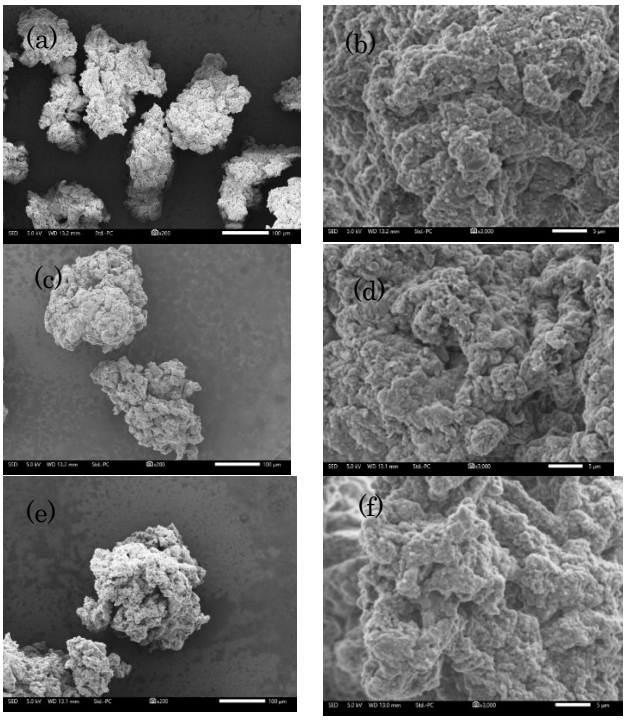

Fig. 3. SEM image of microcapsules for various volume of Tween 20: (a) 0.5 mL (magnification of 200X) (b) 0.5 mL (magnification of 3000X) (c) 2 mL (magnification of 200X), (d) 2 mL (magnification of 3000X), (e) 4 mL (magnification of 200X), (f) 4 mL (magnification 3000X).

3.4. Encapsulation efficiency.

The encapsulation efficiency relates to the amount of active ingredient in the microcapsule. The encapsulation efficiency (%) is presented in Table 3 for the influence of amount of crosslinkers (glutaraldehyde) and Table 4 for

the influence of emulsifying agent (Tween 20). The lowest value of encapsulation efficiency is 48.17 ± 7.2% resulting from 1.25 mL glutaraldehyde dan the highest encapsulation efficiency is 66.49 ± 4.8% resulting from 0.5 mL glutaraldehyde and both for 0.5 mL Tween 20.

Table 3. Encapsulation efficiency for various amount of glutaraldehyde (0.5 mL Tween).

glutaraldehyde (mL)	EE (%)
0.25	55.48±21.6
0.5	66.49±4.8
0.75	50.12±16.1
1.25	48.17±7.2

Encapsulation efficiency decreased with an increase in amount of crosslinking agent. Addition glutaraldehyde from 0.25 to 1.25 resulted in decreasing encapsulation efficiency from 55.58 ± 21.6% to 48.17 ± 7.2%. Deka[27] reported the similar phenomenon. The increase in the crosslinker concentration sufficiently hardened the coacervate particles which resulted in low absorption of oil[27]. There is irregular value of encapsulation efficiency such as in 0.5 mL gutaraldehyde which has higher encapsulation efficiency than 0.25 mL glutaraldehyde. The irregularity of the encapsulation efficiency also reported by Jayanudin[19].

Table 4. Encapsulation efficiency for various amount of Tween 20 (0.5 mL glutaraldehyde).

Tween 20 (mL)	EE (%)
0.5	66.49±4.8
2	62.47±4.3
4	50.70±8.7

From Table 4 it can be seen that increasing the volume of Tween 20 from 0.5 mL to 4 mL would decrease the encapsulation efficiency from 66.49 ± 4.8% to 50.70 ± 8.7%. Sharma[29] reported the similar phenomenon. Efficiency decreases with increasing number of Tween 20. This can be explained as follows. The CMC value reported for Tween 20 is 0.0060% and it is known that it forms micelles in the form of ellipsoidal shape with a core-shell structure. As the concentration of Tween 20 increases, more Tween 20 monomers leave the oil-water interface and form micelles that dissolve oil in water. The large number of micelles is not matched by the amount of polymer, resulting in a large amount of oil that is not encapsulated.

4. Conclusion

Based on the results of the experiment, it can be concluded that the process was successfully loaded RBO on the microcapsules with yield 99,90 ± 1.29%. The resulting microcapsules are micro-sized that is not perfectly spherical, have irregular shape , rough surface and also agglomerated. The highest encapsulation efficiency 66.49 ± 4.8% was obtained on addition of 0.5 mL Tween 20 and 0.5 mL glutaraldehyde.

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Nomenclature

W	weight of microcapsules
W1	weight of microcapsules forming materials
EE	encapsulation efficiency
W2	weight of oil encapsuled
W3	weight of oil added for encapsulation

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