Encapsulation of oryzanol-rich rice bran oil using carrageenan

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Abstract. Experiments have been carried out to encapsulate rice bran oil using carrageenan. Two methods have been tried. In the first method, the chitosan-carrageenan polymer solution was dropped into the NaOH-ethanolate solution. The solid formed is filtered and immersed in a 6% glutaraldehyde solution, then filtered and dried. The resulting solid is clustered and not form granules. In the second method, a solution called A in the form of oil emulsion in carrageenan solution is dropped into chitosan solution and then heated to 70 °C. Glutaraldehyde is added to the solution, and the cross-linking process is continued for 3 hours. The solid is filtered and washed with water then with n-hexane. The resulting solid is washed with ethanol and then dried. Experiments result with variation in the amount of carrageenan showed the best results when using 0.3 g carrageenan and 0.5 mL of oil.

1. Introduction

Public awareness of health has recently increased. One healthy lifestyle is done by consuming functional food, one type of benefit food containing antioxidants. Antioxidant compounds help to protect the cells of the body from damage caused by the oxidation process. One of the ingredients that contain high antioxidants is rice bran oil.

Rice bran oil (RBO) is obtained by extracting rice bran, a byproduct of the rice milling process. Rice bran (8 - 10% by weight of milled dry rice) contains about 14 - 18% oil depending on the bran’s geographical origin, the type of rice, and the treatment during milling [1]. Crude bran oil contains 81.3 - 84.3% triglycerides, 2 - 3% diglycerides, 5 - 6% monoglycerides, 2 - 3% free fatty acids, 0.3% wax, 0.8% glycolipids, 1.6% phospholipids, and 4% unstoppable fraction. The non-saponified fraction contains 0.9 - 2.9% oryzanol and 100 - 1000 mg/kg tocopherol and tocotrienol. Tocopherol and tocotrienol (tocol) are known as vitamin E. Together with oryzanol, tocol is an important part of RBO, which has high economic value as an antioxidant. Oryzanol is a mixture of phenolic compounds that are consisting of at least ten components. Chemically of oryzanol is a mixture of ferulic acid esters of triterpene alcohol (phitosterol) with the main components of cycloartenol to produce of 2,4-methylene cyclo artanol, campesterol, β-sitosterol, and campestanol [2]. The content of ferulic acid esters is mostly found in rice, so it is called oryzanol. The usefulness of oryzanol has been shown in various studies, including its benefits for heart health. This benefit includes reducing cholesterol levels, reducing cholesterol absorption, and reducing the possibility of early atherosclerosis. Some literature also states that oryzanol has higher antioxidant power than vitamin E compounds (tocopherol).
Functional foods containing oryzanol can be processed by fortification. The stability of oryzanol is required during fortification. To get the stability needs to be processed through microencapsulation. Microencapsulation is also intended to facilitate fortification applications. Microencapsulation is when small particles or droplets are surrounded by a coating wall or embedded in a homogeneous or heterogeneous matrix to form small capsules. The coating wall can cover this material in solid, liquid, or gas in a tiny closed capsule. The core material gradually diffuses through the capsule wall, thus offering controlled release properties under the desired conditions. Therefore, microencapsulation technology can be used to deliver bioactive components by enhancing their handling properties. The encapsulation of oil in a micro-capsule prevents oxidation induced by moisture, metal ions, oxygen, and heat [3].

To form a microcapsule, a wall forming material (encapsulant) is needed. The encapsulants can be synthetic polymers or natural biopolymers. The type of encapsulant effects consists of several phenomena: the stability of the microparticles, the efficiency of the process, and the protection of the core material. Natural biopolymers native to Indonesia are abundant. Some natural biopolymers can find from seaweed and plant tubers.

Indonesia is the fourth largest carrageenan-producing seaweed exporter globally, but it has not met the world’s demand for dry seaweed, which is predicted to increase. This shows that the world’s need for carrageenan is expanding as well. The seaweed processing industry, especially carrageenan, is still challenging to develop in Indonesia. Indonesia still imports all of its carrageenan needs for additives in the food, pharmaceutical, and cosmetics industries.

Glucomannan is a polysaccharide biopolymer derived from porang tubers. However, the processing of porang tubers as a source of glucomannan has not been carried out correctly. So far, porang farmers only process tubers by turning them into chips and then selling them to Japan, China, and Korea. Thus the economic value of this porang tuber product is still low.

It appears that the challenge of processing porang is to develop a technology of carrageenan and glucomannan become high-value products. One of them is to become an encapsulant in the oryzanol microencapsulation process. The encapsulant material affects the encapsulation technique used. Generally, the size and shape of the microcapsules that are formed depend on the wall material and the method used to prepare it. Microencapsulation techniques are emulsification, spray drying, coaxial electrospray system, freeze drying, coacervation, in situ polymerization, extrusion, fluidized-bed-coating, and supercritical fluid technology [4].

In this research, coacervation techniques will be used. The coacervation technique is proven to produce microcapsules that have good controlled release characteristics. The use of k-carrageenan as an encapsulant using coacervation techniques has been shown to provide satisfactory results. The use of k-carrageenan has been used in the microencapsulation of pimento oil [5] and neem oil [7].

According to [4], P. dioica essential oil microspheres was prepared by dropping oil-in-water emulsion in a NaOH ethanolic solution. The microsphere was then filtered and immersed in the k-carrageenan solution, filtered again, and kept in glutaric aldehyde vacuum dried at 30°C.

In synthesizing olive oil loaded chitosan/carrageenan complex crosslinked with glutaraldehyde, an oil-water emulsion made from carrageenan solution and olive oil were made first, and then dropping in solution of chitosan in an increase temperature. Glutaraldehyde was the added and act as crosslinker. Coslinked particles were filtered and washed with water, n-haxane before dried [5]. The aims of the work were to study the effect of the different encapsulation process and the effect of the different amount of carrageenan and oil on the processes and produced capsules.

2. Materials and Methods
Carrageenan (from Eucheuma cottonii), glucomannan (from porang flour), chitosan, oryzanol, rice bran oil (RBO), distilled water, buffer solution, Tween 20 and glutaraldehyde.
(i) The chitosan-carrageenan polyelectrolyte complex was prepared by mixing chitosan-carrageenan in a ratio of 3: 1, 2: 1 and 1: 1 with a total biopolymer content of 1%. The solution is dropped into a 1 M ethanolic NaOH solution to form micro spheres. The micro particles were then separated by filtering and rinsed with deionized water. The micro-spheres were filtered and stored in 100 mL of 6% glutaraldehyde solution for 3 hours. The micro-spheres were again filtered and rinsed with deionized water.

(ii) A solution called Solution A were made from dissolving 0.3 g (or some other amount) of carrageenan in a 100 mL of buffer solution and stirred at 250 rpm. Stirring is continued for 15 minutes while adding 0.5 mL (or some other amount) of rice bran oil. Chitosan solution were made from dissolving 0.3 g of chitosan in 100 mL of buffer solution pH 4.6 accompanied by stirring at 250 rpm. Into the chitosan solution, solution A is added by dropping to obtain the polyelectrolyte complexation. The system temperature is increased to 70°C. and stirring is continued until it runs for 1.5 hours. Glutaraldehyde 0.1 mL was added to the polyelectrolyte complex and the system temperature was lowered to 15°C. Stirring continues until it runs for 3 hours. The system temperature was raised to 50°C. The solid is separated from the liquid by filtering. The solid is washed with water then with n-hexane. The solid is washed or not washed with ethanol and then dried.

3. Methods
3.1. Encapsulation method one
Observation of the encapsulation process using method 1 showed that the oil was released from the encapsulation matrix. This can be seen from the presence of oil on the liquid surface as shown in Figure 1.

**Figure 1:** The oil is separated and is on the surface of the solution when the polymer solution is dropped into ethanolic NaOH solution

If the process is continued until solids filtering, solids clustering is obtained as shown in Figure 2.
3.2. Encapsulation method two

Experiments were carried out with the aim to find the difference solids appearance when the solid was washed or without being washed with ethanol. Table 1 shows the results obtained.

Table 1: Results of the encapsulation process using Method 2

| 0.5 mL oil with ethanol washing | Brown brick, Shaped in granules and clumps, Not too dry yet |
|--------------------------------|-------------------------------------------------------------|
| 0.5 mL oil with ethanol washing | Brown brick, Formed finer grains, Pretty dry                |
From the observations made, it can be concluded that when the encapsulation using Method 2 the solid produce were separate granules. It was also concluded that washing with ethanol resulted in a drier capsule. The next processes were then carried out using Method 2 with ethanol washing.

3.3. **Effect of the amount of carrageenan**

On studying the effect of the amount of polymer, an experiment was carried out by varying the amount of carrageenan dissolved in same volume of a buffer solution. The volume of the oil remain constant, i.e. 0.5 mL. The variations are using 0.3, 0.5 and 0.8 g carrageenan in 100 mL of buffer solution. The different amount of carrageenan were affect in the solution A and its emulsion. The results of the effect of different amount of carrageenan can be seen in Table 2.

| No. | Sample | Sample condition |
|-----|--------|-----------------|
| 1.  | 0.3 g carrageenan | - Liquid, non viscous  
|     |                    | - A cloudy white emulsion is formed  
|     |                    | - There are few oil bubbles on the surface |
| 2.  | 0.5 g carrageenan | - It’s little viscous  
|     |                    | - A cloudy white emulsion is formed  
|     |                    | - There are a few oil bubbles on the surface |
| 3.  | 0.8 g carrageenan | - Very viscous  
|     |                    | - A cloudy white emulsion is formed  
|     |                    | - There are not a few oil bubbles on the surface |

When the emulsion were used for the next step it will show a difference in the final encapsulation results as can be seen in Table 3.

Greater amount of carrageenan made the solution more viscous, affecting the time to filtered and ease of drying. The use of 0.3 g carrageenan in 100 mL buffer gave the best results.

3.4. **Effect of the amount of oil**

To study the effect of the amount of oil, experiments were carried out by varying the amount of oil 0.5, 0.75 and 1 mL. The experimental results can be seen in Table 4.
Table 3: Results of the encapsulation process on variations in the amount of carrageenan

| Table 3: Results of the encapsulation process on variations in the amount of carrageenan |
|---|---|
| 1 | 0.3 g carrageenan |
| - | Grains separate perfectly |
| - | Greenish brown in color |
| - | Not viscous |
| - | Easy to filter (short time) |
| - | Easy to dry |
| 2 | 0.5 g carrageenan |
| - | Grains are a bit lumpy |
| - | It's brick red |
| - | A little viscous |
| - | It's a bit difficult to filter (long time) |
| - | Doesn't dry easily |
| 3 | 0.8 g carrageenan |
| - | Grains are a bit lumpy |
| - | It's brick red |
| - | So viscous |
| - | Difficult to filter (long time) |
| - | It's hard to dry |

From Table 4 it can be stated that higher oil loading made the granule harder to dry. Higher oil loading made mean oil droplet diameter increased [8]. This affects the time to dry the granules. A higher oil concentration resulted in a higher droplet mean diameter. As the total solids content remain the same this would lead the lower amount of wall material. The amount of wall material is not enough to fully cover the oil droplets and this insufficiency may result in a decrease in encapsulation efficiency [9]. Oil wet the outer surface of the granule making it difficult to dry.

4. Conclusion
Method 1 did not succeed in forming dry grained solids. The dry encapsulated granules can be formed using Method 2. Higher carrageenan content made the oil-in water emulsion more viscous that affect the time to filtered the wet granules. Higher oil loading made the granule harder to dry. The encapsulation process produced better granules with 0.3 g of carrageenan and 0.5 mL of oil.
Table 4: Encapsulation results on various amounts of oil

| 1   | 0.5 mL oil | - Granules separate perfectly  
|     |            | - Greenish brown in color      
|     |            | - Not viscous                  
|     |            | - Easy to filter (short time)  
|     |            | - Easy to dry                  

| 2   | 0.75 mL oil| - Granules separate perfectly  
|     |           | - Yellowish brown              
|     |           | - Not viscous                  
|     |           | - Easy to filter (short time)  
|     |           | - Hard to dry                  

| 2   | 1 mL oil   | - Granules are a bit lumpy    
|     |           | - Yellowish brown in color    
|     |           | - Not viscous                 
|     |           | - Easy to filter (short time) 
|     |           | - Hard to dry                 

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