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Encapsulation of Rice Bran Oil (RBO) by Complex Coacervation Using Glutaraldehyde as Crosslinking Agent

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ABSTRACT. Rice bran is a significant by-product of the processing of rice. Rice bran oil (RBO) can be extracted from it to provide a very valuable product. It has a high smoke point but is less stable in the heating process. Further research is carried out to increase the stability of rice bran oil in its utilization. Many environmentally friendly methods are being developed in storing and packaging food, including the application of microencapsulation. This study aims to determine the effect of the use of glutaraldehyde crosslinking agent on rice bran oil microencapsulation by utilizing the natural polymers of kappa carrageenan, glucomannan, and chitosan as encapsulants using the coacervation method. Microcapsules were analyzed using complex coacervation for their encapsulation efficiency and yield and were characterized using Fourier transform infrared spectrophotometer (FTIR), scanning electron microscope (SEM), and particle size analyzer (PSA). Results show that increasing the amount of glutaraldehyde tends to increase the yield and efficiency of encapsulation. A yield of 49.20% was obtained by adding 1 mL of glutaraldehyde with an encapsulation efficiency of 48.22%. All samples were irregular shapes and the surfaces were roughed and folded. FTIR spectra showed that all samples indicated the presence of RBO as well as other polymers used in the research: carrageenan, chitosan, and glucomannan. The results of PSA showed that particles are in micron size except for the addition of 1 mL glutaraldehyde.

1. INTRODUCTION

In milled or white rice production, hull and bran layers of rough rice kernel are removed during dehulling and milling processes. The bran comprises 3% - 8% of the kernel and is a valuable by-product of rice processing. The rice bran consists of 10–23 % oil, depending on the rice variety and its grinding degree. Rice bran oil is one of the oil types with high nutrition because, in its contents, there are fatty acids, biologically active components, and antioxidant components such as oryzanol, tocopherol, tocotrienol, phytosterol, polyphenol, and squalene. Rice bran oil (RBO) consists of fat, with 47% of the fat being monounsaturated, 33% polyunsaturated, and 20% saturated [1]. Microencapsulation is a physical process where the active components (core components), such as solid particles, water droplets, or gasses, are packed in a secondary component (wall) in the form of a thin film layer. The process of packaging is conducted to protect a substance to be stored in good condition and release the substance under certain conditions when used [2]. Coating materials in microencapsulation maintain the core material's stability from environmental damage: temperature, pH, light, and humidity [3].

In the microencapsulation process, coating materials like chitosan and kappa-carrageenan were used. Chitosan was used because it is insoluble in water, non-toxic, biocompatible, and biodegradable, which manifests antibacterial properties [4]. Kappa-carrageenan is a good coating material because of its pseudoplastic characteristic, making it possible to act as a plasticizer, round and smooth formation on the microencapsulated, and enhances the adhesion force between the wall and core material. Kappa carrageenan also has desirable properties as an emulsifier, is safe to eat, and is biodegradable [5]. Glucomannan is a polysaccharide biopolymer derived from konjac tubers. It is a β-1, 4 linked polysaccharides composed of a d- glucose (G) and d-mannoses (M) backbone with slightly branched. It is known that kappa carrageenan has synergetic interaction with konjac gum [6]. Glutaraldehyde is a cross-linker that can cross-link with many polymers. Chitosan that cross-links with glutaraldehyde can increase stability by forming an intermediate compound [7].

Complex coacervation is a separation technique involving the solution and colloidal phases, in which the core material is encapsulated with a matrix. This technique produces microcapsules in the form of an emulsion or wet microcapsules, making it requires a drying process to gain product in powder form [3]. The complex coacervation method was chosen as the method used for manufacturing because this method has an encapsulation efficiency rate of 90%, and it is easy to control the release of the core material from the coating. This research was conducted to study the effect of the amount of glutaraldehyde as a crosslinking agent in the microencapsulation of rice bran oil using kappa-carrageenan, glucomannan, and chitosan. The instrument that used in this research are static, clamp, thermometer, beaker glass, electric stove, magnetic stirrer, pipette, hand blender, and oven. Microcapsules were tiny, spherical structures created through the microencapsulation process. Microcapsules were typically spherical in shape, which allows for uniform distribution and dispersion in various products. Microcapsules come in a range of sizes, from a few micrometers to a few millimeters, depending on the application and the encapsulation method. Each microcapsule consists of a core material surrounded by a shell or wall material. The core material is the substance being encapsulated, while the shell material serves as a protective barrier.

Microencapsulation technology is important because it can be related to various fields such as agriculture, industry and medicine, flavors, pesticides, dyes, liquid inks and medicines with the aim of protecting a substance so that it remains stored in good condition and releasing the substance under certain conditions when used. Several studies have been carried out on microencapsulation with rice bran oil, including microencapsulation of Rice Brain Oil with gum arabic polymer and maltodextrin using the spray drying method [8]. In this research, kappa-carrageenan, glucomannan and chitosan polymers were used. The microencapsulation method used is complex coacervation because this method has an encapsulation efficiency level of 90% and is easy to control the release of the core material from the coating.

2. MATERIALS AND METHODS

2.1 Materials

Materials used were rice bran oil (Tangerang), kappa-carrageenan (Surabaya), glucomannan (Malang), Sigma brand chitosan, Sigma brand glutaraldehyde, Merck brand tween 20, aquadest (Surakarta), 96% Ethanol solution (Surakarta), Merck brand acetic acid solution, Merck brand sodium acetate, and Merck brand n-hexane.

2.2 Encapsulation Procedure

The microencapsulation process started with making a polymer solution, made from 0.2 grams of kappa carrageenan and 0.1 grams of glucomannan dissolved in 100 mL of buffer solution. The buffer solution was made with 11.55 ml of acetic acid dissolved in 1000 ml of aquadest and mixed with 16.4 grams of sodium acetate dissolved in 1000 ml of distilled water. The solution then was mixed at 2000 rpm speed for 30 seconds using the hand blender. To the solution, it was then added 0.5 ml of rice bran oil and 0.5 ml of Tween 20 followed by mixing for one minute.

The chitosan solution was made by dissolving 0.3 grams of chitosan in 100 ml of buffer pH 4.6 solution and mixing at 2000 rpm speed for 2 minutes. The polymer solution that has been made previously was then added to the chitosan solution dropwise. The system temperature was then raised to 70℃ and stirred for 1.5 hours continuously. After 1.5 hours, the temperature was lowered to 15℃, and 0.25 mL of glutaraldehyde was added to made particle solution. The temperature was raised to 50℃ and stirred for 3 hours, and a crosslinked particle solution was formed. The solution was then filtered with filter paper washed with distilled water, n-hexane, and 96% ethanol. The sample was put in the oven at 50℃ for 15 hours and formed dried microencapsulated rice bran oil. The materials in the form of chitosan, glucomannan, kappa carrageenan, RBO, and glutaraldehyde were totaled to made microcapsule-forming material.

The experiment was conducted by varying volumes of glutaraldehyde, namely of 0.25 mL, 0.55 mL, 0.75 mL, and 1 mL.

2.3 Yield of Microencapsulation

The yield of the encapsulated powder was calculated based on the ratio of the weight of the encapsulated powder produced and the weight of the initial raw material. The yield was calculated using the following formula 1:

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

where W is the weight of microencapsulation, and W_1 is the weight of microcapsule-forming material.

2.4 FTIR (Fourier Transform Infrared)

Fourier Transformed Infrared Spectroscopy (Shimadzu Q-ATR spectrometer, Japan) was used for analyzed the functional groups. The procedure was carried out by taking the required sample and then placing the sample into the FTIR tool to be shot by infrared light. The results of the FTIR test are in the form of a graph and can be analyzed for the composition of the sample through the peaks. The FTIR analysis of rice bran oil with spectral peaks between 800 and 3000 cm^{-1}.

2.5 SEM (Scanning Electron Microscopic)

Scanning electron microscope (JEOL JSM-6510LA, Japan) was used for analyzed the morphology of the samples. The procedure was done by taking the required sample and then placing the sample into the SEM tool to create the surface of the material using a high-magnification electron microscope. The results of the SEM test are in the form of a sample enlargement image showing the microencapsulation of the sample.

2.6 Encapsulation Efficiency

The encapsulation efficiency can be calculated by the determination of oil load in the microcapsules by the Soxhlet extraction procedure using n-hexane as solvent. The encapsulation efficiency (%) was calculated by the following formula 2:

$$\text{Encapsulation efficiency} = \frac{W_2}{W_3} \text{ X } 100\ \% \quad (2)$$

where W_2 is the weight of encapsulated oil (oil after extracted), and W_3 is the weight of the oil added for encapsulation (oil before extraction).

2.7 PSA (Particle Size Analyzer)

PSA analysis uses a Malvern analytical tool. A particle size analyzer is a tool that can measure the distribution of particle size. PDI Values (Polydispersity Index) describe the distribution of particle size. Good PDI values show good long-term stability.

3 RESULTS AND DISCUSSION

3.1 Yield and Efficiency of Encapsulation Results

Complex coacervation is a phenomenon where cationic and anionic water-soluble polymers interact in water to form a liquid polymer-rich phase called a complex coacervate. This coacervate is used to form the microcapsule shell. Chitosan is the cationic polymer used in this experiment. Anionic water-soluble polymers are used to form the complex coacervate, and k-carrageenan is chosen in this experiment. When a complex coacervate is formed, it is in equilibrium with a dilute solution called the supernatant. The supernatant acts as the continuous phase in which the complex coacervate is dispersed [9]. RBO as the core material is dispersed into the system, each drop or particle of the dispersed core material is spontaneously covered with a thin film of coacervate, as long as the coacervate adsorbs on the surface of the dispersed oil droplets. When this liquid film gels, capsules are formed. "Wet" complex coacervate gel is a very rubbery shell that molds extensively without tearing. To increase the strength of the water-swollen shell and create a thermally irreversible gel structure, the complex coacervate capsule shells are cross-linked with glutaraldehyde [10].

Glutaraldehyde is used as a crosslinking agent in capsules because it has two reactive functional groups that are commonly used as crosslinkers [11]. Kappa-carrageenan is a good choice as a coating material because it has pseudoplastic characteristics, is safe to eat and biodegradable, helps the microcapsule formation process to be smoother and spherical, and increases the adhesion force between the wall and core material [7]. Glucomannan is a biopolymer that is easily soluble in water and forms a gel, and functions as a thickening and gelling agent that can form and stabilize the gel structure, making it can be used as a food thickener and can form a thin layer (film) that is transparent. Hence, it is suitable as a coating.

The results of the microencapsulated samples were used to calculate the yield. Yield is used to determine the efficiency and effectiveness of a process. The higher the yield value produced, the more efficient the process used [12]. Based on the results of calculating the percentage yield and efficiency of the microcapsule process with the coacervation method, the microcapsule yield and efficiency were obtained as can be seen in Table 1.

Table 1. Comparison of Microencapsulation Yield and Efficiency with Glutaraldehyde Volume Variation

Sample	Yield (%)	Efficiency (%)
Glutaraldehyde 0.25 ml	38.98	31.66
Glutaraldehyde 0.50 ml	43.04	48.41
Glutaraldehyde 0.75 ml	28.42	21.07
Glutaraldehyde 1.00 ml	49.20	48.22

The yield and efficiency of microencapsulation are presented in Table 3 for the influence of the amount of crosslinkers (glutaraldehyde). From the table it can be seen that the more glutaraldehyde added, the higher the yield of the encapsulation process. With the increase in the amount of glutaraldehyde, the number of polymers that are cross-linked is greater, which increases yield. As the concentration of glutaraldehyde increased, the encapsulation efficiency improved slightly. This increase in encapsulation efficiency was due to the ability of the microcapsules to retain oil due to the formation of crosslinks. Cross-linking with glutaraldehyde played an important role in hardening, making the microcapsule wall compact and thus retaining the oil.

3.2 FTIR Analysis

FTIR analysis identifies functional groups in samples. The spectra of the materials for microencapsulation, which are carrageenan, chitosan, glucomannan, and RBO, are shown in Figure 1. The FTIR spectrum of RBO shows absorption bands at the peak points of 2928 cm^{-1}; 1742 cm^{-1}; and 1162 cm^{-1}, where these peak points are also mentioned in Fadilah's experiment, which shows the absorption bands at the peak points of 2924 cm^{-1}; 1744 cm^{-1}; and 1162 cm^{-1} for RBO [13]. For the FTIR spectrum of glucomannan, the absorption bands show at the peak points of 1730 cm^{-1}; 867 cm^{-1}; and 781 cm^{-1}. These peak points are ester carbonyl groups (C=O) mainly sourced from the acetyl group of glucomannan appearing at 1732 cm^{-1}, CH bending appeared at 873 and 812 cm^{-1} and are attributed to the β-pyranose form of glucose and mannose, respectively [14].

Moreover, the FTIR spectrum from the carrageenan shows peak points of 1254 cm^{-1}; 1062 cm^{-1}; 925 cm^{-1}; and 840 cm^{-1}, where each peak point indicates sulfate groups, glycosidic bonds, 3,6-anhydrogalactose, and galactose-4-sulfate. Meanwhile, the FTIR spectrum of chitosan shows the presence of peak points at 1654 cm^{-1}; 1594 cm^{-1}; and 1067 cm^{-1}, where each peak point indicates the presence of amide I, amide II, and glycosidic bonds. The amide I peak point is caused by C=O stretching vibrations, while amide II is caused by stretching vibrations combined with N-H bending vibrations [15].

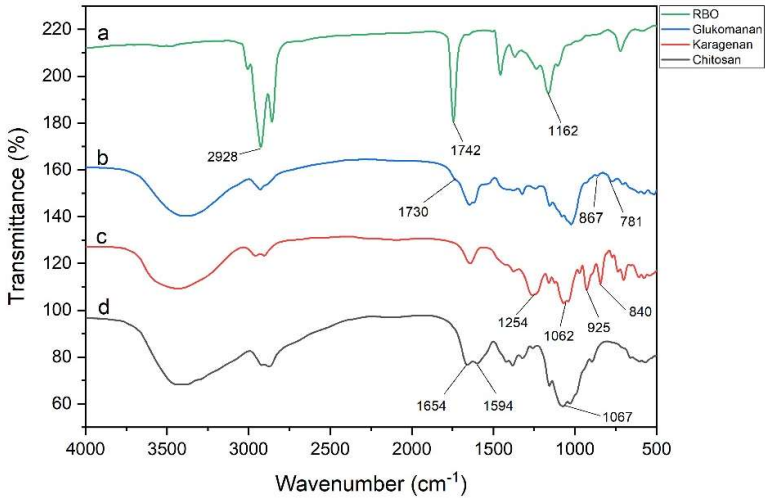


Figure 1. FTIR spectrum of (a) RBO, (b) glucomannan, (c) carrageenan, and (d) chitosan

Fig 1. shows a comparison of the FTIR spectra of the materials for the microencapsulation proces. Fig 2, shows the spectra of microcapsules can be analyzed. Fig 2. shows that there are peaks point of 2923 cm^{-1} in samples a and c, 2919 cm^{-1} in sample b, and 2925 cm^{-1} in sample d as an indication of encapsulated RBO oil. The presence

of carrageenan was indicated at the peak points of 929 cm^{-1} and 845 cm^{-1} for sample a, 927 cm^{-1} and 847 cm^{-1} for sample b, 929 cm^{-1} and 844 cm^{-1} for sample c, and 927 cm^{-1} and 842 cm^{-1} for sample d.

In addition to RBO and carrageenan, glucomannan and chitosan also exist. The presents of glucomannan are indicated by a peak point of 1745 cm^{-1} and 888 cm^{-1} for sample a, 1748 cm^{-1} and 890 cm^{-1} for sample b, 1745 cm^{-1} and 888 cm^{-1} for sample c, and 1744 cm^{-1} and 888 cm^{-1} for sample d. Then there is a peak point of 1633 cm^{-1} for sample a, 1635 cm^{-1} for sample b, 1634 cm^{-1} for sample c, and 1630 cm^{-1} for sample d, indicating chitosan.

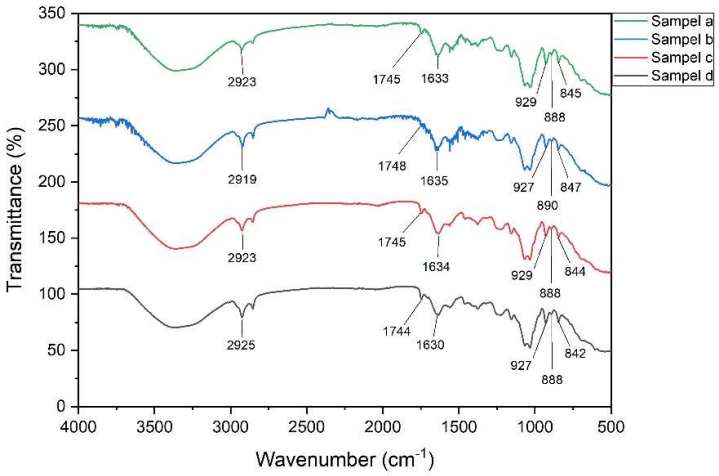


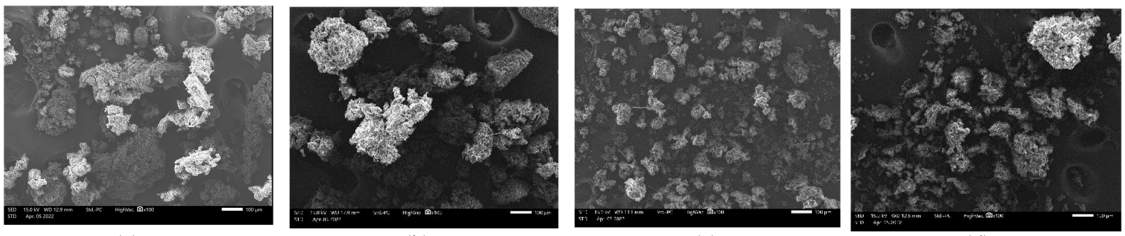
Figure 2. FTIR Spectra for Samples with Glutaraldehyde Variations (a) 0.25 mL, (b) 0.5 mL, (c) 0.75 mL, dan (d) 1 mL

Table 2. Comparison of FTIR Spectra for Samples and Materials

Material	Sample a (cm^{-1})	Sample b (cm^{-1})	Sample c (cm^{-1})	Sample d (cm^{-1})
RBO	2923	2919	2923	2925
Carrageenan	929 & 845	927 & 847	929 & 844	927 & 842
Glucomannan	1745 & 888	1748 & 890	1745 & 888	1744 & 888
Chitosan	1633	1635	1634	1630

3.3 SEM Analysis

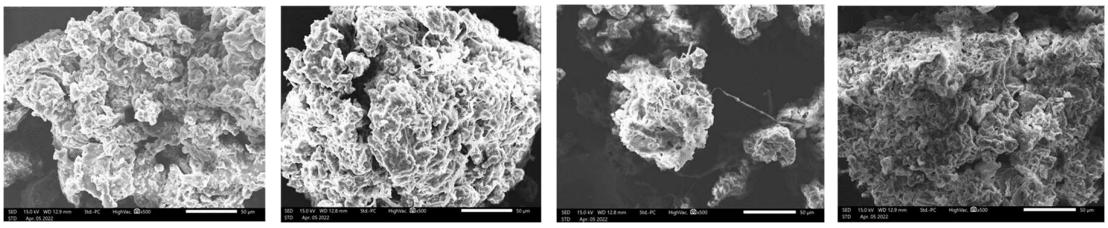
The SEM testing was carried out in a microencapsulated sample of Rice Bran Oil (0.5 mL) coated with kappa-carrageenan (0.2 gram), glucomannan (0.1 gram), and chitosan (0.3 gram) with a variation 0.25 mL, 0. 5 mL, 0.75 mL, and 1 mL of glutaraldehyde. The results of SEM analysis are shown in Figures 3, Figure 4, and Figure 5 for magnification 100x, 500x, and 1000x. Figures show that the four samples have a similar shape. The microcapsules have an irregular shape with rough surfaces. The morphological shape of the microcapsules is not round; this can be seen from the uneven surface shape of the microcapsules which looks textured. This is due to the effect of the coacervation technique carried out to form a combined particulate structure. The morphology of these three samples has a clumpy microcapsule shape due to the high-water content in accordance with the research that has been conducted previously, so the microcapsule shapes are attached [16].



(a) (b) (c) (d)

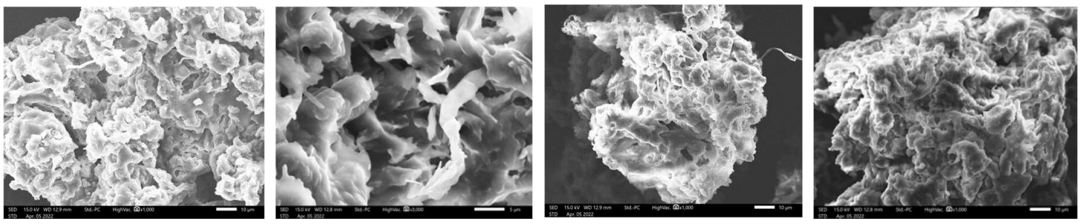
Figure 3. SEM Results (100x) for Samples of Glutaraldehyde Volume Variation; (a) 0.25 ml; (b) 0.5 ml; (c) 0.75

ml; (d) 1 ml



(a) (b) (c) (d)

Figure 4. SEM Results (500x) for Samples of Glutaraldehyde Volume Variation; (a) 0.25 ml; (b) 0.5 ml; (c) 0.75 ml; (d) 1 ml



(a) (b) (c) (d)

Figure 5. SEM Results (1000x) for Samples of Glutaraldehyde Volume Variation; (a) 0.25 ml; (b) 0.5 ml; (c) 0.75 ml; (d) 1 ml

3.4 PSA Analysis (Particle Size Analyzer)

PSA can be used to analyze the particles of a sample which aims to determine the particle size (Polydispersity) and particle size distribution of the sample. The intensity distribution describes how much light is scattered by the particles in the different size bins. Fig 6. shows the PDI (Polydispersity Index) and polydispersity or particle size values are obtained as follows.

Table 3. Comparison of Sample Encapsulation Particle Size with Variations

Sample	PDI	Particle Diameter Range (nm)	Most Particles	
			% Intensity	Particle Diameter (d.nm)
Glutaraldehyde 0.25 ml	0.653	21.04 – 825.0	9.1%	458.7
Glutaraldehyde 0.50 ml	0.639	91.04 – 1106	15.3%	531.2
Glutaraldehyde 0.75 ml	0.541	58.77 – 712.4	13.5%	396.1
Glutaraldehyde 1.00 ml	0.449	712.4 - 1281	31.3%	955.4

From Table 2, the microcapsules with 0.25 mL and 0.5 mL glutaraldehyde were in micron size, with a range of 21 to 712 microns. Meanwhile, adding 0.5 mL and 1 mL glutaraldehyde resulted in a bigger particle size, of more than 100 microns or in millimeters. The polydispersity index value is between 0.449 to 0.653. It indicates that the distribution of particles is running well because this polydispersity value is bigger than 0.3. A PDI value of more than 0.3 indicates a wider and more variative particle size distribution [17]. Particle size and morphology are measured to determine how large the particles formed to obtain the appropriate size.

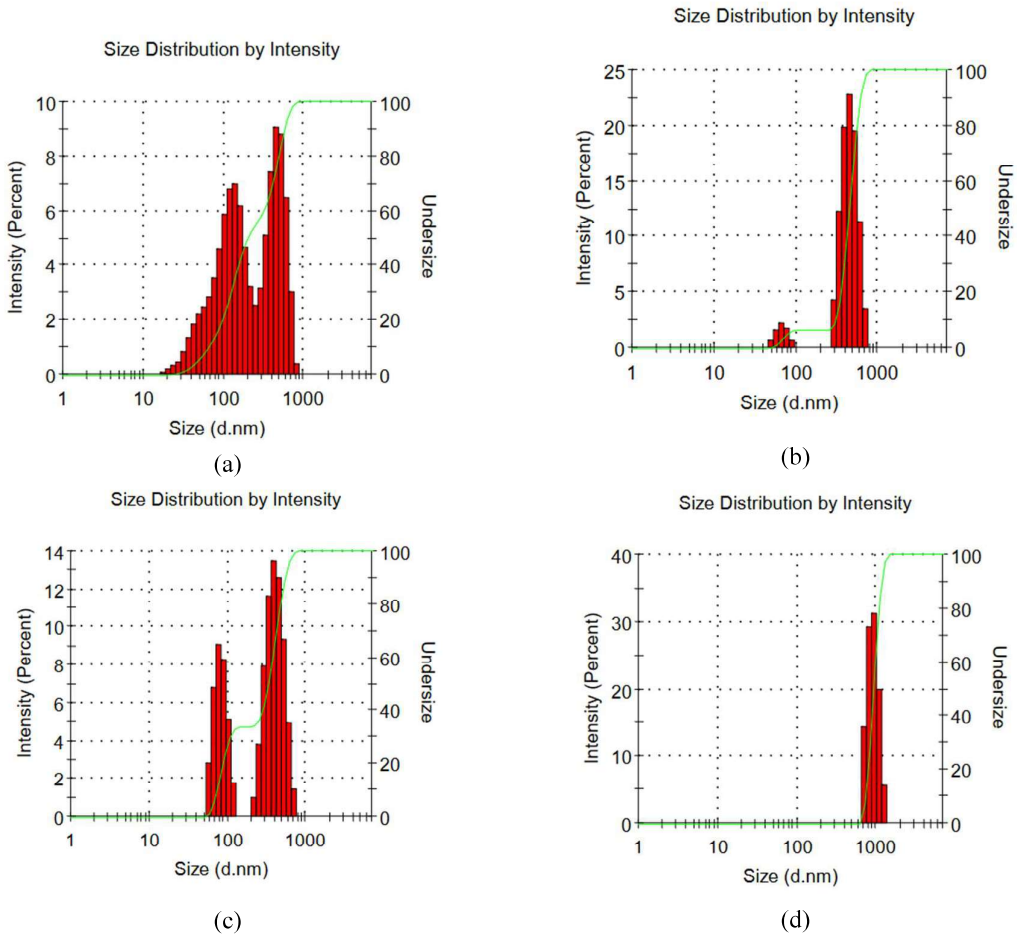


Figure 6. PSA Results of Glutaraldehyde Volume Variation Samples (a) 0.25 ml; (b) 0.5 ml; (c) 0.75 ml; (d) 1 ml

4. CONCLUSION

The addition of glutaraldehyde which can be cross-linked with many polymers affects the yield, encapsulation efficiency, and particles characterized by FTIR, SEM, and PSA. Increasing the amount of glutaraldehyde tends to increase the yield and encapsulation efficiency. A yield of 49.20% was obtained by adding 1 mL of glutaraldehyde with an encapsulation efficiency of 48.22%. FTIR spectra show that all samples indicated the presence of RBO as well as other polymers used in the research: carrageenan, chitosan, and glucomannan. SEM images show that particles have irregular shapes with uneven surface texture. The results of PSA show that particles are in micron size except for the addition of 1 mL glutaraldehyde. Microencapsulation is applied in the fields such as agriculture, industry and medicine, flavours, pesticides, dyes, liquid inks and medicines.

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