Preparation and Evaluation of Plant Extract Microcapsules Using Chitosan

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Abstract: Research on microencapsule formulation and evaluation of ethanol extract by spray drying method was carried out to determine the effect of chitosan addition. It is hoped that from this research, chitosan microcapsule preparations can increase the benefits and stability of the extract. Microcapsule evaluation includes: microcapsule water content test, microcapsule size distribution using a Scanning Electron Microscope (SEM). The microcapsules formed were characterized by antioxidant activity using the 2,2-diphenyl-1-pikrilhidrazil (DPPH) method and total phenol content (folin-ciocalteu method). The results of the morphological evaluation showed that the microcapsules were evenly spherical for all formulas and they had an average moisture content of 4.612 ± 0.02. The anti-oxidant activity of DPPH-SA increased with the increasing concentration of added chitosan. Microencapsules of the extract without chitosan and with the addition of 0-1% chitosan has antioxidant activity of 85,876 ± 1,897% and 86,014 ±0.570 - 86,725 ±0.313. In the TPC (Total Phenol Content) test, the results were 5.00 ± 0.01% and 5.49 ± 0.01-8.98 ±0.02%. Based on the research, it could be concluded that the microencapsulation with the addition of chitosan was able to increase the stability of the extract so that the antioxidant activity and total phenolic content could be higher.

1. Introduction

Microencapsulation is a possibility that provides an effective solution to the challenges faced by the pharmaceutical (Li et al., 2019; G. Zhang et al., 2018), cosmetic (Carvalho et al., 2016), food agriculture (Heidebach et al., 2012; Shahidi and Han, 1993) and textile industries to deliver ingredients in their active form to target locations. One of the microencapsulation methods is the double emulsification method of solvent evaporation. In this method the coating is dissolved in a volatile solvent, the core material to be micro-encapsulated is dissolved in the solvent or dispersed in a polymer coating solution. The polymer solution which has been mixed with the active ingredient solution is emulsified in the surfactant solution. This method can be done in a short time, the cost and processing is relatively cheap and can be used for various core materials, both in the form of water soluble and water insoluble materials (Vengerovich et al., 2017).

Chitosan is a non-toxic, biodegradable and biocompatible amino polysaccharide which makes it a potential encapsulation material for various active ingredients (Muxika et al., 2017; Younes and Rinaudo, 2015). The chitosan coating on food products, for example, provides them with protection from possible antimicrobial attacks (Vinoza and Vavrikova, 2011), antioxidants and a longer shelf life (Cheung et al., 2015). Likewise, its coating on pharmaceutical ingredients has valuable applications in drug preservation and targeted delivery (Kong et al., 2010). In several studies chitosan has also shown good antioxidant activity so that its addition in the encapsulation process greatly improves the benefits of the product (Philibert et al., 2017). Extract is an active ingredient which
sometimes has a lack of taste and smell, so it is necessary to encapsulate it to cover this deficiency. In this research, the ability to add chitosan will be studied in the encapsulation process of extract ingredients and improve its benefits.

2. Procedures

2.1 Materials and research tools

Materials used is Chitosan (the degree of deacetylation 80.24%), petai leaf extract obtained from petai leaves with solvents used ethanol (Merck, 40% purity), arabic gum, maltodextrin, methanol (Merck), DPPH (Sigma-Aldrich, 90% purity), gallic acid (Sigma-Aldrich), sodium carbonate (Merck), folin ciocaltelu (Merck). The tools used in this study were spray drier (Ama Industries), water analyzer (Radwag MAC50), digital ultrasonic cleaner (Branson 5210), UV-Vis spectrophotometer (Shimadzu2480), electric scale (Sartorius), oven (Memmert), rotary evaporator (Scilogex).

2.2 Forming of microencapsules

2.2.1 Formula Design Microencapsules

The design of the extract microencapsulation formula is presented in Table 1.

| Composition          | Formula (%) |
|----------------------|-------------|
|                      | 1  | 2  | 3  | 4  | 5  | 6  |
| Chitosan             | 0  | 0.2| 0.4| 0.6| 0.8| 1.0|
| Extracts             | 2.5| 2.5| 2.5| 2.5| 2.5| 2.5|
| Gum Arabic           | 75 | 75 | 75 | 75 | 75 | 75 |
| Maltodextrin         | 25 | 25 | 25 | 25 | 25 | 25 |

2.2.2 Microencapsulation of extract

A mixture of 40g (20% w/v) commercial maltodextrin, gumarabic and chitosan were dispersed in distilled water. Each ingredient is individually dissolved at 40-60 °C with constant magnetic stirring for 30 minutes to give a final volume of 1000 mL. Chitosan is dissolved separately in acetic acid buffer before being added. The extract (2.5% w based on the main ingredient used) was added to the mixture. The mixture is homogenized using a mini homogenizer (Scilogex) for 5 minutes at 8000-12000 rpm until complete dispersion is achieved. The water slurry, carrier material and extract were spray dried by means of a spray dryer at an output temperature of 80 ± 5°C. The inlet temperature is determined by the output temperature. The feed flow rate is 20 mL/min.

2.3 Evaluation of Microencapsules

2.3.1 Surface Morphology of Extract Microcapsule

The shape and surface morphology of the microcapsules were evaluated using Scanning Electron microscopy (SEM) analysis in the UNDIP integrated laboratory. The extract microcapsules for SEM were obtained by lightly sprinkling the on double adhesive tape, which was affixed to the aluminum snippet. The snippet is then plated with gold with a thickness of 300 Å using a sputter coater and then viewed under SEM with the appropriate magnification obtained.

2.3.2 Antioxidant Activity of Extract Microcapsule

The DPPH Scavenging Activity (1,1 Diphenyl 2-Picryl Hydrazyl -SA) test was carried out by the method of (Banerjee et al., 2005). The 1.0 mL sample microcapsule solution was added 3.0 mL 0.1 mM DPPH solution. The sample's absorbance was measured by spectrophotometer (Shimadzu Japan) at 514 nm. The DPPH-SA is expressed as:
2.3.3 Determination TPC of Extract Microencapsule

The total phenolic content (TPC) of the extract microencapsule was determined using the Shui and Leong method (Shui and Leong, 2006). The sample (0.2 mL), distilled water (15.8 mL) and folin ciocalteu reagent (1 mL) were placed in a closed test tube. After adding 10 mL of sodium carbonate (20% w), the tube was vortexed for 10 minutes and then allowed to react at room temperature for 2 hours. The mixture's absorbance was measured at 765 nm using a spectrophotometer (Shimadzu 2480, Japan) and compared with a blank. The total phenolic content is expressed as gallic acid equivalent (mg GAE / gram dry weight).

\[
\text{DPPH radical scavenging activity (\%) = } \frac{A_{\text{Blank}} - A_{\text{sample}}}{A_{\text{Blank}}} \times 100% \tag{1}
\]

3. Research Results

3.1 Characteristics of Extract Microcapsules

3.1.1 The Surface Morphology of Extract Microencapsule

Observations using SEM showed that the microcapsule particles were spherical. The greater the addition of chitosan concentration, the smoother the surface character. SEM results are presented in Figure 1.

![SEM Results of extract microencapsule](image)

Figure 1. SEM Results of extract microencapsule

3.1.2 The Moisture Content of Extract Microcapsule

The moisture content of the extract microcapsule powder was measured using AOAC method (OFFICIAL METHODS OF ANALYSIS OF AOAC INTERNATIONAL., 2019). Microencapsule extract moisture measurements showed a value in the range between 3.99 ±0.93 and 5.73%. At this humidity the microencapsule content can be stored for a long time.

3.2 Analysis of Extract Microcapsules

3.2.1 The DPPH-SA of Extract Microcapsule
The antioxidant activity of the microcapsule extract was greater with the increase in chitosan which was used as a coating material. DPPH-SA test results are presented in Figure 2.

![Figure 2](image)

**Figure 2.** Antioxidant activity of extract microcapsule

### 3.2.2 The TPC of Extract Microcapsule

The total phenolic content (TPC) of the microcapsule extract was also greater with the addition of chitosan as a coating. These results indicate that the greater the total phenolic content increases the antioxidant activity of the microcapsule extract. TPC assays results are presented in Figure 3.

![Figure 3](image)

**Figure 3.** Total phenol content of extract microcapsule
4. Discussion

4.1 Characteristics of Extract Microcapsules (The Surface Morphology of Extract Microencapsule)

Particle size testing is carried out on all formulas to see the size and shape of the particles. Observation of microcapsule shape and morphology showed that all microcapsule formulas were round. The surface characteristics of the microcapsule have a fairly smooth surface with the increasing concentration of chitosan addition. The microencapsulate extract without chitosan showed wrinkled pores. This is due to the repulsion between the cationic groups Maltodextrin and gum arabic, which results in wrinkled pores only. The pores formed from the microcapsules can be seen in Figure 1. From the observation of the shape and morphology of the microcapsules, it can be seen that from all the formulas F5 and F6 have the best surface shape and character, namely having a rounder surface shape and character and a fairly smooth surface.

The technique used in the manufacture of extract microencapsules in this study was the microencapsulation technique with the spray drying method. In the spray drying method, the material is sprayed and atomized to form droplets into a hot drying medium, then water in the form of droplets will evaporate leaving dry matter (Hidalgo et al., 2018; Osamede Airouyuwa and Kaewmanee, 2019). There are two types of coating materials used, namely arabic gum and maltodextrin with a combination ratio of maltodextrin: gum arab (1:3). With the concentration of the extract added 2.5%. The resulting microencapsulates are brownish green in color. The resulting microencapsulates were analyzed for their antioxidants and phenols using a spectrophotometer.

The choice of maltodextrin as a carrier material is because it can reduce the hygroscopicity of microparticles with a low intake air temperature and a high maltodextrin concentration. It also makes particles colorless and amorphous, tends to be more spherical and has no chemical reactions (Barthold et al., 2019; L. Zhang et al., 2018). Gum arabic is also a good carrier but works better when combined. Encapsulation using whey protein isolate combined with guar gum produces a dry powder that is effectively treated and chemically stabilized, increasing its handling and shelf life (Mehyar et al., 2014). The combination of radiation-depolymerized guar gum and gum arabic shows better retention of the encapsulated flavor than gum arabic alone as a wall material (Sarkar et al., 2012). From previous experiments, it was found that gum arabic and maltodextrin with a combination ratio of maltodextrin: gum arabic (1:3).

The water content of the microencapsulate extracts showed a value ranging between 3.99 ± 0.93 and 5.73% with an average of 4.612 ± 0.02. It’s a sufficiently good moisture content for the microencapsulates to extend their shelf life.

4.2 Analysis of Extract Microcapsules

4.2.1 The DPPH-SA of Extract Microencapsulate

The results showed that the antioxidant activity of the microencapsulated extract was getting better with the addition of the concentration of chitosan as a carrier. DPPH-SA test results are presented in Figure 2. This is because the coated material is better with the addition of chitosan (Shi and Tan, 2002; Tolve et al., 2019). In addition, chitosan has antioxidant activity so that its addition can increase the antioxidant activity of microencapsulate extracts. This activity remains the same for the crude extract and in the encapsulated form; which may be related to the presence of certain compounds identified in the extract with high stability and potential antioxidants.

This study used the DPPH free radical scavenging method for antioxidant capacity testing because this method is known to be faster, practical, accurate, easy to perform, only a small number of samples needed and relatively cheap (Vinsova and Vavrikova, 2011). This method is commonly used to measure the ability of a compound that acts as a free radical scavenger. This method is used because petai leaves have antioxidants (Aisha et al., 2012; Kamisah et al., 2013; Ko et al., 2014). The microencapsulate extract was tested by DPPH and the results were 85.88 ± 1.897% (F1); 86.01 ± 0.570% (F2); 86.15 ± 0.232% (F3); 86.37 ± 0.210% (F4); 86.44 ± 0.570% (F5) and 86.73 ± 0.313%. From the
results of the microencapsule of the highest antioxidant extract at F6 with the addition of 1% chitosan concentration.

4.2.2 The TPC of Extract Microencapsule

The results showed that the highest phenol content was found in crude extracts compared to microencapsule preparations. This occurs because phenol preparations are more in pure preparations which are extracts because phenol is more stable in pure content and reacts to the combination of gum and maltodextrin. TPC assays results are presented in Figure 3.

Arab gum is known to have good emulsification ability because it can reduce the surface tension between the two phases (Sobieralska and Kurek, 2019). Meanwhile, maltodextrin is a starch derivative that is modified by acids or enzymes. The result is a monosaccharide or short chain polymer which then forms a thin film layer to protect and prevent loss of flavor or color components during the drying process. However, maltodextrin has the disadvantage of not having an emulsifying ability and a lack of surface binding activity on the oil-water surface (L. Zhang et al., 2018). This results in a higher maltodextrin ratio with the same ratio of arabic latex produced.

The TPC microencapsule extract was tested and the results were 5.0 ± 0.017% (F1); 5.49 ± 0.01% (F2); 5.96 ± 0.01% (F3); 6.19 ± 0.01% (F4); 7.13 ± 0.01% (F5) and 8.98 ± 0.02%. From the results of the microencapsule of the highest TPC at F6 with the addition of 1% chitosan concentration.

5. Conclusion

Microcapsules extract with the addition of chitosan variations using a simple and reproducible methodology. Capsule formation was confirmed by SEM, and the majority of the microcapsules is presented in the form of a round, the addition of chitosan is able to smooth the surface so as not wrinkled. Generally microcapsules shown to have a protective effect of the extract, so it has antioxidant activity and extract active ingredients protected. So the more the addition of chitosan on mikroencapsul formula provides enhanced antioxidant activity and capable of protecting the active substance.

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