Effect of Arrowroot Nano Starch Preparation Methods on the Characteristics of Temulawak Oleoresin Microcapsules

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Abstract. Active compounds of temulawak often have some disadvantages such as barely dissolve in water, unstable in alkaline and acidic conditions, that need to be overcome by coating with the starch matrix. Starch nanoparticles precipitated with alcohol can serve as coating material because it has a helical hole and the porous structure. The research objective was to determine the effect of starch nanoparticles coating materials on the characteristics of temulawak microcapsule. The treatments tested included types of coating/starch matrix resulted from two different preparations of arrowroot starch nanoparticles namely by butanol and ethanol precipitation with two degrees of hydrolysis. The parameters observed consist of yield, encapsulation efficiency (EE), drug loading (DL), morphology (SEM and TEM), particle size distribution and polydispersity index (PDI), FTIR, and antioxidant activity. The results showed that the yield of microcapsules ranged from 42.91 to 58.31%, with EE from 48.69 to 69.59% and DL from 19.02 to 28.96%. Microcapsule size ranged from micro to nano size having 200 nm in average with rounded shape, and polydispersity index from 0.69 to 0.94. The antioxidant activity showed that microcapsule coated with butanol precipitation starch matrix was higher than that of ethanol precipitation. The FTIR analysis showed a binding of the active ingredient (curcumin) in the matrix as seen with the new absorption peak at a wavelength of 1510 cm⁻¹ as well as two new peaks at 1750 cm⁻¹ and 3730 cm⁻¹.

1. Introduction
Empirically temulawak (Java ginger) rhizome is widely used to increase appetite, improve digestive function, maintain healthy function of heart, joint and bone pain, lowers blood fats, and helps inhibit blood clotting [1]. The active compound of temulawak is curcuminoid and xanthorrhizol. Curcuminoid fraction is the component that gives yellow color with a bitter taste, soluble in acetone, alcohol, acetic glacial, alcohol hydroxide. It has a distinctive aroma, and not toxic. Kurkuminoid of temulawak comprises desmetoxicurcumin and curcumin with structural formula C₂₁H₂₀O₆ and has molecular weight of 368 g/mol [2]. As other medicinal plant, curcumin has some disadvantages namely barely dissolve in water, unstable in alkaline and acidic conditions in the digestive system lead to low bioavailability, and has a short half-life. According to Anand et al. [3] curcumin is slowly absorbed and rapidly metabolized so it has low bioavailability in the blood system. The research reports that after 30 minutes of oral administration of 400 mg curcumin, 90 % curcumin was found in the stomach and small intestine, while only 1 % curcumin found in these organs after 24 hours [4]. Curcumin also has a short half-life. Maiti et al. [5] reported that in smaller doses (1 g/kg curcumin) administered orally to mice appeared to have a half-life for 1.45 hours. Moreover, the solubility of curcumin is also limited to a certain pH range.
One approach to increase the solubility of the active ingredient which are difficult to dissolve is preparing into an oil in water emulsion (o/w), chemical modification and encapsulation [6] and formation of microspheres [7]. Incorporation of active components in the matrix can improve the stability of these components, and can protect it from the acidic environment of the digestive, and ensure release in the small intestine where they are absorbed into the bloodstream [8].

Complex of starch nanoparticles resulted from butanol and ethanol precipitation has a type of crystalline that has helical cavity that can be host for organic and inorganic molecules, including active ingredients. In addition, guests components (active ingredient) with the formation of inclusion complexes will stabilize the active ingredient being shielded from the influence of oxygen or light [9]. According to Kim and Lim [10] the inclusion of molecular complex V - amylose can be used for encapsulation and controlled release of active ingredients of drugs in the pharmaceutical industry or food. Moreover, starch nanoparticles has benefits in terms of higher surface area, lower viscosity at higher concentration and higher entrapment of active ingredients.

The high crystalline fraction of arrowroot starch prepared by lintnerization using HCl was precipitated with butanol and ethanol to produce nano size starch [11]. Nano size particles from arrowroot starch could be applied as encapsulation matrix for herbs extract (sambiloto and temulawak) and lactic acid bacteria [12]. Different methods of nano starch preparation might result in different characteristics of the coating materials. The objective of the research was to determine the effect of starch nanoparticles coating materials on the characteristics of temulawak oleoresin microcapsule.

2. Methodology
This research was conducted at the Laboratory of Indonesian Center for Agricultural Postharvest Research and Development (ICAPRD), and Laboratory of AgroIndustrial Technology Process Laboratory, Bogor Agroindustrial University, from June to October 2013. Raw materials used was temulawak oleoresin and coating material used was arrowroot starch nanoparticles derived from arrowroot starch which experienced double modification process namely acid hydrolysis followed by butanol or ethanol precipitation process.

The raw materials used were temulawak (TL) oleoresin extracted from dried temulawak tubers. Extraction was done with ethanol 70%, using a maceration process for 24 hours followed by evaporation using a vacuum rotary evaporator to produce semi-solid extract. In application as wall material, the extract was then diluted with ethanol 70% until the total dissolved solid content was about 20%.

Starch nanoparticles were produced by butanol and ethanol precipitation of lintnerized starch using phase separation methods developed by Kim and Lim [10] and Ma et al [13] with minor modifications. The Lintnerized starch was prepared by acid hydrolysis that appeared elsewhere [14].

2.1. Preparation of Arrowroot Starch Nanoparticles
Arrowroot starch nanoparticles by butanol precipitation process was prepared according methods Kim and Lim [10] with minor modifications. Lintnerized starch was dissolved in 200 ml of hot distilled water and then was autoclaved at 121°C for 20 min. The solution was cooled to 70°C and about 20 % (v/v) of n-butanol was slowly added to the solution to form a separated butanol phase from the starch solution. The solution was then stirred gently (100 rpm) at 50°C for 3 days and then centrifuged at 5,000 rpm for 20 min. The precipitates were washed with ethanol several times and dried by freeze dryer.

Production of starch NP was carried out via complex formation of lintnerized starch with ethanol precipitation [13]. The lintnerized starch was dispersed with distilled water at ratio of starch: distilled water 1:20 (w/v) and then heated until it was gelatinized (approx. 30 minutes) while stirring with rapid rate on the hotplate stirrer. After that ethanol was added drop wise with a dropping rate of about 3 ml/min for about 60 minutes with rapid stirring. The complex/precipitated was washed several times with ethanol and then filtered, dried with a freeze dryer and analysed.
2.2. Encapsulation Process

The process of encapsulation was as follows: 10 g of starch nanoparticles or mixed starch nanoparticles and maltodextrin 1:2 was dissolved in 150 ml warm distilled water (70°C), stirred with a magnetic stirrer for 30 minutes. The slurry was stood to rehydrate at cold room for a night. The next day, 5 ml of the diluted temulawak extract (total solid 20%), was added to the starch slurry, mixed with a homogenizer (11,000 rpm) for 10 minutes and dried using a spray drying with inlet temperature 170-180°C.

Treatments observed were types of matrix
1= starch nanoparticles (NP) by butanol precipitation (BNP) for 2 hours acid hydrolysis (H2B)
2= starch nanoparticles (NP) by butanol precipitation (BNP) for 24 hours acid hydrolysis (H24B)
3= starch NP by ethanol precipitation (ENP) for 4 hours acid hydrolysis (H4E)
4= starch NP by ethanol precipitation (ENP) for 24 hours acid hydrolysis (H24E)

All experiments were conducted in three replicates.

Analysis of the curcumin loaded starch nanoparticles matrix included yield, encapsulation efficiency (EE) and drug loading (DL), morphology, particle size distribution and polydispersity index, antioxidant activity, and FTIR spectra (ABB MB 3000) at wave number range 400-4500 cm⁻¹.

3. Result And Discussion

3.1 Yield, Encapsulation Efficiency and Drug Loading

The result showed that the range of yield of microcapsule and DL was 42.91-58.31% and 19.02-28.96%, respectively with EE above 60%, except for treatment H2B (Table 1). The result is in line with Paramera et al [14] who did encapsulation of curcumin with OSA starch with the EE value of 56.2 for treatment without emulsifier and 60.4% with the addition of emulsifiers. Other research produces much higher EE value. The variations in EE value is caused differences in the coating material, and active ingredients used. The value of EE with spray drying process in which the material subjected to high temperatures during the encapsulation process also affects the percent of EE. The lower value of drug loading which was opposite to EE showed the high efficiency of encapsulation. Meanwhile, the longer hydrolysis duration which produce finer particle size (Table 2) produce higher yield and EE.

Encapsulation mechanism that occurs depends on the type of coating materials and incorporated active ingredients. According to Yu and Huang [6], the encapsulation with cyclodextrins coating involving Van der Waals bonds and hydrophobic interactions as well as hydrogen bonding contribution of the OH groups from curcumin which stabilized the complex formed. Meanwhile encapsulation using modified starch occurs through hydrogen bonding interactions. The use of starch nanoparticles matrix prepared by butanol precipitation the binding mechanism possibly through bonding van der Waals and hydrophobic interactions as amylose type V has a hydrophobic helix hole [10]. Butanol precipitation method produced nano starch with single helical V-type amylose.

| Butanol precipitation | Yield (%) | EE (%) | Drug loading (%) |
|-----------------------|-----------|--------|------------------|
| Hydrolysis 2 hours    | 42.91     | 48.69  | 23.71            |
| Hydrolysis 24 hours   | 58.31     | 69.59  | 19.02            |
| Ethanol precipitation |           |        |                  |
| Hydrolysis 4 hours    | 46.43     | 63.29  | 28.96            |
| Hydrolysis 24 hours   | 57.08     | 66.23  | 22.05            |

3.2. Morphology

The surface morphology of temulawak oleoresin microcapsules is presented in Figure 1. The morphology observed by SEM revealed that the resulting microcapsules had rounded, curvy and...
somewhat deflated shapes with varying sizes. However, it seemed that different type of matrix and duration of hydrolysis did not affected the surface morphology. However, the particle size distribution measured using PSA showed differently, the longer hydrolysis duration produced smaller particles. The results agree with previous studies by Loksuwan [15] which used lintnerized tapioca. The deflation of the surface structure might be caused by rapid evaporation of the points of fluid during the drying process with spray drying [16]. The TEM observation revealed different pictures (Fig 1 c and 1 d). The longer duration of hydrolysis (24 h) seemed to have broader particle size than that of the shorter hydrolysis. The shorter chain length of the nano starch matrix might affect the microcapsules formed.

Figure 1. Surface morphology (SEM) (a, b) and TEM (c, d) of microcapsules treatments H4E and H24E.

3.3. Particle size Distribution
The particle size distribution of temulawak oleoresin encapsulated with starch nanoparticles prepared by butanol and ethanol precipitation can be seen in Table 2. The produced microcapsule has an average particle size from micro to nanometer between 196.7-258.8 nm from the butanol precipitation methods and 235.6-256.74 nm from ethanol precipitation methods (Table 2).

The PDI value from temulawak oleoresin microcapsules is far to ideal PDI for matrix as proposed by Danhier [17], except for matrix H24B. PDI values below 0.2 represent monodispersity in pharmaceutical formulations because showed monodisperse size distribution. Moreover, Yen [18] stated that the smaller PDI of 0.3 showed the uniformity formula nanoparticles with a narrow distribution. The high variation microcapsules size was often occurs in microcapsules produced by spray drying methods. Moreover, measurement of particle size distribution was also performed on the emulsion formed in dispersion of starch matrix nanoparticles containing temulawak oleoresin after the homogenization steps, before atomization by spray drying, as shown at Figure 2.

Table 2. Average particle size distribution, particle size range and PDI of microcapsules

| Matriks             | Average particle size (nm) | Range of particle size (nm) | PDI  |
|---------------------|----------------------------|----------------------------|------|
|                     |                            | D (10%) | D (50%) | D(90%) |      |
| Butanol precipitation|                            |         |         |        |      |
| Hydrolysis 2 hours  | 258.8±39.0                 | 230.3   | 398.2   | 891.7  | 0.69 |
| Hydrolysis 24 hours | 196.7±26.3                 | 181.3   | 323.7   | 741.5  | 0.39 |
| Ethanol precipitation|                            |         |         |        |      |
| Hydrolysis 4 hours  | 256.7±50.6                 | 323.7   | 387.5   | 1641.5 | 0.88 |
| Hydrolysis 24 hours | 235.6±48.0                 | 204.2   | 545.8   | 698.7  | 0.69 |
3.4. Antioxidant Activity

The antioxidant activity (IC$_{50}$ values) for microcapsule with nanoparticles starch matrix resulted from ethanol precipitation methods was slightly stronger than that of butanol precipitation methods, which is indicated by a lower IC$_{50}$ values (Table 3).

Table 3. Antioxidant activity of microcapsules using NP starch matrix by butanol and ethanol precipitation

| Matrix                  | Curcumin (ppm) | Antioxidant (ppm) | IC$_{50}$ | DPPH  |
|-------------------------|----------------|-------------------|-----------|-------|
| Butanol precipitation   |                |                   |           |       |
| Hydrolysis 2 hours      | 2313.8         | 1184.1            | 44977.6   |       |
| Hydrolysis 24 hours     | 3536.6         | 1090.8            | 29827.1   |       |
| Ethanol precipitation   |                |                   |           |       |
| Hydrolysis 2 hours      | 3029.6         | 1274.6            | 37154.2   |       |
| Hydrolysis 24 hours     | 4107.6         | 1021.8            | 27618.2   |       |

At the concentration of microcapsule of 25-400 ppm, percent free radical inhibition was between 3.04 to 20.48 % for butanol starch nanoparticles matrix. Moreover, the IC$_{50}$ values was 1184.1 ppm and 1090.81 ppm for 2 and 24 hours hydrolysis respectively. Meanwhile, the starch matrix nanoparticles from ethanol precipitation produced almost the same antioxidant inhibition, between 2.09 to 22.44 percent (Table 4). Table 4 showed also that the reduction of free radicals in the treatment of 24 hours hydrolysis was relatively higher than that of 2 hours.

Tabel 4. Percent of free radical reduction and IC$_{50}$ of temulawak oleoresin microcapsule

| Sample                   | Free radical inhibition (%) at concentrations (ppm) | IC$_{50}$ (ppm) |
|--------------------------|-----------------------------------------------------|-----------------|
| Butanol precipitation    |                                                     |                 |
| Hydrolysis 2 hours       | 3.04 4.50 7.30 10.94 18.34  | 1184.1          |
| Hydrolysis 24 hours      | 4.50 5.73 9.32 13.92 20.48  | 1090.8          |
| Etanol precipitation     |                                                     |                 |
| Hydrolysis 4 hours       | 2.09 3.83 5.54 9.41 16.96  | 1274.6          |
| Hydrolysis 24 hours      | 4.92 8.27 12.16 16.31 22.44  | 1021.8          |
3.5. FTIR

Results of the analysis of functional groups showed the difference spectrum between the native starch (Fig 3a) and the nanoparticles starch matrix either using of butanol precipitation (Fig 3b) or the temulawak oleoresin loaded nanostarch matrix (Fig 3c). The differences showed the binding of the active ingredient, in which curcumin occur in the matrix. From the pictures showed the same peaks of the three samples at wave number 1650 cm$^{-1}$ which is an indication of the strong bond that water in starch [13]. Additionally, it seen a new peak at wave number 1510 cm$^{-1}$ which showed the deformation of the amine group (-NH) and 1750 indicate the presence of ester groups (CO). Moreover, there was changes in absorption band narrowed and intense on the spectrum 3400 cm$^{-1}$ indicating the occurrence hydrogen bonding between the hydroxyl group (OH) of curcumin with starch. There was also a shift in the peak of the wave number 1010 to 1050 cm$^{-1}$ which indicate a keto group of curcumin [19].

![Figure 3. FTIR spectra for native arrowroot starch (a), nano starch matrix (b) and temulawak oleoresin loaded nano starch matrix (c)](image)

4. Conclusions

Different types of matrices either starch nanoparticles prepared with butanol and ethanol precipitation produced microcapsules with different characteristics. The yield was 42.91-58.31 %, EE mostly 60 % and DL 19.02 – 28.96%. The morphological surface of the microcapsules was spherical, curvy and somewhat deflated with varying sizes, from 196 to 258 nm. The average particle size of microcapsules from ethanol precipitation matrix was larger than that of butanol precipitation. The antioxidant activity showed that microcapsule coated with butanol precipitation starch matrix was higher than that of ethanol precipitation.

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