

Modification of Cassava Starch with Combination of Steaming and Acid Hydrolysis and Use as Encapsulant in Nanoencapsulation of Cocoa Leaf Crude Extract (*Theobroma cacao* L.)

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ABSTRACT: The utilization of native cassava starch in the food industry is limited. It needs a modified process to increase its utilization. This study aimed to evaluate the effect of a combination of steaming and acid hydrolysis and determine the best temperature and hydrolysis time that is suitable to apply as an encapsulant in the nanoencapsulation process of cocoa leaf crude extract. Modification of cassava starch used 50 °C, 60 °C, and 70 °C temperatures for 30, 60, and 90 minutes with HCl pH 1. The result showed that modified cassava starch produced from a combination of steaming and acid hydrolysis at 70 °C for 30 minutes has the best specifications with 60.48% solubility, 12.38% hygroscopicity, pasting profile (PV=48; BV=3; FV=66; SV=21). Then, it is combined with Arabic gum and used in the nanoencapsulation process using spray drying. This study showed that encapsulation can protect phenolic compounds of cocoa leaf crude extract resulting brownish-red color surrounded by a black circle. Nanocapsule powder has 13.56% moisture content, 350.3 nm particle size, 16.93 zeta potential, and 84.30% encapsulation efficiency. The combination of steaming and acid hydrolysis at 70 °C for 30 minutes produces modified cassava starch which is suitable for use as an encapsulant in the nanoencapsulation process of crude extract of cocoa leaves.

Keywords: modified starch, steaming, acid hydrolysis, nanoencapsulation, cocoa leaf crude extract

INTRODUCTION

Cassava has a high starch content and its existence is quite abundant in Indonesia. The use of native cassava starch as an encapsulant was limited in the food industries and non-food industries because it has several disadvantages such as low water solubility, high hygroscopicity, high viscosity, too sticky, long cooking time, hard-formed, and non-clear pasta (Singh *et al.*, 2007). Therefore, it is necessary to improve the quality of native cassava starch with modification to have properties such as having high water solubility and low hygroscopicity so that it is suitable to be applied as encapsulants (Sun *et al.*, 2010; Wang and Wang, 2010). Modifications were applied with a combination of steaming and acid hydrolysis. Modification with steaming aims to stretch the bonding structure of a solid starch so that it is easier to hydrolyze with acid. The steaming method is also a pretreatment and is expected to reduce the temperature and duration of the acid hydrolysis time (Mucha *et al.*, 2017). After the steaming process is complete, continued with chemical modification with acid hydrolysis. Modification of cassava starch produced from a combination of steaming and acid hydrolysis which has a specification approaching maltodextrin will be used as encapsulants for nanoencapsulation of cocoa leaf crude extract.

Cocoa leaves contain bioactive compounds including alkaloid content (3-5%), gallic catechin gallate catechins (9-14%), epigallocatechin gallate (4-14%), and theobromine (0.05-0.5%). Cocoa leaves also have the

same components as tea leaves (*Camellia sinensis* and *Camellia assamica*) in the form of polyphenols (3.60%); flavonoid glycoside (1.91%); theobromine (1.71%); catechins and tea pigments (Yang *et al.*, 2011). However, bioactive compounds in cocoa leaf extract were unstable against pH, metal ions, exposure to light, temperature, and oxygen so they were easily damaged during storage. Therefore, to maintain the stability of these bioactive compounds, it can be improved with nanoencapsulation technology.

Nanoencapsulation is a coating process of core materials (active ingredients) that were susceptible to damage so to be coated and protected by coating material (encapsulants) and produce nano-sized capsules (1-1000 nm) (Carvajal *et al.*, 2010). Nanoencapsulation produces advantages as expected, such as storage will be better and provide protection against bioactive components such as vitamins, antioxidants, pigments, protein and lipids, and carbohydrates so that it can improve its functional properties and stability (Carvajal *et al.*, 2010).

Various types of natural polymers were known to be used to produce nanoparticles. The ratio concentration of maltodextrin and Arabic gum 1:3 has the best result in instant curcuma products and has a water content of 8.35% (Febriyanti dan Setyowati, 2014). Arabic gum was chosen because the combination of Arabic gum and maltodextrin can be used as a wall material for the encapsulation of onion skin phenolic compounds to

produce the highest encapsulation efficiency, with small particle size and capable of protecting onion skin phenolic compounds (Akdeniz *et al.*, 2017). Maltodextrin and gum Arabic are used as wall materials and was proven to maintain the stability of anthocyanins in the process of microencapsulation using freeze drying technique (Khazei *et al.*, 2014).

Encapsulation techniques with spray drying are an effective way to protect bioactive components from damage. This technique has been used by Saloko *et al.*, (2012) for the nanoencapsulation process of liquid smoke. Therefore, in this study, it is expected that the nanocapsules of cocoa leaf crude extract are produced in powder form and have the ability as natural antioxidants in food products. This study aims to evaluate the effect of combination steaming and acid hydrolysis, determine the best temperature and hydrolysis time suitable to be applied as an encapsulant, and analyze the characteristics of cocoa leaf crude extract nanocapsules and encapsulation efficiency.

MATERIALS AND METHODS

Materials

The main ingredients used in this study were native cassava starch obtained from the Among Roso Menoreh Farmer Group in Boro Kulonprogo Village, Yogyakarta. The cocoa leaves were obtained by smallholders in Langgeran Gunung Kidul Village, Yogyakarta. Other ingredients used were HCl, NaOH, aquadest, Na₂CO₃, a solution of FeCl₃, a solution of folin, ethanol, and Arabic gum TIC (food grade).

Modification of cassava starch with combination steaming and acid hydrolysis

Modification of cassava starch with steaming and acid hydrolysis refers to the method of Xing *et al.*, (2017) with modifications. Twelve grams of cassava starch was suspended in 120 ml of water. The suspension was poured into a steam reactor and the steaming process was conducted at the temperature of 110 °C. When the temperature of the material has reached, the steam reactor is cooling down. Then it was poured into a plastic pan and dried in a cabinet dryer with a temperature of 60 °C for 24 hours.

Cassava starch from the previous steps was then hydrolyzed by acid. The process was done by mixing 30g of steamed starch with 100 ml of HCl solution pH 1. The hydrolysis process was carried out with a hotplate with a temperature variation of 50 °C, 60 °C, and 70 °C with varying times of 30, 60, and 90 minutes. The sample was cooled down to room temperature and neutralized with 0.1 M NaOH until a pH of 7-8 is reached. Then it was washed with distilled water 3-4 times to be transferred to a plastic baking sheet and dried in a 60 °C temperature cabinet for 48 hours. The dried samples were mashed in a blender and sorted using a 60 mesh sieve to obtain a

uniformly sized starch powder. The cassava starch modification produced by a combination of steaming and acid hydrolysis was then analyzed the solubility in water, hygroscopicity, and pasting profile.

Solubility (Chen and Jane, 1994 with modifications)

A total of 0.5 g of modified cassava starch were dissolved in 50 ml of distilled water. Stir using a homogenizer (Ultraturax Basic IKA Werke, Germany) at 4000 rpm for 2 minutes. The modified cassava starch suspension was then placed in a centrifuge bottle and centrifuged at 4000 rpm for 15 minutes. A total of 25 ml of the supernatant was taken, then placed in a weighing bottle and dried in an oven at 105 °C for 48 hours, and the dry weight was obtained.

Hygroscopicity (Zheng *et al.*, 2007 method with modification)

Hygroscopicity testing was carried out by placing 1 gram of sample in an aluminum cup, drying it in an oven for 24 hours, and then weighing it (as a dry weight). Then the samples were conditioned at 96% RH with a saturated K₂SO₄ solution and weighed every 1 hour for 9 weighings. Then the hygroscopicity percentage of all sample calculations.

Pasting profile

RVA (*Rapid Visco Analyzer*) was used to determine the gelatinization profile. This tool is used to measure the temperature of gelatinization and changes in viscosity during heating and cooling. The test is carried out by setting up the tool first, then entering the calculation program by entering the water content value of the sample to be tested. After that, the required sample weight and aquadest weight will be known. After the sample and distilled water are weighed in separate containers, the two are mixed in the canister. After that, put the paddle into the canister, and then push the top of the paddle into the coupling. Testing the gelatinization profile with this RVA takes about 30 minutes per sample.

Preparation of cocoa leaf crude extract

The preparation of cocoa leaf crude extract refers to the study of Osman, (2004). The cocoa leaf was washed with water then blended for 5 minutes, dried at a 50 °C temperature with a cabinet dryer for 10 hours then ground, sieved using a 30 mesh sieve, and produced cocoa leaf powder. Cocoa leaf powder is dissolved with ethanol and macerated at room temperature for 32 hours and every 8 hours is replaced with ethanol solvent then filtered with Whatman paper and evaporated with a solvent with 40 °C temperature of rotary vacuum evaporator at 100 rpm to produce the crude extract.

Nanoencapsulation of cacao leaf crude extract

Preparation of nanoparticle solution by mixing cocoa leaf crude extract with 0.25 g cassava starch modified by a combination of steaming and acid hydrolysis, stirred with a magnetic stirrer at 700 rpm for 30 minutes. Then it is

mixed with 0.75 g Arabic gum and stirred with a magnetic stirrer at 700 rpm for 30 minutes, then homogenized at 4000 rpm for 2 minutes and produced a nanoparticle solution of cocoa leaf crude extract and analyzed the coating of the phenolic compound. The resulting nanoparticle solution was homogenized at a speed of 4000 rpm for 2 minutes. After that, it was dried with a spray dryer and produced nanocapsules powder. The resulting nanocapsules of cocoa leaf crude extract (three samples and each sample three repetitions) were analyzed by coating with phenolic compound, morphological profile with SEM, moisture content, particle size distribution, potential zeta, and encapsulation efficiency.

Coating of phenolic compounds

The phenol staining method used was a modification of the Soloway and Wilen (1952) method, in which phenol staining or painting was carried out using ferric chloride (FeCl₃). By adding 5% FeCl₃, it produces a certain color according to the type of phenolic compound. A yellow, 5% (w/v) FeCl₃ solution was added to the nanoparticle solution with a ratio of 5% FeCl₃ solution: nanoparticle solution of crude cocoa leaf extract (3 drops: 1 ml). The mixture was then stirred slowly for 30 minutes. The colored nanoparticle solution was then viewed with the help of a light microscope using the Optilab application at 400X and 1000X magnification.

Morphological profile with Scanning Electron Microscope (SEM) (Caliskan and Dirim, 2016)

Nanocapsule particles were attached to SEM stubs or sample holders with a diameter of 10 mm using double-sided adhesive tape. Then the sample was coated with gold and viewed at a magnification of 1000 to 10,000 times with a voltage of 20 kV.

Moisture content (AOAC, 2005)

Analysis of water content refers to the AOAC reference (2005). The principle of testing for moisture content is the evaporation of water in a cup containing modified starch samples and crude extract nanocapsules of cocoa leaves after baking at 105 °C for 24 hours. Then it is weighed every 1 hour until it reaches a constant weight with a weighing difference of 0.2 mg.

Particle size distribution (Saloko et al., 2012)

The nanocapsules were dissolved in distilled water as a dispersant, then vortexed for 1 minute and measured using a particle size analyzer with the dynamic light scattering method. The particle size distribution is

determined by the span value. The measurements were taken three times. In addition to determining the particle size distribution, the particle size analyzer tool can be used to determine the potential zeta value.

Encapsulation efficiency (Cvitanovic et al., 2011)

Encapsulation efficiency was calculated based on the ratio of total phenol nanocapsules compared to the nanoparticle solution of crude cocoa leaf extract. The total phenol of the nanocapsule is the total phenol value resulting from spray drying minus the phenol present on the surface of the encapsulant (unconstrained). so that the calculation of the encapsulant surface phenol was carried out using the phenol release approach. To facilitate the calculation, it is also done based on the material balance. Calculation formula:

$$Encapsulation\ efficiency\ (\%) = \frac{(phenol\ nanocapsules) - (surface\ nanocapsules)}{(phenol\ cocoa\ leaf\ crude\ extract)} \times 100$$

Analysis data

This study used a completely randomized design (CRD) factorial pattern of 3 x 3 with 3 repetitions. The treatment factors in this study were the hydrolysis temperatures of 50 °C, 60 °C, and 70 °C and the hydrolysis times of 30, 60, and 90 minutes, respectively. The observed data were then subjected to statistical analysis using ANOVA at the 5% test level. If there is a significant difference, then the analysis is continued with the Duncan Multiple Range Test (DMRT) at a test level of 5%.

RESULT AND DISCUSSION

Analysis of solubility-modified cassava starch in water

The solubility of modified cassava starch with a combination *steaming* dan acid hydrolysis method with a variation of temperature and hydrolysis time is shown in Table 1. There were three samples with three repetitions for each treatment, for a total of twenty-seven samples with a standard deviation of 5%. Modification of cassava starch modified with a combination of *steaming* and acid hydrolysis methods at 70 °C for 30 minutes has the highest solubility of 60.47%. The solubility of modified cassava starch from the best treatment at 70 °C for 30 minutes is 60.47%, lower than the solubility of maltodextrin (98.26%) and higher than native starch (1.45%) (Table 2). Modified cassava starch produced from the combination of *steaming* and acid hydrolysis methods which is close to the specifications of maltodextrin that has a solubility

Table 1. The solubility of modified cassava starch with a combination of *steaming* and acid hydrolysis

Combination Hydrolysis temperature (°C)	Hydrolysis time (minutes)			Average
	30	60	90	
50	26.81 ⁱ	43.60 ^k	43.91 ^k	38.10 ^r
60	34.03 ^j	47.26 ^l	49.94 ^m	43.74 ^s
70	60.48 ^p	56.14 ^o	52.78 ⁿ	56.47 ^t
Average	40.44 ^x	48.99 ^y	48.88 ^y	

The same notation showed no significant difference at the level of significance 5% (p < 0.05)

of 98.26%, will be applied as encapsulants (Robert dan Fredes, 2015). According to Ali *et al.*, (2014), high water solubility values can facilitate the release of flavors or active compounds. Solubility is the ability of a solute to dissolve in a certain solvent under certain conditions to form a homogeneous solution. Solubility is related to the application of the nanocapsules in the final product. The easier it dissolves, the easier it is to handle the process.

Hygroscopicity analysis

Table 3 showed the hygroscopicity of modified cassava starch with a combination of steaming dan acid hydrolysis methods. Modified cassava starch produced with the combination of *steaming* and acid hydrolysis methods at 70 °C for 30 minutes has a hygroscopicity level of 12.38% (Table 3), which is nearly the percentage of the commercial hygroscopicity of maltodextrin 12.40 % (Table 2). It showed one of parameters to be applied as encapsulants of cocoa leaf crude extract, and it is expected to produce nanocapsule powder which is not easily deflated.

According to Zheng *et al.* (2007), hygroscopicity depends on the interaction of the modified group (hydrophilic or hydrophobic) with water. The composition of saccharides from starch hydrolysate will determine its hygroscopicity.

Pasting profile of starch

Analysis pasting profile of starch using RVA. Measurements with RVA include the heating and cooling

process phases at constant stirring (160 rpm). The RVA instrument plots the gelatinization profile curve as the relationship of the viscosity value (cP) on the y-axis to changes in temperature (°C) during the heating and cooling phases on the x-axis. Data obtained from RVA measurements are initial gelatinization temperature or pasting temperature (PT), peak viscosity (PV), breakdown viscosity (BV), setback viscosity (SV), and final viscosity (FV).

The peak viscosity (PV) of combination of steaming and acid hydrolysis starch has a value of 48 cP and lower than steaming starch of 4259 cP and native starch of 9379 cP (Table 4). The decrease in PV is also assumed to be the result of the reorganization of modified cassava starch granules. Variations in peak viscosity are influenced by the amylose content of starch. It is said that the associative bonds of the amylose fraction are responsible for the structure and paste behavior of starch granules (Yadav *et al.*, 2013).

The breakdown viscosity (BV) of modified cassava starch using the steaming method has a BV value of 2615 cP, which is higher than the BV value of modified cassava starch using a combination of steaming and acid hydrolysis methods; the starch resulting from acid hydrolysis alone is 3 cP, and maltodextrin is 5 cP. Native starch has a BV of 6327 cP (Table 4).

Table 2. Solubility (% db), hygroscopicity (%), and moisture content (% db) of Native Starch and Maltodextrin.

Sample type	Solubility (% db)	Hygroscopicity (%)	Moisture content (% db)
Native starch	1.45	7.57	32.18
Maltodextrin	98.26	12.40	8.16

Table 3. The hygroscopicity of modified cassava starch with a combination *steaming* and acid hydrolysis

Combination	Hydrolysis time (minute)			Average	
	Hydrolysis temperature (°C)	30	60		90
50		13.37 ^{de}	12.85 ^{bc}	12.57 ^{ab}	12.93 ^p
60		14.65 ^l	13.05 ^{cd}	14.12 ^h	13.94 ^q
70		12.38 ^{ab}	13.43 ^{def}	12.85 ^{bc}	12.89 ^p
Average		13.47 ^x	13.11 ^y	13.18 ^y	

The same notation showed no significant difference at the level of significance 5% ($p < 0.05$)

Table 4. Pasting profile

Sample	Peak viscosity (cP)	Breakdown viscosity (cP)	Final Viscosity (cP)	Setback (cP)	Pasting temperature (°C)
Native	9379	6327	4365	1313	62.70
Steaming	4259	2615	2291	647	50.10
Acid hydrolysis	35	3	48	16	ND
Modified starch (steaming and acid hydrolysis)	48	3	66	21	ND
Maltodextrin	17	5	14	2	ND

The decrease in the value of BV in modified cassava starch resulting from steaming and acid hydrolysis indicated a more stable paste. According to Yadav *et al.*, (2013), the breakdown is an important factor and influences the application of starch in food. When the starch granules swell and are subjected to heat and shear, the starch undergoes fragmentation and results in a reduction in viscosity, which indicates starch breakdown.

The final viscosity (FV) of modified cassava starch resulting from the steaming combination was 2291 cP, higher than the FV of cassava starch combined with steaming and acid hydrolysis, which had an FV value of 66 cP; acid hydrolysis 48 cP and maltodextrin was 14 cP. Meanwhile, native cassava starch has a final viscosity value of 4365 cP. Final viscosity is a parameter that indicates the ability of starch to form a thick paste or gel after heating or cooling as well as the resistance of the paste (Budijanto and Yuliyanti, 2012).

Setback viscosity (SV) showed the tendency of starch to retrograde. The setback viscosity of modified cassava starch resulting from a combination of steaming and acid hydrolysis was 21 cP higher than starch resulting from acid hydrolysis of 16 cP and maltodextrin of 2 cP but lower than modified cassava starch resulting from steaming of 647 cP and native starch of 1313 cP (Table 4). It indicated a low retrogradation tendency in modified cassava starch resulting from steaming and acid hydrolysis. Pasting temperature (°C) showed temperature the viscosity value begins to read, indicating that starch begin to gelatinize. Modified cassava starch using the steaming method had a pasting temperature value of 50.10 °C, while native starch had a pasting temperature value of 62.70 °C (Table 4). The pasting temperature value of modified cassava starch combined with steaming and acid hydrolysis, acid hydrolysis, and maltodextrin were not detected because they don't form a paste, so the value of the pasting temperature cannot be detected.

Coating of phenolic compounds analysis

Analysis coating of phenolic compounds extracted from cocoa leaf crude extract with FeCl₃ is shown in Figure 1. According to Soloway *et al.*, (1952) adding FeCl₃ 5%

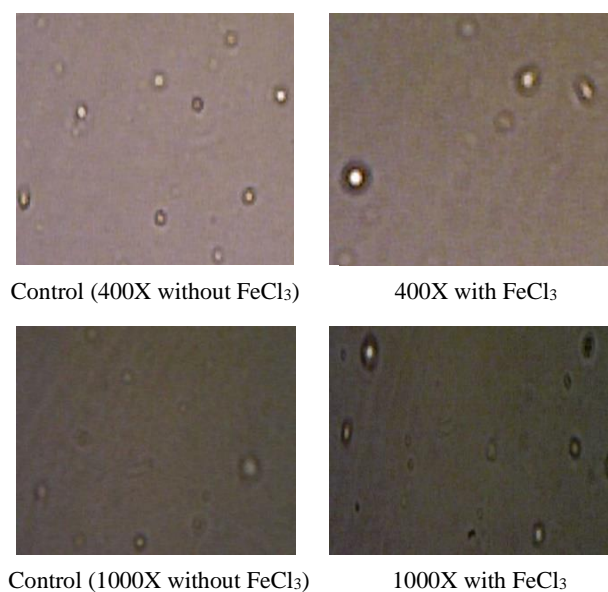


Figure 1. Observation of optimal nanoparticle solution of cocoa leaf crude extract with FeCl₃ 400X, 1000X and control

(b/v) solution in phenolic compounds will form a brownish-red color according to the type of phenol component. The brownish-red color indicates the reaction between ferric chloride which reacts with phenolic compounds while the black circle is a coating material (encapsulant) of modified cassava starch and Arabic gum. This showed that the encapsulation of modified cassava starch and Arabic gum can protect the phenolic compounds of cocoa leaf crude extract.

Particle size distribution analysis

The size distribution of cocoa leaf crude extract nanocapsule particles varied between 10-1000 nm. The size of the nanocapsules of cocoa leaves crude extract is distributed ranging from an average size of 13.23 nm (19.4%), 39.25 nm (29.4%) to 225.2 nm (51.2%). Overall, nanocapsules of cocoa leaf crude extract were 350.2 nm in size (Figure 2). Unevenness is marked by the presence of three peaks of varied sizes. The variety of particle sizes can be caused by agglomeration and results in large particles (Liu *et al.*, 2011).

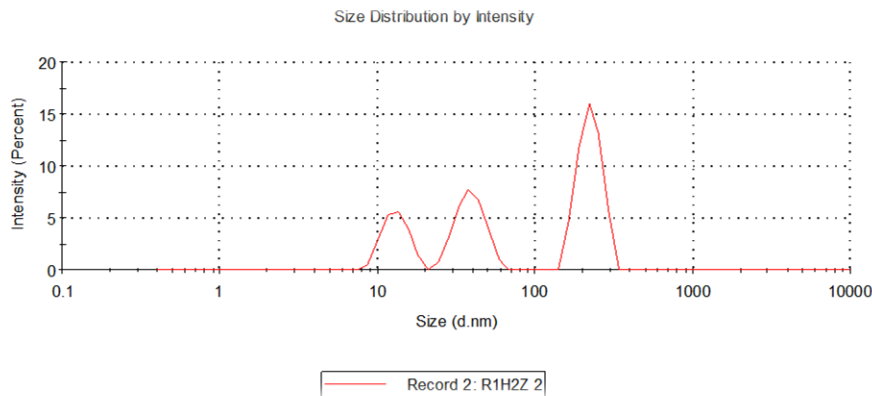


Figure 2. Distribution of nanocapsule particles of cocoa leaf crude extract with PSA (Particle Size Analyzer)

The polydispersity index value in this study is 0.601. It showed that the nanocapsule particles have good distribution (Liu *et al.*, 2011). The average potential zeta value obtained in this study amounted to -16.93. The potential zeta value is less than -30, so it causes the particles gathered or the occurrence of agglomeration and unstable dispersion systems (Liu *et al.*, 2011).

Encapsulation efficiency and moisture content of nanocapsule cocoa leaf crude extract

The encapsulation efficiency of cocoa leaf crude extract with an encapsulant combination of modified cassava starch and Arabic gum is 84.30%. Meanwhile, according to Utami (2015), the encapsulation of phenolic compounds with a combination of maltodextrin encapsulants and Arabic gum has an encapsulation efficiency of 47.36%. It showed the result better than the reference. The completed results of the encapsulation process on phenolic compounds of cocoa leaf crude extract are influenced by the presence of a combination of modified cassava starch and Arabic gum as encapsulants which have a high molecular weight can form the surrounding layer so that it can absorb phenolic compounds from the crude extract of cocoa leaves.

The nanocapsule powder produced from the encapsulation process using a spray dryer with an inlet temperature of 90 °C has a moisture content of 13.56%. Inlet temperature by spray drying is one of the factors that affect the moisture content of the nanocapsule cocoa leaf crude extract. The increase in inlet temperature of spray drying will cause a decrease in the moisture level and water content of the powder (Phisut, 2012).

Morphology profile

The morphology profile of the nanocapsules of cocoa leaf crude extract showed in Figure 3. The morphology profile of nanocapsules of cocoa leaf crude extract has a round form with shrinkage on the surface. It is caused by rapid water evaporation during the spray drying process. In addition, shrinkage also occurs due to uneven bubbles in the nanocapsules.

There is excessive evaporation, which can cause membrane cracks. The cracking or rupture of the wall occurs due to fragility and the inability of the material to resist pressure at high heating temperatures during the spray drying process (Gharsallaoui *et al.*, 2007). According to Harris *et al.*, (2010) the microcapsules produced have the same morphological shape which is round and wavy. It is caused by rapid solvent evaporation during the spray drying process.

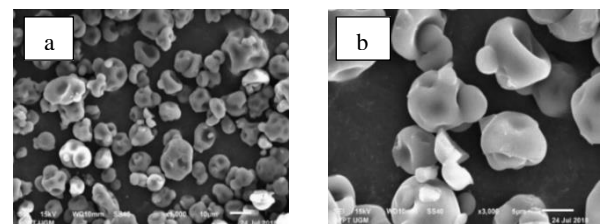


Figure 3. Morphology profile of nanocapsule cocoa leaf crude extract (a) 1000X magnification (b) 3000X magnification

CONCLUSION

The combination of steaming and acid hydrolysis can improve the characteristics of modified cassava starch. The best treatment is a combination of steaming and acid hydrolysis at 70 °C temperature for 30 minutes using HCl pH 1. It was suitable to be used as an encapsulant with a combination of Arabic gum have results the solubility of 60.47% hygroscopicity of 12.38%, water content of 8.86%, pasting profile (peak viscosity=48; breakdown viscosity=3; final viscosity=66; setback=21 and pasting temperature =ND). The use of encapsulants resulted from a combination of modified cassava starch was able to coat the phenolic compounds of cocoa leaf crude extract resulting in a brownish-red color surrounded by a black circle. Nanocapsule powder of cocoa leaf crude extract had a moisture content of 13.56%, a particle size of 350.3 nm, a zeta potential of -16.93, and an encapsulation efficiency of 84.30%. The morphology profile of the nanocapsules was round with a shrinking surface.

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