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Preparation of arrowroot starch nanoparticles by butanol-complex precipitation, and its application as bioactive encapsulation matrix

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Abstract

Arrowroot starch is one of Indonesia's local native starches derived from the *Maranta arundinaceae* tubers. This starch has high amylose content, increasing its potential to produce nano-sized V-amylose starch by butanol-complexed precipitation. One of starch pretreatments is lintnerization to produce crystalline and low DP starch. The aim of this research was to find out the characteristics of arrowroot starch nanoparticles produced by butanol-complex precipitation of lintnerized starch and its application as an encapsulation matrix for herb extract. Treatments involved lintnerized starch concentration of 5 and 10%, resulted from 2 and 24 hours of lintnerization process, and then each starch was precipitated by n-butanol. Preparation of starch nanoparticles by butanol precipitation following lintnerization of arrowroot starch produced nanosize particles ranged from 78.6 nm to more than 538.7 nm (average 261.4 nm), showing non-granular morphologies, and consisting of high porosities, as well as changing its crystalline pattern and thermal properties. The amylose content and solubility increased but enzymatic digestibility decreased to below than 60%. The lower particle size and enzymatic digestibility with high porosity made aroowroot starch nanoparticles suitable as controlled release matrix for bioactive compound. Application of starch nanoparticles as an encapsulation matrix for andrographolide extract showed smooth and sphere morphology with the average size about 200 nm. The FTIR spectra showed carbonyl strecthing band at 1727cm^{-1} from and rographolide.

Introduction

Starch, an abundant, non-toxic, biodegradable, edible, and relatively inexpensive material, has been widely used in various industries such as food, textile, and pharmacy. It is used in many applications including surface sizing, as a food emulsifier, fat replacer, excipients for tabletting and drug delivery carriers (Mahkam, 2010). Arrowroot starch is one of local native starches produced from the arrowroot tuber (*Maranta arundinaceae*) having unique characteristics in terms of high amylose content (29.03-31.34%) (Richana *et al.*, 2000). According to Srichuwong *et al.* (2005), the degree of polymerization (DP) of arrowroot starch naturally has short to medium chains amylopectin, in which the DP 13-30 reaching 68.3% and A-type crystalline pattern. Hizukuri *et al.* (1983) also reported that amylopectin of A-type starch is thought to contain a higher proportion of shorter branched chains. However, like other starches, native arrowroot starch has some limitations including limited solubility, poor processability (high viscosity), and incompatibility with some hydrophobic polymers (Simi and Abraham, 2007).

To expand its utility, starch needs to be modified to improve the characteristics of hydrophobicity, crystallinity and stability to enzymatic and thermal degradation. Preparation into a starch nanoparticle is one kind of modification to enhance starch performance (Xu *et al*., 2010). A starch nanoparticles has benefits in terms of higher surface area, lower viscosity at higher concentration and higher entrapment of active ingredients (Chen *et al*., 2006).

Research on preparation of starch having nano-sized particles by a butanol complex has been carried out by some researchers such as Herbert and Chancy (1994); Kim *et al.* (2009); Kim and Lim (2010). Butanol can only form a complex with amylose and precipitates, but not with amylopectin. Production of nano-sized particles by butanol complex require preparation of starch into shorter and more crystalline amylose, such as acid hydrolysis (lintnerization) or acid-alcohol hydrolysis. Kim *et al*. (2009); Kim and Lim (2010) used amylomaize dextrin prepared by acid-alcohol treatments to produce starch nanocrystals. The yield of the nanoparticle, however, was very low.

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The complex formation was the V-type with a single helix, which has great potential as a nano-carrier for active components for food, drugs and cosmetics (Kim *et al*., 2009). The properties of amylose that could form inclusion complexes with some molecules made amylose suitable as a carrier matrix. Among the molecules that could make inclusion complexes with amylose are iodine, alcohols, fatty acids, aromas (Rondeau-Mouro *et al.*, 2004; Biais *et al.*, 2006), dimethyl sulfoxide (DMSO) (Godet, 1995), as well as drug compounds like salicylic acid, p-aminobenzoic acid and ibuprofen (Lay-Ma *et al.*, 2011).

Herb active ingredient like Andrographolide (AG), generated from herbs of the Andrographis paniculata (local name Sambiloto), is one of materials in traditional medicine with various usages for some medications such as anti-diabetic, anti-malaria, anti-cancer, anti-microbe, anti-oxidants, hepatoprotetives, anti-hipertension and imunomodulator. AG tastes very bitter and barely dissolves in water, unstable at base and acid condition in the digestive system, as well as short half time ($t_{1/2}$ 2 h) (Lai, 2009). Incorporation of AG into the nano-starch matrix will enhance its solubility and bioavailability as well as protects from environment deterioration.

In the present study, the high crystalline fraction of arrowroot starch prepared by lintnerization using HCl was precipitated with butanol to produce nano-sized starch. There is no report on the application of nano-sized starch prepared from butanol complex as encapsulation matrix or carrier for herbs active ingredient, including *Andrographis paniculata* extract.

Materials and Methods

Materials

The raw materials used were of arrowroot (*Maranta arundinaceae*) tubers Creole varieties and then extracted by wet extraction method to produce starch according to common procedure in starch extraction derived from tubers. The chemical characteristics of native starch were as follows: moisture content 11.29%, ash 0.19%, lipid 0.46%, protein 0.12%, amylose 37.23% and reducing sugar 55.78 ppm.

Andrographolide (AG) was extracted from sambiloto (*Andrographis paniculata*) dried leaves with ethanol 70%, using a maceration process for 24 hours and then evaporated by using a vacuum rotary evaporator to produce semi-solid extract. The extract was then diluted with ethanol 70% until the dissolved total solid content was about 20%. Chemicals used in the process included HCl, 1-butanol, ethanol, HCl, NaOH; all were of analytical grade from Merck and used as received.

Preparation of starch nanoparticles

Starch nanoparticles produced by butanol-complexed precipitation of lintnerized starch carried out using phase separation methods developed by Kim and Lim (2010) with minor modifications. The Lintnerized starch was prepared by using acid hydrolysis that appeared elsewhere (Jayakody and Hoover, 2002; Faridah *et al.*, 2010). The arrowroot starch was hydrolyzed slowly in the acid solution HCl 2.2 N with ratio (1:2) at 35^{o}C, and incubated in a shaker water bath and shaking at 120 rpm for 2 h (H2) and 24 h (H24). The slurry was neutralized using NaOH 1N until pH 6.0-7.0 and then washed with distilled water and ethanol, and oven dried at 40^{o}C for overnight.

About 5% (B5) and 10% (B10) of lintnerized starch dissolved in 200 ml of hot distilled water and then was autoclaved at 121^{o}C for 20 min. The solution was cooled to 70^{o}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^{o}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. The weight of the precipitates was measured to calculate the yield of the nano-sized particles. All the experiment conducted in three replicates, and the data were expressed as means ± standard deviation.

Physicochemical properties of arrowroot starch nanoparticles

Morphology of starch nanoparticles were determined using a JSM/6510LA Analytical Scanning Electron Microscope (JEOL) with a 20 kV voltage and micrographs. The particle size distribution was determined using a dynamic light scattering detector (Delsa Nano, C Beckman Coulter). Distribution of molecular weight of starch nanoparticles were eluted by Gel Permeation Chromatography (GPC) using Toyopearl Gel HW-65S (Tosoh Co., Japan) with column size 2.6 cm ID x 100 cm, according to the procedures reported by Sunarti *et al.* (2001) and Ozturk *et al.* (2009). Solubility and swelling power were conducted at room temperature (30^{o}C) using the method reported by Leach *et al.* (1959). Enzymatic digestibility (*in vitro*) of starch was estimated by the procedure described by Spence and Jane (1999) using pancreatic amylase. Total amylose content was determined using colorimetric methods according to Perez and Juliano (1978). The crystalline structure of

native starch and starch nanoparticles was observed using X-ray diffractometer (Maxima-X, XRD-7000, Shimadzu, Japan) with Cu-Ka radiation (λ = 1,5406 Å) operated at 35 kV. The scanned range of theta degree (2θ) was from 2–35°. The thermal behaviour of the starch was monitored using differential scanning calorimetry Perkin-Elmer DSC (Perkin-Elmer Co., Norwalk, CT).

Application as andrographolide encapsulation matrix

The starch nanoparticle with the lowest digestibility were applied as an encapsulation matrix for andrographolide herbs extract. The process of encapsulation was as follows: 10 g starch nanoparticles was dissolved in 150 ml warm distilled water (70°C), stirred with a magnetic stirrer for 30 minutes, and stood to rehydrate at cold room for a night. The next day, 5 ml of the diluted andrographolid 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. Analysis of the andrographolide loaded starch nanoparticles matrix included morphology (SEM and TEM), particle size distribution and FTIR spectra (ABB MB 3000, at wave number range 400-4500 cm^{-1}.

Result and Discussion

Morphology

The lintnerization of arrowroot starch produced hydrolyzed starch with a lower chain length; and increased its crystallinity since acid attacked the amorphous regions and remained in the crystalline regions. According to Palma-Rodriguez *et al.* (2012) acid hydrolysis decreased amylopectin chain length with an increase in the short chains. The results proved that the degree of polymerization (DP) of lintnerized starch from 2 h and 24 h lintnerization were 495.35 ± 13.40 and 103.6 ± 10.03, respectivelly. Previously we have reported that lintnerization did not change the starch granule morphology (Winarti *et al*., 2014), but after complexed by butanol, showed non-granular morphology as shown in Figure 1 (a-d). The morphology of the starch-butanol complex showed sphere particles but formed agglomeration and still adhered to each other. The morphology also showed high porosity. The existence of pores in the particles showed the ability to absorb other particles, such as active ingredients.

Figure 1 also showed that a starch complex, from 24 h of lintnerization, revealed much finer structure than that of 2 h as emphasized from the TEM profile (Figure 1 e-f). Moreover, the average particle size also revealed much lower size as shown at Table 1. DP and crystallinity of the lintnerized starch might affect the resulted starch complex. According to Kim *et al.* (2009) and Kim and Lim (2010), the DP of starch affects the size of the nano-sized particles produced by butanol precipitation.

Table 1. Particle size, size distribution and PDI of arrowroot starch nanoparticles

Sample[1]	Average particle size (nm)	Size range			PDI
		D(10%)	D(50%)	D(90%)[2]	
H2B5	436.4 ± 111.8	396.4	468.2	683.4	0.527
H2B10	367.4 ± 31.9	324.1	382.5	550.6	0.584
H24B5	155.9 ± 19.3	144.7	152.9	290.2	0.361
H24B10	134.0 ± 30.8	119.3	135.0	187.4	0.528

[1]5% (B5) and 10% (B10) of lintnerized starch prepared for 2 h (H2) and 24 h (H24) followed by complex precipitation with n-butanol. [2]Cumulative diameter at 10%, 50% and 90%

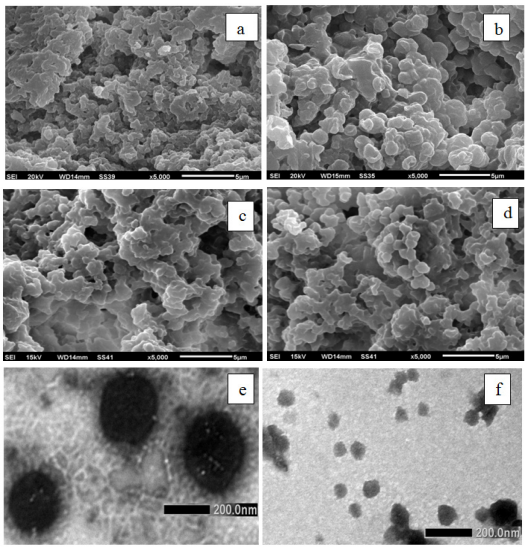

Figure 1. Starch morphology of starch nanoparticles by SEM (a-d) and TEM (e-f). 5%(B5) and 10% (B10) of Lintnerized starch produced from 2 h (H2) and 24 h (H24). (a) & (e) H2B5, (b) & (f) H24B5, (c) H2B10, and (d) H24B10.

Particle size distribution

The particle size of the starch nanoparticles prepared from lintnerized starch varied from nanoparticles to microparticles, as shown in Table 1. The range of particles was from 78.6 nm to more than 538.7 nm. However, from the distribution curve it would be found that the average particle size (cumulative 50%) was at nano size (lower than 300 nm). The nano-sized particles of amylomaize dextrin (less than 200 nm) were also reported by Kim and Lim (2010). Gallant *et al*. (1997) reported that preparation of the nano-scale starch particles having crystallinity is feasible because the starch granule is inherently composed of nano-scale crystalline blocklets, in a range of diameters from 20 to 500 nm depending on its botanical origin and the location in granule.

Other researchers mentioned that starch nanocrystal resulted from acid hydrolysis for several days or several weeks could have particle size less than 100 nm however the yield was very low (Kim

Table 2. The yield, solubility and swelling power of native and starch nanoparticles at room temperature[1,2]

Sample	Yield (%)[3]	Solubility (%)	Swelling Power (%)
Native	-	3.50 ± 0.42	2.26 ± 0.22
H2B5	20.65 ± 3.45[a]	11.31 ± 1.59[a]	7.92 ± 0.67[b]
H2B10	27.38 ± 4.98[b]	16.89 ± 1.65[b]	7.16 ± 0.59[b]
H24B5	21.22 ± 4.21[a]	9.44 ± 1.16[a]	6.27 ± 0.23[b]
H24B10	23.8 ± 3.67[ab]	9.43 ± 1.87[a]	5.28 ± 0.59[a]

[1]5% (B5) and 10% (B10) of lintnerized starch prepared for 2 h (H2) and 24 h (H24) followed by complex precipitation with n-butanol. [2]Results are mean ± standard error of mean value (n = 3). Means have different superscript letters in the same column are significantly different (P < 0.05). [3]based on lintnerized starch used.

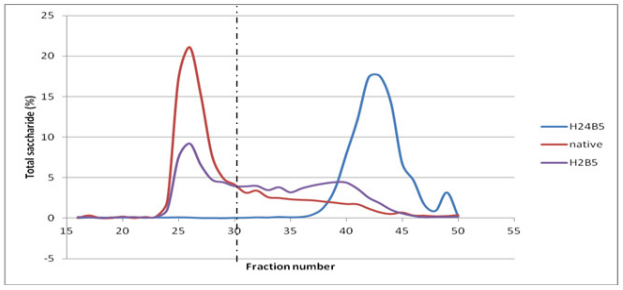

Figure 2. GPC profile of native and arrowroot starch nanoparticles using Toyopearl gel (HW 65S) Tosoh Co, Japan

et al., 2009; Le Corre *et al.*, 2012). Samples prepared from 24 h lintnerization indicated lower particle size than that of 2 h lintnerization. As already mentioned, longer duration of lintnerization produce shorter chain length and lower molecular weight as well as a higher crystalline region.

The polydispersity index (PDI) showed that the dispersity of starch nanoparticles ranged from 0.361 to 0.584. Higher value indicated that the size was more varied or less homogenous. The high PDI also indicated that the nanoparticles starch mostly still agglomerated as also shown from SEM profile at Figure 1 (a-d).

Molecular weight distribution

Molecular weight distribution of native starch by Gel Permeation Chromatography (GPC) showed a bimodal distribution with a shifting the composition of fraction I (FN 15-30) which consist of amylopectin and fraction II (FN 30-50) as amylose fraction as well as oligosaccharides with low molecular weight, between native and starch nanoparticles (Figure 2). The figure revealed that the percentage of carbohydrate of fraction II increased from 29.79% to became 56.46% and 98.65% for starch nanoparticles prepared from 2 h and 24 h lintnerization, respectively. The butanol complex prepared from 24 h lintnerization only had one fraction (FN 37-50) indicating that amylopectin (fraction I) degraded to a smaller size molecule (short amylose fraction) during lintnerization and butanol complexation. Saibene and Seetharaman (2010) mention that the elution peak shifted towards higher fraction numbers with increasing lintnerization time, indicating a reduction

Table 3. Enzymatic digestibility and amylose content of starch nanoparticles[1,2]

Sample	Enzymatic digestibility (%)	Amylose content (%)
Native	74.12±0.11[b]	35.68±1.15[a]
H2B5	64.63±0.12[ab]	52.62±1.51[b]
H2B10	57.63±0.13[a]	38.15±2.23[a]
H24B5	59.08±0.16[a]	35.60±0.42[a]
H24B10	53.68±0.15[a]	34.86±1.41[a]

[1]5% (B5) and 10% (B10) of lintnerized starch prepared for 2 h (H2) and 24 h (H24) followed by complex precipitation with n-butanol. [2]Results are mean ± standard error of mean value (n = 3). Means have different superscript letters in the same column are significantly different (P < 0.05).

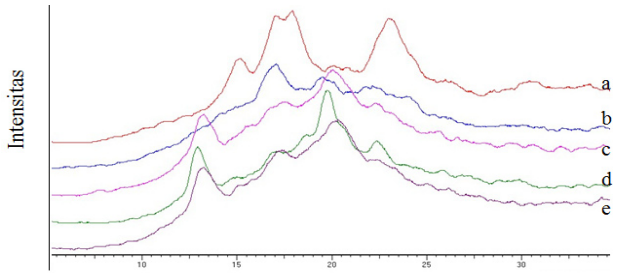

Figure 3. Crystalline pattern of native arrowroot starch (a) and starch nanoparticles: H2B5 (b); H2B10 (c); H24B5 (d) and H24B10 (e)

of the molecular size. Moreover, Mizukami et al. (1999) said amylose fraction had much smaller molecules (about one-third in size) than those of the whole amyloses.

Solubility and swelling power

The solubility and swelling power (SP) of the starch nanoparticles produced changes more significantly than that of native and lintnerized starch, as seen in Table 2. The solubility of starch nanoparticles at room temperature (9.43–16.89%) increased compared to the native starch (4.15 ± 0.63%), and lintnerized starch (3.87–7.41%). Moreover, the SP (5.28–7.92%) increased from 2.48 ± 0.15% (native) and lintnerized starch (1.91–3.03%) (Winarti *et al.*, 2014). Lopez *et al.* (2010) mentioned that acid modification decreases swelling power of starch granules. According to Palma-Rodriguez *et al.* (2010), acid-treated starch in which amylose is leaching and short chain amylopectin increasing, would provoke a structure with more branches but less ability to swell. This also explained the decreasing SP value.

Enzymatic digestibility and amylose content

The enzymatic digestibility of the nanosize starch was lower compared to native arrowroot starch, as seen in Table 3. Lintnerization and butanol precipitation reduced the enzymatic digestibility. However, Table 3 also revealed that lintnerization affected the enzymatic digestibility more than butanol treatment. Native starch had 74.12% digestibility and decresed to 71.63 and 57.63% after 2 h lintnerization at 5 and 10% concentration; and 59.08 and 53.68% at 24 h lintnerization at 5 and 10% concentration,

Table 4. Thermal properties of native and related arrowroot starch nanoparticles

Samples	T_{onset} (°C)	T_{peak} (°C)	Enthalpy (J/g)
Native starch	40.61	93.61	231.34
H2B5	43.00	86.55	381.55
H2B10	41.22	84.24	310.54
H24B5	46.99	93.38	359.82
H24B10	46.48	92.24	366.92

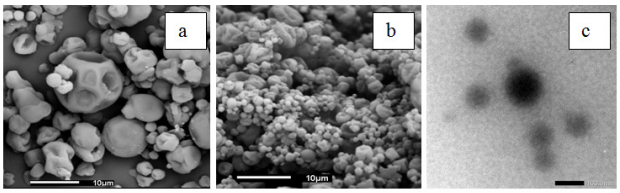

Figure 4. Morphology (SEM) of andrographolide extract encapsulated in starch nanoparticle matrix prepared by butanol precipitation from treatment H2B5 (a) and H24B5 (b) and TEM of H24B5 (c)

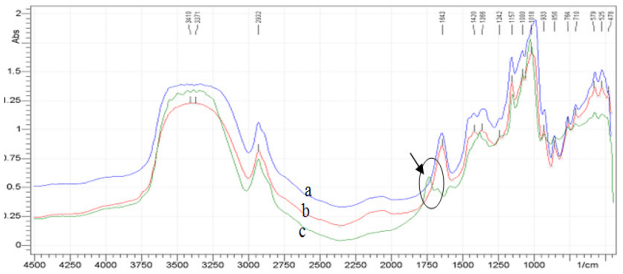

Figure 5. FTIR spectra for native arrowroot starch (a), nanostarch matrix H24B5 (b) and andrographolide loaded nanostarch matrix (c)

respectively. The crystalline fraction of lintnerized starch produced by 24 hours, was much higher than that of 2 h lintnerization and became more resistant to enzymatic digestion. These results showed that higher crystallinity increase the enzymatic resistence. The crystalline regions at high amylose corn starch hydrolyzed in acid for 24 h are more resistant to enzyme attack than that of unhydrolyzed starch (Nagahata *et al.*, 2010).

The amylose content of starch nanoparticles shown at Tables 3. The butanol precipitation increased the level of amylose, as butanol only formed complex with amylose. Moreover, the degree of hydrolysis affect the amylose content in which the longer the hydrolysis the level of amylose tend to decrease. 24 h hydrolysis might resulted too short dextrin chain to form complex with butanol. Godet *et al.* (1995) mentioned that the dextrin chain that were able to form complex with butanol should have chain length and linearity favorable to complex formation.

X-ray diffraction patterns of arrowroot starch nanoparticles

Native arrowroot starch had A-type crystallinity. This type of crystal was characterized by peaks at 2 tetha (2θ), at 15°,17°, 18° and 23°, respectively as shown in Figure 3. The crystallinity of native arrowroot starch was 23.31%. Acid hydrolysis and butanol complexation increased the crystalinity degree to 28.36%, 31.29%, 45.12% and 39.15% respectively for butanol complex from 2 h and 24 h lintnerization, at concentration 5% and 10% each.

The crystalline type shifted from A-type to become V-type as shown at Figure 3 as seen the strong peak at 20° (2 tetha), except for treatment H2B5 (b). According to Osella *et al*. (2005) the peak at 20° is attributed to a well-formed "V"-structure. Moreover, Kim and Lim (2010) said that a starch-butanol complex by butanol precipitation has V-type amylose. Besides, Ma *et al.* (2008) mentioned that the shifting of crystalline type caused the disruption of molecular order that caused loss of granule integrity of starch during gelatinization that lead to the destruction of A-type crystallinity. The molecular inclusions in V-amylose complexes may be used for encapsulation and controlled delivery of various substances in the pharmacology and food system (Shimoni *et al.*, 2007). Moreover the crystalline arrangement of modified-starches can be important when they are used as encapsulating agents, due to the possibility to produce linkages among the remaining polymer chains and a wall material with improved characteristics as encapsulating agent (Palma-Rodriguez *et al*., 2012).

Thermal properties

Thermal properties of the arrowroot starch nanoparticle were analyzed using DSC. Treatment of acid hydrolysis might have had an effect on thermal properties. As shown in Table 4, sample treated with acid followed by butanol precipitation revealed higher onset temperature (T_o) but lower peak temperature (T_p) and higher enthalpy. This might have occured because although lintnerization produced a higher proportion of the crystalline part, butanol precipitation reduced crystallinity and produced more amorphous parts, therefore, reducing the peak temperature. Moreover, freeze drying process resulted higher portions of amorphous regions. Higher enthalpy was shown from all samples compared the native starch. According to Le Corre *et al.* (2012), several factors could increase the melting temperature of semi-crystalline polymer including an increase crystallinity, more perfect crystal and a decrease in plasticizer content.

Application as encapsulation matrix

The starch nanoparticle by butanol precipitation was applied as an encapsulation matrix for an andrographolide herb extract using the spray drying process. Results showed that the morphology of the

encapsulated andrographolide produced a spherical form without cracking at the outer surface of nanocapsules, as shown in Figure 4. The products used matrix from treatment 24 h lintnerization showed much lower size and more homogenous compared to 2 h matrix. The SEM result was proven by TEM and the size distribution.

The average particle size resulted from the matrix prepared from 24 h lintnerization, exhibited much lower average particles size and PDI than that from 2 h lintnerization. The average particle size was 130.2 ± 36.2 nm compared to 257.4 ± 63.9 nm for 24 hour and 2 hour lintnerization, respectively; with the range of particles size was 116.2 to 541.4 nm and 152.2 to 620.3 nm respectively. The homogenity of particle resulted from 24 hour lintnerization revealed much higher as shown from the lower PDI (0.309), compared to 2 hour lintnerization (0.518). The PDI value from andrographolide loaded nanostarch matrix especially for H24 hour lintnerization is close to ideal PDI for matrix as proposed by Danhier *et al.* (2009) that PDI values about 0.2 represent monodispersity in pharmaceutical formulations because showed monodisperse size distribution.

FTIR spectra

The FTIR spectra of native starch is almost similar to starch nanoparticles produced by butanol complex as shown at Figure 5. There are several absorbancies bands at 1159, 1082, and 1014 cm^{-1}, which are attributed to C-O bond stretching. In andrographolide loaded nanostarch matrix, however, revealed different spectra indicating the interaction between nanostarch matrix and andrographolide extract. Andrographolide shows carbonyl strecthing band at 1727cm^{-1} (Lai, 2009) as also observed from the spectra (Figure 5). Besides, there were some spectra bands showing –OH stretching vibration at 3319-3402 cm^{-1} due to presence of three –OH groups. The aliphatic C-H stretching vibration was observed at 2848-2990 cm^{-1}. While, the $=CH_2$ was observed at 1674 cm^{-1} and C-C was observed at 1031 cm^{-1} (Shariff *et al*., 2007).

Conclusion

Precipitation of lintnerized arrowroot starch using n-butanol resulted starch nanoparticle having non-granular and porous morphology with the average particle size about 261.4 nm and the shifting crystallinity pattern from A-type becoming V-type as well as lower enzymatic digestibility. Application of starch nanoparticles as an encapsulation matrix of andrographolide extract showed smooth and sphere morphology with size distribution less than 300 nm. Starch nanoparticles prepared from 24 h lintnerization revealed better characteristics than that of 2h. Arrowroot starch nanoparticles produced from butanol precipitation could be applied as controlled release matrix of hydrophobic active ingredients like herbs extract.

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