Effect of Acid Hydrolysis on the Characteristics of Andrographolide-Loaded Arrowroot Starch Nanoparticles

Christina Winarti and Nur Richana
Indonesian Center for Agricultural Postharvest Research and Development, Ministry of Agriculture, Bogor 16114, Indonesia
Email: christina.winarti01@gmail.com

Titi Candra Sunarti
Department of Agroindustrial Technology, Bogor Agricultural University, PO Box 220, Bogor 16002, Indonesia
Email: titibogor@yahoo.com

***Abstract*—Active coumpund of *Andrographis paniculata* which have lots pharmacological activities has limitations need to overcome such as encapsulating in starch matrix. The aim of the research was to find out the effect of acid hydrolysis level on the characteristics of andrographolide encapsulated in starch nanoparticle matrix. Treatments observed were types of matrix (A): A1=starch nanoparticles (NP) and A2=mixed starch NP and maltodekxtrin (MD) and the duration of hydrolysis (B): 2 hours (B1) and 24 hours (B2). Result showed that the yield of microcapsule ranged from 62.56-69.365%, encapsulation eficiency 45.06-59.02%, drug loading 18.9-24.2%, particle size 9.12-23.65μm with round sphere forms. The highest antioxidant activity was microcapsule using 24h NP matrix but for the inhibition to α-glucosidase was 2h lintnerization. The FTIR spectra showed interaction between nanostarch matrix and andrographolide as carbonyl strecthing band at 1727cm^{-1}.**

***Index Terms*—sambiloto (*andrographis paniculata*) extract, arrowroot starch, microcapsule, starch nanoparticles**

I. Introduction

Andrographis paniculata (local name Sambiloto) is one of medicinal plants with various usages for some medications such as anti-diabetic, anti-malaria, anticancer, anti-microbe, anti-oxidants, hepatoprotetives, anti-hipertension, anti-inflammation [1]-[3] and anti-HIV as well as imunomodulator [4]. The active ingredients of sambiloto is Andrographolide (AG). AG have some characteristics which limits its applications including very bitter taste, barely dissolves in water, unstable at base and acid condition in the digestive system, as well as short half time ½ 2 hour [5]. These characteristics cause low digestibility and bioavailability of AG in digestive system.

One approach to increase the solubility of the active ingredient which is difficult to dissolve namely 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]. It also prevents unwanted flavor perception (bitter, astringent).

In this research AG is incorporated into the nano-starch matrix prepared from arrowroot starch. Arrowroot starch is one of local native starches produced from the arrowroot tuber (*Maranta arundinaceae)* having unique characteristics in terms of high amylose content. Furthermore, its A-type amylopectin structure thought to contain a higher proportion of shorter branched chains [9] which potential as wall matrix to entrap active ingredients.

Starch nanoparticles have 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 to produce nanosized starch [10], [11]. However, the yield of starch nanoparticles was very low. Moreover, mixing starch NP with commercial modified starch like maltodextrin will reduce the cost. Their characteristics if applied wall material in encapsulated product may also be influenced by the composition of wall material.

Acid hydrolysis affected the characteristics of the produced nanosized particles. Application of nanosized particles as encapsulation materials might affect the nanoencapsulated products [10]. The aim of the research was to find out the effect of acid hydrolysis level on the characteristics of sambiloto/andrographolide extract encapsulated in arrowroot starch nanoparticle matrix.

II. Methodology

The raw materials used were sambiloto (*Andrographis paniculata*) extract and arowroot starch nanoparticles. AG was extracted from sambiloto dried leaves with ethanol 70%, using a maceration process for 24 hours and

Manuscript received January 17, 2015; revised May 3, 2015.

doi: 10.18178/ijfe.1.1.29-33

then evaporated by using a vacuum rotary evaporator to produce semi-solid extract. In application as encapsulated wall material, the extract was then diluted with ethanol 70% until the total dissolved solid content was about 20%.

Starch nanoparticles produced by butanol complexed precipitation of lintnerized starch carried out using phase separation methods developed by Kim and Lim [11] with minor modifications. The Lintnerized starch was prepared by using acid hydrolysis that appeared elsewhere [12], [13].

A. Preparation of Arrowroot Starch Nanoparticles

Lintnerized starch was dissolved in 200ml 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 (100rpm) at 50°C for 3 days and then centrifuged at 5,000rpm for 20min. The precipitates were washed with ethanol several times and dried by freeze dryer.

B. Encapsulation Process

The process of encapsulation was as follows: 10g of starch nanoparticles or mixed starch nanoparticles and maltodextrin 1: 2 was dissolved in 150ml 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, 5ml of the diluted sambiloto extract (total solid 20%), was added to the starch slurry, mixed with a homogenizer (11.000rpm) for 10 minutes and dried using a spray drying with inlet temperature 170-180°C.

Treatments observed were types of matrix (A) in which A1=starch nanoparticles (NP) and A2=mixed starch NP and maltodextrin (MD) and the duration of acid hydrolysis (B) for 2 hours (B1) and 24 hours (B2). All the experiments were conducted in three replicates

Analysis of the andrographolide loaded starch nanoparticles matrix included yield, encapsulation eficiency (EE) and drug loading (DL) [14], morphology, particle size distribution and polydispersity index, antioxidant activity [15], inhibition to α-glucosidase [16] and FTIR spectra (ABB MB 3000) at wave number range 400-4500 cm^{-1},

III. Result and Discussion

A. Yield, Encapsulation Efficiency and Drug Loading

The type of matrix used affected on the yield of the resulting microcapsules in which 2 hours hydrolysis matrix resulted in a greater yield than that of 24 hours hydrolysis. The average yield produced ranged from 62.560 to 69.365% (Table I). However, matrix resulted from the treatment of 24 hours starch hydrolysis tend to have higher EE and DL than that of 2 hours hydrolysis. The encapsulation efficiency (EE) ranged between 45.06-59.02% and Drug Loading (DL) 18.9-24.2 % (Table I). The duration of acid hydrolysis seem to affect much on the EE and DL of the microcapsule. The higher EE and DL of microcapsule using 24 hours hydrolysis may related to the lower particle size that enhance the surface area.

The successful of oil encapsulation could be characterized by minimum surface oil content in the microcapsule produced and maximum retention of core material in the particle [17]. The more the amount of core material added, encapsulation efficiency is lower. Some parameters that affect EE including wall ratio: core, the spray dryer inlet temperature, the efficiency encapsulated ranged between 12.1 and 82.2 % [18].

TABLE I. Microcapsule Yield, Encapsulation Efficiency and Drug Loading of Microcapsules Using Different Kind of Matrix

Sample	Yield (%)	Encapsulation Efficiency (%)	Drug loading (%)
NP starch matrix			
H 2	65.465	45.061	18.765
H 24	63.740	59.024	24.200
Mixed matrix			
H 2 + MD	69.365	46,463	19.050
H 24 + MD	62.560	57.323	23.503

B. Morphology

Result showed that the morphology of encapsulated andrographolide produced spherical form without cracking at the outer surface of nanocapsules (Fig. 1). Smooth shape without cracks showed that matrix prepared from hydrolyzed starch followed by precipitation with butanol has resulted sufficiently elastic matrix. The resulted spherical forms are in accordance with previous studies namely Rocha [19] which encapsulated lycopene using modified starch.

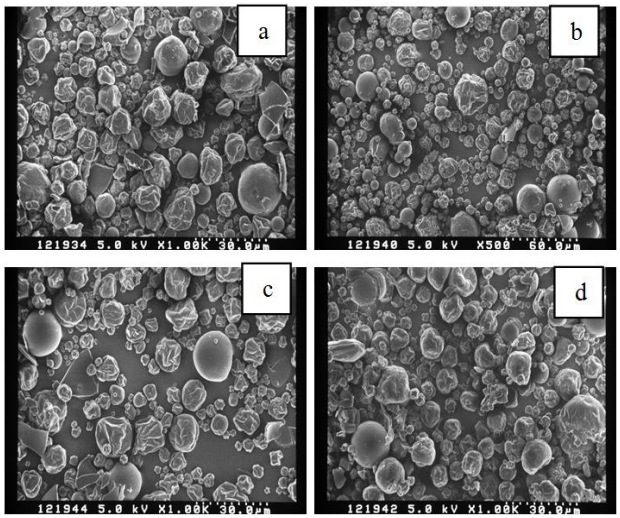

Figure 1. Morphology of andrographolide encapsulated in starch nanoparticle matrix treatment A1B1 (a), A2B1 (b), A1B2 (c), A2B2 (d)

The morphology measured by SEM revealed that the resulting microcapsules morphology was rounded, curvy and somewhat deflated with varying sizes (Fig. 1). However, different type matrix and duration of hydrolysis seem did not affected the surface morphology. The results agree with previous studies namely Loksuwan [20] which used lintnerized tapioca. The deflation of the surface structure caused by rapid evaporation of the points of

fluid during the drying process with spray drying [21], [22].

C. Particles Size Distribution

The particle size distribution of microcapsule, measured by Partcle Size Analyzer, shown at Table II. The average particle size resulted from the matrix prepared from 24 hour lintnerization, exhibited lower average particles size and more uniform than that from 2 hour lintnerization for both two types of matrix. Moreover, the polydispersity index (PDI) for 24 hour hydrolysis also smaller showing the more uniformity/homogenity of particle size, especially for NP matriks. The homogenity of particle resulted from 24 hour lintnerization revealed much higher as shown from the lower PDI (about 0.3), compared to 2 hour lintnerization (about 0.5). The PDI value from andrographolide matrix is close to ideal PDI for matrix as proposed by Dahnier [23] that PDI values below 0.2 represent monodispersity in pharmaceutical formulations because showed monodisperse size distribution. Moreover, [24] stated that the smaller PDI 0.3 shows the uniformity formula nanoparticles with a narrow distribution. Particle size of 173nm has binding capacity 80% while the size of 426nm is only 59% [25].

TABLE II. AVERAGE PARTICLE SIZE AND POLYDISPERSITY INDEX OF AG ENCAPSULATED IN ARROWROOT STARCH

Sample	Average particle size (μm)	Size range (μm)	PDI
NP matrix			
H2	12.928 ± 2.69	1.60 - 43.96	0.498
H24	9.123 ± 1.50	0.45 – 17.22	0.309
Mixed matriks			
H2 + MD	15.066 ± 3.66	0.36 – 61.50	0.526
H24 + MD	14.84 ± 1.36	0.36 – 43.96	0.528

D. Antioxidant Activity

The antioxidant activity was measured by analyzing the free radical inhibition activity of AG extract ranged from 4.1 to 21.29% at a concentration of 100-1600ppm, while the microcapsules with NP starch matrix derived from 2 hours and 24 hydrolysis hours reached 24.17 and 30.18%, respectively at concentration of 1600ppm. The IC_{50} values for these two treatments were 3573.38 and 2722.35ppm, respectively (Table III). While using mixed starch showed lower antioxidant activity.

TABLE III. FREE RADICAL INHIBITION AND IC_{50} OF AG ENCAPSULATED IN ARROWROOT STARCH NANOPARTICLES

Matrix	Free radical inhibition (%) at several concentration (ppm)					IC_{50} (ppm)
	100	200	400	800	1600	
NP matrix						
- H2	3.53	5.53	10.17	16.57	24.17	3573.38
- H24	5.15	8.81	13.69	19.45	30.18	2722.35
Mixed matrix						
- H2+MD	3.84	7.07	11.72	17.92	21.45	4050.91
- H24+MD	4.17	7.67	12.27	18.33	22.08	4010.64

Compared to other studies the result is relatively lower. The microcapsules containing only about 5% of AG extract, that caused the low value of antioxidant activity. Lin [26] stated that the reduction of free radical activity of the AG extract ranged from 48.0 to 66.8% at a concentration of 1-50μg/mL, whereas the research of Arifullah [3] at 10-22% at a concentration of 10-160μg/mL (ppm). The differences are due to differences in geographic origin of the plant. The extract of andrografolid of potential as a substitute for synthetic antioxidants on the current market [2].

The higher antioxidant capacity of microcapsul using starch NP matix caused by the lower particle size that could bind more active ingredient. Smaller size provide greater surface area so they have more active side that lead to the higher binding capacity.

1) Inhibition to α-glucosidase

The results of the inhibition activity to α-glucosidase enzyme in the microcapsules showed a fairly good inhibitory activity. Table IV also showed that the inhibition activity of α-glucosidase in microcapsules with nanoparticles starch matrix from treatment 2 and 24 hours hydrolysis was higher than that of the mixed matrix with MD. This is likely due to the synergistic between AG extract with nanoparticles that are resistant starch (indicated by the low enzymatic digestibility). Moreover, MD is highly hidrophylic that lead to high enzymatic digestibility. Previous study reported that arrowroot starch NP has lower enzymatic digestibility compared to native starch [10]. Resistant starch is a starch that is hard to digest or it has a hypoglycemic activity, just like AG extracts does [27]. In fact, a recent study [28] examined the use of modified starches with a high fraction of Slow Digestion Starch (SDS) and Resistant Starch (RS) has been used as a carrier for a variety of components including herbs medicine to increase its benefits in lowering blood glucose levels and glycemic index and colon health. So, the arrowroot starch NP matrix could also be applied as slow release matrix and lowering glycemic index as well.

TABLE IV. INHIBITION TO A-GLUCOSIDACE OF AG ENCAPSULATED IN ARROWROOT STARCH NANOPARTICLES

Sampel	Inhibition (%) at concentration (ppm)			
	75	150	300	600
NP Matrix				
H2	24.24	26.49	35.45	58.51
H24	7.12	18.99	17.26	38.62
Mixed matrix				
H2 + MD	42.68	4.99	12.14	25.93
H24 + MD	8.04	16.07	9.03	17.39

E. FTIR Spectra

The FTIR spectra of native arrowroot starch is almost similar to the starch nanoparticles produced by butanol complex as shown at Fig. 2(a), and Fig. 2(b). There are

several absorbancies bands at 1159, 1082, and 1014cm^{-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} [5] as also observed from the spectra (Fig. 2(c)). The absorbancies peak at 1727cm^{-1} is typical of andrographolide. From the figure it was clear that AG has entrapped in nanostarch matrix.

Moreover, there were some spectra bands showing –OH stretching vibration at 3319-3402cm^{-1} due to presence of three –OH groups. The aliphatic C-H stretching vibration was observed at 2848-2990cm^{-1}. While, the =CH 2 was observed at 1674cm^{-1} and C-C was observed at 1031cm^{-1} as reported by [29].

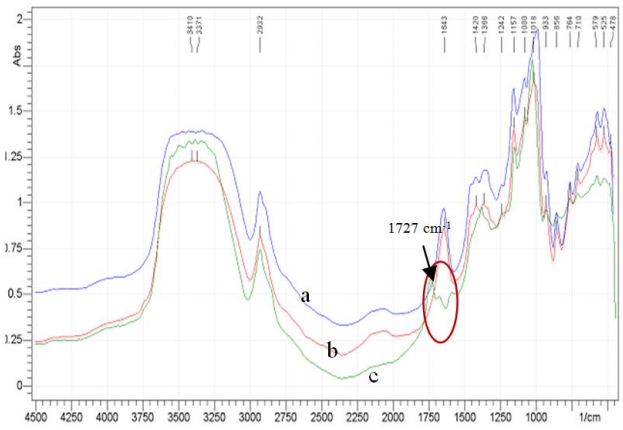

Figure 2. FTIR spectra for native arrowroot starch (a), nanostarch matrix (b) and andrographolide loaded nanostarch matrix (c)

IV. Conclusion

Different types of matrices produced microapsule with different characteristics. The use of nanoparticles starch matrix provides higher antioxidant activity and inhibition of α-glucosidase than that of the mixed matrix between NP and maltodextrin. While the appearance of the SEM image and the particle size distribution relativelly similar. The addition of maltodextrin into starch NP as wall material is an alternative to lower the cost and still provide good characteristics of microcapsul.

Acknowledgment

Thanks for the financial support by the Indonesian Agency for Agricultural Research and Development, Ministry of Agriculture, through KKP3T project in fiscal year 2012.

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Christina Winarti was born in Jogjakarta, Indonesia on April 24, 1968. She graduated from Gadjah Mada University from Department of Post Harvest Technology in 1992 and completed her Master Degree from Graduate School of International Development (GSID) Nagoya University. Meanwhile she got PhD from Department of Agroindustrial Technology, Bogor Agricultural University, Indonesia, in 2014. Currently, she is working as a researcher at Indonesian Agency for Agricultural Research and Development (ICAPRD), Ministry of Agriculture.
She is conducting research on postharvest technology especially in processing and application of biomaterial. Her research interest is on biomaterial engineering including bio-nanotechnology and nutraceuticals.

Nur Richana was born in Central Java, Indonesia on July, 25, 1954. She has obtained a BSc degree in Gajah Mada University and her M.Sc and Ph.D degree in the year 2006 from Bogor Agriculture University. She is working as a researcher in postharvest technology, at Indonesian Agency for Agricultural Research and Development, Ministry of Agriculture as a researcher since 1983. She also College research Professor at Bogor Agriculture University and Gajah Mada University. She has published several research articles in the field of bioprocess engineering for food and biofuels and postharvest technology. Her research interest includes enzymology, fermentation and production of biofuels.

Titi Candra Sunarti was born in December 19, 1966 in Bogor City, Indonesia. She graduated her PhD in 2001 from Division of Applied Bioscience and Biotechnology, Graduate School of Bioresources, Mie University, Japan; and finished her undergraduated and master program from Study Program of Agroindustrial Technology, Bogor Agricultural University, Indonesia on 1994.
She works as lecturer at Department of Agroindustrial Technology, Bogor Agricultural University since 1991. Her publications are mainly about carbohydrate and its utilization, such as International Food Research Journal (2014), Carbohydrate Polymer (2012), and Procedia Chemistry (2012). Her research interests are focused on the exploring and utilization of tropical carbohydrate sources for agro-industry.
Dr. Titi C Sunarti is also member of Indonesian Society for Microbiology (PERMI), Indonesian Society for Food Science (PATPI) and Indonesian Agroindustry Association (AGRIN); and Editor for Indonesian Journal of Agroindustrial Technology.