CHARACTERIZATION OF CURCUMIN LOADED STARCH NANOPARTICLES FROM NATIVE CASSAVA AND POTATO STARCHES

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ABSTRACT

The objective of this study was to investigate the characteristics of curcumin loaded starch nanoparticles, formulated from native cassava and potato starches using the nanoprecipitation method. Curcumin was loaded during the formation of starch nanoparticles at a certain ratio. The results showed that curcumin loaded in the starch nanoparticles from 1% potato starch solution achieved the highest loading efficiency, loading ability, and solubility. The curcumin loaded starch nanoparticles from 2% native cassava starch and 2% native potato starch performed the higher antioxidant ability and direct UV irradiation protection. TEM analysis demonstrated that curcumin loaded starch nanoparticles had a spherical shape with the mean diameter of 80 ± 8 nm. This means curcumin was successfully loaded into starches by applying the simple nanoprecipitation method.

Keywords: Curcumin, starch, starch nanoparticles, nanoprecipitation method.

1. INTRODUCTION

Curcumin (1,7-bis(4-hydroxy-3-methoxy-phenyl)-1,6-heptadiene-3,5-dione) is a natural polyphenol present in the rhizomes of turmeric (*Curcuma longa*). It is also the main component in curcuminoids extracted from turmeric, which is accounting for 60-80% [1]. Curcumin exhibits numerous valuable properties including antioxidant, anti-inflammatory, antiproliferative, anti-invasive, and antiangiogenic activities [2]. In spite of these promising properties in both biomedical and functional food, the applications of curcumin are limited because of its low water solubility that results from inter and intra hydrogen bonding [3]. In alkaline solution (pH > 7), higher solubility of curcumin is observed, while crystals of 10-50 microns are formed in an aqueous acidic solution (pH < 7). However, pure curcumin is considered highly unstable to chemical degradation in an aqueous alkaline solution [4]. Other environmental factors such as UV irradiation and heat also contribute to the decomposition of curcumin [5]. Therefore, free curcumin molecules have resulted in poor bioavailability and clinical efficacy. To enhance water solubility, stability and bioavailability of curcumin, many methods have been researched in which polymer-based nanocarrier was widely investigated.

Being one of the most available natural polymers, starch is a well-known and inexpensive polysaccharide which has received significant attention in drug delivery applications as they are hydrophilic, biodegradable, and biocompatible with tissue and cells [6]. Chin *et al*. [7] has created starch nanoparticles from native sago by a simple nanoprecipitation method under controlled conditions. These methods were simple, fast and easy to perform and successfully created starch nanoparticles. Recently, debranched starch has been widely used to fabricate starch nanoparticles, which lower particle size and improve the encapsulation capacity of bioactive compounds [8]. However, the structure and characteristics of starch nanoparticles were found to be affected by the degree of polymerization of starch chains and fabrication method [8]. Cassava and potato starches are two kinds of root starches with different morphology, structure and physicochemical properties, in which the cassava starch had a small particle size and exhibited the A-type crystalline structure, and the potato starch had a bigger particle size and exhibited the B-type crystalline structure. These differences might affect the formation, characteristics and encapsulation capacity of starch nanoparticles. Until now, no study on the encapsulation of curcumin using debranched cassava or potato starch has been done. In addition, the different starch concentration in the solution and various ratios of curcumin and starch might affect the encapsulation efficiency and properties of the nanoparticles. Therefore, the objectives of this study are to investigate the characteristics (size, shape, loading efficiency, UV protection, antioxidant ability) of curcumin loaded starch nanoparticles, formulated from native cassava and potato starches with different starch concentration using the nanoprecipitation method.

2. MATERIALS AND METHODS

2.1. Materials

Cassava and potato starches were isolated from raw cassava and potato tubes using the conventional method, including grinding, sieving, centrifuging and drying steps as previously described by Hung *et a*l. [9]. After drying, the moisture content of starches was in a range of 11-14%. Total starch, amylose content and DPn of cassava starch were 98.6%, 17.0% and 1400 glucose units, respectively, whereas those of potato starch were 98.2%, 27.4% and 1520 glucose units, respectively [9]. Curcumin for synthesis was purchased from Merck (Germany). Other chemicals were bought from chemicals stores.

2.2. Loading curcumin into starch nanoparticles

Curcumin was completely dissolved in absolute ethanol at the concentration of 0.125 mg/mL. Native cassava and potato starches were mixed with distilled water at the concentration of 1% (w/v) or 2% (w/v) and heated at 80 ^oC for completely dissolving. Then, the starch solutions were cooled down to room temperature, and a certain volume of starch solution was dropped-wise into curcumin solution while stirring at around 1,000 rpm with the ratio of 0.05 grams of curcumin per one gram of starch (5% wt). The curcumin-loaded starch nanoparticles were collected by centrifugation at 8,500 rpm for 15 min. After that, they were rewashed with absolute ethanol to remove excess free curcumin that adhered to the surface of starch nanoparticles.

2.3. Determination of loading efficiency and ability

Loading efficiency and capacity of curcumin loaded starch nanoparticles were determined according to the method of Chin *et al.* [7] with minor modification. Two milligrams of each sample were added into 2 mL of distilled water and treated in a bath type sonicator for 30 min at 80 °C to extract curcumin. Afterwards, 10 mL of absolute ethanol was added and mixed well with suspension. The mixture was then centrifuged at 3,000 rpm for 10 min, and the pellet in the centrifuge tube was extracted again with an additional 10 mL of absolute ethanol. The supernatant of two times was collected and combined after all. Curcumin concentration was determined by UV-vis spectrophotometry at 419 nm according to the following calibration curve:

$$Y = 0.147X + 0.0069, \qquad R^2 = 0.999$$

(Where X is the curcumin concentration in µg/mL and Y is the absorbance at 419 nm)

Loading efficiency was determined by the ratio of loaded curcumin to the original curcumin weight in the preparation step and calculated by the following equation:

$$Loading\ efficiency = \frac{\text{Mass of detected curcumin}}{\text{Mass of original curcumin}} \times 100$$

Loading ability was calculated as the weight of curcumin loaded on the weight of starch following the formula:

$$Loading\ ability = \frac{\text{Mass of detected curcumin}}{\text{Mass of starch nanoparticles}} \times 100$$

2.4. Morphology determination

The samples were photographed their images using the Transmittance Electron Microscope according to the method of Li *et al*. [10].

2.5. Water solubility

Cold water solubility (CWS) was determined by the method of Eastman & Moore [11] with some modification. For each sample, 0.5 g of curcumin loaded starch nanoparticles was added to 10 mL of distilled water at room temperature. The mixture was vortexed for 15 min. Then, the suspension was centrifuged at 1,500 rpm for 20 min before 5 mL of the supernatant was transferred into a pre-weighed dried container. The CSW (%) was calculated by multiplying the dry weight of the collected supernatant (g) by 2000. The solubility of curcumin was calculated by the mean of CWS of starches (%) and loading ability of each sample.

2.6. UV irradiation stability

The UV photolysis protection ability of curcumin loaded starch nanoparticles was examined based on the method of Li *et al*. [12]. A UV lamp (Spectroline Longlife^{TM} Filter Highest Ultraviolet Intensity) was applied to mimic the UV rays of sunlight of 285 and 365 nm. Four Petri dishes corresponding to different recorded times of 3, 6, 12 and 24 h were prepared for 0.01 g of each sample. For pure curcumin, 0.2 mg of free curcumin was weighed in a petri dish. All samples were then irradiated under UV illumination at the wavelengths of 285 nm or 365 nm at room temperature in a dark box for 24 h. The distance between the bottom of the Petri dishes and the UV lamp was approximately 10 cm. After treatment, the starch nanoparticles and pure curcumin was dissolved in the distilled water and ethanol, respectively and measured the amounts of remained curcumin as described in section 2.3. The remained curcumin after irradiation was used to calculate the stability of samples.

2.7. Antioxidant ability

The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay was used to determine the antioxidant ability of curcumin loaded starch nanoparticles following the method reported by Huang *et al*. [13] with minor modification.

The DPPH radical scavenging activity was calculated according to the equation:

$$DPPH\ radical\ scavenging\ activity\ (\%) = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100$$

(Where A_{blank} and A_{sample} are the absorbance of DPPH solutions added with water and sample solution respectively at the wavelength of 517 nm).

2.8. Statistical analysis

Statistical analysis was conducted with IBM SPSS 20 for Windows. All data are expressed as mean and standard deviation (SD). Comparison among samples was performed using One-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Differences are considered significant at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Loading efficiency and ability

Table 1 shows the results regarding loading efficiency and loading ability of curcumin into starch nanoparticles. The loading efficiency of samples was in a range of 28.72% - 57.58%, and their loading ability was in a range of 14.38 - 28.82 mg/g. There were significant differences in loading efficiency as well as loading ability among 4 samples. The sample of 1% native potato starch was evaluated as the highest performance on loading efficiency (57.58%) and loading ability (28.82 mg/g), while the lowest one was the sample of 2% native potato starch (28.72% and 14.38 mg/g, respectively). Regarding the starch nanoparticles from native cassava starch, 1% native cassava sample performed higher loading efficiency and ability than 2% native cassava sample, similar to those from native potato starch. The higher concentration of starch had the lower loading efficiency and loading ability because the starch paste of 2% was more agglomeration and higher viscosity than that of 1%, which hindered the interaction between curcumin and the hydroxyl groups of starch. In addition, the starch nanoparticles fabricated from the 2% native cassava starch had higher loading efficiency and loading capacity because cassava starch had lower viscosity than did the potato starch [9], resulting in easier encapsulation of curcumin at high starch concentration. Li *et al*. [12] reported that the maximum loading efficiency was on the sample with a ratio of 3:100 (3% wt) between curcumin and soluble starch (97.76% loading efficiency). This variation might be owing to the different starting materials between the two studies. The low loading efficiency and loading ability of starch nanoparticles were reflected by the aggregation of starch nanoparticles. When curcumin was loaded into starch nanoparticles, the solution turned from transparent to opaque yellowish with a significant amount of aggregation.

Table 1. Loading efficiency and loading ability of curcumin in starch nanoparticles

Group	Loading efficiency (%)	Loading ability (mg/g)
1% Native cassava	45.84 ± 6.87^{bc}	22.94 ± 3.44^{bc}
2% Native cassava	42.78 ± 1.05^{bcd}	21.41 ± 0.53^{bcd}
1% Native potato	57.58 ± 2.33^{ab}	28.82 ± 1.17^{ab}
2% Native potato	28.72 ± 1.70^{d}	14.38 ± 0.85^{d}

Data are expressed as mean ± SD (n = 2), data with different superscripts in the same column are significantly different ($p < 0.05$) analyzed by ANOVA.

3.2. Morphology properties

The sample of 1% native cassava starch was chosen to capture by TEM for its representative morphology. Figure 1 shows a TEM micrograph of curcumin loaded starch nanoparticles from 1% cassava starch carrier. Curcumin loaded cassava starch nanoparticles were observed to be spherical and 80 ± 8 nm in size. The size and shape of curcumin loaded starch nanoparticles from 1% cassava starch in this study were smaller than the mean particle size of 87 nm, as reported by Chin *et al.* [7].

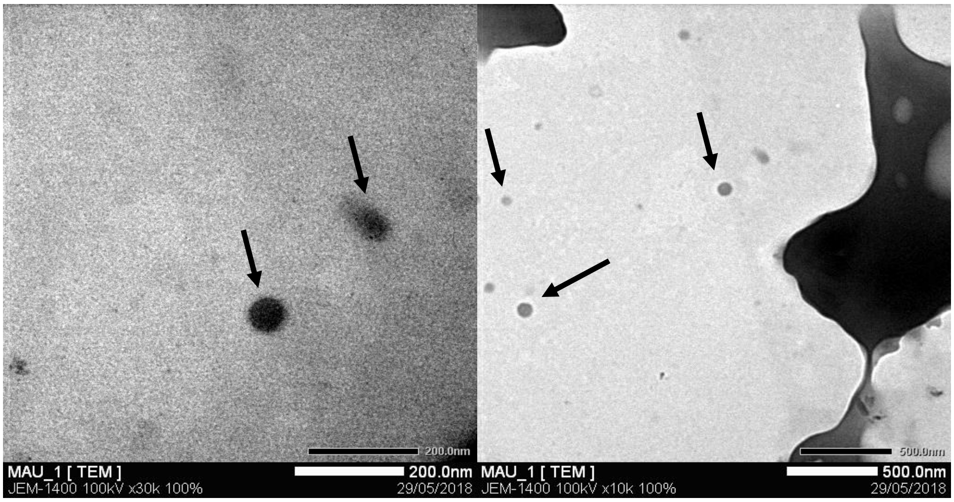


Figure 1. TEM micrographs of curcumin loaded 1% cassava sample

3.3. Water solubility

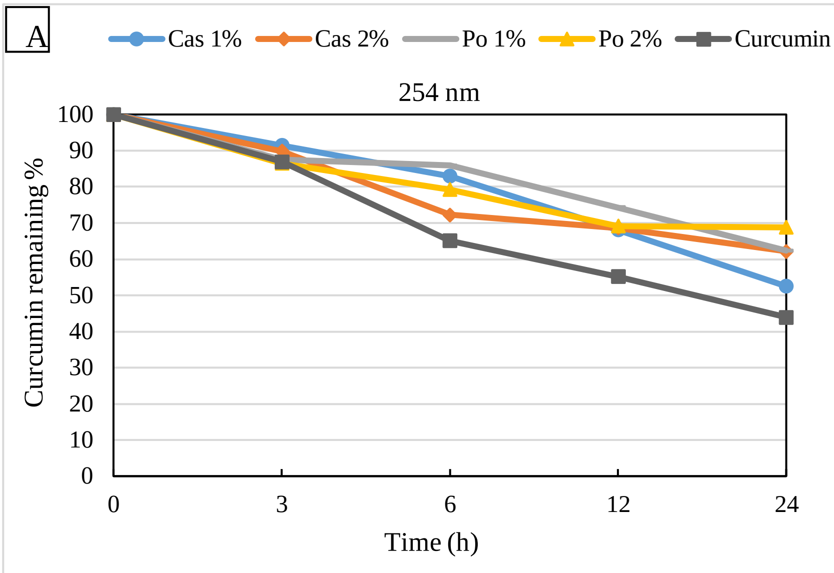
Table 2 shows the solubility of curcumin loaded in the starch nanoparticles in cold water. The solubility of curcumin in the starch nanoparticles was determined in a range of 0.22-0.38 mg/mL. Sample of 1% native potato starch yielded the highest solubility (0.38 mg/mL). The water solubility of models is one of the crucial characteristics that decide the effectiveness of nanoparticles. The curcumin loaded in raw native cassava and raw native potato had no significant difference in cold water solubility. Aditya *et al.* [14] reported that the solubility of native curcumin in water was 0.39 ± 0.05 µg/mL because both intermolecular and intramolecular hydrogen bonding was formed when curcumin is in crystal phase inhibiting curcumin dissolving in water. Thus, the solubility of curcumin loaded in starch nanoparticles from 1% potato starch increased by 1000 times compared to pure curcumin. The possible reason is that the matrix of starch can capture the curcumin and prevent the crystallization of curcumin which maintains the amorphous state [10].

Table 2. The cold water solubility of curcumin loaded starch nanoparticles (mg/mL)

Group	CWS of curcumin (mg/mL)
1% Native cassava	0.26
2% Native cassava	0.28
1% Native potato	0.38
2% Native potato	0.22

3.4. UV irradiation stability

The proportion of curcumin remaining in samples under treatment with UV irradiation at the wavelengths of 254 and 365 nm was recorded at 0, 3, 6, 12 and 24 h. Figures 2A and 2B show the positive protection ability of curcumin loaded starch nanoparticles from UV irradiation with a wavelength of 254 nm and 365 nm, respectively. At 365 nm of UV irradiation, all curcumin loaded starch nanoparticles protected curcumin better than free curcumin, remaining only 54.19% after 24 h exposure. The curcumin remaining decreased when the time increased. Most samples retained about 70 - 80% of the curcumin at the end of the test. The results in this study are consistent with the results found by Li *et al*. [12], who reported that the starch nanoparticles fabricated from the soluble starch retained more than 80% of the curcumin during 24 h of UV irradiation treatment. The protection of starch nanoparticles to the curcumin under UV photolysis treatment was due to the encapsulation and possible hydrogen bonding between starch and curcumin.



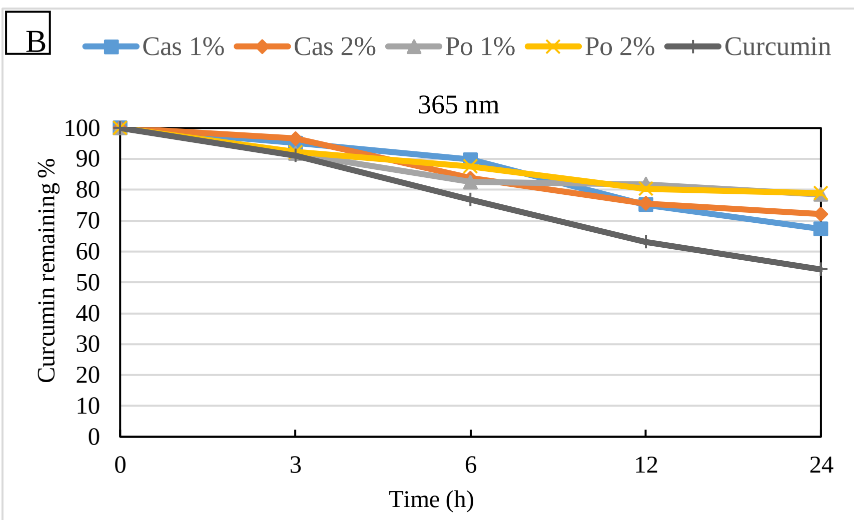


Figure 2. Stability of curcumin in the starch nanoparticles under UV irradiation at 254 nm (A), and under UV irradiation at 365 nm (B)

Curcumin loaded starch nanoparticles performed lower protection at a wavelength of 254 nm compared to those at a wavelength of 365 nm. At this wavelength, the curcumin remaining in all starch nanoparticles was in the range of 52-69%. Degradation under UV treatment for a long period is the weakness of pure curcumin. Therefore, the formulation of curcumin loaded starch nanoparticles was expected to bring effective protection against UV irradiation.

3.5. Antioxidant ability of curcumin loaded starch nanoparticles

DPPH assay used to determine antioxidant characteristic of curcumin loaded starch nanoparticles is illustrated in Figure 3.

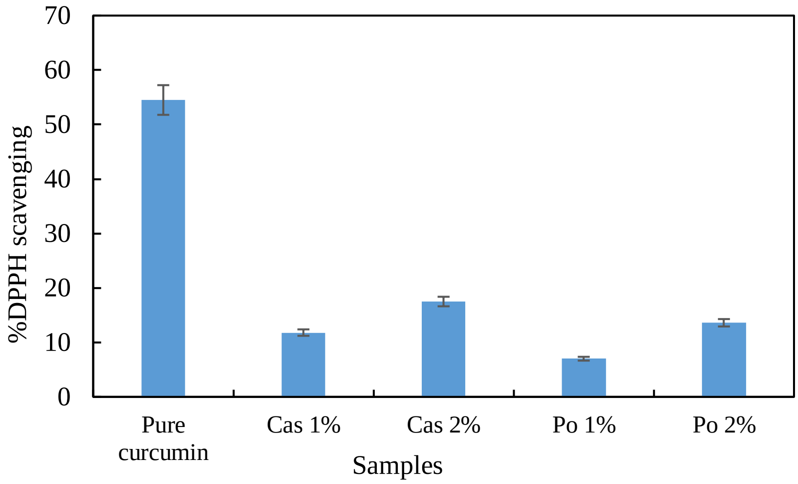


Figure 3. DPPH radical scavenging activity of curcumin loaded starch nanoparticles

The DPPH radical scavenging of curcumin loaded starch nanoparticles was in a range of 6.99% - 17.49%, which was lower than that of free curcumin. The curcumin loaded starch nanoparticles from 2% native cassava starch (17.49%) or 2% native potato starch exhibited higher DPPH radical scavenging than those of 1% native starch. The results indicated that the curcumin was tightly bound by starch nanoparticles from 1% native starch more than 2% native starch, resulting in lower amounts of curcumin released into free form, therefore, lower antioxidant activity. Antioxidant ability is the important factor that decides the usefulness of curcumin derivatives. Consequently, it is necessary for curcumin loaded starch nanoparticles to retain their high antioxidant ability. However, the results regarding the antioxidant ability of samples were not good when comparing to pure curcumin. It could be that the curcumin loaded starch nanoparticles required time to release curcuminoid fully to achieve the maximum antioxidant ability because the curcumin was bounded in the starch matrix. Therefore, 30 min might not be the optimal time to reach the maximum antioxidant ability of curcumin loaded starch nanoparticles. Nonetheless, the antioxidant ability of samples seemed largely retained in starch nanoparticles.

4. CONCLUSION

In summary, curcumin loaded starch nanoparticles brought more significant effects on loading efficiency, loading ability, solubility, UV protection and antioxidant ability. In this study, the formulation between curcumin and 1% native potato starch reached the highest loading efficiency, loading ability, solubility characteristics. Curcumin loaded debranched cassava nanoparticles showed a mean size of about 80 ± 8 nm with a spherical shape.

Compared with the equivalent amount of free curcumin, the solubility of curcumin loaded starch nanoparticles was improved. However, samples could not be completely dissolved in water at room temperature due to the aggregation, resulting in the antioxidant ability of these nanoparticles was significantly decreased. Nonetheless, the UV irradiation stability was enhanced in curcumin loaded starch nanoparticles, which offers potential applications in industrial production scale for this functional food product.

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TÓM TẮT

MỘT SỐ TÍNH CHẤT CỦA CÁC HẠT TINH BỘT DẠNG NANO
CÓ CHỨA CURCUMIN TẠO THÀNH TỪ TINH BỘT SẮN VÀ TINH BỘT KHOAI TÂY

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Mục tiêu của nghiên cứu này là xác định các đặc tính của các hạt nano tinh bột có chứa curcumin được tạo thành từ tinh bột sắn và tinh bột khoai tây tự nhiên sử dụng phương pháp kết tủa nano. Curcumin được nạp vào bên trong hạt nano tinh bột trong quá trình hình thành các hạt này ở các hàm lượng khác nhau. Kết quả cho thấy rằng curcumin được nạp vào các hạt nano tinh bột khi sử dụng nồng độ 1% tinh bột khoai tây có hiệu quả nạp, khả năng nạp và độ hòa tan cao nhất. Trong khi đó, các hạt nano tinh bột có chứa curcumin tạo thành từ 2% tinh bột khoai tây hoặc 2% tinh bột sắn có khả năng kháng oxy hóa và khả năng bảo vệ khỏi tia UV cao hơn. Kết quả chụp trên kính hiển vi điện tử truyền qua (TEM) cho thấy các hạt nano tinh bột có chứa curcumin có dạng hình cầu và có kích thước trong khoảng 80 ± 8 nm. Kết quả này cho thấy curcumin đã được nạp thành công vào hạt nano tinh bột bằng cách sử dụng phương pháp kết tủa nano đơn giản.

Từ khóa: Curcumin, tinh bột, hạt nano tinh bột, phương pháp kết tủa nano.