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Preparation and characterization of tapioca starch-chitosan hybrid material by non-solvent precipitation method

*Apinyaporn Boonsakulthongchai, Ampa Jimtaisong**

Program in Cosmetic Science, School of Cosmetic Science, Mae Fah Luang University, Chiang Rai 57100, Thailand

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ABSTRACT

Tapioca starch has potential application in agriculture, foods, pharmaceuticals, and cosmetics. However, tapioca starch has high moisture absorption and microbial contamination. Hybridization of tapioca starch with chitosan is an alternative method for modification of its properties. In this work, tapioca starch-chitosan hybrid material was prepared by precipitation method. Ethanol was used as anti-solvent agent. The characteristics of tapioca starch-chitosan hybrid material (TS-CSP) were investigated by DSC, FT-IR, TGA, XRD, and SEM techniques. TS-CSP exhibited quite similar thermal stability to tapioca starch evaluated by TGA. DSC result showed new compound was formed. FT-IR techniques showed the interaction between hydroxyl group of tapioca starch and amino group of chitosan molecule. SEM image of the TS-CSP showed round and polygonal shape with thin layer of chitosan film on its surface. XRD pattern of TS-CSP showed an A-type starch diffraction peak with less crystalline structure after being mixed with amorphous chitosan. Moreover, TS-CSP has anti-bacterial property studied by disk diffusion method. It showed inhibition effect against *S. aureus* TISTR 746, *E. coli* TISTR 527, and *P. aeruginosa* TISTR 1287 with inhibition zone of 8, 8, and 9 mm, respectively.

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* *Corresponding author*: Tel.: Tel.: +66-89-430-3232
E-mail address: ampa@mfu.ac.th

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INTRODUCTION

Starch is a natural polysaccharide, which derived from plants, e.g., corn, potato, rice, sorghum, tapioca, and wheat (Smith, 2001). Morphology and particle size of starches are different depend on their nature source, which affect their properties (Smith, 2001). Tapioca starch granule has size of 2-40 microns (Moorthy, 2002) and possesses good flow property. Tapioca starch has smooth feeling, which is suitable for use as a cosmetic ingredient. However, its use in cosmetics is limited due to moisture absorption which leads to coagulation of starch particles, hard flowing or spreading, and also microorganism contamination. In recent year, modified starches have wide spread interest in cosmetic industry because the limitations of native starches have been improved. Modified starches are abundantly used in many industries such as food, pharmaceutical, cosmetic, and paint industry. It is prepared by using physical or chemical method, which may use many chemicals and may be unsafe for human skin. Thus, an aspect of using environmentally friendly conditions with less chemicals has been widely studied recently.

Hybridization is the way to improve the stability and properties of natural starch. It is a blending and balancing of two polymers to make efficient materials with completely new properties and functions. Many works reported that blending natural starch with chitosan as hybrid material can improve anti-bacterial effect and other properties, i.e., water vapour permeability, hardness, stiffness, and flexibility of blend film (Bourtoom and Chinnan., 2008; Chang et al., 2010; Liu et al., 2009; Ma et al., 2008; Wang et al., 2007; Xu et al., 2005). Chitosan is a natural polymer, which has considered attention for its unique properties. It is biodegradable, biocompatible, and non-toxic polymer that derived from partial de-acetylation of chitin. Chitosan has excellent film forming ability, antimicrobial activity (Martins et al., 2014), chelation, adsorption and complexes with oppositely charged polymers (Polk et al., 1994; Liu et al., 1997). Thus, chitosan is an interesting candidate for hybridizing with starch. Moreover, it has been reported that, precipitation of starch paste solution with ethanol resulted in starch micro/nanoparticle (Ma et al., 2008). Ethanol precipitation is a method using ethanol as an anti-solvent by directly dropping into dissolved starch to produce non-solvent precipitation of starch nanoparticles with sizes between 10 nm to 1µm and larger aggregates (Saari et al., 2017). The process is mild, simple, fast and reproducible for preparing precipitates of natural polymer.

Thus, it is of interest to prepare the hybrid material of tapioca starch and chitosan using an eco-friendly method. The preparation of tapioca starch-chitosan (TS-CSP) hybrid material by precipitation method, which using ethanol as anti-solvent agent was described in this paper. Hybrid material was characterized by FT-IR, DSC, TGA, SEM, and XRD techniques. Effect of chitosan in term of anti-bacterial activity of TS-CSP hybrid material was also reported.

MATERIALS AND METHODS

Chemicals and reagents

Tapioca starch (TS) was of Bangkok Inter Food Co., Ltd, Thailand. Commercial grade of chitosan flake (CS) with average molecular weight of about 1,500 kDa was a gift from Marine Bio Resources Co., Ltd, Thailand. Ethyl alcohol was of Zenith Science (Thailand). Water was purified with a Milli-Q water purification system (Millipore, Bedford, USA). All other chemicals and solvents in this study were of analytical grade.

Preparation of tapioca starch-chitosan hybrid material

Tapioca starch-chitosan hybrid material (TS-CSP) was prepared using precipitation method with slight modifications (Ma et al., 2008). Tapioca starch solution (5% w/w) was prepared by dispersing the required amount into DI water. Chitosan solution (1, 2, 5, and 10% w/w) was prepared by dissolving the required concentration into 1% v/v acetic acid solution. Tapioca starch and chitosan solution were then mixed well for 1 h prior to the reaction. Ethyl alcohol (70% v/v, 150 ml) was used as an anti-solvent agent. It was slowly dropped into the mixture of tapioca starch-chitosan with constant stir at 45°C for 1h. Then, the suspension was cooled down to room temperature. Ethyl alcohol (70% v/v, 150 ml) was re-dropped again into the mixture with constant stir for 1h. The mixture was left overnight for separation. The precipitates of TS-CSP hybrid material were collected by suction filtration, dried at 40°C for 24 h in an oven. The dried samples were washed with distilled water until pH of the wash solution was about 4-5, dried at 40°C for 24 h in an oven. The dried samples were then ground into powder form using a mortar and pestle set.

Fourier transform infrared spectroscopy (FT-IR)

The samples were characterized by Fourier Transform Infrared Spectroscopy (FT-IR, Spectrum GX; PerkinElmer, FT-IR Spectro GX, USA) using a diffuse reflectance. The determination was carried out under a transmittance mode at 4000-400 cm^{-1}, resolution of 4 cm^{-1} and scan number of 16 times/sample.

Scanning electron microscope (SEM)

The surface analysis was performed on samples fixed on copper supports. The surface structure was examined by using a Scanning Electron Microscope, SEM (JEOL/JSM-IT300, Japan). The morphology of the samples was determined by SE1 detector, spot size: 250, ETH at 15 kV, Mag: 1,000, and 10,000.

X-ray diffraction (XRD)

The nature and alter crystallizations of the samples were determined by XRD (PANalytical/X'Pert Pro MPD, the Netherlands). The samples were carried out using CuKα, step angle and scan speed of 0.001° and 2°/min, 15 mA, 30 kV in range of 5° to 40°.

Differential scanning calorimetry (DSC)

The samples about 2-5 mg were prepared into alumina crucibles and blank alumina crucible was used as a control. The hybrid materials were examined by differential scanning calorimeters (Mettler Toledo 822e, Germany) at heat rate of 10°C/min, temperature 25 to 500°C under N_2 atmosphere.

Thermal gravimetric analysis (TGA) and differential thermal gravimetric (DTG) analysis

TGA studies were analyzed by thermo-gravimetric analysis (Mettler Toledo 822e, Germany) at heating rate of 10°C/min, temperature 25 to 500°C under N_2 atmosphere. Samples about 2-5 mg were prepared into alumina crucible and blank alumina crucible was used as control.

Anti-bacterial test

Anti-bacterial activities of TS-CSP hybrid materials were assayed by disk diffusion method. A microorganism which commonly found in cosmetic products, *Staphylococcus aureus* TISTR 746 (*S. aureus*), *Escherichia coli* TISTR 527 (*E. coli*), and *Pseudomonas aeruginosa* TISTR 1287 (*P. aeruginosa*) were used for screening antibacterial effect of TS-CSP hybrid material.

RESULTS AND DISCUSSION

Preparation of tapioca starch-chitosan hybrid material.

The tapioca starch-chitosan hybrid material (TS-CSP) was prepared by using 70% ethanol and it has fine texture with pale yellow colour, Figure 1. The physical mixture of tapioca starch and chitosan (TS-CS) was also prepared in order to study the effect of ethanol solvent. It was found that a product with uneven hard film was obtained. The results pointed out that by adding the anti-solvent ethanol, the small microgranule of starch will be formed (Xu et al., 2014; Joye and McClements, 2013)

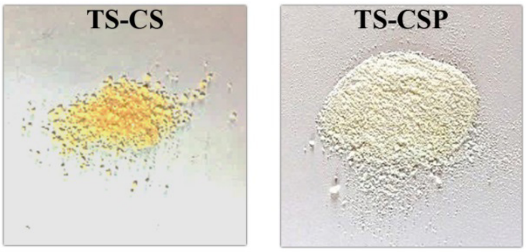

Figure 1. Appearances of TS-CS mixture and TS-CSP hybrid materials.

The effect of chitosan on the physical properties of hybrid material was investigated. The TS-CSP prepared using 5% w/w of starch with different concentration of chitosan (1, 2, 5, 10% w/w) are shown in Figure 2. Higher concentration of chitosan led to fine particle with slightly pale-yellow film of chitosan covering on the surface.

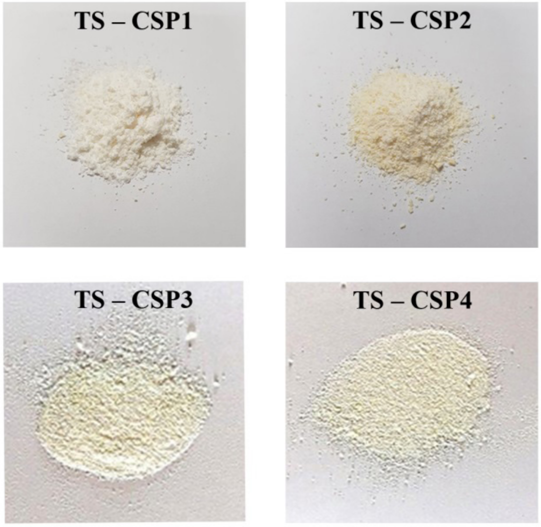

Figure 2. Appearances of TS-CSP hybrid materials prepared with different starch: chitosan ratio.

TS-CSP1 had fine particle with smooth feeling but it had relatively low % yields, Table 1. The TS-CSP3 was selected as optimal condition because of easier prepared and grinded into fine particle with relatively high % yields.

Table 1. Hybrid material prepared with different ratio of starch: chitosan.

Sample code	Starch : Chitosan ratio	Yield (%)
TS - CSP1	5:1	43.05
TS - CSP2	5:2	50.58
TS - CSP3	5:5	52.81
TS - CSP4	5:10	47.34

Fourier transform infrared spectroscopy (FT-IR)

The FT-IR spectra of native tapioca starch, chitosan, and TS-CSP3 are presented in Figure 3. In the spectrum of native tapioca starch, the broad band between 3000 to 3600 cm^{-1} and the peak at 2933 cm^{-1} correspond to OH and CH stretching. The peaks at 1647 cm^{-1}, 1428 cm^{-1}, and 1364 cm^{-1} refer to OH and CH_2 of starch. And several discernible absorbencies around 1149 cm^{-1}, 1078 cm^{-1}, and 1021 cm^{-1} are attributed to CO bond and CC bond (Goheen and Wool, 1991), linking to C-O-C group of hexose ring (Shi et al., 2012). Moreover, absorbencies at 930 cm^{-1}, 861 cm^{-1}, 765 cm^{-1}, and 566 cm^{-1} are the characteristic absorption bands of native starch which due to the entire anhydroglucose ring stretching vibrations (Mano et al., 2003).

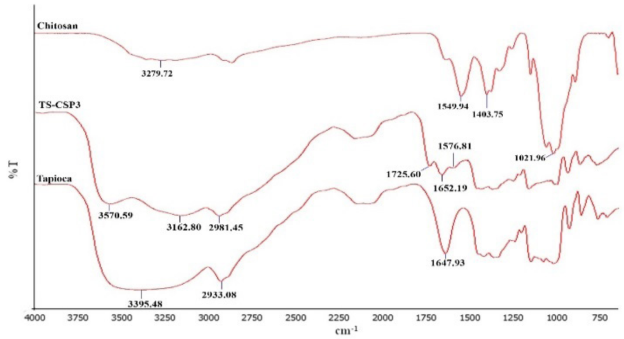

Figure 3. FT-IR spectra of tapioca starch, chitosan, and TS-CSP3 hybrid material.

TS-CSP3 exhibited quite similar spectrum to that of the parent starch. Characteristic bands at 3162 cm^{-1} and 2981 cm^{-1} of TS-CSP3 were shift from 3395 cm^{-1} and 2933 cm^{-1} of native starch, respectively. A band at 1652 cm^{-1} was shift from 1647 cm^{-1}. The new peaks at 3570 cm^{-1} was OH group of starch, indicating to the intermolecular formation of hydrogen bond (Dai et al., 2009). Peaks at 1725 cm^{-1} and 1576 cm^{-1} correspond to carbonyl group and NH bending (amide II) of chitosan (Bourtoom and Chinnan., 2008), respectively. The results indicated that interactions were present between the hydroxyl groups of starch and the amino groups of chitosan (Meenakshi et al., 2002).

Scanning electron microscope (SEM)

The surface morphology of TS-CSP3 hybrid material was analysed by using SEM, Figure 4. Tapioca starch has round and truncated shape with smooth surface (Moorthy, 2002). The TS-CSP3 hybrid material still showed round and truncated shape with thin layer of chitosan film on the surface.

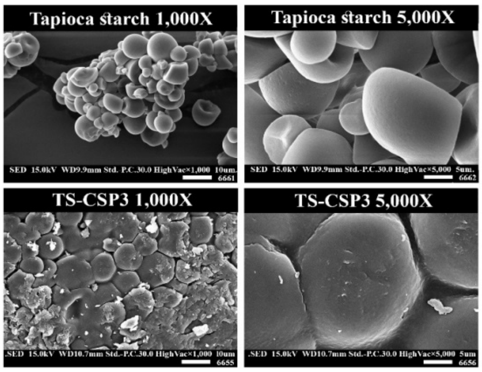

Figure 4. SEM images of native tapioca starch, TS-CSP3 hybrid material (1,000X and 5,000X).

X-ray diffraction (XRD)

XRD pattern of tapioca starch, chitosan, and TS-CSP3 are shown in Figure 5. XRD pattern of native tapioca starch showed strong diffraction peak at Bragg angles 2θ of 15.19, 17.23, 18.06, 19.9, 23.04°, respectively which was an A type pattern (Hizukuri, 1960). Chitosan has amorphous pattern, which showed main diffraction peaks at $2\theta = 20.5°$ with less intensity and no dominant peak. (El-Barghouthi et al., 2008). After TS-CSP3 hybrid material was prepared, the intensity of A-type starch diffraction peak was decreased and less crystalline structure after being mixed with amorphous chitosan. The decrease in the intensity of hybrid materials could be explained by molecular characteristic miscibility of starch and chitosan.

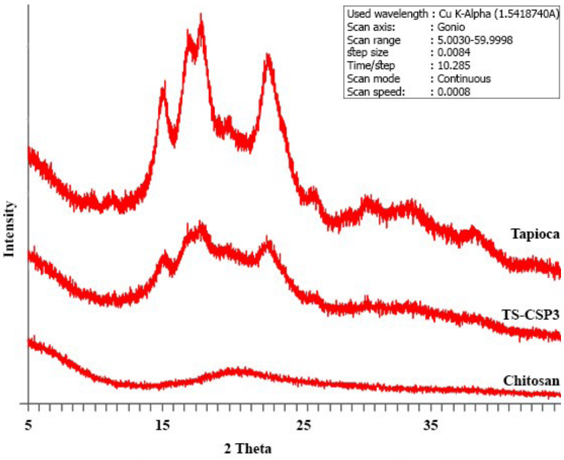

Figure 5. XRD patterns of tapioca starch, chitosan, and TS-CSP3 hybrid material.

Differential scanning calorimetry (DSC)

DSC curve of native tapioca starch, Figure 6, showed the first endothermic peak at 113°C, which may due to a loss of moisture. Other endothermic peaks of native tapioca starch were in the range of 270-350°C which may be the induction of decomposition of starch. For TS-CSP3 hybrid material, it possessed quite similar pattern to that of the parent starch. The first endothermic peak of TS-CSP3 hybrid material is at 103°C which different from its parent starch. The result confirmed that a slightly changing in DSC curve of TS-CSP3 hybrid material is the interaction of precipitated tapioca starch and chitosan, which may present between the hydroxyl groups of tapioca starch and the amino groups of chitosan.

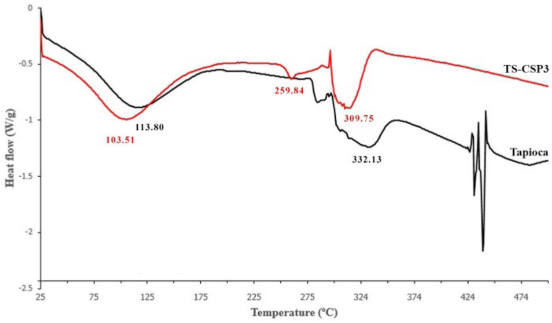

Figure 6. DSC results of tapioca starch and TS-CSP3 hybrid material.

Thermal gravimetric analysis (TGA) and differential thermal gravimetric (DTG) analysis

TGA curves and DTG curves of tapioca starch, chitosan, and TS-CSP3 hybrid material are shown in Figure 7. TGA curve of TS-CSP3 is similar to that of native tapioca starch. It showed the change of temperature at about 100-120°C and at 280-340°C which may due to moisture evaporation and decomposition, respectively.

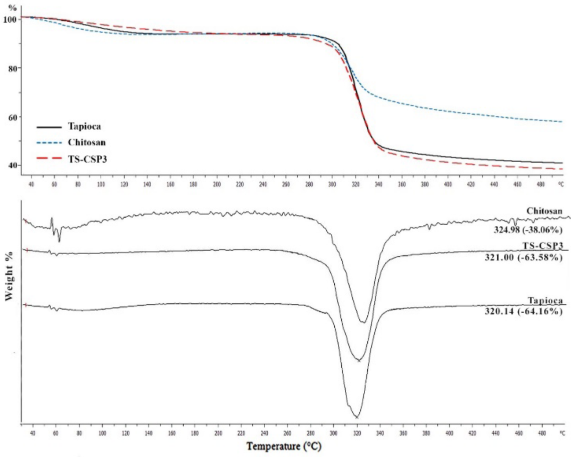

Figure 7. Thermal gravimetric analysis (TGA) and differential thermal gravimetric (DTG) analysis of tapioca starch, chitosan, and TS-CSP3 hybrid material.

The mass loss of samples was decreased at onset temperature. At the maximum mass loss of samples, the temperature was detected, there are decomposition temperature, T_{max} as the peak temperature that showed in DTG curve. Table 2 shows total weight loss of sample. The total weight loss of tapioca starch and chitosan from 260-360° C is 64, and 38% wt., respectively. The weight loss of TS-CSP3 hybrid material equal to 63% wt. which was slightly changed from the parent starch. Therefore, it indicated that the thermal degradation of TS-CSP3 hybrid material was slightly reduced after precipitated of TS-CSP3 hybrid material was prepared.

Table 2. Total weight loss of tapioca starch, chitosan, and TS-CSP3 hybrid material in range of 260-360°C

Samples	Weight loss (% wt.)
Tapioca starch	64.16
Chitosan	38.06
TS-CSP3 hybrid material	63.58

Anti-bacterial test

Anti-microbial activities of TS-CSP3 hybrid material were performed by disk diffusion method. Microorganisms for screening anti-bacterial effect are *Staphylococcus aureus* TISTR 746 (*S. aureus*), *Escherichia coli* TISTR 527 (*E. coli*), and *Pseudomonas aeruginosa* TISTR 1287 (*P. aeruginosa*), which commonly found in cosmetic products. A disc with 30µg/ml of tetracycline (30 µl) was used as positive control, 100 mg/ml of tapioca starch (30 µl), chitosan, and 1000 mg/ml of starch-chitosan hybrid materials (30 µl) were placed on disc as sample. Inhibition zone data of hybrid material is shown in Table 3. It can be seen that a negative control and tapioca starch solution did not show any inhibition effect. Grow of inhibition of positive control (Tetracycline) was around 22-28 mm. Chitosan exhibit zone of inhibition around 9-9.3 mm. The TS-CSP3 hybrid material exhibit zone of inhibition around 7-9 mm. This anti-microbial test suggested that anti-microbial activity may contribute from the chitosan which had anti-microbial effect (Liu et al., 2009).

Table 3. Inhibition zone diameter of TS-CSP3 hybrid material

Microorganism	Inhibition zone (mm)				
	Negative control (DI water)	Positive control (Tetracycline)	Tapioca Starch	Chitosan	TS-CSP3
S. aureus	0	27.7	0	9.0	7.7
E. coli	0	25.3	0	9.3	7.7
P. aeruginosa	0	22.3	0	9.0	8.7

CONCLUSIONS

TS-CSP hybrid material was successfully prepared by precipitation method using 70% ethanol as anti-solvent agent. The appropriated ratio of tapioca starch and chitosan is 5:5 %w/w. The results of characterization by SEM, XRD, DSC, and TGA indicated that physicochemical characteristics of TS-CSP3 were different from its parent starch. FT-IR spectrum of TS-CSP3 hybrid material indicated that interaction may present between the hydroxyl groups of starch and the amino groups of chitosan. Antibacterial activity of the TS-CSP3 hybrid material showed inhibition effect against the growth of *E. coli*, *P. aeruginosa*, and *S. aureus* which will be useful in food and cosmetic applications.

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