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Encapsulation Efficiency of Oolong Tea Chitosan Nanoparticles for Cosmetic Applications

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Abstract

Antioxidant activities of aqueous Oolong tea extracts, produced under different extraction conditions, including stirring at 80 °C for 5 and 10 min and soaking in hot water (80 °C) for 5 and 10 min, were investigated. Extract produced from 5 min stirring at 80 °C showed antioxidant activities with19.28 mM $FeSO_4$, for ferrous reducing power, and 27.39 mM trolox equivalent, for ABTS. HPLC analysis of this extract revealed the presence of caffeine, epicathechin gallate, epicatechin, and cathechin. To enhance stability and penetration of the extract, chitosan (CS) - tripolyphosphate (TPP) nanoparticles were developed by the ionic gelation method. Tea extract-loaded CS-TPP nanoparticles were successfully prepared by mixing chitosan, TPP, and extract in a ratio of 6:1:3. The size of nanoparticles was observed in the range of 200 - 400 nm. For encapsulation efficiency, optimized nanoparticles gave significant efficiency of 79 %EE. In addition, the extract could absorb UV in the range of UVA (250 - 380 nm.) Comparison of UVA protection between Oolong tea extract and CS-TPP loaded Oolong tea extract showed that Oolong tea extract had a higher ability to protect UVA than CS-TPP loaded Oolong tea extract did.

Keywords: Oolong tea, nanoencapsulation, chitosan, ionic gelation method, UV protection

Introduction

Tea is a plant that has been cultivated for a long time in Chiang Rai province. Chiang Rai province has an estimated area of 45,600 hectares which is used for the cultivation of tea, representing more than half the amount of the tea-cultivating area of Thailand, approximately 97,000 hectares. Tea is mostly cultivated in the north of Thailand, in areas such as Chiang Mai, Phayao, Phrae, Nan, and Chiang Rai. Assam tea is an important cash crop for the country, especially in Chiang Rai; residents and various hill tribes are employed in the growing of tea. Nowadays, many people are paying attention to tea consumption as a healthy food choice and value the production of tea. Tea in the north of Thailand is an imported species from China, due to the climate of Thailand, and comes from the species *Camellia sinensis*, which can be divided in 2 types: 1. *Camellia sinensis* var. *Sinensis* (Chinese tea); the trunk has small, dark green leaves. The tree grows more slowly than Assam. In addition, it is low-temperature resistant, and has environment variability. 2. *Camellia sinensis* var. *Assimica* (Assam tea) is a single-trunk tree with a single large green leaf, and adapts to the environment well. The tea (*Camillia sinensis*) is one of the most popular beverages in the world, owing to its pleasing aroma, taste, and putative positive physiological functions. Tea is well known to be rich in flavonoids, or polyphenols, providing beneficial effects, particularly antioxidant activity, e.g., free radical scavenging and metal chelating abilities [1,2]. Oolong tea is a fermented tea, prepared by firing the leaves after rolling for a short time. The fermentation process of Oolong tea results in oxidation of the polyphenols, which obtains more complexes of polyphenols. It has been reported that the aqueous extract of Oolong tea exhibits strong radical scavenging activities, which are associated with a high content of catechins (C), epicathechin (EC), epicathechin gallate (ECG), and epigallocathechin gallate (EGCG). Furthermore, it has been indicated

that the ability to scavenge free radicals of Oolong tea polyphenols is due to the phenolic hydroxyl group attached to the flavan-3-ol structure [3]. Recently, ultraviolet (UV) radiation reaching the earth's surface has been increasing because of the depletion of the ozone layer. UV radiation exposure on mammalian skin induces a number of biological responses, resulting in the development of erythema, edema, sunburn cells, photo ageing, and skin cancer. Ultraviolet radiation consists of UVA (320 - 400 nm), UVB (280 - 320 nm), and UVC (200 - 280 nm). The major components of tea have been the subject of researches into anti-inflammatory and antioxidant effects against UV irradiation [4]. Nowadays, the financial crisis in Thailand has had an effect on agriculture. Therefore, tea products must be more diverse, in order to increase profitability in a competitive market, such as through advertising, or using attractive packaging; tea product value depends on capitalism. Moreover, farmers have relatively little knowledge of the market, and cannot plan their marketing. Therefore, it is essential that farmers receive supporting knowledge about applying products from tea, in order to increase the value their tea. Furthermore, Phenolic compounds in Oolong tea can be generally degraded under extreme temperatures or light, or when they are applied to cosmetic products or to human skin. Therefore, chitosan was selected to encapsulate the extract due to its humectant effect on skin, biocompatibility, non-toxicity, and biodegradability. This allowed for protection of, and control of the release of, Oolong tea extract, which provides benefits for cosmetic applications [5-7]. Therefore, the aim of this work is to study Oolong tea extract and to develop tea extract chitosan nanoparticles to enhance stability and penetration of the skin.

Materials and methods

Plant materials

Oolong tea (*Camillia sinensis*) was purchased from Chui Fong Tea, Chiang Rai province. Dr. Angkana Inta, Department of Biology, Faculty of Science Chiang Mai University, confirmed the species of tea. Oolong tea used in this study was collected during October, 2014.

Chemicals

2,2'-Azino-bis-3-ethylbenzothiazoline-6-sulfophonic acid (ABTS), acetic acid, ascorbic acid, chitosan, 1,1-diphenyl-2-picryl-hydrazil (DPPH), ethanol, Folin-Ciocateu reagent, gallic acid, hydrochloric acid (HCl), potassium persulphate (K_2SO_8), iron(III)chloride ($FeCl_3$), iron(II)sulphate ($FeSO_4$), sodium acetate (NaOAc), sodium carbonate (Na_2CO_3), sodium tripolyphosphate (STP), 2,4,6-trispyridin-2-yl-1,3,5-triazine (TPTZ), and trolox were purchased from Sigma-Aldrich, Prathum Wan, Bangkok, Thailand.

Extraction of Oolong tea

Oolong tea powder (4 g) was extracted with distilled water (200 ml) under 4 conditions, stirring in a beaker (5 and 10 min) and soaking in a thermos flask (5 and 10 min) in hot water (80 °C). Then, the extracts were filtered, rapidly freeze-dried for 24 h, and stored at 4 °C until further analysis.

Determination of antioxidant activities

ABTS radical scavenging assay

For the ABTS assay, the ABTS solutions was prepared by mixing 7 mM ABTS solution (10 ml) with 140 mM K_2SO_8 solution (88 µl); this was kept for 14 h at room temperature in the dark. The solution was then diluted with ethanol to obtain an absorbance of 0.70 ± 0.02 units at 734 nm using a spectrophotometer. Fresh ABTS solution was prepared for each assay. The extracts (1 % v/v, 100 µl) were allowed to react with 2 ml of the ABTS solution, and the absorbance was measured at 734 nm after 1 min incubation using a spectrophotometer. All samples were analyzed in triplicate. Trolox was used as a standard in this study. The capability to scavenge ABTS radicals was calculated by the following equation and expressed as a Trolox equivalent;

$$\% \text{ ABTS radical scavenging activity} = [(A_o - A_s)/A_o] \times 100 \quad (1)$$

A_o is the absorbance of the control, and A_s is the absorbance of the sample.

Ferric reducing antioxidant power (FRAP) assay

FRAP working reagent was prepared by mixing 100 ml acetate buffer solution (pH 3.6) with 0.0312 g of TPTZ in 10 ml of HCl and 10 ml of $FeCl_3$. In brief, distilled water (90 µl) was mixed with FRAP working reagent (900 µl) and 1 % v/v of extract solution (30 µl) was added. The reaction was incubated at 37 °C for 10 min and absorbance at 597 nm was measured. All samples were analyzed in triplicate. $FeSO_4$ was used as a standard in this study.

UV-protection test of Oolong tea extract

The UV absorption of the extract was determined using a UV-Visible spectrophotometer. The extract powder (1 mg) was dissolved in 1:1 ethanol and water (3 ml) and then the absorbance measured in the range of 200 - 600 nm. Sun protection factor and Boots star rating of the gel containing 3 % w/w extract was measured by an Optometrics LLC/SPF 290F.

HPLC analysis of Oolong tea extract

Chemical compositions of Oolong tea extract were analyzed with a HPLC Waters Alliance 2695 with PDA and ELSD. The column used was the EPS C-18 reversed-phase. The mobile phase consisted of water and acetonitrile in a ratio of 87:13 v/v. Elution was performed at a flow rate of 2 ml/min. Absorption detection was performed at 210 nm. The sample injection volume was 20 µl.

Preparation of chitosan (CS)-tripolyphosphate (TPP) nanoparticles

Chitosan solution (0.50 % w/v) was mixed with acetic acid solution at room temperature, filtered, and then used to prepare the CS-TPP wall material by mixing with TPP solution (0.8 % w/v) in various ratios and stirring for 1 h at room temperature (**Table 1**). The concentration of chitosan varied with TPP solution in the optimized ratio. Moreover, the extract was loaded in optimal CS-TPP wall material in various conditions. All samples were analyzed in triplicate and the particle size and zetapotential were measured by a Zetasizer Nano ZS90 analyzer. The morphology of the microspheres was examined using a Transmission Electron Microscope (TEM).

Encapsulation efficiency of nanoparticles

Two hundred µl of CS-TPP loaded Oolong tea extract determined entrapment efficiency. The catechin content was analyzed by the modified Folin-Ciocalteu method. In brief, 200 µl of samples were mixed with 140 µl of 0.2 N Folin-Ciocalteu reagents, 2.4 ml of deionised water, and 420 µl of sodium carbonate (20 % w/v). The mixture was placed in the dark at ambient conditions for 1 h, to which 910 µl of deionised water was added, and absorbance was measured using a UV-vis spectrophotometer at 765 nm. All samples were analyzed in triplicate. Encapsulation efficiency (EE) was calculated using equation;

$$EE\ (\%) = (TC - FC)/TC \times 100 \quad (2)$$

where, TC is the total amount of catechin and FC is the free amount of catechin in the supernatant.

Table 1 Preparation of Cosmetic Product (gel) from Oolong Tea extract and Chitosan (CS)-Tripolyphosphate (TPP) Nanoparticles loaded with Oolong Tea extract.

No.	International Nomenclature of Cosmetic Ingredients	% w/w	% w/w
1	Deionization water	qs to 100	qs to 100
2	Disodium EDTA	0.1	0.1
3	Propylene glycol	8.0	8.0
4	Ammonium Acryloyldim	1.5	1.5
5	Pentylene Glycol	1.0	1.0
6	Oolong tea extract	3.0	-

Measurement of gel from CS-TPP nanoparticle loaded Oolong tea extract and gel from Oolong tea extract

The morphologies of the gel from CS-TPP nanoparticle loaded Oolong tea extract and gel from Oolong tea extract were examined using TEM.

Sun protection factor (SPF) and Boots star rating of the UV absorption of gel from CS-TPP nanoparticle loaded Oolong tea extract and gel from Oolong tea extract was measured by an Optometrics LLC/SPF 290F.

Results and discussion

HPLC analysis of Oolong tea extract

From HPLC analysis, several phenolic compounds were found. The retention time of, cathechin, epicathechin, and epicathechin gallate were 3.160, 3.637, 5.241, and 5.714, respectively (**Figure 1**). The relationship between the quality and the chemical components in tea indicated that caffeine and polyphenols play important roles in the quality of tea. Especially, catechins, the main component of polyphenols in tea leaf, are well known for their antioxidant properties.

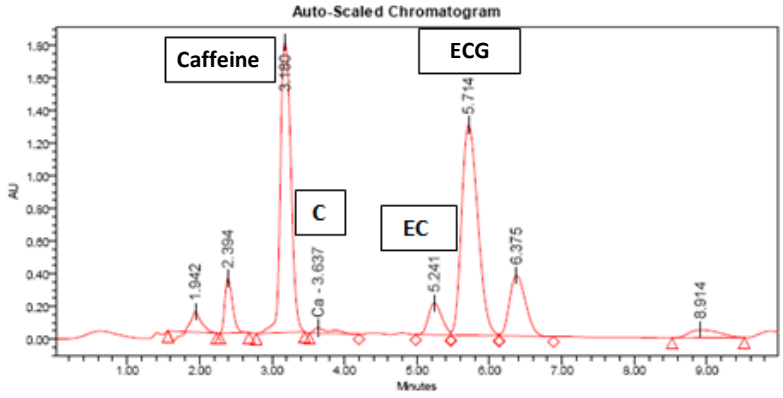

Figure 1 HPLC Analysis of 5 min stirring Oolong tea extract.

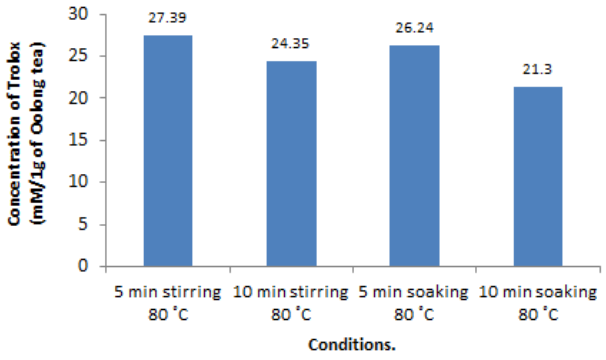

Figure 2 ABTS radical scavenging of Oolong tea extracts.

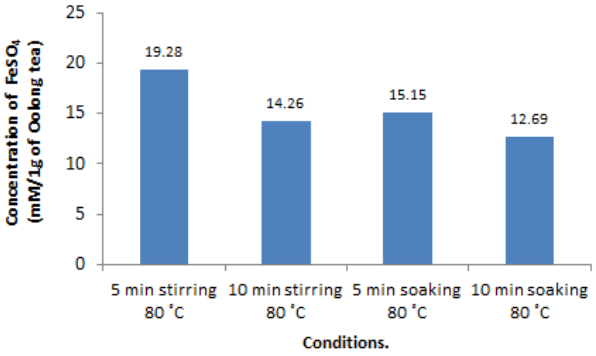

Figure 3 Ferric reducing antioxidant power of Oolong tea extracts.

The antioxidant capacities of Oolong tea have be widely studied to be benefit to human. The antioxidant activities of Oolong tea extracts were determined by ABTS radical scavenging and FRAP assays. The results showed that the 5 min stirring extract showed the highest antioxidant activity. Moreover, it also displayed the highest significance in both ABTS scavenging activity, with 27.39 mM trolox equivalent, and FRAP, with 19.28 mM of $FeSO_4$ per gram of extract (**Figures 2** and **3**). Therefore, the extract obtained from stirring at 80 °C for 5 min was chosen for further study.

UV protection analysis

In this experiment, the results showed that the extract had the ability to absorb UV in wavelengths between 200 - 400 nm, which is a UVA range, as shown in **Figure 4**. The ability of 3 % w/w Oolong tea gel to protect against UV was determined by using a SPF analyzer. **Table 2** showed that the gel could protect UVA with a Boots star rating value of 5.

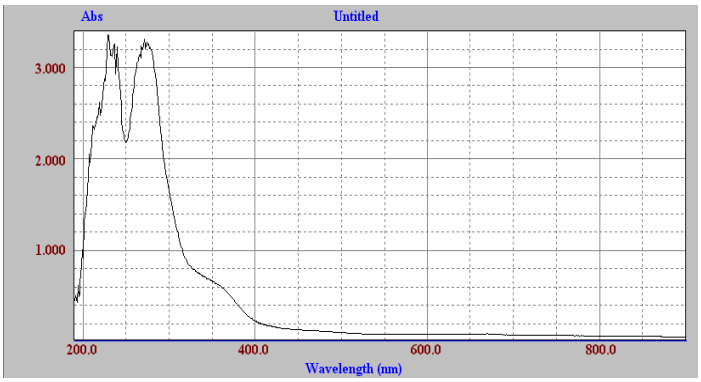

Figure 4 UV-visible analysis of Oolong tea extract.

Table 2 UV protection analysis of 3 % w/w Oolong tea gel.

	Value	STDV
UVA/UVB	5.164	11
Boots star rating	5	Ultra

Preparation of Chitosan (CS)-tripolyphosphate (TPP) nanoparticles

In general, particle size distribution and morphology are the most important parameters of characterization of nanoparticles. Nanoencapsulation of drugs involves forming drug loaded particles with diameters ranging from 1 to 1000 nm. Nanoparticles are defined as solid, submicron-sized drug carriers that may or may not be biodegradable. The major application of nanoparticles is in active release and active targeting. It has been found that particle size affects the active release. Smaller particles offer larger surface area and subsequently there is a compromise between a small size and maximum stability of nanoparticles. The measurement of the zeta potential allows for predictions about the storage stability of colloidal dispersion as both a large negative or positive zeta potential will repel, whereas those having low zeta potential values will combine and flocculate [8]. The CS-TPP nanoparticles exhibited a positive zeta potential from the structure of chitosan polymer, which is expected to enhance the stability of the nanoparticle suspension and prevent particle aggregation. Therefore, in this experiment the ratio of chitosan and TPP was studied as chitosan solution (0.30 % w/v) was mixed with TPP solution (0.8 % w/v) in various ratios (1:1 - 1:10) and stirred for 1 h at room temperature. The ratio of chitosan and TPP at 6:1 provided the smallest particle size of wall material (140.1 nm) with zeta potential at 54.8 mV as shown in **Table 3**.

Table 3 Size and zeta potential of ratio CS-TPP.

Ratio (CS-TPP)	Size (nm)	Zeta potential (mV)
1:1	820.0	-8.63
1:2	789.7	-8.45
1:3	181.9	44.8
1:4	176.9	49.5
1:5	159.6	53.7
1:6	140.1	54.8
1:7	180.1	62.5
1:8	184.1	63.0
1:9	188.6	61.0
1:10	450.4	43.7

The effect of the chitosan concentration on the extract encapsulation was studied. Chitosan with various concentrations (0.05 - 0.30 % w/v) was mixed with TPP in a ratio of 6:1. The results showed that 0.30 % w/v of chitosan solution provided the smallest particle size with a 263 nm (zeta potential value 54.7 mV). The particle size of tea-loaded CS-TPP decreased as the concentration of chitosan increased (**Table 4**) because the molecules of chitosan are cationic polysaccharides. When attached with molecules of acetic acid the particle size will be decrease.

Table 4 Size and zeta potential of various concentration of Chitosan.

Concentration of Chitosan (% w/v)	Size (nm)	Zeta potential (mV)
0.05	1683	5.93
0.10	1418	26.1
0.15	1066	51.8
0.20	797.8	51.8
0.25	661.7	53.2
0.30	263	54.7

This CS-TPP wall material was then loaded with extract in various ratios (1:1, 1:2 and 1:3). Oolong tea-loaded CS-TPP nanoparticle was successfully prepared by applying the extract and CS-TPP wall in the ratio of 1:3. This provided the smallest size of tea-loaded nanoparticle with 429.4 nm and 40.6 mV of zeta value as shown in **Table 5**. The CS-TPP wall was then loaded with extract in a ratio of 1:3 (extract and CS-TPP).

Table 5 Size and zeta potential of ratio Oolong tea extract: CS-TPP.

Ratio of Oolong tea extract: CS-TPP	Size (nm)	Zeta potential (mV)
1:1	1767	-7.58
1:2	1141	28.3
1:3	429.4	40.6

Characterization of chitosan (CS)-tripolyphosphate (TPP) nanoparticles

TEM was used to investigate the shape and size of Oolong CS-TPP nanoparticles. The TEM image of the particles is shown in **Figure 5**. The overall dimension of Oolong tea loaded CS-TPP nanoparticles was showed 429.4 nm in **Figure 6**, with a spherical shape.

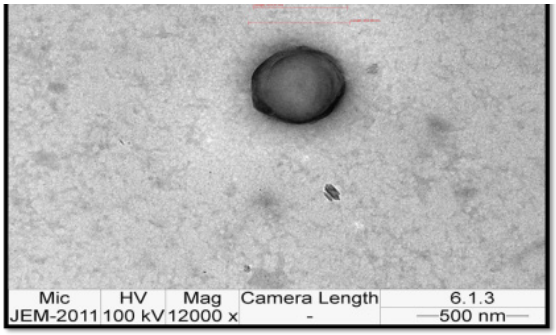

Figure 5 TEM analyses of CS-TPP 0.30 % w/v of chitosan solution TPP and extract in ratio of 6:1:3.

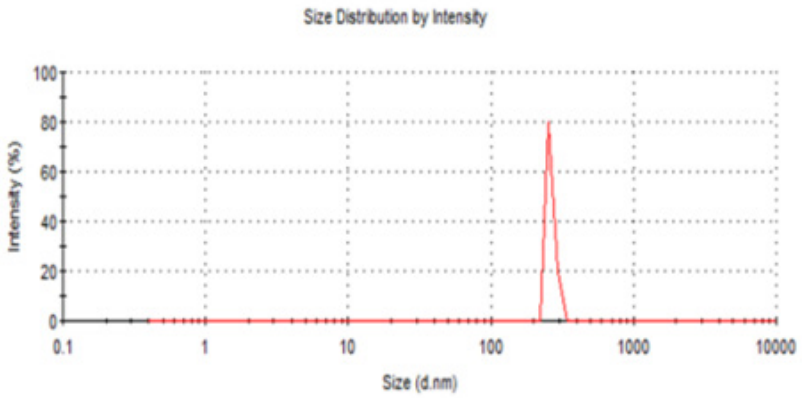

Figure 6 Size distribution of Oolong tea loaded CS-TPP nanoparticles.

Encapsulation efficiency of nanoparticles

The encapsulation efficiency of CS-TPP loaded Oolong tea extract 79 % was observed at pH 4.9, which may account for the competitive interaction between phenolic (OH^-) catechin and PO_4^{3-} groups of TPP for the protonated amino groups of CS. The above results suggest that pH and the concentration of TPP may play significant roles during particle formation, and also in the encapsulation of bioactivity. Previous studies used this method to calculate the encapsulation efficiency of CS particles [9-12].

Measurement of gel from CS-TPP nanoparticle loaded Oolong tea extract and gel from Oolong tea extract

In this experiment, the ability to protect from UV of 3 % CS-TPP loaded Oolong tea extract gel was determined by an SPF analyzer, compared with the gel from Oolong tea extract. The gel from CS-TPP nanoparticle loaded Oolong tea extract could protect from UVA with PA+, SPF 1.05±0.01, UVA/UVB ratio 1.488, and critical wavelength 390 nm. In addition, comparison of UVA protection between oolong tea extract and CS-TPP loaded Oolong tea extract gel showed that Oolong tea extract gel had a higher ability to protect from UVA than CS-TPP loaded Oolong tea extract gel did.

Conclusions

The results of this study indicated that Oolong tea extract obtained from stirring at 80 °C for 5 min contained significant antioxidant activity. CS-TTP nanoparticles loaded with Oolong tea extract were prepared by the ionic gelation method. The formation of CS-TPP nanoparticles loaded with Oolong tea extract could be achieved by using chitosan (0.30 % w/v) and TPP in the ratio of 6:1. It was observed that the smallest nanoparticle size, with a range between 200 - 400 nm, was achieved by using Oolong tea extract and chitosan at a ratio of 1:3. For encapsulation efficiency, optimized nanoparticles gave significant efficiency of 79 %EE. In addition, the extract could absorb UV in the range of 250 - 380 nm. Comparison of UVA protection between Oolong tea extract gel and CS-TPP loaded Oolong tea extract gel showed that Oolong tea extract gel had a greater ability to protect from UVA than CS-TPP loaded Oolong tea extract gel did.

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