Encapsulation of Phenolic Compound from Star Fruit with Chitosan Nanoparticle

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Abstract

The use of chitosan for the encapsulation of active components has gained interest in the last years due to its mucous adhesiveness, non-toxicity, biocompatibility and biodegradability. The benefits of encapsulating active agents in a polymer matrix include their protection from the surrounding medium or processing conditions and their controlled release.In this study chitosan nanoparticles were obtained for the encapsulation of phenolic compound. Chitosan anoparticles were prepared by ionic gelation of chitosan and sodium tripolyphosphate. X-ray diffraction, FTIR, and TEM were used to structurally characterize these chitosan nanoparticle products. The penolic compound were added to the sodium tripolyphosphate solution and this was added dropwise to the chitosan solution while stirring. The effect of the encapsulating systems on the phenolic compound stability and its release properties was analyzed.The products obtained allowed to control the release of phenolic compound and therefore these encapsulating methods are a promising technique for nutraceutical and cosmetic applications.

Keyword : chitosan nanoparticle, phenolic compound,

1. Introduction

Encapsulation is a process in which thin films, generally of polymeric materials are applied to little solid particles, liquid or gases droplets. This method is used to trap active components and release them under controlled conditions. Several materials have been encapsulated in the food industry, among others, aminoacids, vitamins, minerals, antioxidants, colorants, enzymes and sweeteners[1]. Because of the many benefits offered by encapsulation,entrapped microorganisms can be used to advantage for producing dairy products such as yoghurt, cheese and frozen milk products, as well as for biomass production. The use of encapsulated microorganisms reduced the incubation time by 50% and 60% for fresh fermentedcheese production[2] and cream fermentation [3] , respectively. Sheu and Marshall[4] reported that about 40% more lactobacilli survived freezingof ice cream when they were entrapped in calcium alginate than when they were not entrapped. Moreover, encapsulation protectedthe microorganisms in batch-frozen and continuously frozen ice milk mixes[4]. The benefits of encapsulating active agents in a polymer matrix include their protection from the surrounding medium or processing conditions and their controlled release [5]

Chitosan is receiving a lot of interest in the encapsulation of active compounds due to its biocompatibility and low toxicity [6] In order to improve the use efficiency of active agent and reduce the cost of production, active agent immobilization technology is applied.

In recent years, nanotechnology has showed a significant attraction to the preparation of immobilized enzymes. Under the scale of nano, nano-materials have characteristics such as magnetism and large surface area, etc. Recently, chitosan nanoparticle have been prepared to improve its antimicrobial activity[7], and mainly used as drug carrier as reported in previous studies [8]. chitosan nanoparticles had been prepared, characterized, and used to adsorb eosin Y, acid orange from aqueous solutions [9-10]. Nanoparticles are made of natural or artificial polymers ranging min size from 10–1000 nm [11]. .Nanoparticles display unique physical

and chemical features because of effects such as the quantum size effect, mini size effect, surface effect and macro-quantum tunnel effect. Hence the aim of the present study is to investigate for the encapsulation of star fruit extract with chitosan nanoparticle

Star fruit is grown in the tropic and sub-tropic regions of the world. It is quite a popular fruit and largely planted in Southeast Asia and many other countries. It is usually consumed fresh or made into fruit juice or juice drinks. Leong & Shui [12] reported that star fruit is a good source of natural antioxidants, and the antioxidants in star fruit were found to be proanthocyanidins, -epicatechin and vitamin C [12]

Because extract studies involve several variables, the factorial experimental design was applied to extract systems. Factorial designs are widely used to investigate the effects of experimental factors and the interactions between those factors, that is, how the effect of one factor varies with the level of the other factors in a response. The advantages of factorial experiments include the relatively low cost, a reduced number of experiments, and increased possibilities to evaluate interactions among the variables. They are few papers reported in the literature on the application of factorial experiment in wastewater treatment, such as application of the central composite design and response surface methodology to the advance treatment of olive oil processing wastewater using Fenton's peroxidation [13], empirical modeling of *Eucalyptus* wood processing [14] and the optimization of ozone treatment for colour and COD removal of acid dye effluent using central composite design experiment [15]. In this study, star fruit was chosen for the purpose of investigating its extract phenol compound A 2^3 full factorial design with 20 assay at room temperature was used to evaluate the importance of solvent ratio (X1), temperature (X2),and extract time (X3).

2. Materials and methods

2.1. Chemicals and reagents

Folin–Ciocalteu reagent, sodium hidroxide, sodium tripolyphosphate and acetic acid from Merck (Darmstadt, Germany), gallic acid from Kalbe Farma.Chitosan, from laboratory analytical chemistry State Univercity of Jakarta.

2.2. Sample preparation and optimazing extraction

Several batches of star fruits were purchased from a local supermarket or wholesale center. Fresh star fruit obtained from the market was homogenized using a blender, centrifuged and filtered under vacuum. The liquid portion (juice) was used directly for antioxidant capacity and total phenolic assays.The extraction of phenolic compound from the dried powder was carried out at different ratios of solvents acetone:water (45%, 50%, and 55, respectively), to obtain suitable extraction solvent and ratio of solvent to water. Under the selected extraction solvent,different extraction temperatures (75, 85 and 90^0 C, respectively) were compared. Under the selected solvent and temperature, different extraction times (40,45,50 min, respectively), were applied for obtaining a suitable extraction time. The extraction was carried out in sealed glass bottles, which were placed into a water bath at a preset temperature.

2.3. Total phenolic compound content by Folin–Ciocalteau assay

Total phenolics were determined using Folin–Ciocalteau reagents.Gallic acid standard solution (2.0 mg/ml) was prepared by accurately weighing 0.01 g and dissolving 50 ml of distilled water. The solution was then diluted to give with concentrations working standard solutions of 1.5, 1.0, 0.5, 0.2, and 0.1 mg/ml. Forty microlitres of juice or gallic acid standard was mixed with 1.8 ml of Folin–Ciocalteu reagent (previously diluted 10-fold with distilled water and allowed to stand at room temperature for 5 min, and then 1.2 ml of sodium bicarbonate (7.5%) was added to the mixture. After standing 60 min at room temperature,

absorbance was measuredat 765 nm. Results are expressed as mg/g gallic acid equivalents (GAE).

2.4. The factorial design

The high, middle and low levels defined for the 2^3 factorial design werelisted in Table 1. The low, middle and high levels for the factors were selected according to some preliminary experiments. The factorial design matrix and total phemolic compound measured in each factorial experiment is shown in Table 2, with the low (−1),middle (0) and high (+1) levels as specified in Table 1. Total phenolic compound was determined as average of three parallel experiments. The order in which the experiments were made was randomized to avoid systematic errors. The results were analyzed with the SPSS versi 16.0 software, and the main effects and interactions between factors were determined.

Table 1 Factors and levels used in the factorial design

Factors	symbol	low	middle	high
temperature	X1	75	85	90
aceton(%)	X2	45	50	55
Contact time(min)	X3	40	45	50

2.5 Preparation of chitosan nanoparticles

Chitosan beads 500 mg was dissolved in 100 ml acetic acid 1% (v/v) to obtain chitosan solution, and raised to pH 4.6-4.8 with 10 N NaOH. STPP(1%) with pH 6.0 was slowly dropped into a chitosan solution. Under magnetic stirring at room temperature, 1 ml STPP 1% solution was added dropwise to 25 ml of chitosan solution. The mixture was stirred for a further 20 min followed by sonification. The resulting suspension was subsequently centrifuged at 12.000 rpm for 10 min. We added additional STPP into supernatant after centrifugation, and observed a milky emulsion, which implied that not at all the chitosan had been converted to nanoparticles. The mixture was stirred again for a further 20 min followed by sonification. The resulting suspension was subsequently centrifuged at 12.000 rpm for 10 min. The chitosan nanoparticles were obtained by freezing the emulsion at -4^0C. The frozen emulsion was then thawed in the atmosphere and the nanoparticles were precipitated. The weight of the collected nanoparticles mg. 20 min and then dried at 100 °C for 96 h.The Chi-tosan nanoparticle was characterized by X-Ray Diffractometry, Fourier Transform Infrared spectrometry and TEM

2.6 Encapsulation total phenol compound with chitosan nanoparticle

For doping of the total phenolic compound, the method is as follows: 0.5 g of chitosan nanoparticle was added in 25mL of total phenolic compound solution (10% w/v), and stirred vigorously at room-temperature for 24 h. The solid was then filtered, extensively washed with distilled water, and dried at 50 ◦C for1 day.

2.7. Stability of totalphenolic compounds and encapsulation efficiency

The total phenolic compound encapsulated in microspheres were quantified by Folin–Ciocalteu method after dissolving 30 mg of CNP in 20 mL of deionized water. This procedure was done after the obtention of microspheres and 3 months later in order to evaluate the stability of the encapsulated total phenolic compound over time.

Different matrix destabilizing agents (pH, temperature and chelating agents) were tested. Ten beads of control and CNP coated samples were placed in test tubes containing 10 ml of the destabilizing agent: HCl 0.1 N, NaOH 0.1 N and sodium citrate 1 and 10% w/v as a calcium chelator were assayed at ambient temperature, and distilled water was tested at 50

and 100^0 C. To facilitate the observation of the structure disintegration of beads, a colorant(methyl violet) was used as the active component. Observations were performed for 24 h.The release of the active agent in water was quantified by total polyphenols using Folin-Ciocalteau method. The assay was carried out sinking. 10 beads in test tubes with 5 ml of distilled water under continuous agitation. Control and coated chitosan humid beads were tested by triplicate. Measurements were performed at different times between 10 min and 48 h. Percentage release and effective diffusion coefficients (D) were calculated. The amount of lyophilized extract loaded in beads was estimated by dissolving a known amount of capsules in sodium citrate (10% w/v) during 20 min for control capsules and 90 min for chitosan coated beads in an Orbit- Environ Shaker (Lab-Line Instruments, USA) at 37^0 C and 125 rpm. The concentrations of lyophilized extractloaded in the beads were determined by Folin-Ciocalteau method. A blank of sodium citrate solution was also performed.The percentage of loading efficiency was calculated with the following equation:

$$\text{Loading efficiency (\%)} = \frac{L}{L0} \times 100\%$$

where L is the amount of extract determined on the solution of sodium citrate and L0 is the initial amount of extract dissolved in CNP solution.

3.Result

3.1 . Models for extract total phenolic compounds from starfruit

So far, one has at least demonstrated that extract total phenolic compounds depends on solvent ratio . A mere investigation of the individual effects of the amount of each of them upon the adsorption efficiency is not sufficient to elucidate such an interaction. This is why the use of a factorial 3^3 experiment design, by varying extract time and the temperature, could be very useful to also investigate the interactions between the major parameters involved. The results obtained after 20 attempts are summarized in Table 2.

Table 2 Design Experimental of the dependent variables to the extract parameters.

Experiment	X1	X2	X3	Total phenol (mg/g)
1	60.00	90.00	35.00	16.42
2	50.00	80.00	61.82	17.21
3	63.18	63.18	55.00	15.84
4	50.00	63.18	45.00	14.86
5	50.00	96.82	45.00	18.04
6	50.00	80.00	45.00	18.13
7	40.00	90.00	35.00	16.34
8	60.00	70.00	35.00	12.11
9	60.00	90.00	55.00	18.32
10	40.00	70.00	35.00	9.92
11	50.00	80.00	45.00	18.06
12	50.00	80.00	45.00	18.16
13	50.00	80.00	28.18	13.27
14	40.00	70.00	55.00	14.32
15	60.00	70.00	55.00	12.75
16	50.00	80.00	45.00	18.12
17	50.00	80.00	45.00	18.08
18	33.18	80.00	45.00	12.48
19	50.00	80.00	45.00	18.07
20	66.82	80.00	45.00	12.26

The mixture design for the first approach suggested 20 experimental points, each corresponding to a mixture composition. For each point an average total phenolic compound

under compression was determined. These numerical results are represented in a triangular graph, Fig. 1, which shows the level curves of total phenolic content as a function of the composition, obtained from a quadratic regression. The final form of the polynomial model that describes the polyphenol content is the following

$$R1 = 18.10 + 0.21X1 + 1.70X2 + 0.96X3 + 0.24X1X2 - 0.17X1X3 - 0.46X2X3 - 2.02X1^2 - 0.58X2^2 - 1.01X3^2$$

The positive coefficients, corresponding to the interactions between aceton concentration and temperature X1X2 , represent the synergetic effect of the mixture of components on the expected value for total phenol content . In addition, aceton (%) and temperature had positive linear effects on total phenolic yields (Fig. 1). The optimal condition determined by RSA was at ethanol concentration of 50 %, extraction temperature of 85 °C and time of 40 min with a maximal yield of total phenolic compounds of 23.65 mg/g of dry powder. .Fig. 1 is a response surface plot showing the effect of aceton concentration and temperature on the total phenolic content at the fixed time of 45 min..

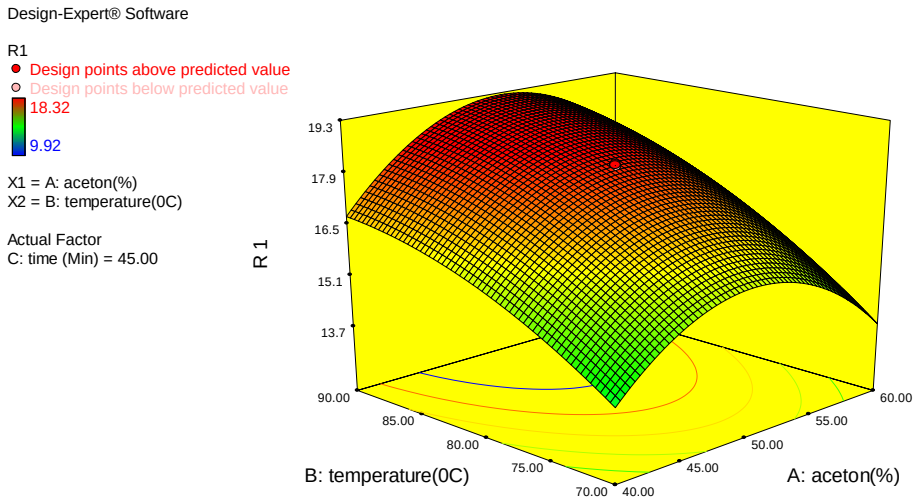

Fig. 1. Surface plot of polyphneolic yield (R1) as a function of concentration of aceton and temperature at extraction time of 45 min

Fig. 2 reports the iso-response curves for aceton and nanopowder maintaining extract time at the fixed value of 45 min. Analysis of this graph indicated that the highest total phenol content hvalue are obtained with high levels of temperature and low levels of aceton... Thus, the chosen independent variables ranged as follows: X1 = % aceton : 35–55(wvv); X2 = % temperature : 7.8-90^{0}C

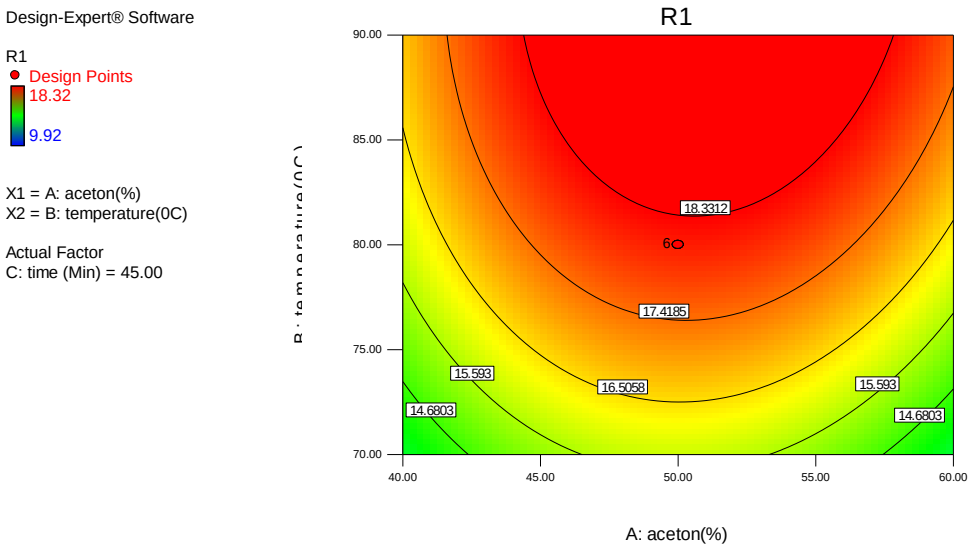

Fig. 2 Iso-response curves of formulation of nanopackaging encapsulation efficiency as a function of the factors aceton (*X*1) and temperature (*X*2), maintaining extract time (*X*3) at 45 min.

3.2 Characterization chitosan nanoparticle

3..2.1The FTIR Analysis

The FTIR spectra of nanoparticle chitosan are shown inf Fig 3. A characteristic band at 3406cm^{-1} is attributed to –NH_2 and OH groups stretching vibration and the band for amide I at 1655 cm^{-1} is seen in the infrared soectrum chitosan. Whereas in the FTIR spectra of nanoparticle chitosan the peak of 1655 cm^{-1} disapperar and 2 new peaks at 1639 cm^{-1} and 1542 cm^{-1} appears. The disappearance of the band could be attributed to the linkage between the phosphoric and ammonium ions. The nanoparticle chitosan also showed a peak for P = O at 1155 cm^{-1}. Qi et al [17] observed similar results in their study of the formation nanoparticle chitosan and chitosan film treated with phosphate.

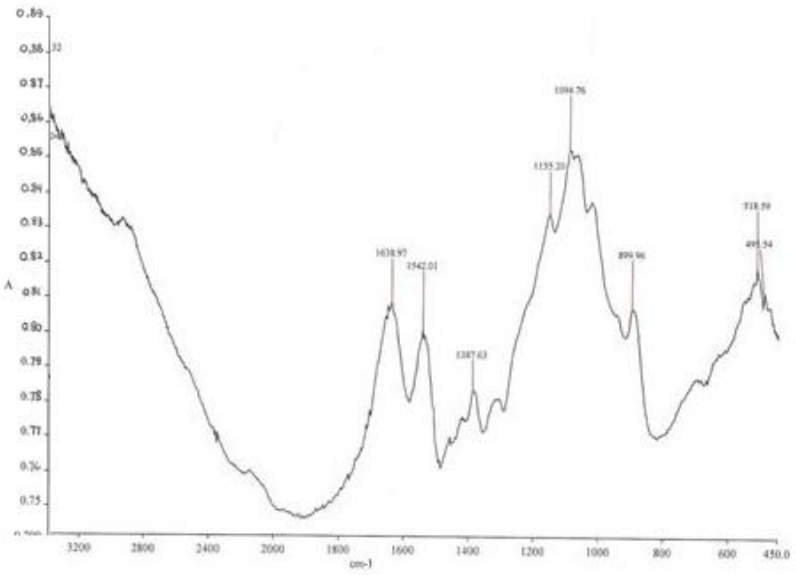

Fig 3 IR Spectra of chitosan nanoparticle

Chitosan with a pKa of 6.3 is polycationic when dissolved in acid and presents –NH_3^+ sites. STPP ($Na_5P_3O_{10}$) dissolved in water dissociates to give both hydroxyl and phosphoric ions. Since the cross linking of chitosan would be dependant on the availability of

the cationic sites and the negatively charged species, it was expected that the pH of STPP would play a significant role in same. pH would bring about a change on the extent and type cross linking. Hence in the present study, pH conditions pH 8.6 were used for reaction. At pH 8.6 both OH^- and phosphoric ions were prsent and may compete each other to interact with the $– NH_3^+$ of chitosan.

3. 2.2XRD Analysis

X-ray diffraction pattern of chitosan nanoparticle are shown in Fig 4.However, no peak is found in the difractogram of chitosan nanoparticle. The XRD pf chitosan nanoparticle is characteristic of an amoorphous polymer. The crystal structure of the amorphous chitosan nanoparticle has been destroyed after crosslinked with sodium tripolyphosphate.

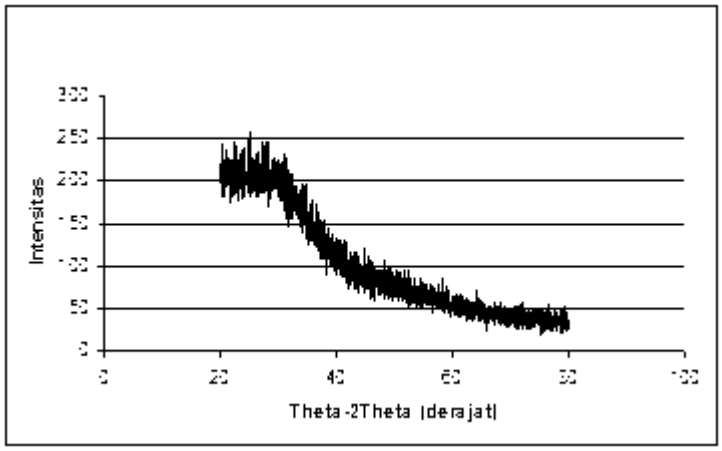

Fig 4. X-ray powder diffraction patterns of chitosan nanoparticle

Fig. 5 depicts the TEM image of chitosan nanoparticle along with SAED particles shown in inset. The average diameter of particles was about 9.1-100 nm. Zhi et al[18] was reported ,when NaOH was selected as the precipitator, the average diameter chitosan nanoparticle was 32-142 nm.

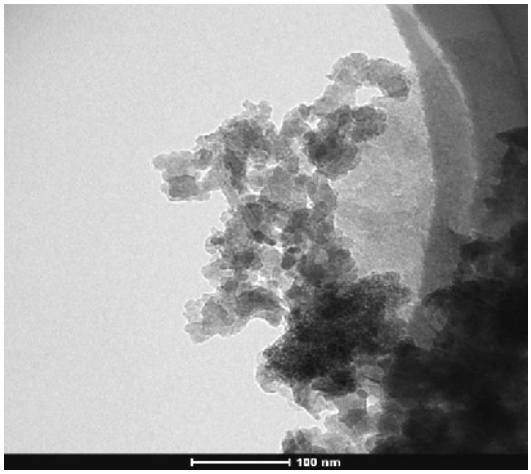

Fig.5. TEM imagesand size distribution of chitosan nanoparticle

3.3. Stability of polyphenolic compounds and encapsulation efficiency

The total polyphenol content in the obtained chitosan nanoparticle was measured to determine the encapsulation efficiency. In addition, this was also calculated after one week to study the stability of the encapsulated polyphenols over time. The encapsulation efficiency

was near 100%, after one week the polyphenol content was 100%. Therefore, chitosan nanoparticle maintainedthe stability of the polyphenols over time and are a goodvehicle for the encapsulation of these compounds. Kosaraju et al. (2006) also observed that the encapsulation of olive leaf extract by the spray-drying process did not lead to the inactivation of polyphenolic. Zhen- et al (2007) report that chitosan nanoparticle- could improve the enzyme activity by 13.17% than that of the free neutral lipase. Deladino et al. (2008) studies showed that beads without chitosan released around 50% of the polyphenol content and beads with chitosan released around 35% at 3.5 h, which is significantly lower than the released amount

3.4. Release studies

Although the pH of the skin is 5.5, the pH of cosmetic formulations can range between 5.5 and 7 (Meyer Rosen, 2005). The release studies of phenol were performed in two buffers with different pH values (pH 5.0 and 6.0). In both cases, 90% of polyphenols was delivered from phenol 3 h,while phenol-CNP slowed down the release, being at this same time 56% in pH 5.0 and 47% in pH 6.0. The higher release in pH 5.0 was due to the solubility of chitosan hydrochloride at this pH. Deladino et al. [12](prepared chitosan–alginate beads for the encapsulation of yerba mate. Their studies showed that beads without chitosan released around 50%of the polyphenol content and beads with chitosan released around 35% at 3.5 h, which is significantly lower than the released amount in the present study.

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