

TANNIN CONTENT OF ARECA NUT AFTER NANOCHITOSAN COATING ON DRYING METHODS

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ABSTRACT

Objective: This research aimed to investigate the effect of nanochitosan (NC) coating in different drying methods on the tannin content of areca nut.

Methods: Areca nut was obtained from the seed of Duncan's multiple range test (DMRT). Chitosan was isolated from shrimp shell waste through deproteination, demineralization, depigmentation, and deacetylation. NC was made using the ionic gelation method. The treatment used three levels of concentrations; K0 (uncoated-control), K1 (NC 1.5 g/l), and K2 (NC 3 g/l), and it employed two drying methods, namely, P1 (sun drying) and P2 (oven drying). Tannin content measured by spectrophotometry method. The data were analyzed by analysis of variance univariate two factors and followed by Duncan's Multiple Range Test (DMRT).

Results: The result showed that the NC-coated areca nut has a higher tannin content compared with the uncoated areca nut. The level of NC concentration had a significantly different effect ($p 0.02 < 0.05$), whereas the drying method gave no significant effect ($p 0.81 > 0.05$) and there was no interaction between NC concentration and drying method ($p 0.89 > 0.05$). The tannin content in areca nut in K0 (control) treatment was 17.44%, K1 (NC 1.5 g/l) was 18.11%, and K2 (NC 3 g/l) was 18.83%.

Conclusion: Application of NC as a coating on areca nut can prevent the loss of tannin throughout the drying process. Dried areca nut with K2 treatment (NC 3 g/l) has the highest tannin content as it reaches 18.83%.

Keywords: Tannin, Areca nut, Nanochitosan, Coating.

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INTRODUCTION

Areca nut, also known as betel nut, is a seed of *Areca catechu* L. which is widely used in various fields including for medicinal purposes because of its characteristic: Antiparasitic effects, antioxidant effects, antibacterial and antifungal effects, anti-inflammatory and analgesic effects, regulating effects on blood glucose and lipids, and anti-allergic effects [1]. This plant is commonly found in Pacific Island and South Asia [2]. In Indonesia, dried areca nut becomes one of the valuable export commodities, whose quality is determined by water content and chemical compound. However, the quality of areca nut has begun to decline, especially in chemical compounds due to improper postharvest handling, so a right method for handling postharvest of areca nut is needed to prevent quality degradation.

Tannin, especially condensed tannin, has been identified as the characteristic constituent in areca nut [1]. Tannins are secondary metabolites produced by higher plants [3] and classified as a large polyphenol group that can be found in plants [4]. Furthermore, they have several physiological, biochemical, and pharmacological activities, such as antimicrobial, antioxidant, antiviral, antitumor, enzyme inhibition, chemical defense, and radical scavenging [5].

Drying is an essential process in post-harvest to preserve and prevent the degradation quality of plant materials [6] and it has a crucial effect on the tannin content [7]. During the drying process, the declining quality of plant substances due to physical damage, microorganism contamination, and transformation in chemical components becomes a serious issue due to its big impacts on the selling price.

Recently, there are many alternative methods used in the post-harvest process including coating. The coating is an effective method to extend shelf life [8] and maintain the chemical compounds during post-harvest. This coating uses edible materials or in other words, the materials can be

consumed by humans. The most commonly used edible coating material is chitosan which can be extracted from crustacean shells such as shrimp, crabs, and other marine crustaceans [9]. The previous studies have shown the effect of using chitosan as a coating base. Azimzadeh and Jahadi [10] reported that the use of chitosan as an edible coating can extend shelf life and improve the stability of dried cashews and indicates antifungal activity in oranges [11]. Varasteh *et al.* [12] also reported that chitosan coating can delay the decrease of anthocyanin content in pomegranate.

To improve the effectiveness of coating materials, they were modified into nanosize [13]. The modification of chitosan to nanochitosan (NC) as the base coating material can improve the ability of chitosan, both as absorbent, antifungal, and antibacterial and its role as a carrier of essential compounds [14]. This study aims to determine the effect of NC coating in different drying methods on the tannin content of areca nut.

METHODS

Plant material

Areca catechu L. var. *Betara* was obtained from Betara Subdistrict, Tanjung Jabung Barat District, Jambi Province, Indonesia. Ten fruits of *Areca catechu* L. var. *Betara* were prepared for each treatment and repetition. They have been sorted based on the similar size and color. Then, the areca fruits were divided into two parts and the seeds (nuts) were pulled out from the rind.

Chitosan isolation

Chitosan was isolated from shrimp shell waste through four steps [15]: Deproteinization, demineralization, depigmentation, and deacetylation.

Deproteinization

Shrimp shell powder was mixed with 4% NaOH solution at a ratio of 1:10 (g/ml). The mixture was heated at 70°C for 2 h while stirring, then

it was filtered and rinsed with aquadest until neutral. The solid formed was cooled and dried at 80°C in the oven for ±6 h.

Demineralization

The result of deproteinization was mixed with 1.5 M HCl solution at a ratio of 1:15 (g/ml). The mixture was stirred for 1 h without heating then filtered and rinsed with aquadest until neutral. The solid formed was dried in the oven at 70°C for ±6 h.

Depigmentation

The result of demineralization was mixed with 4% NaOCl solution at a ratio of 1:10 (g/ml). The mixture was stirred for 1 h without heating, then it was filtered and rinsed with aquadest until getting a neutral pH. The solid formed was dried at 70°C for ±6 h. The result of depigmentation was called chitin.

Deacetylation

Chitin powder was mixed with 50% NaOH solution at a ratio of 1:10 (g/ml). The mixture was heated at 90°C for 2 h then filtered and rinsed with aquadest. The solid formed was dried at 80°C for ±6 h. The result of deacetylation was called chitosan.

NC formation

NC was prepared using an ionic gelation method based on electrostatic interactions between the amine group of chitosan and polyanion triphosphate (TPP) [16] with two different levels of chitosan concentration; K1 (1.5 g/l) and K2 (3 g/l).

Chitosan 1.5 g and 3 g were, respectively, dissolved in 100 ml of 1% acetic acid. An 800 ml of aquadest was added and homogenized for 2 h with a magnetic stirrer 3700 rpm. A 20 µl emulsifier (Tween 80 0.1%) was sprayed on the solution and homogenized for 30 min, then 200 ml TPP 0.1% was added dropwise into the chitosan solution to form a nanoparticle suspension. The stirring was continued for 1 h so that the cross-linking process occurred perfectly and the resulting particles remain stable.

The particle size of the NC solution was then measured using particle size analyzer (PSA).

Application of NC

The areca nut for each treatment and replication was placed on a tray and sprayed with 1.5 g/l (K1) and 3 g/l (K2) of NC coating solution. The control treatment (K0) was not given with NC coating solution.

Drying treatment

The areca nut that has been coated with NC and the control treatment was dried using two methods; P1 (sun drying for 6 h/day for 4 days) and P2 (oven drying at 60°C for 46 h).

Tannin analysis

The tannin content was analyzed after the drying with the ultraviolet-visible spectrophotometric using the Folin-Ciocalteu reagent and pyrogallol standard curves.

Statistical analysis

The data were analyzed with the SPSS software using analysis of variance (ANOVA) univariate two factors and followed by DMRT (Duncan's multiple range test (DMRT)).

RESULTS

NC particle size

The results of measurements using PSA are presented in Table 1.

Tannin content

The results of tannin content measurements using spectrophotometric are shown in Table 2.

ANOVA analysis results on 95% confidence level of tannin content in areca nut after drying showed that the concentration of NC had a significantly different effect (p 0.02 < 0.05), whereas for the drying method gave no significant effect (p 0.81 > 0.05) and there was no interaction between NC concentration and the drying method (p 0.89 > 0.05). Followed by the DMRT test result, it has a significant effect on each treatment K0, K1, and K2.

DISCUSSION

NC particle size

Based on the result of the particle size in Table 1, it can be seen that the NC coating solution made of the ionic gelation method has a particle size of 2 nm and its polydispersity index (PI) was 0.371 meaning that the NC particle size is homogeneously dispersed (monodispersed) and no aggregation occurs. According to Bera [17], the PI is used to describe the uniformity of particle size distribution. This index has a range of values between 0.0 (0.0–0.5: monodispersed) and 1.0 (0.6–1.0: polydispersed) [18].

According to D value, it is known that 10% of the sample size is below 1.6 nm, 50% of the sample size is below 1.8 nm, and 90% of the sample size is below 2.4 nm. D value is used to indicate the distribution of particle size based on its cumulative percentage (0–100%) [19].

Tannin content

Bedi and Scully [20] stated that the tannin content of the areca nut is 11–26%. In this research, fresh areca nut was analyzed and it contained 20.73% tannins and reduced after the drying treatment. Based on Table 2, it can be seen that the level of concentration has a significant effect on the tannin content in areca nut than the dried areca nut without coating. However, it has no significant difference in the used drying methods and no interaction between NC concentration and the drying methods.

The drying methods play a crucial role in tannin content [7]. Betoret *et al.* [21] mentioned that the drying process involves structural and biochemical changes, which can affect the functionality of bioactive compounds. Tannin is one of the bioactive compounds and the large polyphenolic group in plants.

Dried areca nut with NC coating which dried by sunlight or oven has a higher tannin content than the dried areca nut without coating. It is probably due to the oxidation of tannins and their exposure to oxygen [22]. Some physiological transformations have been observed in pre-harvest and post-harvest chitosan coating applications, including the change in total phenolic content and enzyme activities such as superoxide dismutase, polyphenol oxidase (PPO), and peroxidase (POD) [23].

Enzymatic oxidation of tannin can be attributed to the browning damage [24,25] that can decrease the quality of areca nut involving the loss of tannin. The browning process is mainly due to the activities of PPO and POD [26]. Pen and Jiang [27] reported that using chitosan as a coating can improve the quality and extend the shelf life of fresh-cut Chinese water chestnut by reducing respiration rate and inhibiting activities of PPO and POD. Ghasemnezhad *et al.* [28] also reported that the chitosan coating can decrease the activity of PPO by suppressed monophenolase and diphenolase actions.

Areca nut with the highest concentration of NC 3 g/l (K2) has the larger tannin content 18.83% than K1 (1.5 g/l) 18.11%. The use of

Table 1: Nanochitosan characterization

Sample	Distribution result	D (10%)	D (50%)	D (90%)	Polydispersity index
Nanochitosan coating solution	2 nm	1.6 nm	1.8 nm	2.4 nm	0.371

Table 2: Statistical result of tannin content of dried areca nut

Level of concentration	Tannin content (%)
Fresh areca nut	20.73
*K0 (control)	17.44 ^a
*K1 (NC 1.5 g/l)	18.11 ^b
*K2 (NC 3 g/l)	18.83 ^c

*Different letters (a,b,c) indicate statistically significant differences (p 0.02 < 0.05); NC: Nanochitosan

nanoparticle materials can induce high reactivity, effective catalyst in plant metabolism, improve penetration into the cell, and increase plant activity [29]. NC application as a coating has a selective permeability to gasses (CO₂ and O₂) [30], it can reduce the oxidation of phenolic compound including tannin. Decreases in polyphenol oxidation after chitosan application have been reported in *Luffa cylindrica* (sponge gourd) [31] and *Fragaria ananassa* (strawberry) [32] but it has never been reported on areca nut because the use of chitosan as a coating on areca nut has never been done before. This study revealed a decrease in tannin degradation on areca nut after applying nanochitosan coating.

According to Wang and Gao [33], chitosan-coated fruit revealed a lower rate of decrease in total phenolic compound compared with non-coated fruit probably due to lower permeability of oxygen and lower activity of enzymes. Moreover, the use of chitosan or NC as the coating material can extend shelf life, delayed the decline in sensory quality by reducing the respiration rate, and showed high antimicrobial activities [34].

CONCLUSION

Application of NC as a coating on areca nut can prevent the loss of tannin throughout the drying process. Dried areca nut with K2 treatment (NC concentration 0.3 g/l) has the highest tannin content of 18.83%.

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AUTHORS' CONTRIBUTIONS

Fevi Mawadhah Putri designed and carried out the experiment, wrote the manuscript under supervised by Dr. Erma Prihastanti, M.Si and Dr. Endah Dwi Hastuti, M.Si. All authors discussed the results and contributed to the final manuscript.

CONFLICTS OF INTEREST

There are no conflicts of interest in this research.

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