

## The Radical Scavenging Activity and Thermal Stability of Cinnamon Extract-Loaded Nanoparticles

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## Abstract

The cinnamon extract-loaded nanoparticles with high phenolic content and antioxidant activity were successfully developed in a previous study. This study aimed to investigate the radical scavenging activity in 2.2-diphenyl-1-picrylhydrazyl system and stability of the nanoparticles under heat treatment. This study is important for directing the application of the nanoparticles in foods in the future. The thermal stability test was conducted using two different methods, which were the combination of relatively lower temperature (20-100°C) with a long time treatment (up to 120 hours) and the combination of relatively higher temperature (110-150°C) with a short time treatment (equal to or less than 2 hours). The results show that the cinnamon extract-loaded nanoparticles exhibited a radical scavenging activity of the nanoparticles. The thermal treatment caused a significant degradation on the phenolic content and antioxidant activity of the nanoparticles. The energy activation (Ea) of the phenolic content and antioxidant activity was found at 35.17 kJ mol<sup>-1</sup> and 27.91 kJ mol<sup>-1</sup>, respectively. This study suggests that the cinnamon extract-loaded nanoparticles might be preferably incorporated into foods minimally involving heat exposure during their manufacture.

Keywords: antioxidant; cinnamon; nanoparticle; phenols; thermal stability

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## **INTRODUCTION**

Sustainable agriculture is gaining high attention nowadays to face the challenges of global food demand and encounter civilization's nutritional needs. Every part of food system, including farmers, food industries, distributors, retailers, consumers and waste managers can play a significant role in developing a sustainable agricultural system. To ensure sustainable agriculture, several factors should be taken into account, namely environmental conservation, agricultural production, farm profit and community well-being. As agreed by many

scholars, sustainable agriculture system is highly important to achieve food security of a nation, not only for today but also for the future (Flora, 2018; Pandey, 2018; Scott et al., 2018). According to Mc Carthy et al. (2018), food security can be defined as the condition that "all people at all times have access to sufficient, safe, nutritious food to maintain a healthy and active life". The understanding of the statement of "nutritious food to maintain a healthy and active life" nowadays continuously evolves, and thus scientists develop foods with a specific function for health (also known as functional food) (Muhammad and

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Dewettinck, 2017). Functional food can be created by adding some functional ingredients, such as herb and spice extracts, in food, in either free or encapsulated form (McClements et al., 2018). Extract of cinnamon (*Cinnamomum sp*) containing a high level of phenols is widely recognized to have beneficial health effects such as anti-cancer, anti-tumor, antioxidant activity, as well as prevention of inflammation-mediated neurodegenerative diseases, inhibiting the angiogenesis involved in cancer and decrease cholesterol levels (Ribeiro-Santos et al., 2017).

In recent years, encapsulation in nano-scale has been widely developed by scientists. In the food industry, nano-sized materials are typically designed for the delivery of active ingredients in food (Katouzian and Jafari, 2016; Sodano et al., 2016). Nanoparticulate delivery systems, e.g. micelles, liposomes, nanoemulsions and biopolymeric nanoparticles, are some examples of applications of nanoscience and nanotechnology in the food industry (Chau et al., 2007). A nanoparticle is a term used for both nanocapsules nanospheres (Konan et al., 2002). and Nanocapsules are vesicular systems, in which the bioactive compound is entrapped in a unique polymer membrane or wall material, while nanospheres are homogeneous dispersions of bioactive compounds in a matrix system (Ezhilarasi et al., 2013). Both nanocapsule and nanosphere are included as composite nanoparticles, which can be defined as particles formed from the active component and one or more excipients (Jove and McClements, 2013). Nanoencapsulation of bioactive compounds and the incorporation of the nanomaterial in a food matrix have gained considerable importance since those factors may have substantial impacts on the improvement of bioavailability, biological activity and stability, release control and mask of undesirable odors and flavours of bioactive compounds (Handford et al., 2014; Joye et al., 2014; Esfanjani and Jafari, 2016).

In our previous study, cinnamon extractloaded nanoparticles made of shellac-xanthan complexes have been successfully fabricated using anti-solvent precipitation method (Muhammad et al., 2020). In that study, we found that the nanoparticles had a spheroidal morphology with a particle size of about 150 nm. The cinnamon nanoparticles had a high level of polyphenols and exhibited a high antioxidant activity. Shellac-xanthan gum complexes showed their potentials as a carrier of the cinnamon extract as polyphenols can be released at the targeted site in the digestion system (i.e. intestine) triggered by alkaline pH. The nanoparticles have even been applied in chocolates and cocoa drinks aiming at improving their phenolic content and antioxidant activity (Muhammad et al., 2018; Muhammad et al., 2019). Moreover, a potential synergistic effect between cinnamon and cocoa was found (Muhammad et al., 2017). The improvement of phenolic content and antioxidant activity of foods can be achieved by adding the cinnamon extractloaded nanoparticles into food formula and this is important since it offers an opportunity to create a healthier food product.

Nevertheless, the application of the cinnamon extract-loaded nanoparticles in various food needs some considerations. In fact, most of food products are produced by involving heat treatment. Heat treatment might significantly decrease the phenolic content and antioxidant activity of the nanoparticles as many literature have discussed the sensitivity of phenolic and antioxidant compounds to thermal process. An antioxidant can be defined as a molecule or species that slows down or prevents the oxidation of another molecule. There are two main modes of action of antioxidant, namely Hydrogen-Atom Transfer (HAT) and Single Electron Transfer (SET). In the HAT mechanism, an antioxidant component (abbreviated as an aromatic component [Ar] and a hydroxy component [OH]) donates an H-atom to an unstable free radical, and in this process produces a more stable free-radical species. In the SET, an antioxidant component transfers one electron to reduce any target compound, including metals, carbonyls and radicals (Craft et al., 2012). According to Ross et al. (2011), phenols are sensitive to non-enzymatic condensation and polymerization at elevated temperature, resulting in decreased content. As the antioxidant activity of a material highly correlates with its phenolic content, a degradation of phenols can result in a significant decrease in antioxidant activity. Those facts suggest the importance to understand the stability of the cinnamon-loaded nanoparticles upon heat treatment.

This study, therefore, aimed to investigate the stability of the cinnamon-loaded nanoparticles under heat treatment. As the cinnamon extractloaded nanoparticles is potential source of antioxidant activity, a study on its potency as a radical scavenging activity was also included.

#### MATERIALS AND METHOD

# Preparation of cinnamon-loaded shellac nanoparticles

Cinnamon-loaded shellac nanoparticles were prepared by anti-solvent precipitation method according to our previous study (Muhammad et al., 2020). Cinnamon bark (*Cinnamomum burmannii* Blume) was ground into powder and then subjected to extraction in ethanol with a ratio of 1:10 for 48 hours in ambient temperature ( $\pm 20^{\circ}$ C). After the cinnamon powder residue was separated using a vacuum filter, the cinnamon extract was then mixed with 2% w/w ethanolic solution of shellac powder (SSB 55 Astra FP, SSB Stroever GmbH & Co. KG (Bremen, Germany) in various proportions (0% to 50% (w/w)). The mixture was slowly injected to xanthan gum solution (0.3% w/w) at a ratio of 1:3 (w/w) using a syringe. Afterward, the homogenous mixture was subjected to rotary evaporation to remove ethanol and to form colloidal nanoparticles. The schematic diagram of the preparation of cinnamon-loaded nanoparticles by anti-solvent precipitation method is presented in Figure 1.



Figure 1. Preparation of cinnamon-loaded nanoparticles by anti-solvent precipitation method

## DPPH (2,2-*diphenyl*-1-*picrylhydrazyl*) radical scavenging activity analysis

The protocol proposed by Muhammad et al. (2017) was used for the DPPH assay. Briefly, 100  $\mu$ l of colloidal nanoparticles were added to 4 ml of 0.01 mM DPPH in methanol, and then maintained in dark condition for 30 min.

Subsequently, the absorbance was measured at 517 nm using a UV-visible spectrophotometer. The DPPH radical scavenging activity of the nanoparticles was measured at 100  $\mu$ g ml<sup>-1</sup> and 200  $\mu$ g ml<sup>-1</sup>. BHA (butylated hydroxyl anisole) and epicatechin were used as references. The DPPH radical inhibition was calculated using Eq. 1.

#### Thermal stability test

Prior to the test, the colloidal cinnamon extract-loaded nanoparticles were lyophilized to obtain dry powder of cinnamon extract-loaded nanoparticles. Thermal stability tests towards phenolic content and antioxidant activity of cinnamon-loaded nanoparticles were performed using two different protocols. The first test was adopted from the method of Zheng et al. (2011) with a slight modification. The samples were placed in a glass tube and then maintained in the dark for 120 hours at different temperature (20, 40, 60, 80 and 100°C). The effect of temperature on the stability of the cinnamon polyphenols and the antioxidant activity was assessed. The total phenolic content was analyzed using the Folin-Ciocalteu method while the phosphomolybdenum method was used to determine the antioxidant activity, following the protocol of Muhammad et al. (2020). The polyphenol and antioxidant activity retention values were calculated using Eq. 2.

Retention = 
$$\frac{\text{the content of cinnamon polyphenol or antioxidant retained}}{\text{the initial content of cinnamon polyphenol or antioxidant}} \times 100\%$$
 ......(Eq. 2)

The second method was conducted to examine the degradation kinetics of the cinnamon polyphenol and antioxidant activity following the protocol of Oancea et al. (2017). The samples were placed at distinct temperatures in the range of 110°C to 150°C for 2 hours. The samples were analyzed every 30 min, and finally, the degradation kinetics were calculated using the Arrhenius equation (Eq. 3):

$$k = Ae^{\frac{-Ea}{RT}} \dots (Eq. 3)$$

where k is the reaction constant (min-1), A is the Arrhenius' constant (min-1), Ea is the activation energy (J mol<sup>-1</sup>), R is the universal gas constant (8.3144 J mol<sup>-1</sup> K<sup>-1</sup>) and T is the absolute temperature (K).

#### **Statistical analysis**

Analysis of variance (one-way ANOVA) was performed using SPSS Statistics 22 to find the significant differences (p<0.05) among the length of the thermal treatments. DMRT (Duncan's Multiple Range Test) was used as a post-hoc test when significant differences were found.

#### **RESULTS AND DISCUSSION**

#### **Radical scavenging activity**

The radical scavenging activities of cinnamonloaded shellac nanoparticles were tested using DPPH radical system. This typical assay is based on the reduction of the radical by hydrogen atom transfer from H<sup>-</sup> donors. DPPH method for determining radical scavenging activity is considered simple and highly sensitive as well as give reproducible results (Hidalgo et al., 2010). Figure 2 shows that without cinnamon extract loading, the nanoparticles did not exhibit DPPH radical scavenging activity, strongly indicating that cinnamon extract played a significant role in the radical scavenging activity of the nanoparticles.



Figure 2. DPPH radical scavenging activity of nanoparticles loaded with various levels of cinnamon

Note: Mean values with a different uppercase letter indicate a significant difference (p<0.05) at the concentration of 100 µg ml<sup>-1</sup> and 200 µg ml<sup>-1</sup>. Mean values with a different lowercase letter indicate a significant difference (p<0.05) at the different cinnamon loading at similar concentration

The radical scavenging activity of the nanoparticles was highly correlated with the cinnamon loading agreeing our previous findings (Muhammad et al., 2020). As shown, the higher level of cinnamon loading led to a higher DPPH radical scavenging activity. Our previous study has shown that the antioxidant activity of cinnamon extracts is highly correlated with their phenolic content (Muhammad et al., 2017). Gallic acid, p-hydroxybenzoic acid, p-hydroxybenzal dehyde, protocatechuic acid, salicylic acid, syringic acid, vanillic acid, vanillin, caffeic acid, quercetin, tannic acid, chlorogenic acid, ferulic acid, p-coumaric acid, cinnamic acid, sinapic acid and eugenol have been found as phenolic constituents in cinnamon (Muhammad and Dewettinck, 2018). Interestingly, the antioxidant activities of the nanoparticles loaded with 50% of cinnamon were comparable with BHA when tested at a concentration of 100 µg ml<sup>-1</sup> and 200 µg ml<sup>-1</sup>. This indicates that the nanoparticles have a great potency as natural antioxidants, replacing synthetic antioxidant agents. However, as many studies have presented the instability of phenols against thermal treatment, a study focusing on the

impact of heat treatment on the phenolic content of the nanoparticles is required.

#### Thermal stability test

The effect of temperature on the stability of the phenolic content and antioxidant activity is presented in Figure 3. In general, the stability of the polyphenols and the antioxidant activity of the cinnamon nanoparticles at a lower temperature were better than at higher temperatures. The polyphenol retention of the nanoparticles during the heat treatment at 60°C and 80°C for 120 hours were about 90% and 70%, respectively. This manifestation was also well-demonstrated in the study of Zheng et al. (2011), who worked with bayberry polyphenol encapsulated in ethylcellulose matrices. They found that after heat treatments at 50°C and 90°C for 60 hours were given, the polyphenol retentions were about 85% and 60%. Exposure to elevated temperatures led a reduction in polyphenols and other to antioxidant compounds due to their sensitivity to heat. Theoretically, at high temperature, nonenzymatic condensation and polymerization of phenolic compounds occur, resulting in a decreased phenolic content (Ross et al., 2011).



Figure 3. Retention of polyphenol (A) and antioxidant activity (B) of the of shellac-xanthan gum colloidal nanoparticles containing cinnamon oleoresin in different temperature

This experiment used five different temperatures and five incubation times (20°C to 100°C; 0 to 120 hour(s)) to provide sufficient data for further analysis examining the correlation coefficient. This is important to provide an evidence of the correlation between phenolic content and antioxidant activity. It was found that the correlation coefficient between the retention of polyphenols and that of antioxidant activity was found at 0.770 and significant at 0.01. It means that the degradation of antioxidant activity of the nanoparticles is highly associated with the decrease of phenolic content during heat treatment. Yet, it is noteworthy in Figure 3 that at the temperature of  $60^{\circ}$ C, about 90% of polyphenols were retained, but at the same time, the antioxidant activity decreased by about 25%. This might be because some phenols underwent a transition to their epimers, resulting in a different level of antioxidant activity than expected.

To confirm the thermal stability of the nanoparticles in terms of phenolic content and

antioxidant activity, a test at higher temperatures (110-150°C) and shorter period (0-120 min) was performed. Expectedly, a substantial degradation of phenolic content and antioxidant activity was observed as a function of temperature and time (Table 1). For instance, after a heating process for 120 min at 110°C, the total phenolic content decreased from 164.7 mg EE g<sup>-1</sup> to 131.4 mg EE

 $g^{-1}$  dry weight, while the antioxidant activity decreased from 128.8 mg TAE  $g^{-1}$  to 100.5 mg TAE  $g^{-1}$  dry weight. At a higher temperature (i.e. 150°C for 10 min), the total phenolic content and the antioxidant activity of the nanoparticles dropped from 164.7 mg EE  $g^{-1}$  to 86.7 mg EE  $g^{-1}$  dry weight and from 128.8 mg TAE  $g^{-1}$  to 75 mg TAE  $g^{-1}$  dry weight, respectively.

Temperature	Heating time	Total phenolic content	Antioxidant activity
(°C)	(min)	(mg EE g <sup>-1</sup> dry weight)	(mg TAE g <sup>-1</sup> dry weight)
110	0	$164.7\pm0.1^{a}$	$128.8\pm0.4^{\rm a}$
	30	$143.9\pm1.3^{\rm b}$	$113.4\pm5.1^{b}$
	60	$144.1 \pm 1.0^{\mathrm{b}}$	$107.2\pm0.1^{\mathrm{bc}}$
	90	$143.9\pm1.1^{\rm b}$	$109.9\pm3.0^{\rm b}$
	120	$131.4\pm2.0^{cd}$	$100.5\pm5.3^{cd}$
120	0	$164.7\pm0.1^{\mathrm{a}}$	$128.8\pm0.4^{\rm a}$
	30	$144.2\pm4.7^{\rm b}$	$111.0 \pm 6.3^{b}$
	60	$127.1 \pm 1.4^{d}$	$95.8\pm0.1^{de}$
	90	$129.6\pm0.9^{\rm d}$	$92.8\pm0.3^{\rm e}$
	120	$124.8\pm4.1^{\text{de}}$	$90.4\pm0.4^{efg}$
130	0	$164.7\pm0.1^{a}$	$128.8\pm0.4^{\rm a}$
	30	$136.8\pm5.4^{\rm c}$	$106.0\pm0.1^{\mathrm{bc}}$
	60	$128.3\pm0.1^{\text{d}}$	$93.1 \pm 0.6^{\text{e}}$
	90	$117.0\pm1.9^{\rm fg}$	$92.3\pm0.7^{ef}$
	120	$120.7\pm0.1^{\text{ef}}$	$88.9\pm0.6^{efgh}$
140	0	$164.7\pm0.1^{a}$	$128.8\pm0.4^{\rm a}$
	30	$131.5\pm1.2^{\mathrm{cd}}$	$96.4 \pm 1.1^{de}$
	60	$118.5\pm0.6^{\rm ef}$	$84.9 \pm 1.0^{fgh}$
	90	$117.8\pm0.6^{\rm fg}$	$82.6\pm0.7^{\rm hi}$
	120	$119.9\pm0.5^{\rm ef}$	$83.7\pm0.3^{gh}$
150	0	$164.7\pm0.1^{\mathrm{a}}$	$128.8\pm0.4^{\rm a}$
	30	$128.7\pm0.2^{\rm d}$	$84.1\pm2.3^{\mathrm{gh}}$
	60	$111.7\pm0.9^{\text{gh}}$	$75.8\pm0.2^{\rm ij}$
	90	$107.9\pm0.3^{\rm h}$	$71.7\pm0.2^{\mathrm{j}}$
	120	$86.7\pm0.1^{\rm i}$	$73.0\pm0.4^{j}$

Table 1. Degradation of phenolic content and antioxidant activity of cinnamon loaded nanoparticles

Note: Mean values with the same letter do not differ significantly (p<0.05) in the same column

In addition to understand the degradation of phenolic content and antioxidant activity during the thermal treatment, the data shown in Table 1 were useful to calculate the degradation kinetics of the polyphenol and antioxidant activity. Kinetics study of thermal degradation of phenolic content is pivotal to give a better insight on the degradation process in detail, which could help in circumventing the undesired thermal degradation of phenols and antioxidant activity. In this study, the degradation kinetics were modelled using first-order kinetics following the study of Oancea et al. (2017). The kinetic parameters are useful to designate the changes in the quality during thermal processing and to predict the thermal stability of nanoparticles. The estimated kinetic parameters describing the heat-induced changes in phenolic content and antioxidant activity are presented in Table 1. It is shown that the rate constants of the loss of phenolic compounds and antioxidant activity increased with temperature. The corresponding activation energies were 35 kJ mol<sup>-1</sup> and 28 kJ mol<sup>-1</sup> for phenolic content and antioxidant activity, respectively (Table 2). The Arrhenius plot for the temperature dependence of the rate constant *k* for polyphenols content and antioxidant activity of the cinnamon-loaded nanoparticles is given in Figure 4. The results obtained in this study were comparable with the findings previously reported by different research groups. Zorić et al. (2014) reported that activation energies for cyanidin-3-glucosylrutinoside and neochlorogenic acid of freeze-dried sour cherry Marasca paste during thermal treatment using the heating temperature of 80-120°C and the processing time of 5-50 min were 42 kJ mol<sup>-1</sup> and 27 kJ mol<sup>-1</sup>, respectively. In a study by Karaaslan et al. (2014), the activation energy of total phenolic content degradation during a thermal process in the range of 55-75°C was reported at 21.9-36.2 kJ mol<sup>-1</sup>, depending on the type of pre-treatment of pomegranate arils before the thermal process.

 

 Table 2. Estimated kinetic parameters of polyphenols content and antioxidant activity of shellacxanthan gum colloidal nanoparticles containing cinnamon

Parameter	Temperature (°C)	Rate constant $k . 10^{-3}$ (min <sup>-1</sup> )	Ea (kJ mol <sup>-1</sup> )
Phenolic content	110	1.88	35.17
	120	2.31	
	130	2.59	
	140	2.65	
	150	5.35	
Antioxidant activity	110	2.07	27.91
	120	2.95	
	130	3.09	
	140	3.59	
	150	4.73	



Figure 4. Arrhenius plot for the temperature dependence of the rate constant k for polyphenols content and antioxidant activity of engineered cinnamon nanoparticles

From the thermal stability test and the kinetics study, it is noticeable that a high temperature results in the loss of the cinnamon polyphenol content and antioxidant activity of the nanoparticles. It can be understood as it was found in our previous study that the cinnamon-loaded nanoparticles have a low encapsulation efficiency (Muhammad et al., 2020). Polyphenols surrounding on the surface of the nanoparticles significantly degraded upon heat treatment as the phenols were not covered by the wall material. Despite of the low encapsulation efficiency, heat treatment typically induces bioactive compound degradation, even if it has been fully encapsulated. For instance, it has been reported that the activation energy of encapsulated lutein was also quite low at the level of 44.81 kJ mol<sup>-1</sup>, respectively when it was observed in the range of 70°C to 90°C (da Silva et al., 2017). Aside from the significant impact on the degradation of phenolic content and antioxidant activity, a heat treatment may also change the structure of the

nano-/microcapsule, leading to the loss of its functionality. As shown by Doost et al. (2018), a heat exposure led to a significant increase in the particle size of colloidal nanoparticles loaded with quercetin. These results suggest that the cinnamon extract-loaded nanoparticles might be preferably incorporated into foods, which are manufactured with minimal involvement of a heat treatment.

### CONCLUSIONS

To conclude, the level of antioxidant activity of nanoparticles depends on the percentage of cinnamon extract loaded in the nanoparticles. A significant decrease in the phenolic content and antioxidant activity of the nanoparticles happened upon thermal treatment, suggesting that the cinnamon extract-loaded nanoparticles might be preferably added into foods with minimal heat exposure during their manufacture. A further study focusing on the application of the cinnamon extract-loaded in various food products aiming at improving their phenolic content and antioxidant activity might be an interesting topic for the future research, following the nowadays global food demand on healthy foods.

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