Stability and functionality of xanthan gum–shellac nanoparticles for the encapsulation of cinnamon bark extract

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ARTICLE INFO

ABSTRACT

The aim of this study was to prepare stable shellac nanoparticles containing a cinnamon bark extract using xanthan gum by anti-solvent precipitation. The nanoparticles were characterized in terms of their gastric pH stability, surface charge, particle size and morphology. The effect of the cinnamon extract loading on the properties of the nanoparticles, including the encapsulation efficiency and antioxidant properties, were also investigated. Ultimately, the release behaviour and the thermal stability of the nanoparticles were established. The results showed that xanthan gum can stabilise shellac nanoparticles at gastric pH by electro-steric stabilisation. The morphological analysis of the nanoparticles by Cryo-SEM showed that spherical particles with a smooth outer surface were formed. A decrease in encapsulation efficiency was observed when a higher level of cinnamon extract loading was used. The bioparticles fortified with cinnamon extract exhibited antioxidant activity and ferric-reducing antioxidant power at the level of 185 mg tannic acid equivalent and of 127 mM ascorbic acid equivalent per gram dry weight of nanoparticles, respectively. From the release study, it was shown that more than 90% of cinnamon polyphenol was released at the intestinal pH. Nanoencapsulation effectively improved the thermal stability of the polyphenol-rich cinnamon extract. The polyphenol retention after heat treatment (90 °C, 20 min) was still higher than 90%. This study presents the formulation of cinnamon extract containing nanoparticles, which may be applicable in the food industry as a prospective antioxidant agent.

1. Introduction

Bioactive phytochemicals have gained considerable importance in the food and pharmaceutical industries following many studies about their potential health benefits (Assadpour & Jafari, 2018). Cinnamon (*Cinnamomum Sp.*) contains various phytochemicals such as cinnamaldehyde and polyphenols. These bioactive compounds have been widely acknowledged to have anti-tumor, anti-inflammatory, anti-diabetic, anti-hyperglycemic and antioxidant properties (Muhammad & Dewettinck, 2017; Muhammad, Praseptiangga, Van de Walle, & Dewettinck, 2017; Ribeiro-Santos et al., 2017; Sedaghat Doost, Dewettinck, Devlieghere, & Van der Meeren, 2018a). Due to its bioactive potential, cinnamon has been used to improve the total phenolic content and antioxidant activity of foods such as coffee, chocolate and yoghurt (Durak, Gawlik-Dziki, & Pecio, 2014; Ilmi, Praseptiangga, & Muhammad, 2017; Muhammad & Dewettinck, 2017; Muhammad, Saputro, Rottiers, Van de Walle, & Dewettinck, 2018; Muhammad et al., 2019; Shori & Baba, 2011).

However, polyphenol-rich cinnamon extract is poorly soluble in water, and consequently, it has low oral bioavailability (Helal, Tagliazucchi, Verzelloni, & Conte, 2014). The preparation of nanoparticles that have a small and uniform particle size distribution has been acknowledged as an effective way of improving the bioavailability and biological activity as well as to control the release of bioactive compounds (Esfahani, Jafari, Jafarpour, & Dehnad, 2019; Ghasemi et al., 2017, 2018; Joye, Davidov-Pardo, & McClements, 2014; Katouzian & Jafari, 2016). Nanoparticle fabrication may also be useful in preventing the degradation of the phenolic content and bioactivity of

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polyphenol-rich cinnamon extract due to light, heat, and oxygen (Joye & McClements, 2014; Teixeira, Ozdemir, Hill, & Gomes, 2013). As far as is known, studies into the synthesis and stability of nanocapsules containing polyphenol-rich cinnamon extract are still scarce up till now.

Among the existing methods, anti-solvent precipitation is considered as an efficient technique for synthesizing nanocapsules due to its low cost and easy operation (Esfanjani & Jafari, 2016; Sedaghat Doost et al., 2019a). In this method, a solution of a hydrophobic compound dissolved in an organic solvent is added to an anti-solvent (aqueous phase). The nanoparticles are formed by three main steps: (a) generation of supersaturation; (b) nucleation; and (c) growth of nuclei (Joye & McClements, 2013; Sedaghat Doost, Muhammad, Stevens, Dewettinck, & Van der Meeren, 2018b).

In the last several years, special emphasis has been given to the functionality of shellac as a base material for nanoparticles. Shellac is a hard, tough and amorphous resin consisting of polyhydroxy polycarboxylic esters, lactones and anhydrides secreted by lac insects (*Kerria lacca*) (Patel et al., 2013). It has interesting properties as a nanocapsule wall material given its tremendous film-forming properties such as excellent gloss, low gas permeability and the ability to prevent moisture transfer. Moreover, shellac is of natural origin, biodegradable, odourless in cold conditions, non-toxic, physiologically harmless and generally recognised as safe (so-called "GRAS' status in the USA). For these reasons, shellac is an acceptable material for the food and pharmaceutical industries, particularly for the coating of phytochemicals and controlled drug delivery purposes (Byun, Ward, & Whiteside, 2012; Farag & Leopold, 2011; Poovarodom & Permyanwattana, 2015). Recently, a study performed by Sedaghat Doost et al. (2018b) successfully encapsulated quercetin as a bioactive compound within core-shell nanoparticles containing shellac and almond gum. In similar works, Sun et al. (2017) and Chen et al. (2018) studied the fabrication and characterization of binary composite particles based on zein and shellac.

Nevertheless, shellac is restricted in its application as a delivery system for bioactive compounds because it tends to aggregate at an acidic pH (1.2), which might be solved by the incorporation of a stabiliser (Patel, Heussen, Hazekamp, & Velikov, 2011). A nano-particulate carrier of bioactive compounds targeting the colon has to survive the harsh acidic conditions of the stomach; otherwise, the distribution, release, and bioavailability of the compound can be uncontrolled (Jafari & McClements, 2017; Park, Saravanakumar, Kim, & Kwon, 2010). Natural polymers have been widely acknowledged to have many functions in foods, including as a stabilising agent (Manuhara, Praseptiangga, Muhammad, & Maimuni, 2016; Muhammad et al., 2019; Praseptiangga, Giovani, Manuhara, & Muhammad, 2017; Sedaghat Doost et al., 2019b). Xanthan gum, a non-toxic biopolymer produced by *Xanthomonas campestris* through fermentation, has been widely used as a thickening and stabilising agent in various products, including emulsion systems and micro particles. The main chain of xanthan gum is based on a linear backbone of 1,4-linked β-D-glucose (Garcıa-Ochoa, Santos, Casas, & Gomez, 2000; Palaniraj & Jayaraman, 2011).

Thus, the purpose of this study was to investigate the functionality of xanthan gum in stabilising shellac nanoparticles containing cinnamon extract. The novelty of this work lay in the functionality of xanthan gum in shellac nanoparticles and the use of a spice extract instead of a single compound as the core material for the nanocapsules. The use of an herb or spice extract with high antioxidant properties is preferable for the manufacture of polyphenol- and antioxidant-rich foods compared to using a large amount of a single antioxidant as it may have an adverse effect on health (Shahidi & Ambigaipalan, 2015). In this study, therefore, the synthesis of nanocapsules containing cinnamon extract was conducted. The physicochemical and antioxidant properties as well as the release behaviour and thermal stability of the nanoparticles were also evaluated.

Table 1
Antioxidant properties of cinnamon extract.

Parameter	Value	Unit (per gram of dry weight)
Total phenolic compound	273 ± 8	mg epicatechin equivalent
Total flavonoid	25 ± 1	mg quercetin equivalent
Total antioxidant activity	208 ± 4	mg tannic acid equivalent
Ferric reducing antioxidant power activity	208 ± 3	mmol L^{-1} ascorbic acid equivalent

2. Materials and methods

2.1. Materials

Cinnamon bark (*Cinnamomum burmannii* Blume) was collected from Mount Kerinci, Indonesia. Fine shellac powder (SSB 55 Astra FP) and xanthan gum (Satiaxane CX 931) were provided by SSB Stroever GmbH & Co. KG (Bremen, Germany) and Cargill France SAS (France), respectively.

2.2. Preparation of polyphenol-rich cinnamon extract

Briefly, 10 g of cinnamon powder was subjected to extraction in 100 ml of ethanol before being stirred for 48 h at 20 °C. Next, the mixture was filtered by vacuum (Laboport, KNF Neuberger, Inc., USA) (Muhammad et al., 2017). The antioxidant properties of the cinnamon extract after the removal of the solvent are given in Table 1.

2.3. Preparation of colloidal nanoparticles

Colloidal nanoparticles were prepared by anti-solvent precipitation, according to our previous work (Sedaghat Doost et al., 2018b). In brief, 2% (w/w) of shellac powder was dissolved in ethanol using a magnetic stirrer. Xanthan gum was prepared separately in distilled water at different concentration levels (0–0.5% (w/w)) to examine the minimum concentration of xanthan gum required to stabilise the shellac at pH 1.2. The shellac solution was injected into the xanthan gum solution using a syringe at a shellac-to-xanthan gum ratio of 1:3 (w/w), and then mixed using a magnetic stirrer to obtain a homogeneous mixture. Next, colloidal nanoparticles were formed by removing the ethanol through rotary evaporation (Laborota 4000 Heidolph, Germany). To prepare the nanoparticles containing cinnamon, the solvent (ethanol) was partially replaced by cinnamon extract in various proportions (12.5%–50% (w/w)).

2.4. Characterization of colloidal nanoparticles

2.4.1. Determination of particle size and surface charge

Photon correlation spectroscopy (PCS100M, Malvern Instrument Ltd, UK) was used to determine the z-average particle size, while a Zetasizer IIC (Malvern Instrument Ltd., UK) was utilised to measure the ζ-potential of the colloidal nanoparticles. A few drops of the colloidal dispersion was appropriately diluted prior to the analysis of the particle size and ζ-potential. The z-average particle diameter was obtained by a cumulant analysis of the light that was scattered at an angle of 150°. The measurements were reported as the averages of 3 separate injections, with three readings made per injection. The measurements were carried out at 25 °C.

2.4.2. Morphological characterization

A JEOL JSM 7100F scanning electron microscope equipped with a PP3010T Cryo-SEM preparation system (Oxford Instruments, UK) was used to investigate the shape of the synthesised particles. A few milligrams of lyophilised colloidal nanoparticles were rehydrated in a few drops of water. The samples were placed on the cryo-specimen holder, and then cryo-fixed in slush nitrogen (-210 °C). Subsequently, the

sample was transferred to the cryo-unit in a vitrified state. After the sample was fractured, it was sublimated (20 min, −70 °C) and sputter-coated with platinum (4 min, 0.5 mbar). Finally, the sample was transferred to the microscope, where it was observed at −140 °C.

2.4.3. Determination of encapsulation efficiency and loading capacity

The encapsulation efficiency was determined by measuring the difference between the phenolic content on the surface of the nanoparticles and the total phenolic yield in the dispersion, according to Zheng, Ding, Zhang, and Sun (2011) with minor modifications. To dissolve and determine the phenolic content on the surface of the nanoparticles, 20 mg of the sample were added to distilled water (10 ml). Next, the mixture was centrifuged (Sigma 4K15 Sartorius AG, Germany) at 13102 g for 30 min. The supernatant was collected and the total phenolic content was analysed using Folin-Ciocalteu reagent (see Section 2.4.4). To determine the total phenolic yield, 20 mg of particles were dissolved in 10 ml of buffer solution at pH 7.4. The encapsulation efficiency was calculated using Eq. (1).

Encapsulation efficiency

$$= \frac{\text{total phenolic yield} - \text{phenolic content on the surface}}{\text{total phenolic yield}} \times 100\%$$

(Eq. 1)

The loading capacity was determined according to Teixeira et al. (2013) (Eq. (2)).

Loading capacity

$$= \frac{\text{total phenolic yield} - \text{phenolic content on the surface}}{\text{amount of particles produced}} \times 100\%$$

(Eq. 2)

2.4.4. Analysis of total phenolic content

The total phenolic content was determined using the Folin–Ciocalteu method, as described by Udayaprakash et al. (2015), whereby 0.2 ml of extract solution was mixed with 0.2 ml of Folin–Ciocalteu reagent and 1 ml of distilled water. After incubation for 6 min, 2.5 ml of 7% Na_2CO_3 were added. Afterwards, the mixture was maintained at ambient temperature (± 20 °C) for 90 min, and then, the absorbance was measured at 760 nm using a UV–visible spectrophotometer (Varian Cary 50 Bio, Agilent Technology). The total phenolic content was expressed as milligrams of epicatechin equivalent (ECE) per gram of dry weight of nanoparticles.

2.4.5. Total antioxidant content assay

The total antioxidant content was assayed by using the phosphomolybdenum method by Udayaprakash et al. (2015). The analysis was conducted after the wall material had been solubilised (see Section 2.4.3). The reagent solution for performing the assay was created by mixing 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate at a ratio of 1:1:1. Next, 0.5 ml of the sample was added to 4.5 ml of the reagent solution. The solution was incubated at 95 °C for 90 min using a water bath. The absorbance was measured at 695 nm using a UV–visible spectrophotometer after the sample reached room temperature. The total antioxidant content was stated as milligrams of tannic acid equivalent per gram of dry weight (mg TAE/g DW) of the nanoparticles.

2.4.6. FRAP (Ferric Reducing Antioxidant Power) assay

Phosphate buffer (0.2 M, pH 7, 2.5 ml) was added to the colloidal nanoparticles (1 ml), and then mixed with potassium ferricyanide (1%, 2.5 ml). The solution was incubated at 50 °C for 30 min before the addition of 2.5 ml of 10% trichloroacetic acid. In order to separate the larger aggregates, the solution was centrifuged for 10 min. The supernatant was mixed well with distilled water and 0.1% $FeCl_3$ at a ratio of 5:5:1, and then, the absorbance was measured at 700 nm using a

UV–visible spectrophotometer (Udayaprakash et al., 2015). A standard plot of ascorbic acid was used to measure the FRAP activity of the samples. The FRAP activity was expressed as mmol L^{-1} of ascorbic acid equivalent per gram dry weight (mmol L^{-1} AAE/g DW) of the nanoparticles.

2.4.7. pH-responsive study

The protocol of Patel et al. (2011) was used to test the release of phenolic compounds from the shellac colloidal nanoparticles at different pH conditions. An aqueous medium (25 ml at pH 1.2) was prepared using 0.1 M HCl. Cinnamon extract and the nanoparticles were separately introduced into the release medium, which was slowly stirred for 2 h at 37 °C in the absence of light. Immediately afterwards, a few drops of NaOH were added to change the pH to 7.4, and the incubation was continued for another 2 h. Periodically, a sample was taken, and the total phenolic content was analysed by means of the Folin–Ciocalteu method (see Section 2.4.4).

2.4.8. Stability test

The test for the thermal stability of the cinnamon extract (free and nanoencapsulated forms) was conducted in a water bath at a temperature of 90 °C, according to the protocol of Sedaghat Doost et al. (2018b). The samples were collected after 20 min of heat treatment. The total phenolic content and antioxidant activity of the samples were analysed (see Section 2.4.4 and Section 2.4.5, respectively) before and after the heat treatment.

2.5. Experimental design and statistical analysis

The parameters that were considered to be affecting the characteristics of the nanoparticles were the xanthan gum concentration, the cinnamon loading and the storage temperature. The effect of each compositional and operational parameter on the characteristics of the nanoparticles was studied based on a single factor experiment with 5 (five) levels, except for the study on the effect of the xanthan gum concentration on the particle size, the ζ-potential, the encapsulation efficiency and the loading capacity of the nanoparticles. The experiment was performed by means of a completely randomized design (CRD), and the results represented the means of three replicates. A data analysis was performed by SPSS Statistics 23 using an analysis of variance (one-way ANOVA). A DMRT (Duncan's multiple range test) and T-test were performed to analyse the differences between the means. A significance level of 5% was applied.

3. Results and discussion

3.1. Preparation of stable shellac colloidal particles

As shellac tends to aggregate at an acidic pH, xanthan gum was incorporated into the anti-solvent phase to stabilise the system. It was even found that an instant aggregation of some parts of shellac occurred during the injection of the solvent phase into the anti-solvent phase when xanthan gum was absent. The instant aggregation could be prevented by the incorporation of 0.1% of xanthan gum. A minimum concentration of 0.3% of xanthan gum was required to prevent aggregation at a pH of 1.2. As the pH decreased, the negative charges present within the chemical structure of the xanthan gum were screened and thus, xanthan gum at a concentration below 0.3% was inadequate to induce repulsion against aggregation. While the uncontrolled aggregation was occurring, particles of irregular shape and size were formed (Fig. 1). At an acidic pH, H^+ ions will tend to accumulate on negatively-charged nanoparticles and the ζ-potential value may become less negative. In terms of the oral delivery system for bioactive compounds, the aggregation of nanoparticles is undesired since the release of the bioactive compounds can be uncontrolled. To get a better insight into the stabilisation effect of xanthan gum on
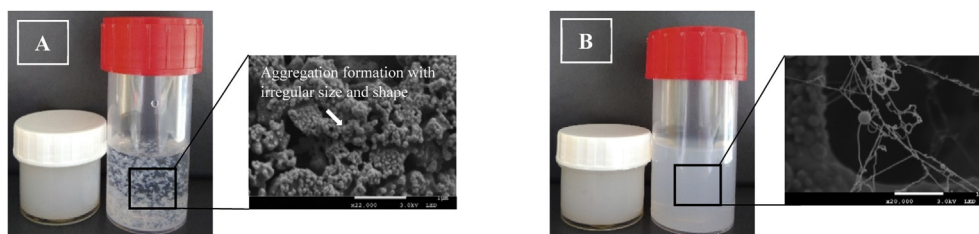
Fig. 1. Image of the colloidal particles at neutral pH (small container) and pH 1.2 (large container): (A) without the use of xanthan gum and (B) with the presence of 0.3% xanthan gum. Insets: Microscopic image of the shellac colloidal dispersion obtained by Cryo-SEM.
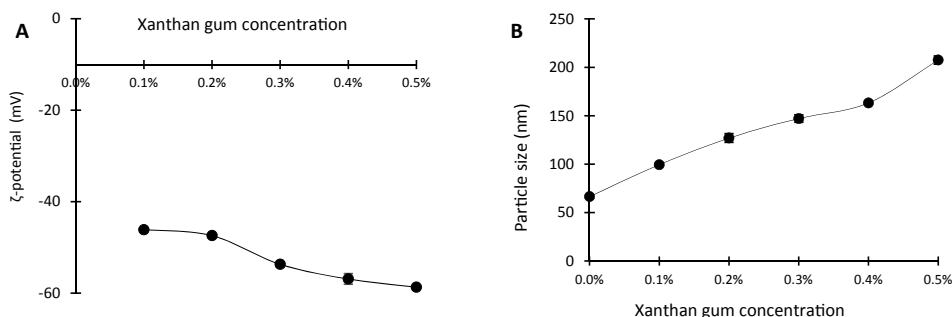

Fig. 2. The effect of xanthan gum incorporation on the zeta-potential (A) and (B) particle size of the shellac colloidal nanoparticles at neutral pH.

shellac nanoparticles, the ζ-potential of the nanoparticles was evaluated.

As shown in Fig. 2, the incorporation of a higher concentration of xanthan gum had a substantial impact on the ζ-potential of the nanoparticles. As xanthan gum is a negatively-charged biopolymer with a high surface charge density, the higher percentage of xanthan gum led to more negative ζ-potential values. The highly significant correlation between the incorporation of xanthan gum and the ζ-potential was verified by a Pearson's correlation analysis (-0.983), which showed that the correlation was significant at $p < 0.01$, which was in agreement with a previous study by Patel et al. (2011).

It should be noted that the concentration of xanthan gum that was required to stabilise the shellac nanoparticles at pH 1.2 was lower than that of almond gum, which was previously reported to be at a concentration of 0.7% (Sedaghat Doost et al., 2018b). According to Joye and McClements (2013), the stabilisation effect of different types of gums is determined by their composition, molecular weight and chain length. The difference in the charge density of xanthan and almond gum seems to play a significant role in the stabilisation effect. For instance, in this study the ζ-potential of xanthan gum at a concentration of 0.5% was found to be -59 mV, while, at a similar concentration, almond gum resulted in a ζ-potential of -32 mV (Sedaghat Doost et al., 2018b).

Increasing the xanthan gum concentration resulted in a significant growth in the size of the particles (with a correlation coefficient of 0.975) (Fig. 2). The growth in particle size indicated that more xanthan gum molecules were adsorbed onto the colloidal shellac particles (Patel et al., 2011). In a previous study, it was shown by transmission electron microscopy that the shellac was surrounded by almond gum, which led to the stabilisation of the formed nanoparticles (Sedaghat Doost et al., 2019a). The ability of the xanthan gum to cover the surface of the shellac particles was due to the hydrogen bonding between the shellac and xanthan gum, as reported by Patel et al. (2013). The adsorption of an amphiphilic stabiliser at the surface of the hydrophobic colloidal particles is the basis for a steric stabilisation effect (Cerrutti, de Souza, Castellan, Ruggiero, & Frollini, 2012).

A rise in the size dispersity, which is a measure of the distribution of width in the samples, from 0.14 to 0.46 was observed when the xanthan gum concentration was increased from 0 to 0.5%. An increase in size dispersity indicated the formation of agglomerates of nanoparticles, possibly due to a higher viscosity upon the introduction of more

xanthan gum, which might hamper the diffusion between the solvent and anti-solvent (Joye & McClements, 2013; Kakran, Sahoo, Li, & Judeh, 2012). Rheological experiments confirmed that a higher concentration of xanthan gum caused a higher apparent viscosity at all the tested shear rate levels (not shown).

3.2. Impact of cinnamon extract loading and gum concentration on the characteristics of the nanoparticles

Cinnamon extract was loaded into the shellac-xanthan gum complex, and the effect of this on the characteristics of the nanoparticles is shown in Table 2. The nanoparticles with a higher level of cinnamon extract loading tended to induce a bigger particle size and less negative charges. The latter indicated that the cinnamon extract was at least partly present at the surface of the shellac-xanthan gum system. To challenge this hypothesis, a study investigating the effect of the proportion of cinnamon extract on the encapsulation efficiency was carried out.

The phenolic content was used as the parameter for the encapsulation efficiency since polyphenols are acknowledged to be the main constituent offering health benefits (Gruenwald, Freder, & Armbruester, 2010; Li et al., 2013; Lu et al., 2011; Muthenna, Raghu, Kumar, Surekha, & Reddy, 2014; Ribeiro-Santos et al., 2017). It was shown that about 30% of cinnamon polyphenols were encapsulated within the nanoparticles and hence, the rest might have been associated with the surface of the nanoparticles. Cinnamon extract does not consist of only one compound but is rich in structurally diverse phytochemically active constituents, such as cinnamic acid, sinapic acid, p-coumaric acid, cafeic acid, protocatechuic acid, quercetin, kaempferol, gallic acid, vanillic acid, syringic acid, ferulic acid, quercetin-3-rhamnoside, tannic acid, caffeic acid, chlorogenic acid, rosmarinic acid and salicylic acid (Muhammad & Dewettinck, 2017). Some of these cinnamon extract constituents are highly soluble in water. During the nanoparticle formation (precipitation of shellac in the evaporation process), some of these compounds might have been in a solute state in the water, and thus, could not be entrapped in the colloidal shellac nanoparticles. When the colloid was lyophylised, the compounds were deposited onto the surface of the nanoparticles. This fact implies that the constituents of a spice extract can have an important influence on the encapsulation efficiency.

Table 2
Characteristics of nanoparticles loaded with different concentrations of cinnamon extract and xanthan gum.

Cinnamon extract (%)	Xanthan gum (%)	Z-average particle size (nm)	ζ-potential (mV)	Encapsulation Efficiency (%)	Loading Capacity (%)
0	0.3	149 ± 3 [a]	−53.6 ± 0.4[a]		
12.5		159 ± 5[ab]	−45.3 ± 0.5[bc]	33.6 ± 0.5[a]	2.4 ± 0.1[a]
25		157 ± 5[ab]	−43.8 ± 1.4[c]	31.7 ± 1.3[ab]	2.9 ± 0.1[b]
37.5		154 ± 6[ab]	−45.1 ± 0.7[bc]	32.0 ± 1.0[ab]	3.6 ± 0.1[c]
50		162 ± 6[b]	−46.1 ± 0.7[b]	29.7 ± 1.8[b]	3.5 ± 0.1[c]
12.5	0.3	159 ± 5[a]	−45.3 ± 0.5[a]	33.6 ± 0.5[a]	2.4 ± 0.1[a]
	0.4	225 ± 8[b]	−48.5 ± 1.0[b]	27.1 ± 2.9[b]	2.0 ± 0.3[a]
	0.5	241 ± 5[c]	−53.6 ± 1.3[c]	27.9 ± 2.7[b]	2.1 ± 0.3[a]

Mean values with the same letter do not differ significantly ($p > 0.05$) in the same column. Statistical analysis was performed separately for each combination for each experimental set up (different concentration of cinnamon loading and different level of xanthan gum).

The encapsulation efficiency was also significantly affected by the initial amount of cinnamon extract, where the encapsulation efficiency was lower at higher concentrations of the cinnamon extract. This result was in accordance with the finding of Xu and Du (2003), who worked with bovine serum albumin (BSA) and chitosan. They discovered that a low BSA loading led to high encapsulation efficiency, while a high BSA loading resulted in low encapsulation efficiency. When a smaller ratio of cinnamon extract to shellac was employed, the shellac had a higher chance of entrapping the cinnamon extract. As expected, at a higher initial cinnamon extract concentration, the actual amount of phenolic compounds entrapped in the nanoparticles was higher. Therefore, the loading capacity increased (Table 2).

Confirming the results from the previous experiment (Section 3.1), Table 2 also shows that the incorporation of a higher concentration of xanthan gum led to larger particles and more negative surface charges. The drawback in increasing the xanthan gum concentration was that it led to a significant decrease in the encapsulation efficiency. This could have been due to an increase in the viscosity. This result was in agreement with the negative relationship between the viscosity and encapsulation efficiency in the fabrication of nanoparticles, as reported by Gan and Wang (2007) as well as Kraisit, Limmatvapirat, Nunthanid, Sriamornsak, and Luangtana-anan (2013). The reduction in the encapsulation efficiency was directly proportional to the loading capacity.

3.3. Microstructural characterization

A microstructural visualisation was performed to study the surface morphology of the shellac-xanthan gum nanoparticles containing cinnamon extract. To study the morphology of the nanoparticles, they were initially freeze-dried and then, a small amount of water was added to rehydrate the particles. The freeze-drying step was conducted to make the colloidal nanoparticles denser and, therefore, easier to observe. The rehydration step was carried out to reverse the form of the colloidal nanoparticles. It was found that the nanoparticles were spherical with a smooth surface. There was no significant difference in the morphology between the blank nanoparticles (Fig. 1B) and the nanoparticles loaded with cinnamon extract (Fig. 3). This result was in accordance with the previously published literature explaining that nanoparticles, produced using anti-solvent precipitation, are often spherical (Joye & McClements, 2013; Sedaghat Doost et al., 2018b). Fig. 3 shows that the particles containing cinnamon extract in shellac-xanthan gum were of nanoscale size. The size corresponded well with the information on the particle size obtained by photon correlation spectroscopy, which indicated an intensity-weighted particle size distribution ranging from 73 to 366 nm.
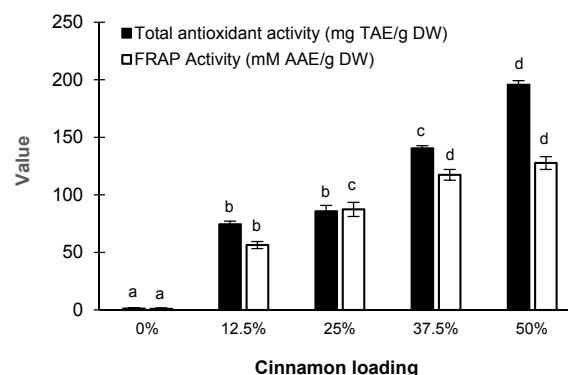
3.4. Antioxidant activity

As there is no single method that can provide unequivocal results, two different assays (phospomolybdenum and FRAP methods) were
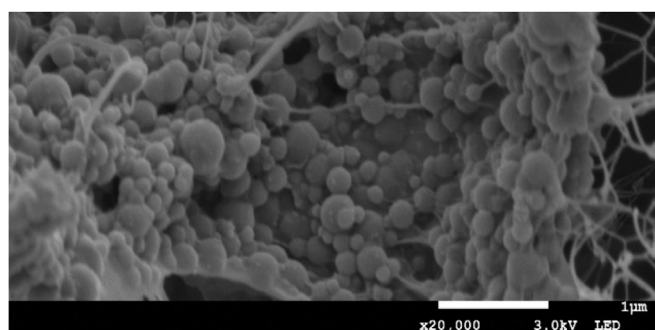
Fig. 3. Typical morphology of shellac nanoparticles incorporated with 0.3% xanthan gum loaded with 50% cinnamon extract.

used to investigate the antioxidant potency of the nanoparticles. In the phosphomolybdenum method, the formation of a phosphomolybdenum complex is based on the reduction of Mo (VI) to Mo (V) by the sample analyte, and the subsequent formation of a green complex at an acidic pH. The FRAP assay measures the ability for the reduction of Fe^{3+} to Fe^{2+}. Fig. 4 shows that without the cinnamon extract loading, the nanoparticles did not exhibit antioxidant activity when evaluated in both assays, thereby strongly indicating that cinnamon extract plays a significant role on the antioxidant activity of the nanoparticle system. The antioxidant activity of the nanoparticles was highly correlated with the cinnamon loading. As shown in both assays, a higher level of cinnamon loading led to a higher antioxidant activity. A previous study showed that the antioxidant activity of cinnamon extract is highly correlated with its phenolic content (Muhammad et al., 2017). This result indicates that the nanoparticles have a great potential as a natural

Fig. 4. Total antioxidant activity assayed using phospomolybdenum method and Ferric Reducing Antioxidant Power (FRAP) of nanoparticles stabilised with xanthan gum (0.3%) loaded with various levels of cinnamon. Mean values within each antioxidant assay with different lowercase letter differ significantly ($p < 0.05$).
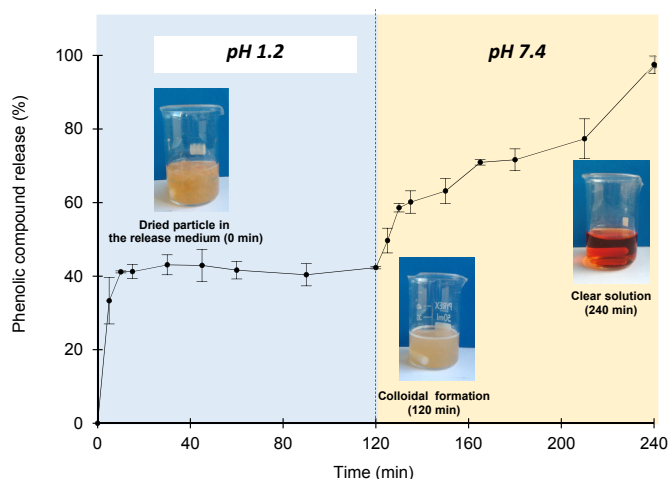
Fig. 5. Release profile of cinnamon phenolic compounds from freeze-dried colloidal nanoparticles in a release medium (2 h at pH 1.2 followed by incubation for 2 h at pH 7.4). Insets: physical appearance of freeze-dried nanoparticles during the treatment.

antioxidant to replace synthetic antioxidants.

3.5. pH-responsive release

Fig. 5 illustrates the release profile of the phenolic compounds of cinnamon extract from the freeze-dried nanoparticles. There was a burst release of around 40% in the first 15 min, which was associated with the loosely attached polyphenols on the surface of the nanoparticles. Afterwards, the release level remained constant. The dried nanoparticles at the low pH changed to a turbid colloidal form. When the pH was increased to 7.4, a sharp rise in the release of the phenolic content from the particles was observed. It was then followed by a gradual increase to more than 90% at the end of the study. This indicated that at pH 7.4, the shellac had more solubility, as could be also visibly observed from the decreased turbidity. As shown, in the first 120 min, the dried nanoparticles changed to a turbid colloidal form in the release medium. In the next 120 min at pH 7.4, the hazy colloidal appearance changed into a clear, brown-coloured solution (Fig. 5, insets). This pH-responsive behaviour indicated that the shellac-xanthan gum system can play an important role in preventing the discharge of the content and subsequently, controlling the release of polyphenol in the digestion system. Once the bioactive compounds are perfectly encapsulated in a shellac gum system, it can be expected that an uncontrolled burst release can be prohibited, and then, the bioactive compounds can be released in the targeted site triggered by the intestinal alkaline pH.
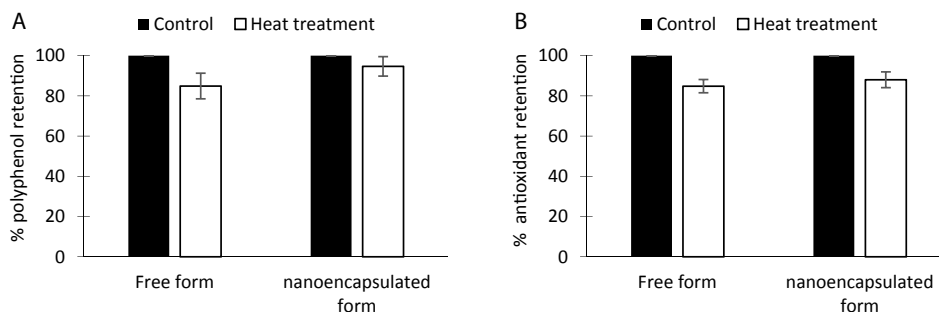
3.6. Thermal stability test

As polyphenols are prone to degradation upon heat treatment during processing, the thermal resistance of cinnamon extract in its free form and in its nanoencapsulated form was investigated. It is useful to draw a conclusion on the effectiveness of nanoencapsulation to prevent polyphenol degradation. As shown in Fig. 6, after the heat treatment, the polyphenol retention of the free cinnamon extract was about 84%, while that of the nanoencapsulated cinnamon extract was 94%. This indicated that nanoencapsulation can really have an impact on the thermal stability of polyphenols. However, in this study, nanoencapsulation could not perfectly prevent the polyphenols of cinnamon from degrading upon heat treatment. This was probably because some of the polyphenols of cinnamon were not well-encapsulated, as shown in the encapsulation efficiency test. The nanoencapsulated cinnamon extract also had better antioxidant activity retention as compared to the free cinnamon extract, at the level of 88% and 85%, respectively. This was reasonable since the antioxidant activity of cinnamon extract is directly proportional to its polyphenol content (Muhammad et al., 2017). Thus, based on the result of the thermal stability test, it was concluded that nanoencapsulation can significantly improve the stability of polyphenol and antioxidant compounds upon heat treatment.

4. Conclusions

This work confirmed the potential use of xanthan gum to stabilise shellac colloidal nanoparticles at a low pH. Upon the addition of cinnamon polyphenols, about a third of the initial load was encapsulated within the core of the shellac-xanthan gum nanoparticles, while the remaining part was attached to the surface of the nanoparticles. The encapsulated cinnamon polyphenols were completely released in alkaline pH conditions. The nanoparticles containing cinnamon extract exhibited high antioxidant activity, indicating that the shellac-xanthan gum nanoparticle system may be used as a carrier of polyphenols for improving the health-promoting properties of food. Nanoencapsulation effectively improved the thermal stability of the polyphenol-rich cinnamon extract. Further studies are required to improve the encapsulation efficiency of engineered cinnamon nanoparticles.

Conflicts of interest

The authors declare that they have no conflict of interest.

Fig. 6. The retention of polyphenols (A) and antioxidant activity (B) cinnamon extract upon heat treatment (90 °C, 20 min).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodhyd.2019.105377.

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