RESEARCH ARTICLE Asian Journal of Dairy and Food Research, Volume 44 Issue 4: 578-585 (August 2025)

Preparation and Characterization of Propolis (*Trigona sp.*) Extract-loaded Chitosan Tripolyphosphate Nanoparticles

Puspita Sari[1], Lusi Karlina Watiningsih[1], Boy Arief Fachri[2], Mukhammad Fauzi[1], Sutarsi[3], Rayya Rumaisha-Zuhriansyah[4], Dina Mustika Rini[5]

10.18805/ajdfr.DRF-483

ABSTRACT

Background: Propolis contains various bioactive compounds, including polyphenols, with diverse biological properties. However, its use is hampered by poor water solubility, instability in aqueous materials and limited absorption within the body. To overcome these limitations, the development of propolis nanoparticles has been proposed. Chitosan is widely utilized to prepare chitosan-based nanoparticles. It readily undergoes gelation upon interaction with polyanions, such as sodium tripolyphosphate (TPP). Chitosan-TPP nanoparticles, serving as wall materials, are prepared through the ionic gelation method. This study aims to encapsulate propolis extract in chitosan nanoparticles (propolis extract-loaded nanoparticles, PE-NP) using chitosan of different molecular weights (low and medium molecular weight, LMW and MMW), varying chitosan concentrations and different volumes of propolis extract. Furthermore, the PE-NPs were characterized by their physical characteristics and antioxidative properties.

Methods: PE-NPs were prepared using the ionic gelation method, in which chitosan and cross-linker TPP were used as wall materials (chitosan nanoparticles). The formulation was prepared by varying the molecular weight of chitosan (LMW and MMW), chitosan concentrations (0.1, 0.2 and 0.3%) and propolis extract volumes (0.1, 0.2 and 0.3 ml). The physical characteristics of PE-NP, including pH, turbidity, encapsulation efficiency, particle size, polydispersity index and zeta potential, as well as its antioxidative properties, were evaluated.

Result: The resulting PE-NP revealed low encapsulation efficiency (23.937-41.192%) and pH in the range of 3.9-4.8. PE-NP also presented antioxidant capacity that polyphenolic compounds, particularly flavonoids and phenolic acids, contributed to. The suspension of PE-NP had a particle size of 350.000-488.280 nm with PDI values higher than 0.3 (heterogeneous dispersion) and positive zeta potential (39.340-48.200 mV), resulting in stable nanoparticles. The PE-NP produced has antioxidant properties, making it suitable for use as a functional ingredient in the food industry.

Key words: Antioxidant capacity, Chitosan-TPP nanoparticle, Ionic gelation, Propolis extract.

INTRODUCTION

Propolis, also known as bee glue, is a complex substance produced primarily by *Apis mellifera* L bees. It is composed of resin and other materials, such as lipophilic material from leaves, mucilage, gum and latex, that bees collect from various plant sources, including leaves, buds and exudates. These materials are enzymatically processed by bee saliva and mixed with beeswax (Zaccaria *et al.*, 2017). Propolis comprises over 500 chemical constituents, including flavonoids, phenolic and polyphenolic compounds, terpenes, terpenoids, coumarins, steroids, amino acids and aromatic acids (Zullkiflee *et al.*, 2022). Notable polyphenolic compounds in propolis include pinocembrin, crysin, galangin, caffeic acid phenethyl ester (CAPE), caffeic acid, 1,1-dimethylallylcaffeate (DMAC), with lower amounts of quercetin, apigenin, naringenin and kaempferol (Gregoris and Stevanato, 2010). Propolis exhibits various biological properties, including antiapoptotic, antibacterial, anticancer, antiinflammatory, antioxidant, antiviral and antidiabetic activities (Zullkiflee *et al.*, 2022). In addition, a previous study investigated the anticytotoxic and antioxidant properties of propolis from different places in Indonesia (Hasan *et al.*, 2014).

The use of propolis extract is limited by its poor solubility in water, instability in an aqueous solution (do Nascimento

[1]Department of Agricultural Products Technology, Faculty of Agricultural Technology, Universitas Jember, Jember 68121, Indonesia.
[2]Department of Chemical Engineering, Faculty of Engineering, Universitas Jember, Jember 68121, Indonesia.
[3]Department of Agricultural Engineering, Faculty of Agricultural Technology, Universitas Jember 68121, Jember, Indonesia.
[4]Department of Food Science and Technology, Faculty of Agricultural Technology, IPB University, Bogor 16680, Indonesia.
[5]Department of Food Technology, Faculty of Engineering, Universitas Pembangunan Nasional "Veteran" Jawa Timur, Surabaya 60294, Indonesia.

Corresponding Author: Puspita Sari, Department of Agricultural Products Technology, Faculty of Agricultural Technology, Universitas Jember, Jember 68121, Indonesia.
Email: puspitasari.ftp@unej.ac.id
ORCIDs: 0000-0002-3897-2759, 0000-0002-0279-0670, 0000-0002-5969-4233, 0009-0008-7699-1281.

How to cite this article: Sari, P., Watiningsih, L.K., Fachri, B.A., Fauzi, M., Sutarsi, Zuhriansyah, R.R. and Rini, D.M. (2025). Preparation and Characterization of Propolis (*Trigona sp.*) Extract-loaded Chitosan Tripolyphosphate Nanoparticles. *Asian Journal of Dairy and Food Research*. **44(4):** 578-585. doi: 10.18805/ajdfr.DRF-483.

Submitted: 23-11-2024 **Accepted:** 17-04-2025 **Online:** 11-06-2025

et al., 2022) and limited absorption within the body, which are major challenges associated with the use of propolis in its raw form or as extracts (Justino *et al.*, 2023). A promising approach to address these limitations is the development of propolis nanoparticles (Justino *et al.*, 2023; Kim *et al.*, 2023; Shahab-Navaei and Asoodeh, 2023). The reduced particle size and increased surface-to-volume ratio of nanoparticles enhance their reactivity, effectively addressing several challenges posed by raw propolis (Kazemi *et al.*, 2019). Nanoparticles provide an improved delivery system for bioactive materials by enhancing their bioavailability, solubility, stability and absorption (Kim *et al.*, 2022).

Chitosan has been found for extensive use in pharmaceutical and food applications due to its beneficial characteristics, including hydrophilicity, biodegradability, biocompatibility, non-toxicity (Desai, 2016), cationic properties and affordability, while considered safe and cost-effective. This polysaccharide is widely used in the preparation of chitosan-based nanoparticles (Alishahi *et al.*, 2011; Kim *et al.*, 2022; Kim *et al.*, 2023). Various methods, such as emulsion-based cross-linking, complex coacervation, droplet coalescence, reverse micellar, solvent diffusion/evaporation and ionic/ionotropic gelation methods, can be employed to produce chitosan nanoparticles (Desai, 2016). Among these, the ionic/ionotropic gelation method is often favored due to its simplicity, non-toxicity and inexpensive. This method relies on electrostatic interactions between oppositely charged polymers, typically involving polycationic chitosan and a polyanion such as sodium tripolyphosphate (TPP) (Cota-Arriola *et al.*, 2013). Factors influencing the properties of physically cross-linked polysaccharide-based nanoparticles include the polysaccharide concentration, the molecular weight of the polysaccharide, the cross-linker to polysaccharide ratio and the pH (Jonassen *et al.*, 2012). Chitosan nanoparticles have been employed as an encapsulating agent for propolis extract. Some researchers have investigated propolis nanoparticles using polysaccharide chitosan (Kim *et al.*, 2023; Parolia *et al.*, 2020). In this study, we encapsulated propolis extract in chitosan nanoparticles (propolis extract-loaded nanoparticles, PE-NP) using low and medium molecular weight chitosan (LMW and MMW) and TPP *via* ionic gelation as wall materials. We examined the physical characteristics and antioxidative properties of PE-NP prepared with the different molecular weights of chitosan, varying chitosan concentrations and different volumes of propolis extracts.

MATERIALS AND METHODS

Materials and chemicals

The present study was carried out in the laboratories of the Department of Agricultural Products Technology, Faculty of Agricultural Technology, Universitas Jember from July 2023 to February 2024. Raw propolis from *Trigona* sp. was sourced from the Research Institute for Non-Timber Forest Products (Mataram, Lombok, Indonesia) and stored at -20°C to maintain its integrity until further treatment. Chemicals and reagents used for nanoparticle preparation and analysis were purchased from Merck (Darmstadt, Germany) and Sigma-Aldrich (St. Louis, MO).

Preparation of propolis extracts

Raw propolis was extracted following the method described by Cottica *et al.* (2015), with minor modifications. Frozen raw propolis was size-reduced using a dry blender. Ten g of propolis was mixed with 96% ethanol (100 ml) and stirred continuously for 24 h at 37°C. The resulting mixture was filtered through Whatman No. 1 filter paper. This extraction process was repeated three times and the filtrates were combined and vacuum evaporated at 50°C to obtain a concentrated extract.

Preparation of PE-NP

PE-NP was prepared using a modified method based on the ionic gelation technique described by Alishahi *et al.* (2011) and Kim *et al.* (2022). Briefly, LMW and MMW chitosan were dissolved separately in acetic acid to obtain chitosan solution at 0.1, 0.2 and 0.3% (w/v) concentrations. These solutions were stirred overnight at room temperature with a magnetic stirrer and filtered under a vacuum to remove insoluble residues. TPP was dissolved in water at the same concentrations (0.1, 0.2 and 0.3%, w/v) and also filtered to ensure clarity. Under magnetic stirring at 500 rpm and room temperature, 1.8 ml of TPP solution was added to the 9 ml of chitosan solution in a drop-wise manner with micropipette and the formation of chitosan-TPP nanoparticles began spontaneously *via* the TPP ionic gelation mechanism. The concentration of TPP used corresponded to the concentration of chitosan. Different volumes of propolis extract (0.1, 0.2 and 0.3 ml) were dropwise added into the chitosan nanosuspensions. The mixture was gently stirred at 500 rpm for 5 min at room temperature to promote cross-linking. The resulting nanosuspension was subjected to further analysis.

Physical characterization

The pH was measured at room temperature with a pH Meter (Bakhti *et al.*, 2025). The turbidity of nanoparticle suspensions was assessed by measuring their light absorbance at 750 nm using a spectrophotometer (Eratte *et al.*, 2014). Particle characterization was performed using a Zetasizer Nano ZS (Malvern, Worcestershire, UK) to determine parameters such as mean particle size (z-average), particle size distribution expressed as polydispersity index (PDI) and zeta potential (Alishahi *et al.*, 2011; Kim *et al.*, 2022). The phenolic encapsulation efficiency of the nanoparticles was evaluated based on the method of Alishahi *et al.* (2011), with slight modification. The encapsulation efficiency of nanoparticles was determined by centrifuging the suspensions at 18000 rpm for 30 min. The phenolic content in the supernatant, representing unencapsulated phenolic (free phenolic), was quantified using the Folin-Ciocalteau method (Singleton and Rossi, 1965; Arkan *et al.*, 2024).

Antioxidative properties

The total phenolic content was measured using the Folin-Ciocalteau method described by Singleton and Rossi (1965); Arkan *et al.* (2024), with absorbance measured at 765 nm using a UV-VIS spectrophotometer. The results were expressed as µg gallic acid equivalent per ml of nanoparticle suspension (µg GAE/ml); Where GAE= Gallic acid equivalent. Antioxidant capacities were determined using the DPPH (2,2-diphenyl-picrylhydrazyl) method (Chen *et al.*, 2006; Mudoi and Das, 2024) with modifications, which assesses the radical scavenging ability of the sample. Absorbance was recorded at 517 nm using a UV-VIS spectrophotometer and the antioxidant capacities were expressed as Trolox equivalent antioxidant capacity (TEAC, mmol TE/ml); Where TE= Trolox equivalent.

RESULTS AND DISCUSSION

Propolis is rich in bioactive compounds, including polyphenols, with various biological activities. However, its application is limited by low water solubility, instability in aqueous environments and limited absorption within the body. A nanoparticle-based approach represents a promising strategy to overcome these limitations by enhancing the bioavailability, solubility, stability and absorption of these compounds. This study focuses on encapsulating propolis extract in chitosan nanoparticles, PE-NP and evaluating their physical characteristics and antioxidative properties.

Preparation of PE-NP

Chitosan, a weak base composed of D-glucosamine groups, is insoluble in neutral and alkaline pH solutions. However, in acidic environments, its amino groups are protonated, transforming chitosan into a water-soluble polysaccharide and a positively charged polysaccharide with high charge density, as each D-glucosamine unit carries a single positive charge (Desai, 2016). This polysaccharide is widely used to prepare chitosan-based nanoparticles (Alishahi *et al.*, 2011; Kim *et al.*, 2022; Kim *et al.*, 2023). Chitosan rapidly forms a gel when interacting with polyanions, a process known as ionic gelation, which involves the establishment of inter and intramolecular cross-linking mediated by polyanions. In this study, chitosan-TPP nanoparticles, as the wall materials, are prepared using the ionic gelation technique. This process occurs through the electrostatic interaction between the polycationic chitosan and an anionic cross-linker, TPP. During nanoparticle preparation, TPP interacts with the amino groups of chitosan to form ionically cross-linked chitosan. Once mixed in dilute acetic acid, chitosan and STTP produced nanoparticles with a positive surface charge (Alishahi *et al.*, 2011; Kim *et al.*, 2022). The parameters that can affect the characteristics of PE-NP include the molecular weight of the chitosan, the chitosan concentration, the pH and the encapsulant concentration (propolis extract).

Chitosan-TPP nanoparticles were produced as wall materials to encapsulate propolis's phenolic compounds. These nanoparticles could form ionic and hydrogen bonds with phenolic compounds in propolis extract, as described by Alishahi *et al.* (2011), which explained the mechanism of the interaction of vitamin C with chitosan-TPP nanoparticles. Moreover, encapsulants can be incorporated into nanoparticulate systems through three primary mechanisms: electrostatic interaction, physical encapsulation and surface adsorption (Alishahi *et al.*, 2011; Yang and Hon, 2009).

Physical characteristics

The formation of chitosan-TPP nanoparticles via the ionic gelation technique relies on multiple electrostatic interactions between the positively charged amino groups of chitosan and negatively charged TPP, resulting in an opalescent solution. An opalescent visual indicates nanoparticle synthesis (Li *et al.*, 2003). An opalescent suspension revealed turbidity of nanoparticle suspensions. The turbidity of nanoparticle suspensions was evaluated by measuring their light absorbance at 750 nm using a spectrophotometer, with higher absorbance values indicating higher suspension turbidity. The absorbance (or turbidity) values of PE-NP are presented in Table 1. The absorbance (or turbidity) values increased with increasing chitosan concentration, at both LMW and MMW chitosan. This increase is attributed to the enhanced electrostatic interactions between chitosan and its polyanion (TPP), which form more cross-links, resulting in a more opalescent solution and higher absorbance value. Nanoparticle suspensions prepared using LMW chitosan have lower absorbance (or turbidity) values than MMW chitosan, as shown in Table 1.

The pH levels of the nanoparticle suspensions in all treatment variations are 3.9-4.8 (Table 1). The pH of nanoparticle suspensions is influenced by chitosan concentration and the addition of propolis extract. A higher chitosan concentration decreases the pH values due to the addition of a more acidic chitosan solution. The pH is influenced by the acetic acid solution used to dissolve the chitosan. As the concentration of chitosan increases, an equivalent increase in the concentration of acetic acid is required, resulting in a proportional increase in the use of acetic acid and a decrease in the pH of the suspension. The addition of higher-volume extracts slightly decreases the pH values. Propolis extract contains caffeic acid, phenolic acid and their esters, which are weak acids (Gregoris and Stevanato, 2010; Zullkiflee *et al.*, 2022). Chitosan-based nanoparticles effective at pH 4.5 provide better physical stability, particle size and distribution characteristics (Kammona and Kiparissides, 2012).

Chitosan-TPP nanoparticles may interact with phenolic compounds present in propolis extracts by means of hydrogen bonding and ionic interactions. Table 1 presents the encapsulation efficiency of phenolic compounds in propolis extract loaded into the nanoparticulate system.

The values range from 30.680-41.192% for nanoparticle suspensions using LMW chitosan and 23.937-36.350% for nanoparticle suspensions using MMW chitosan. The encapsulation efficiency values of this study tend to be low compared to other studies using different active compounds. Nanoparticles loaded with quercetin have an encapsulation efficiency of 48.5% (Tan *et al.*, 2011). Similarly, vitamin C-loaded nanoparticles achieved an encapsulation efficiency in the range of 30 to 40% when formulated with chitosan of MW 65, 90, 250 and 450 kDa and increasing to approximately 70% with chitosan of MW 110 kDa (Alishahi *et al.*, 2011). In addition, nanoparticles encapsulating polyphenolic compounds derived from *Rosa canina* showed an encapsulation efficiency of 46% (Stoica *et al.*, 2013).

The encapsulation efficiency increases as the chitosan concentration increases, at both LMW and MMW chitosan. The more chitosan and TPP complexes formed, the greater the ability of nanoparticles to entrap or bond interaction with active compounds (Nastiti and Siahaan, 2015). The encapsulation efficiency of nanoparticle suspensions using LMW chitosan is higher than that of MMW chitosan. These results are the same findings of Alishahi *et al.* (2011); Yang and Hon (2009). Chitosan with a lower molecular weight has shorter polymer chains, facilitating easier protonation of its free amino groups. This increased protonation capacity promotes greater adsorption of the phenolic compounds present in propolis extract, primarily through hydrogen or ionic bonding interactions (Alishahi *et al.*, 2011; Yang and Hon, 2009). The larger chitosan molecules lead to higher viscosity and thicker solutions, which can reduce the encapsulation efficiency (Wu *et al.*, 2005).

Antioxidative properties

The total phenolic content of PE-NP was quantified by a colorimetric assay using the Folin-Ciocalteu reagent, with results standardized to gallic acid equivalents (GAE). Fig 1 shows the standard curve plotted to estimate total phenolic content in the PE-NP with regression equation y = 9.6348x - 0.0009 and R^2 = 0.9978. The results obtained were expressed in µg GAE/ml of sample. Fig 2 displays the total phenolic content of PE-NP with the addition of 0.1, 0.2 and 0.3 ml propolis extracts. The propolis extract used in this study had a total phenolic content of 1.939 mg GAE/ml. The total phenolic content of nanoparticle samples with the addition of 0.1 ml propolis extract ranged from 21.049 to 23.021 µg GAE/ml, the addition of 0.2 ml propolis extract ranged from 37.188 to 40.976 µg GAE/ml and 0.3 mL propolis extract ranged from 54.625 to 59.711 µg GAE/ml, in both LMW and MMW chitosan. The total phenolic content was found to increase with the addition of more propolis extract. Another researcher also reported similar results (Shahab-Navaei and Asoodeh, 2023), stating that adding more propolis extracts increased phenolic content in nanoparticles. The total phenolic content in nanoparticle samples using LMW and MWM chitosan at the same chitosan concentration have relatively the same values. Total phenolic content decreased slightly with increasing chitosan concentration. Chitosan does not contain phenolic compounds (Nadia *et al.*, 2014). Zullkiflee *et al.* (2022) stated that propolis contains many flavonoids and phenolic compounds, including phenolic acid. Crysin, pinocembrin, caffeic acid, caffeic acid phenethyl ester (CAPE), 1,1-

Table 1: Physical characteristics of propolis extract-loaded nanoparticles, PE-NP (mean±SD, n=3).

Chitosan (%)	Propolis extract (ml)	Turbidity (AU)	pH	Encapsulation efficiency (%)
Low molecular weight/LMW chitosan				
0.1	0.1	0.021±0.003	4.8±0.000	30.680±0.445
	0.2	0.054±0.002	4.6±0.000	34.964±0.119
	0.3	0.076±0.007	4.5±0.000	36.403±0.122
0.2	0.1	0.031±0.000	4.6±0.000	34.542±1.778
	0.2	0.059±0.005	4.4±0.000	35.389±0.585
	0.3	0.095±0.004	4.2±0.000	39.802±1.294
0.3	0.1	0.058±0.003	4.1±0.000	37.832±0.176
	0.2	0.081±0.002	4.0±0.000	38.912±0.210
	0.3	0.134±0.002	3.9±0.000	41.192±0.393
Medium molecular weight/MMW chitosan				
0.1	0.1	0.042±0.002	4.8±0.000	23.937±1.713
	0.2	0.085±0.005	4.6±0.000	28.640±0.844
	0.3	0.153±0.004	4.5±0.000	29.951±0.703
0.2	0.1	0.058±0.002	4.6±0.000	26.547±1.590
	0.2	0.105±0.005	4.4±0.000	29.468±1.521
	0.3	0.161±0.004	4.2±0.000	32.769±1.587
0.3	0.1	0.085±0.003	4.1±0.000	30.613±1.746
	0.2	0.135±0.003	4.0±0.000	34.254±1.045
	0.3	0.173±0.003	3.9±0.000	36.350±1.122

dimethylallylcaffeate (DMAC) and galangin have been identified as the main phenolic constituents of propolis, with smaller amounts of apigenin, quercetin, kaempferol and naringenin also detected (Gregoris and Stevanato, 2010). Propolis *Trigona* sp. from Indonesia has a phenolic content of 104-498 µg/g and a flavonoid content of 0.405-129.265 µg/g (Hasan *et al.*, 2014). Another researcher also stated that propolis extract from Indonesia has a total phenolic content of 140.32 µg GAE/g and a total flavonoid content of 12.088 µg QE/g (Hariyanto, 2017).

The DPPH assays were used to evaluate the antioxidant capacity of PE-NP, with results expressed as mmol of Trolox equivalent per ml of sample. The antioxidant capacity of nanoparticle samples with the addition of 0.1 ml propolis extract ranged from 0.0030 to 0.0032 mmol TE/ml, the addition of 0.2 ml propolis extract ranged from 0.0047 to 0.0051 mmol TE/ml and 0.3 mL propolis extract ranged from 0.0066 to 0.0072 mmol TE/ml, in both LMW and MMW chitosan (Fig 3). The findings demonstrate that a greater volume of propolis extract enhances the ability to neutralize free radicals, as evidenced by an increase in antioxidant capacity. Shahab-Navaei and Asoodeh (2023); do Nascimento *et al.* (2016) also explained the same results, namely that adding a higher amount of propolis extract increased the antioxidant capacity of nanoparticles. Increased chitosan concentration and using LMW and MMW chitosan resulted in not-too-different antioxidant capacity values.

Propolis is known to be rich in polyphenolic compounds, particularly flavonoids and phenolic acids. Besides flavonoids, propolis also contains aromatic acids and esters, aldehydes and ketones, terpenoids and phenylpropanoids, steroids, amino acids, polysaccharides and many other organic and inorganic compounds (Zulhendri *et al.*, 2021; Zullkiflee *et al.*, 2022). The antioxidant capacity of PE-NP is attributed to the presence of phenolic compounds, especially flavonoids and phenolic acids. Previous studies have demonstrated that propolis extract or

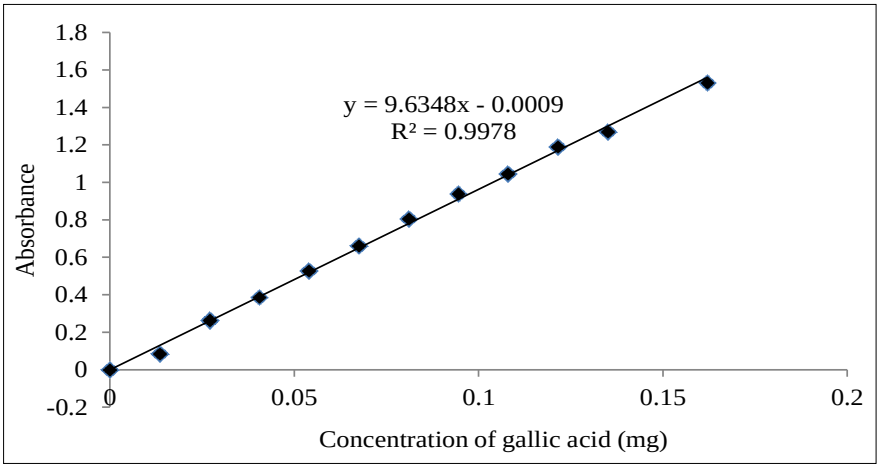
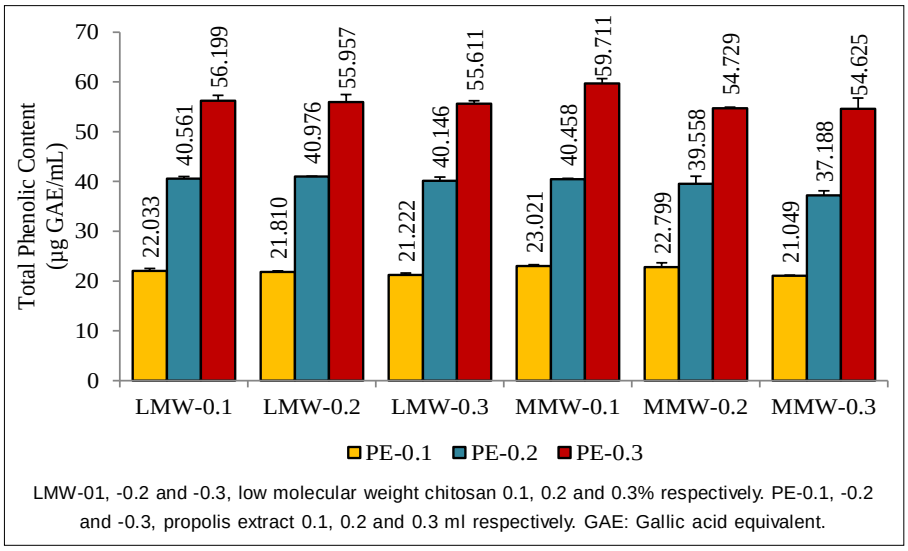


Fig 1: Standard calibration curve of gallic acid.



LMW-01, -0.2 and -0.3, low molecular weight chitosan 0.1, 0.2 and 0.3% respectively. PE-0.1, -0.2 and -0.3, propolis extract 0.1, 0.2 and 0.3 ml respectively. GAE: Gallic acid equivalent.

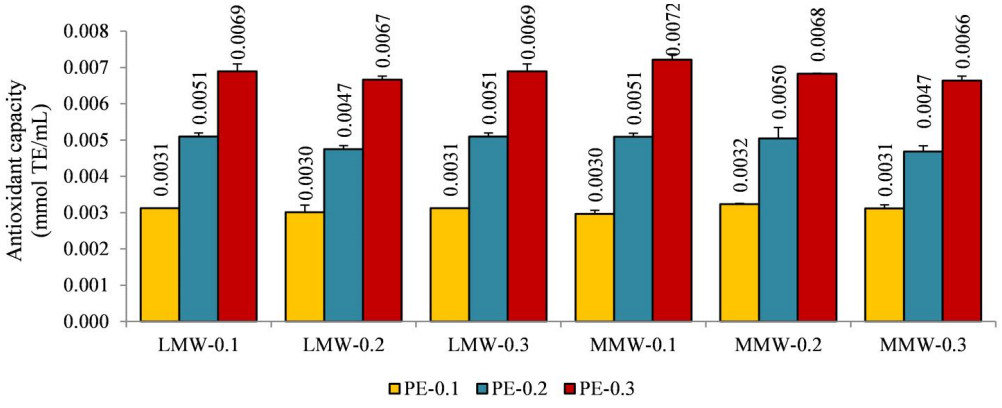
Fig 2: Total phenolic content of propolis extract-loaded nanoparticles, PE-NP.

its fractions contain phenolic compounds capable of exerting antioxidant effects (Cottica *et al.*, 2015; Hasan *et al.*, 2014). Additionally, a positive correlation between total phenolic content and antioxidant activity in propolis samples has been reported (da Silva *et al.*, 2006). However, other bioactive compounds, such as terpenoids, may also contribute to the overall antioxidant potential of propolis. Therefore, while phenolic compounds play a significant role in antioxidant activity, other compounds like terpenoids may also contribute and their influence should be considered. Antioxidants are generally divided into two categories based on their mechanism of action: preventive and chain-breaking. Phenolic compounds in propolis function as chain-breaking antioxidants by neutralizing peroxyl radicals involved in chain propagation. This reaction shortens the chain length and demonstrates antioxidant efficacy (Shahab-Navaei and Asoodeh, 2023). Beyond its antioxidant properties, propolis exhibits significant antiviral, antibacterial, antifungal, antiparasitic, antiapoptotic, anticancer, antiinflammatory and antidiabetic activities (da Silva *et al.*, 2006; Zulhendri *et al.*, 2021; Zullkiflee *et al.*, 2022).

Particle characteristics

PE-NP suspensions using LMW chitosan (concentrations of 0.1 and 0.2%) and MMW (concentration of 0.1%) with the addition of 3 mL propolis extract are selected for assay particle characteristics (size, PDI and zeta potential). These nanoparticle formulas have a stable appearance and better encapsulation efficiency. The suspensions remained stable and well-dispersed, with no aggregations or precipitation observed after seven days of storage. In addition, 0.3 mL of propolis extract has the highest antioxidant capacity. The results of particle characterization of PE-NP, including size, PDI and zeta potential, are presented in Table 2.

Nanoparticles generally have particle sizes below 1000 nm and particle sizes below 500 nm have better characteristics (Buzea *et al.*, 2007). The particle size of PE-NP ranged within the nanometric scale, measuring between 350.00 and 488.280 nm, with a particle size distribution or PDI varying from 0.305 to 0.482 (Table 2). Compared with the other research of Kim *et al.* (2023), nanoparticles loaded with propolis extract, using chitosan and hyaluronic acid, resulted in particle sizes ranging from 357.5 nm to 378.3 nm, with the PDI ranging from 0.173 to 0.424. The results also revealed that increased chitosan concentration and molecular weight increased particle size and PDI due to nanoparticle suspensions that may produce aggregates. Hence, the particle size is relatively non-uniform and heterogeneous. Moreover, Yang and Hon (2009) explained that using a higher molecular weight of chitosan resulted in a larger nanoparticle size. This is due to an increase in the viscosity, so particle formation is more difficult and implies the formation of larger particles. PE-NP suspensions have PDI values higher than 0.3 (Table 2) and



LMW-01, -0.2 and -0.3, low molecular weight chitosan 0.1, 0.2 and 0.3% respectively. PE-0.1, -0.2 and -0.3, propolis extract 0.1, 0.2 and 0.3 ml respectively. TE: Trolox equivalent.

Fig 3: Antioxidant capacity of propolis extract-loaded nanoparticles, PE-NP.

Table 2: Particle size, particle distribution and zeta potential of propolis extract-loaded nanoparticles, PE-NP (mean±SD, n=3).

Chitosan (%)	Propolis extract (ml)	Particle size (nm)	Particle size distribution/ polydispersity index (PDI)	Zeta potential (mV)
Low molecular weight/LMW chitosan				
0.1	0.3	350.000±4.910	0.305±0.010	39.340±0.814
0.2	0.3	478.000±7.130	0.348±0.047	48.200±0.208
Medium molecular weight/MMW chitosan				
0.1	0.3	488.280±4.291	0.482±0.022	42.540±1.002

are heterogeneous dispersion. Kim *et al.* (2023) stated that a PDI value greater than 0.3 indicates heterogeneity, which may be attributed to the presence of chitosan aggregates. According to Lu *et al.* (2011), the PDI with ranging values of 0-0.25 has a homogeneous size distribution. Moreover, the PDI can range between 0 and 1, with values close to 0 indicating a homogeneous dispersion system and above 0.5 indicating high heterogeneity (Avadi *et al.*, 2010). Nanoparticles with a particle size of 100-300 nm generally have a PDI of less than 0.3, while nanoparticles with more than 500 nm have a PDI of more than 0.5 (NanoComposix, 2015). Nanoparticles with a homogeneous particle size distribution are more physically stable, so they do not cause particles to aggregate (Avadi *et al.*, 2010).

PE-NP suspensions have a positive zeta potential of 39.340 to 48.200 mV (Table 2), revealing that PE-NP is stable. According to do Nascimento *et al.* (2016), a zeta potential with high absolute values, either negative or positive (±30 mV), indicates a stable suspension, as the repulsive forces between particles inhibit the aggregation of nanoparticles. The nanoparticle suspensions showed stability and dispersion, with no observed tendency towards aggregation. Moreover, dispersion with low zeta potential makes it easier for particles to interact and form aggregates (NanoComposix, 2015). The increase in chitosan concentration and molecular weight also increased positive zeta potential. In contrast to previous findings by Kouchak *et al.* (2012), insulin-loaded nanoparticles with a larger molecular weight of chitosan resulted in lower zeta potential values.

CONCLUSION

In this study, an ethanolic extract of propolis was prepared from raw propolis from *Trigona* sp. obtained from Mataram, Lombok, Indonesia. LMW and MMW chitosan and TPP were successfully used to prepare propolis extract-loaded chitosan nanoparticles (PE-NP) based on the ionic gelation method. Chitosan-TPP nanoparticles could encapsulate phenolic compounds in propolis extract with particular physical characteristics, resulting in low encapsulation efficiency of 23.937-41.192% and pH of suspensions in the range of 3.9-4.8. PE-NP also presented antioxidant capacity that was contributed to phenolic compounds in propolis. The suspension of nanoparticles had a particle size of 350.00-488.280 nm with PDI values higher than 0,3 (heterogeneous dispersion) and positive zeta potential (39.340-48.200 mV), which revealed stable nanoparticles. Consequently, the synthesized nanoparticles exhibit antioxidant properties, making them suitable for use as functional ingredients in the food industry.

ACKNOWLEDGEMENT

We would like to thank Universitas Jember for the research funding (Hibah Kelompok Riset-KeRis).

Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from using this content.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

REFERENCES

Alishahi, A., Mirvaghefi, A., Tehrani, M.R., Farahmand, H., Shojaosadati, S.A., Dorkoosh, F.A., Elsabee, M.Z. (2011). Shelf life and delivery enhancement of vitamin C using chitosan nanoparticles. *Food Chemistry*. **126(3):** 935-940.

Arkan, N.D., Setyawardani, T., Sumarmono, J., Naufalin, R., Santosa, S.S., Rahardjo, A.H.D. (2024). Effect of spice powder on physicochemical characteristics, functional properties and microbiological quality in soft cheese. *Asian Journal of Dairy and Food Research*. **43(3):** 396-403. doi: 10.8805/ajdfr.DRF-374.

Avadi, M.R., Sadeghi, A.M.M., Mohammadpour, N., Abedin, S., Atyabi, F., Dinarvand, R., Rafiee-Tehrani, M. (2010). Preparation and characterization of insulin nanoparticles using chitosan and Arabic gum with ionic gelation method. *Nanomedicine: Nanotechnology, Biology and Medicine*. **6(1):** 58-63.

Bakhti, S., Bekada, A., Bouzouina, M., Benabdelmoumene, D. (2025). Pomegranate peel extract fortified yogurt: Effect on physicochemical, microbiological and sensory quality of functional dairy product. *Asian Journal of Dairy and Food Research*. **44(1):** 8-15. doi: 10.18805/ajdfr.DRF-426.

Buzea, C., Pacheco, I.I., Robbie, K. (2007). Nanomaterials and Nanoparticles: Sources and Toxicity. *Biointerphases*. **2(4):** MR17-MR71.

Chen, F.A., Wu, A.B., Shieh, P., Kuo, D.H., Hsieh, C.Y. (2006). Evaluation of the antioxidant activity of *Ruellia tuberosa*. *Food Chemistry*. **94(1):** 14-18.

Cota-Arriola, O., Onofre Cortez-Rocha, M., Burgos-Hernández, A., Marina Ezquerra-Brauer, J., Plascencia-Jatomea, M. (2013). Controlled release matrices and micro/nanoparticles of chitosan with antimicrobial potential: Development of new strategies for microbial control in agriculture. *Journal of the Science of Food and Agriculture*. **93(7):** 1525-1536.

Cottica, S.M., Sabik, H., Antoine, C., Fortin, J., Graveline, N., Visentainer, J.V., Britten, M. (2015). Characterization of Canadian propolis fractions obtained from two-step sequential extraction. *LWT-Food Science and Technology*. **60(1):** 609-614.

da Silva, J.F.M., de Souza, M.C., Matta, S.R., de Andrade, M.R., Vidal, F.V.N. (2006). Correlation analysis between phenolic levels of Brazilian propolis extracts and their antimicrobial and antioxidant activities. *Food Chemistry*. **99(3):** 431-435.

Desai, K.G.H. (2016). Chitosan nanoparticles prepared by ionotropic gelation: An overview of recent advances. *Critical Reviews in Therapeutic Drug Carrier Systems*. **33(2):** 107-158.

do Nascimento, T.G., da Silva, P.F., Azevedo, L.F., da Rocha, L.G., de Moraes Porto, I.C.C., Lima e Moura, T.F.A., *et al.* (2016). Polymeric nanoparticles of Brazilian red propolis extract: Preparation, characterization, antioxidant and leishmanicidal activity. *Nanoscale Research Letters*. **11(1).** doi: 10.1186/s11671-016-1517-3.

do Nascimento, T.G., de Almeida, C.P., da Conceição, M.M., dos Santos Silva, A., de Almeida, L.M., de Freitas, J.M.D., *et al.* (2022). Caseinates loaded with Brazilian red propolis extract: Preparation, protein-flavonoids interaction, antioxidant and antibacterial activities. *Journal of Thermal Analysis and Calorimetry*. **147(2):** 1329-1343.

Eratte, D., Wang, B., Dowling, K., Barrow, C.J., Adhikari, B.P. (2014). Complex coacervation with whey protein isolate and gum arabic for the microencapsulation of omega-3 rich tuna oil. *Food and Function*. **5(11):** 2743-2750.

Gregoris, E. and Stevanato, R. (2010). Correlations between polyphenolic composition and antioxidant activity of Venetian propolis. *Food and Chemical Toxicology*. **48(1):** 76-82.

Hariyanto, R.A.B. (2017). Penentuan Kandungan Fenolik, Flavonoid Dan Aktivitas Antioksidan Ekstrak Propolis Trigona Sp [Unpublished bachelor thesis, Institut Teknologi Sepuluh November, Indonesia].

Hasan, A.E.Z., Mangunwidjaja, D., Sunarti, T.C., Suparno, O., Setiyono, A. (2014). Investigating the antioxidant and anticytotoxic activities of propolis collected from five regions of Indonesia and their abilities to induce apoptosis. *Emirates Journal of Food and Agriculture*. **26(5):** 390-398.

Jonassen, H., Kjøniksen, A.L., Hiorth, M. (2012). Effects of ionic strength on the size and compactness of chitosan nanoparticles. *Colloid and Polymer Science*. **290(10):** 919-929.

Justino, I.A., Marincek, A., Ferreira, I.R.S., Amaral, R.L.F., Fontanezi, B.B., Aldana-Mejía, J.A., Bastos, J.K., Marcato, P.D. (2023). Brazilian red propolis extract free and encapsulated into polymeric nanoparticles against ovarian cancer: Formulation, characterisation and biological assays in 2D and 3D models. *Journal of Pharmacy and Pharmacology*. **75(6):** 806-818.

Kammona, O. and Kiparissides, C. (2012). Recent advances in nanocarrier-based mucosal delivery of biomolecules. *Journal of Controlled Release*. **161(3):** 781-794.

Kazemi, F., Divsalar, A., Saboury, A.A., Seyedarabi, A. (2019). Propolis nanoparticles prevent structural changes in human hemoglobin during glycation and fructation. *Colloids and Surfaces B: Biointerfaces*. **177:** 188-195.

Kim, D.H., Jeong, E.W., Baek, Y., Lee, H.G. (2023). Development of propolis extract-loaded nanoparticles with chitosan and hyaluronic acid for improving solubility and stability. *LWT-Food Science and Technology*. **181:** 114738.

Kim, E.S., Baek, Y., Yoo, H.J., Lee, J.S., Lee, H.G. (2022). Chitosan-tripolyphosphate nanoparticles prepared by Ionic gelation improve the antioxidant activities of astaxanthin in the *in vitro* and *in vivo* model. *Antioxidants*. **11(3).** doi: 10.3390/antiox11030479.

Kouchak, M., Avadi, M., Abbaspour, M., Jahangiri, A., Boldaji, S.K. (2012). Effect of different molecular weights of chitosan on preparation and characterization of insulin loaded nanoparticles by ion gelation method. *International Journal of Drug Development and Research*. **4(2):** 272-277.

Li, X.W., Lee, D.K.L., Chan, A.S.C., Alpar, H.O. (2003). Sustained expression in mammalian cells with DNA complexed with chitosan nanoparticles. *Biochimica et Biophysica Acta-Gene Structure and Expression*. **1630(1):** 7-18.

Lu, W., Luo, Y., Chang, G., Sun, X. (2011). Synthesis of functional SiO 2-coated graphene oxide nanosheets decorated with Ag nanoparticles for H_2O_2 and glucose detection. *Biosensors and Bioelectronics*. **26(12):** 4791-4797.

Mudoi, T. and Das, P. (2024). Nutritional composition, mineral content and antioxidant properties of unpolished red rice cultivars of Assam, India. *Asian Journal of Dairy and Food Research*. **43(2):** 301-305. doi: 10.18805/ajdfr.DR-1751.

Nadia, L.M.H., Suptijah, P., Ibrahim, B. (2014). Production and characterization chitosan nano from black tiger shrimp with ionic gelation methods. *Journal Pengolahan Hasil Perikanan Indonesia*. **17(2):** 119-126.

NanoComposix. (2015). NanoComposix's Guide To Dynamic Light Scattering Measurement and Analysis. NanoComposix: San Diego.

Nastiti and Siahaan, P. (2015). Effect of chitosan molecular weight on BSA (bovine serum albumin) encapsulation efficiency using Na-TPP crosslink agent. *Journal Kimia Sains Dan Aplikasi*. **18(3):** 104-109.

Parolia, A., Kumar, H., Ramamurthy, S., Davamani, F., Pau, A. (2020). Effectiveness of chitosan-propolis nanoparticle against *Enterococcus faecalis* biofilms in the root canal. *BMC Oral Health*. **20(1).** doi: 10.1186/s12903-020-01330-0.

Shahab-Navaei, F. and Asoodeh, A. (2023). Synthesis of optimized propolis solid lipid nanoparticles with desirable antimicrobial, antioxidant and anti-cancer properties. *Scientific Reports*. **13(1):** 1-14.

Singleton, V.L. and Rossi, J.A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*. **16(3):** 144-158.

Stoica, R., Şomoghi, R., Ion, R.M. (2013). Preparation of chitosan-tripolyphosphate nanoparticles for the encapsulation of polyphenols extracted from *Rose hips*. *Journal of Nanomaterials and Biostructures*. **8(3):** 955-963.

Tan, Q., Liu, W., Guo, C., Zhai, G. (2011). Preparation and evaluation of quercetin-loaded lecithin-chitosan nanoparticles for topical delivery. *International Journal of Nanomedicine*. **6:** 1621-1630.

Wu, Y., Yang, W., Wang, C., Hu, J., Fu, S. (2005). Chitosan nanoparticles as a novel delivery system for ammonium glycyrrhizinate. *International Journal of Pharmaceutics*. **295(1-2):** 235-245.

Yang, H.C. and Hon, M.H. (2009). The effect of the molecular weight of chitosan nanoparticles and its application on drug delivery. *Microchemical Journal*. **92(1):** 87-91.

Zaccaria, V., Curti, V., Di Lorenzo, A., Baldi, A., Maccario, C., Sommatis, S., Mocchi, R., Daglia, M. (2017). Effect of green and brown propolis extracts on the expression levels of microrna, mrnas and proteins, related to oxidative stress and inflammation. *Nutrients*. **9(10):** 1-17.

Zulhendri, F., Chandrasekaran, K., Kowacz, M., Ravalia, M., Kripal, K., Fearnley, J., Perera, C.O. (2021). Antiviral, antibacterial, antifungal and antiparasitic properties of propolis: A review. *Foods*. **10(6):** 1360.

Zullkiflee, N., Taha, H., Usman, A. (2022). Propolis: Its role and efficacy in human health and diseases. *Molecules*. **27(18):** 6120. https://doi.org/10.3390/molecules271 86120.