

Incorporation of green-synthesized ZnONPs in chitosan coating on the internal quality of chicken eggs

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ABSTRACT

Chicken eggs as one of nutritious food have limiting shelf life. Incorporating ZnONP derived from green synthesis methods using plant extract in composite coating with chitosan might help extending the chicken eggs shelf life. The research involved the synthesis of ZnO nanoparticles via ethanolic *Mentha piperita* extract, incorporation in chitosan then followed by coating application on chicken eggs. The coated eggs were stored for 6 weeks. The coating treatments include chitosan 1%; chitosan + ZnONP 0.05%; chitosan + ZnONP 0.1% and control. Parameter observed involved weight loss; Haugh Unit, egg yolk index, pH, air sac diameter and microbial test. The results indicated that The ZnONP derived from ethanolic *Mentha piperita* extract, which contained 96.69 ± 0.06 mg EAG/g phenol, yielded relatively homogenous nanoparticles ranging from 50.38 to 99.87 nm. The integration of ZnO nanoparticles into the chitosan matrix as a coating material can reduce egg spoilage, thereby extending their shelf life. Egg coated over six weeks storage showed that the chitosan-based coating reduced the weight loss, enhanced the Haugh units value, and egg yolk index and maintain pH compared to control. Incorporation of ZnONP in chitosan did not enhance coating performance significantly compared to chitosan itself.

Introduction

Chicken eggs are nutritious food ingredients that are susceptible to quality degradation that limit shelf life. Egg quality is determined by internal and external factors. Chicken genetics, feed, disease, chicken age, temperature, humidity, storage, transport, bacterial contamination) plays critical roles on egg quality (Sonale *et al.*, 2025). Sustainable poultry production by implementing eco-friendly farming principles to proper post-harvest handling will produce high quality eggs with enhanced shelf life. Other external method to enhance eggs's shelf life is by applying polymer coating. One method to prevent eggs's internal degradation is by applying polymer coating. The most developed and applied egg-coating material is chitosan. Chitosan, a crustaceae derived polysaccharide, can perform good film properties and have permeability to CO₂ and O₂ with preferable mechanical properties. Chitosan, on the other hand, has some problems that make it less useful as a film and coating material. This material is fragile, poor water resistance, and does not block UV radiation well since it is hydrophilic (Kumar *et al.*, 2023). Incorporation of other material such as essential oil, plant extract or nanomaterial such as ZnO can increase their performance.

Green synthesis of nanoparticles is an innovative way to make nanomaterials that focuses on eco friendly methods. Traditional methods of making nanoparticles often use harmful chemicals and a lot of energy. On the other hand, green synthesis uses natural biological processes to make nanoparticles safely and sustainably (Huston *et al.*, 2021). This method lessens the impact on the environment while enhancing the biocompatibility of the resulting nanoparticles, making them suitable for a variety of uses, particularly in environmental remediation and medicine (Hano and Abbasi, 2022). One important method for producing ZnO nanoparticles is the green synthesis approach, which makes use of biological processes. Plant extracts serve as reducing and capping agents because of their diverse phytochemical constituents, which react with precursor salts to form

stable nanoparticles. These constituents include proteins, amino acids, organic acids, vitamins, and a variety of secondary metabolites, including flavonoids, alkaloids, polyphenols, terpenoids, and polysaccharides (Singh *et al.*, 2016). According to other studies, polyphenols are thought to be the most crucial chemical elements in the creation and maintenance of nanoparticles. According to Kharisova *et al.* (2013), polyphenols have the ability to obstruct subsequent reactions and particle aggregation processes, resulting in the formation of particles with stable properties.

ZnONP can reduce oxidative stress, shield cells from damage, and lessen the effects of dangerous free radicals. ZnONP increases the shelf life of coated products by acting as an active antibacterial agent in the coating solution. An effective antibacterial packaging substitute might be offered by the use of coating materials that incorporate natural ingredients like chitosan (Souza *et al.*, 2021; Alamdari *et al.*, 2022). Although chitosan has many advantages as a coating material, its poor mechanical and barrier qualities may restrict its use. Therefore, to improve these qualities and address these shortcomings, reinforcing materials—including nanomaterials—are usually used (Pires *et al.*, 2021).

Numerous studies has examined the utilization of ZnO nanoparticles in biopolymer composites for active food packaging to extend food shelf life by suppressing microbial growth (Al-Naamani *et al.*, 2016; Noshirvani *et al.*, 2017). Although there have been no documented uses for eggs, some researchers have proposed using chitosan zinc oxide packaging composites to increase the fresh meat's shelf life (Souza *et al.*, 2021; Sasidharan *et al.*, 2024). This study shows that a composite coating of ZnO nanoparticles and chitosan can prolong the shelf life of poultry meat, providing a workable substitute for sustainable and eco-friendly antimicrobial food packaging.

The purpose of this study was to investigate the environmentally friendly synthesis of ZnO nanoparticles as an antibacterial agent to prolong the shelf life of chicken eggs using polyphenol-rich plant extracts, specifically *Mentha piperita* extract. Alamdari *et al.* (2022) have carried out

the green synthesis of ZnONP from *Mentha* plants and its composite with chitosan for use as a packaging material. *Mentha pulegium* extract as a natural stabilizing and reducing agent. Compared to pure chitosan films, the resulting nanocomposite film has better mechanical, thermal, and barrier qualities. The incorporation of ZnO nanoparticles, which improve the film's durability and strength, is responsible for the augmentation. The ZnO/chitosan nanocomposite film exhibits strong antibacterial activity against a variety of microorganisms. According to Vieira *et al.* (2023), employing green tea extract to produce ZnO nanoparticles in an environmentally friendly manner has a notable photocatalytic efficacy, and have antibacterial properties. Moreover, Basumatary *et al.* (2023) mentioned that incorporating ZnO nanoparticles in chitosan composite increase the antibacterial activity against food borne bacteria.

Chicken eggs are nutrient-dense but possess a limited shelf life of up to 14 days at ambient temperature. Coating applications have shown an improvement in the shelf life of eggs. Coated eggs are anticipated to exhibit superior internal quality and extended shelf life during storage in comparison to uncoated eggs (Suresh *et al.*, 2015; Xu *et al.*, 2018; Winarti *et al.*, 2021; Caner *et al.*, 2022; Shurmasti *et al.*, 2023). Shurmasti *et al.* (2023) indicate that the use of an edible coating composed of chitosan and polyvinyl alcohol can extend the shelf life of eggs by 2-3 weeks at room temperature. Combining chitosan with hydrophobic materials, such as essential oils like basil oil (de Araújo *et al.* 2023), rosemary oil (Baighout *et al.*, 2023), and turmeric oil (Hejazian *et al.*, 2023), increases its effectiveness. There is currently no documentation on the use of ZnONP to enhance egg quality and shelf life. This study aimed to identify the optimal combination of chitosan and ZnONP solutions to enhance the shelf life and quality of eggs during storage by employing ZnONP's antibacterial qualities as an active ingredient.

Materials and methods

Materials

Zn(NO₃)₂·4H₂O from Sigma Aldrich as a precursor, ethanol technically grade solvent for mint leaves extraction sourced from the local market as a reducing agent, NaOH from Sigma Aldrich were the materials employed during preparation of ZnO nanoparticles synthetis. Chitosan from Sigma Aldrich and the eggs utilized are fresh eggs from layer farmers in Banten, with a weight of 60-65 g per egg, and were used during the coating process.

Preparation of ZnO nanoparticles

Mint leaves, thoroughly washed with water, were extracted using ethanol solvent via the maceration method. The residual solvent was eliminated via a vacuum evaporator at a temperature of 40°C. The mint leaf extract paste was subsequently diluted in distilled water at a 1:10 (w/v) ratio for further synthesis. The obtained total phenolic content of the mint extract was 96.69±0.06 mg EAG/g.

Synthesis of ZnO nanoparticles

The synthesis of ZnO nanoparticles using mint leaves extract was conducted at temperatures of 70°C and 90°C. The procedure for synthesizing ZnO nanoparticles utilizing mint leaves extract is as follows: A 0.050 M Zinc salt solution was placed in a three-necked flask and heated on a magnetic stirrer hot plate until a specific temperature was attained, with a stirring speed of 500 rpm. Mint leaves extract was introduced into the zinc salt solution at a 1:2 (v/v) ratio, followed by the incremental addition of 1 M NaOH until a pH of 8 was achieved. The mixed solution was subjected to a steady temperature and stirring speed for 3 hours, followed by cooling. The solids generated during the reaction were purified via centrifugation at 10,000 rpm for 5 minutes, followed by two washes of the

precipitation with ethanol. The precipitate was subsequently dried in an oven at 80°C for 2 hours, followed by calcination at 400°C for 2 hours. The desiccated materials were pulverized with a mortar for analysis.

Characterization of ZnO nanoparticles

ZnO nanoparticle crystal produced was analysed the size distribution of ZnO nanoparticles using Particle Size Analyser, Nano ZS (ZEN3600, England). The ZnO sample was placing in a cuvette and diluted with 3 mL of distilled water, and measuring its particles at 25 °C. The micro structure characteristics of ZnO nanoparticles was identified using A scanning electronic microscope (SEM) (Hitachi SU-3500, Japan) with magnitude 50.000 times.

Preparation of coating solution

A chitosan-derived coating material was synthesized by dissolving chitosan at a 1% concentration in a 1% acetic acid solution. ZnO nanoparticle powder, obtained through green synthesis with mint extract, was included into the chitosan dispersion formulation. The concentration of ZnO nanoparticles included was approximately 0.1% and 0.05% of the volume of the chitosan-based coating solution. The formulation of the egg coating is detailed in Table 1.

Table 1. Composition of egg coating formula.

Samples	Composition of coating formula
Control	No coating treatment
Chitosan 1%	Coating solution of chitosan 1%
CZn 0.05%	Coating solution of chitosan 1% + ZnO NP 0.05%
CZn 0.1%	Coating solution of chitosan 1% + ZnO NP 0.1%

Application of coating on eggs

The coating application technique on egg samples is executed according to the Winarti *et al.* (2023) method with several modifications. The used eggs were 1-day-old, 57–65 gram specimens from layer farmers in Banten. The following is the procedure for carrying out egg coating: In accordance with Table 1, 126 classified eggs were split up into three processing divisions.

The following process was used to create colloidal chitosan: 500 milliliters of 1% Glacial acetic acid were used to dissolve 5 grams of chitosan. The solution was then homogenized for 30 minutes at 40°C with a magnetic stirrer. After that, ZnO NP crystals were added to the chitosan mixture while stirring at concentrations of 0.1% and 0.05% (w/v). A magnetic stirrer was then used to homogenize the resulting chitosan solution after adding 2% glycerol. A 2% emulsifier (Tween 80) was then added, and the mixture was once more homogenized using a magnetic stirrer at 70°C for half an hour. The coating solution was made suitable for use as an egg coating material by allowing it to reach a temperature of 30°C. Soaking techniques were used to apply the coating material. Both the coated and uncoated samples were kept at room temperature (29–31°C) for six weeks.

Observation were conducted weekly until the end of storage. Six eggs were monitored for each treatment weekly.

Egg characterization

Weight loss

Calculated based on the difference in weight of the egg during storage compared to its total weight on day 0 and multiplied by 100 percent (Suresh *et al.*, 2015; Xu *et al.*, 2018).

Haugh unit

HU determination was done according to Purwati *et al.* (2015) and Suresh *et al.* (2015), which involved the measurement of egg weight and albumen height. The egg was weighed with a digital scale, then broken and placed on a flat glass surface. Furthermore, the albumen height was measured using a vernier caliper. The formula for measuring HU is as follows:

$$HU = 100 * \log (H + 7.57 - 1.7 * W^{0.37}) \dots\dots\dots(1)$$

HU: Haugh Unit; H: Albumen height (mm); W: Egg weight (g)

Egg yolk index

The egg yolk index was determined by comparing the height (mm) and width (mm) of the egg yolk (Shurmasti *et al.*, 2023).

pH

The egg white was separated from the egg yolk, and then its pH was measured using a pH meter (Suresh *et al.*, 2015).

Air sac diameter

The air sac diameter was determined by fracturing the blunt end of the egg, which houses the air cavity. Subsequently, the diameter of the air cavity was measured from the inner membrane of the shell using a caliper (Winarti *et al.*, 2021).

Microbial testing

Microbial test conducted on selected sample of all four treatments of control (non coated eggs); chitosan and chitosan-ZnONP coated egg for total microbes (TPC) and E coli content (SNI 2897-2008)

Data analysis

Data obtained were analyzed by analysis of variance (F-test) at the 5% level, followed by Duncan's Multiple Range Test (DMRT) when significant differences were detected.

Results

ZnO NPs characteristics

The particle size of ZnO nanoparticles produced under basic circumstances exhibited greater uniformity but still somewhat big, with an average dimension of 387.1 nm (Fig. 1a). Under acidic circumstances, the majority of particles produced were larger than 1 millimeter. The distribution of particles measuring less than 100 nm remained minimal, constituting less than 1% (Fig. 1b).

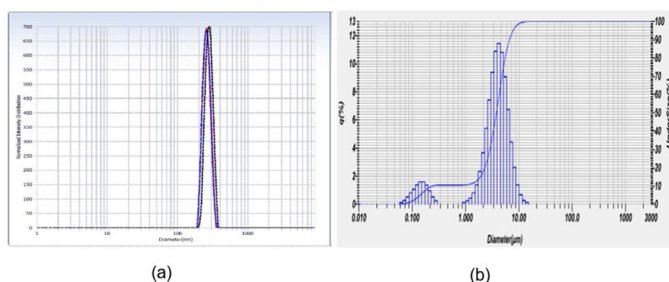


Fig. 1 . The distribution of ZnO particle diameter at varying temperatures and pH levels: (a) temperature 90°C, pH 8, (b) temperature 60°C, pH 5.

The synthesis of ZnO nanoparticles utilizing mint leaves extract at pH 8, with an extract-precursor ratio of 1:2 (v/v) at temperatures of 70°C and

90°C, yielded spherical morphology (Fig. 2). At a reaction temperature of 90°C, the particle morphology is comparatively more uniform than that formed at a reaction temperature of 70°C. The synthesis of ZnO nanoparticles at 70°C yields particles ranging from 50.38 to 65.24 nm (Fig. 2a and b), whereas synthesis at 90°C results in particles measuring between 50.38 and 99.87 nm (Fig. 2c and d).

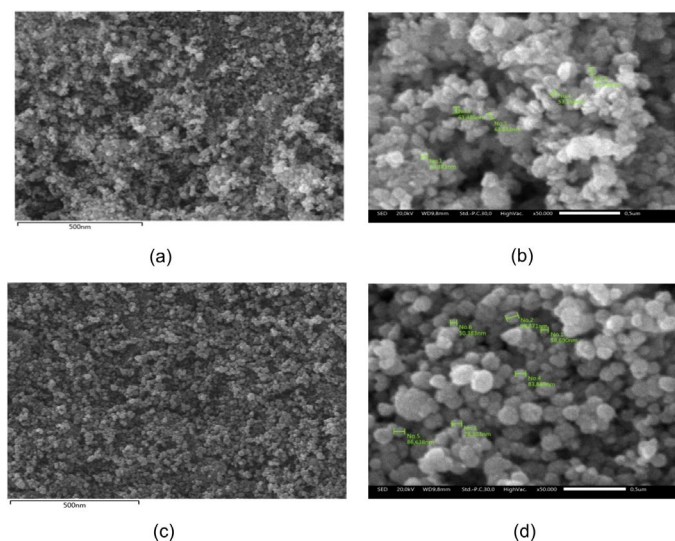


Fig. 2. Results of microstructure analysis with SEM (magnification 50,000) and particle distribution of ZnO nanoparticles synthesized using 1% mint leaf extract with a precursor concentration of 0.05 M and an extract-precursor ratio of 1:2; (a) and (b) reaction temperature 70°C; (c) and (d) reaction temperature 90°C.

Effect of coating on internal quality of eggs

Weight loss

Throughout the 6-week storage duration, a steady increase in weight loss was observed. Treatment with ZnONPs has shown efficacy in mitigating weight loss, with elevated zinc concentrations (CZn 0.1%) yielding superior outcomes compared to lower concentrations (CZn 0.05%). The chitosan and control almost have similar weight loss value (Fig. 3). In the first four weeks, although not statistically significant, the control group regularly demonstrated superior weight loss. However, at the 5th and 6th weeks, this gap reached statistical significance. CZn 0.1% exhibited minimal weight loss, succeeded by CZn 0.05%, while the control group incurred the maximum loss. During the sixth week, the CZn 0.1% treatment exhibited a weight reduction of 5.51%, whereas the control group experienced a reduction of 6.84%. Incorporation of ZnONP showed greater effect in reducing weight loss during storage compared with a reduced amount (0.05%).

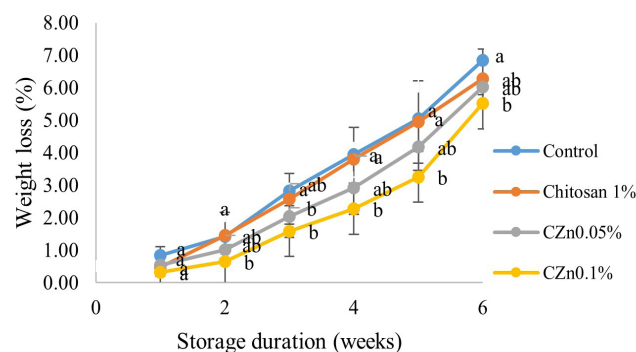


Fig. 3. Effect of coating on egg weight loss during storage.

Haugh unit

The CZn 0.1% treatment exhibited a superior HU value compared

to both the control, Chitosan 1% and CZn 0.05% treatments. There was a substantial difference between the treatment and the control during storage weeks 2 and 6 (Fig 4). HU standards were classified into C quality (< 31), B quality (31 – 60), A quality (60-72), and AA quality (>72). After the sixth week, the HU value for Chitosan and CZn 0.1% coated egg were 32.44 and 34.66 (Grade B), compared to 24.69 for the control (Grade C).

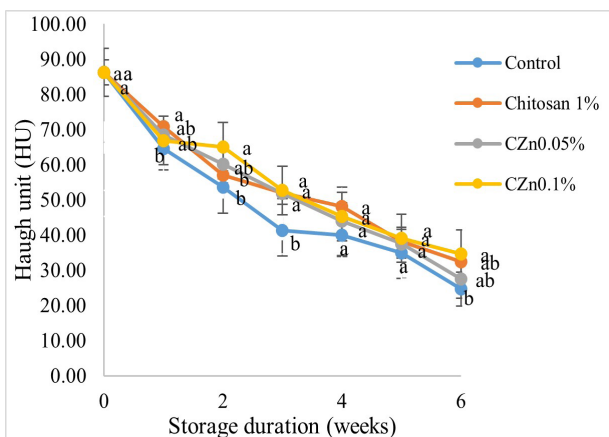


Fig. 4. The effect of coating on the Haugh unit of eggs during storage.

Egg yolk index

From the 4th to the 6th week of storage, the coated eggs exhibited a markedly reduced yolk index in comparison to the control (Fig. 5). The concentration of CZn 0.05% exhibited a superior protective effect relative to the higher concentration of 0.1%. The increased concentration of ZnO diminished the quality of the yolk. During the sixth week, the CZn 0.1% treatment yielded the lowest yolk index value of 0.105. The control and CZn 0.5% treatments yielded values of 0.127 and 0.142, respectively.

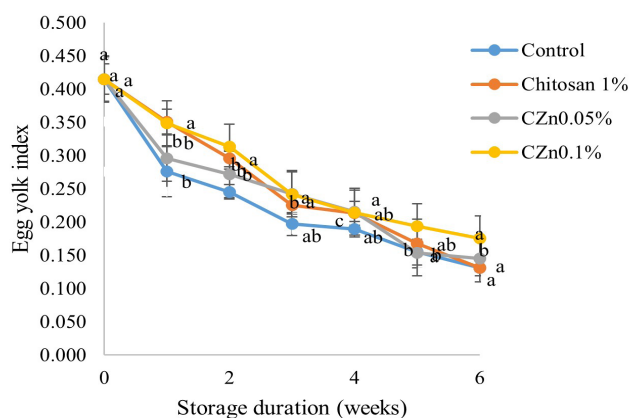


Fig. 5. Effect of coating on egg yolk index during storage.

pH

Fig. 6 indicates that all coating treatments, chitosan, CZn 0.05% and CZn 0.1%, exhibited a slower pH increase relative to the control. The non coated eggs showed higher pH at the end of storage.

Air sac diameter

The coated eggs treated with chitosan and zinc chitosan both at concentrations of 0.05% and 0.1% exhibited no significant influence on the enlargement of the egg air sac diameter throughout a 6-week storage period as compared to the control (Fig. 7). The longer the duration the air sac diameter increasing.

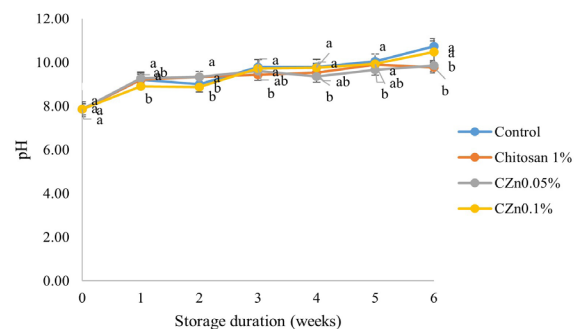


Fig. 6. Effect of coating on egg pH during storage.

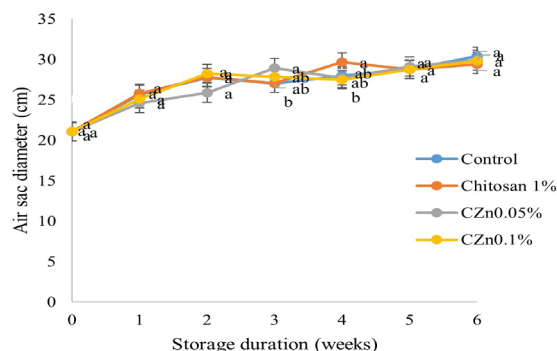


Fig. 7. Effect of coating on the diameter of air sac in eggs during storage.

Microbial testing

Table 2 showed that the coating treatment on eggshells significantly reduced the total microbial count compared to the uncoated control. Incorporation of ZnO NPs decrease the load of microbes in the surface of eggshell.

Table 2. Effect of coating on TPC and E coli in egg shell.

No.	Treatments	TPC (Cfu/g)	<i>E. coli</i> APM coliform/gr
1	Control	6.1×10^7	$< 10^1$
2	Chitosan 1%	2.6×10^5	$< 10^1$
3	Chitosan 1% + ZnONP 0.05%	1.6×10^5	$< 10^1$
4	Chitosan 1% + ZnONP 0.1%	1.8×10^5	$< 10^1$

Discussion

The synthesis of ZnO nanoparticles with mint leaf extract occurred in an acidic environment, as the pH of the precursor solution was 5, while the pH of the plant extract, obtained with water solvent, ranged from 2 to 3. Under acidic circumstances, the nanoparticle synthesis process exhibits reduced efficiency, resulting in the production of bigger particles. The increased particle size may result from the agglomeration process, as positively charged metal particles more readily absorb organic material from negatively charged reductants, complicating the management of particle aggregation rates. Moreover, acidic circumstances might alter the chemical structure and reactivity of the reductant, resulting in the formation of alkoxide ions. A drop in the acidity level of the solution during the reaction may lead to the redissolution of alkoxide ions, preventing the formation of a precipitate (Rahayu *et al.*, 2020). Previous investigations have demonstrated that the ideal pH for the synthesis of ZnO nanoparticles utilizing plant extracts is 8 (Zare *et al.*, 2017). Therefore, the subsequent synthesis process of ZnO nanoparticles was carried out in a reaction with a basic atmosphere (pH 8).

The variation in particle morphology is intricately linked to the reaction rate that transpires. An increase in reaction temperature correlates

with an elevated rate of nanoparticle production. Reduced metal ions that have undergone nucleation cannot undergo particle growth due to the rapid reaction rate, resulting in a very homogeneous particle morphology (Song *et al.*, 2009). The concentration of plant extracts employed as reducing and capping agents influences the morphology of the resultant nanoparticles (Jeevanandam *et al.*, 2018).

Green-synthesized ZnONPs provide safe, biocompatible, and environmentally sustainable food-grade coatings, with controlled size and morphology for optimal barrier and antimicrobial performance. The ZnONP produced using mint extract of the research have particle size from 50.38-99.87 nm (Fig. 2). Moreover, research by Ahmad *et al.* (2023) showed that ZnONPs synthesized from mint extract contain (Zn) Zinc, (C) Carbon, and (O) Oxygen. These compounds revealed the presence of metallic zinc acetate as main material used in the synthesis of ZnO NPs. The utilization of chitosan and its combination with ZnONPs composite for preserving the interior quality of eggs has not been extensively documented. In order to help preserve the internal balance of eggs and, eventually, egg quality, chitosan can create a semi-permeable membrane that permits selective gas exchange while serving as a barrier to gases and other substances. According to the findings, the interior quality of the chitosan-ZnONP-coated eggs was better than that of the control group.

Nanoparticles, not exceeding 100 nm in size, are appropriate for coating eggshells characterized by heterogeneous pore widths between 10 and 70 nm (Muhammad and Dewi, 2020; Phan and Haes, 2019). With smaller pore diameters enhancing gas barrier capabilities, these nanoparticles obstruct the pores, reducing permeability and creating a physical barrier against microbial contamination and water vapor (Jafarzadeh and Jafari, 2021). Furthermore, ZnO-based nanoparticles demonstrate antibacterial properties, suppressing microbial proliferation (Rahayu *et al.*, 2020). This is partially attributable to the characteristics of chitosan, which possesses favorable permeability qualities, and its amalgamation with zinc oxide can diminish thickness, solubility, water vapor permeability, and transparency (Dordevic *et al.*, 2024). The composite layer of chitosan and ZnO nanoparticles can extend the shelf life of eggs due to its superior antibacterial properties and diminished water content, hence promoting sustainable food preservation. According to Fatoni *et al.* (2021), the average diameter of the inhibitory zone for chitosan-ZnO nanoparticles (1:2) against *Escherichia coli* was 18.3 ± 0.4 mm and 15.7 ± 1.0 mm at concentrations of 0.5% and 0.25% (w/v), respectively.

The coated eggs show less weight loss compared to uncoated eggs. The application of chitosan is more successful in sealing the pores of the eggshell and creating a solid outer layer that acts as a barrier, thereby substantially reducing weight loss (Xu *et al.*, 2018). Its ability to obstruct pores and exhibit antibacterial properties makes the application of chitosan coating with zinc effective. In a study on egg coating using a CH/PVA composite, Shurmasti *et al.* (2023) showed less weight loss. The efficacy of the coating layer as a barrier in mitigating weight loss is contingent upon its mechanical qualities. The decrease in egg weight during storage occurred due to the evaporation of water and the release of carbon dioxide from the albumen through the eggshell (Suresh *et al.*, 2015).

Coated eggs exhibited a superior Haugh unit value relative to uncoated eggs. The HU value of the research result during observations week 4 (28 days) attained 43.9-43.25. The reduction in Haugh unit value after storage is attributed to albumen depletion, caused by elevated quantities of clusterin and ovoinhibitor, together with the disruption of ovalbumin structure (Xu *et al.*, 2018).

The addition of Zinc, specifically at a concentration of 0.1%, can maintain high HU levels during storage, particularly in the latter weeks of observation (M5 and M6). ZnONP protects eggs from deterioration during storage by acting as an antioxidant and antibacterial. Zinc preserves the integrity of the cell membrane, sustaining the structure of the vitelline membrane that separates the yolk from the egg white, thus preventing their amalgamation, which can diminish the HU value. The enzymes that break down the proteins in egg whites may be inhibited by zinc. This can

aid in maintaining the viscosity of egg white, an essential component in HU measurements.

The coating treatment did not substantially influence the yolk index during the initial storage period up to three weeks. Increased levels may cause more pronounced pH changes, hence accelerating the degradation of egg yolk quality. The ZnO coating influences the egg's internal environment by altering the eggshell's gas permeability. Elevated concentrations (0.1%) may considerably impair gas exchange, changing the composition or structure of the egg yolk and lowering the egg yolk index. Applying a coating to the egg surface can preserve the quality of the contents, enhance shell strength, and diminish microbial presence on the eggshell. Higher concentrations may cause greater pH changes, thus accelerating the decline in egg yolk quality. ZnO coating also affects the gas permeability of the eggshell which can affect the internal environment of the egg. Elevated concentrations (0.1%) may excessively impair gas exchange, leading to alterations in the structure or content of the egg yolk and a subsequent decrease in the egg yolk index. Coating the egg's surface can improve its strength, reduce the amount of microorganisms on it, and maintain the quality of its contents (Rachtanapun *et al.*, 2022). The constant flow of water from the albumen to the yolk through the vitelline membrane, which is fueled by osmotic pressure, is responsible for the liquefaction and flattening of the egg yolk, which lowers the egg yolk index value during storage. As the ovomucin-lysozyme complex breaks down, the albumen's viscosity decreases, which is correlated with the osmotic pressure between the albumen and the egg yolk. Xu *et al.* (2018) indicate that chitosan egg coating can mitigate CO₂ and water vapor loss, decelerate structural alterations in albumen, and diminish the osmotic pressure increase between albumen and egg yolk, thereby enhancing the quality of the egg yolk.

The coating treatment did not significantly affect the pH of albumin. The application of CZn0.05% coating was more efficacious in maintaining a low (stable) pH throughout the storage duration, particularly in the last weeks of storage. Zinc likely aids in the stabilization of egg pH during storage. The stability of pH is essential for maintaining the quality of egg whites. The viscosity of the egg white changes when the pH of the egg becomes more acidic. Shurmasti *et al.* (2023) indicate storage induces a modification in the acidity of the albumen, resulting in a reduction in viscosity due to the disintegration of the albumen..

The duration of storage correlates linearly with the increase in the diameter of the air sac although statistically did not significantly affect. The volume of the air cell in the egg enlarges with prolonged storage. According to Winarti *et al.* (2021), the enlargement of the air cavity happens as a result of the egg weight decreasing during storage due to gas release and water evaporation. Long-term egg storage causes increased fluid loss, which lowers the volume of the egg's contents and causes the air cell to expand. This enlargement of the air cavity occurs due to a decrease in egg weight caused by the process of water evaporation and gas release during storage. When eggs are stored longer, fluid loss becomes more significant, so that the volume of the egg contents shrinks and enlarges the air cavity inside.

The results of the analysis of total microbes and *E. coli* showed that coating treatment with chitosan and chitosan incorporated with ZnONP can inhibit microbial growth on the surface of the eggshell (eggshell) with the test results of TPC enumeration for control, chitosan; CZnONP 0.05% and CZnONP 0.1% are 6.1×10^7 ; 2.6×10^5 ; 1.6×10^5 ; 1.8×10^5 respectively (Table 2). The results of a study by Ahmed (2017) showed $< 10^1$; it can be assumed that *E. coli* is not present in the eggshell. The integration of coating materials employing natural components such as chitosan (Souza *et al.*, 2021; Alamdari *et al.*, 2022) may serve as an effective antibacterial packaging solution.

Conclusion

The polyphenol content in the ethanolic extract of *Mentha piperita*

produced relatively homogeneous nanoparticles with sizes ranging from 50.38 to 99.87 nm. The integration of ZnO nanoparticles into the chitosan matrix as a coating material can reduce egg spoilage, thereby extending their shelf life. The analytical results indicated that the application of the coating solution significantly affect weight, Haugh units, pH and egg yolk index during the 6-weeks storage period at ambient temperature. However, incorporation of ZnONP in chitosan have no significant impact in extending the internal quality compared to chitosan itself. These findings suggest that while incorporation of ZnONP has potential as preservation agent, the formula needs to be optimized to achieve maximum effectiveness.

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Conflict of interest

The authors have no conflict of interest to declare.

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