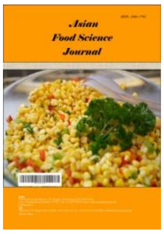

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Effects of Addition of TiO_2 Edible Coatings and Storage Periods on the Chicken Eggs Quality

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Authors' contributions

This work was carried out in collaboration among all authors. Authors IT and KUAA designed the study, performed the statistical analysis and wrote the protocol authors IT and MWA wrote the first draft of the manuscript. Authors AA and MWA managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

The study aims to determine the effect of addition TiO_2 solution on edible coatings of nanocomposite casein-chitosan on the internal chicken eggs quality observed at 7 and 14 days of storage. The design of the study was a Complete Randomized Factorial Design using 2 factors and the length of storage of chicken eggs. The data was analyzed using the Analysis of Variance (ANOVA) and continued with Duncan's Multiple Range Test if there is a significant difference. Edible coatings made from casein-chitosan with the difference between addition of 3, 5, 10 mL of TiO_2 1% then applicated of the solution as coating on eggs. The egg stored at 25 ℃ for 7 and 14 days then observed in the study i.e yolk index, albumen index, Haugh Unit, yolk color, yolk pH, albumen pH, and Total Plate Count (TPC). The results showed that the yolk index ranged from 0.25-0.40; albumen index 0.03-0.08; Haugh unit 48.68-80.42; yolk color 7-9; pH yolk 8.17-9.20; pH Albumen 8.17-9.20; TPC 0.7×10^5-1.1×10^5 cfu/mL. Edible coatings of casein-chitosan with 5 mL TiO_2 solution were able to reduce the decrease in the internal quality of chicken eggs observed at 7 to 14 days of storage. However, all treatments in this study showed better results in the 7 days storage period.

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Keywords: Edible coatings; casein; chitosan; TiO_2; internal quality of eggs.

1. INTRODUCTION

Eggs are human food that is almost perfect protein food, available rich nutrition, providing biological value such as vitamins, minerals and fatty acids that are needed every day for the growth and maintenance of body tissues [1]. Nevertheless, the storage of eggs can change and tend to degrade egg quality. The main factors that affect egg damage are temperature conditions, relative humidity, and storage time [2]. An egg contains complete amino acids, most minerals, and high calories. But, the longer storage time of eggs and the absence of special handling can decrease internal eggs quality. It is caused by physical and microbiological damage as well as evaporation of water, carbon dioxide, ammonia, nitrogen, and hydrogen sulfide from inside the egg [3]. Hence, preservation is needed to avoid damage, maintain internal quality and extend the shelf life of chicken eggs. Edible coatings on eggs are one of the efforts to extend its shelf life. It aims to maintain the internal chicken eggs quality. The use of *edible coatings* as preservation is by giving an additional layer to eggs or other food products made from natural basic materials, i.e. polysaccharides, proteins, and lipids [4]. It is easy to apply and protect eggs from damage or contamination. Chitosan and casein are the fundamental ingredients of making edible coatings. Chitosan has powerful and compact properties due to the presence of a matrix formed from polysaccharides. It has a good enough water permeability value to increase the shelf life of fresh products [5,6]. In addition, chitosan is elastic, flexible, and hard to tear. It has hydrophobic properties and is difficult to break by high heat. Casein-chitosan edible coatings are also formulated with other elastic materials such as lipid groups (glycerol) and TiO_2. The compound of TiO_2 has anti-UV properties that increase hydrogen bonding and cohesion strength in composite solutions, have emulsion stability and form properties in optimal film layers at concentrations of 0.1% [7]. TiO_2 belongs to a hydrophobic inorganic nanoparticles category that can form hydrogen bonds with protein biopolymer chain polymers. Due to its capability, TiO_2 can create complex pathways that reduce the transport of oxygen and water vapor when crossing the protein/nanomaterial matrix [8]. TiO_2 is known as photocatalyst which is widely used to decipher environmental problems such as industrial waste pollution containing colour substances, phenols, and other substances since it has an active and stable crust to biological and chemical processes. Therefore, the objectives of this study were to investigate the effect of the addition of TiO_2 edible coatings and storage on the chicken eggs quality (yolk index, albumen index, Haugh unit, yolk colour, yolk and albumen pH, and Total Plate Count).

2. MATERIALS AND METHODS

2.1 Materials

The material used in the study was a solution of edible coatings of nanocomposite casein-chitosan with TiO_2 1% difference in the volume solution applied to chicken eggs. The chicken eggs used were obtained from one of the farms in UPR Sumber Mina Dau, Sumbersekar Village, Dau Subdistrict, Malang Regency. The eggs used as samples were Lohmann Brown strain (MB402), 26 weeks old.

2.2 Methods

2.2.1 Preparation and application of edible coatings in eggs

The eggs used in the study consisted of 80 clean and not cracked eggs with a uniform weight; ten eggs were then coated with edible coating solutions separately. The edible coating was made from casein-chitosan (1:4 w/w) and TiO_2 1%. The egg coating was done by dipping it into the solution. The study used TiO_2 1%, added with different variations in the volume of casein-chitosan 3 mL (E1), 5 mL (E2), and 10 mL (E3), and applied as a coating on eggs. The eggs were kept at 25 ^{o}C for 7 and 14 days; the yolk index, albumen index, Haugh Unit, yolk colour, yolk pH, Albumen pH, and Total Plate Count (TPC) were then observed in the study. All experiments were performed in four replications.

Determination of Yolk index: The yolk index was calculated using the formula:

$$\mathrm{YI} = \frac{\text{Height of yolk (mm)}}{\text{Average diameter of yolk (mm)}}$$

Determination of Albumen Index: The albumen index was calculated using the formula:

$$AI = \frac{\text{Height of albumen (mm)}}{\text{Average diameter of albumen (mm)}}$$

Haugh Unit: Haugh unit was calculated using the formula [9]

$$Haugh\ unit = 100 \log (\mathrm{H} + 7.57 - 1.7\ \mathrm{W}^{0.37})$$

Where: H = Thick Albumen height (mm)
W = Egg weight (g)

Yolk Color: Yolk color was determined by Harmayanda et al. [10] is compared on the scale displayed by DSM Roche yolk color fan (Fig. 1.) after the egg was cracked open.

Fig. 1. Yolk color fan

Yolk and Albumen pH: The pH of the yolk and Albumen [9] were measured with a pH meter. About 5 mL to 10 mL of each sample is taken and put in a beaker glass. The pH meter was first standardized using buffer solution of pH 4 and pH 7. The electrode was rinsed with distilled water and then dipped into the sample to get the pH value.

Total Plate Count: Buffered peptone water 1% sterile as much as 225 mL was prepared into a sterile Erlenmeyer flask containing 25 mL of sample, then homogenized for 1 to 2 minutes. 1 mL of the suspension at the 10^{-1} dilution was transferred with a sterile pipette into the 9 mL BPW solution to obtain a 10^{-2} dilution. The procedures were repeated to obtain dilutions of 10^{-3}, 10^{-4}, 10^{-5}, and so on. About 15 mL to 20 mL of Standard Methods Agar (SMA) at a temperature of 45 °C ± 1 °C were added to the Petri dish that already contained 1 mL of suspension (Duplo). Samples were incubated at 34-36 °C for 24 hours to 48 hours by placing the Petri dish upside down. After that, the average colony from the Petri dish was calculated.

2.3 Statistical Analysis

Data were processed by Analysis of Variance (ANOVA). If the analysis happens to show a significant difference ($p<0.05$) or a very highly significant difference ($p<0.01$), then the Duncan's Multiple Range Test was applied.

3. RESULTS AND DISCUSSION

The internal condition of chicken eggs on a storage period of 7 and 14 days is presented in Fig. 2. The effect of the addition of TiO_2 1% on edible coatings casein-chitosan on the internal quality of chicken eggs is revealed in [1].

3.1 Internal Condition of Eggs

After 7 and 14 days of storage, the control egg underwent visible physical changes in the yolk and albumen sections (E0D7 and E0D14). The yolk and albumen on the control eggs were more significant to widen than eggs coated in edible coatings. According to Riawan et al. [11], the longer the storage time, it will result in much evaporation of liquids and gases in the eggs hence decreasing the egg quality. Nonetheless, this study influences the rate of decline in the internal quality of chicken eggs. However, the seven-day storage period of chicken eggs is better than the 14-day storage.

3.1.1 Yolk and albumen index

The treatment on the sample showed a highly significant difference ($p<0.01$) to the yolk index and a significant difference ($p<0.05$) to the albumen index. After 14 days of storage, 10 mL of TiO_2 1% solution in edible coatings of nanocomposites casein-chitosan turned out to be efficient to maintain the yolk index value compared to other treatments during the same storage period. TiO_2 solution in edible coatings of nanocomposites casein-chitosan affects its microstructure hence the chitosan matrix granules can be spread evenly on the edible coatings solution [12]. These properties become the basis that edible coatings can cover the eggshell evenly until it covers the pore. Hence, it can suppress the transfer of water vapour in the egg. This causes the osmotic pressure of yolk and albumen to remain stable, so the transfer of fluid from albumen to the yolk is not too large. Those conditions can reduce the changes in yolk diameter and height during storage.

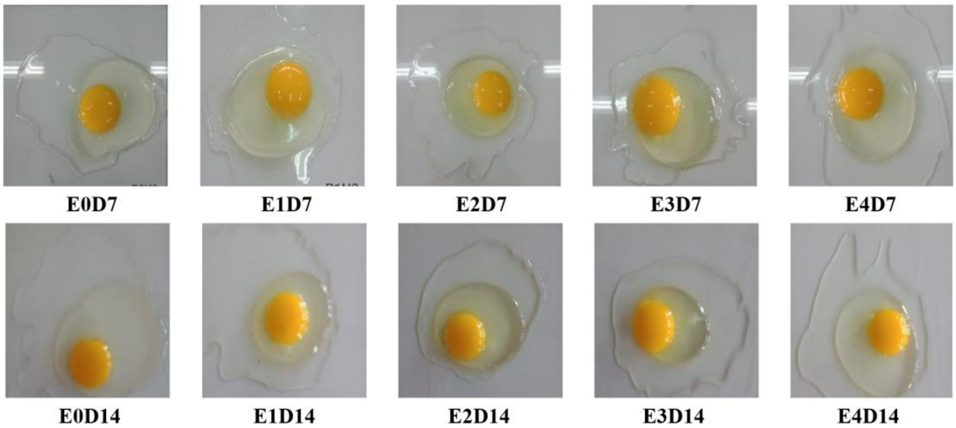

Fig. 2. Internal eggs condition storage for 7 and 14 days

During the 7-day storage period, 3 mL of TiO_2 1% solution in edible coatings of nanocomposites casein-chitosan could produce a higher albumen index value compared to other treatments in the same storage period. However, the addition of 5 mL of TiO_2 1% solution to the edible coatings of nanocomposites casein-chitosan was the most effective solution to maintain the albumen index value compared to other treatments in the same 14-days storage period. The well-covered eggshell pores by edible coating layers prevented the evaporation of water and CO_2 from the inside. Accordingly, ovomucin on the albumen did not suffer any damages. Hence, the albumen viscosity can be well-maintained. Widyastuti and Daydeva [13] stated that if albumen loses CO_2 and changes pH, ovomucin then loses the ability to maintain viscosity. When the albumen liquid becomes more dilute, the height will decrease, which leads to the albumen index decrease.

3.1.2 Yolk color

The treatment on the sample showed a significant difference ($p<0.05$) to the resulting yolk colour value. In the 7-day storage period observation, the addition of 10 mL of TiO_2 1% solution into edible coatings of nanocomposites casein-chitosan could produce a higher yolk colour if compared with other treatments in the same storage period. However, in the 14-day storage period monitoring, the addition of 3 mL of TiO_2 solution as edible coatings was more efficient at maintaining yolk colour compared to other treatments in the same storage period.

The yolk colour is affected by the types of pigments, namely carotene and riboflavin classified as lipochrome and liochrome contained in the rations consumed [14]. But, it was ruled out from this study because the rations and strains of chicken used were the same types; hence the yolk discolouration is influenced by other factors. According to Shinn [15], egg yolk index and yolk colour can be affected by conjugated linoleic acid (CLA), the only lipid supplement. CLA egg supplementation increased saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA) and also decreased monounsaturated fatty acids (MUFA) of the egg yolk.

3.1.3 Yolk and albumen pH

The difference in treatment in each sample showed an insignificant difference ($p>0.05$) to the yolk pH value. In the 14-day storage observation, 3 mL of TiO_2 1% solution added to edible coatings of nanocomposites casein-chitosan was more efficient to maintain yolk pH values when compared to other treatments in the same storage period. The buffer system mechanism in the yolk was still in good condition, so there was no significant change in the resulting yolk pH value. The changes in yolk pH occurred slowly while the value was not too large. The increase in pH value of yolk was due to several possibilities. Moreover, Mota et al. [16] explained that during storage, alkaline ions such as sodium, potassium, and magnesium in the egg move the albumen fluid towards the yolk leading to an increase in yolk pH value.

The treatment on the sample showed a significant difference ($p<0.05$) to the resulting albumen pH value. Observations within the storage period of 7 and 14 days showed that the addition of 5 mL of TiO_2 1% solution to edible nanocomposites casein-chitosan could produce a lower albumen pH value and be efficient in maintaining albumen pH value compared to other treatments in the same storage period. TiO_2 1% solution, as the constituent ingredients of edible nanocomposites casein-chitosan, can cover the eggshell pores evenly. It causes gas, moisture, and other materials from albumen not to come out of the egg through the eggshell pores, in which the buffer system on albumen remains good. According to Chen et al. [17], albumen in chicken eggs, covered by coatings from chitosan with the addition of different TiO_2 levels during the 20-day storage period, can be more stable in pH 8.0-8.2 compared to the control treatment at pH > 9.

3.1.4 Haugh unit

The different treatments in the sample showed a highly significant difference ($p<0.01$) to Haugh Unit value (Fig. 3). Observations on the chicken egg made during the 7-day storage period showed that the addition of 3 mL of TiO_2 1% solution in edible coatings of nanocomposites casein-chitosan could produce a higher Haugh Unit value compared to other treatments in the same storage period. However, in the 14-days storage period, the addition of TiO_2 1% 3mL solution to edible coatings of nanocomposites casein-chitosan was more efficient in maintaining the Haugh Unit value when compared with other treatments in the same storage period. Edible coatings of casein-chitosan with the addition of TiO_2 1% solution is effective to prevent the discharge of water vapour and gas from inside the eggs. Film coatings formed of chitosan coatings with protein and TiO_2 nano-therapies have strong moisture permeability resistance [18]. Hence, the Haugh Unit value does not decrease rapidly during the storage process.

3.1.5 TPC

The treatment on the sample showed an insignificant difference ($p>0.05$) to the resulting TPC value (Table 1). This might be due to several factors. Chitosan has a good antimicrobial activity against bacteria and fungi, characterized by low water activity value (a_w) that the film layer made, so bacteria, yeast, and incessant have lower chances to grow properly [19]. During the 14-day storage, a TPC value exceeded the maximum limit of microbial contamination following SNI standards of 1×10^5 CFU/mL. Moreover, nanoparticles are not sufficient to inhibit the growth of both gram-positive and negative bacteria (*S. aureus and E. coli*) in UV-insinuated conditions or not. Yet, in nanohybrid, Ag-TiO_2 showed better antimicrobial activity. Therefore, it is necessary to use additional ingredients to increase antimicrobial activity in the edible coatings solution used [20].

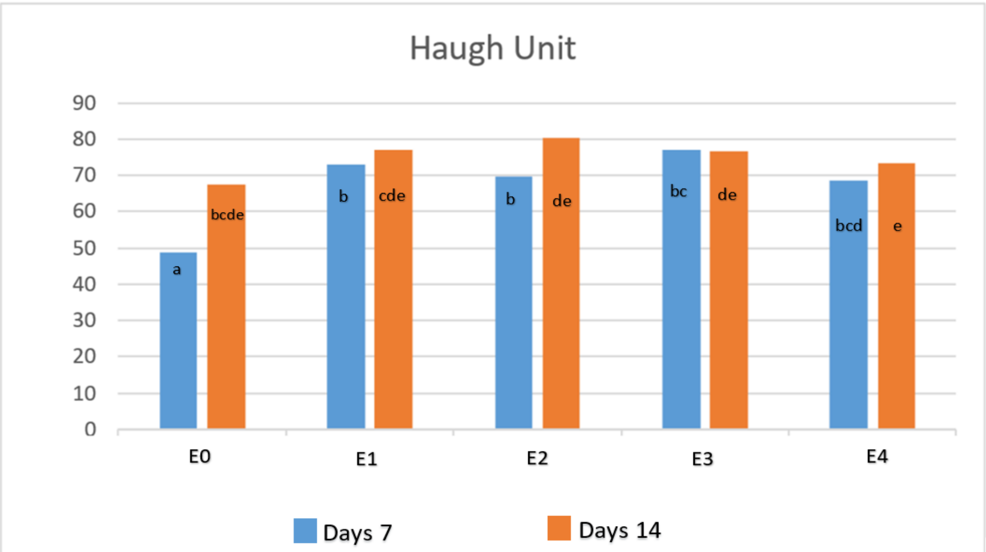

Fig. 3. Haugh unit for 7 and 14-day storage period

Description: [a,b,c,d,e] Different superscripts in the same column show a highly significant difference ($p<0.01$) to the Haugh Unit

Table 1. Average of yolk index, albumen index, yolk color, yolk pH, albumen pH

Trait	Time (days)	Group				
		E0	**E1**	**E2**	**E3**	**E4**
Yolk Index	7	0.32 ± 0.01^{b}	0.39 ± 0.02^{de}	0.39 ± 0.02^{e}	0.40 ± 0.01^{e}	0.39 ± 0.01^{de}
	14	0.25 ± 0.01^{a}	0.38 ± 0.02^{de}	0.35 ± 0.01^{bc}	0.36 ± 0.02^{cd}	0.40 ± 0.03^{e}
Albumen Index	7	$0.05 \pm 0,01^{b}$	0.07 ± 0.01^{cd}	$0.08 \pm 0,01^{d}$	0.07 ± 0.01^{cd}	0.07 ± 0.01^{cd}
	14	0.03 ± 0.01^{a}	0.06 ± 0.01^{bc}	0.06 ± 0.01^{bc}	$0.08 \pm 0,01^{d}$	0.06 ± 0.01^{bc}
Yolk Color	7	7.25 ± 0.95^{b}	7.25 ± 0.5^{b}	7.5 ± 0.58^{b}	8.75 ± 0.5^{cd}	9 ± 0.816^{d}
	14	6.25 ± 0.5^{a}	7 ± 0.82^{ab}	7.75 ± 0.5^{bc}	7.25 ± 0.5^{b}	$7.5 \pm 0,58^{b}$
Yolk pH	7	6.2 ± 0.14	6.32 ± 0.19	6,2 ± 0.41	6.27 ± 0.09	6.4 ± 0.29
	14	6.5 ± 0.29	6.5 ± 0.28	6.45 ± 0.19	6.55 ± 0.64	6.52 ± 0.50
Albumen pH	7	9.15 ± 0.17^{e}	8.32 ± 0.15^{ab}	8.37 ± 0.12^{ab}	8.17 ± 0.15^{a}	8.27 ± 0.09^{ab}
	14	9.2 ± 0.16^{e}	8.85 ± 0.17^{d}	$8.65 \pm 0,10^{cd}$	8.45 ± 0.10^{bc}	8.72 ± 0.20^{d}
TPC	7	0.9 ± 0.4	0.8 ± 0.4	0.8 ± 0.4	0.7± 0.5	0.8 ± 0.5
($x10^5$ cfu/ml)	14	1.2 ± 0.1	1.1 ± 0.4	1.1 ± 0.3	1.0 ± 0.6	0.9 ± 0.6

Means in the same row with different superscripted letters are significant difference ($p<0.01$) to the yolk index and highly significant difference ($p< 0.05$) abumen index, yolk color and albumen pH

4. CONCLUSION

The study results were that edible coatings of casein-chitosan with the addition of 5 mL of TiO_2 1% solution could maintain the internal quality of chicken eggs observed at a storage period of 7 to 14 days. The observation included the yolk-albumen index, Haugh Unit, albumen pH value, and yolk colour. From all the observations made on the sample, the storage period of 7 days showed a better result.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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