Original Research Article

Effects of addition of TiO_2 edible coatings and storage on the chicken eggs quality

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 or very significant difference. This study was conducted between April to September 2021 at the Faculty of Animal Science, Universitas Brawijaya. Edible coatings made from casein-chitosan with 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 TPC. The results showed that the yolk index ranged from 0,2523-0,4032; albumen index 0,0327-0,0825; haugh unit 48,6862-80.4220; yolk color 7-9; pH yolk 8,175-9,2; pH Albumen 8,175-9,2; Total Plate Count $0,7\times10^5$-$1,1\times10^5$ cfu/l. Edible coatings of casein-chitosan with 5 mL TiO_2 solution were able to reduce the decrease in 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.

Comment [MOU1]: Delete: "or very significant"

Comment [MOU2]: Delete it

Comment [MOU3]: replace haugh with Haugh, as it was introduced by Raymond Haugh. Make changes through ought the manuscript.

Comment [MOU4]: What is TPC, at least justify the abbreviation for first time

Comment [MOU5]: The reviewer think the authors made typing mistake y using "comma" instead of "point". Moreover, the reviewer suggests the authors to maintain 3 digits only (for example 0.33; 14.8; 103). Please make changes through ought the manuscript.

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Keywords: edible coatings, casein, chitosan, TiO_2, internal quality of eggs

1. INTRODUCTION

Eggs are human food and as almost perfect protein food, available rich nutrition, biological value as vitamins, minerals and fatty acids that are needed every day for the growth and maintenance of body tissues [1]. Nevertheless, the storage of egg can change and tend to degrade egg quality. The main factors that directly affect egg damage are temperature conditions, relative humidity, and storage time [2]. An egg contains complete amino acids and mostly minerals as well as 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, microbiological damage as well as evaporation of water, carbon dioxide, ammonia, nitrogen, and hydrogen sulfide from inside the egg [3]. Hence, the preservation is needed to avoid damage, maintain internal quality and extend the shelf life of chicken eggs. Edible coatings on eggs is one of the efforts to extend its shelf life. It aims to maintain the internal chicken eggs quality. The use of *edible coatings* is preservation 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 basic ingredients of making *edible coatings.* Chitosan has strong 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 produce [5,6]. In addition, chitosan is elastic, flexible, and difficult to tear. It has hydrophobic properties and is difficult to break by high heat. Casein-chitosan edible coatings are also formulated with other materials

such as lipid groups (glycerol) which are elastic and TiO_2. The compound of TiO_2 as anti-UV properties which can 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 include in the category of inorganic nanoparticles hydrophobic that can form hydrogen bonds with protein biopolymer chain polymers. Thus, creating 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 namely industrial waste pollution containing color substances, phenols, and its kind because of these compounds have an active and stable crusty to biological and chemical processes. Therefore, the objectives of this study were to investigate the effect of addition of TiO_2 edible coatings and storage on the chicken eggs quality (yolk index, albumen index, haugh unit, yolk color, yolk and albumen pH, and Total Plate Count).

2. MATERIAL AND METHODS

Comment [MOU7]: MM section must be improved and its is not clear to reviewer that if the coating was applied on the egg shell or only on the edible part of eggs (after cracked and opened)

2.1 Material

The material used in the study was a solution of edible coatings 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 taken from the age of the layer and the harvest on the same day.

Comment [MOU8]: Reviewer suggests the authors to explain experimental design in detail. Such as layer breed, age of the laying hens, environmental parameters of the layer farm. Total numbers of eggs collected and how many eggs were divided per treatment?, Eggs were washed before applying the coatings?, etc…..

Table 1. Treatment of addition of TiO_2 edible coatings and storage on the chicken eggs

Sample	Treatment	Stored for days
E0D7	Eggs without coating edible coatings (control)	7
E1D7	Eggs coated edible coatings of casein-chitosan	7
E2D7	Eggs coated edible coatings of casein-chitosan +TiO_2 1% solution as much as 3 mL (v/v)	7
E3D7	Eggs coated edible coatings of casein-chitosan +TiO_2 1% solution as much as 5 mL (v/v)	7
E4D7	Eggs coated edible coatings of casein-chitosan +TiO_2 1% solution as much as 10 mL (v/v)	7
E0D14	Eggs without coating edible coatings (control)	14
E1D14	Eggs coated edible coatings of casein-chitosan	14
E2D14	Eggs coated edible coatings of casein-chitosan +TiO_2 1% solution as much as 3 mL (v/v)	14
E3D14	Eggs coated edible coatings of casein-chitosan +TiO_2 1% solution as much as 5 mL (v/v)	14
E4D14	Eggs coated edible coatings of casein-chitosan +TiO_2 1% solution as much as 10 mL (v/v)	14

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2.2. Methods

2.2.1 Preparation and Application of edible coatings in eggs

Edible coating was made casein-chitosan (1:4 w/w), TiO_2 1% and egg coated the coating solution by dipping. The study was based on TiO_2 1% were added difference variations in the volume solution of edible coating casein-chitosan (3mL, 5mL, and 10mL) then applicated of the solution as coating on eggs. The egg stored at 25 ^{o}C 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). All experiments were perfomed in four replications. The treatments used in this study is shown in Table 1.

Comment [MOU10]: replace "perfomed" with "performed"

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

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

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

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

2.2.1.3 Haugh unit
Haugh unit was calculated using the formula [9]

$$Haugh\ unit = 100 \log (\text{H} + 7{,}57 - 1{,}7\ \text{W}^{0{,}37})$$

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

2.2.1.4 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

2.2.1.5 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.

2.2.1.6 Total Plate Count
Buffered peptone water 1% sterile was prepared 225 mL of into a sterile erlenmeyer flask which containing 25 mL of sample, homogenize 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 then make dilutions 10^{-3}, 10^{-4}, 10^{-5} etc. 15 mL to 20 mL of Standard Methods Agar (SMA) at a temperature of 45°C ± 1°C were added to petri dish that already contains 1 mL of suspension (duplo). Samples were incubated at a temperature of 34-36°C for 24 h to 48 h by placing the petri dish upside down. After that, calculate the average colony from the petri dish.

3. Statistical analysis
Data processed by Analysis of Variance (ANOVA). If the analysis shows a difference that is real difference (P<0.05) or very real difference (P<0.01), then using Duncan's Multiple Range Test.

3. RESULTS AND DISCUSSION

The internal chicken eggs condition on a storage period of 7 and 14 days is presented in Figure 2. While the difference effect of TiO_2 1% volume on edible coatings casein-chitosan on the internal chicken eggs quality is revealed in Table 2 and Table 3.

Comment [MOU11]: delete and replace commas with point

Comment [MOU12]: replace "roche" with "Roche"

Comment [MOU13]: Why the authors did not calibrate the pH meter with standard solution of pH 10? Reviewer is asking this because the pH value of albumen is always greater than 8 or near to 10

Comment [MOU14]: Replace "erlenmeyer" with "Erlenmeyer", as it is the name of the scientist. Please revise it thorough ought the manuscript.

Comment [MOU15]: replace "petri" with "Petri". Please revise it through ought the manuscript.

Comment [MOU16]: Reviewer suggests the authors to rewrite this section and do not right real difference or very real difference. It can be replaced with scientific words such as significant difference or highly significant difference.

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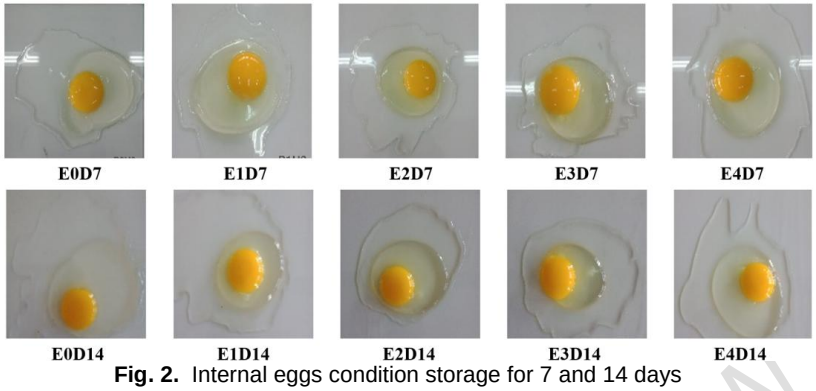


Fig. 2. Internal eggs condition storage for 7 and 14 days

Table 2. Average of yolk index and albumen index

Sample	Yolk Index	Albumen Index
E0D7	0,3206 ± 0,0155^{b}	0,0520 ± 0,0047^{b}
E1D7	0,3888 ± 0,0246^{de}	0,0754 ± 0,0134^{cd}
E2D7	0,3946 ± 0,0210^{e}	0,0803 ± 0,0141^{d}
E3D7	0,4032 ± 0,0087^{e}	0,0710 ± 0,0055 ^{cd}
E4D7	0,3891 ± 0,0160^{de}	0,0693 ± 0,0055^{cd}
E0D14	0,2523 ± 0,0082^{a}	0,0327 ± 0,0070^{a}
E1D14	0,3853 ± 0,0226^{de}	0,0644 ± 0,0078^{bc}
E2D14	0,3510 ± 0,0144^{bc}	0,0632 ± 0,0136^{bc}
E3D14	0,3598 ± 0,0208^{cd}	0,0825 ± 0,0086^{d}
E4D14	0,4008 ± 0,0348^{e}	0,0614 ± 0,0112^{bc}

Description: ^{a,b,c,d,e} Different superscripts in the same column show a very noticeable difference (P<0.01) to the yolk index and show a noticeable difference (P<0.05) to the albumen index

Comment [MOU18]: please replace it with highly significant or significant, respectively

3.1 Internal Condition of eggs

After 7 and 14 days of storage, the control egg underwent physical changes that were clearly visible in the yolk and albumen sections (E0D7 and E0D14). Yolk and Albumen on the control egg are more significant to widen than chicken eggs coated in edible coatings. According to Riawan et al [11], that the longer storage time will result in a lot of evaporation of liquids and gases in the egg, hence it will decrease the egg quality. In fact, this study has an influence on the rate of decline in internal chicken eggs quality. But the storage period of chicken eggs for 7 days is better than 14 days.

3.1.1 Yolk and Albumen Index

The treatment on the sample had a very significant difference (P<0.01) to yolk index and significant difference (P<0.05) to albumen index. After 14 days storage showed that the addition of TiO_2 1% solution in amount of 10 mL in edible coatings nanocomposites casein-chitosan was more efficient in maintaining yolk index value compared to other treatments during the same storage period. TiO_2 solution to edible coatings of nanocomposites casein-chitosan affects its microstructure, hence the chitosan matrix granules can be spread evenly on 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 vapor in the egg. This causes the osmotic pressure of yolk and albumen to remain stable, so the transfer of fluid from albumen to yolk is not too large. Those conditions can reduce the changes of yolk diameter and height during storage.

During the storage period of eggs for 7 days showed that the addition of TiO_2 1% solution of 3 mL in edible coatings fo nanocomposites casein-chitosan was able to produce a higher albumen index value compared to other treatments in the same storage period. However, the 14-days storage period of chicken eggs showed that the

addition of TiO_2 1% 5 ml solution to edible coatings of nanocomposites casein-chitosan was more efficient in maintaining albumen index value compared to other treatments in the same storage period. The good covered eggshel pores by layers of edible coatings did not result in evaporation of water and CO_2 from inside the egg. As a result, ovomucin on albumen did not suffer damage, hence the viscosity of Albumen was maintained. Widyastuti and Daydeva [13] stated that if Albumen loses CO_2 and changes pH, then ovomucin loses the ability to maintain viscosity. The more dilute the Albumen liquid then the height decreases which results in a decrease in the eggs quality of Albumen index.

Table 3. Average of yolk color, yolk pH and albumen pH

Sample	Yolk Color	Yolk pH	Albumen pH
E0D7	$7,25 \pm 0,9574^{b}$	$6,2 \pm 0,1414$	$9,15 \pm 0.1732^{e}$
E1D7	$7,25 \pm 0,5^{b}$	$6,325 \pm 0,1893$	$8,325 \pm 0,1500^{ab}$
E2D7	$7,5 \pm 0,5774^{b}$	$6,2 \pm 0,4082$	$8,375 \pm 0.1258^{ab}$
E3D7	$8,75 \pm 0,5^{cd}$	$6,275 \pm 0,0957$	$8,175 \pm 0,1500^{a}$
E4D7	9 ± 0.8165^{d}	$6,4 \pm 0,2944$	$8,275 \pm 0.0957^{ab}$
E0D14	$6,25 \pm 0,5^{a}$	$6,5 \pm 0,2944$	$9,2 \pm 0.1633^{e}$
E1D14	7 ± 0.8165^{ab}	$6,5 \pm 0,2828$	$8,85 \pm 0.1732^{d}$
E2D14	$7,75 \pm 0,5^{bc}$	$6,45 \pm 0,1915$	$8,65 \pm 0,1000^{cd}$
E3D14	$7,25 \pm 0,5^{b}$	$6,55 \pm 0,6455$	$8,45 \pm 0,1000^{bc}$
E4D14	$7,5 \pm 0,5774^{b}$	$6,525 \pm 0,5058$	$8,725 \pm 0.2062^{d}$

Description: ^{a,b,c,d,e} Different superscripts in the same column show a very noticeable difference ($P<0.01$) to the yolk index and show a noticeable difference ($P<0.05$) to the yolk color and pH albumen.

3.1.2 Yolk Color

The treatment on the sample provides significant difference ($P<0.05$) to the resulting yolk color value. Observations of the 7-day storage period of chicken eggs showed that the addition of a 1% 10 mL TiO_2 solution to edible coatings of nanocomposites casein-chitosan was able to produce a higher yolk color compared to other treatments in the same storage period. However, the 14-day storage period of chicken eggs showed that the addition of a 3 mL TiO2 solution to edible coatings was more efficient at maintaining yolk color compared to other treatments in the same storage period.

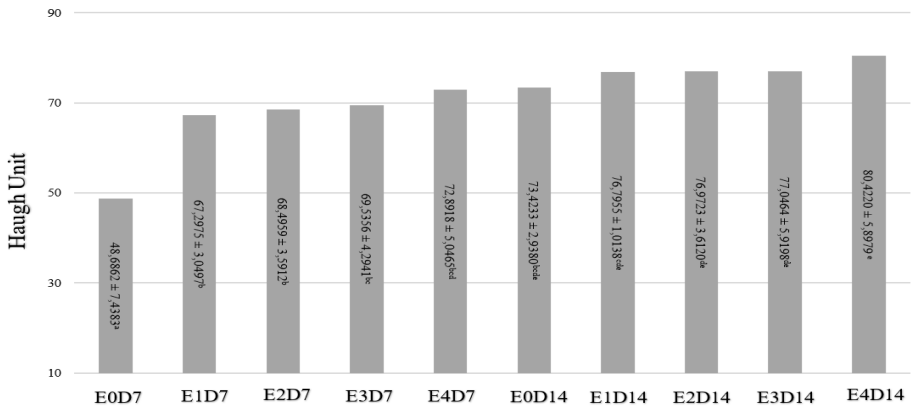
The color of the yolk is affected by the types of pigments namely carotene and riboflavin classified as lipochrome and lichrome contained in the rations consumed [14]. But in this study, it was ruled out because the rations and strains of chicken used have the same type, hence the yolk discoloration is influenced by other factors. According to Shinn [15], egg yolk index and yolk color can be affected by conjugated linoleic acid (CLA) which is the only lipid supplement. CLA egg supplementation induced an increase in egg yolk saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA) and a decrease in monounsaturated fatty acids (MUFA).

3.1.3 Yolk and Albumen pH

The difference in treatment in each sample gives an unsignificant difference ($P>0.05$) to the yolk pH value. The storage period of chicken eggs for 14 days showed that the addition of TiO_2 1% solution of 3 mL in edible nanocomposites casein-chitosan was more efficient in maintaining yolk pH values compared to other treatments in the same storage period. The buffer system mechanism in yolk is still in good condition, so there is no major change in the resulting yolk pH value. The change in yolk pH occurs slowly and the value is not too large. The increase in pH value of yolk is due to several possibilities. Moreover, Mota et al. [16] explained that during storage in chicken eggs, alkaline ions such as sodium, potassium, and magnesium can move the albumen fluid towards the yolk leading to an increase in yolk pH value.

The treatment on the sample makes significant difference ($P<0.05$) to the resulting albumen pH value. Observations made during the storage period of chicken eggs for 7 and 14 days showed that the addition of 5 mL TiO_2 1% solution to edible nanocomposites casein-chitosan was able to produce a lower pH value of albumen and was efficient in maintaining albumen pH value compared to other treatments in the same

storage period. TiO_2 1% solution as well as the constituent ingredients of edible nanocomposites casein-chitosan can cover the egg shell pores evenly. That causes gas, moisture, and other materials from Albumen not to come out of the egg through the eggshell pores, hence the buffer system on Albumen is in a good condition. According to Chen et al [17] stated that chicken eggs that have been coated by coatings from chitosan with the addition of different TiO_2 levels during the 20-day storage period, Albumen can be more stable in pH 8.0-8.2 compared to control treatment chicken eggs pH >9.



Description: [a,b,c,d,e] Different superscripts in the same column show a very noticeable difference (P<0.01) to the haugh unit

Figure 3. Haugh unit for 7 and 14 days

3.1.4 Haugh Unit

The difference in treatment the sample had a very significant difference (P<0.01) to haugh unit value (Figure 3). Observations made during the storage period of chicken eggs for 7 days showed that the addition of TiO_2 1% solution of 3 mL in edible coatings of nanocomposites casein-chitosan was able to produce a higher haugh unit value compared to other treatments applied in the same storage period. However, the 14 days storage period of chicken eggs showed that the addition of TiO_2 1% ml solution to edible coatings of nanocomposites casein-chitosan was more efficient at maintaining the haugh unit value compared to other treatments in the same storage period. Edible coatings of casein-chitosan with the addition of TiO_2 1% solution is effective in preventing the discharge of water vapor and gas from inside chicken eggs. Film coatings formed of chitosan coatings with protein and TiO_2 nanotherapies 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 provides an unsignificant difference (P>0.05) to TPC value which is resulted. This might due to several factors. While chitosan has good antimicrobial activity against bacteria and fungi characterized by low water activity value (a_w) the film layer made, hence small chances bacteria, yeast and incessant can grow properly [19]. Although the results of the variety analysis of 10 samples did not make an authentic difference (P>0.05) to the TPC value, there is a difference in the magnitude of the TPC value in each sample Table 4. During the 14-day storage period has a TPC value exceeding maximum limit of microbial contamination in accordance with SNI standards of 1×10^5 cfu/ml. Moreover, nanoparticles are not yet sufficient to inhibit the growth of both gram-positive and negative bacteria (*S. aureus and E. coli*) in UV-insinuated conditions or not, but in nanohybrid Ag-TiO2 showed better antimicrobial

activity. Hence, it is necessary to add other ingredients which increase antimicrobial activity in the edible coatings solution used [20].

Table 4. Average of Total Plate Count

Treatment	Total Plate Count (x10^5 cfu/ml)
E0D7	0,9 ± 0,4
E1D7	0,8 ± 0,4
E2D7	0,8 ± 0,4
E3D7	0,7± 0,5
E4D7	0,8 ± 0,5
E0D14	1,2 ± 0,1
E1D14	1,1 ± 0,4
E2D14	1,1 ± 0,3
E3D14	1,0 ± 0,6
E4D14	0,9 ± 0,6

4. CONCLUSION

The results of the study is concluded that edible coatings of casein-chitosan with the addition of TiO_2 1% solution as much as 5 mL can maintain the internal chicken eggs race quality observed at a storage period of 7 to 14 days including yolk-albumen index, haugh unit, pH of albumen and yolk color. But, from all the observations made on the sample, the better results were shown in observations made during the storage period of 7 days.

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Comment [MOU19]: The authors can follow similar format while citing references. Choose either APA or other formats for citation…

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