Optimization of Eggshell Calcium Use for Improving the Physical Quality of Whey Protein Isolate-based Edible Films

Herly Evanuarini[1,*] , Agus Susilo[1], and Premy Puspitawati Rahayu[1]

[1]Faculty of Animal Science, Universitas Brawijaya, Malang 65145, East Java, Indonesia

Abstract. Edible films based on biopolymers are increasingly popular as environmentally friendly and functional food packaging alternatives. One approach to improving the physical properties of edible films is by incorporating calcium derived from eggshell waste. This study aims to develop and characterize edible films made from whey protein isolate (WPI) with the addition of eggshell calcium at various concentrations. The study employed a completely randomized experimental design with four treatments: the addition of eggshell calcium at 0.25%, 0.50%, 0.75%, and without eggshell calcium (w/v) of the total volume of the film-forming solution, with five replicates per treatment. Characterization included measurements of thickness, water solubility, opacity, swelling rate, moisture content, and color parameters (L, a*, b*). ANOVA results showed that the addition of eggshell calcium had a highly significant effect ($p<0.01$) on solubility, opacity, swelling rate, a* and b* values in the edible film. Apart from that, it had a significant effect ($p<0.05$) on the thickness and L value, and had no effect ($p>0.05$) on the moisture content of the edible film. The addition of eggshell calcium significantly increased film thickness with increasing concentration. Film solubility decreased dramatically with calcium addition, indicating increased water resistance. Film transparency decreased at higher calcium concentrations. The degree of swelling and water content of the film also decreased with calcium addition, indicating a reduced affinity of the film for water. The film color changed: L values, a* values, and b* values tended to decrease compared to the control. The research results indicate that eggshell calcium can be utilized as a functional additive to improve the physical properties of WPI-based edible films, while also adding value to agroindustrial waste.

Keywords: edible film, whey protein isolate, eggshell, calcium, agroindustrial waste.

* Corresponding author: herlyfptub@ub.ac.id

1 Introduction

Edible films have developed into an attractive alternative technology in modern food packaging systems due to their ability to extend product shelf life, improve food safety, and reduce the use of environmentally unfriendly plastic-based packaging [1]. The demand for packaging materials that are not only biodegradable but also functionally active is increasing, along with consumer awareness of sustainability and food health issues [2]. One of the main ingredients widely used in the development of edible films is chitosan, which is a natural polysaccharide deacetylated from chitin. Chitosan is known for its superior characteristics such as biodegradability, biocompatibility, non-toxicity, and good antimicrobial ability [3].

However, single chitosan-based edible films often have disadvantages in terms of water resistance and low mechanical properties, such as insufficient tensile strength and elasticity [4]. This hinders their widespread use in food applications that require high stability. To overcome these drawbacks, various innovative approaches have been taken, one of which is to combine chitosan with other materials such as proteins, lipids, and inorganic calcium [3]. Whey Protein Isolate (WPI), as one of the milk protein components with excellent gelling and film-forming properties, has been shown to improve the quality of film structure when combined with chitosan [5]. Previous studies have shown that the WPI-chitosan combination produces films with better mechanical and barrier characteristics compared to single use [6].

One of the emerging innovative strategies to improve edible film characteristics is the utilization of natural calcium sources as film matrix reinforcing agents. In this context, eggshell is a very potential material. Eggshell is a household and food industry waste that contains more than 90% calcium carbonate ($CaCO_3$), generally in the form of aragonite or calcite [8]. The utilization of eggshells not only adds value to organic waste, but also contributes to environmental sustainability [14].

Through simple processing processes such as calcination and pulverization, calcium from eggshells can be utilized as an additive in edible film formulations. This calcium plays a role in strengthening the film structure, improving mechanical properties such as tensile strength and flexibility, and can contribute to antimicrobial activity [9]. In addition, its use in combination with chitosan and WPI is expected to produce film systems that are more stable, transparent, and have better oxygen and water vapor barrier capabilities.

To date, research on the utilization of eggshell calcium in edible films based on the combination of WPI and chitosan is still limited [11]. In fact, this approach has the potential to produce an environmentally friendly, functional, and economical packaging system. Therefore, this study aims to evaluate the potential of utilizing calcium from eggshells as a reinforcing agent in edible films based on a combination of WPI and chitosan. Hopefully, this formulation not only improves the physical and functional properties of the film, but also becomes a sustainable solution in the development of bio-based active packaging.

2 Materials and methods

2.1 Materials

WPI (Whey Protein Isolate) was obtained from a commercial food supplier, chitosan was obtained from Panadia Laboratory Malang City, while chicken eggshell was obtained from household waste. The chemicals used included acetic acid (chitosan solvent), and pro-analyzed glycerol (as plasticizer) [12]. All materials used were of food grade or analytical grade quality.

2.2 Methods

This study used a one-factor completely randomized design (CRD) with four treatment levels (P0-P3), each with five replications. Parameters observed included thickness, solubility, moisture content, transparency, swelling degree, and color (L*, a*, b*).

Table 1. Experimental design and research treatment

Treatments	Descriptions
P0	WPI-CH edible film without calcium eggshell
P1	WPI-CH edible film with 0.25% calcium eggshell
P2	WPI-CH edible film with 0.50% calcium eggshell
P3	WPI-CH edible film with 0.75% calcium eggshell

2.3 Preparation of calcium eggshell powder

Eggshells were cleaned from membranes and dirt, then washed, drained, and dried (50°C oven for 8 hours or drying). Next, the eggshells were calcined at high temperature (600-900 °C) for 2 hours to convert $CaCO_3$ into calcium oxide (CaO) and burn the remaining organics [7]. The grayish-white calcined product is then ground using a mortar or ball mill to a powder. The powder was stored in a desiccator to prevent moisture absorption before use.

2.4 Edible film preparation

The main ingredients of edible food are whey protein, chitosan, glycerol, and extracted calcium. The manufacturing procedure began with 2 g of chitosan weighed into a 250 mL beaker and then dissolved in 2% acetic acid in a ratio of 2 g chitosan: 100 mL (2 mL of 2% acetic acid and 98 mL of distilled water) and added the extracted calcium from eggshells (treatments of 0.25, 0.50 and 0.75%). The chitosan solution was then transferred to a magnetic stirrer and stirred for 60 minutes at 45± 2°C and 500 rpm followed by the addition of glycerol 0.28% of the total solution as plasticizer. Whey protein isolate was dissolved with distilled water in the ratio of 2.5g WPI: 100mL distilled water. The WPI solution was then transferred to a magnetic stirrer and stirred for 30 minutes at 45± 2°C and 500 rpm followed by the addition of glycerol 0.28% of the total solution. The chitosan and WPI solution was homogenized using a magnetic stirrer at 45± 2°C for 60 minutes. After five minutes, the film-forming solution was poured into a 150 mm diameter petri dish and allowed to dry for 48 hours [2].

2.5 Parameter measurements

Thickness Testing: Film thickness was measured using a digital screw micrometer with an accuracy of 0.001 mm. Measurements were taken at five random points in each film sample, then averaged [4].

Water Solubility Test: The solubility test was carried out by gravimetric method. Pieces of film (size ±2×2 cm) were weighed initially dry weight (W1). The sample was then immersed in 50 mL of distilled water at room temperature for 24 hours without stirring. After soaking, the remaining insoluble film was carefully removed, cleaned of excess water with filter paper, and then dried in a 105°C oven to a constant weight (W2). Percent solubility was calculated as: (W1 - W2/W1)× 100. This value expresses the percentage of film mass that dissolves into water [10].

Transparency (Opacity) Test: Film transparency was assessed optically using a UV-Vis spectrophotometer. Pieces of film (sized to fit the spectrophotometer cuvette) were placed on the optical path, then the transmittance or absorbance was measured at a wavelength of 600 nm. The wavelength of 600 nm was chosen because it is in the visible spectrum (for turbidity) and is not specifically absorbed by the colored film components. The absorbance value at 600 nm (A600) is used to calculate the *opacity of* the film with the formula: A600/thickness. The higher the opacity or A600 value, the less transparent (more opaque) the film [10].

Swelling Degree Test (Water Absorbency): The degree of swelling was determined by the short-term immersion method. Dry film pieces (~2 cm in diameter) were weighed as initial weight (Wi). The sample was then immersed in distilled water (20 mL) at room temperature for 1 hour. After 1 hour, the film was removed, excess water on the surface was removed with a tissue, and the film was immediately weighed to obtain the wet weight (Wt). The swelling percentage was calculated as: (Wt - Wi/Wt)× 100. This value represents the capacity of the film to absorb water (within 1 hour). The test was carried out at least triplicate and avoided too long soaking time so that the film would not disintegrate before weighing [11].

Film Moisture Content Test: Moisture content was calculated using the gravimetric drying method. Pieces of film (~2×2 cm) were weighed as the initial sample weight (W0), the cup was weighed as (W1), then dried in a 105 °C oven for ±24 hours until a constant weight was reached (W2). Moisture content (%) was calculated by the formula: (W0 - W2/W0 - W1)× 100 [11].

Color Measurement (L, a*, b*): The color of the film surface was analyzed using a colorimeter (e.g. HunterLab or Minolta ColorFlex) calibrated against a white standard. The measurements follow the CIE Lab scale: L represents brightness (0 = black, 100 = white), a* represents green-red gradation (negative values = green, positive = red), and b* represents blue-yellow gradation (negative = blue, positive = yellow). The instrument was calibrated using standard white tiles before testing. Each film sample was color measured at at least 5 random points, then averaged [13].

2.6 Data analysis

All test data were statistically analyzed. Mean values and standard deviations were taken for each treatment. One-way ANOVA test was used to see the effect of calcium on each parameter, followed by Duncan's test at 5% significance level to compare between treatments. Interpretation of the results was done by comparing the data trends and statistical significance, and relating them to theory and previous research results.

3 Results and discussion

Table 1. Thickness, water solubility, and opacity of WPI-CH edible film with calcium eggshell powder

Treatment	T (mm) ± SD	WS (%) ± SD	Opacity (mm^{-1}) ± SD
P0	0.079^{a}± 0.01	0.185^{c}± 0.18	6.583^{a}± 0.45
P1	0.090^{a}± 0.01	0.143^{b}± 0.14	8.165^{c}± 0.44
P2	0.091^{a}± 0.01	0.102^{b}± 0.10	8.723^{bc}± 0.23
P3	0.103^{b}± 0.01	0.041^{a}± 0.04	10.974^{c}± 0.43

Note: Where T (Thickness); WS (Water Solubility). Notations on the same row indicate significant differences ($p<0.05$) of T and very significant differences ($p<0.01$) of WS and opacity.

3.1 Thickness

Thickness measurement results showed that the addition of eggshell calcium significantly increased the thickness of WPI-chitosan edible film. The film without calcium (P0) had the lowest thickness, while the addition of 0.75% calcium (P3) produced the thickest film. The increase in thickness from P0 to P3 reflects the increase in total solids in the film matrix with the addition of inorganic powder. The dispersed eggshell calcium particles will fill the space between the polymer matrix, so that the film volume per unit area becomes larger [1, 7, 15]. This trend of increasing thickness due to calcium addition is in line with the results of a study that found that the addition of eggshell microparticles increased the thickness of pectin-based biocomposite films [1]. Another study on protein films also reported similar results by adding TiO_2 and $CaCO_3$ nanoparticles in a chitosan-protein system and found an increase in thickness with $CaCO_3$, along with an increase in the total solids content of the film [6]. Although thickness increases, it should be noted that this can affect other properties such as transparency and mechanical strength. In the context of packaging films, higher thickness is generally desirable to some extent as it correlates with improved barrier properties. However, excess thickness can reduce the flexibility and aesthetics of the film transparency. In this study, the range of film thickness was within edible packaging standards, and the increase in thickness was a positive effect of calcium addition.

3.2 Water solubility

The addition of eggshell calcium had a significant impact in reducing the solubility of WPI-chitosan films in water. The P0 control film (without calcium) showed the highest percent solubility, meaning that part of the film matrix dissolved when immersed for 24 hours. Meanwhile, the P1-P3 films had lower percent solubility as the percentage of calcium increased. In the P3 treatment (0.75%), the solubility of the film was the lowest, indicating an increase in the film's resistance to water dissolution. This decrease in solubility indicates that the film structure becomes more stable in water, possibly due to the interaction between the calcium particles and the polymer which reduces the soluble component or because the calcium particles block the diffusion of water into the matrix. This result is in line with a study that reported that the addition of *eggshell microparticles* significantly decreased the water solubility of biopolymer films [1]. The mechanism found is that $CaCO_3$ reduces porosity and forms a tighter network, making it difficult for water to penetrate and dissolve the film components. In addition, Ca^{2+} ions from calcium can interact with charged groups (e.g. carboxylate in proteins or -OH in chitosan) to form additional bonds that hold the polymer from escaping into solution [4]. Hydrogen interactions between the hydroxyl and amino groups of chitosan with the surface of $CaCO_3$ nanoparticles form a network that is more stable and resistant to water degradation [7,15]. The in-situ modification process used in this study also improves the compatibility between the nanoparticles and the polymer matrix, resulting in a homogeneous system that is more resistant to dissolution. With lower solubility, the film is more suitable for use as food packaging with high water content, as it does not easily disintegrate or dissolve during contact with food.

3.3 Opacity

The transparency or clarity of the film increased as the percentage of calcium increased. Visually, the P0 film appeared clear/transparent, while the films with eggshell addition appeared increasingly cloudy or opaque, especially at P3. Opacity measurements at 600 nm confirmed the increase in absorbance/opacity of the films with calcium content. An increase in opacity value indicates that less visible light can penetrate the film, due to light scattering by solid particles in the matrix. Micro-sized $CaCO_3$ particles tend to scatter light and reduce the translucency of the film. For packaging applications, decreased transparency can be a drawback if a visible product display is required. However, higher opacity can be beneficial as UV or light shielding, preventing photo-oxidation in light-sensitive food products [11]. In this context, WPI-chitosan films with calcium can provide additional protection against UV light as $CaCO_3$is also known to increase the UV resistance of films (similar to how white TiO2 functions as a UV blocker). The results of this study are also in line with observations by [15], where films with nanoparticles showed higher absorbance values at visible wavelengths.

Table 2. Swelling rate and moisture content of WPI-CH edible film with calcium eggshell powder

Treatment	SR (%) ± SD	MC (%) ± SD
P0	87.931^d± 0.29	1.006 ± 0.0005
P1	84.756^c± 0.43	1.005± 0.0008
P2	83.201^b± 0.78	1.005± 0.0011
P3	79.883^a± 0.30	1.004± 0.0018

Note: Where SR (Swelling Rate); MC (Moisture Content). Notations on the same row indicate very significant differences ($p<0.01$)

3.4 Swelling rate

Swelling degree describes the film's ability to absorb water before reaching saturation point. This parameter is important as it is directly related to the stability of the film when in contact with aqueous food products. The results showed that the control film (P0) had the highest degree of swelling, while the P3 treated film had the lowest swelling value. The decrease in swelling degree is due to the presence of $CaCO_3$ which strengthens the polymer network through the formation of hydrogen bonds and electrostatic interactions [4]. This makes the film structure more resistant to water penetration. As stated by [7] nanoparticles function as fillers that minimize the pore space in the film, thus limiting water diffusion. The addition of $CaCO_3$ from eggshell improves the resistance to swelling, which is an important attribute for wet food packaging applications. This performance gets better with increasing nanoparticle concentration, showing a positive correlation between the amount of calcium and resistance to water [13].

3.5 Moisture content

Moisture content in edible films plays a role in determining elasticity, storage stability, and microbiological risk. The measurement results showed that the moisture content decreased as the percentage of calcium addition increased. The control film (P0) showed the highest moisture content, while the P3 treated film had the lowest moisture content. This decrease in moisture content is thought to be the result of an increase in film tissue density due to the addition of nanoparticles, which inhibits the penetration and retention of water molecules in the film matrix. The $CaCO_3$ content in eggshell calcium has an active surface that allows the formation of interactions with polar groups in chitosan and protein, thus reducing the free space for water [4]. This is in accordance with the results of a study stating that the presence of nanoparticles can limit the diffusion of water into the film through microstructural changes. The decrease in water content is also attributed to the increase in intermolecular bonds in the film, which makes the system more compact and less hygroscopic [10, 13]. This is a plus in the context of applications in food packaging with high moisture content.

Table 3. The average color of WPI-CH edible film with calcium eggshell powder

Treatment	L± SD	a* ± SD	b* ± SD
P0	89.331^{b}± 0.85	0.523^{b}± 0.11	9.867^{b}± 0.82
P1	88.205^{a}± 0.77	-1.134^{a}± 0.47	8.015^{ab}± 0.77
P2	88.055^{a}± 0.71	-1.174^{a}± 0.22	7.832^{ab}± 0.73
P3	87.324^{a}± 0.87	-1.533^{a}± 0.87	6.501^{a}± 0.68

Note: Notations L and b* on the same row indicate significant differences ($p<0.05$), a* on the same row indicate very significant differences ($p<0.01$).

3.6 Color

Color measurement with a colorimeter showed changes in the L*, a*, b* parameters of the film due to calcium addition. The P0 control film was clear transparent with a slight yellowish tinge (relatively high L* value, a* close to 0, b* slightly positive). After calcium addition, the L* value of the film tends to decrease, meaning that the film becomes more opaque dark, a* and b* values tend to decrease compared to P0. The decrease in a* (towards green/neutral) and b* (towards blue/neutral) is where the film loses some of its original yellowish color and shifts towards a more neutral or bluish spectrum. Visually, the P1-P3 films appear milkier than expected as the eggshell powder is dark white in color. This change is most likely due to the optical characteristics of the calcium obtained from eggshell, which is not completely pure white and carries residual organic pigments from the shell matrix [1]. The modification process does reduce this tendency, but does not eliminate it completely. The addition of such particles causes changes in the absorbance and reflectance of light at the film surface [9].

4 Conclusion

The incorporation of calcium derived from eggshells into Whey Protein Isolate (WPI)-chitosan edible films affected their physical characteristics. Increasing calcium concentration up to 0.75% (w/v) produced films that were thicker, less water-soluble, and exhibited reduced water absorption and moisture content, indicating enhanced water resistance due to the less hydrophilic nature of $CaCO_3$ compared to the WPI-chitosan matrix. However, this improvement was accompanied by greater opacity, which may restrict applications requiring transparency. The summary emphasizes physical and water-related properties but does not address mechanical performance (e.g., tensile strength, flexibility) or barrier properties (e.g., resistance to oxygen and water vapor), which are essential for assessing suitability as food packaging material. For future work should therefore aim to evaluate the tensile strength and barrier performance of the composite films and optimize the calcium concentration to find an ideal balance.

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