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EFFECT OF CHITOSAN COATING ON CHICKEN EGG QUALITY DURING SHELF LIFE

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Table of Contents

1. Introduction

Eggs have long been a fundamental part of the human diet for centuries due to their exceptional nutritional profile and versatile functional properties. Eggs are packed with excellent protein quality, essential long-chain fatty acids, vital micronutrients, and key vitamins The wide range of eggs' functional properties such as foaming, gelling, emulsifying, coagulating, coloring, and flavoring contribute significantly to the industry from savory dishes to sweet treats. Global egg production and consumption have steadily increase over the years as the widespread availability and high digestibility of eggs meet the needs of diverse populations. However, eggs are highly perishable and susceptible to quality degradation due to various environmental factors. Therefore, it is essential to explore and identify effective strategies to preserve egg shelf life. Coatings have gained increasing attention as an effective approach to extend egg shelf life in the recent years. This innovation could contribute the maintenance of egg freshness and lower the risk of breaking during handling. Chitosan is a promising material that has been extensively studied for coating of eggs. The material could offer a strong barrier to gases, microbes and also biodegradable, eco-friendly. With researches continue, chitosan protective layer is anticipated to take on more important role in improving food safety, reducing food waste, and enhancing overall sustainability in egg production and distribution. The concern over different regulations across countries could be significant. US, Canada, Australia, and Japan adopt hot water washing followed by refrigerated storage while many European and Asian countries avoid washing eggs and store them at ambient temperature to maintain the natural barrier. Different countries, therefore, mandate tailored approaches that depend on the requirements. This approach could represent the future of the egg industry, enabling the production of high-quality products and enhancing consumer quality of life, while promoting sustainability in the long run.

2. Aim

The aim of this thesis is to investigate the effects of chitosan coating alone and in combination with sorbitol as a plasticizer on egg shelf life. Chicken eggs size M were selected for this study. Egg quality was evaluated based on parameters such as weight loss (WL), Haugh unit (HU), yolk index (YI), white index (WI), and air cell height (AC). In the first experiment, different concentrations of chitosan coating (1.5%, 2.0%, 2.5%, 3.0%) were evaluated. In the second experiment, the coatings contain sorbitol at varying concentrations (1.0%, 2.0%, 3.0%) with a fixed chitosan concentration of 2.0%. Both experiments were conducted in 4 weeks period at room temperature. The investigation assessed the coating's effectiveness and identified the potential concentration for industrial application.

3. Literature review

3.1 Egg characteristic and protective mechanism

3.1.1 Egg natural protective barrier

Egg has main protective barriers: membrane, shell and cuticle. These barriers play an important role in protecting egg against penetration of harmful microorganisms (Baron and Jan, 2011). The inner shell membrane showed the greatest barrier effectiveness, followed by the egg shell and the outer membrane (Lifshitz et al., 1964).

3.1.1.1 The cuticle

The cuticle is the outermost thin layer covering the eggshell of an egg and it serves as the first protective barrier against physical damage and microbial contamination (Kulshreshtha et al., 2018). This layer fills and partly seals the pores in the shell, could extends into pores up to 50 μm (Kulshreshtha et al., 2018; Sparks and Board, 1984). The cuticle is mainly made up of mucoproteins (glycoproteins) with a small amount of polysaccharides and lipids (Baker and Balch, 1962; Hasiak et al., 1970; Rose-Martel et al., 2012; Wedral et al., 1974). The mucoprotein and lipid content make the surface resistant to water and limit water loss, thereby protecting the egg from external factors (Baron and Jan, 2011). Low quality cuticle can expose the egg to a higher risk of microbial penetration. It also negatively influences its water vapour conductance and compromise the egg's protective function (Peebles and Brake, 1986). Moreover, several cuticle proteins, such as ovocalyxin-25 (OCX-25), ovocalyxin-32 (OCX-32), ovocalyxin-36 (OCX-36), clusterin, and lysozyme C, have been shown to be effective against pathogens (Chien et al., 2008; Gautron et al., 2001; Hincke et al., 2000). Hen genetics, diet, housing, and egg processing can all influence the cuticle quality (Bain et al., 2013; Board and Halls, 1973; Rodríguez-Navarro et al., 2013). The composition of the cuticle changes rapidly after laying. Within 24 hours after laying, the cuticle dries and the eggshell becomes significantly more permeable (Rodríguez-Navarro et al., 2013).

3.1.1.2 The eggshell

The eggshell is the rigid, semi-permeable, mineralized covering of an egg that serves as the second protective barrier after the cuticle (Sharaf Eddin and Tahergorabi, 2019). Shell mineralization begins in the distal isthmus, where calcification occurs on organic aggregates present on the outer membrane surface. In the uterus, the calcite crystals grow outward for about 20 hours, forming the mammillary (inner) and palisade (outer) layers. Mature eggshell consists

of approximately 95% calcium carbonate and 3.5% organic matrix composed mainly of glycoproteins and proteoglycans (Hincke et al., 2000). A study using extracts of eggshell soluble matrix (0.1 mg/ml) demonstrated inhibitory effects against microorganisms such as *Pseudomonas aeruginosa, Bacillus cereus*, and *Staphylococcus aureus* for more than 8 hours. On the other hand, the same protein extracts suppressed the growth of *Salmonella enteritidis* and *Escherichia coli* only temporarily, for approximately 4 hours (Aygun, 2017). The eggshell contains about 6000 to 17 000 per egg with diameters ranging from 0.022 to 0.054 mm. The presence of pores decreases shelf life by enhancing trans-shell diffusion of gases and moisture, thereby promoting dehydration and structural degradation of albumen proteins (Gabriela da Silva Pires et al., 2020).

3.1.1.3 Membranes

The hen's egg contains two membranes beneath the shell. The outer membrane is located adjacent to the calcified shell, while the inner membrane is located next to the egg white. The outer shell membrane consists of three layers: one of keratin fibers and two of mucin fibers. The inner membrane contains two layers that are mainly composed of keratin and mucin fibers (Lifshitz et al., 1964). The membranes was reported to have significant amount of keratin (Baker and Balch, 1962). Lifshitz et al (1964) found that the inner shell membrane prevented bacterial movement better than the outer membrane. Mineral analysis revealed the inner shell membrane has a significant amount of calcium. This finding suggests that calcium plays an important structural role in stabilizing the shell matrix. This was further supported by changes in membrane permeability to radioactive amino acids in a study by (Wedral et al., 1974). The components in the membranes, such as glycoproteins, lysozyme, β-N-acetylglucosaminidase, and ovotransferrin can provide antimicrobial protection (Ahlborn and Sheldon, 2005; Gautron et al., 2001; Hincke et al., 2000).

3.2.2 Egg's internal physicochemical changes during storage

Internal changes are largely dependent on storage duration. As storage time increases, egg quality progressively declines and leads to deterioration. These alterations are typically the thinning of the albumen, an increase in pH, rupture of the vitelline membrane, and a rise in yolk water content, among other physicochemical modifications (Omana et al., 2011; Wang et al., 2019; Yuceer and Caner, 2014). Freshly laid eggs contain approximately 1.44-2.05 mg of CO_2 per gram of albumen and this can rapidly drop only within the first few hours after laying (Biladeau and Keener, 2009; Keener et al., 2001). In the albumen, the weak carbonic acid

gradually decomposes, releasing additional CO_2 and water. This process leads to thinning of the egg white and an increase in albumen pH (Mountney, 2017). Permeation of water vapor and CO_2 through the egg shell also contributes to an increase in the internal pH of the egg (Grashorn, 2016). A statistically significant negative correlation ($P \leq 0.01$) has been reported between the ovomucin content and albumen pH under varying storage temperatures (Wang et al., 2019). The albumen viscosity decrease as storage time increases due to the binding capacity between ovomucin and lysozyme diminishes (Acker and Ternes, 1994). The breakdown of amino acid chains releases water bound to large protein molecules, further degrades the viscosity of the thick albumen (Gabriela da Silva Pires et al., 2020). Extended storage period results in the loss of vitelline membrane integrity as a consequence of glycoprotein II degradation. The gradual degradation of the vitelline membrane facilitates the water migration from the albumen to the yolk, further flattening the yolk (Bermudez-Aguirre and Niemira, 2023; Ragni et al., 2007). Beside the storage duration, several other factors influence shell egg quality includes hen age, storage temperature, and relative humidity (Wang et al., 2019).

3.2 Egg quality

The quality of eggs is commonly evaluated through two main approaches: external and internal assessment. The external quality of table eggs is determined by examining characteristics such as shell color, strength, and the presence of cracks (Sharaf Eddin et al., 2019). Meanwhile, internal assessment focuses on the condition of the albumen and yolk, considering parameters such as height, viscosity, color, pH, and the presence of internal defects (Bermudez-Aguirre and Niemira, 2023; Sharaf Eddin et al., 2019).

3.2.1 External assessment

3.2.1.1 Shell breaking strength and thickness

The external quality of egg relies mainly on shell thickness and breaking strength (Bermudez-Aguirre and Niemira, 2023). Shell strength is evaluated through the amount of force required to crack the eggshell and the unit is expressed in Newtons (N) or kilogram-force (kgf). Shell strength has an average value of about 4.2 N and a minimum acceptable range between 3.0 and 3.5 N (Bermudez-Aguirre and Niemira, 2023). Shell strength is evaluated by pressing the egg between two pressure plates until the shell cracks. A low shell strength increases the likelihood of economic losses and microbial contamination. Shell strength is strongly correlated with organization of the shell matrix and the shell thickness (Sharaf Eddin et al., 2019). The shell thickness typically ranges from 0.2 to 0.57 mm, with an average value of 0.4 mm and the

minimum acceptable thickness is 0.3 mm (Gabriela da Silva Pires et al., 2020). A positive correlation has been reported between thickness uniformity and breaking strength. Consistent shell thickness has shown to enhance mechanical resistance (Dong et al., 2017). These parameters are important as they are able to determine the fragility and viability of the egg during handling, packaging, and storage.

3.2.1.2 Egg crack

Cracked eggs increase a significant risk of microbial contamination, quality deterioration and show major economic losses in egg production (Sharaf Eddin and Tahergorabi, 2019). Careful visual inspection and handling are essential for crack detection and prevention. Even a hairline crack can increase the risk of bacterial penetration and reduce egg's shelf life (Zhao et al., 2010). Shell cracking is influenced by poor shell quality, genetic factors, improper manual handling, wash water quality, and inappropriate storage temperature (Jindal & Sritham, 2003). Automated systems such as high-speed electronic grading machines and acoustic resonance frequency analysis have been implemented for rapid and accurate identification of shell cracks (Sharaf Eddin and Tahergorabi, 2019).

3.2.2 Internal assessment

3.2.2.1 Haugh unit

In most studies, Haugh unit (HU) is a standard indicator of egg freshness (Gabriela da Silva Pires et al., 2020). HU value depends on the height of the thick albumen and the egg's weight (R.r, 1937). The decrease in HU values indicates a decline in egg internal quality. This mainly results from albumen weakening due to H_2CO_3 breakdown, increased pH, and the resulting watery egg white (Eke et al., 2013; Yuceer and Caner, 2014).

Table 1: Egg's quality classification based on Haugh Unit
(Source: own editing based on USDA, 2020)

HU	Grade
HU > 72	AA
71 > HU > 60	A
59 > HU > 31	B
HU < 30	C

Temperature and storage duration significantly affect the Haugh unit. Fikiin et al (2020) reported that the Haugh unit (HU) remained nearly constant for approximately 20 days when eggs were stored at 10 °C. Whereas, storage at 30 °C caused the HU to drop sharply from 85 to 35.

3.2.2.2 Yolk index

Yolk index (YI) is another parameter can be used to assess egg freshness. YI is defined as the ratio of yolk height to its corresponding diameter. The values above 0.38 correspond to extra-fresh eggs, values between 0.28 and 0.38 indicate fresh eggs and values below 0.28 are classified as regular eggs (Bermudez-Aguirre and Niemira, 2023). The YI is highly depends on yolk viscosity and vitelline membrane integrity (Bermudez-Aguirre and Niemira, 2023; Li et al., 2017). YI values decrease progressively as they degrade over time (Bermudez-Aguirre and Niemira, 2023). Vitelline membrane is influenced by storage time, temperature, relative humidity, and egg handling practices (Bermudez-Aguirre and Niemira, 2023). Experimental data indicate that the vitelline membrane strength declines sharply from 463.40 g in fresh eggs to 162.80 g after one week of storage at 25 °C (Kirunda and McKee, 2000).

3.2.2.3 Weight, air cell and pH

Weight loss serves as an indicator of egg quality and shelf-life stability (Gabriela da Silva Pires et al., 2020; Xu et al., 2018). Prolonged storage leads to moisture evaporation through the eggshell pores, which results in weight loss and progressive internal quality decline (Caner and Yuceer, 2015; Yüceer and Caner, 2020). Air cell is also affected by prolonged storage, typically increasing in size as moisture and carbon dioxide are lost through the shell. According to EU classification criteria, Grade A eggs must have an air cell no greater than 6 mm. Although regulations presume stability over 28 days, the author demonstrates that it is maintained only when eggs are stored at 6 °C. At 15-22 °C, the air cell size increases rapidly, risking non-compliance with EU standards (Grashorn, 2016). The rise in pH can also serve as an indicator of freshness. During storage, the albumen pH rises from approximately 7.6-7.9 to as high as 9.27, while yolk pH slightly increases from around 6.0 to 6.5 (Bermudez-Aguirre and Niemira, 2023; Yüceer et al., 2022; Yüceer and Caner, 2020).

3.2.2.4 Microbilogical aspects

Besides the physicochemical parameters, the microbiological aspect of eggs must also be considered. The microbial contamination directly affects egg quality and safety and commercial eggs must be free of spoilage microorganisms and pathogenic bacteria. However, maintaining microbial safety is difficult as eggs provide nutrients that favor microbial growth (de Souza et al., 2015). Microbes such as *Pseudomonas, Acinetobacter, Proteus, Aeromonas, Alcaligenes, Escherichia, Micrococcus, Serratia, Enterobacter,* and *Flavobacterium* typically cause

spoilage in eggs. Whereas *Salmonella spp., Staphylococcus aureus, Campylobacter jejuni, Listeria monocytogenes,* and *Yersinia enterocolitica* are the most common pathogenic bacteria found in eggs (Aragon-Alegro et al., 2005). Among these pathogens, *Salmonella enteritidis* remains a significant concern due to its strong association with egg-related salmonellosis. Due to the high risk of contamination with *Salmonella spp*., the FDA has classified shell eggs as a potentially hazardous food (Keener, 2017). Egg contamination can be classified as endogenic (vertical) or exogenic (horizontal) (Grashorn, 2016). Endogenic contamination happens during egg formation in infected hens, mainly caused by *Salmonella spp*., with contamination rates ranging from 0 to 8.1% (Cox et al., 2000; Sharaf Eddin and Tahergorabi, 2019). Exogenic contamination happens more common after laying due to contact with feces or environmental bacteria such as *Streptococcus, Staphylococcus, Aerococcus, and Escherichia* (Reu et al., 2008). Factors such as shell surface contamination, cracks, and abnormal calcification promote the risk of microbial penetration. Conversely, attributes such as egg weight, shell porosity, and cuticle thickness were found to have no significant effect on contamination levels (Messens et al., 2007).

3.3 Egg preservation methods

The inherent structural fragility and the progressive degradation of functional and nutritional constituents during storage of egg remain a major drawback. In addition, the physicochemical properties of the egg's main components are highly variable and sensitive to environmental conditions. Given the increasing consumer demand for high-quality products, eggs that are microbiologically safe, physically intact, and nutritionally preserved is critical. Therefore, the development of scientifically validated measures is critical. Pasteurization is defined as any process or treatment that reduces the most resistant microorganisms of public health concern to levels that no longer pose a risk under normal distribution and storage conditions (NACMCF, 2006). Any pasteurization or treatment method intended to inactivate *Salmonella enteritidis* in shell eggs or egg products must achieve at least a 5-log pathogen reduction (FDA, 2022b). Sanitation and disinfection are milder processes than pasteurization. These treatments usually achieve only 2 to 3-log microbial reductions (99-99.9%). Sanitation involves general hygienic practices during egg handling and hatchery (Bermudez-Aguirre and Niemira, 2023). Disinfection usually refers to surface decontamination using chemical agents such as chlorine (Georgescu et al., 2017). Different preservation processes can be categorized as either traditional or novel methods, which may be thermal or non-thermal treatments.

3.3.1 Traditional methods

Traditional pasteurization methods for shell eggs, such as hot water immersion and hot, humid air treatment rely on thermal processing. Although effective in reducing microbial loads, these methods require long processing times and high energy usage. However, heat exposure can also affect egg quality and create difficulties in uniformly heating cold spots at the same time (Bermudez-Aguirre and Niemira, 2023). Therefore, the length and temperatures of the treatments must be properly controlled.

3.3.1.1 Hot water immersion

Hot water immersion is the most common thermal method has been utilized to pasteurize eggs. The organic debris such as feces, feathers, and soil can protect microorganisms and reduce the efficacy of pasteurization so the eggs need to be washed before treatment (Bermudez-Aguirre and Niemira, 2023; Keener, 2017). As required by the United States Code of Federal Regulations, the wash water used must be at least 32.2 °C and 6.7 °C warmer than the egg's internal temperature (USDA, 2008). The cleaned eggs are then submerged in a water bath maintained at a controlled temperature. However, the alteration of texture such as protein coagulation still remains the primary concern during heat treatment. Some of these proteins can be denatured at temperatures as low as 60 °C (Hou et al., 1996). Eggs are typically pasteurized at 57 °C for a few minutes to avoid visible coagulation, however, a decline in whipping ability has been reported even at this temperature (Caner et al., 2025; Kuang et al., 2023). Eggs pasteurized in a circulating water bath at 57 °C for 25 minutes achieved approximately a 3-log reduction in *Salmonella enteritidis ATCC 13076*. Longer duration than 30 minutes at 57 °C was reported to cause egg white denaturation. The heating regimen had no significant effect ($P > 0.05$) on the Haugh unit, pH, yolk index, or color. However, significant changes ($P < 0.05$) were observed in egg white viscosity, turbidity, and hue, suggesting partial protein denaturation (Hou et al., 1996). Another study of immersion heating by Schuman et al (1997) demonstrated complete inactivation of *S. Enteritidis* for 50-57.5 minutes at 58 °C or 65-75 minutes at 57 °C. Due to prolonged heating Haugh unit values increased, while yolk index and albumen pH remained unaffected in both studies. Based on the experiments, the extended exposure (>35 minutes) at either temperature (57 °C or 58 °C) reduced albumen clarity and foaming functionality. Lower temperatures (e.g., 57 °C) appeared to cause less damage, but brief exposure above 57.2 °C still resulted in a decline in foaming capacity (Schuman et al., 1997).

3.3.1.2 Hot / humid air

Hot/humid air treatment is another conventional method for shell egg pasteurization. Hou et al (1996) applied a hot air oven with forced-air circulation set at 55 °C to pasteurize shell egg and identified this as the maximum temperature that could be used without inducing denaturation of egg components. At this temperature, a 5-log reduction of *Salmonella* was achieved after 180 minutes with significant inactivation observed between 50 and 180 minutes (Hou et al., 1996). However, such prolonged heating is not practical for commercial use, as it may may favor microbial growth and cause drying or coagulation of the shell inner membranes and outer albumen layers (Keener, 2017). In 2010, Pasquali et al (2010) examined a hot air treatment to pasteurize shell eggs and reported no significant changes in key quality traits (pH, turbidity, breaking strength, and yolk index) compared to untreated eggs after 28 days of storage at 20 °C. The treatment achieved up to a 1.9-log reduction of *S. Enteritidis* on the eggshell surface. However, in this experiment, the use of a custom-built hot air apparatus delivering two 8-second pulses at 600 °C, separated by a 30-second burst of cold air may create difference compared to conventional method (Pasquali et al., 2010). Hot air treatment reduced *S. Enteritidis ATCC 13076* more effectively than hot water immersion but the long heating time restricts its commercial application (Hou et al., 1996). Such prolonged processing times could increase production costs and further reduce the feasibility for large-scale egg pasteurization. This limitation led to the development of a combined approach, involving rapid initial heating via water-bath treatment followed by prolonged incubation using hot air. The combination of water-bath treatment at 57°C for 25 min with hot-air heating at 55°C for 60 min achieves a 7-log reduction in *S. enteritidis ATCC 13076* without affecting key quality parameters such as Haugh unit, pH, yolk index, or color. However, statistically significant differences ($p < 0.05$) were detected in egg white viscosity, turbidity, and hue value. Lysozyme is an antimicrobial protein in egg white, and most of its loss occurs during water-bath heating. Reducing this loss can improve the safety of shell eggs. Therefore, the combined method reduced the water-bath step to only 25 minutes could prevent major lysozyme loss and diminish changes in turbidity and viscosity (Hou et al., 1996).

3.3.2 Novel methods:

Due to the limitations of conventional technologies, there is a growing need for improved approaches. Numerous studies have been conducted to meet industrial demands for more effective, economical, energy-efficient and rapid processing methods. Some emerging technologies incorporate heat, while others rely on non-thermal applications.

3.2.2.1 Thermal technologies

3.2.2.1.1 Microwave

Microwave techniques have been explored as a potential method for treating shell eggs in recent years. In the United States, the frequencies of 915 and 2450 MHz are used as they effectively penetrate the internal components of eggs (Keener, 2017). The microwave method is quite simple and economical. This method uses non-ionizing electromagnetic waves to produce uniform volumetric heating inside the egg. The heat is primarily generated through the oscillation of polar molecules such as water (Bermudez-Aguirre and Niemira, 2023; FDA Health, 2024; Souza et al., 2019). The technique mainly relies on thermal effects but some studies suggest a potential contribution from non-thermal mechanisms. Non-thermal effects may come from electroporation as it disrupts microbial cells through electrical mechanisms (Bermudez-Aguirre and Niemira, 2023). A study demonstrated more than 5-log reduction of Salmonella typhimurium (from 7 to 1.2 log CFU/mL) using microwave power level 9 for 20 seconds (Shenga et al., 2010). The method's main limitation is its penetration depth. Microwave energy penetration changes based on the dielectric constant (ε′) and loss factor (ε″) of the food components (Bermudez-Aguirre and Niemira, 2023). The albumen has higher dielectric constants and loss factors so the heating rate is faster than the yolk in a microwave environment. However, laboratory experiments on in-shell eggs revealed that the heating rates of the albumen and yolk were comparable (Dev et al., 2008). The penetration and heating uniformity also greatly depend on egg geometry and the compositional characteristics of the yolk and albumen (Bermudez-Aguirre and Niemira, 2023; Keener and Misra, 2016). The author proposed in-shell heating parameters of 3.5 (± 0.26), 7 (± 0.46), and 9 (± 0.52) minutes to reach pasteurization temperatures at power densities of 2.0, 1.0, and 0.75 W, respectively, with no cracks or deformation observed on the shell surface (Dev et al., 2008). To this day, in-shell egg microwave pasteurization has not been widely commercialized. There is an interesting case of a South African patent that used microwave pasteurisation to treat eggs for 30 to 40 minutes. The process achieved a 2 to 5-log reduction in microbial load but there were some alterations in the albumen (Geveke et al., 2017; Ismail et al., 2025).

3.2.2.2 Non-Thermal technologies

3.2.2.2.1 UV light

Light-based technologies play an important role in food pasteurization, with ultraviolet (UV) light being one of the most widely used methods. UV light has three types: UV-A (315-400

nm), UV-B (280-315 nm), and UV-C (200-280 nm). Among these types, UV-C has the strongest germicidal effect and is commonly used for disinfection. It is highly effective as most microorganisms absorb UV light best at around 254 nm (Keener, 2017). UV-C exposure leads the formation of thymine dimers, disrupting DNA replication and transcription and consequently killing the microorganism (Keener, 2017; Souza et al., 2019). Two methods are currently available for UV-C delivery: continuous and pulsed light. In the continuous mode, UV light is emitted steadily without interruption, whereas the pulsed mode releases intense microsecond- to millisecond- intermittent pulses. Pulsed UV light is preferred for microbial inactivation as these pulses deliver much higher energy in a short time compared to continuous mode (Souza et al., 2019). Numerous studies have confirmed the efficacy of UV-C treatment in reducing microbial loads (De Reu et al., 2006; Keklik et al., 2010; Rodriguez-Romo and Yousef, 2005). According to Keklik et al (2010), the treatment caused no negative effects on shell strength, cuticle structure, or albumen height. Similarly, Mattioli et al (2020) found that UV-C treatment maintained both shell strength and egg weight. During the experiment, fatty acid and cholesterol profiles are preserved, while antioxidant losses were minimal (~4%) and cholesterol oxidation products (COPs) remained low (Mattioli et al., 2020). These results highlight UV light as promising non-thermal approaches. UV-C treatment offers great potential as it is inexpensive, chemical-free, and causes no thermal damage. The method is widely applied in water and surface disinfection (Souza et al., 2019). Still, its commercialization for eggs remains limited due to its penetration capacity and the complex geometry of eggshells.

3.2.2.2.2 Ozone

Ozone (O_3) is a highly reactive gas that rapidly decomposes into oxygen, leaving no chemical residues (Jadhav et al., 2021; Keener, 2017; Souza et al., 2019). It is produced by exposing air or oxygen to high-energy sources such as corona discharge, electrochemical processes, or UV radiation, with the electric discharge method being the most common (Souza et al., 2019; Tapp and Rice, 2012). Ozone is highly unstable and requires on-demand generation and immediate application (Jadhav et al., 2021; Keener, 2017; Souza et al., 2019). This raises additional operational costs compared to more stable sanitizers such as chlorine (Bott, 1991). The strong oxidizing properties of ozone make it an effective disinfectant in water, air, and food systems. Its bactericidal activity results from the oxidation of cell membranes, lipids, proteins, and nucleic acids, leading to cell death (Keener, 2017). Within just 10 minutes of exposure, high-concentration ozone at 101.3 kPa achieved over a 5-log reduction of *Salmonella Enteritidis* on egg surfaces (Rodriguez-Romo and Yousef, 2005). Similarly, Perry and Yousef (2013) reached

comparable microbial reductions with 160 g/m³ ozone in a vacuum-sealed chamber for 60 minutes. Ozone treatment at 6 ppm for 4-5 minutes maintains Grade AA quality after 4 weeks and extends egg freshness by at least 2 week (Yüceer and Caner, 2020). Ozone treatment is also reported to help maintain a lower albumen pH throughout storage and resulted in significantly higher total soluble solids in the yolk compared to the control group. Excessive ozone exposure, however, may oxidize internal components and reduce tocopherols, carotenoids, and cholesterol (Mattioli et al., 2020). While the controlled applications could maintain the functional properties (HU, YI, pH, viscosity) during storage (Yüceer et al., 2016; Yüceer and Caner, 2020). Ozone is Generally Recognized as Safe (GRAS) as it is non-toxic, residue-free. Ozone shows strong potential for commercial egg processing when carefully controlled and combined with other preservation techniques. However, most evidence remains at the laboratory scale (Jadhav et al., 2021; Food and Drug Administration, 2001).

3.2.2.2.3 Irradiation

Irradiation is a non-thermal preservation method that uses ionizing radiation: gamma rays, X-rays, or high-speed electrons to control microbial contamination without raising food temperature (Souza et al., 2019). The method causes oxidative degradation of DNA and other vital cellular components to inactivate microbes (Castell-Perez and Moreira, 2021). The radiation dose is the amount of ionizing radiation absorbed by a food product and expressed in kilograys (kGy). For shell eggs, the United States Food and Drug Administration has approved a maximum dose of 3 kGy of gamma rays since 2000 (Food and Drug Administration, 2000). However, irradiated eggs is not currently permitted in Europe. In the EU, irradiation is only allowed for dried herbs, spices, and vegetable seasonings (EC, 1999). Radioactive isotopes such as cobalt-60 and cesium-137 are typically used to emit high-energy gamma photons (Jadhav et al., 2021; Souza et al., 2019). Penetration depth is a critical concern in the application of irradiation. Eggs should be irradiated from the side rather than the top or bottom to avoid uneven exposure and quality loss (Kim et al., 2011). Doses above 3 kGy are required for complete Salmonella inactivation but they may reduce sensory quality. Whereas, doses ≤ 2 kGy generally maintain acceptable characteristics (Mészáros et al., 2006; Min et al., 2005). The incorporation of other treatments may enhance the effectiveness of the process. Combination of irradiation with chitosan coatings has successfully achieved inactivation at even lower doses at 1.86 kGy (Yun et al., 2012). However, large-scale adoption is still limited by high operational costs, uneven dose control, regulatory restrictions, and consumer concerns associating irradiation with nuclear risks (Kim et al., 2011).

3.2.2.2.4 Edible coating

Edible coating is a promising way to preserve eggs, forming an outer sealing layer that controls the transfer of moisture and oxygen (Caner et al., 2022). Many researches have shown that coated eggs have better internal quality and lower shell breakage rates (Caner, 2005; Caner and Cansiz, 2008; Xie et al., 2002). A significantly higher air permeability was reported in uncoated eggs (Kopacic et al., 2018). Beyond their effectiveness in preserving egg quality, coatings also enhance the visual appeal of eggs by giving the shell a brighter appearance (Gabriela da Silva Pires et al., 2020). Since appearance is a key factor influencing consumer purchasing decisions, this improvement can increase the market attractiveness of coated eggs (Caner, 2005).

The application of edible coatings is relatively simple and can be carried out by spraying, enrobing, electrostatic spraying, or dipping eggs into the coating solution. Coatings may be applied as a single layer or as multilayer films, depending on the requirement. The method of application depends on the viscosity and texture of the coating solution, egg surface characteristics, and available equipment (Sharaf Eddin and Tahergorabi, 2019). Dipping and spraying are the most common: the dipping method requires no specialized machinery but necessitates proper draining afterwards, while the spraying method ensures more uniform coverage and lower risk of contamination (Sharaf Eddin and Tahergorabi, 2019; Zhong et al., 2014). The adhesion of the coating to the eggshell surface is also an important factor. Based on previous studies, the effectiveness of coatings depends greatly on their composition, structural properties, and the storage conditions under which they are applied (Lin and Zhao, 2007; Sharaf Eddin and Tahergorabi, 2019).

Although edible coating demonstrates significant potential, several technical and regulatory limitations still hinder large-scale adoption. Coatings must be non-toxic, approved for food applications, and applied under strict hygienic conditions in accordance with both USDA and EU regulations. Many commonly used coating materials such as milk, soy, fish, peanut, and wheat derivatives are allergens. Therefore, clear labeling and careful formulation control is a must. Specialized materials, additional processing steps, and quality assurance can increase the production costs (Sharaf Eddin et al., 2019). Due to these limitations, innovative approaches are needed to optimize coating processes, balance cost efficiency but ensure high-quality, safe products at the same time.

Most previous preservation methods have focused on reducing microbial loads while edible coatings mainly maintain the internal quality of eggs. This approach is particularly valuable due to increasing consumer preference for freshness and minimal processing. The edible coatings can be effectively combined with other preservation techniques to ensure both safety and freshness better.

Based on the material, the coatings can be categorized into three main types: proteins, lipids, and polysaccharides coatings (Sharaf Eddin et al., 2019). Among these coatings, polysaccharides are particularly preferred for their low thickness, high flexibility, and excellent transparency (Pavlath and Orts, 2009). The following section reviews existing coating materials, their applications, and discusses their potential in egg preservation and shelf-life extension.

3.2.2.2.4.1 Protein-based coating

Protein-based coatings can be prepared from proteins of either plant or animal origin. Common plant protein sources include soy protein, wheat gluten, and zein, while animal proteins are obtained from milk, fish and pork (Sharaf Eddin et al., 2019). Among these, whey and zein provide effective barriers to the permeation of oxygen, carbon dioxide, volatile compounds, and lipids (Sharaf Eddin and Tahergorabi, 2019). Among whey protein isolate and whey protein concentrate, zein coatings exhibited the best performance in maintaining Haugh unit (HU), yolk index (YI), and albumen viscosity (Caner and Yüceer, 2015). Whey protein isolate and whey protein concentrate coatings were found to be more effective in preserving egg weight during storage. All coatings, however, significantly enhanced shell strength ($P< 0.05$). Similarly, Xie et al (2002) reported that eggs coated with soy protein isolate, whey protein isolate, wheat gluten, or carboxymethyl cellulose had greater puncture strength than uncoated controls. Beside the finding that were effective, soy protein appeared less efficient than other protein sources, showing minimal or even lower quality values compared with uncoated eggs (Khattak, Sharma, & Sanghi, 2016). Gelatin is typically obtained from porcine collagen through partial hydrolysis but its application is limited due to religious restrictions and concerns regarding bovine spongiform encephalopathy (Gennadios, 2002).

Although proteins form effective barriers, their inherent hydrophilic nature exhibit poor water resistance and mechanical durability. To overcome these drawbacks, modification techniques such as denaturation, aggregation, plasticization, and chemical cross-linking are often applied

(Sharaf Eddin and Tahergorabi, 2019; Vieira et al., 2011). The tensile strength of protein-based edible coatings can be improved through chemical cross-linking reactions with aldehydes. Proper pH and transition temperature adjustment is essential before proteins are used in edible coatings as it determine the shape of protein molecules (Sharaf Eddin and Tahergorabi, 2019). Elevated extrusion temperatures led to improved mechanical properties and enhanced water vapor transfer in protein-corn oil composite films (Nur Hanani et al., 2014). Incorporation of plasticizers could also enhance the flexibility and adhesion of protein coatings (Assis and Britto, 2014; Wan et al., 2005). Plasticizers are non-volatile compounds with glycerol, sorbitol, and polyethylene glycol are among the most used in formulations. Plasticizers less hygroscopic than glycerol, such as sorbitol, can augment the water barrier properties of protein-based coatings (Wan et al., 2005).

3.2.2.2.4.2 Lipid-based coating

Neutral lipids, fatty acids, waxes, and resins are traditional lipid-based materials for edible coatings (Lin and Zhao, 2007). Lipid-based coatings are hydrophobic nature and have low water vapor permeability, which result from their low-polar molecular structure. However, these coatings tend to be opaque and inflexible, limiting their mechanical adaptability and visual transparency compared to other biopolymer coatings. Lipids such as waxes and oils are applied either as individual coatings, emulsions, or bilayer (Martin-Belloso and Fortuny, 2010). In bilayer coatings, a lipid layer is added over a polysaccharide or protein base, while in emulsions, the lipid phase is entrapped within the polymer matrix (Lin and Zhao, 2007; Martin-Belloso and Fortuny, 2010). Although bilayers typically provide a more uniform and effective barrier, they require two application steps while emulsions can be applied in one (Martin-Belloso and Fortuny, 2010). In addition to their barrier properties, oil-coated eggs exhibit a glossier surface than uncoated eggs, as verified by instrumental measurements and consumer assessments (Gabriela da Silva Pires et al., 2020).

Among lipids, waxes are the most frequently used in edible coatings (Cindric et al., 2007). The high melting point and specific gravity of lipids can enhance the mechanical and tensile strength of the coating (Sharaf Eddin et al., 2019). Several types of waxes have been investigated for coating, including carnauba, beeswax, and candelilla waxes (Aguirre-Joya et al., 2019; de S. Dantas et al., 2013; Eyng et al., 2021; Mudannayaka et al., 2016). The storage temperature a key determinant of coating performance. The plastic behavior of waxes varies with temperature as they remain flexible at room temperature but become brittle at lower temperatures (Sharaf

Eddin and Tahergorabi, 2019). In one study, wax-coated eggs maintained AA-Grade quality for at least eight weeks and experienced negligible water loss compared with 5% loss in uncoated eggs. The coated eggs also retained 87% of their CO_2 content and the shell strength increased by 4-10% (Biladeau and Keener, 2009).

Edible oils represent another important material used in the formulation of lipid-based coatings. Nongtaodum et al (2013) have shown coconut, palm, rice bran, and soybean oil coating could preserve internal quality of eggs, maintaining Grade A quality for up to four weeks longer than uncoated eggs. Oil coating treatments from other sources: mineral, canola, corn, grape seed, olive, soybean, and sunflower oils were found to be equally effective in maintaining internal egg quality during five weeks of storage at 25 °C, reducing weight loss to below 0.8% and extending shelf life by at least three weeks compared with uncoated eggs (Ryu et al., 2011). The study further noted that soybean oil represents a more practical coating option due to its low cost. The coating performance depends on the viscosity of the oil. Oils with viscosities 11 14, 18, 22, 26 cP were found to be more effective than those with 7 cP, resulting in higher Haugh unit values and lower moisture loss (Waimaleongora-Ek et al., 2009). The performance of lipid-based coatings is influenced by storage temperature. Without refrigeration, uncoated eggs and mineral oil–coated eggs declined from AA to C and B Grades, respectively, after five weeks of storage based on Haugh unit measurements (Jirangrat et al., 2010). Meanwhile, when stored at 4 °C, mineral oil–coated eggs maintained their AA Grade for at least 15 weeks, with only 1.19% weight loss. Nevertheless, these coatings may increase the lipid content of eggs, which could pose limitations for large-scale commercial or nutritional applications (Sharaf Eddin et al., 2019).

Resin has potential as a coating material. Shellac is a natural resin that can be obtained from the secretions of insects Laccifer lacca. Musa et al (2011) reported that a 5% shellac coating reduced quality deterioration in eggs during 30 days of storage at 40 °C. Wood resins are frequently altered through chemical modification to enhance their performance (Sharaf Eddin and Tahergorabi, 2019). Processes such as hydrogenation, isomerisation and polymerisation increase their resistance to oxidation and discoloration while also enhancing their thermoplastic characteristics.

3.2.2.2.4.3 Polysaccharide-based coatings

Water-soluble, gel-forming polysaccharides are commonly used in edible coatings to extend food shelf life (Sharaf Eddin and Tahergorabi, 2019). They form viscous, uniform films that improve coating adhesion and overall mechanical stability (Molavi et al., 2015; Sharaf Eddin and Tahergorabi, 2019). Polysaccharide-based coatings provide poor moisture barriers but allow selective permeability to oxygen and carbon dioxide (Sharaf Eddin and Tahergorabi, 2019). Polysaccharide-based coatings are typically derived from plant sources such as cellulose and its derivatives (pectin, starch), seaweed extracts (alginates, carrageenan) or crustacean shells (chitosan) is obtained, as well as certain mucilage compounds (Martin-Belloso and Fortuny, 2010)

Cellulose is one of the polysaccharide used in edible coatings due to its high proportion of hydroxyl groups, which can form stable intra- and intermolecular hydrogen bonds that enhance structural strength (Molavi et al., 2015; Suppakul et al., 2010). Cellulose can be chemically modified by altering the levels of methoxyl, hydroxypropyl, and carboxymethyl groups. These processes improve the water retention capacity, reduces dissolution temperature, enhances gelation potential, and increases electrolyte sensitivity (Molavi et al., 2015). In one study, methylcellulose and hydroxypropyl methylcellulose coatings with polyethylene glycol-400 as a plasticizer effectively reduced weight loss and maintained egg quality parameters such as pH and Haugh unit (HU) during storage, allowing eggs to retain Grade A quality for up to 28 days (Suppakul et al., 2010).

Starch is another polysaccharide commonly used in the production of polysaccharide-based edible coatings (Falguera et al., 2011). The mechanical and crystalline properties of starch-based coatings depend on the amylose-to-amylopectin ratio as well as the source of starch (Gennadios et al., 1997). Native starches have limited industrial use due to their poor water dispersibility, gelation and retrogradation tendency upon cooling. To improve functionality, starches are modified by disrupting hydrogen bonds through molecular weight reduction, resulting in lower gelatinization temperatures and greater stability under refrigeration (Sharaf Eddin and Tahergorabi, 2019). Starch modification can be achieved through acid hydrolysis or chlorinated starches production with the chlorinated starches often preferred due to its greater resistance to thickening and improved digestibility (Falguera et al., 2011). Sweet potato starch demonstrates excellent film-forming ability, comparable to or superior to potato starch (Issa et

al., 2017, 2018). (Sharaf Eddin and Tahergorabi, 2019) found that sweet potato starch-based coatings containing 4.0% and 6.0% thyme essential oil effectively preserved egg internal quality for two weeks longer than uncoated eggs during storage at 25 °C.

Chitosan, derived from shellfish waste, exhibits excellent oxygen barrier properties and possesses inherent antimicrobial activity (Sharaf Eddin and Tahergorabi, 2019). Numerous studies have consistently demonstrated chitosan effectiveness as a coating in preserving quality and extending shelf life (Caner and Cansiz, 2008; Elsabee and Abdou, 2013; Kopacic et al., 2018; Pujols et al., 2014; Wardy et al., 2013; Xu et al., 2018; Yuceer and Caner, 2014). Other polysaccharide such as alginate, carrageenan, gellan gum, gum arabic, pectin, chia seed mucilage, and pullulan have also demonstrated excellent barrier properties and proven effective in extending shelf life (De Leo et al., 2018; Hejazian et al., 2023; López-Díaz and Méndez-Lagunas, 2023; Morsy et al., 2015; Pan et al., 2023; Ruan et al., 2023; Sariyel et al., 2022; Tammina et al., 2025). Application of 10% gum arabic coating has reduced CO_2 loss through the eggshell and improved the stability of egg weight, specific gravity, and albumen pH (Sariyel et al., 2022). Whereas, chia seed mucilage with turmeric essential oil coating showed promising by minimizing moisture and CO_2 loss while maintaining Haugh unit and yolk index values. Formulations containing 0.6–0.7% chia mucilage with turmeric extract were the most effective as the coating preserves egg freshness for 30–40 days at room temperature (Hejazian et al., 2023). Pullulan forms edible coatings with excellent oxygen barrier properties, low permeability, and good mechanical strength (Singh et al., 2008). Coating with pullulan has shown promise by reducing weight loss during 10 weeks of storage at both room and refrigerated temperatures (Morsy et al., 2015).

3.4 Chitosan coating

Chitosan is a deacetylated form of chitin, obtained from crustaceans waste or insects. Chitosan has attracted considerable attention due to its excellent oxygen barrier and antimicrobial properties (Dutta et al., 2009; Elsabee and Abdou, 2013; Ezazi et al., 2021; Kopacic et al., 2018). Its application is widely studied to preserve egg quality and extend shelf life (Bhale et al., 2003; Caner et al., 2022; Caner and Cansiz, 2008; Kopacic et al., 2018; Pujols et al., 2014; Torrico et al., 2010; Yuceer and Caner, 2014). Without coating, the eggs had already degraded to Grade B by the 2nd week, whereas the 8% chitosan coating was able to maintain Grade A quality for at least 4 weeks at room temperature (Caner et al., 2022). According to Pujols et al (2014), α- and β-chitosan emulsions showed comparable barrier efficiency, maintaining less

than 2% weight loss and extending shelf life for up to three weeks at 25 °C. The coating is considered highly promising as its raw materials is biocompatible, biodegradable, and naturally abundant (Caner et al., 2022). The efficiency of chitosan is affected by sources of chitosan, concentration, molecular weight, number of coating layers, and combination with other materials. Higher chitosan concentrations and multiple coating applications have been shown to better preserve internal egg quality (Bhale et al., 2003; Caner et al., 2022; Xu et al., 2018). The coating with 8% of chitosan exhibited the strongest barrier properties, outperforming the 4% and 1% coatings (Caner et al., 2022). Application of three coating layers resulted in a thicker barrier and better preservation compared to one or two layers (Xu et al., 2018). Research on chitosan coatings of varying molecular weights showed that low-molecular-weight chitosan (470 kDa) was most effective in minimizing weight loss, compared with medium and high molecular weights (Bhale et al., 2003). While preparing the solution, it is also important to consider the characteristics of chitosan. chitosan is not soluble in water without acetic acid or at pH values exceeding 5, it may be regarded as the hydrophobic material (Zhang et al., 2014). Storage conditions strongly affect the performance of the coating. At 4 °C, eggs coated with 1% chitosan maintained Grade A quality up to the 20th week with an average Haugh Unit of 59.99 ± 2.4, whereas at 25 °C, the same coating degraded to Grade B by the third week, with an average HU of 48.81 ± 5.8 (Wardy et al., 2013).

Various combinations have been explored with chitosan-based coatings. The incorporation of oil can enhance the hydrophobicity of chitosan-based coatings, improving their barrier properties (Wardy et al., 2013). The use of mineral oil has been shown to effectively maintain internal egg quality (weight loss, HU, YI, and albumen pH) at 25 °C for up to 5 weeks (Torrico et al., 2010). Later publication of Wardy et al (2013) explored soybean oil can provide comparable protective effects while serving as a more economical alternative with shorter drying times, successfully extending egg shelf life by approximately 5 weeks at room temperature and up to 15 weeks under refrigeration compared to uncoated eggs. Substances such as montmorillonite nanocomposites can reinforce the coating layer and provide enhanced shell strength by sealing micro-cracks and pores on the eggshell surface (Caner et al., 2022). The antimicrobial properties of the coating can be enhanced by incorporation of lysozyme. Addition 10% to 60% of lysozyme can enhance the effectiveness of chitosan coating (Yuceer and Caner, 2014). Propolis can also improve the coating performance due to its excellent antimicrobial, antiviral, and antioxidant properties (Ezazi et al., 2021). Chitosan coating with the addition of lactic acid has proved effective in extending egg shelf life without compromising

consumer perception or acceptability (Caner and Cansiz, 2008). Proper proportion and interaction between coating materials are key to performance. Moderate levels of chitosan and propolis effectively reduced weight loss by limiting water evaporation and bacterial penetration (Ezazi et al., 2021). A blended coating of 4% chitosan and 5% polyvinyl alcohol at a 25:75 ratio showed excellent performance based on results of weight loss ($0.57 \pm 0.08\%$), stable albumen pH (8.30 ± 0.04), and favorable Haugh Unit (61.00 ± 0.07) and yolk index (0.37 ± 0.02) values.

4. Materials and methods

4.1 Materials

A total of 420 freshly laid, unwashed, brown-shelled eggs size M without any physical damage were obtained from the farm at Capriovus Ltd. (Szigetcsép, Hungary). Two experiments were conducted to evaluate the effect of edible coatings on egg quality during storage. 210 eggs were used in experiment I, and 210 eggs in experiment II. The components of the coatings were collected from various suppliers in Budapest, Hungary, including Chitosan powder (GymBeam), and Acetic acid (Spar) and Sorbitol (Natural nutrition).

4.2 Methods

The first experiment examined the effect of chitosan coatings on extending egg shelf life, the second investigated the combination of sorbitol and chitosan coatings on egg quality during shelf life. Coating was performed using the dipping method, where eggs were fully immersed in the solution, rotated 2-3 times, and then placed on metal net to air-dry.

4.2.1 Experiment I: Effect of Chitosan on egg quality during shelf life

The experiment consisted of five groups: uncoated control samples and chitosan-coated samples with concentrations of 1.5%, 2.0%, and 2.5%, 3.0%. All chitosan coating solutions were prepared using 0.5% acetic acid as the solvent (1000 mL per batch). The powder was first mixed with distilled water before being blended with the acid solution to reach the desired concentration. The prepared mixtures were covered with aluminium foil and left to rest for 24 hours before use. Coating was performed using the dipping method, where eggs were fully immersed in the solution, and then placed on metal net to air-dry for approximately 3 hours.

4.2.2 Experiment II: Effect of the combination of sorbitol and chitosan coatings on egg quality during shelf life

In the second experiment, there were five treatment groups: uncoated control samples, 2.0% chitosan-coated samples with different concentrations of sorbitol 1.0%, 2.0%, 3.0% and only chitosan 2.0%. The chitosan coating solution was prepared using the same procedure as in the first experiment, and then added with sorbitol concentration of 1.0, 2.0 and 3.0%. Eggs were then coated using the same dipping method as for chitosan, and then placed on metal net to air-dry, which required approximately 3 hours for complete drying.

4.3 Measurement of egg quality

The eggs were stored at 25 °C and analyzed weekly for quality parameters, including weight loss, air cell height, white index (WI), yolk index (YI), and Haugh unit (HU). Shelflife evaluation was conducted in a 4-week storage period.

4.3.1 Weight loss

The weight of each egg using a digital analytical balance (Kern PFB, Kern & Sohn Gmbh, Balingen-Frommern, Germany) with a ± 0.01 g accuracy. Egg weights were recorded at weekly intervals throughout the storage period to evaluate weight loss and changes in internal quality. The weight loss was determined by using the formula below (Pham et al., 2024):

$$\text{Weight loss (\%)} = \left(\frac{W_i - W_f}{W_i}\right) * 100 \quad (1)$$

Where:

W_i: the initial egg weight (g)

W_f: the egg weight at the time of measurement (g)

4.3.2 Air cell

The air cell was measured by gently breaking the broader end (air cell side) of the egg and measuring its depth using a digital caliper (Mitutoyo 500-150-30, AOS Absolute Digimatic, Japan). According to Grade A standards, fresh eggs should have an air cell no greater than 6 mm, which served as the reference criterion for evaluation.

4.3.3 Yolk Index

The yolk's horizontal and vertical diameters, along with its height, were measured using a digital caliper (Mitutoyo 500-150-30, AOS Absolute Digimatic, Japan). The yolk index (YI) was then calculated using the following formula of Sharp and Powell (Sharp and Powell, 2002):

$$YI = \frac{\text{Yolk height}}{\text{Yolk width}} \times 10 \quad (2)$$

4.3.4 White index

Measuring was perfomed using a electronic caliper (Mitutoyo 500-150-30, AOS Absolute Digimatic, Japan) was used to measure. The dimensions of the egg white to be recorded include its height and its horizontal and vertical diameters. Following the method described by Tung, the albumen height was taken 10 mm away from the yolk margin (Pham et al., 2024). The white index (WI) was then calculated using the following formula (R.r, 1937):

$$WI=\frac{\text{White height}}{\text{White width}} \times 100 \quad (3)$$

4.3.5 Haugh Unit

The Haugh unit (HU) was then calculated according to the following equation:

$$HU=100 \times \log (H-1.7\times W^{0.37}+7.6) \quad (4)$$

Where

H: the measured albumen height (mm)

W: the egg weight (g)

4.4 Data Analysis

Microsoft Excel was used to analyzed the the results of the experiments. Data were visualized through line graphs with the x-axis is the storage weeks and the y-axis indicated the corresponding quality indices. Standard deviations were displayed as error bars at each data point to reflect measurement variability. For each experiment, a total of five graphs were generated, corresponding to the evaluated quality parameters: weight loss, air cell height, white index (WI), yolk index (YI), and Haugh unit (HU).

5. Result and discussion

5.1 Experiment I: Effect of Chitosan on egg quality during shelf life

5.1.1 Weight loss measurement

Figure 1: Weight loss measurement of experiment I
(Source: own work)

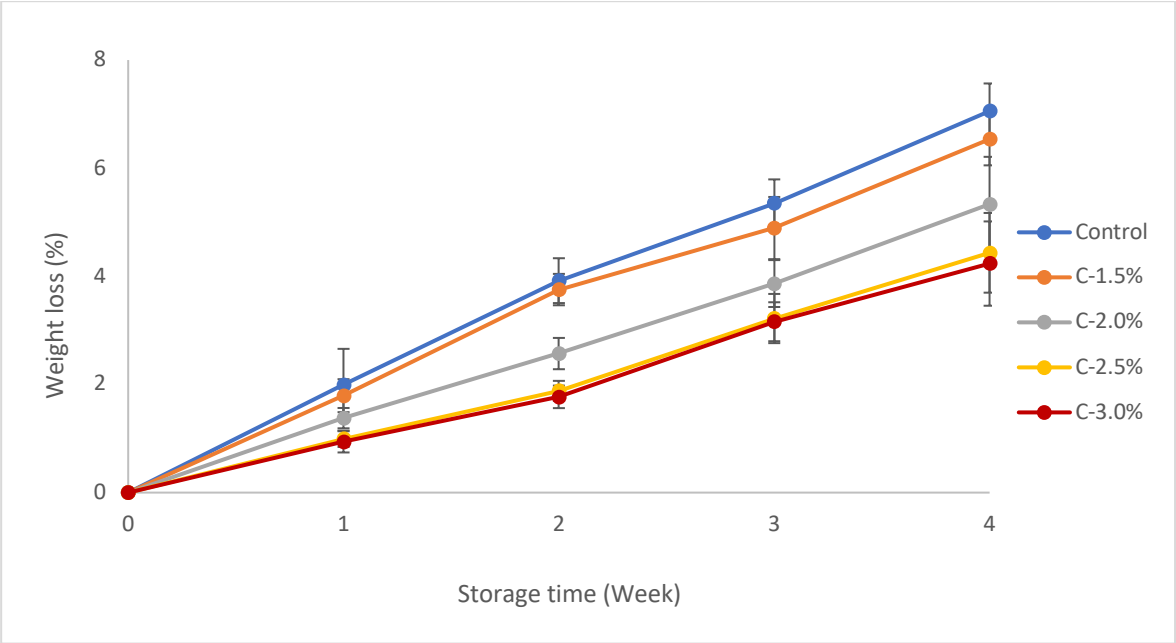
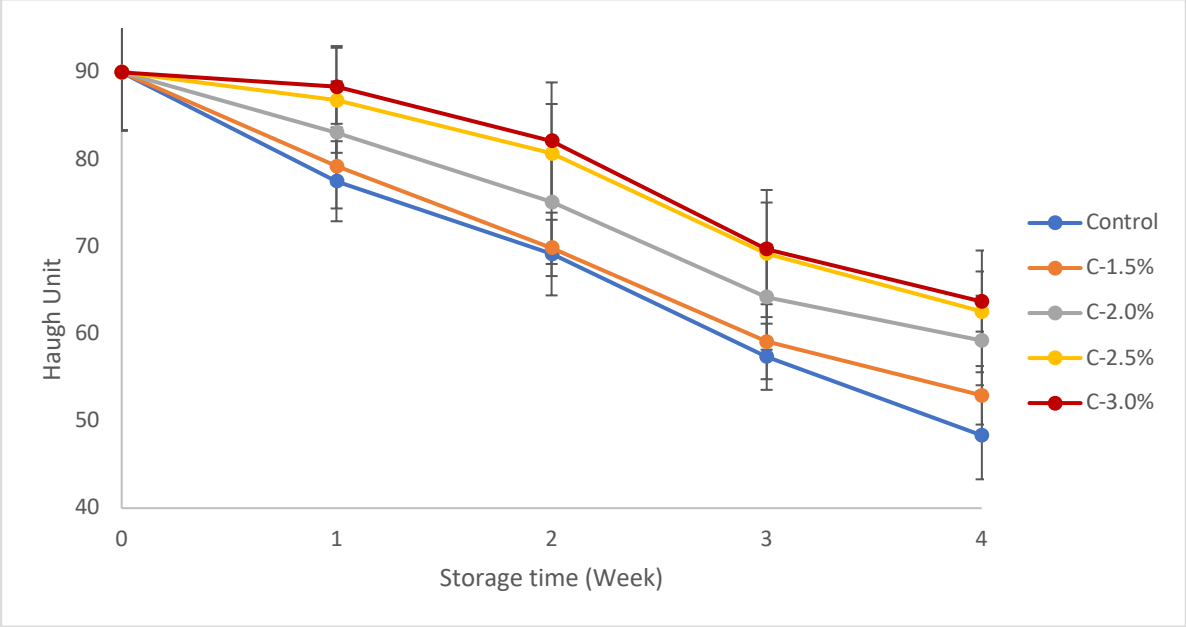


Figure 1 illustrates the weight loss patterns of both the control and coated egg samples. All samples exhibited a similar trend with progressive increase in weight loss over time. Gradual evaporation of moisture through the pores of the eggshell is considered to be the main reason causing weight loss (Caner and Yüceer, 2015; Yüceer and Caner, 2020). The control group exhibited the highest weight loss with an average of 7.05 ± 0.51% at the end of the experiment. After 4 weeks of shelf life at room temperature, the chitosan coating at 1.5% showed only slight better compared to the control with an average of 6.53 ± 0.48%, indicating that this concentration was not effective. Whereas, the 2.0% of chitosan coating demonstrated a noticeable lower weight loss of 5.33 ± 0.88%, suggesting better protection of egg quality. The 3.0% chitosan coating achieved the highest efficiency in minimizing the weight loss of 4.24 ± 0.78%, while the 2.5% concentration showed comparable results to 3.0% at 4.43 ± 0.74%.

5.1.2 Haugh Unit measurement

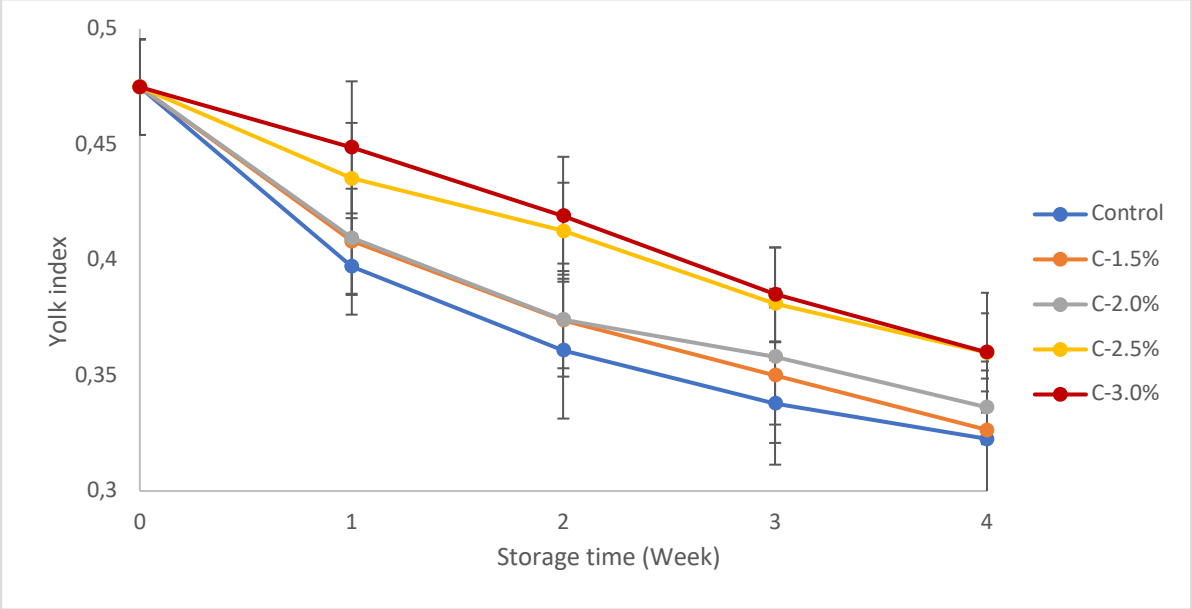
Figure 2: Haugh Unit measurement of experiment I
(Source: own work)



The Haugh Unit is an indicator for detecting early-stage egg quality deterioration after laying (Caner et al., 2022). The Haugh Unit results followed a similar trend to the weight loss measurements. The sample coated with 1.5% chitosan had a higher value in HU than the control, but no significant difference was detected by the end of the fourth week. The control sample (uncoated) had already declined to Grade B by the second week of storage. Using lower chitosan concentration of 1.0%, the study by Wardy et al (2013) showed a similar trend, where the low chitosan concentration had results comparable to the control and proved ineffective in preserving egg quality. The 2.0% chitosan coating performed better, maintaining higher Haugh Unit values compared to the control with average HU of 59.23 ± 5.13 at the end of the experiment. The best results were observed for the 2.5% (62,58 ± 6.98) and 3.0% (63,70 ± 3.45) coatings, with the 2.5% concentration showing only a slightly lower efficiency than the 3%. Coatings with chitosan of 2.0%, 2.5%, and 3.0% concentrations maintained Grade A quality up to the fourth week and extended the shelf life by at least 2 weeks compared to the uncoated samples based on Haugh Unit values. The results showed that increasing chitosan concentration enhances egg quality preservation, consistent with the previous study (Caner et al., 2022).

5.1.3 Yolk Index measurement

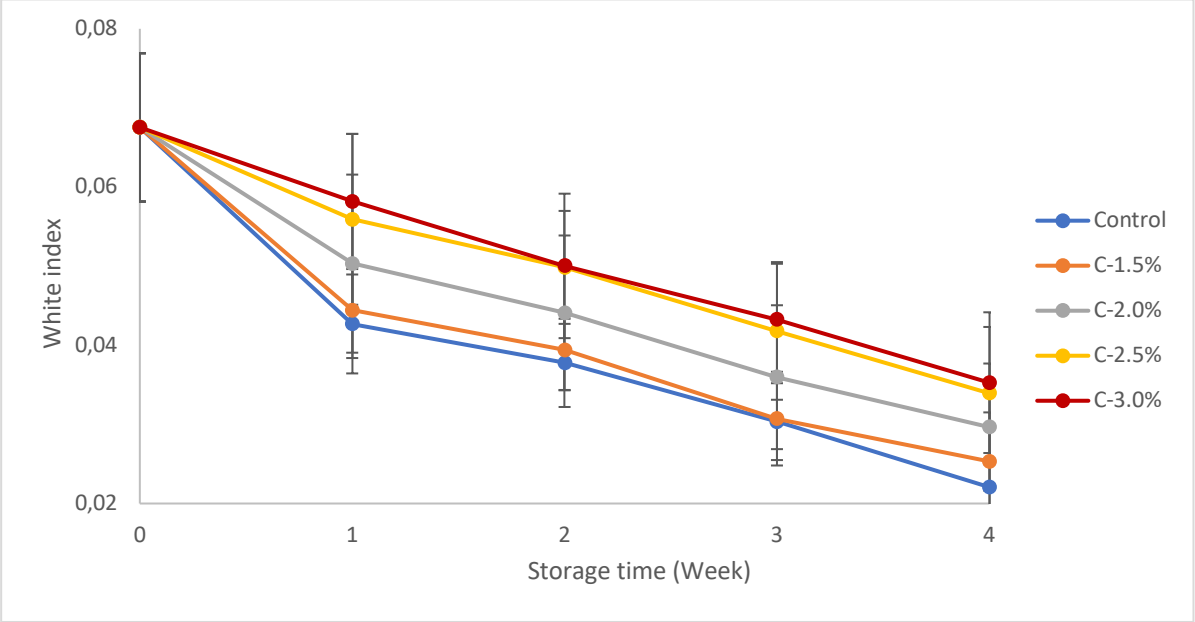
Figure 3: Yolk Index measurement of experiment I
(Source: own work)



The control sample still exhibited the lowest YI values throughout the 4-week storage period at reduction of 0.15 unit. As shown in Figure 3, the YI of 2.0% chitosan (0.33 ± 0.016) coating produced results comparable to the 1.5% chitosan coating (0.32 ± 0.029). Although 1.5% and 2.0% chitosan coating performed slightly better than control sample, it still follows the same overall trend. Coatings with 2.5% and 3% concentration of chitosan demonstrated the best performance, maintaining significantly higher yolk index values than the other samples at 0.36 ± 0.026 and 0.36 ± 0.017 respectively, clearly indicating their superior effectiveness in preserving yolk quality.

5.1.4 White Index measurement

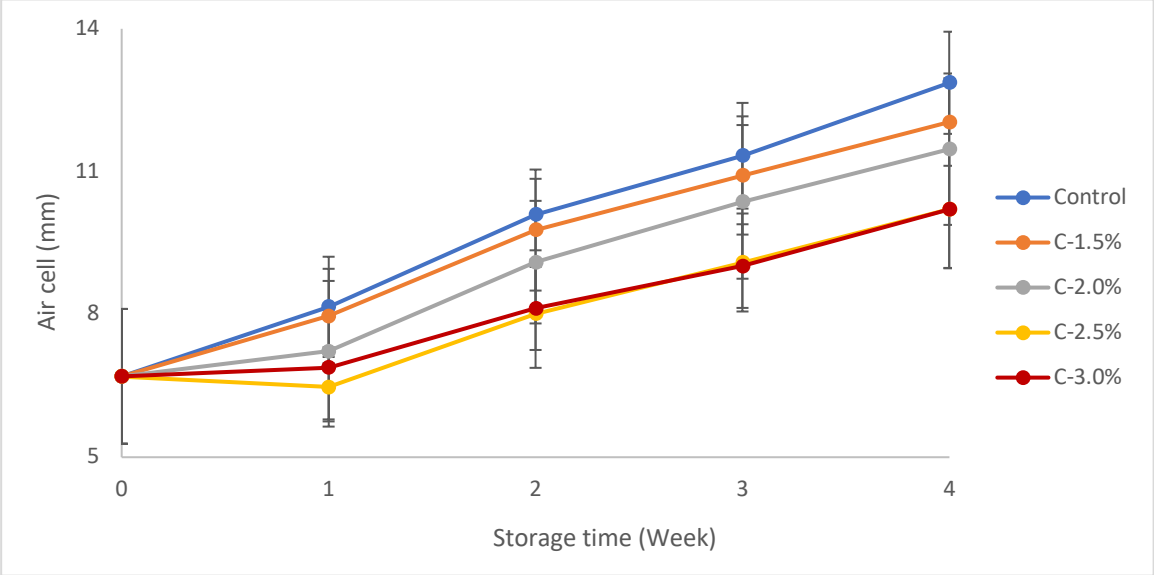
Figure 4: White Index measurement of experiment I
(Source: own work)



The white index measurements also reflected a similar pattern to the previous parameters. The control and 1.5% coated eggs showed a sharp reduction in white index values. This suggests weaker albumen structure and faster quality loss. Meanwhile, the 2.0% coating slightly slower decline at average WI of 0.029 ± 0.008 at the end of 4th week. The highest preservation efficiency was again recorded for the 2.5% and 3% coatings (0.034 ± 0.008 and 0.035 ± 0.009), which retained the albumen's structure and viscosity more effectively throughout storage, with minimal difference between the two concentrations. Studies evaluating the White Index (WI) are relatively limited, however, since the observed trends are consistent with other quality parameters, the results obtained in this study can be considered reliable.

5.1.5 Air cell measurement

Figure 5: Air cell measurement of experiment I
(Source: own work)

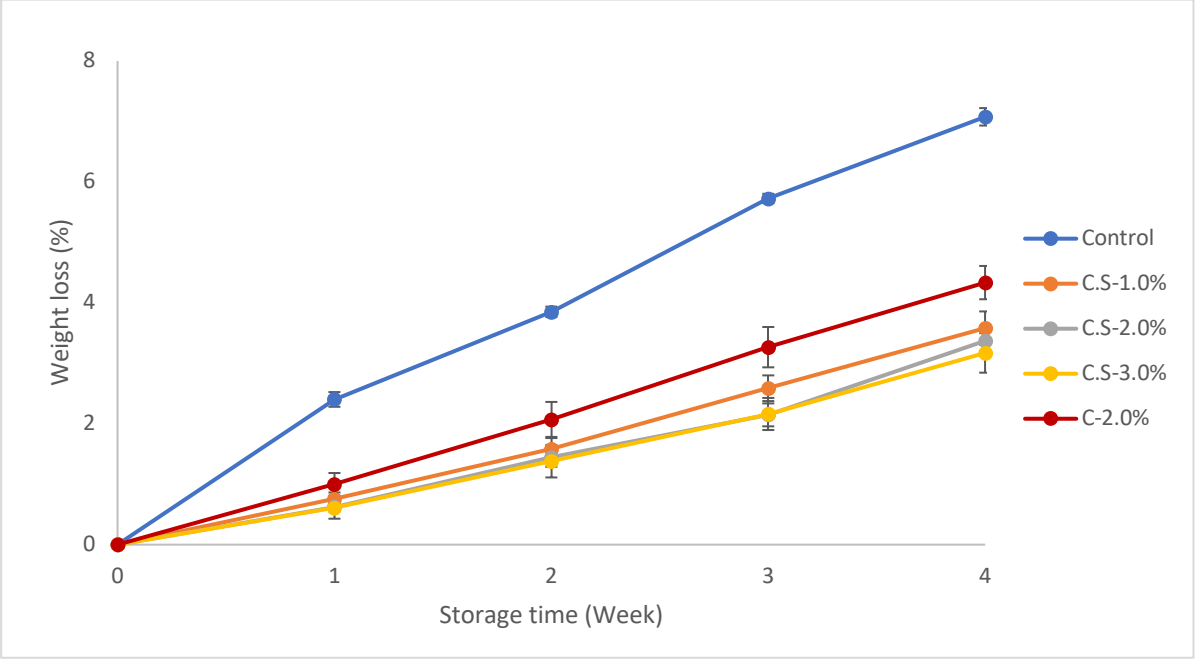


The control and 1.5% coated samples exhibited the greatest increase in air cell height, with an average air cell of 12.87 ± 1.07 and 12.04 ± 0.91 at the end of the experiment. The results show inherent limitation of these barriers to moisture and CO_2. The 2.0% chitosan coating increased more moderately with 11.47 ± 1.59, suggesting improved gas retention compared to the lower concentrations. The results of air cell at final week of 2.5% and 3.0% chitosan concentration were 10.21 ± 1.24 and 10.20 ± 1.23, showing the most stable results. The results indicated these coatings effectively minimizing internal gas exchange and slowing the enlargement of the air cell. The negligible difference further supports 2.5% chitosan as an optimal coating level for maintaining egg freshness during storage. The air cell is a parameter that has not been extensively evaluated in coating studies. Although the requirement of air cell height for fresh eggs should not exceed 6 mm, the initial measurements in this study were already approximately 6.5–7 mm.

5.2 Experiment II: Effect of the combination of sorbitol and chitosan coatings on egg quality during shelf life

5.2.1 Weight loss measurement

Figure 6: Weight loss measurement of experiment II
(Source: own work)

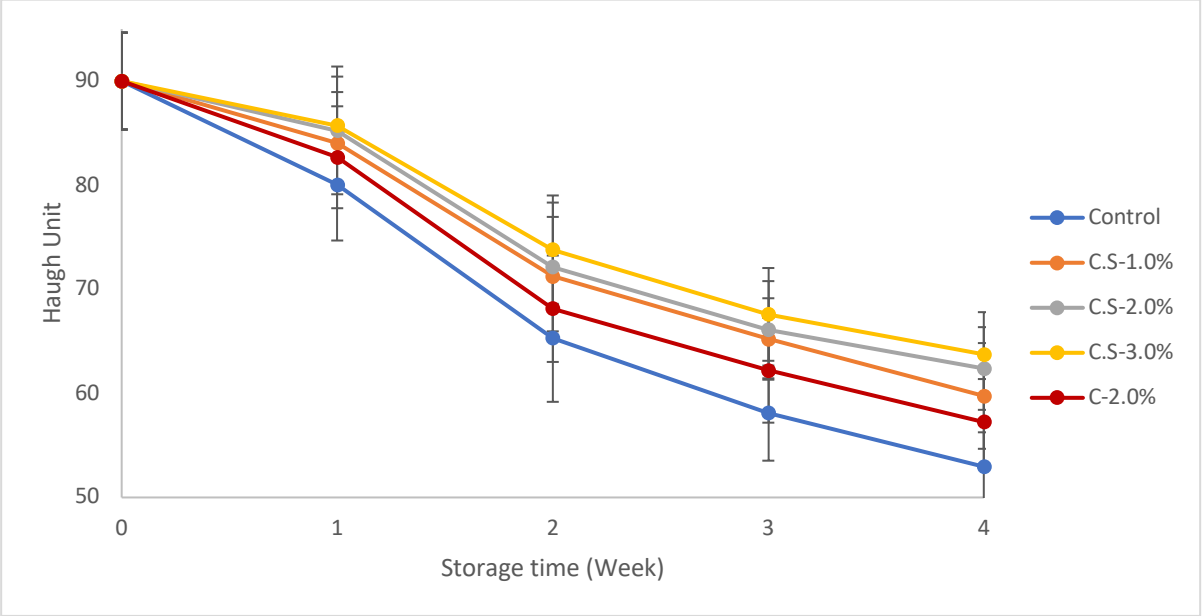


The Figure 6 shows that the control sample without any coating exhibited highest weight loss compared to the coated samples. The results are consistent with the findings of Caner et al (2022), who reported that uncoated eggs (control) exhibited significantly lower quality parameters compared to those coated with chitosan containing a plasticizer. The incorporation of plasticizer improved the coating performance, maintaining the weight better than the samples coated with chitosan alone. In Caner et al (2022)'s study, glycerol was used as the plasticizer, while in the present experiment sorbitol was applied. Although the specific plasticizer differs, both compounds enhanced the coating's flexibility and moisture retention capacity. Notably, sorbitol has been shown in several studies to provide slightly superior performance to glycerol (Kim et al., 2008; McHugh and Krochta, 2002). This can be attributed to sorbitol's higher molecular weight and lower hydrophilicity, which makes sorbitol-plasticized films having lower gas and water vapor permeability than those containing glycerol (McHugh and Krochta, 2002; Thomazine et al., 2005; Wan et al., 2005). Among the sorbitol-coated samples, the 3.0% concentration with an average weight loss of 3,17 ± 0,33% demonstrated the highest effectiveness in reducing weight loss, followed closely by the 2.0% (3,37 ± 0,23%) and 1.0%

(3,58 ± 0,28%) coatings, which also showed notable improvements compared to the control (7.07 ± 0,14%) after 4 weeks of storage. This suggests that the effects observed align closely with previous findings while demonstrating sorbitol's potential as an effective plasticizer in chitosan-based coatings.

5.2.2 Haugh Unit measurement

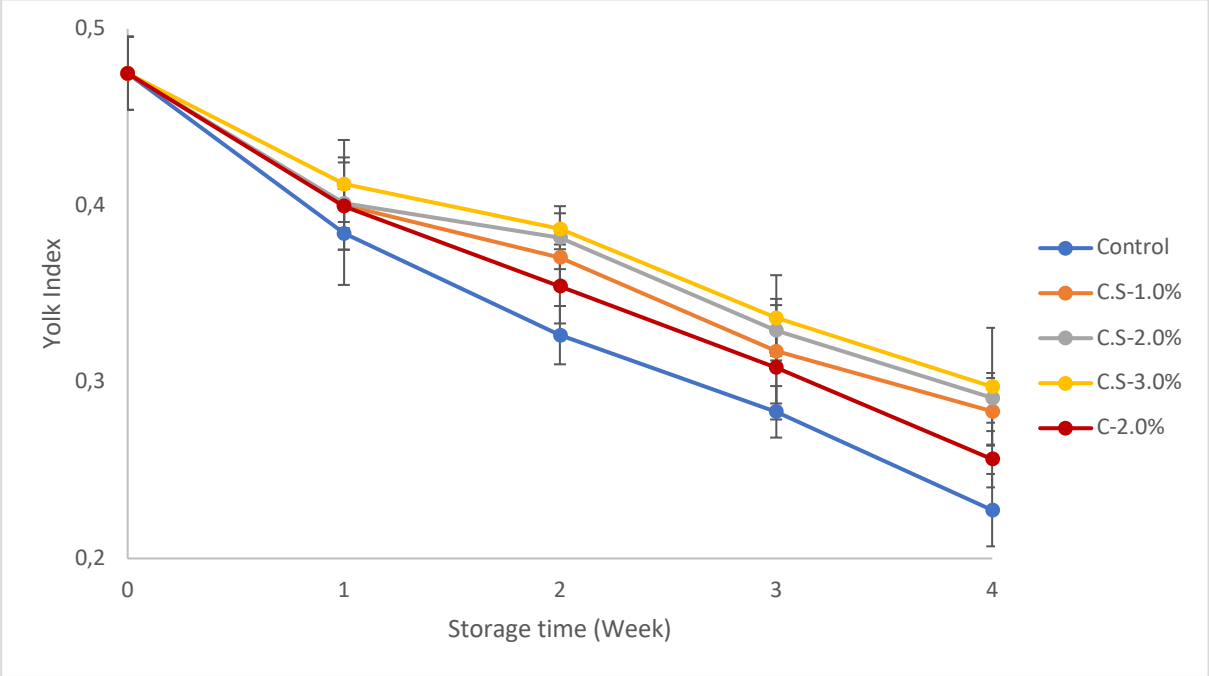
Figure 7: Haugh Unit measurement of experiment II
(Source: own work)



The Figure 7 illustrates that the four coated groups showed relatively comparable effectiveness. The control group exhibited the most drastic decline with much lower Haugh Unit values throughout the 4-week storage period compared to the other four groups. The Haugh Unit value of 2.0% chitosan-sorbitol 59.75 ± 5.07 observed in this study follows a similar trend as previous research using 1.0% chitosan-glycerol with an average HU of 55.4 ± 1.5. Both coatings maintaining Grade A quality until the third week (Caner et al., 2022). Caner, Coşkun and Yüceer (2022) reported 4.0% chitosan coating preserved Grade A only up to the third week. Whereas, in the present study, the 2.0% chitosan coating maintained Grade A quality until the fourth week. This improvement may be attributed to variations in acetic acid concentration or differences in plasticizer formulation.

5.2.3 Yolk Index measurement

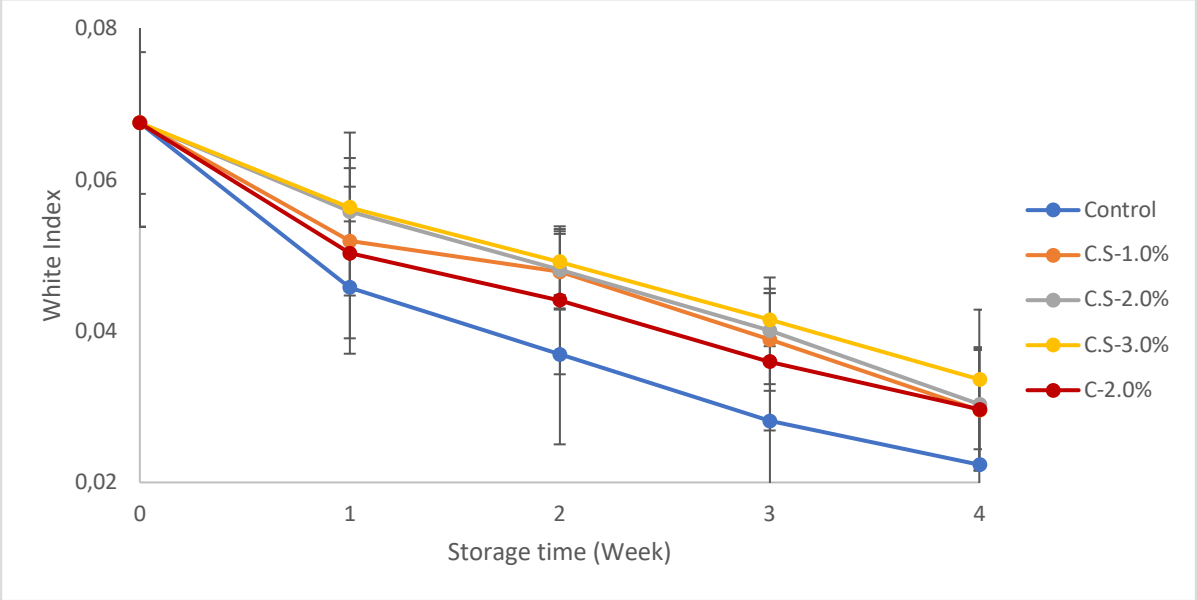
Figure 8: Yolk Index measurement of experiment II
(Source: own work)



As indicated above, Yolk index (YI) values within the range of 0.28–0.38 are generally recognized fresh egg. The measurement of yolk index followed the same overall pattern as observed in the previous parameters. The results from the referenced study showed that this freshness level could be maintained for up to four weeks in coated samples. Similarly, in the present experiment, eggs coated with 2.0% chitosan and supplemented with 1.0%, 2.0%, and 3.0% sorbitol maintained YI values of 0.28 ± 0.019, 0.29 ± 0.014, and 0.30 ± 0.033, respectively, at the end of the fourth week. Better results were observed when the concentration of sorbitol increased, indicating that higher sorbitol levels more effectively preserved the internal quality of the eggs. The 3.0% sorbitol–chitosan coating maintained the highest yolk index values throughout the 4-week storage period, followed by the 2.0% and 1.0% sorbitol concentration. From the Figure 8, the sample without plasticizer exhibited considerably lower values. Meanwhile, both the chitosan-only and control samples could no longer be classified as fresh, with final YI values of 0.25 ± 0.016 and 0.22 ± 0.020, respectively.

5.2.4 White Index measurement

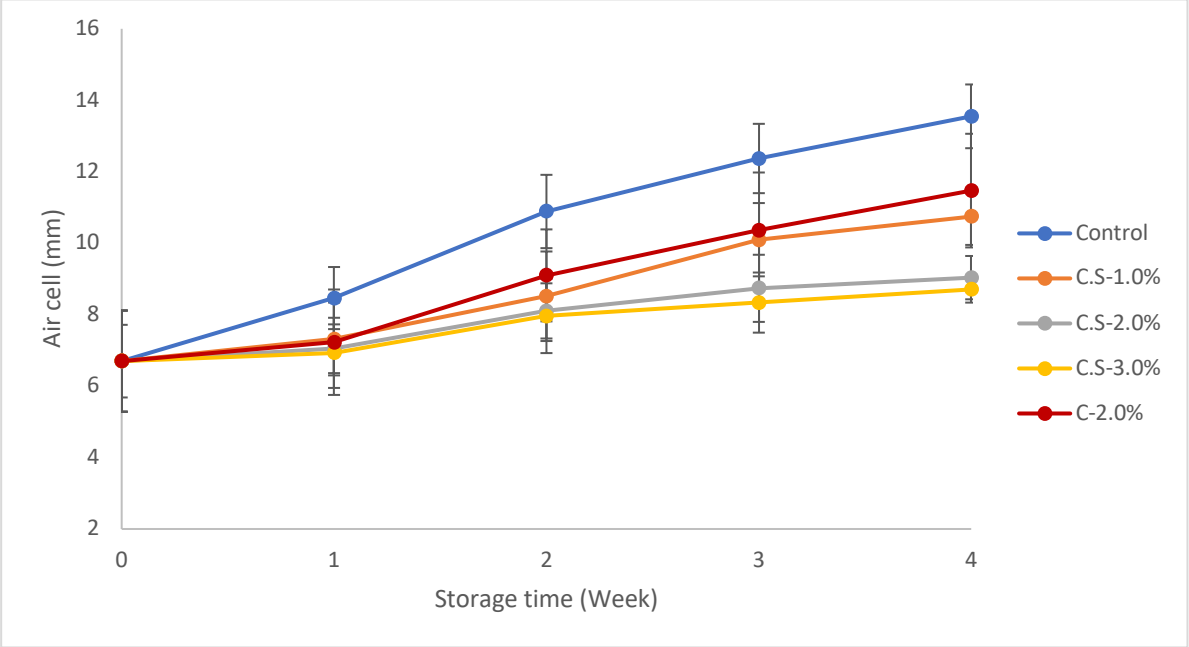
Figure 9: White Index measurement of experiment II
(Source: own work)



From the Figure 9, three coatings contain sorbitol exhibited comparable performance, while the coating contains only chitosan has slightly lower values. Based on the data obtained, the addition of sorbitol enhanced the coating's ability to maintain albumen firmness and prevent quality degradation. The control group exhibited a rapid decline in white index values throughout the storage period out of all samples. The White Index (WI) results were consistent with other quality parameters. At the end of the fourth week, the WI values for the control, chitosan-only (2.0%), and chitosan coatings (2.0%) incorporated with 1.0%, 2.0%, and 3.0% sorbitol were 0.022 ± 0.008, 0.029 ± 0.008, 0.029 ± 0.008, 0.030 ± 0.007, and 0.034 ± 0.009, respectively.

5.2.5 Air cell measurement

Figure 10: Air cell measurement of experiment II
(Source: own work)



The Figure 10 demonstrated that the incorporation of 2.0% and 3.0% sorbitol achieved the best performance out of all samples with the lowest air cell height throughout the storage period. The samples coated with only chitosan and the one with 1.0% sorbitol showed relatively similar results, indicating limited effectiveness in preventing gas exchange. Without coating, the control group displayed the most pronounced increase in air cell height and reflected rapid moisture and CO_2 loss during storage. At the end of the fourth week, the AC values for the control, chitosan-only (2.0%), and chitosan coatings (2.0%) incorporated with 1.0%, 2.0%, and 3.0% sorbitol were 13.55 ± 0.89, 11.47 ± 1.59, 10.74 ± 0.79, 9.04 ± 0.61, and 8.71 ± 0.37, respectively. These findings confirm that higher sorbitol concentrations enhance the coating's barrier properties, effectively reducing air cell enlargement and preserving egg freshness for a longer duration.

6. Conclusion

Based on the results obtained from Haugh Unit, yolk index, white index, air cell, and weight loss, it can be concluded that higher concentrations of chitosan produced more effective preservation results. Both 2.5% and 3.0% chitosan coatings demonstrated comparable performance, indicating that 2.5% can be considered more economical. Whereas, 1.5% chitosan showed less effect in egg quality compared to 2.0%, 2.5%, and 3.0% of chitosan. The addition of sorbitol as a plasticizer significantly enhanced coating. Higher sorbitol concentrations enhance the barrier properties. Based on the availability, biodegradability, safety, and low cost of chitosan, it has potential for industrial-scale application in egg preservation.

7. Suggestion

For large-scale adoption, futher development on the chitosan coating formulas and its application process is needed. Future research could explore:

Experiment in combination other natural biopolymers or nanoparticles (e.g., alginate, gelatin, TiO_2, ZnO) to enhance antimicrobial activity and mechanical stability.

Sensory and consumer acceptance studies to ensure that coating application does not affect the visual or sensory qualities of eggs.

Shelf-life modeling and cost–benefit analysis to determine the economic feasibility of implementation in commercial egg production lines.

Alternative plasticizers or crosslinking agents that maintain coating flexibility while minimizing cost and potential environmental impact.

8. Summary

Egg is an important food source that provides essential nutrients while being widely available and relatively affordable. Its importance necessitates suitable approaches to better maintain egg quality and reduce economic losses. Various methods have been developed to either lower microbial loads on eggs or through approaches such as coating to maintain key quality parameters. Coating has gained increasing attention due to its biodegradable, non-toxic, and safe nature with specific properties that make it a suitable for extending egg shelf life. Among the main categories of coating materials, polysaccharides are notable for their excellent barrier properties, with chitosan being extensively studied for its effectiveness. It is not only eco-friendly but also cost-effective and has antimicrobial properties, making it a potential coating material.

This study investigates the effects of chitosan coatings at four concentrations (1.5%, 2.0%, 2.5%, and 3.0%) and 2.0% chitosan combined with sorbitol as a plasticizer at three concentrations (1.0%, 2.0%, and 3.0%) on extending egg shelf life. The coating was applied using the dipping method, and placed on metal net to air-dry. The applied coating provides a protective surface over the shell, sealing the pores, lowering gas exchange, reducing breakage, and maintaining egg quality better than uncoated control. Egg qualities were evaluated based on weight loss (WL), Haugh unit (HU), yolk index (YI), white index (WI), and air cell height (AC) during 4 weeks at 25 °C. Based on HU values, eggs coated with 1.5% chitosan extended freshness by one additional week, while those with higher concentrations or with sorbitol extended it by at least two weeks. All parameters followed a similar trend: higher concentrations of chitosan and sorbitol had better internal quality preservation. In the chitosan-only treatments, all concentrations except 1.5% showed improvement over the uncoated control. The 2.0%, 2.5%, and 3.0% coatings successfully maintained grade A egg quality after four weeks of storage. Furthermore, the addition of sorbitol as a plasticizer to 2.0% chitosan markedly enhanced the coating efficiency, showing better results than chitosan alone and retaining grade A quality until the end of the experiment. The 2.5% chitosan coating showed performance comparable to 3.0%, suggesting it as a more economical option for large-scale application. The incoporation of 3.0% among all sorbitol treatments provided the best preservation effects even though overall performance of different concentration of sorbitol had no significant difference.

frOverall, this study highlights the effectiveness of chitosan-based coatings in extending egg shelf life by providing an efficient barrier to moisture and CO2 transfer. Chitosan has attracted considerable attention due to its excellent oxygen barrier and antimicrobial properties. The combinations with other materials should be investigated to improve the properties of the coating layer. Research could explore the combination of chitosan with oils to enhance hydrophobicity and improve its moisture barrier properties. By combining with antimicrobial components, the coating could potentially prevent microbial growth and better maintain egg quality. Chitosan is cost-effective but the additional procedures and requirements associated with its application can increase production costs. Therefore, it is essential to find a balanced approach that maintains both egg safety and quality while remaining economically viable.

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10. List of tables and figures

10.1 List of figures

10.2 List of tables

DECLARATION

on authenticity and public assess of thesis

Student name:	Duong Hanh Hoa
Student's Neptune code:	CE3WG1
Title of thesis:	Effect of chitosan coating on chicken egg quality during shelf life
Year of publication:	2025
Name of the consultant's institute:	Institute of Food Science and Technology
Name of consultant's department:	Department of Livestock Product and Food Preservation Technology

I declare that the final thesis submitted by me is an individual, original work of my own intellectual creation. I have clearly indicated the parts of my thesis or dissertation which I have taken from other authors' work and have included them in the bibliography. Furthermore, I declare that the artificial intelligence tools (e.g. text generation, linguistic correction, translation, data analysis) used during the preparation of the thesis did not substitute my own research and creative work; their use was indicated either in the list of sources or in the methodology section, and I acted in accordance with professional and ethical expectations.

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Name of the student:	Duong Hanh Hoa
Neptun ID:	CE3WG1
Level of program (mark with X):	☒ BSc/BA ☐ MSc/MA ☐ Doctoral School (PhD) ☐ Other: .....
Name and code of the subject*:	ELTUD
Title of the work:	Effect of Chitosan Coating on Chicken Egg Quality During Shelf Life

* Not required to be completed in the case of a doctoral dissertation.

2. Declaration on the Use of AI

I, the undersigned, fully aware of my ethical responsibility, make the following declaration:

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3. Details of Artificial Intelligence Usage

TABLE I: Assistant or Minor Usage (e.g., translation, language proofreading, brainstorming, etc.)

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Purpose of Use	Name and Version of the AI Tool Used	Affected Section (if not applicable to the entire text)
Polishing texts, fixed grammar	Grammarly, Chat GPT 4.0	

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