MASTER THESIS

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Effect of edible coating on egg quality during storage

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1.1.Introduction:

My academic focus as a master's student in Food Safety and Quality Engineering, has involved studying different methods for preserving and maintaining the quality of food while also learning to assess and control food quality. Having gained insights into innovative strategies during my studies, I am now eager to apply this knowledge in the field of egg preservation, where issues like spoilage and economic losses are important.

In 2021, the EU exhibited robust egg production for consumption, estimated at 96 billion eggs. The Eurostats (2023) data illustrates a steady upward trend in production from 2013 to 2021. The European Commission (2019) predicts that Member States' egg production will rise by 9% by 2030. In 2018, the EU 28 (European Union 28 countries) produced 9.3% of global eggs. Because of its export orientation, the EU 28 has achieved self-sufficiency rates ranging from 102 to 104% in recent years. The EU 28 countries' exports of eggs and egg products totaled 219 thousand tons in 2018, making them the world's second largest exporter. In contrast, imports of eggs and egg products were significantly lower, totaling 27 thousand tons in 2018.

The global egg market is a particularly important economical market, which primarily deals with chicken eggs, is significant, with revenues of US$26.91 billion in 2023 (Statista, 2023). It is expected to grow at a rate of 7.71% annually between 2023 and 2028. China is the leading contributor to this market, generating US$20.05 billion in revenue in 2023 (Statista, 2023). This statistical observation emphasize the extensive consumption of eggs and their economic significance within daily dietary practices (Statista, 2023).

In the context of egg quality, healthy poultry lay eggs with sterile liquid content, protected by membranes covering eggshells and albumen. These membranes temporarily safeguard against microbial invasion through the eggshell's pores. However, these defense mechanisms are susceptible to microbial reproduction and destruction, resulting in physical and chemical changes in eggs (Techer et al., 2014) .in order to maintain the egg quality ,studies (Derelioğlu & Turgay, 2019; Eddin & Tahergorabi, 2019) have predominantly focused on microbiological quality and safety, leading to use of edible coatings, designed to seal eggshell pores, prevent gas exchange with the external environment, and maintain internal quality during storage.

1.2.The goal of the thesis work:

The aim of the study is to comprehensively evaluate the effect of edible coating on egg quality by investigating how cassava starch-based coatings affect the egg quality during storage. Specifically, I studied the impact of different cassava starch concentrations in the coating solution on egg quality over four weeks. Additionally, and evaluated the performance of the most effective coating on various egg size classes, focusing on quality parameters like Haugh unit, weight loss, yolk index, albumen index, air cell size.

In this thesis work, two experiments were conducted, involving more than 400 eggs in total:

In the first experiment, I evaluated the influence of three different concentrations of cassava starch in the coating solution on egg quality during storage, which by calculating the effects on egg quality parametres over a period of four weeks, I was able to identify the most suitable concentration that maintain egg quality and extends shelf life.

In the second experiment I applied the most suitable coating chosen from the previous first experiment to different egg size classes. Through assessing various quality parameters, I was able to understand the effect of coating and eggs of varying sizes on the overall egg quality preservation.

In summary the aim was to evaluate the effectiveness of cassava coating and how varied sizes of egg affect the effectiveness of coating, my experiments results share information about the potential benefits of cassava starch-based coatings for maintaining egg quality and extending shelf life.

1.3.Literature overview:

1.4.General Overview of Eggs:

Eggs are considered a nutritional and important food for humans, serving as a rich source of essential lipids, proteins, vitamins, and minerals. Moreover, they prove to be an important ingredients in various food products, providing a combination of nutritional, sensory, and functional attributes, including emulsifying, coagulating, and gelling properties. Despite these advantages, eggs are prone to spoilage and contamination.

Not only they hold a nutritional importance but they also have an important economical market, which by 2028, the European market is expected to reach a weight of 9.74 billion kg, with an estimated 3.1% volume growth in 2024. It is anticipated that each person will consume around 10.0 kg of eggs in 2023 (Statista, 2023). With a significant laying hen population exceeding 350 million hen and an annual egg yield surpassing 6.7 million tons, the European Union regulation regarding egg products to provide a good quality food product and protect the consumers safety eggs (European Commission, 2022), also emphasize the importance of exploring preservation methods for.

In summary, the egg market is an important sector that plays a crucial role in diets worldwide. Its continued growth reflects an importance in exploring technologies to maintain it quality.

1.5.Nutritional Value of Eggs:

This table below provides the nutritional composition of whole fresh raw egg (U.S. DA, 2024a) .

Table 1: nutritional composition of whole fresh raw egg

Nutrient	**Amount per 100g**
Water	74.6g
Energy	155 kcal (649 kJ)
Protein	12.6g

Total Lipid (Fat)	10.6g
Ash	1.08g
Carbohydrate	1.12g
Total Saturated Fatty Acids	3.27g
Total Monounsaturated Fatty Acids	4.08g
Total Polyunsaturated Fatty Acids	1.41g

1.6.Egg composition:

The egg is covered by a shell that is porous, having a thickness of about 0.2–0.4 mm (Springer, 2009). The shells of chicken eggs can be white-yellow or brown, while duck eggs may appear greenish to white. The shells of various wild bird eggs often have characteristic spots. Inside the shell, there are two closely adhering membranes (inner and outer) that separate at the large end, forming an air space called the air cell. The air cell is usually around 5 mm in diameter in fresh eggs, which expands as eggs are stored, serving as an indicator of egg age (Zhao et al., 2024). The egg white, also known as albumen, is a gel-like liquid with a faint straw tint, consisting of three fractions with different thicknesses. The yolk is a thin but strong layer of albumen called the chalaziferous layer. This layer branches on opposite sides of the yolk, resembling twisted rope-like cords that function as anchors to keep the yolk centered. The germinal disc (blastoderm) is located at the top of a club-shaped latebra on one side of the yolk. The yolk itself consists of alternating layers of dark- and light-colored material arranged concentrically. When an egg is opened, the chalazae remain attached to the yolk. On average, a chicken egg weighs around 58 g, with its primary components being water (approximately 74%), protein (around 12%), and lipids (about 11%) (U.S. DA, 2024a) .

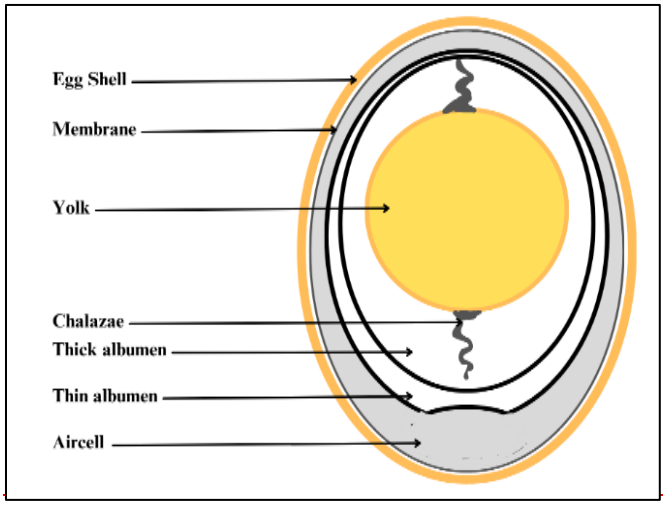

Figure 1: structure of the egg

An egg is comprised of several distinct components, each with different characteristics that are listed below :

1.6.1. Air cell:

As an egg age, it undergoes a natural process known as transpiration, where water vapor is gradually lost through tiny pores in the eggshell. These pores function as passages for gas exchange, and with the aging of the egg, the transpiration rate increases. The loss of moisture is balanced by air seeping into the egg through the shell, causing the air cell inside to expand. This balance between water vapor leaving and air entering through the porous shell leads to changes in the overall density of the egg. Consequently, when the egg is placed in water, the larger end rises to shallower depths over time. Factors like temperature, humidity, and the inherent porosity of the eggshell influence the transpiration rate, making the observation of changes in the air cell size a valuable indicator of an egg's freshness (Jalili-Firoozinezhad et al., 2020).

The presence and size of the air cell, especially at the larger end of the egg, serve as dynamic indicators influenced by the cooling and contraction of the egg contents after laying. This phenomenon is crucial in the grading system for chicken eggs, evaluated through candling. Initially, a very fresh egg, labeled as grade AA, has a small air cell. As the air cell expands, accompanied by a decline in egg quality, the grading shifts from AA to A and eventually to B (mcgee, 2007).

1.6.2. Shell:

The formation of eggshells occurs in two stages: the uterus and the isthmus. Eggshell membranes are generated in the isthmus, with inner thin and exterior thick layers made up mostly of proteins like collagen. The eggshell, which acts as both a biochemical and physical barrier, forms in the uterus (shell gland) of laying chickens. This complex biomineralization process involves the secretion of numerous proteins and minerals (Zhao et al., 2024). The composition includes both inorganic elements like caco3 crystals and organic components such as glyco- and phospho-proteins and proteoglycans. Different eggshell matrix proteins, including as ovocalyxin, ovocleidin, and osteopontin, regulate the form and development of calcite crystals (Elhamouly et al., 2023).

1.6.3. Membrane:

The eggshell membrane (ESM) is sited between the albumen's enclosing membrane and the inside of eggshell. ESM takes an important part in mineralizing the eggshell and it defends the mineralization process of the albumen. The ESM's inner and outer membranes have been identified by scanning electron microscopy (SEM). SEM photographs indicate that their outer layers are typically 50–70 μm thick, whereas those on inside range from 15 to 30 μm. Both layers possess tangled structures of woven fibers whose diameters vary from 0.1 to 7 μm. More particularly, outside layer fibers range in size from 1 to 7 μm while those of inside layer vary between .01 and three micrometers. The inner layers are denser than outer ones, with gaps among fibers that render them permeable to gas or water at pore sizes near about five micrometers. ESM lies amidst this albumin and shell encompassing two layers such as Outer ESM and Inner ESM. Furthermore, SEM images reveal how the mammillary layer looks like, palisade layer with its respective locations above the mammillary body region, and even the egg shell membrane itself which covers them underneath it all.Amino acid analysis of ESI has indicated that it consists over twenty amino acids(Park et al.,2016).

1.6.4. Egg white (Albumen):

The egg white, or ´albumen´, is a mixture of water, proteins, and carbohydrates, serving as a secondary protective layer against bacterial penetration into the yolk. The egg white has four layers with varying viscosities, named based on their viscosity and position relative to the yolk: outer

thin, outer thick, inner thin, and inner thick (chalaziferous) layers. The thick portions, rich in ovomucin, exhibit high viscosity, while the overall egg white behaves pseudoplastically, with apparent viscosity decreasing with increasing temperature until fluidity is lost around 60 °C. During shearing, filamentous superaggregates of thick parts break down, leading to lower viscosities in thin portions (Jalili-Firoozinezhad et al., 2020) .

1.6.5. Egg yolk:

Egg yolk is rich with important nutrients like phospholipids, vitamins, minerals, and lutein, making it easily absorbed by the body. Its composition includes about 50% water, 30% lipids, 16% proteins(U.S. DA, 2024b), and some other minor substances. When we separate egg yolk using a gentle spinning process, we get two parts: a liquid part (plasma) and a solid part (granules).

In the food industry, egg yolk is a key ingredient because it can help in some useful recipes, like making bakery products smooth and thick. That is why it commonly found in products like mayonnaise, salad dressing, ice cream, and baked goods (Jalili-Firoozinezhad et al., 2020).

In industrial context, ensuring the safety of egg yolk consumption involves the implementation of heat treatment to eliminate potentially harmful microorganisms. However, this process poses a challenge due to the heat sensitivity of egg yolk proteins and fats. Exposure to temperatures above 65 °C can induce protein denaturation and fat breakdown, leading to the formation of clumps and alterations in texture. Consequently, there is a need to explore and develop alternative smethods, preserving its structural integrity and functional properties during industrial processing. (Zhao et al., 2024) .

1.7.Challenges in egg preservation spoilage:

Eggs, commonly found in our kitchens, are delicate and prone to spoilage. To keep them fresh, refrigeration is crucial, a practice followed in various countries like the United States, Australia, Japan, Sweden, and the Netherlands (Cader et al., 2014). The majority of eggs (approximately 90%) are sterile upon laying, but there is a possibility of contamination (Techer et al., 2014). Unfortunately, eggs also provide an optimal environment for the growth of both spoilage and pathogenic microorganisms. The rate of egg spoilage is influenced by factors such as nutrient availability, temperature, storage conditions, and handling procedures (Cader et al., 2014) .

According to EU legislation, Article 13 of Commission Regulation (EC) No 589/2008, enacted on 23 June 2008, provides detailed instructions for the implementation of Council Regulation (EC) No 1234/2007 concerning marketing standards for eggs. This article specifically addresses the indication of the date of minimum durability. According to Article 3(1) (5) of Directive 2000/13/EC, the date of minimum durability must be set within 28 days after the eggs are laid. If the laying period is specified, the calculation of the date of minimum durability should commence from the initial day of that period (Commission Regulation, 2008).

That main spoilage risk are the following:

1.7.1. Microbial contamination:

Eggs can be contaminated by various pathogenic microorganisms, presenting a significant food safety concern. The microflora inhabiting the eggshell comprises Gram-positive bacteria such as *Staphylococcus, Streptococcus, Aerococcus, and Micrococcus*, (Techer et al., 2014) with minor presence of Gram-negative bacteria including *Salmonella, Escherichia, and Alcaligenes* sp., as well as Gram-positive bacteria like Bacillus. Studies have reported varying levels of mesophilic aerobic microbiota on eggshell surfaces, ranging from $10^{3.8}$ to $10^{6.3}$ colony-forming units per egg, with an average level of approximately $10^{4.5}$ cfu/egg (Liu et al., 2021). While Gram-positive bacteria dominate the eggshell surface flora, the contamination of egg contents primarily involves Gram-negative bacteria, which are known to better resist natural egg defenses. These bacteria possess characteristics facilitating penetration through eggshell and membranes, resistance to albumen's growth-inhibiting properties, and various enzymatic activities that break down complex nitrogen and carbon sources in egg fluids, promoting bacterial growth. The primary event of egg spoilage manifests as a rotten egg, characterized by colored shells (black, blue, pink, red, green) and a foul odor. Bacteria implicated in this spoilage include *Pseudomonas, Proteus, Alcaligenes, Enterobacter, Serratia, Stenotrophomonas, Cloaca, Acinetobacter, Moraxella, and Citrobacter spp*. Other spoilage events lead to yellow pigmentation of shell membranes, attributed to Flavobacterium or *Cytophaga* species (Techer et al., 2014). Contamination can occur through vertical and horizontal routes. Vertical contamination happens during egg formation in the ovary or oviduct, while horizontal transmission occurs post-lay when bacteria penetrate through the shell. The cloaca in laying hens is where their intestines, urinary system, and reproductive system all come together. Because everything shares this one exit point, called the cloaca, it can sometimes

cause the outside of eggs to get dirty or contaminated. The drop in egg temperature after oviposition creates negative pressure inside the egg, potentially facilitating bacterial penetration. However, the presence of pathogenic bacteria on the eggshell is not always linked to fecal contamination.

Concerning viral contamination, table eggs are rarely associated with transmitting viral foodborne diseases. Eggs from flocks infected with avian influenza can yield contaminated eggs, but the risk of viral transmission to consumers is extremely low. Although there are sporadic reports of Hepatitis E virus transmission through contaminated eggs, the egg contents are not conducive to the replication of human foodborne viruses, making this mode of transmission unlikely (Chousalkar et al., 2021) .

In a previous study, Chousalkar et al., (2021) found that, spoilage bacteria commonly found in eggs were identified. *Staphylococcus* spp., *Bacillus* spp., *and Stenotrophomonas* spp. were identified as the most abundant bacteria. *Staphylococcus* spp., *and Bacillus* spp. were prevalent in the egg contents, with *Staphylococcus* spp. Known for contaminating various foods like milk, meat, eggs, and fish. The production of Staphylococcal enterotoxin or invasive enzymes by these bacteria can result in severe symptoms, including diarrhea and vomiting, and may even lead to fatal outcomes in extreme cases.

Bacillus spp. displayed robust protease activity, a crucial factor contributing to their survival within eggs (Liu et al., 2021) . The majority of the isolated spoilage bacteria exhibited potent enzyme-producing capabilities, a vital adaptation for their existence in eggs. Alkalinity tolerance tests revealed that spoilage bacteria could withstand alkaline conditions, creating favorable conditions for their growth in eggs. Some strains demonstrated enhanced adaptability to alkaline environments over time, indicating genetic flexibility and variability. Comparisons of growth curves showed similar trends within genera, such as *Bacillus* spp. and *Staphylococcus* spp., highlighting their resilience to alkaline conditions. The pH of egg whites experiences a rapid increase after laying, impacting the growth conditions for bacteria.

1.7.2. Aging process:

In regular handling conditions, signs of compromised egg quality include changes like an increase in the air cell volume and a decrease in weight. Aging involve water loss through the eggshell,

liquefaction, a rise in the egg white's pH, flattening of the egg yolk, and weakening of the vitelline membrane (Anton, 2007) . These changes occur due to gas exchange between the internal content of eggs and its surroundings, along with the movement of iron or water between the egg white and yolk. With the yolk having less hydration, water moves from the egg white to the yolk during storage, contributing to liquefaction (Yimenu et al., 2017) .

During storage, the albumen undergoes physical deterioration, transitioning from a thick, gelatinous structure to a thin, white gel due to increased pH resulting from CO^2 loss through the shell. This deterioration leads to decreased viscosity as the albumen loses its original structure, attributed to the destabilization of the ovomucin–lysozyme complex. Separating the beta fraction of ovomucin from the complex causes the thinning of the albumen (Caner & Cansiz, 2008).

The transfer of mineral cations from the egg white to yolk can enhance this process, especially at higher storage temperatures. The yolk index, representing the yolk's spherical nature and quality, decreases over time during storage at higher temperatures but remains constant at lower temperatures (Stadelman & Cotterill, 2017) .

1.8.Common methods of eggs preservation:

Ensuring effective egg preservation is crucial for reducing economic losses and extending shelf life, providing consistent access to fresh and safe to consume eggs for consumers. Due to the natural perishability of eggs, it is essential to employ strategies like refrigeration to reduce financial losses and enhance food security. These preservation techniques also play a vital role in extending the shelf life of eggs, ensuring their quality and safety for an extended period.

In this discussion, we will look at different egg preservation methods, each with its own set of characteristics, advantages, and disadvantages. Numerous studies were conducted to determine the efficacy of these methods and their potential contributions to the advancement of food preservation practices. Furthermore, previous research examples will be used to show how these preservation techniques can be applied and produce results.

1.8.1. Edible coating:

Edible coatings play a crucial role in preserving the quality and safety of various food products, including eggs. Different application methods, such as spraying, electrostatic spraying, or dipping, can be employed based on factors like viscosity, texture, and equipment availability. Dipping and spraying are common methods, with dipping being cost-effective and easy, while spraying offers uniform coating and reduces the risk of contamination (Sharaf Eddin et al., 2019) .

Achieving a uniform coating is essential for maintaining food safety and quality. Good adhesion between the product surface and the coating solution is crucial. The effectiveness of an edible coating depends on its barrier properties to moisture, oxygen, and carbon dioxide, influenced by the chemical composition, structure of coating-forming polymers, characteristics of the produce, and storage conditions.

There is different types of edible coating based on different grades, during my literature reaserch I classified them as the following:

1.8.2. Protein-based Edible Coatings:

Proteins derived from plant and animal sources, such as gelatin, fish proteins, milk proteins, and egg products, are commonly used in edible coatings. Gelatin, obtained from collagen, is widely used, although alternatives like fish proteins and milk proteins are explored due to concerns about porcine gelatin (Sharaf Eddin et al., 2019) . Protein-based coatings are preferred for their superior barrier properties against oxygen, carbon dioxide, aroma, and lipids. However, they may have limitations such as poor resistance to water vapor and hydrophilic properties.

Protein-based coatings represent a diverse and versatile class of materials used in the egg market. These coatings offer numerous advantages such as biocompatibility, biodegradability, and functional properties. Among the several types of protein-based coatings available, these are the widely used examples:

1.8.3. Whey protein:

The ability of whey-based films to form a cohesive layer is contingent upon the thermal denaturation of whey protein within an aqueous solution. Heating induces modifications in the three-dimensional structure of whey protein, exposing internal hydrophobic and SH (sulfhydryl) groups, thereby promoting hydrophobic interactions and intermolecular S-S (disulfide) bonding upon drying. Achieving a uniform film is facilitated by adjusting the pH of the film-forming solution in whey protein concentrate (WPC) to 6.6 and heating it to 75°C Furthermore, ultraviolet (UV) radiation and ultrasound (US) treatments have been shown to enhance the properties of whey-based films. Incorporating plasticizers into these films renders them flexible. While whey proteins inherently exhibit poor moisture barrier properties due to their hydrophilic nature, the addition of a plasticizer results in flexible, transparent, and flavorless films (Hauzoukim & Mohanty, 2020) .

Characteristics of solvent-cast water-soluble protein (WP) films: The essential attributes of edible films revolve around their mechanical, barrier, and visual properties, as these aspects dictate their applicability and usability. Similar to conventional plastic film packaging, the primary mechanical properties of interest include tensile strength, elastic modulus, and percent elongation. Meanwhile, key barrier properties encompass film oxygen permeability and water vapor transmission rate.

Additional properties like oil and aroma permeability are relevant for certain applications. Among appearance attributes, transparency, color, and gloss are paramount (Jooyandeh, 2011) .

It mechanical properties such as tensile strength, elastic modulus, percent elongation, and resiliency serve as indicators of protein-protein interactions within whey protein (WP) film matrices. Tensile strength refers to the maximum force applied to a film per unit original cross-sectional area before breakage. Elongation represents the distance the film can stretch before breaking, divided by the original film length. Resiliency, on the other hand, denotes the overall toughness of the film and can be estimated by multiplying tensile strength by percent elongation. These properties can be adjusted to produce more flexible, stretchable, and resilient films by altering the protein's state or adding plasticizers. Increased cross-linking during denaturation results in stronger and stiffer films with greater elongation compared to films made with WP in its native form. However, cross-linking also permits greater deformation of the films. The amount and type of plasticizer in a WP film further influence tensile properties, with plasticizer efficiency contingent upon factors such as size, shape, and compatibility with the protein (Jooyandeh, 2011) .

In the study conducted by P. G. Da S. Pires et al., (2021), the application of whey protein coating with plasticizers proved instrumental in extending the shelf life of eggs. This innovative approach significantly mitigated weight loss in the eggs, preserving their freshness and internal quality over an extended storage duration of up to 42 days at 20 °C. After a duration of 42 days, it was observed that uncoated eggs exhibited a higher weight loss percentage (5.4%) in comparison to those coated with WPC+GLY (glycerol) (3.8%), WPC+SOR (sorbitol) (3.3%), and WPC+PRO (propylene glycol) (3.9%). Additionally, uncoated eggs displayed a Haugh Unit (HU) value of 58.46 (B), whereas coated eggs demonstrated elevated values: WPC+GLY – 66.58 (A), WPC+SOR – 68.79 (A), and WPC + PRO – 71.53 (A). Moreover, the coating effectively stabilized the pH levels of both albumen and yolk, ensuring consistent freshness throughout the storage period. Additionally, the whey protein coating enhanced the Yolk Index (YI), indicating superior yolk quality preservation, particularly evident after 14 days of storage.

In de Araújo Soares et al., (2021)study, the use of whey protein coating with plasticizer extended the shelf life of eggs by up to 8 weeks when stored at room temperature (25 ± 3 °C and 70 ± 5% RH). Coated eggs showed significantly lower weight loss (11.65%) compared to uncoated eggs

(14.505%) after 8 weeks, indicating reduced moisture transfer and improved shelf life. Additionally, the coating helped maintain high Haugh Unit (HU) values, with coated eggs retaining grade AA quality for up to 3 weeks, compared to uncoated eggs which deteriorated rapidly. The yolk index (YI) was also better preserved in coated eggs, lasting up to 3 weeks with a YI of 0.39, while uncoated eggs degraded within 1 week. Furthermore, the protein-based coating contributed to maintaining albumen and yolk pH closer to fresh levels for a longer duration, indicating better internal quality preservation. The coating also enhanced foam stability (>75%) for up to 5 weeks, suggesting a potential reduction in the need for refrigeration without compromising egg quality.

1.8.4. Corn Protein:

The utilization of corn zein for eggshell coating presents an appealing option due to its superior barrier properties against moisture and oxygen compared to other proteins. Zein, a class of alcohol-soluble proteins known as prolamins, is found in the endosperm of corn. Constituting 50% or more of the total endosperm protein, zein primarily serves as a nitrogen storage source for the germinating embryo. This protein is organized into small, dense bodies embedded within the glutelin protein matrix and distributed in the outer layers of the corn endosperm. Extraction of zein can be achieved through aqueous alcohol, resulting in a granular powder upon drying (Padua & Wang, 2002).

Extensive research has been conducted on the formation of films using corn zein, derived from the prolamin fraction of corn proteins, and wheat gluten, a combination of wheat protein's prolamin and glutelin fractions. Gluten films exhibit notable oxygen barrier properties along with resistance to water vapor and strong mechanical characteristics. Introducing nonpolar hydrophobic substances such as mineral oil into these films has been found to reduce water vapor permeability by up to 25% compared to control groups. Thermal treatment has shown promise in enhancing the mechanical properties of gluten-based films through covalent crosslinking of gliadin polypeptides. Additionally, applying wheat gluten to eggshells has been shown to bolster shell strength and reduce microbial contamination (Hauzoukim & Mohanty, 2020) .

In the study by Entezari et al. (2022), the application of zein protein coating with plasticizer extended the shelf life of eggs significantly. Coated eggs showed reduced weight loss throughout the 28-day storage period, with zein-coated and zein-extract coated eggs exhibiting weight losses

ranging from 0.16% to 1.45% and from 0.16% to 1.53%, respectively, compared to the uncoated control eggs' range of 0.12% to 1.85%. Additionally, both zein-coated and zein-extract coated eggs maintained higher Haugh Unit (HU) values, indicating better albumen quality and freshness, with HU values ranging from 86.34% to 92.43% and 86.93% to 90.71%, respectively, compared to control eggs' range of 77.78% to 93.85%. The yolk index values were also higher in coated eggs, suggesting superior yolk quality preservation, with values ranging from 32.64 to 45.04 for zein-coated eggs and from 32.64 to 43.36 for zein-extract coated eggs, compared to control eggs' range of 32.55 to 42.71. Furthermore, the coatings effectively reduced Salmonella enteritidis contamination on the eggshells, with both types of coatings showing a reduction in Salmonella populations by day 28, while a significant reduction did not occur in the control group. Overall, the zein protein coating with plasticizer demonstrated efficacy in extending the shelf life of eggs by reducing weight loss, maintaining albumen and yolk quality, and reducing bacterial contamination.

1.8.5. Rice protein:

In a study by P. G. S. Pires et al. (2019a), the use of rice protein coating, either alone or combined with propolis at concentrations of 5% or 10%, extended the shelf life of eggs by reducing weight loss during storage, with coated eggs experiencing 4.11% to 4.40% weight loss compared to 5.39% in uncoated eggs. Additionally, the coatings maintained higher Haugh unit (HU) values, indicative of better albumen quality, throughout the 6-week storage period, with the best results observed in eggs coated with RPC (Rice Protein Concentrate) combined with 10% propolis. Furthermore, coated eggs exhibited higher yolk index (YI) values, indicating superior yolk quality preservation, especially in eggs coated with RPC and propolis. The coatings also helped maintain lower pH levels in both albumen and yolk, contributing to better internal quality preservation. While the coatings influenced eggshell color, with propolis-coated eggs showing darker shells, there were no significant differences in eggshell breaking strength among treatments. Overall, the study concludes that coatings based on rice protein and propolis effectively preserved various quality parameters of eggs, thus extending their shelf life, and potentially reducing economic losses in the egg industry during storage at room temperature.

In P. G. S. Pires et al. (2020) study, the use of rice protein concentrate (RPC) coatings enriched with essential oils (tea tree, copaiba, or thymo) significantly reduced weight loss in eggs during a 6-week storage period at 20°C. The cumulative weight loss of coated eggs ranged from 4.23% to

4.10%, compared to 5.43% in uncoated eggs at the end of the storage period. This indicates a notable reduction in weight loss, with coated eggs maintaining their weight within acceptable ranges up to 4 weeks of storage, as opposed to uncoated eggs which exceeded the acceptable weight loss threshold of 3.46% by week 4. Additionally, the coatings helped preserve the albumen quality, as indicated by higher Haugh unit (HU) values compared to uncoated eggs throughout the storage period. Eggs coated with RPC maintained grade "AA" quality for up to 6 weeks, whereas uncoated eggs degraded to grade "A" after 3 weeks and to grade "B" after 6 weeks. Furthermore, the yolk index of coated eggs remained higher compared to uncoated eggs, indicating better yolk quality preservation. The study suggests that RPC-based coatings, especially when combined with essential oils, can effectively extend the shelf life of eggs by preserving their internal quality parameters, such as weight, albumen quality, and yolk quality, thus potentially reducing economic losses in the egg industry during storage.

1.8.6. Gelatin coating:

Gelatin, widely used in the food industry, shows potential for preserving eggs, although research on its application in egg preservation is limited. Its film-forming ability, barrier properties, and biocompatibility suggest promise for egg coating. While its efficacy in egg preservation requires further investigation, gelatin's proven record of efficiancy in food preservation suggests it could extend the shelf life of eggs. Further research is needed to fully assess gelatin's effectiveness in preserving egg quality, offering potential advancements in egg preservation techniques.

Gelatin, derived from collagen through processes like acidic, alkali, or enzymatic hydrolysis, is composed of protein. Widely utilized in the food industry, gelatin boasts favorable physical and chemical properties. Its exceptional film-forming ability, transparency, and biodegradability render it a popular choice for edible films with promising applications. However, gelatin films (GF) are susceptible to moisture and exhibit poor waterproof performance .Consequently, they are often blended with other materials to enhance moisture permeability and mechanical properties (Wang et al., 2021).

1.8.7. Lipid-based Edible Coatings:

Lipid-based coatings, which are more commonly applied to fresh fruits and vegetables, utilize waxes like carnauba wax, polyethylene wax, beeswax, and candellia wax. These coatings are hydrophobic and prevent moisture loss from being produced (Sharaf Eddin et al., 2019). Lipid-based coatings were used in eggs in different research that used different techniques that may include emulsions, and storage temperature is a critical factor affecting their efficacy. .

1.8.8. Wax coating:

Beeswax (BW) is a complex chemical compound consisting of fatty acids, hydrocarbons, and esters, produced by wax glands in honey bees. It finds extensive application in the food industry for coating and packaging due to its versatility, providing plasticity, waterproofing, resistance to moisture, antioxidant properties, and capability to deliver active ingredients (Sun et al., 2021) .

According to Biladeau & Keener. (2009), eggs coated with wax exhibited remarkable preservation of AA quality for an extended duration of at least 8 weeks. Moreover, these coated eggs showed minimal water loss through the shell, with only 5% compared to uncoated eggs. Additionally, the wax-coated eggs displayed an impressive 4–10% increase in shell strength compared to their uncoated counterparts. These results proves the effectiveness of wax coatings in prolonging the quality and durability of eggs, offering enhanced protection against moisture loss, and bolstering structural integrity.

In Eyng et al.(2021) study, the application of carnauba wax coating on eggs significantly reduced weight loss during storage, with coated eggs showing 46.1% lower weight loss compared to uncoated eggs. This reduction in weight loss contributed to a shelf life extension of up to 28 days. Furthermore, eggs coated with carnauba wax exhibited higher specific gravity, Haugh unit, and yolk index values, indicating improved internal quality retention. Coating with carnauba wax functioned as a physical barrier, reducing the transfer of moisture and carbon dioxide through the eggshell pores, thereby minimizing structural changes in the albumen and yolk. The preservation effect of carnauba wax was more pronounced at higher storage temperatures (25°C), suggesting its efficacy in maintaining egg quality under adverse conditions. Additionally, carnauba wax coating demonstrated potential in minimizing lipid oxidation in the egg yolk, with eggs coated with 15%

wax showing lower malondialdehyde concentrations compared to those coated with 12% wax or left uncoated. However, the antioxidant capacity of carnauba wax remains debated in the literature, with further research needed to elucidate its mechanism of action in egg preservation.

In the study conducted by Dewage & Abeyrathne. (2021), the use of mineral oil and plant waxes as coating materials significantly impacted various quality parameters of eggs stored at room temperature. Eggs coated with mineral oil, "boomi" wax, and "dawul kurundu" wax exhibited significantly lower weight loss compared to non-coated eggs over a 6-week storage period. Additionally, mineral oil-coated eggs maintained a higher Haugh unit (HU) value, indicating better albumen quality, for a longer duration compared to non-coated and wax-coated eggs. Coated eggs also showed lower albumen and yolk pH levels, indicating reduced gas permeability through the eggshell. Furthermore, mineral oil-coated eggs exhibited a significantly lesser increase in air sac volume compared to non-coated eggs, suggesting better preservation of internal egg structure. However, while both "boomi" wax and "dawul kurundu" wax coatings improved internal quality parameters compared to non-coated eggs, they were found to be less effective than mineral oil. Overall, the use of mineral oil as a coating material showed the most promising results in extending the shelf life of eggs stored at room temperature.

1.8.9. Vegetable oils:

According to a study conducted by Nongtaodum et al. (2013), coconut, rice bran, soybean, and palm oil were evaluated as coating materials for preserving the internal quality and extending the shelf life of coated eggs. The results showed that eggs coated with these oils remained of high quality for at least 4 weeks longer than uncoated or glycerol-coated eggs when stored for 5 weeks at 25 °C. Throughout the storage period, all oil-coated eggs maintained AA grade for 3 weeks and A grade for 5 weeks, with less than 0.5% weight loss. Furthermore, oil-coated eggs exhibited a glossier appearance compared to non-coated eggs, as assessed through instrumental analysis and consumer evaluations. Consumer perception of surface glossiness and odor of oil-coated eggs was found to be acceptable. Given the abundance and widespread consumption of coconut, rice bran, and palm oils worldwide, the study suggests their potential application as coating materials to preserve egg quality and minimize weight loss during storage without refrigeration.

According to Ndife, (2020) , the vegetable oil treatment yielded superior results. It provided better protection against deterioration for fresh shell eggs compared to untreated eggs. Among the various oil treatments, cold vegetable oil coating proved to be the most effective, significantly extending the shelf life of the studied egg samples by preserving their quality parameters. Additionally, the addition of antibiotics to cold vegetable oil enhanced protection against bacterial growth in shell eggs. However, sensory evaluation indicated poor acceptance of hot oil coating.

1.8.10. Mineral oil:

Waimaleongora-Ek et al. (2009) conducted research on the efficacy of mineral oil coating in preserving egg quality, finding that it extended shelf life by at least 3 weeks compared to noncoated eggs at 25°C, a significant benefit considering the substantial egg production in the United States. Notable differences were observed among mineral oil viscosities during the 5-week storage, with mineral oil proving most effective, significantly reducing weight loss, and extending shelf life by 3 weeks. Initial consumer acceptance studies indicated that eggs coated with mineral oil were equally acceptable as fresh non-coated eggs after 5 weeks. Further research is ongoing to assess shelf life under commercial conditions and sensory quality of mineral oil-coated eggs.

According to D. R. Jones et al. (2018), the coating of eggs with mineral oil has demonstrated a significant effect on extending their shelf life and preserving quality during storage. The study revealed that eggs coated with mineral oil and stored at 4°C exhibited the least weight loss over a 15-week storage period compared to other treatments, with cumulative percentage weight losses as low as 0.33%. Moreover, the physical quality of these eggs, including shell strength, Haugh unit, yolk shape measurements, and vitelline membrane strength and deformation, was better maintained compared to unwashed eggs stored at either 4°C or 22°C. Notably, eggs stored at 22°C without mineral oil coating showed rapid deterioration in quality, indicating the effectiveness of mineral oil coating in preserving egg quality. While the exact extension of shelf life in terms of specific duration was not explicitly stated, the substantial differences in weight loss and quality preservation between treatments suggest a notable extension of shelf life in eggs coated with mineral oil and stored at refrigerated temperatures.

1.8.11. Polysaccharide-based edible coatings:

Polysaccharide-based coatings, often used in confectionery, desserts, and bakery items, include water-soluble-gel forming polysaccharides like cellulose, chitosan, and starch. Cellulose, the most common, is known for stable hydrogen bonding and mechanical strength. Chitosan, derived from chitin, offers excellent oxygen barrier properties and some antimicrobial activity. Starch-based coatings depend on factors like amylose and amylopectin content and can enhance the shelf life of eggs. (Sharaf Eddin et al., 2019) .

1.8.12. Cassava starch coating:

Cassava starch is characterized by its properties, including high starch yield and low cost. When combined with carboxymethyl cellulose (CMC), these characteristics are further enhanced. CMC, being soluble in water, complements cassava starch by improving mechanical properties and moisture resistance in food packaging applications. This combination overcomes the limitations of native starch, such as low mechanical strength and high water permeability. Additionally, CMC's presence promotes compatibility with cassava starch, resulting in improved moisture resistance and cross-linking.

Homsaard et al. (2021) study demonstrated that the application of cassava starch-based coatings led to a significant reduction in microbial contamination on eggshells compared to uncoated eggs. Specifically, coated eggs showed a mean count of total aerobic mesophilic bacteria ranging from 0.70 to 0.91 log10 CFU/ml at 35 days of storage, while uncoated eggs had a count of 3.17 log10 CFU/ml. Moreover, eggs coated with cassava starch exhibited notably less weight loss (ranging from 1.49% to 5.08%) compared to uncoated eggs (ranging from 2.92% to 8.08%) from day 14 to day 35 of storage.

According to Rachtanapun et al. (2022a) , the use of cassava resulted in notable benefits. Both coated and uncoated eggs retained their freshness, maintaining a grade AA rating during storage for 4 weeks at 4°C. Specifically, the CS/CMC/paraffin coating effectively prevented egg weight loss during low-temperature storage at 4°C. Furthermore, this egg coating material demonstrated efficacy in maintaining Haugh unit (HU) and minimizing weight loss during storage at 25°C for 4 weeks. The CS/CMC/paraffin coating combined with low-temperature storage (4°C) not only

preserved HU but also reduced egg weight loss and prevented microbial contamination within the eggshell. Moreover, the freshness of eggs preserved by the coating material did not compromise their nutritional value. As a result, this innovative egg coating technology, characterized by high transparency and low cost of edible polymers, offers a promising solution for egg production, storage, and distribution.

According to Oliveira et al. (2022), the coating applied to the eggs, consisting of cassava starch combined with essential oils (CS+GIN, CS+LEM, and CS+TAH), led to a significant reduction in microbial contamination. Throughout the storage period, the count of total aerobic mesophilic bacteria on coated eggshells was notably lower compared to uncoated eggs. Specifically, at 35 days of storage, the mean counts ranged from 0.70 to 0.91 log10 CFU/ml for coated eggs, while uncoated eggs had a count of 3.17 log10 CFU/ml. Additionally, the coating contributed to reduced weight loss in eggs, with coated eggs exhibiting significantly less weight loss (ranging from 1.49% to 5.08%) compared to uncoated eggs (ranging from 2.92% to 8.08%) from day 14 to day 35 of storage. These results suggest that the shelf life of the eggs was extended by at least 14 days, as coated eggs maintained lower microbial contamination and reduced weight loss beyond this period compared to uncoated eggs.

According to Mota et al. (2017), the effects of coating on eggs during a 28-day storage period were investigated. It was found that while egg weight remained consistent across various treatments, weight loss of stored eggs was higher compared to fresh eggs, with no significant difference among the stored treatments. Coatings of cassava or yam starches did not effectively mitigate weight loss during storage. Additionally, there was a decrease in albumen percentages and an increase in yolk percentages in stored eggs compared to fresh ones, with no significant differences among the treatments. Regarding internal quality, only eggs stored at 5°C showed no significant difference from fresh eggs in Haugh Units values, indicating better maintenance of internal quality. However, eggs stored under other conditions experienced a decline in Haugh Units values over time, with coatings of cassava and yam starches resulting in lower Haugh Units values, suggesting decreased internal quality. Yolk color remained stable during storage, with no significant effects of coatings observed. Notably, the albumen pH of uncoated eggs stored at 25°C was higher than fresh eggs, while those stored at 5°C and coated with yam starch exhibited lower pH values, indicating better quality maintenance.

1.8.13. Chitosan coating:

Chitosan, derived from chitin, is a cationic linear polysaccharide widely employed in agriculture, food, biomedicine, and environmental industries. Its numerous functional groups, including amino groups, contribute to its positive charges, making it highly versatile. As the second most abundant biopolymer in nature, chitosan exhibits remarkable film-forming abilities and advantageous properties such as biodegradability, biocompatibility, low oxygen permeability, good mechanical strength, mucoadhesiveness, and derivability from inexpensive biomass (Wu et al., 2013) .Furthermore, its non-toxicity and low permeability to oxygen, along with excellent film-forming abilities under acidic conditions, make it an ideal material for film production. Chitosan also possesses antimicrobial and antifungal properties, rendering it effective against a wide range of pathogenic and spoilage microorganisms (Derelioğlu & Turgay, 2019) .

In Caner et al. (2022) research, chitosan coating exhibited remarkable efficacy in preserving the quality of fresh eggs. The study explored various concentrations of chitosan coatings, incorporating MMT as nanomaterials to enhance the eggs' functional properties and retain their freshness. These coatings effectively maintained internal qualities, reducing food losses by sealing pores on the shell surface and minimizing mass transfer. Different concentrations of chitosan proved economically and environmentally favorable, preserving functional characteristics such as ph, HU, YI, TS, and RWC during storage. Notably, chitosan coatings, especially at 8% concentration and combined with MMT, significantly enhanced shell puncture strength, extending the eggs' shelf life by 2–3 weeks compared to controls. Furthermore, chitosan coatings, particularly at higher concentrations and combined with MMT, effectively covered micro-cracks and holes in the shell, maintaining shell strength and stability.

In the study conducted by Rachtanapun et al. (2022b), the use of wax coating consisting of chitosan (CS), carboxymethyl cellulose (CMC), and paraffin (6/1/0.5 w/v%) significantly extended the shelf life of eggs. Specifically, the coating maintained the Haugh unit (HU) values corresponding to grade AA for 4 weeks at 25°C, while uncoated eggs deteriorated to grade B within the same period. Additionally, coated eggs exhibited lower weight loss compared to uncoated eggs at all storage temperatures (4°C, 25°C, and 30°C), with a particularly notable reduction in weight loss observed at low temperatures. Furthermore, the coating material effectively prevented microbial

contamination, as evidenced by the absence of detectable microbial counts in coated eggs stored at 30°C after 4 weeks, while uncoated eggs had a total microbial count of 728 cfu/ml. The albumen pH of coated eggs stored at 30°C remained lower than that of uncoated eggs, indicating reduced gas permeability and maintaining a more stable pH environment.

1.8.14. Pullulan edible coating:

Pullulan, a polysaccharide produced by the fungus *Aureobasidium pullulans,* is colorless, tasteless, and exhibits low oxygen permeability. Pullulan-based coatings have been shown to preserve internal quality, extend shelf life, and reduce weight loss in eggs during storage (Sharaf Eddin et al., 2019).

Pullulan-based coatings offer excellent adhesive properties, high mechanical strength, and inertness to food ingredients. They are known for their lack of color, taste, and odor, and their limited permeability to oxygen and carbon dioxide gases. Moreover, pullulan is a challenging carbon source for food spoilage microbes, further enhancing its suitability as an edible "active" coating (Ganduri, 2020).

In Morsy et al. (2015) study, pullulan coating proved to be effective in preserving the internal quality of eggs. The research showed that eggs coated with pullulan exhibited an extended shelf life of at least 2 weeks when stored at 25°C, compared to non-coated eggs. Furthermore, after 10 weeks of storage, there were no significant differences observed in weight loss, Haugh unit, and yolk index between the two pullulan treatments. However, the use of pullulan combined with nisin as a coating material showed advantages in reducing microbial contamination during storage.

1.8.15. Refrigeration:

Various methods are implemented to mitigate microbial contamination on eggshells, with common practices including washing and cold temperature storage. Notably, egg washing is prevalent in the USA, Japan, and Australia, whereas it is discouraged or prohibited in the UK and EU. The current regulations governing egg handling in the EU, particularly EC Regulation No. 589/2008 (Fikiin et al., 2020), require careful revaluation to establish clear and specific guidelines regarding temperature and humidity control throughout the handling, storage, transportation, and distribution processes of raw eggs. It is essential to consider and implement measures that address the food

safety and quality aspects influenced by temperature and humidity variations to a greater extent. Additionally, efforts should be focused on aligning the significant differences in codes and practices related to egg handling between Europe, the USA, and other regions globally, thereby eliminating unnecessary trade barriers (Fikiin et al., 2020) .The storage conditions for eggs in the downstream supply chain exhibit considerable variability, with the USA mandating temperatures at or below 7.2°C, while other countries recommend storage between 0−22°C. In contrast, some countries, like Australia, lack specific regulations and often adhere to guidelines established by others. Cold storage plays a dual role in enhancing the shelf life of eggs and suppressing the proliferation of mesophilic microorganisms. The shelf life of eggs typically ranges from 21–35 days (Chousalkar et al., 2021), contingent upon the country of origin, with environmental factors such as temperature and relative humidity influencing egg quality. Maintaining eggs at 4°C has been shown to stabilize albumin quality and egg weight over a 21-day period (Chousalkar et al., 2021), whereas storage at higher temperatures resulted in deterioration.

Beyond extending shelf life, cold storage serves to curtail bacterial replication on contaminated eggshell surfaces, particularly addressing the cross-contamination risk posed by *Salmonella*. Condensation on eggshells, stemming from their removal from chilled environments, presents a challenge in the egg supply chain. This condensate, potentially laden with bacteria, poses a risk of cross-contamination in kitchen settings, especially in high relative humidity conditions.

Refrigeration experiments conducted by (Khan et al., 2021) revealed that ambient temperature significantly impacts the growth and survival of *Salmonella Typhimurium* in eggs. Specifically, the temperature variations influenced the virulence of *Salmonella Typhimurium*, leading to salmonellosis when inoculated eggs were stored at ambient temperature. In vitro analysis of gene expression demonstrated that genes related to metabolism, stress response, virulence, and colonization were down-regulated in both the egg albumen and on the egg surface. Moreover, in vivo experiments conducted on mice infected with *Salmonella*-contaminated egg wash and albumen stored at ambient temperature showed the onset of *Salmonella* shedding in feces by day 15 post-infection. Mild heat treatment emerges as a practical measure to reduce *Salmonella* contamination on both eggshell surfaces and shell pores.

In Shin et al. (2012) investigation on the effect of various refrigeration temperatures on the quality of shell eggs, significant differences were observed in Haugh Units (HU), albumen pH , and yolk index (YI) based on storage temperature. Eggs stored below 2.2°C maintained HU measurements above 79, indicative of higher quality, while storage at higher temperatures led to decreased HU measurements due to protein changes and moisture exchange. However, storage below freezing temperatures exhibited no significant difference in HU values compared to slightly higher temperatures. The ideal storage temperature range for maintaining shell egg quality was determined to be between -1.1°C and 2.2°C. Furthermore, the study found a tendency for HU values to decrease with storage time, with eggs stored below 3.9°C exhibiting a slower rate of decline. Additionally, albumen pH increased over time, but no specific trend was observed with storage temperature. Overall, a lower temperature limit for egg storage was recommended between 0.6°C and 2.2°C to preserve AA quality and minimize quality deterioration during refrigerated storage, especially for prolonged durations exceeding 3 weeks in commercial settings. Therefore, egg processors and retailers are advised to maintain eggs within this temperature range to ensure optimal quality preservation.

1.9.Methods of eggs quality evaluation:

1.9.1. Weight loss:

Because of the aging process and the porous structure of the eggshell, the weight of eggs decrease over time during storage due to the loss of gases and moisture, which serves as an indicator of egg quality. Analyzing egg weight loss is a straightforward and non-destructive process, entailing the periodic monitoring of egg weight over time intervals.

1.9.2. Albumen pH:

The quality of albumen in eggs is a significant indicator of food safety. If fresh eggs have watery albumen, it may suggest bacterial contamination. Factors affecting the initial quality of albumen include hen genotype, age, size, breed, diet, water intake, environmental conditions, and health status. After laying, eggs undergo physicochemical and functional changes, such as pH increase, weight loss, evaporation, and microbial contamination, primarily due to increased gas exchange, especially carbon dioxide, and moisture through eggshell pores and cracks. The albumen's

deterioration during storage is influenced by storage conditions, including temperature and humidity, and eggshell characteristics (Nematinia & Abdanan Mehdizadeh, 2018) .

The typical pH range of freshly laid egg albumen is between 7.6 and 8.5 (Eddin & Tahergorabi, 2019) . In another research it was declared that pH of the eggs, during storage for 10 days at room temperature, was increased from 7.78 to 9.26 (Eddin & Tahergorabi, 2019). Following egg laying, the loss of CO^2 through the eggshell pores induces changes in the bicarbonate buffer system. Consequently, there is an increase in albumen pH during storage due to the ongoing breakdown of constituents in egg white and potential alterations in the bicarbonate buffer system. However, it is essential to consider that variations in egg size, initial egg quality, and storage conditions (such as temperature, humidity, and duration) may influence albumen pH both before and after storage.

The procedure described by Eddin & Tahergorabi (2019) depend on centrifuge tubes, thin and thick albumens were combined and homogenized for 20 seconds using a laboratory homogenizer. Subsequently, the pH of the albumen mixture is measured with a pH meter.

1.9.3. Haugh unit:

The Haugh unit, developed by Raymond Haugh in 1937 (D. Jones, 2012), has emerged as the predominant measure of albumen or interior egg quality, often regarded as the "gold standard" in determining egg quality. This metric correlates egg weight with the thickness of the dense albumen (Eisen et al., 1962). Typically, Haugh unit measurement involves a destructive test performed on a grading sample of eggs within a batch.

1.9.4. Yolk index:

The yolk index (YI) serves as an indirect measure of the yolk vitelline membrane's strength, representing the ratio of yolk height (h in mm) to yolk width (d in mm) The yolk index, which is calculated by dividing the yolk height by the yolk diameter, serves as an indicator of egg freshness.. It is a key parameter for assessing egg freshness, with a standard range typically falling between 0.30 and 0.50 in fresh eggs. According to P. G. S. Pires et al., (2019b), a high-quality fresh egg typically has a Yolk Index (YI) of approximately 0.45. Storage duration notably impacts the YI, in uncoated eggs, where the YI decreased from 0.48 to 0.38 after 3 weeks of storage. However, as eggs age, their YI tends to decrease. A higher YI indicates better yolk quality.

1.9.5. Albumen index

The albumen egg index is a metric that assesses the inner quality of eggs based on albumen properties. It can be determined nondestructively using a variety of methods. Ultrasonic waves can be used to measure albumen index in fresh chicken eggs, yielding values of 0.117 ± 0.014 (Febria et al., 2022) and 0.106 according to (Heiman & Carver, 1936). Furthermore, Fourier transform near-infrared spectroscopy has been used to estimate thick albumen height, with a high correlation between spectral data and albumen height (Crawford & Hayward-Piatkovskyi, 2022). Furthermore, ovomucin content in albumen, which contributes to gelation, varies with storage duration, influencing freshness indices such as the Haugh unit and yolk index (Yang & Geveke, 2020).

1.9.6. Air cell size:

The air cell in an egg is a key sign of freshness and quality. Traditional methods for determining egg freshness include the Haugh Unit test, which is damaging and time-consuming (Rho et al., 2023). To solve this, non-destructive approaches have been investigated, such as thermal imaging to determine air cell size for freshness prediction (Nakaguchi & Ahamed, 2022). Furthermore, developments such as deep learning algorithms have been used to analyze air cell changes in eggs quickly and non-invasively, allowing for longer expiry dates based on freshness estimates (Gu et al., 2022). Monitoring the hatching process entails precisely evaluating air cell changes, with research focused on segmentation algorithms for egg-candling photos to improve hatching efficiency (OA et al., 2018). Furthermore, the size of the air cell during incubation is highly connected to the eggshell temperature and weight loss, highlighting the significance of air cell dynamics during the incubation period.

1.10. Materials and methods

1.11. Materials:

This study used cassava starch powder with a dry purity of 99% obtained from Hunorganic Ltd. (Budapest, Hungary). Gelatin (purity ≥99%), sorbitol (purity ≥99%), and glycerol (purity of 99%) were acquired from Szilasfood Ltd (Kistarcsa, Hungary), Parma Produkt Ltd (Budapest, Hungary), and Budai Szent Klara Pharmacy. The equipment used for the experiment consisted of an electronic balance (Kern PFB, Kern & Sohn Gmbh, Balingen-Frommern, Germany), a heated plate, mixing utensils, and a digital caliper. The electronic balance was used to correctly weigh the materials used

in the studies. Cleaning materials were employed to maintain a clean and sanitary environment during the experiment. The hot plate was used to heat and prepare the coating. Mixing utensils were used to stir and blend the materials. Finally, a digital caliper was used to measure the dimensions of the samples accurately.

1.12. First experiment: Evaluating the effect of different starch concentrations in the coating solution on the quality of egg during storage

The first experiment was designed to evaluate the impact of varying starch concentrations in the coating solution on the quality of fresh chicken eggs during storage. Given that cassava starch coating on egg is a relatively new, it is essential to conduct research to determine the suitable cassava coating formula for preserving egg quality effectively.

The experiments took place at The Hungarian University of Agriculture and Life Sciences (MATE) at the Buda campus in Budapest, Hungary. A total of 250 eggs were utilized in the experiment divided into four groups; a control group without any coating S2 group with 2% cassava starch S3 group with 3% cassava starch and S4 group, with 4% cassava starch.

250 eggs were inspected for any damage and then categorized into four groups, each containing 60 eggs. Additionally, 10 eggs were set aside for initial recordings. There groups were dipped in different coating solution (2, 3, 4% of cassava starch) for 15 seconds except for the control group. The coated eggs were then left to dry at room temperature for 1 hour. And finally, the eggs were placed in a container and stored for up to 4 weeks.

Cassava starch was totally gelatinized at 95 °C in 30 minutes. After 1 hour of hydration at room temperature, gelatin was heated to 70 °C for 30 minutes. The coating elements were combined in the following order: gelatinized cassava starch, gelatin, sorbitol, and glycerol. The coating solution was utilized once it had cooled to room temperature. In order to conduct experiments, the eggs were first washed in tap water (16 °c) and then dried with a towel. Eggs were randomly divided into four groups, including control, s2, s3, and s4 groups. Each group had 60 eggs. Then, the three groups (s2, s3, and s4) were immersed in the selected coating solution for 15 s and dried for 1 h at room temperature. During the experiment, eggs were placed in fiber-molded containers where they were stored for up to four weeks.

Table 2: coating formulas for different groups

Group	Coating Solution
Control	No coating
S2	2% cassava starch, 0.5% gelatin, 3% sorbitol, 0.5% glycerol
S3	3% cassava starch, 0.5% gelatin, 3% sorbitol, 0.5% glycerol
S4	4% cassava starch, 0.5% gelatin, 3% sorbitol, 0.5% glycerol

1.13. Second experiment: evaluating the effect of Cassava Starch Coating on Various Egg Sizes.

In the second experiment, we applied the best coating obtained from the first experiment to different egg size classes and evaluated its impact on egg quality parameters such as Haugh units, yolk index, albumen index, and air cell size. This experiment aimed to evaluate the performance of the coating on eggs of different

Materials

A total of 322 commercially available eggs were used in the experiment and divided into four size groups (small, medium, large, and extra-large eggs).Subgroups were made within each size category for both coated and uncoated eggs.

Table 3: the experiment egg weight classes

Class	Weight (g)
Small (S)	**<53**
Medium (M)	**53-63**
Large (XL)	**63<**

The 322 eggs were inspected to ensure they were free from any damage and then divided into 3 groups of different size classes each coated and uncoated. They were first weighed and then dipped

in each coating solution, for 15 seconds except for the control group which received no coating. The coated eggs were then left to dry at room temperature for 1 hour. And finally, the eggs were placed in a molded fiber container and stored for up to 4 weeks.

The coating solution, had 4% starch, 0.5% gelatin, 3% sorbitol, and 0.5% glycerol.

1.14. Methods

1.14.1. Egg quality parameters

1.14.2. Weight loss

All eggs were weighed before being stored and measured every week. Egg weights were recorded with ±0.01 g accuracy on a digital electronic scale (Kern PFB, Kern & Sohn Gmbh, Balingen-Frommern, Germany). For the coated samples, the weight of the egg after coating was used as the initial weight. The difference from the initial egg weight and each interval was used to calculate the weight loss (%).

The weight loss of eggs during storage is calculated as follows (Hoover, 2022) :

$$Weight\ Loss\ (\%) = \frac{Initial\ Weight\ of the\ egg\ at\ Day\ 0\ (g)\ -\ Weight\ of the\ egg\ after\ Storage\ (g)}{Initial\ Weight\ of\ the\ egg\ at\ Day\ 0\ (g)} \times 100$$

1.14.3. Air cell size

To measure the air cell size in an egg, a small portion of the eggshell is carefully removed to allow for the insertion of a digital caliper. The caliper is then used to measure the depth of the air cell within the egg. This method provides a direct and accurate measurement of the air cell size, which can be used to evaluate egg quality and freshness.

1.14.4. Haugh Unit, yolk index and albumen index

During each week, the eggs were weighted and cracked on a transparent glass surface, and the height, the length and width of the egg yolk were measured using a digital caliper. The albumen height was measured at middle region of the thick albumen. Then the measured data was used to calculate according to the following equations

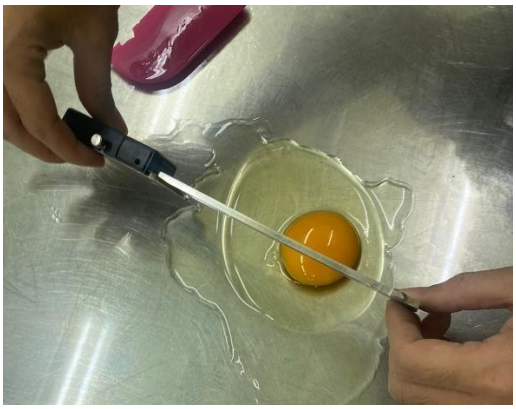

Figure 2: Albumen width measurement

1.14.5. Haugh Unit

The Haugh units is calculated as follows: (Eisen et al., 1962)

$$\boldsymbol{HU = 100 \cdot log(H + 7.57 - 1.7W^{0.37})}$$

Here, H is the observed height of the albumen in millimeters, and W is the observed weight of the egg in grams.

The Haugh Unit grading system typically includes the following classifications (P. G. Da S. Pires et al., 2021):

- Grade AA: Eggs with Haugh Unit values of 72 or higher are considered best quality. These eggs have thick and firm albumen, indicating superior quality.
- Grade A: Eggs with Haugh Unit values between 60 and 71 are slightly lower than Grade AA, Grade A eggs still have relatively high-quality albumen.
- Grade B: Eggs with Haugh Unit values between 60 and 31 have thinner albumen and are often considered lower in quality compared to Grade AA and Grade A eggs.
- Grade C: Haugh Unit value below 31 are considere bad quality

1.14.6. Yolk index

The YI is calculated using the formula:

$$YI = \frac{h}{d}$$

Where YI is the yolk index, h is the yolk height in millimeters, and d is the yolk width in millimeters.

1.14.7. Albumen index

The AI is calculated using the formula (Heiman & Carver, 1936):

$$AI = \frac{h}{d}$$

Where AI is the albumen index, h is the albumen height in millimeters, and d is the albumen width in millimeters.

1.14.8. Albumen pH

After extracting the albumen from the yolk, a glass rod was used to mix the thin and thick albumen before measuring, the pH of the homogenized sample was determined by using a pH meter.

1.14.9. Statistical analysis

For the statistical analysis, IBM SPSS software was used to conduct a two-way ANOVA analysis. This software was chosen for its robust capabilities in handling complex statistical procedures, allowing for a comprehensive examination of the data and interactions between variables in the study. The two-way ANOVA analysis helped the understanding of the effects of different variables (time, treatment, egg size) on the dependent variable (Haugh unit, yolk index …), with a significance level of $p < 0.05$, with the aim understanding the relationships and significance of the factors under investigation.

1.15. Results and discussion

1.16. First experiment: Evaluating the effect of different cassava starch concentrations in the coating solution on the quality of egg during storage

1.16.1. Weight loss:

Egg weight loss primarily occurs due to air loss through the porous shell, affecting all eggs. In our experiment control eggs experienced more weight loss than those coated with cassava starch (Figure 3). Interestingly, eggs coated with cassava starch showed consistent weight loss patterns during the first two weeks of storage. However, starting from the third week of shelf life, higher

concentrations of cassava starch in the coating showed effectiveness in reducing weight loss compared to others.

FAO regulations (FAO UN, 2003) consider that the reduction of egg weight from 2-3% during storage is acceptable. Following that, only the S4 group meets the standard in the 4th week, with a weight loss of 2.84% within the acceptable range throughout storage. On the other hand, both the S3 and S2 groups surpass the acceptable weight loss threshold by the third week, indicating that S2 and S3 coatings may be less effective than S4,and finally the control group was the first to exceeded the acceptable weight loss limit by the second week.

Similarly the statistical analysis in Table 4 supports the conclusion that storage time was the most influential factor on egg weight loss during the experiment. The results show that the effect of time on weight loss was significant, with an F-value of 1416.685. The effect of treatment was also significant, with an F-value of 384.222, suggesting that the treatment had a significant impact on weight loss. The interaction effect between time and treatment was also significant, with an F-value of 71.154, showing that the combination of time and treatment had a combined effect on weight loss. Overall, the results suggest that time and treatment was the most critical factor affecting egg weight loss.

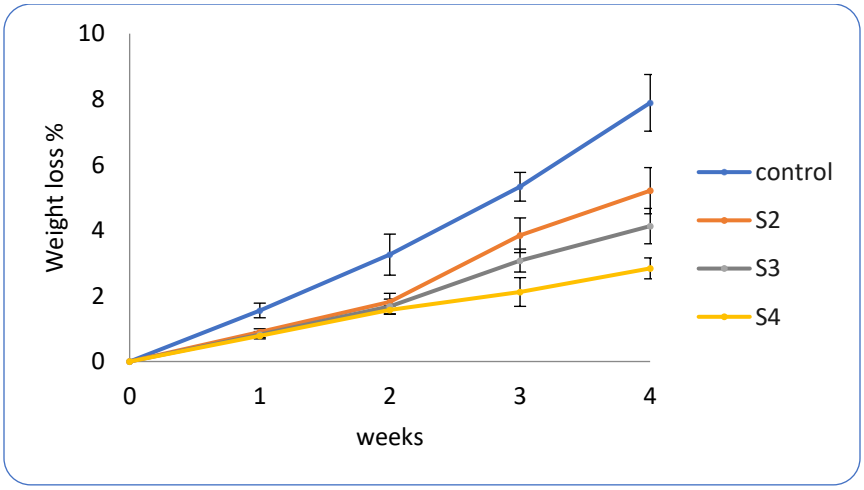

Figure 3 : first experiment Weight loss of egg samples during storage

Table 4: First experiment weigh loss ANOVA two way results

Tests of Between-Subjects Effects

Dependent Variable: Weight loss

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	854,241[a]	19	44,960	403,855	,000
Intercept	1089,698	1	1089,698	9788,253	,000
TIME	630,862	4	157,715	1416,685	,000
TREATMENT	128,323	3	42,774	384,222	,000
TIME * TREATMENT	95,056	12	7,921	71,154	,000
Error	20,039	180	,111		
Total	1963,977	200			
Corrected Total	874,279	199			

a. R Squared = ,977 (Adjusted R Squared = ,975)

Our results are in agreement with those reported by Homsaard et al. (2021), who observed a progressive increase in weight loss from 1.5% to 6.50% in non-coated eggs after 4 weeks of storage at 28°C, in our study, coated eggs, particularly those treated with cassava starch, demonstrated more favorable weight preservation outcomes with 2.84% compared to 4.86% in Homsaard et al. (2021) study. The coated eggs, especially those with cassava starch treatment, showed significantly lower weight loss rates. The result of this work is consistent with the results of Homsaard et al. (2021) that coating materials effectively inhibited moisture evaporation and subsequent weight loss.

Similarly, Rachtanapun et al. (2022) found that cassava starch coatings enhanced egg freshness and quality, resulting in weight losses of 2.8%, 3.3%, and 4.6% for uncoated eggs at 4°C, 25°C, and 30°C, respectively at the fourth week of storage. In contrast, our coated eggs consistently displayed lower weight loss percentages across different cassava starch concentrations. Additionally, the report of Oliveira et al. (2022) showed that coated eggs kept lower weight loss percentages compared to uncoated eggs after 35 days of storage at 20°C. Our results supports those

conclusion, that the cassava starch-coated eggs experience significantly less weight loss than uncoated eggs.

1.16.2. Haugh Unit:

In our study, the HU values of the control group decreased gradually as time passed during storage (figure 4). They started at 86.15 (grade AA) in the first week and dropped to 40.97 (grade B) by the fourth week. Similarly, although at a slower pace than the control group, the HU values of eggs coated with cassava starch (S2, S3, and S4) also decreased over time. By the fourth week, the coated eggs had HU values ranging from 59.15 (grade B) for S1 coating to 68.20 (grade A) for the S4 coating, showing that the eggs' quality was better than that of the control group, especially when using the S4 coating.

The statistical analysis in the annex 1 shows that the storage time and treatment had a significant effect on the Haugh unit of the eggs. The results indicate that time was the most influential factor, with an F-value of 197.939, suggesting that the length of time had a substantial impact on the Haugh unit, the effect of treatment was also significant, with an F-value of 76.609, indicating that different treatments had an important impact on the Haugh unit. Overall, the results suggest that time and treatment were the most critical factor affecting the Haugh unit, followed by the effect of treatment.

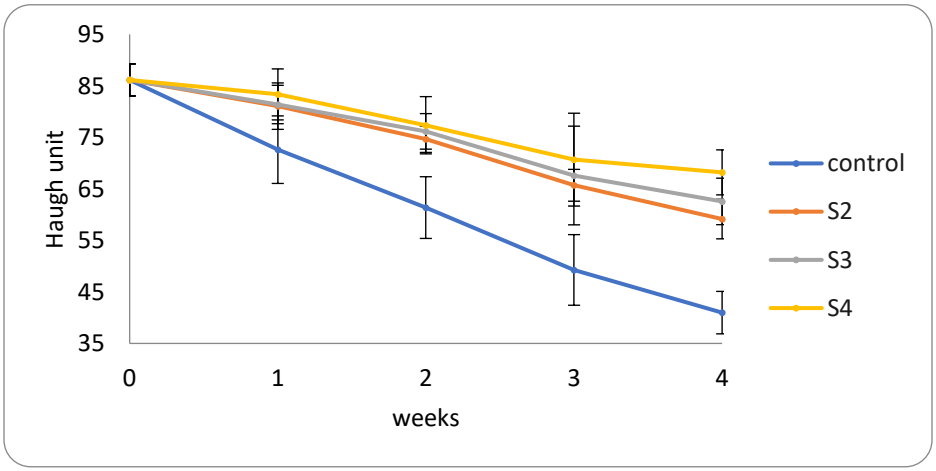

Figure 4 : first experiment Haugh unit of egg samples during storage

Our study revealed that the HU values of our coated eggs remained higher throughout the storage period, in a close range to the results of Rachtanapun et al. (2022), who observed a decrease in HU

values from 95 (grade AA) to 75 for uncoated eggs in the first week of storage. Additionally, after four weeks, uncoated eggs deteriorated to grade B (HU 45), while the coated eggs kept a grade AA HU value of 73 at 25°C.

Similarly, Oliveira et al. (2022) found that after 35 days of storage, coated eggs retained superior HU values compared to uncoated eggs. Specifically, at 20°C, coated eggs were classified as grade A (HU 70.61 ± 5.35), while uncoated eggs were categorized as grade B (HU 51.60 ± 4.28). In summary, our research demonstrates that utilizing coatings based on cassava starch can effectively maintain higher Haugh unit values, thereby enhancing the overall freshness and quality of eggs during storage.

1.16.3. Yolk index:

One important metric for determining the freshness of eggs is the yolk index, which typically ranges from 0.30 to 0.50 in fresh eggs. In our study (figure 5), the control group exhibited a progressive decline in yolk index values throughout the storage period, decreasing from 0.38 in the first week to 0.24 by the fourth week. Similarly, the yolk index values for eggs coated with cassava starch (S2, S3, and S4) also decreased over time, although at a slower pace than the control group.

Comparatively, after four weeks, the yolk index values for coated eggs were 0.27 for the S2 group and 0.30 for S3 and S4, indicating better retention of yolk quality compared to the control group.

While the yolk index values of the control group deteriorated from good to inferior quality within four weeks based on grading standards, the coated eggs, particularly those with S4 and S3 coatings, maintained higher yolk index values within the range indicative of good quality throughout the storage period. Overall, the S4 coating formulation was found to better preserve egg yolk quality than the uncoated control group and other coating types like S2.

The statistical analysis for the Yolk Index (YI) in the annex 2 indicates that both time and treatment significantly impact the Yolk Index of eggs. Time was the most influential factor, with an F-value of 132.273, suggesting a substantial effect on the Yolk Index. Treatment also had a significant effect, with an F-value of 19.701, indicating an important impact on the Yolk Index. The interaction effect between time and treatment was also significant, with an F-value of 2.042, highlighting the

combined influence of these factors on the Yolk Index. Overall, the results emphasize the importance of both time and treatment when maintaining the eggs quality.

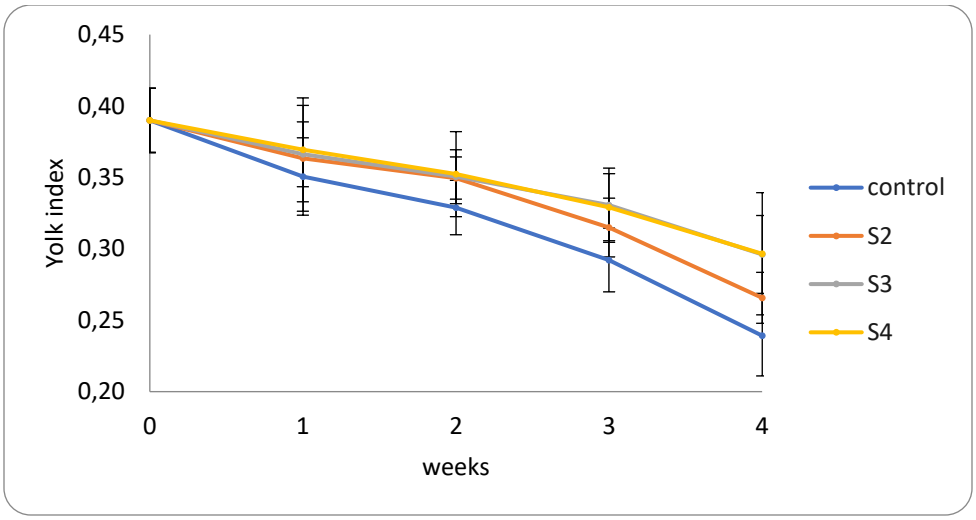

Figure 5: first experiment yolk index of egg samples during storage

Comparing our results to those presented by Oliveira et al.(2022), where the yolk index decreased during egg storage and reached a similar level between eggs treated with cassava starch (mean 0.36 ± 0.03), our study aligns with these results by showing parallel trends in preserving yolk quality. Both studies reveal that coated eggs retained higher yolk index values than uncoated eggs throughout the storage duration, highlighting the enhanced preservation of yolk quality in coated eggs.

1.16.4. Albumen index:

In the study (figure 6), the control group displayed a steady decline in white index values from week 0 to week 4, suggesting a gradual deterioration in albumen quality. Interestingly, treatment groups S2, S3, and S4 exhibited similar trends but maintained slightly higher white index values throughout the experimental period compared to the control. Particularly, group S4 showed consistently higher values from week 2 onwards, implying potential efficacy of the treatments in preserving albumen quality.

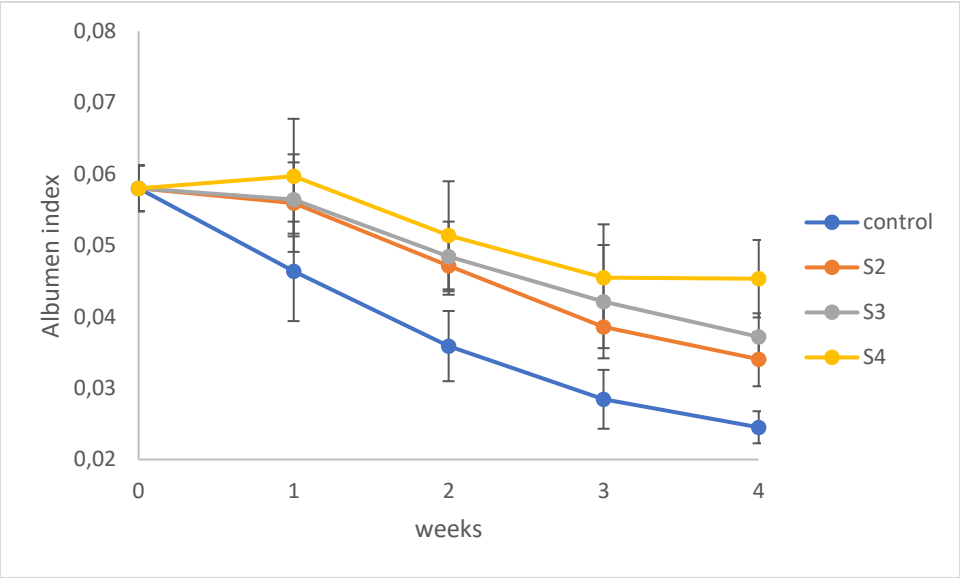

Figure 6: first experiment albumen index of egg samples during storage

The statistical analysis reported in Annex 3 demonstrates that the time variable has a considerable effect on the albumen index, with an f-value of 107.099, showing that the albumen index changes significantly over time. The treatment variable has an important impact on the albumen index, as indicated by an f-value of 40.157. Overall, the results indicate that both time and treatment have a considerable effect on the albumen index, as well as a significant interaction between the two.

1.16.5. Air cell size:

One of the most important metrics for determining how fresh an egg is its air cell size, greater air cell sizes correspond to lower egg quality. In our investigation (figure 7), the air cell size of the control group increased gradually over the storage period, from 5.93 in the first week to 9.69 by the fourth week. In a similar vein, eggs coated with cassava starch (S2, S3, and S4) showed an increase in air cell sizes over time, but at varying rates depending on the coating composition. In comparison to the control group, the coated eggs' air cell sizes at the fourth week ranged from 7.926 to 8.246, suggesting comparatively improved air cell size retention.

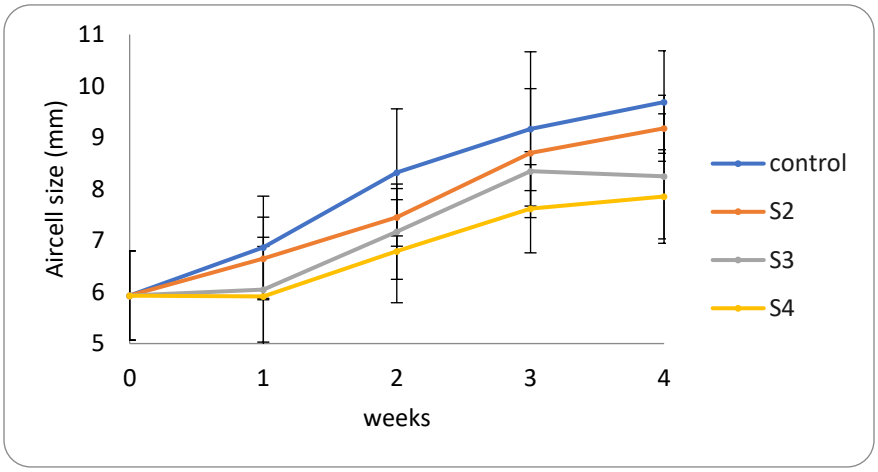

Figure 7: first experiment air cell of egg samples during storage

When grading criteria are considered, the air cell sizes in the control group in our study gradually grew throughout the storage period, reaching values that suggest a decline in freshness and quality. On the other hand, coated eggs especially those coated with S4 maintained smaller air cell sizes over the course of the storage period, indicating improved freshness preservation.

The statistical analysis for the dependent variable of air cell size is presented in the annex 4. The results indicate that the corrected model has a significant effect on air cell size, with an f-value of 17.331 and a significance level of 0.000. The intercept has a high f-value of 12529.172, indicating that the mean air cell size is significantly different from zero. The time variable has a significant effect on air cell size, with an f-value of 66.753 and a significance level of 0.000, indicating that the air cell size changes significantly over time. The treatment variable also has a significant effect on air cell size, with an f-value of 13.750 and a significance level of 0.000, indicating that different treatments have a significant effect on air cell size. Overall, the analysis suggests that both time and treatment have significant effects on aircell size.

1.16.6. Albumen pH

Egg albumen pH is a critical determinant of egg quality and freshness; freshly laid eggs typically have a pH range of 7.6 to 8.5. To comprehend the effects of various coating treatments on albumen pH, we tracked the pH levels of albumen during a storage period in our study.

Our results (figure 8) revealed that the albumen pH levels in all groups gradually increased over the duration of the storage period. This is consistent with previous research, which indicated that

albumen pH rises during storage due to continuous egg white ingredient breakdown and potential alterations through the porous eggshell. The albumen pH of the control group gradually increased throughout the trial, rising from 9.014 in the first week to 9.366 in the fourth. Though the S4 coating generated the best results, eggs coated with cassava starch (S2, S3, and S4) showed a similar trend of rising pH levels over time.

Our results are consistent with the projected pattern of rising pH levels during storage length when compared to those of Eddin & Tahergorabi, (2019), who observed an increase in egg albumen pH from 7.78 to 9.26 during a 10-day storage period at ambient temperature. However, because experimental conditions and processes vary, direct comparisons may not always produce the same results.

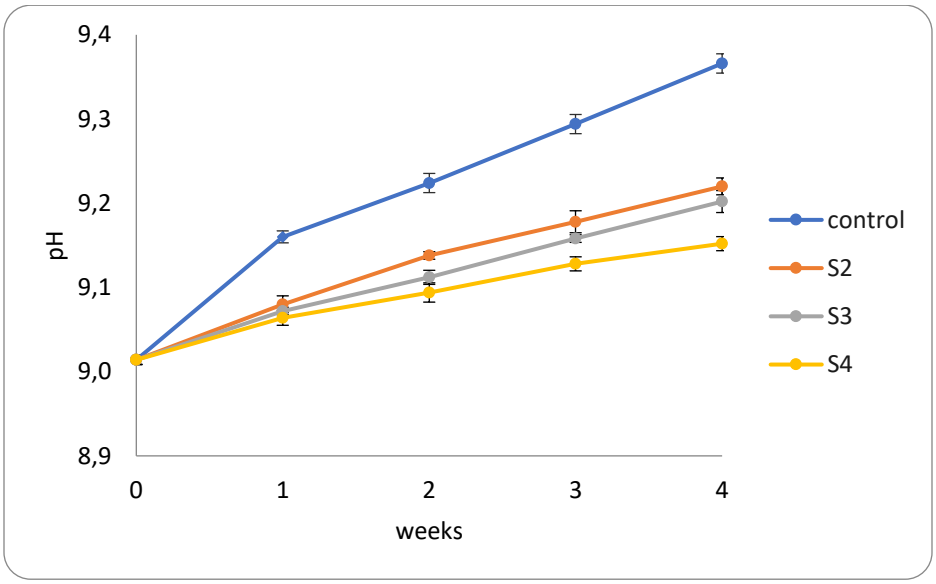

Figure 8: first experiment albumen pH of Egg samples during storage

1.17. Second experiment: evaluation of the effect of cassava starch coating on various egg sizes during storage

In the second experiment, after determining the most suitable coating formula concentration from the first experiment, the focus shifted to applying this suitable coating to eggs of varied sizes (small, medium, extra-large) to investigate its impact on various quality parameters over a 4-week period. By measuring different quality parameters weekly, such as weight loss, Haugh unit and yolk index… the goal was to assess how the cassava starch coating influenced the quality and freshness of eggs of varying sizes during storage. This experiment aimed to provide insights into the

effectiveness of the cassava starch coating across different egg sizes and its ability to maintain egg quality over an extended period, contributing valuable information for egg producers and researchers in the field of food science and storage

Given the experimental design focusing on evaluating different egg sizes, direct comparison of our results with existing research results is challenging. This is mainly due to our study's unique nature, which explores the impact of cassava coating on various egg sizes, a specific aspect that seems lacking in available online research sources.

1.17.1. Visual inspection:

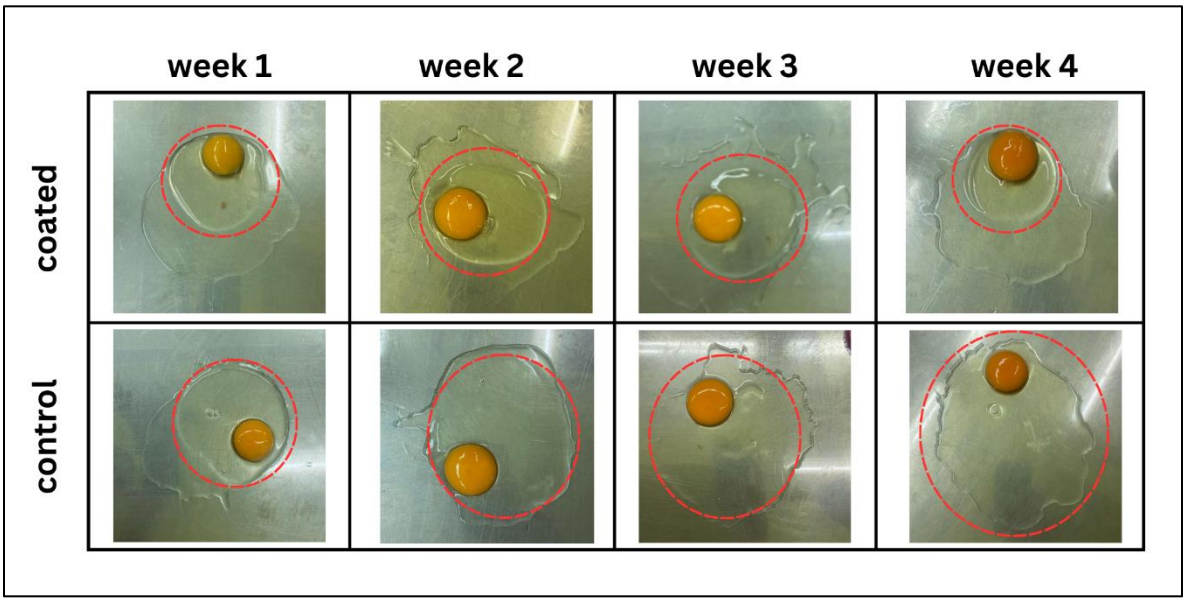

Figure 9: second experiment albumen of egg samples during storage

During the second experiment (figure 9), a noticeable difference was observed between the control eggs and the coated eggs in terms of albumen thickness. The control eggs exhibited a rapid loss of albumen integrity, resulting in a thinner consistency over time. In contrast, the coated eggs demonstrated a remarkable preservation of albumen thickness, maintaining their structural integrity throughout the experiment. This visual observation shows the potential benefits of the cassava coating in enhancing the quality and freshness of eggs.

1.17.2. Weight loss:

The analysis of the data (figure 10) reveals several key trends and implications regarding the impact of egg size and experimental conditions on weight loss.

Across all sizes of eggs (S, M, XL), a consistent pattern of weight loss is observed over time. Interestingly, the control groups consistently exhibit higher weight loss compared to the experimental groups. This suggests that the treatment of cassava starch coating influence the rate of weight loss in eggs.

Larger eggs (XL) tend to experience higher weight loss compared to smaller eggs (S, M) across both control and experimental conditions. This discrepancy is possibly attributed to variations in initial egg weight and surface area.

The control groups consistently demonstrate lower weight loss compared to their respective control groups. This disparity indicates that experimental conditions, notably the cassava starch coating, may have influenced the rate of weight loss. Factors such as temperature, humidity, or storage conditions may contribute to this difference.

FAO regulations (FAO UN, 2003) consider that the reduction of egg weight from 2-3% during storage is acceptable. Following that, only the S and M groups meets the standard in the 4^{th} week, on the other hand, both the XL group exceed the acceptable weight loss threshold by the third week.in contrast all the uncoated groups exceed the acceptable weight loss by the 2^{nd} week.

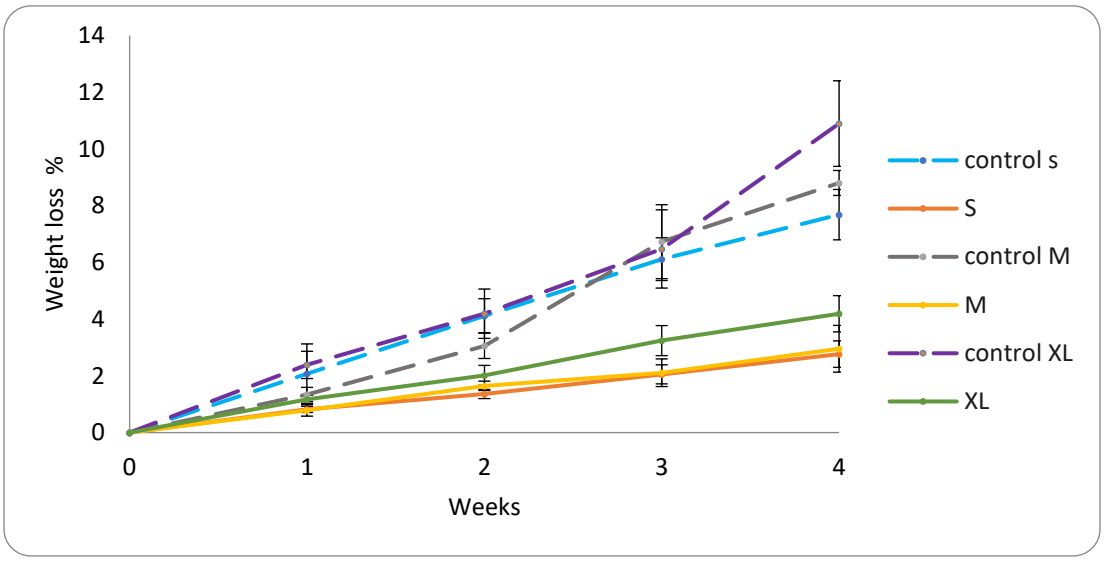

Figure 10: second experiment weight loss of egg samples during storage

In comparison to prior experiments, coated eggs lost less weight than untreated eggs. A study (Rachtanapun et al., 2021) found that coating AA-grade eggs with cassava starch, gelling agents, and waxes resulted in a 2.4% weight decrease in a close range to our results for coated eggs after 4 weeks.

Based on Table 5, the treatment factor has the highest F-value, followed by egg size and time. Which means that the treatment has the greatest impact on weight loss, followed by time and egg size. The results conclude that the treatment is effective at preserving quality over time. However, it is important to note that egg size also plays a significant role in weight loss, with larger egg sizes having a bigger weight loss.

Table 5: ANOVA two way test results for experiment 2 weight loss

Tests of Between-Subjects Effects								
Dependent Variable: Weight loss								
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
Corrected Model	2053,701[a]	26	78,988	179,788	,000	,951	4674,497	1,000
Intercept	2255,941	1	2255,941	5134,823	,000	,955	5134,823	1,000

TIME	1438,349	4	359,587	818,468	,000	,931	3273,873	1,000
SIZE	32,399	2	16,200	36,873	,000	,233	73,745	1,000
TREATMENT	630,472	1	630,472	1435,038	,000	,855	1435,038	1,000
TIME * SIZE	42,280	8	5,285	12,029	,000	,284	96,235	1,000
TIME * TREATMENT	193,172	3	64,391	146,562	,000	,644	439,686	1,000
SIZE * TREATMENT	,407	2	,203	,463	,630	,004	,925	,125
TIME * SIZE * TREATMENT	25,692	6	4,282	9,746	,000	,194	58,478	1,000
Error	106,760	243	,439					
Total	5071,727	270						
Corrected Total	2160,461	269						
a. R Squared = ,951 (Adjusted R Squared = ,945)								
b. Computed using alpha = ,05								

1.17.3. Haugh Unit:

The Haugh unit values of eggs in our study (figure 11) coated with cassava starch consistently exceeded those of uncoated eggs (control) across all sizes (S, M, XL) over the 4-week period. This indicates the effectiveness of the cassava starch coating in maintaining the freshness and internal quality of the eggs.after 4 weeks Larger eggs (XL) generally exhibited lower Haugh unit values having a “B” grade compared to smaller eggs (S, M) that maintained “A” grade during storage in coated groups, while uncoated groups all reach grade “B” quality with control XL size having the lowest value, suggesting a size-dependent trend possibly influenced by egg composition and structure. Which is supported by the statistical analysis in annex 5 where we found that the most significant factor affecting the haugh unit is the treatment with the highest F-value of 422.393 followed by the factor of time 368,198 and finally the factor of size by a value of 189,898.

The sustained higher Haugh unit values in eggs with cassava starch coating suggest the effectiveness of the protective barrier that preserves the structural integrity and freshness of the

eggs. These results emphasize the positive impact of cassava starch coating on egg quality, displaying its potential to prolong shelf life and enhance freshness.

In comparison to prior research (Pham et al., 2023), coated eggs had higher Haugh unit values than uncoated eggs, indicating greater albumen quality and freshness. Which is supported by a study on egg shelf life that found that coated eggs had higher Haugh unit values than untreated eggs throughout storage. Another study (Oliveira et al., 2022) found that eggs covered with cassava starch and essential oils had better interior quality, as shown by higher Haugh unit values than uncoated eggs.

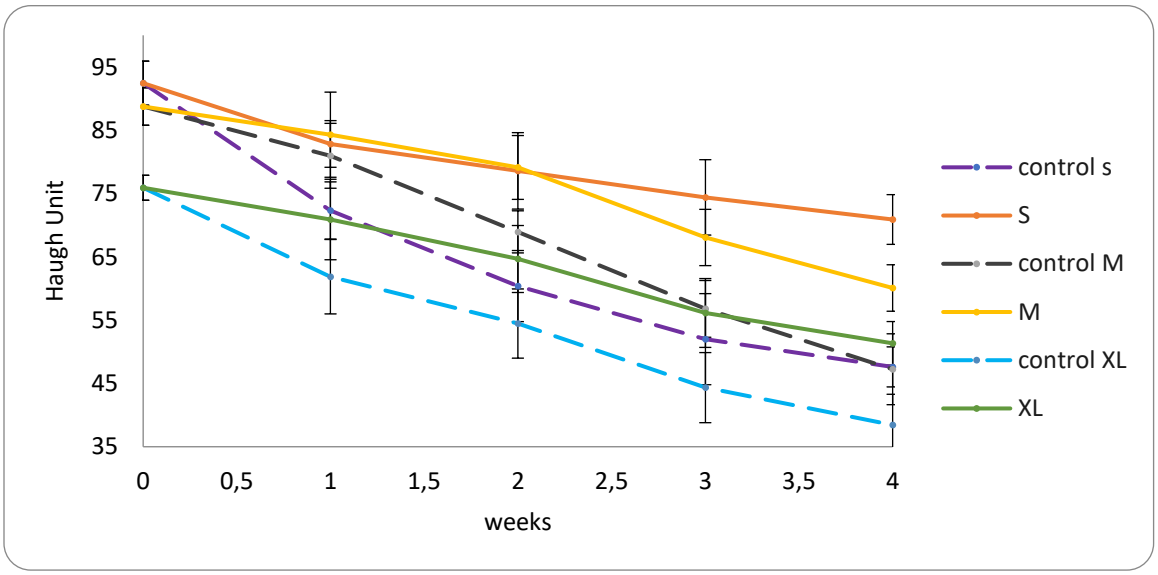

Figure 11: second experiment Haugh unit of egg samples during storage

1.17.4. Yolk index:

According to figure 12 the yolk index values of eggs with cassava starch coating consistently outperformed those of uncoated eggs (control) across all sizes (S, M, XL) during the 4-week observation period. This suggests that the cassava starch coating positively influenced the yolk index, indicating better yolk quality and integrity. Larger eggs (XL) generally displayed lower yolk index values compared to smaller eggs (S, M) in both control and experimental groups, indicating a potential size-related influence on yolk composition and structure. This observation is supported by annex 6 where we found that the most significant factor affecting the yolk index was the

treatment with an F-value of 911,595 followed by the factor of time with an F-value 298,448 and finally there was a significant effect of the size factor with a value of 155,448 on the yolk index values

The sustained higher yolk index values in eggs with cassava starch coating imply that the coating may have contributed to maintaining yolk quality and structure. These results highlight the beneficial impact of cassava starch coating on the yolk index, underscoring its potential to enhance yolk quality and freshness.

When compared to the results of earlier studies, we find that we had aligned results coated eggs had higher yolk index values than uncoated eggs, indicating greater yolk quality and freshness. A study (Elm et al., 2023) found that covering eggs with carnauba wax enhanced their yolk index values during storage, indicating greater egg quality preservation. A study combining cassava starch, methyl celluloses, and waxes (Rachtanapun et al., 2021) discovered that coated eggs had higher Haugh unit values and better internal quality, indicating improved yolk quality compared to untreated eggs.

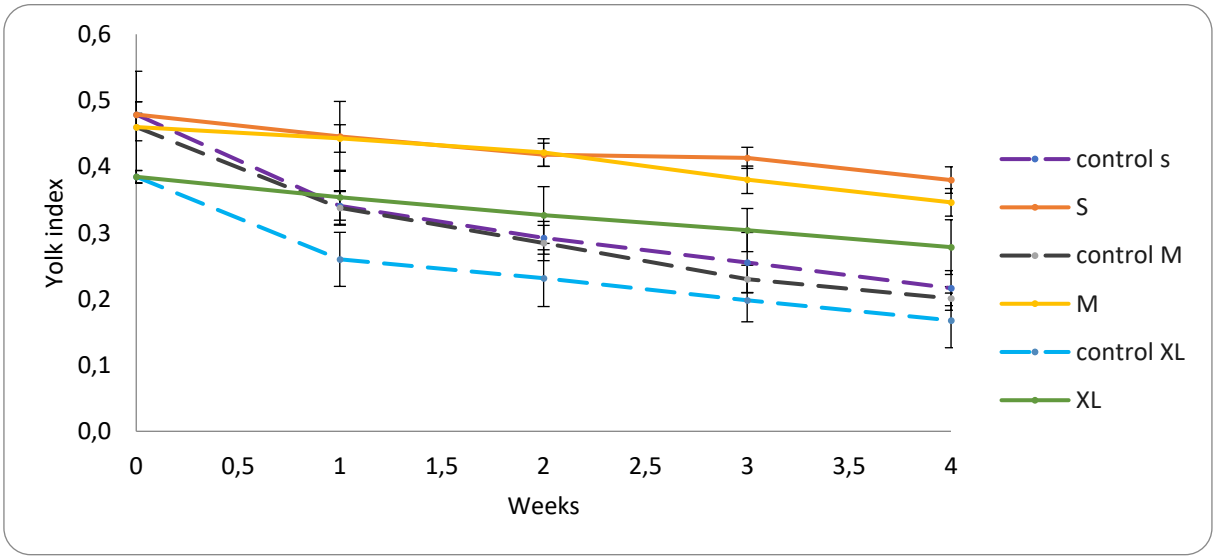

Figure 12: second experiment Haugh unit of egg samples during storage

1.17.5. Albumen index:

The white albumen index values of eggs with cassava starch coating in figure 13 consistently surpassed those of uncoated eggs (control) across all sizes (S, M, XL) during the 4-week period.

This indicates that the cassava starch coating positively influenced the white albumen index, suggesting improved albumen quality preservation and integrity. Larger eggs (XL) generally exhibited lower white albumen index values compared to smaller eggs (S, M) in both control and experimental groups, indicating a potential size-related influence on albumen composition and structure. Which is proven in the statistical analysis in annex 7 that the time was the most significant factor affecting the egg quality with an F-value of 209,144 followed by the factor of treatment with and F-value of 183.042 and finally the egg size with an F-value of 73.961

The sustained higher white albumen index values in eggs with cassava starch coating suggest that the coating have played a role in maintaining the quality and structure of the albumen. These results underscore the beneficial impact of cassava starch coating on the white albumen index, highlighting its potential to enhance albumen quality and freshness.

When compared to data from other studies, coated eggs had higher albumen quality than uncoated eggs. A study found that coating eggs with cassava and yam starches improves albumen quality retention during storage, supporting the usefulness of starch coatings in protecting egg quality (Mota et al., 2017). A study on biodegradable egg coverings indicated that coated eggs had higher albumen height and quality than uncoated eggs, indicating a positive impact on egg quality preservation (da S. Oliveira et al., 2020).

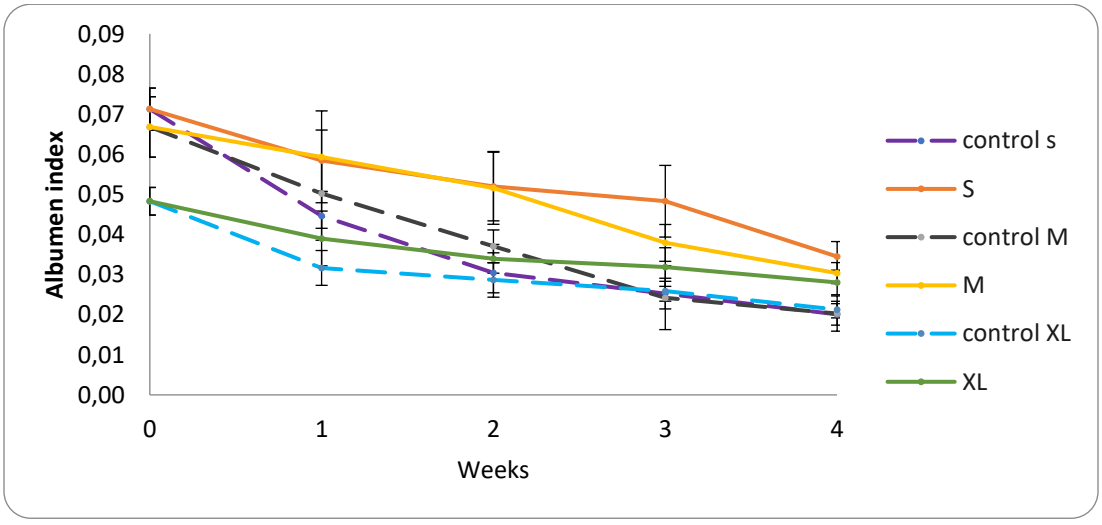

Figure 13: second albumen index of egg samples during storage

1.17.6. Air cell size:

The air cell depth values of eggs with cassava starch coating were compared to those without coating (control) across all sizes (S, M, XL) over the 4-week period. The air cell depth is an important parameter that reflects the age and quality of an egg. Generally, as eggs age, the air cell becomes larger due to moisture loss and air entering through the shell. In this study (figure 14), the air cell depth in eggs with cassava starch coating showed variations compared to uncoated eggs where coated egg had lower values than uncoated eggs and as eggs size is smaller the air cell size is smaller. The differences observed in air cell depth between the control and experimental groups indicate the effectiveness of the coating on egg quality, which is proven in The statistical study in Annex 8 shows that time is the most significant factor impacting egg quality, with an F-value of 200.148. This was followed by treatment (F-value = 136.518) and egg size (F-value = 47.032).

When compared to the results of other studies, our results are aligned with previous researches. According to a study (Elm et al., 2023) , coatings can have an impact on aircell size and overall egg quality. Research suggests that applying carnauba wax coatings to eggs can prevent weight loss and improve internal quality retention, which is affecting the aircell size positively (. A study on the impact of storage temperature on coated eggs (Rachtanapun et al., 2022b) indicated that the coating material helped preserve egg quality and maintain consistent aircell size throughout time.

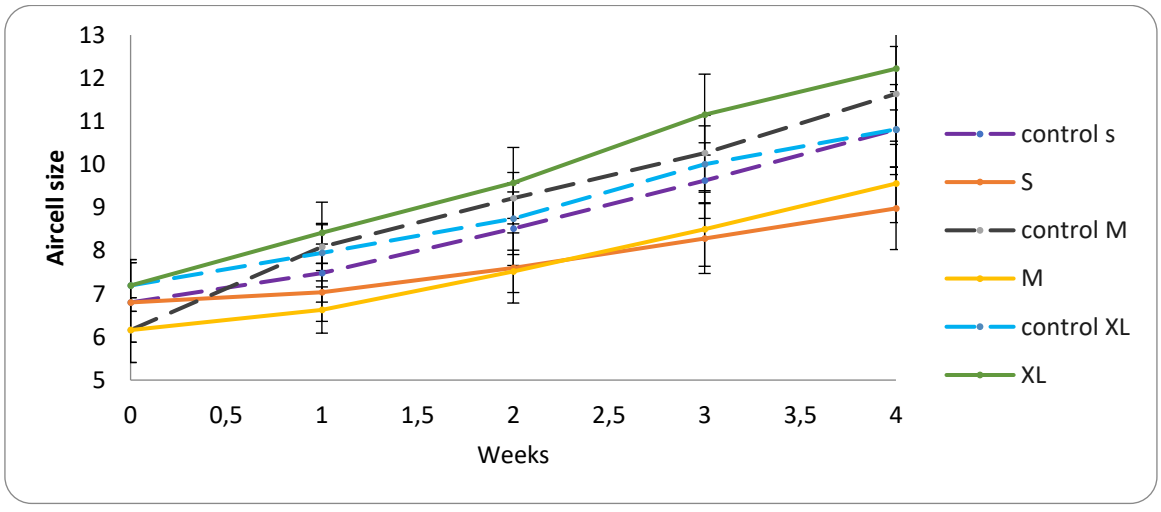

Figure 14: second experiment air cell of egg samples during storage

1.18. Summary

1.19. First experiment: Evaluating the effect of starch concentrations in the coating solution on the quality of egg during storage

The first experiment aimed to choose the suitable cassava starch concentration for the coating in order to maintain the chicken eggs quality. The experiments involved using over 250 commercial eggs classified into 4 sample groups: control group with no coating and three coated group dipped in 2, 3, and 4% of cassava starch coating to assess the impact on egg quality over a 4-week storage period. Results indicated that higher cassava starch concentrations led to reduced weight loss in eggs, with the S4 group meeting acceptable weight loss standards throughout storage of 4 weeks. Additionally, eggs coated with cassava starch maintained higher Haugh Unit (HU) values, indicating better freshness compared to uncoated eggs, particularly S4, showed improved quality retention over time maintaining grade **"A"** quality over 4 weeks of storage. Furthermore, the coated eggs exhibited better preservation of albumen quality and aircell size compared to the control group. Overall, the study demonstrated that cassava starch coatings effectively preserved egg quality parameters, potentially extending the shelf life of eggs especially for the s4 coating group that we concluded to be the most suitable coating.

In summary, the results from our study generally align with various research results mentioned before, which demonstrate that cassava coating is indeed effective in preserving egg quality .Our research support the effectiveness of cassava coating as a valuable preservation method for maintaining egg quality, although all coated eggs exhibited significant efficacy in maintaining egg quality, the results indicate that the S4 coating was the most suitable. Over the course of the study, the S4 group consistently displayed superior performance in preserving freshness and internal quality when contrasted with the other coated egg cohorts.

1.20. Second experiment: Evaluation of cassava starch coating on various egg sizes during storage

The second experiment aimed to assess the impact of cassava starch coatings on various quality parameter across different egg sizes (S, M, XL), including Haugh unit values, yolk index, white albumen index, and air cell size, over a 4-week period. The study reaffirmed that cassava starch coating consistently preserved egg quality over 4 weeks, with coated S and M eggs at grade "A" and XL at grade "B", outperforming uncoated eggs with lower HU values and graded "B". Additionally, the yolk index and white albumen index showed better preservation in eggs with the

coating, suggesting improved yolk and albumen quality retention. For example, the air cell size, a crucial indicator of egg aging, also exhibited lower values in coated than uncoated eggs. Interestingly, our study found that larger eggs (XL) had a faster deterioration process that is possibly due to their larger eggshell surface area. This finding suggests that larger eggs may be in more need of the coating to preserve their quality and freshness.

1.21. Conclusion

Overall, from both experiments, it is evident that cassava starch is indeed effective in prolonging the shelf life of eggs. The results highlight the benefits of using cassava starch coatings to reduce weight loss, maintain freshness parameters, and maintain the quality of eggs. The second experiment showed that larger eggs undergo a faster aging process emphasizes the importance of applying coatings, especially considering that larger eggs are more valuable. This further emphasizes the potential of cassava starch as a valuable tool in the egg industry for maintain the product quality and extending shelf life.

Recommendations for Future Research:

1. **Developing non-destructive testing techniques for eggs:** while the current evaluation methods used in our study were efficient, there is a need for non-destructive testing techniques to assess egg quality. These methods can measure egg quality without destructing the eggs, which is less time consuming and more sustainable and environmental friendly.

2. **Incorporating gel agents or antimicrobial agents into coatings:** exploring the addition of gel agents or antimicrobial agents to cassava starch coatings can potentially enhance the protective properties of the coating, therefore the coating can extend the shelf life of eggs and maintain the overall quality.

3. **Investigating different coating application techniques:** exploring various coating application methods, such as dipping or spraying, can help determine the most effective and efficient way to apply cassava starch coatings to eggs.

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1.23. List of figures:

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1.25. Annexes:

First experiment

ANNEX 1 :

Tests of Between-Subjects Effects

Dependent Variable: Haugh unit

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	30161.393[a]	19	1587.442	58.080	.000
Intercept	1029042.884	1	1029042.884	37649.716	.000
TIME	21640.264	4	5410.066	197.939	.000
TREATMENT	6281.646	3	2093.882	76.609	.000
TIME * TREATMENT	2239.483	12	186.624	6.828	.000
Error	4919.764	180	27.332		
Total	1064124.041	200			
Corrected Total	35081.157	199			

a. R Squared = .860 (Adjusted R Squared = .845)

ANNEX 2 :

Tests of Between-Subjects Effects

Dependent Variable: Yolk index

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	0.365[a]	19	0.019	32.247	0.000
Intercept	22.741	1	22.741	38205.509	0.000
TIME	0.315	4	0.079	132.273	0.000
TREATMENT	0.035	3	0.012	19.701	0.000
TIME * TREATMENT	0.015	12	0.001	2.042	0.023
Error	0.107	180	0.001		
Total	23.213	200			
Corrected Total	0.472	199			

a. R Squared = .773 (Adjusted R Squared = .749)

ANNEX 3 :

Tests of Between-Subjects Effects

Dependent Variable: Albumen index

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	0.022[a]	19	0.001	30.772	0.000
Intercept	0.343	1	0.343	9292.554	0.000
TIME	0.016	4	0.004	107.099	0.000
TREATMENT	0.004	3	0.001	40.157	0.000
TIME * TREATMENT	0.001	12	0.000	2.984	0.001
Error	0.007	180	3.689E-005		
Total	0.371	200			
Corrected Total	0.028	199			

a. R Squared = .765 (Adjusted R Squared = .740)

ANNEX 4 :

Tests of Between-Subjects Effects

Dependent Variable: Aircell size

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	276.704[a]	19	14.563	17.331	0.000
Intercept	10528.311	1	10528.311	12529.172	0.000
TIME	224.371	4	56.093	66.753	0.000
TREATMENT	34.662	3	11.554	13.750	0.000
TIME * TREATMENT	17.672	12	1.473	1.753	0.059
Error	151.255	180	.840		
Total	10956.270	200			
Corrected Total	427.959	199			

a. R Squared = .647 (Adjusted R Squared = .609)

Second experiment

ANNEX 5 :

Tests of Between-Subjects Effects

Dependent Variable: Haugh unit

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
Corrected Model	54660.776[a]	26	2102.338	81.523	.000	.897	2119.596	1.000
Intercept	1203207.076	1	1203207.076	46657.096	.000	.995	46657.096	1.000
TIME	37980.842	4	9495.211	368.198	.000	.858	1472.794	1.000
SIZE	9794.296	2	4897.148	189.898	.000	.610	379.796	1.000
TREATMENT	10892.786	1	10892.786	422.393	.000	.635	422.393	1.000
TIME * SIZE	1114.218	8	139.277	5.401	.000	.151	43.206	.999
TIME * TREATMENT	602.972	3	200.991	7.794	.000	.088	23.382	.989
SIZE * TREATMENT	1305.973	2	652.987	25.321	.000	.172	50.642	1.000
TIME * SIZE * TREATMENT	231.388	6	38.565	1.495	.180	.036	8.973	.575
Error	6266.556	243	25.788					
Total	1242269.757	270						
Corrected Total	60927.332	269						

a. R Squared = .897 (Adjusted R Squared = .886)

b. Computed using alpha = .05

ANNEX 6 :

Tests of Between-Subjects Effects

Dependent Variable: Yolk index

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
Corrected Model	1.966[a]	26	.076	78.375	.000	.893	2037.743	1.000
Intercept	30.579	1	30.579	31688.490	.000	.992	31688.490	1.000
TIME	1.152	4	.288	298.448	.000	.831	1193.793	1.000
SIZE	.301	2	.150	155.844	.000	.562	311.687	1.000
TREATMENT	.880	1	.880	911.595	.000	.790	911.595	1.000
TIME * SIZE	.019	8	.002	2.515	.012	.076	20.119	.907
TIME * TREATMENT	.020	3	.007	6.775	.000	.077	20.326	.975
SIZE * TREATMENT	.015	2	.008	7.972	.000	.062	15.944	.954
TIME * SIZE * TREATMENT	.005	6	.001	.823	.553	.020	4.937	.324
Error	.234	243	.001					
Total	31.545	270						
Corrected Total	2.201	269						

a. R Squared = .893 (Adjusted R Squared = .882)

b. Computed using alpha = .05

ANNEX 7 :

Tests of Between-Subjects Effects

Dependent Variable: Albumen index

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
Corrected Model	.053[a]	26	.002	42.784	.000	.821	1112.378	1.000
Intercept	.436	1	.436	9112.081	.000	.974	9112.081	1.000
TIME	.040	4	.010	209.144	.000	.775	836.576	1.000
SIZE	.007	2	.004	73.961	.000	.378	147.921	1.000
TREATMENT	.009	1	.009	183.042	.000	.430	183.042	1.000
TIME * SIZE	.004	8	.000	9.784	.000	.244	78.273	1.000
TIME * TREATMENT	7.458E-005	3	2.486E-005	.519	.669	.006	1.558	.156
SIZE * TREATMENT	.001	2	.001	14.791	.000	.109	29.583	.999
TIME * SIZE * TREATMENT	.000	6	6.069E-005	1.268	.273	.030	7.609	.495
Error	.012	243	4.786E-005					
Total	.477	270						
Corrected Total	.065	269						

a. R Squared = .821 (Adjusted R Squared = .802)

b. Computed using alpha = .05

ANNEX 8 :

Tests of Between-Subjects Effects

Dependent Variable: Albumen index

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
Corrected Model	.053[a]	26	.002	42.784	.000	.821	1112.378	1.000
Intercept	.436	1	.436	9112.081	.000	.974	9112.081	1.000
TIME	.040	4	.010	209.144	.000	.775	836.576	1.000
SIZE	.007	2	.004	73.961	.000	.378	147.921	1.000
TREATMENT	.009	1	.009	183.042	.000	.430	183.042	1.000
TIME * SIZE	.004	8	.000	9.784	.000	.244	78.273	1.000
TIME * TREATMENT	7.458E-005	3	2.486E-005	.519	.669	.006	1.558	.156
SIZE * TREATMENT	.001	2	.001	14.791	.000	.109	29.583	.999
TIME * SIZE * TREATMENT	.000	6	6.069E-005	1.268	.273	.030	7.609	.495
Error	.012	243	4.786E-005					
Total	.477	270						
Corrected Total	.065	269						

a. R Squared = .821 (Adjusted R Squared = .802)

b. Computed using alpha = .05

DECLARATION

the public access and authenticity of the thesis/dissertation/portfolio[1]

Student's name:	Hamza Khabir
Student's Neptun code:	FNXMBO
Title of thesis:	Effect of edible coating on egg quality
Year of publication:	2024
Name of the consultant's institute:	Hungarian University of Agriculture and Life Sciences
Name of consultant's deparment:	Department of Livestock Product and Food Preservation Technology

I declare that the final thesis/thesis/dissertation/portfolio 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.

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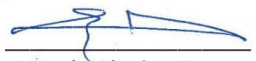

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STATEMENT ON CONSULTATION PRACTICES

As a supervisor of master student Hamza Khabir (Student's neptun ID: FNXMBO), I here declare that the final master's thesis has been reviewed by me, the student was informed about the requirements of literary sources management and its legal and ethical rules.

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