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Effect of edible coating on egg quality during storage

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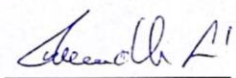
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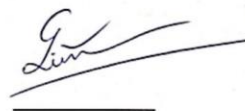
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1.1.Introduction:

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. Furthermore, I conducted a second experiment to test the most efficient coating on various egg size classes using parameters such as Haugh unit, weight loss, yolk index, air cell size, as well as albumen index..

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

For the first experiment, I tested how cassava starch, at three different concentrations in the coating solution, influenced the quality of eggs stored for four weeks. The most suitable concentration that is determined by different parameters and that is able to maintain quality in eggs and prolong shelf life is used for the second experiment.

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 commonly known as one of the most nutritious and important foods for humans, which makes them a great source of vital lipids, proteins, vitamins, and minerals. Furthermore, they are the essential ingredients in different food products, which enrich their nutritional, sensory, and functional properties like emulsifying, coagulating, and gelling. 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). The supply outlook for the year 2023 is highly likely with estimated single-person consumption of 10.0 kg in a year (Statista, 2023). The EU has a laying hen population significantly higher than 350 million and its egg production amounts to 6.7 million tons annually; hereby the EU regulation as was articulated by the European Commission, (2022) emphasize the need to develop new ways to preserve eggs to meet both the quality and safety of food products.

The egg market with its considerable significance is a sector no one in the world can ignore and it goes without saying that it has an impact on everyone's diet on a global scale. Its ongoing development constitutes the exploration of technical means for sustaining sustainable production level.

1.5.Nutritional Value of Eggs:

The 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 shell contains two membranes (inner and outer) that are inseparably close to each other. At its large end they form a cavity, the size of which can be compared to the air cell. Generally, an air cell is around 5 mm in diameter when fresh eggs are stored, which increases in size as eggs are stored, and it serves as an indication of egg age (Zhao et al., 2024) . The egg white, which is also called albumen, is a translucent colored gel like liquid with three fractions, which are thin in consistency and extract residue after centrifugation. The second layer or vitellium's thicker segment is the chalaziferous layer which is the thin but strong part of albumen. This region, from opposite sides of the yolk, resembles twisted rope-like cord with the function to keep a yolk centered. The germinal disc (blastoderm) is found at the top of a pointed endho (larva) of a latebra on one side of the yolk. In eggs the yolk is made of dark- and light-colored layers that are arranged in the form of a circle alternatively. The chalazae is one of the most important yolk parts it allows to hold the yolk securely until a chick weighs down. An average chicken egg weighs approximately 58 g and has three main parts that are water (around 74%) that is the most of it, protein (about 12%) and lastly the lipids (around 11%) (U.S. DA, 2024a).

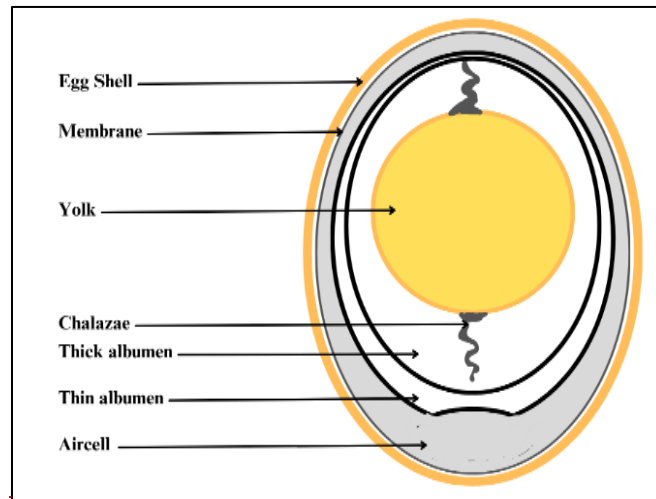


Figure 1: structure of the egg

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

1.6.1. Air cell:

As the egg ages, it undergoes a natural procedure, which is the process referred to as transpiration, where water vapor goes out through tiny holes on the eggshell. This pore acts as a channel for gas exchange and, as the egg ages, transpiration rate increases. The loss of moisture is humidified by air leaking through the shell into the egg, in turn expanding the air cell inside. This equilibrium of air leaving and entering through the porous shell results in modifications of the total density of the egg. Hence, when the egg is immersed in water, the wider end sinks down deeper into the water. The temperature, humidity and the eggshell porosity determines the transpiration rate. The air cell size difference therefore could be of great help to indicate the eggs freshness increases (Jalili-Firoozinezhad et al. 2020). (Jalili-Firoozinezhad et al., 2020).

It is evident that the air cell (especially the one which is bigger at the bigger end) during the cooling and contraction process is a very good indicator of the process of cooling and contraction of the egg after laying. This is the essence of the egg-grading system, which uses candling as a tool for evaluation. At first, a very fresh egg with an AA grade has a small air cell. Alongside air cell's expansion, which is accompanied by a decrease in egg quality, the grades also shift from AA to A and then 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 is a complex biomineralization process, which involves the secretion of a variety of proteins and minerals (Zhao et al., 2024). Thus, the composition comprises two components, inorganic elements such as CaCO_3 crystals besides organic ones including glyco- and phospho-proteins and proteoglycans. Other than calcium, the major constituent of eggshell matrix proteins such as ovocalyxin, ovocleidin, and osteopontin play a role in the development and shape 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, also known as 'albumen', is a chemical compound containing water, proteins and carbohydrates with a second protective role, regulating microorganisms to reach the yolk. The egg white has four layers with varying viscosities, named based on their viscosity and position relative

to the yolk: epithelial, stratum corneum, granular, and cellular layers (Chalaziferous). The thick portions where ovomucin is abundant show high viscosity; meanwhile, the whole egg white behaves like a pseudoplastic with the apparent viscosity decreasing with increasing temperature up to the loss of fluidity around 60 °C. In the process of shearing, the filamentous superaggregates of thick parts break down which leads to the lower viscosities of the thin portions (Jalili-Firoozinezhad et al., 2020) .

1.6.5. Egg yolk:

Egg yolk is an ideal health booster because it combines phospholipids (rufawind), vitamins, minerals, and lutein which the body easily absorbs. The composition of it equals about 50% water, 30% lipids, 16% proteins (U.S. DA, 2024b), and a few other insignificant substances. When we separate egg yolk using a gentle spinning process, we get two parts: a two-part fluid kind, called plasma, and one solid kind named grains.

In the food industry, egg yolk is a key ingredient because it can help in different 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 the industrial sense heat treatment is an essential measure to make consuming the egg yolk safe by deactivating any dangerous microorganisms that may have survived the previous process. In this connection, how to deal with heat sensitivity of egg yolk compounds, like proteins and fats, represents one of the most pressing issues. Degradation of protein structure, lipid breakdown and consequently clump formation are among the chemical reactions causing a change in the texture at temperatures exceeding 65 °C. 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 is a main ingredient that can be found all over the house as they are fragile and perishable. Their freshness can be maintained only through the use of refrigeration that is widely practiced in countries such as the United States, Australia, Japan, Sweden and the Netherlands (Cader et al., 2014). Most eggs (90%) are sterile once they are laid, but contamination is possible (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) .

Article 13 of Commission Regulation (EC) No 589/2008 which was adopted on 23rd June 2008 by the EU Law , contains the details of how to implement the regulation of EU Council Regulation (EC) No 1234/2007 regarding the advertising rules for eggs. This article briefly explains about the types of eggs and the date of expiry, which should be within the duration of the egg sets that to be at least 28 days after laying. If a laying period is stated, then the date of minimum durability should be calculated from the initial day of that period (Commission Regulation, 2008).

That main spoilage risk are the following:

1.7.1. Microbial contamination:

Contamination with various pathogenic microorganisms is one of the major food safety problems associated with eggs. The bacteria living inside the surface of the eggshell can be classified into Self-deciphering bacteria as *Staphylococcus*, *Streptococcus*, *Aerococcus*, and *Micrococcus*, (Techer et al., 2014) with minor presence of Self-deciphering bacteria such as *Salmonella*, *Escherichia*, and *Alcaligenes* sp., as well Mesophilic aerobic microbiota levels can vary significantly on eggshell surfaces and may range from $10^{3.8}$ to $10^{6.3}$ cfu/egg with average levels around $10^{4.5}$ cfu/egg as reported by Liu et al., (2021). Even though the bacterial flora of eggshells predominantly belongs to Gram-positive bacteria, the internal content of eggs is almost contaminated by Gram-negative bacteria known for their greater sustainability to the natural defenses of eggs. Bacteria contain the characteristics enabling them to penetrate eggshell and membranes, the capacity resisting proteins in the albumen containing growth-inhibiting substances, and other enzyme activities breaking down compounds of nitrogen and carbon in the egg fluids into complex diets, which results in bacterial growth. The egg spoilage starts with the decaying egg color that changes the entire spectrum from black to red, blue, green, and pink. Additionally, the egg produces a horrible smell - egg rot. 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. often recognized as the most likely foods to be a source of contamination such as milk, meat, eggs, and fish. The bacteria that have capabilities to form Staphylococcal enterotoxin or transfer invasive enzymes as medium can result in severe indigestion with symptoms like diarrhea and vomiting. Sometimes, the consequences may even be organ failure leading to death.

Bacillus spp. exhibited the impressive capacity of protease activities, one of most essential elements determining to maintain their life in eggs (Liu et al., 2021) .Most of the spoilage bacteria we isolated from the eggs had a strong enzyme-producing potential which is to survive and their strategy for life. We subjected the pathogens to alkaline resistance tests, and results showed that certain bacteria were able to tolerate high alkaline pH, while others were not affected. Thus, the eggs became a good habitat for the growth of those spoilage bacteria. Water supply allowed some strains to progressively develop adaptability to alkaline environment. This is an indication of the

genetic flexibility and the variability that most bacteria populations demonstrate. As for growth-curve comparisons, the species of different genera as *Bacillus*, spp. displayed the same pattern of growth particularly, streptococci and staphylococci members suggest that microbes can endure alkalic environments magnificently. Egg whites' pH rises dramatically after chicken lay eggs that generally have positive effects on 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) .

In the storage process, the albumen loses its physical integrity and transforms into a thin white gel as the pH value of the shell increases which is a side-effect of air loss due to exposure. The deterioration is thus based on the loss on viscosity of the albumen, which departs from its conformational structure that was due to the unbundling 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:

The effective prevention of eggs is not only essential in reducing economic losses but it is also a guarantee for extended shelf life as well as continuity in the availability of fresh and safe to ingest eggs to consumers. The last one is natural decay of eggs that is why it is vital to apply refrigeration and to mitigate financial losses and food security. These techniques of preservation not only perform a crucial duty of keeping the freshness of eggs but also enhance their life span and quality.

In this discussion, we will divide the egg preservation into several methods and will analyze each one of them through its own unique features, pros and cons.

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 serve as a key element for ensuring that quality and security of different food products such as eggs. Varying application methods like spraying, electrostatic spraying or dipping can depend on factors like viscosity, texture, and equipment that is available. Surface coating can be achieved through dipping and spraying amongst other methods, however, dipping is fast, easy, and cheap while spraying is considered the best since it provides uniformity and at the same time reduces the risk of contamination (Sharaf Eddin et al. , 2019).

There is great importance in having a uniform and even coating for the food safety and quality maintenance. In this situation, adhesion of the coat between the product surface and the coating solution is of utmost importance. Edible coating performance depends on its water, air, and carbon dioxide shielding power, which takes into consideration both chemical compounds and the structure of the polymer layer forming the coating, as well as the qualities of the produce, and the 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:

Edible protein coating compositions are made of pure protein products which are extracted from plants and animals for example gelatin, fish proteins, milk proteins, and egg products. Gelatin, obtained from collagen, is a prevalent area of research, some alternatives to fish proteins and milk proteins have been explored by researchers, as some concerns have been raised about the 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 unique property of whey films to consolidate togetherates relies on the mechanism of thermal denaturation of protein in aqueous solution. Heating causes structural changes in whey proteins which move the internal sites of hydrophobic and SH (sulfhydryl) groups whereby these hydrophobic interactions and intermolecular S-S (disulfide) bonding would form during 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. Incorporation of plasticizers in this process provides these films with flexibleness. On the one hand, while solutions of whey proteins intrinsically have weak water barrier performance due to their naturally hydrophilic character, the rest of the solution appears as a flexible, transparent, and flavorless film upon the addition of the plasticizer (Hauzoukim & Mohanty, 2020) .

Characteristics of solvent-cast water-soluble protein (WP) films: These properties of the edible films, which are particularly related to their mechanical, barrier and visual characteristics, actually

define the demand for these films for use in specific situations. For instance, dimensional stability, tensile strength, Young's modulus and elongation are the basic mechanical properties as with thin plastic film packaging oxygen barrier materials. Whereas there are some main barrier properties, such as oxygen permeability of films and water vapor transmission rate. Besides this, specific properties, like being oil and aroma permeable, are important for some purposes. 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 their 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), has shown that coating eggs in whey protein mixed with plasticizer increases the shelf life of eggs. This unique approach greatly reduced the incidence of weight loss in the eggs, so that by the end of storage period of up to 42 days at 20 °C, the eggs freshness and inner properties were preserved. 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). Additionally, this coating stabilized the pH of proteins, such as albumen and yolk, preventing them from changing their characteristics substantially over the storage time. Therefore, the whey protein wrapping led the Yolk Index (YI) to be increased,

suggesting better yolk quality which the YI was proven more efficient in recording these preservations at day 14.

In de Araújo Soares et al., (2021) study, egg longevity was extended for up to 8 weeks by utilization of whey protein coating with plasticizer when stored at room temperature (25 ± 3 °C and $70 \pm 5\%$ RH). The resultant weight loss remained 11.65% lower for coated eggs than 14.505% for uncoated ones, suggesting a smaller volume of water vapor that transited through them and a longer shelf life extension. Furthermore, coating was able to maintain most Haugh Unit (HU) values ranging between high 2 to low 2; coated eggs maintained AA quality grade up to 3 weeks compared to uncoated eggs of deteriorated to D grade in 3 weeks. The coatings prolonged the shelflife of eggs better than those without. They coated egg yolk aged up to 3 weeks with a YI of more than half. But uncoated eggs degraded within 1 week. Additionally, the protein-based casing was in charge of holding the musical compositions inside after eliminating the degradation caused by the external variations, hence the internal stability was guaranteed. This coating was also responsible for foam stability ($>75\%$) up to 5 weeks, which may be one of the reasons that eggs maintained good quality with a probable lower requirement of refrigeration.

1.8.4. Corn Protein:

The possibility of applying zein, which is extracted from corn to the eggshell coating is a very appealing option due to its favourable quality compared to other proteins as a barrier to moisture and oxygen. Through alcohol-soluble proteins, a group of prolamins, in the endosperm of corn called zein is found. 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. By aqueous alcohol extraction, zein can be obtained as granular powder after drying (Padua & Wang, 2002).

The preparation of protein films is an area of corn zein (the prolamin fraction of corn protein) and wheat gluten (an outcome of wheat protein's prolamin and glutelin) synthesis, after extensive research. 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. Proving to be beneficial, thermal treatment has been able to reinforce the mechanical properties of gliadin theoretically through covalent crosslinking. Furthermore, the improvement of eggshells by the addition of wheat gluten renders shells more stable and also reduces the possibility of microbial contamination (Hauzoukim & Mohanty, 2020) .

In the study by Entezari et al. (2022), zein protein coating was applied, with the addition of plasticizer, with eggs' lifetime being extended greatly. In this regard, coated eggs demonstrated an average weight loss ranging from 0.16% to 1.45% and 0.16% to 1.53% in regards of zein-coated and zein-extract coated eggs over the 28-day storage period respectively, while the uncoated controlled eggs experienced weight losses ranging from 0.12% to 1. Furthermore, the zein-coated and zein-extract coated eggs maintained a high Haugh Unit (HU) value. Therefore, a range of 86.34% to 92.43% and 86.93% to 90.71% were recorded, indicating enhanced albumen quality and freshness in comparison to control eggs with an HU range of 77. 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. In addition, the coatings greatly reduced the water permeability of the eggshells, while both types of treatments showed reduction of *Salmonella* populations on the eggshells by the end of the 28-day exposure, with the control group showing no statistical difference in poultry eggs bacterial content. All in all, zein led to an improvement in quality of the eggs which allowed prolonged keeping period, preserving albumen and yolk quality and the minimum possible number of bacteria contamination.

1.8.5. Rice protein:

In a study by P. G. S. Pires et al. (2019a), rice protein coating had two effects; mostly extended shelf life of eggs and at the same time reduction in weight loss during storage when combined with 5% or 10% of propolis coating the eggs by 4.11 to 4.40% compared to the 5.39% in the uncoated eggs. Besides, the coatings had higher preservation capability of Haugh unit (HU) values during the 6 weeks of storage period, with the best performance seen in egg coated with RPC (Rice Protein Concentrate), particularly when propolis was added at 10% amount. Moreover, coating eggs with their corresponding materials showed higher (YI) scores, meaning better yolk quality retentive performance, particularly for the ones covered with RPC and propolis. It has been found that pH is held at a lower level which is a factor of the external coatings and for maintaining more adequate

quality inside of whole eggs. Propolis and artificial coatings changed the color of eggshells, with the propolis-coated eggs showing more opaque shells. However, there were no statistically significant differences between these coatings and untreated eggs in terms of their shell strength. 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, through test of rice protein concentrate (RPC) coatings with essential oils (tea tree oil, copaiba oil or thyme oil) it was found that the losses in weight of eggs were significantly reduced during a 6-week storage period at 20°C. The egg weight loss regarded to coated eggs ranged between 4.23% and 4.10%, whereas uncoated eggs had around 5.43% weight loss on the end of storage. 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. Furthermore, coatings served as a protective barrier and this was demonstrated through higher Haugh unit (HU) values, a quality measure for the albumen, in the coated egg samples compared to the uncoated egg samples. Shelled already eggs fresh preserved in the RPC maintained the "AA" quality level much longer than eggs merely packed in the carton without RPC, for up to 6 weeks it remained a higher grade, followed by 3 weeks and finally to the worst condition after 6 weeks. Therefore, the coating of nonconventional egg had significantly better data, with coating eggs holding the yolk index ones for a longer period than the simple egg. The paper shows that RPC coating blends offered good efficacy, particularly when used together with essential oils, on the shelf life of eggs by saving their attributes pertaining to weight of egg, quality of egg white and that of a yolk. This can possibly be an answer to the economic losses that can take place during storage in the egg industry.

1.8.6. Gelatin coating:

As gelatin, being the most commonly used stabilizer in food, gets an interest for egg preservation, yet there is almost no information about this connotation in egg preservation research. The feature of forming a film, its barrier properties, and biocompatibility likely will give a positive effect to the coating process operation. While its ability in egg preservation has not been experimented, it has saved its reputation in food preservation so it could maybe be the one to extend the shelf life of eggs. More studies have to be done to evaluate the efficiency of using gelatin in egg

conservation, thus usage of gelatin in egg conservation would be expected to give a new perspective in egg conservation techniques.

The gelatin widely used in the industry is a protein derivative of collagen that is usually processed into a gelatin with the use of acidic, alkali, and enzymatic hydrolysis chemical methods. One of the most common functionalities of this substance in food production is represented by an assortment of desirable physical and chemical properties. Although great nonetheless, this film's specific traits, such as film-forming ability, transparency, and bio-compostability, that make it very popular used for edible films with good applications. While it has a risk of a water absorption from the environment, they are breathable films. In this way, they are often joined with other materials to improve moisture transmittance and mechanical characters, and are normally used with traditional textiles like cotton and polyester (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. It was found that the preservation effect of carnauba was maximum under storage temperatures of 25°C, which implies that carnauba is a superior wax in guarding against alterations in egg quality under adverse conditions. To add, the other benefit is that carnauba wax coating shows its potential for reducing lipid oxidation in the egg yolk in which egg coated by 15% wax mostly contain lower levels of malondialdehyde and much less damages than those coated with 12% wax or not coated. Even though the antioxidant capacity of carnauba wax is still a point of contention in the scientific community, a detailed research is needed to clear all the doubts and establish its role in preserving eggs.

In the study conducted by Dewage & Abeyrathne. (2021), the use of paraffin and vegetable waxes as the coating material had a major influence on different parameters of storages eggs at room temperature. The eggs containing wet that classify as mineral oil, "boomi" wax and "dawul kurundu" wax have good weight loss which is encountered by the non-coated eggs when stored for six weeks. Further, oiled eggs scored higher Hough unit (HU) all the while, denoting better albumen quality, than both un-oiled and wax coated eggs. The eggs that were coated had lower amounts of albumen and yolk with pH that indicated that gas was not quickly leaking from the shell. Additionally, the former have displayed a breath-taking lesser increase in air sac volume compared to non-coated eggs, and this indicates a superior preservation of the internal structure of egg. Yet, the boomi wax and dawul kurundu coatings were more successful in reducing the internal quality parameters compared with the non-coated samples, but they were less effective at mineral oil. Generally, chemical oil as binder noted the most viable use for enhancement in the shelf life of the 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 3 weeks of storage, all oil coated eggs maintained AA grade levels, and the A grade grade could be maintained for up to 5 weeks, less than 0.5% in weight loss. Aside from that, the eggs that were oiled were shinier than the non-oiled ones, as evaluated both by using the instruments' analysis and consumer assessments. Consumers were found to be willing to accept the oil-glossy skin and the egg odor of egg samples with oil coating. Coconut, rice bran, and palm oils that are readily available and widely used entail their possible utilization as coating materials for maintaining freshness of eggs and reducing degradation through storage.

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) had done a study on the effectiveness of mineral oil coating in preserving egg quality, which found that this approach extended the shelf life of the eggs by at least three weeks at 25°C when compared to the noncoated ones. There were some contrasts between the viscosity of minerals oil during 5 weeks of store and that oil was the best one, decreasing weight loss and extending the shelf life by 3 weeks. In the initial consumer acceptance study, the trials were repeated with oil coating on the eggs and these were equally acceptable after 5 weeks as non-coated eggs. Currently, in addition to the shelf life studies stored in laboratories, shelf life in real commercial conditions is being studied to define sensory quality of ghee-coated eggs as well.

According to D. R. Jones et al. (2018), he proved that egg coating with mineral oil to improve the shelf life of the quality the product during the storage has big positive result. Data showed that eggs coated with mineral oil and stored at 4°C were the ones that contributed the least to the overall weight loss in 15 weeks compared to the performed experiments, with a highest percentage of 0.33% loss of the initial weight. Additionally, the Haugh units, shell cutout strength, and the three yolk shape measurements were maintained without any significant change when the eggs were stored at this condition. Not less importantly, eggs kept at 22 °C and had no mineral oil coating showed a dramatic decrease in quality which proved that the functionality of the mineral oil in keeping eggs quality was spot on. It was not specifically clear about how much prolongation had taken place in terms of certain particular period. However, the discernible change in weight reduction and quality preservation of eggs that had been treated with mineral oil and stored in refrigerator implies some extent increase in shelf-life.

1.8.11. Polysaccharide-based edible coatings:

Polysaccharide based coatings which is often seen in confectionery, desserts, and bakery items usually consists of water-soluble-gel which includes cellulose, chitosan, and starch. Cellulose, which is the predominant one, creates a sort of hydrogen coupling that is brittle. Chitan for the prevention of oxygen barrier properties is good and can even produce a small amount of antimicrobial activity. The prolonged shelf-life of eggs can be attributed to the starches used in the coatings walls of amylose and amylopectin and the additional factors influencing their function (Sharaf Eddin et al., 2019) .

1.8.12. Cassava starch coating:

As starch properties pertain to cassava, it shows a high amount of starch yield and also cheap cost; thus, this kind of starch is preferred in the food production. The blends of modified CMC (carboxymethyl cellulose) are brought together to achieve a superior performance. Through the molecular dispersion in water based environments, CMC together with cassava starch creates blockage of starch granules, thereby results in increasing moisture resistance properties in food packaging applications. The blends have the advantages of native starch (for example, high strength and low water permeability) while at the same time naturally addressing the drawbacks of native

starch. Also, CMC was incorporated into mix until its adherence to cassava starch, thus facilitating its water resistance and cross-linking.

Homsaard et al. (2021) study demonstrated that, the application of cassava starch was found to substantially decrease bacterial contamination on eggshells than the untreated ones. This study illustrated that, at 35 days of storage, the uncoated eggs registered a total mean aerobic mesophilic bacteria count of 3.17 log₁₀ CFU/ml, while the coated eggs had much lower counts fluctuating between 0.70 to 0.91 log₁₀ CFU/ml. Furthermore, coating fresh eggs with cassava starch has been demonstrated to produce egg whites with only low to moderately significant weight loss compared to uncoated egg whites which show significantly greater loss over time – day 14 to day 35 storage.

According to Rachtanapun et al. (2022a) , the cassava inspired large-scale benefits. Coated and uncoated eggs maintained the same quality during storage for four week at 4°C, thus, the freshness as well as the grade AA rating did not change. The ice-preserving effect of the CS/CMC/paraffin coating was clearly shown under low-temperature storage at 4°C (no weight loss). Strengthening this case with the evidence is the coating material exhibited efficacy for holding HU constant and minimizing loss of weight during storage at 25 ° C within 4 weeks. The covering of the microparticle composite hydrogel film with paraffin and low temperature storage at 4°C elicited halve eggs, delay egg weight loss, and avoid microbial contamination. Another thing is that the eggs preserved using the coating materials did not lose their nutritious qualities due to the freshness that comes with these type of preservation materials. Consequently, this interesting egg-coating technology stands on transparent and economical edible polymers basis and brings a hope for an efficient egg production, storing and transportation.

According to Oliveira et al. (2022), the coating plated on the surfaces of the eggs, resulted in a marked reduction in the microbial load of the treated eggs (CS+GIN, CS+LEM, and CS+TAH). After the period of storage, the total count of aerobic mesophilic bacteria on coating eggshells manifested lower values as compared to the uncoated eggs. In particular, egg forms coated with it at 35% stored had the mean log₁₀ CFU ranged from 0.70 and 0.91; while uncoated eggs were 3.17 log₁₀ CFU/ml. Moreover, the coating also contributed to the significant drop in the egg weight loss, with the coated eggs emerging with about 1.49-5.08% weight loss across the study period compared with uncoated eggs that experience 2.92%-8.08% weight loss. Such findings should be

interpreted as that the shelf life of eggs covered with edible coatings was extended by 14 days at least because those had maintaining lower microbial pressures and weight loss below this period in comparison with uncoated ones.

According to Mota et al. (2017), It was found that the egg weight remained constant regardless of the different treatments while there was a higher loss of egg weight when it was stored compared to fresh eggs, with margins of the stored treatments were not significantly different. 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. Pertaining to the internal quality, only the eggs that were stored at the temperature of 5°C demonstrated no statistically significant difference in Haugh Units values in comparison with fresh eggs, which evidences better retention of the internal quality. Nevertheless, under variable conditions, eggs with coatings of cassava and yam starches record a decline in the Haugh Units values over time, which indicates a decrease in the internal quality. The yolk color remained stable during storage, but coatings did not have any effects. Specifically, the albumen pH of uncoated eggs kept at 25°C was high as compared to fresh eggs, and at 5°C and coated with yam starch was low indicating good quality retention.

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:

A polysaccharide, called pullulan, that is produced by the filamentous fungus *Aureobasidium pullulans* is transparent, tasteless and is low in oxygen transmission. Bringing on the coating in eggs with pullulan-based helps basically to preserve the internal quality of egg, prolongs shelf life and reduce weight loss in egg during storage (Sharaf Eddin et al., 2019).

Pullulan-coatings make good secondary barrier follows from their excellent adhesive properties, high mechanical strength, and inertness to food ingredients. They are the unique ones as they are devoid of color, denom, and flavor to mention a few, and the lack of permeability of oxygen and carbon dioxide gases. Furthermore, pullulan is regarded as a microbes' limited carbon source

available for the spoilage of foods, thus in a certain sense it is a great material of "active" edible coating (Ganduri, 2020).

In Morsy et al. (2015) study, it revealed that pullulan coating to be a great way of keeping the inner quality of eggs unharmed. The result was that eggs, which had pullulan covering, and at 25°C stored, had a shelf-life of at least 2 weeks. This was different from those not having pullulan. In addition, these two storage treatments of pullulan were not significantly different in 'ounces lost', Haugh unit, and yolk index after 10 weeks storing. On the other hand, when pullulan and nisin are used jointly as coating agent motion the loss of microbial viability is demonstrated during storage.

1.8.15. Refrigeration:

Several different measures are used to confront microbial contamination on with a common practice of washing and storing them cold. Among the other countries that undertake egg washing, accounting for the USA, Japan, and Australia, the UK and EU are on the other side of the spectrum, being either opposed or even banned such practice. Currently, the EU egg regulations, including especially EC Regulation No. is the controlling factor over egg-handling in EU. Codes 589/2008 (Fikiin et al., 2020) appears to be shrouded in ambiguity and complexity and thus might be relooked from the perspective of temperature and humidity regulation as well as other measures of quality at each raw egg handling step. It is necessary to identify and apply techniques that assure food safety and find the way to solve problems connected with temperature and humidity changes which are in greater percentage as well. Additionally, attention should be paid to the harmonization of different codes and standards by connecting breeding and processing egg handling processes with those from all over the world thus creating fewer trade barriers (Fikiin et al., 2020). The transport condition of eggs in the retail supply chain is greatly diverse. The United States mandates temperatures of not more than 7.2 degrees Celsius while other countries recommend storage between,0 On the one hand, certain countries, such as Australia, can be roofless and apply laws built by other countries (or reference them). Cold storage as vegetative and contagious factors both play their pivotal role in prolonging the eggs shelf life and suppressing mesophilic microorganisms proliferation. The shelf life of eggs from various countries usually spans 21-35 days (Chousalkar et al., 2021) but is determined by factors such as temperature and relative humidity in keeping with egg quality. The egg held at temperature 4°C had been found to curb degradation of albumin quality

and weight in egg for a period of 21 days (Chousalkar et al., 2021), whereas preservation in higher temperatures implied lower egg weight and protein quality.

Alongside its ability to delay spoilage, refrigeration helps to slow down the multiplication of bacteria on shell-tainted surfaces, thus diminishing the risk of cross-contamination among foods with this bacterial pathogen. Condensation on eggshells, which comes to be from removing them out of the refrigeration, leads a complication in egg supply chain. These clouds, which can carry bacteria as well, would be seen as a potential cross-contamination source in the kitchen because of the high relative humidity there.

Observations of microbes growth and survival in the recent research studies (Khan et al., 2021) have identified that ambient temperature is one of the critical factors affecting *Salmonella Typhimurium* in eggs. In particular, the temperature differences instigated a relationship which gave a virulence to *Salmonella Typhimurium* which resulted in the salmonellosis when the eggs were inoculated at ambient temperature. As an in vitro expression analysis shown, eggs related to metabolism, stress response, virulence and colonization, in both albumen and outer membrane, are underexpressed. Similarly, mouse experiments of eating egg wash and albumen that have bacteria contaminant showed *Salmonella* shedding at the end of the 15th day after infection. Mild heat treatment, which is a plausible strategy against *Salmonella*'s contamination on both the eggshell surfaces and shell pores, will be taken into account.

In Shin et al. (2012) investigation, which study the effect of different refrigeration temperatures on the quality of shell eggs, there are obvious differences in Haugh Units (HU), albumen pH, and yolk index (YI) i.e. based on storage temperature. Keeping eggs below -30°C, in this manner, we observed that HU readings were above 79% suggesting high quality, but put eggs under higher temperatures, this led to HU readings decreased because of to protein changes and moisture exchange. On the other hand, the storage temperatures which had exposed them to the freezing temperatures did not cause an improvement in their HU value while their counterparts that had them stored at slightly higher temperatures exhibited an improvement in their HU value. The actual storage temperature range for the holding of shell eggs to be in top-tier quality was established to be between -1.1°C and 2.2°C. In addition to this, the fact that the values of HU has a decline tendency with storage time and the decline rate for the stored eggs below the 3.9°C was slower became clear.

In the same way, albumen pH also increased over the time, yet no exactly steady pattern was outlined with the conservation storage temperature. Overall, a temperature interval from 0.6 degree Celsius to 2.2 degree Celsius was recommended as a limit to avoid any AA (loss of baby(ies)'s age and appearance) and to speed up quality deterioration during 3 weeks of refrigerated storage in commercial models. Thus, the eggs processor and retailers must keep the eggs at the inside range to have the longest intact periods of eggs.

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. Features impacting the primary of albumen quality can be split into hen variances such as their genotype, age, size, breed, diet, water intake, environmental conditions, and their health status. As an allantoic complex takes shape, these eggs begin to pursue physicochemical and functional changes that include an increase in pH, weight loss, evaporation and microbial contamination, primarily through increasing gaseous exchange between carbon dioxide and moisture through airshell pores and shell cracks. The albumen's deterioration during storage is influenced by storage conditions, including temperature and humidity, and eggshell characteristics (Nematnia & 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 CO² through the eggshell pores induces changes in the bicarbonate buffer system. On the same note, as a result of metabolites breakdown during storage, egg white becomes more alkaline(increase in albumen pH). In addition, interferences in the bicarbonate buffer system may also occur. Nevertheless, it should be remembered that besides differences in size of egg and quality

of initial egg and their storage conditions (temperature, humidity and time elapsed) can lead to albumen pH changing 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 the year 1937 as cited in (D. Jones, 2012), has over time stood out as the emerging measure of albumen quality, which is commonly called the “golden standard” in determining egg quality. Egg weight is highly related to albumen thickness a physical parameter of the egg quality according to Eisen et al. (1962). Usually for Haugh unit measurement, a destructive test on a grading sample of eggs is needed.

1.9.4. Yolk index:

Yolk index (YI) is the ratio of top yolk to the diameter of the yolk which is an indirect measure of the vitelline of the yolk membrane as a strength. It is also called as the egg freshness measure as the standard range is between 0.30 and 0.50 for fresh eggs. As per P. G. S. Pires and others (2019), the ideal fresh egg is one with a YI which is about 0.45. The time of storage, for uncoated eggs, was observed to sharply cut down the YI which decreased from 0.48 to 0.38 after a period of 3 weeks. Nevertheless, with age, the YI score of the egg is subject to decreases. It means that a higher YI is a sign of 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 $\geq 99\%$), sorbitol (purity $\geq 99\%$), 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. These 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) :

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

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)

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

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 considered 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.

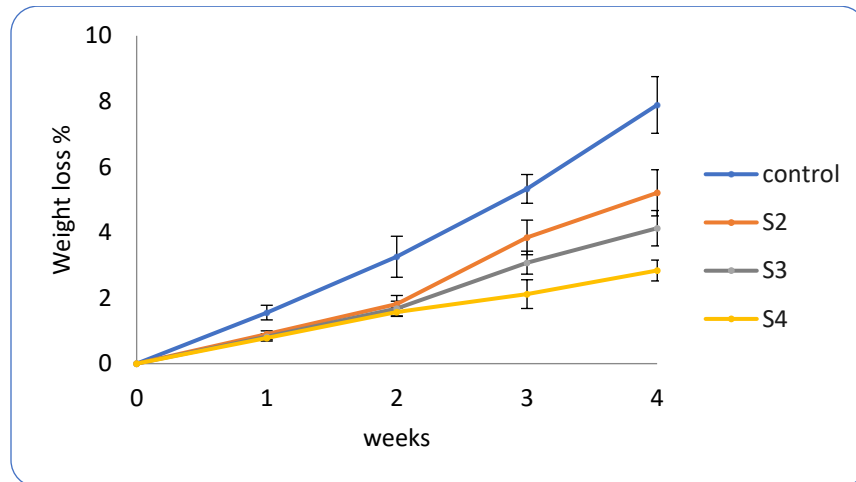


Figure 3 : first experiment Weight loss of egg samples during storage

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.

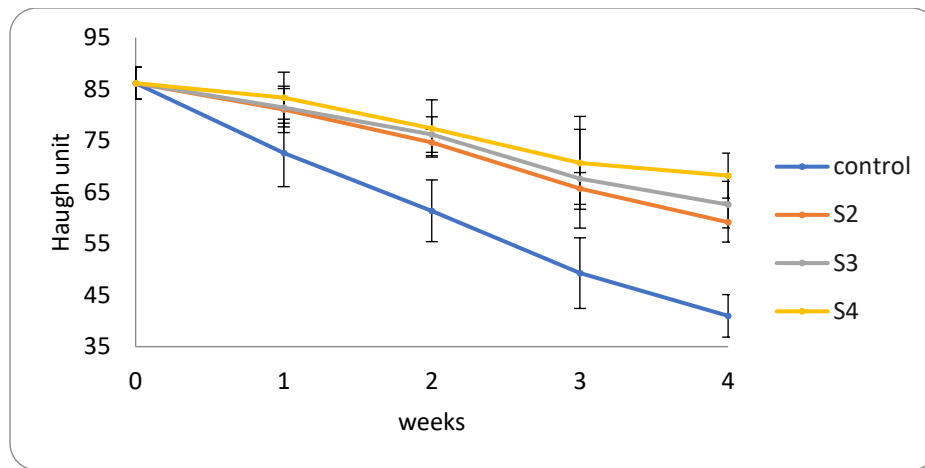


Figure 4 : first experiment Haugh unit of egg samples during storage

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.

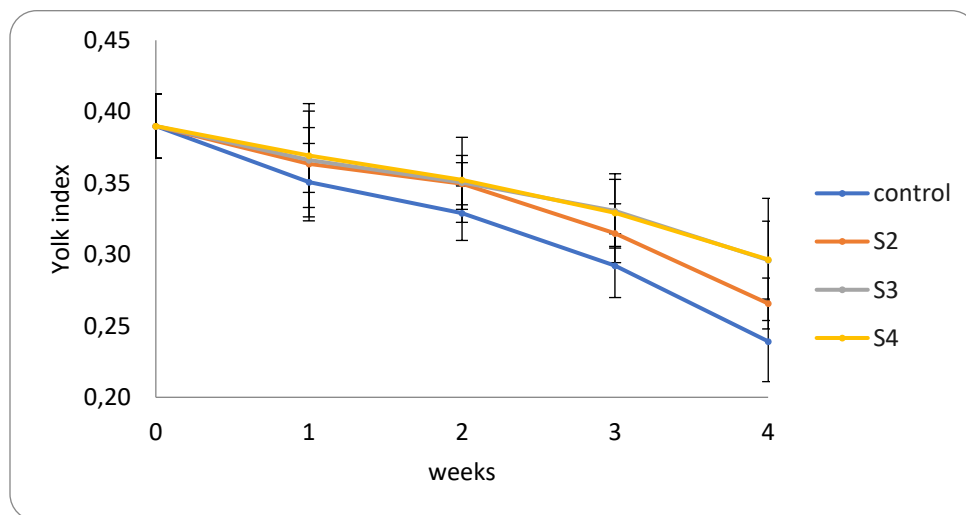


Figure 5: first experiment yolk index of egg samples during storage

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.

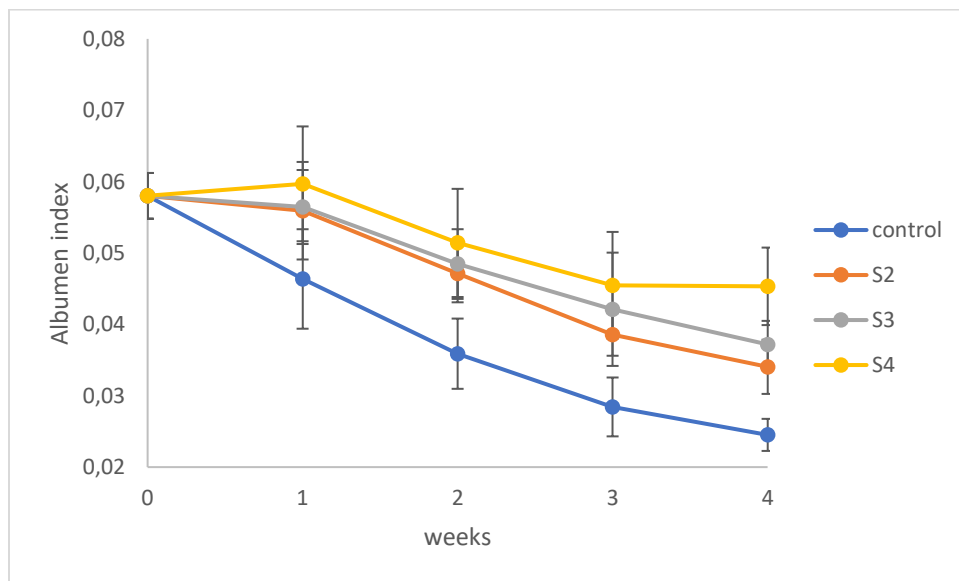


Figure 6: first experiment albumen index of egg samples during storage

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.

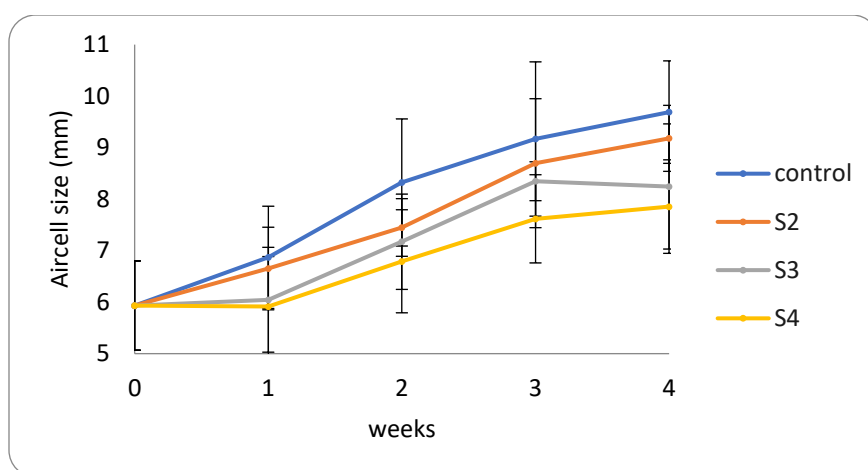


Figure 7: first experiment air cell of egg samples during storage

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 air cell 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.

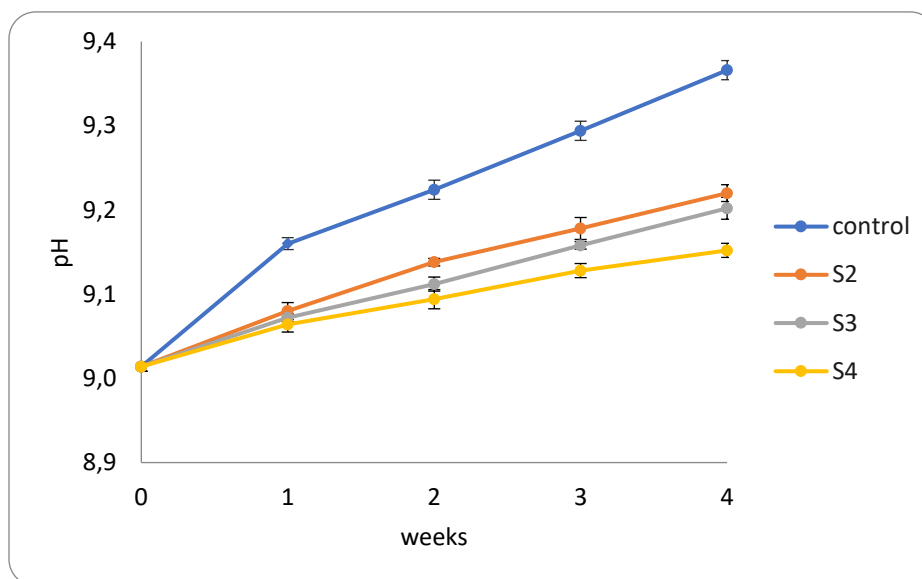


Figure 8: first experiment albumen pH of Egg samples during storage

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:

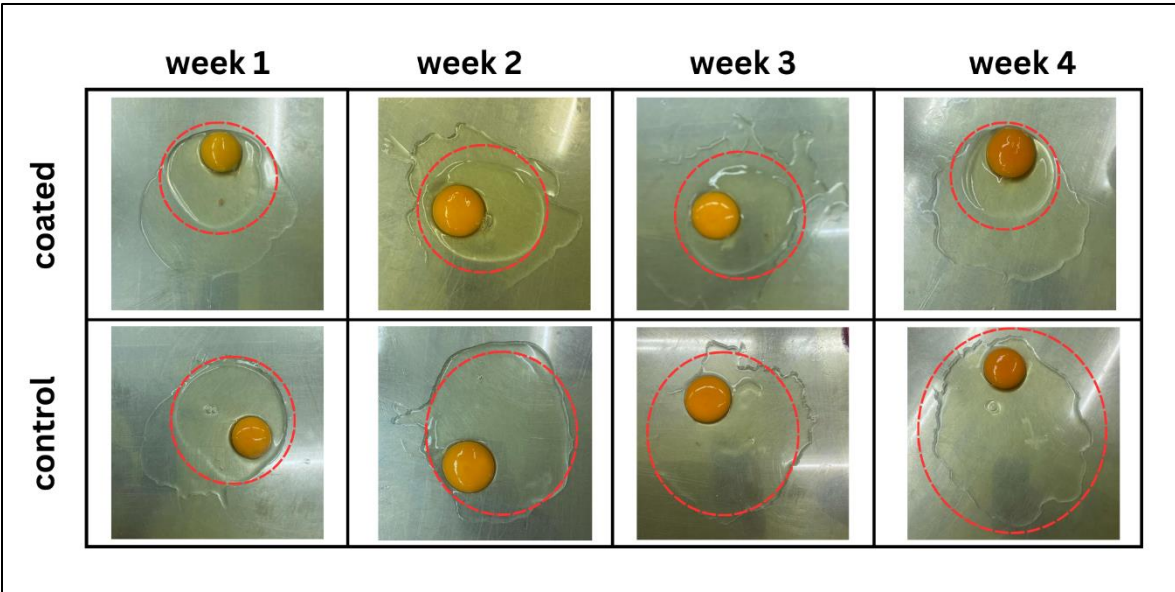


Figure 9: second experiment albumen of egg samples during storage

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 4th 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 2nd week.

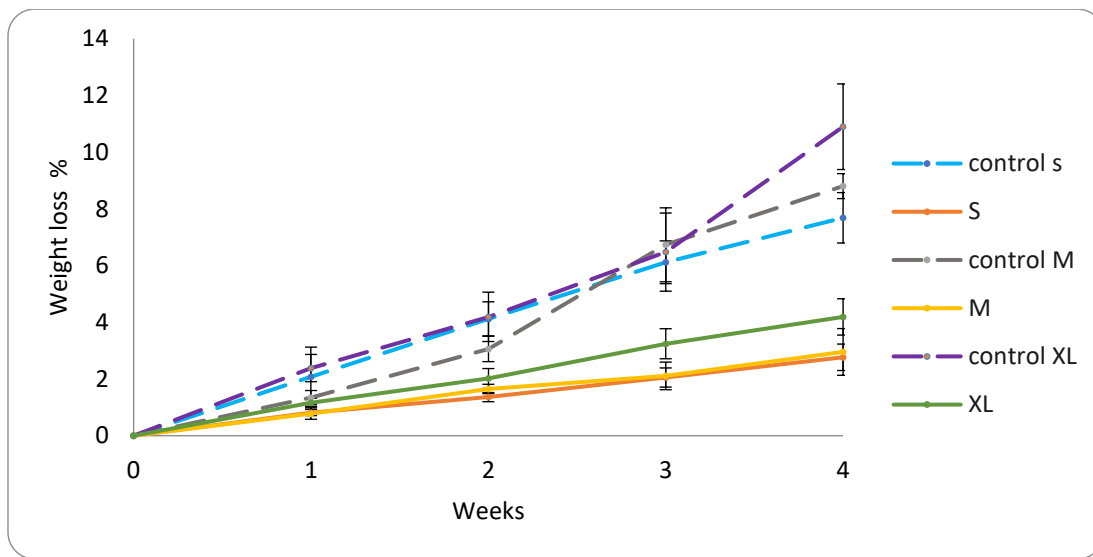


Figure 10: second experiment weight loss of egg samples during storage

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.

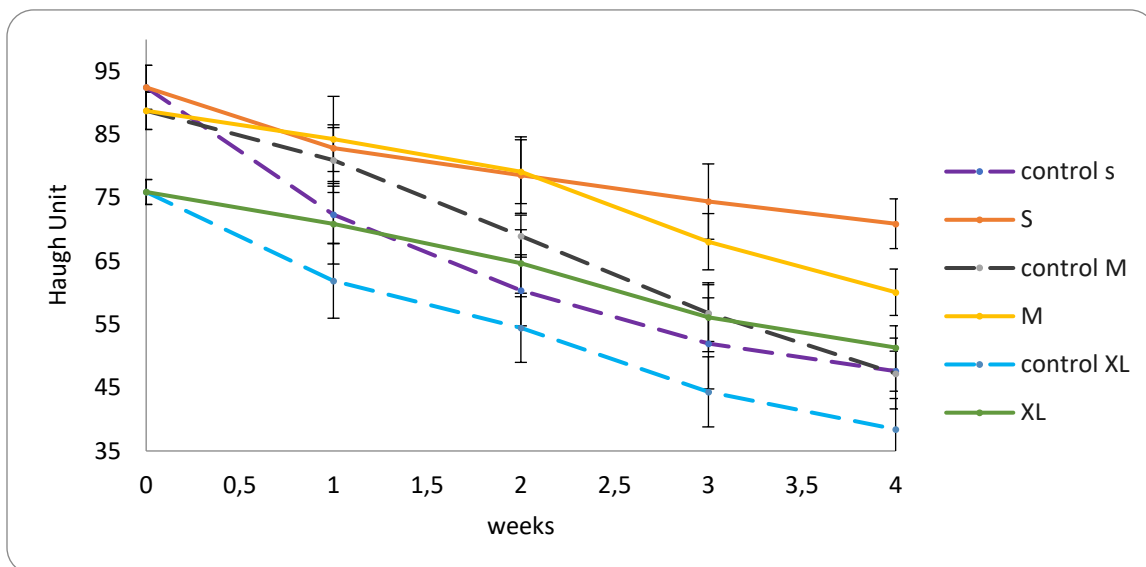


Figure 11: second experiment Haugh unit of egg samples during storage

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.

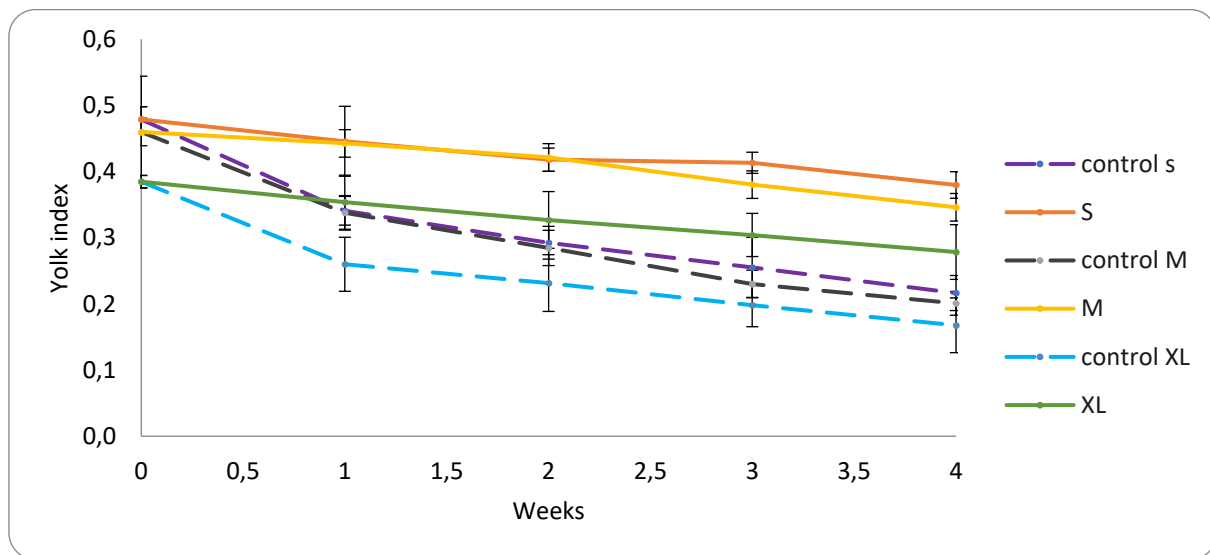


Figure 12: second experiment Haugh unit of egg samples during storage

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).

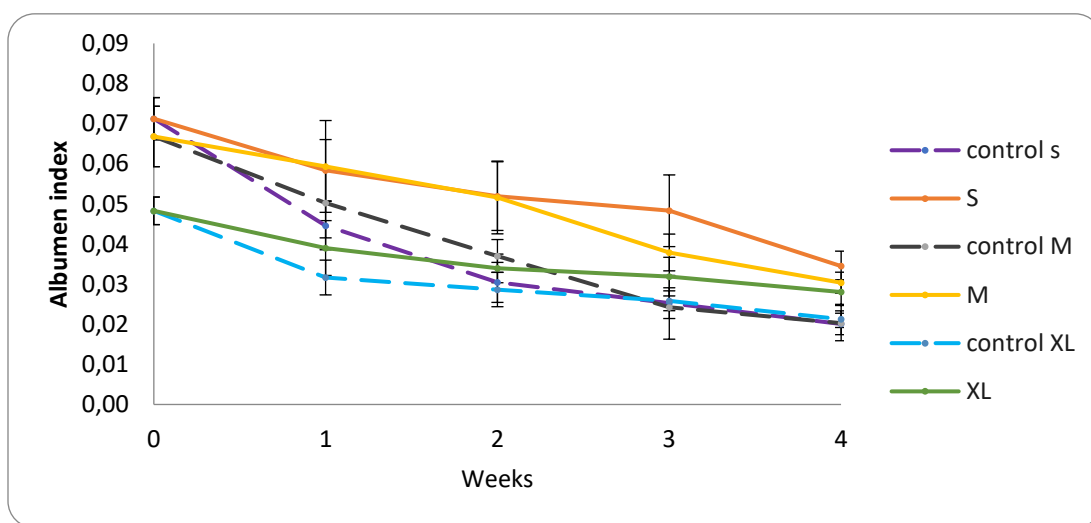


Figure 13: second albumen index of egg samples during storage

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.

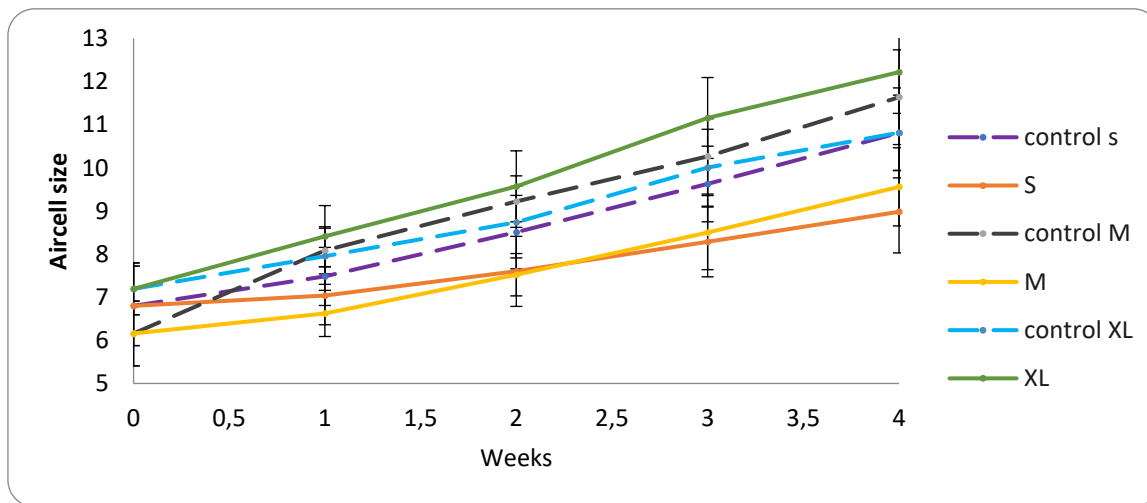


Figure 14: second experiment air cell of egg samples during storage

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

In conclusion, both experiments have proven that starch from cassava can be applied as the preservative to prolong the shelf life of eggs. The test carried out deceit us the advantages of using cassava starch film in order to preclude moisture loss, maintain freshness criteria and maintain products quality. During the second experiment, the samples of bigger eggs had the quicker aging process that confirmed the necessity of creating the coatings — especially in the case of larger eggs that might be worthier. It provides more evidence of the promising nature of this material for use in the egg sector because of maintaining quality devoid of any compromise and having a long lasting 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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1.25. 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 *	7.458E-005	3	2.486E-005	.519	.669	.006	1.558	.156
TREATMENT SIZE *	.001	2	.001	14.791	.000	.109	29.583	.999
TREATMENT TIME * SIZE *	.000	6	6.069E-005	1.268	.273	.030	7.609	.495
TREATMENT 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 *	7.458E-005	3	2.486E-005	.519	.669	.006	1.558	.156
TREATMENT SIZE *	.001	2	.001	14.791	.000	.109	29.583	.999
TREATMENT TIME * SIZE *	.000	6	6.069E-005	1.268	.273	.030	7.609	.495
TREATMENT 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

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