



Hungarian University of Agriculture and Life Sciences

**Techno-Functional and Sensory Properties of Nutritionally Enhanced Liquid Eggs**

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## LIST OF ABBREVIATIONS

Abbreviation	Definition
$a^*$	Redness - greenness
ANOVA	Analysis of variance
$b^*$	Yellowness - blueness
BCAAs	branched-chain amino acids
EO	Essential oils
EVOO	Extra virgin olive oil
FAO	Food and Agriculture organization
FRAP	Ferric Reducing Antioxidant Power
HDL	High density lipoprotein
LDL	Low density lipoproteins
K	consistency index
$L^*$	Lightness
LWE	Liquid whole egg
LEW	Liquid egg white
n	Flow behavior index
PDCAAS	Protein digestibility-corrected amino acid score
SPSS	Statistical package for social science
$\dot{\gamma}$	Shear rate (1/s)
$\tau$	Shear stress (Pa)
$\tau_0$	Yield stress (Pa)

## 1. INTRODUCTION

Eggs are among the most versatile and nutritionally valuable ingredients in the food industry, widely used for their unique functional properties such as emulsification, foaming, coagulation, and gelation. Liquid whole egg, which combines both yolk and albumen in a standardized, pasteurized form, is especially valued in commercial food manufacturing for its consistent quality and ease of use (McNamara, 2013). It plays a critical role in bakery products, emulsified sauces, dairy analogs, ready meals, and confectionery, offering emulsifying, foaming, gelling, thickening, and binding properties that are difficult to replicate with synthetic or single-function additives.

Despite these advantages, the functional performance of liquid whole egg can be variable and is often affected by multiple factors such as pasteurization temperature, storage time, and protein denaturation (Puglisi & Fernandez, 2022). Additionally, there is an increasing demand from the food industry for egg-based ingredients that not only retain their traditional roles but also perform optimally under modern processing conditions, including high-shear mixing, heat treatment, and prolonged storage. These challenges create a need for the enhancement of liquid egg systems in a way that preserves their natural composition while improving functional reliability and product quality (Hintono et al., 2023).

Techno-functional enhancement refers to the targeted improvement of physical and functional properties of food ingredients to better meet specific technological and sensory requirements (Manthei et al., 2023). In case of liquid egg, this may include increasing viscosity, strengthening gelation or foaming capacity, stabilizing emulsions, or improving thermal stability. Such enhancements are often achieved through the addition of natural biopolymers, proteins, or hydrocolloids, or by modifying processing parameters (Mine, 1995). Recent trends in food science encourage the use of natural, clean-label fortifiers such as plant-based proteins, dietary fibers, or food-grade gums which align with consumer preferences for minimally processed and health-conscious foods (Grant et al., 2021).

Protein fortification is a particularly promising approach for enhancing the techno-functional behavior of liquid egg. Proteins, both animal and plant-based, can interact with egg proteins to form new structural networks that improve viscosity, emulsion stability, and gel strength. When incorporated into liquid egg systems, these proteins may contribute to enhanced water-binding

capacity, improved emulsification, and more stable foam formation, depending on their structure, solubility, and interaction with endogenous egg proteins (Tian et al., 2024).

However, introducing external proteins or hydrocolloids into the liquid egg matrix can also alter its rheological and sensory properties (Matsuoka et al., 2019). Thus, it is essential to study the impact of these modifications systematically considering not only their functional benefits but also their influence on product appearance, mouthfeel, and consumer acceptance. Moreover, understanding how these additives interact with egg proteins under different thermal treatments and over storage time is key to optimizing the formulation for industrial use.

This research aims to explore the techno-functional enhancement of liquid whole egg through the incorporation of selected protein fortifiers, focusing on their impact on pH, color and rheological behavior, over time and under varying storage and pasteurization conditions. It also aims to determine the optimal conditions under which these enhancements can be maximized without compromising the natural structure or sensory profile of the liquid egg.

The research is relevant not only from a technological standpoint but also in terms of sustainability and innovation. The development of improved egg-based formulations with different proteins could provide a competitive advantage in the food market. Furthermore, this work contributes to the broader scientific understanding of protein-protein interactions in mixed systems and offers insights into how formulation strategies can be used to tailor food textures and functionalities.

In summary, this thesis aims to address the growing need for improved functional performance in liquid egg products by applying protein and oils fortification strategies and analyzing their impact through a multidisciplinary lens. By doing so, it seeks to develop a liquid egg system with enhanced techno-functional properties that can meet the evolving demands of food manufacturing, while supporting innovation, nutritional value, and consumer appeal.

## 1.1 Objectives

The overall aim of this research is to develop functionally enhanced and sensory-acceptable fortified liquid egg products by systematically exploring the combined effects of protein or oil additives and pasteurization, with a focus on rheological behavior, pH, color and customer acceptance. The study also aims to assess how these modifications are influenced by pasteurization temperatures and storage duration. To achieve this, the following specific objectives have been identified:

- To investigate the effect of different concentrations of different protein powders and different oils on the techno-functional properties of liquid egg products, including viscosity, yield stress, flow behavior, and gelation characteristics.
- To investigate the influence of pasteurization at different temperatures (50°C, 55°C, and 60°C for egg white and 60°C, 65°C, 70°C for whole egg) on the structure-function relationships of fortified liquid egg systems, especially in terms of protein interactions, and stability.
- To perform sensory analysis on the most promising formulations to evaluate potential changes in texture, mouthfeel, or appearance that may affect consumer acceptance.
- To determine the optimal combination of protein concentration and processing conditions that achieves significant improvement in functional properties while maintaining the natural characteristics of liquid whole egg.

## 2. LITERATURE REVIEW

### 2.1 Eggs: composition, nutritional values, production and consuming

#### 2.1.1 Composition of whole egg

The primary function of an egg is to supply the essential nutrients and conditions required for the embryo's development. This role is facilitated by the egg's intricate structure and composition. Progressing from the innermost to the outermost components, in the sequence of their formation, the parts of the egg include the yolk, vitelline membrane, albumen (egg white), shell membranes, and the shell. A detailed depiction of the egg's structure is provided in Figure 1.

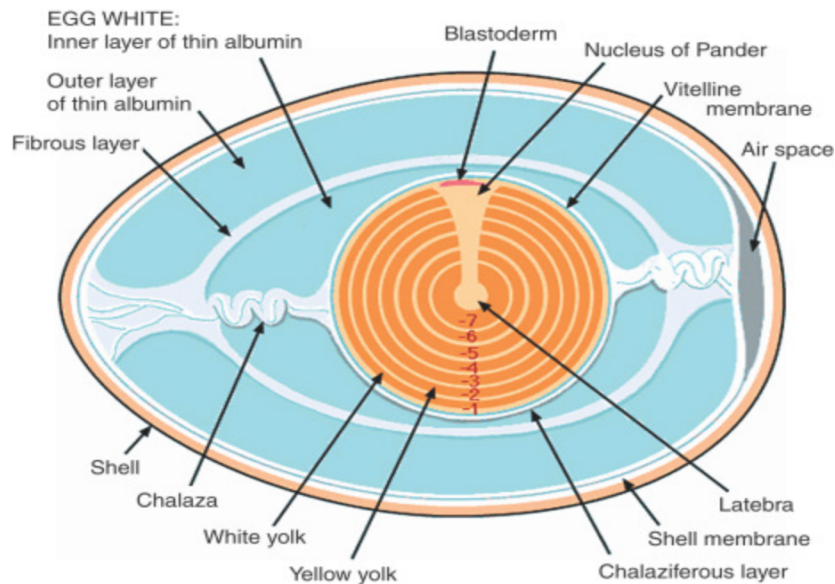


Figure 1: A detailed depiction of the egg's structure (Nys & Guyot, 2011)

Eggs are composed of three main parts: the shell, the egg white, and yolk. Each of these components has distinct structures and nutrient profiles that contribute to the egg's overall functionality, nutritional value, and applications in both food science and biology (Nys & Guyot, 2011). The eggshell is primarily made up of calcium carbonate (about 94-97%), along with small amounts of magnesium carbonate and calcium phosphate. It provides structural protection to the egg and a barrier against microbial contamination. It is also porous, allowing gases and moisture to pass in and out, which is crucial for embryo development if the egg is fertilized (Nys & Guyot, 2011). The shell is covered by a cuticle layer that helps protect against bacteria which is called

cuticle. As for egg white, it constitutes about 60% of the egg's weight and is about 90% water, with the rest being primarily proteins (Hester, 2018). Key proteins in the egg white are ovalbumin, ovotransferrin, lysozyme and avidin. The last component of egg is egg yolk, *it* comprises about 30-35% of the egg's weight and contains most of the egg's lipids and vitamins (Abeyrathne et al., 2013). It consists of approximately 50% water, with the remaining content made up of lipids, proteins, and nutrients. The yolk is rich in lipids, including triglycerides, phospholipids and cholesterol. These lipids give the yolk its creamy texture and contribute to the richness in foods (Oladimeji & Gebhardt, 2023). It also contains proteins, including vitellin and lipovitellin, aid in providing nutrition for a developing embryo. The yolk is nutrient-dense, containing vitamins A, D, E, and K, as well as B-complex vitamins. It also provides minerals such as phosphorus, iron, calcium, and zinc. Another important component of egg yolk is carotenoids which gives it its yellow color, these pigments (like lutein and zeaxanthin) have antioxidant properties beneficial for eye health (Réhault-Godbert et al., 2019).

### 2.1.2 Egg production and consumption

Eggs have been a staple of human diets for years, valued for their nutritional density, culinary versatility, and ease of availability. Historical evidence suggests that humans began domesticating birds like chickens for egg production as early as 8,000 years ago in Southeast Asia and India. Ancient Egyptians and Romans recognized the value of eggs, incorporating them into daily meals and rituals (Zaheer, 2015). The industrial revolution saw the mechanization of egg production, leading to the standardization of sizes and grades. In the 20th century, eggs became a symbol of economic recovery and protein-rich diets, particularly during wartime when they were emphasized as a vital source of nutrition. Today, eggs are consumed worldwide, boiled or scrambled preparations to integral components of complex dishes like meringues and pastries (Niakousari et al., 2019). Beyond their culinary uses, eggs have played a role in cultural and symbolic contexts, representing fertility and rebirth in various traditions and celebrations, such as Easter (Newall, 1984).

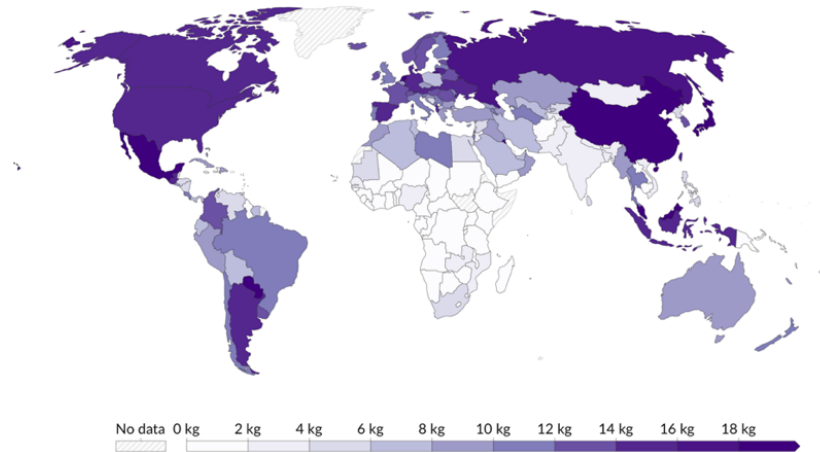


Figure 2: The average per capita egg consumption, measured in kilograms per year (in shell weight) of hen eggs worldwide in 2017, source FAO data accessed in February 2025.

Egg consumption varies significantly across the globe, reflecting cultural, economic, and dietary differences. Eggs are an affordable source of high-quality protein, essential vitamins, and minerals, making them a dietary staple in many regions (Zaheer, 2015). Per capita egg consumption is highest in countries like Japan, China, and Mexico, where eggs are integrated into daily meals in diverse forms, such as steamed, fried, or incorporated into traditional dishes (Henchion et al., 2021). For instance, in Japan, eggs are a key ingredient in dishes like tamago sushi and ramen, while in Mexico, eggs are central to breakfast dishes like huevos rancheros. In developed countries like the United States and European nations, eggs are consumed widely, not only as a meal but also as ingredients in baked goods, pasta, and processed foods (Henchion et al., 2021). The rise of health-conscious diets has further increased egg consumption, with a focus on their nutritional benefits, such as being a low-calorie source of protein and containing important nutrients like choline and vitamin D. However, in some regions of Africa and parts of Southeast Asia, egg consumption remains relatively low, often due to limited access, economic constraints, or cultural preferences for other protein sources (Magdelaine, 2011).

Global trends show an overall increase in egg consumption, driven by population growth, urbanization, and shifting dietary patterns toward protein-rich foods. Additionally, campaigns promoting eggs as a cost-effective way to combat malnutrition have boosted their popularity in developing countries (Magdelaine, 2011). Innovations in egg production, such as enriched eggs with higher omega-3 content or organic options, have also influenced consumption patterns in markets with a focus on health and sustainability (Usturoi et al., 2025).

### 2.1.3 Nutritional values of eggs

Proteins represent a critical nutritional component of eggs, contributing significantly to their dietary value. The amino acid profile and composition of egg proteins are among the most bioavailable to the human body, second only to breast milk (Réhault-Godbert et al., 2019). However, it is important to note that the consumption of raw eggs may reduce protein digestibility due to the presence of trypsin inhibitors, which interfere with protein breakdown. The denaturation of egg proteins through cooking mitigates this issue by altering their structure, thereby enhancing their digestibility (Farjami et al., 2021).

Eggs are considered a complete source of protein because they contain all nine essential amino acids in balanced proportions. An average large egg contains approximately 70 calories and 6 grams of protein, the high biological value of egg protein makes it easily digestible and highly effective for muscle repair and growth, as well as other metabolic functions. It also has 5 grams of fat, which includes both saturated and unsaturated fats (Watkins, 1995). Around 2 grams of these fats are monounsaturated, and about 1.5 grams are polyunsaturated. The yolk is also one of the few natural sources of cholesterol, containing about 186 milligrams per large egg (Watkins, 1995). While dietary cholesterol was once a concern, recent research shows that for most people, cholesterol intake from eggs does not significantly affect blood cholesterol levels, studies show they generally raise high density lipoprotein (HDL) levels without significantly impacting heart health for most people. It is also rich in micronutrients, such as: vitamins B12, riboflavin, K, E and vitamin D which are important for bone health, immune function, and blood clotting (Li et al., 2020). Eggs provide essential minerals like iron, zinc, phosphorus, selenium and choline, a nutrient essential for brain health and cell membrane structure (Myers & Ruxton, 2023).

Egg production and consumption have become vital components of global food systems, due to eggs' rich nutritional profile, economic viability, and relatively low environmental footprint compared to other animal protein sources. Worldwide egg production reached around 90 million metric tons annually, with the majority produced by China, the United States, India, and Japan. China alone accounts for more than a third of global egg production, producing nearly 40 million metric tons per year, driven by its large population and dietary preferences that incorporate eggs in various traditional dishes (Abín et al., 2018).

Table 1: Shows the nutritional values in 100g of egg white and egg yolk (Eunice C., Li-Chan, William D. Powrie, Shuryo Nakai, 1995)

Component	Egg white	Egg yolk
Energy	58 kcal	362 kcal
Protein	12.8 g	16.1 g
Fat	0.3 g	31.7 g
Carbohydrate	0.7	0.3
Water	85.5 g	50.7 g
Phosphorus	12 mg	570 mg
Calcium	13 mg	80 mg
Potassium	120 mg	85 mg
Magnesium	12 mg	16 mg
Sodium	125 mg	30 mg
Iron	0.24 mg	4.10 mg
Vitamin A	0 µg	1100 µg
Vitamin D	0 µg	60 µg
Vitamin E	0 µg	3 µg
Vitamin B1	3 µg	120 µg
Vitamin B2	200 µg	320 µg
Vitamin B6	0.006 µg	0.065 µg
Vitamin B12	0.30 µg	2.80 µg
Folic Acid	16 µg	150 µg
Cholesterol	0 mg	1190 mg

The United States and European Union also have substantial egg production, though production systems and regulatory standards vary widely, with some regions focusing on cage-free and organic production to meet consumer demand for higher animal welfare standards (Abín et al., 2018). The global rise in egg consumption aligns with trends in population growth, urbanization, and rising income levels, particularly in developing countries where eggs are increasingly recognized as an affordable, high-quality protein source. Per capita egg consumption shows considerable regional variation. For example, Japan has among the highest per capita egg consumption globally, with each person consuming an estimated 330 eggs per year, reflecting cultural preferences and integration into daily diets. Meanwhile, countries in Africa tend to have lower per capita consumption due to economic constraints and lower domestic production capacity (Guyonnet, 2023).

Trade in eggs and egg products is also significant, particularly in regions with high production and limited domestic demand. The European Union, the United States, and some Asian countries are major exporters of egg products like dried egg powders and liquid eggs, which are used extensively in food manufacturing and have longer shelf lives than shell eggs (Michele Suman, et al., 2013).

Exported egg products help balance global supply and demand but are subject to trade restrictions and tariffs, as well as health and safety regulations that vary between countries. As global demand for eggs continues to rise, the industry faces challenges to maintain sustainable production practices, uphold food safety, and adapt to evolving consumer expectations, especially regarding animal welfare and environmental impact (Michele Suman, et al., 2013).

Eggs are consumed in various forms across cultures, from boiled or scrambled to components in processed foods. In addition, egg-based products like liquid eggs, egg powders, and specialty egg products (such as omega-3 enriched eggs) cater to diverse dietary needs and culinary applications. An egg is composed of three main parts: the shell, the egg white (albumen), and the yolk. The shell is the egg's outermost structure, providing physical protection (Gautron et al., 2022). It is primarily made of calcium carbonate and is semi-permeable, allowing gases and moisture to pass through. The shell also has a protective cuticle layer to prevent bacterial contamination (Gautron et al., 2022). The egg white makes up about 60% of the egg's weight and consists primarily of water and proteins. Major proteins in egg white include ovalbumin, ovotransferrin, ovomucoid, and lysozyme. These proteins contribute to the egg's functional properties, such as foaming, gelation, and water-binding (Abeyrathne et al., 2013). The yolk constitutes about 30% of the egg's weight and contains a significant amount of lipids, including triglycerides, phospholipids, and cholesterol. It is also rich in proteins, vitamin, and minerals like iron, phosphorus, and calcium. The yolk is responsible for the emulsifying properties of eggs, as it contains lecithin, a natural emulsifier.

Eggs offer numerous health benefits due to their rich nutritional profile (Lechevalier et al., 2011). Because they are an excellent source of high-quality protein, eggs support muscle repair and growth, making them valuable for athletes and those focused on maintaining muscle mass. Additionally, eggs are rich in antioxidants like lutein and zeaxanthin, which help protect eye health by reducing the risk of age-related macular degeneration and cataracts (Santos et al., 2021).

Eggs are known in food science for their multifunctional properties, which enhance texture, stability, and appearance in a wide range of food products. Some of eggs properties are (Filipiak–Florkiewicz et al., 2017):

1. **Foaming:** Egg whites can trap air when whipped, forming a foam that can be stabilized by the proteins in the egg. This property is essential for products like meringues, soufflés, and angel food cakes.

2. Emulsification: The yolk's lecithin acts as an emulsifier, helping to mix fat and water phases in foods. This property is critical in mayonnaise, sauces, and dressings.
3. Coagulation and Gelation: Upon heating, egg proteins denature and form a gel network, which gives structure to products such as custards, quiches, and baked goods.
4. Binding and Adhesion: Eggs help bind ingredients together, enhancing the structure and stability of products like meatloaf, burgers, and batters.
5. Color and Flavor: Eggs contribute a rich, yellow color and mild flavor to baked products, custards, and other dishes.
6. Water Binding: Egg proteins can absorb and retain water, which helps maintain moisture in baked goods and prolong shelf life.
- 7.

## 2.2 Egg proteins

Eggs are an exceptional source of high-quality proteins, contributing to their widespread use in nutrition and food industries. Egg proteins are characterized by their excellent amino acid profile, digestibility, and functional properties. These proteins are distributed across the various parts of the egg: the egg white (albumen), yolk, and shell membranes (Guha et al., 2019).

### 2.2.1 Egg white proteins

The egg white, constituting about 60% of the egg's total weight and contains the majority of its protein content. It is primarily composed of 90% water and 10% proteins (Guha et al., 2019).

Ovalbumin, the main protein in egg white, is synthesized in the oviduct of laying hens and belongs to the serpin family. It is a key reference protein in biochemistry due to its high purity and versatility, serving as a carrier, stabilizer, blocking agent, or standard material. Ovalbumin has a molecular weight of 45 kDa and consists of 386 amino acids (M. et al., 2013). Unlike most proteins, it lacks a classical N-terminal signal sequence but has three post-translational modification sites near the N-terminus, including an acetylated glycine residue as its N-terminal amino acid and proline as its C-terminal amino acid (Zemser et al., 1994). Ovalbumin owns a unique amino acid composition compared to other proteins. In solution, it is easily denatured by agitation, but it demonstrates relative resistance to heat. During storage, it undergoes a conformational change, converting into a more heat-stable form known as S-ovalbumin. This transformation enhances its thermal stability, making it a subject of interest for both fundamental

research and industrial applications (Hincke, 1995). The key proteins in egg white and their denaturation temperature are as follows:

Table 2: the key proteins in egg white and their denaturation temperature (Ahmed et al., 2007)

Protein	Percentage in egg white	Denaturation temperature (°C)
Ovalbumin	54	84
Ovotransferrin	12	61
Ovomucoid	11	77
Lysozyme	3.4	75
Ovomucin	3.5	-
G2 globulin	4.0	92.5
G3 globulin	4.0	-
Ovoinhibitor	1.5	-
Ovoglycoprotein	1	-
Ovoflavoprotein	0.8	-
Ovomacroglobulin	0.5	-
Avidin	0.05	85
Cystatin	0.05	-

Ovotransferrin is a glycoprotein composed of 686 amino acids, with a molecular weight of 76 kDa. Initially it was named as conalbumin, it was renamed ovotransferrin upon the discovery of its ability to bind iron (Wu & Acero-Lopez, 2012). This protein shares a similar amino acid sequence with human serum transferrin and contains 15 disulfide bonds, which contribute to its structural stability (Wu & Acero-Lopez, 2012). Each ovotransferrin molecule can bind and transport two iron ions, playing a crucial role in iron metabolism (Superti et al., 2007). It exists in two primary forms: the apo-form (iron-free) and the holo-form (iron-bound) (Superti et al., 2007). The chemical and physical properties of these forms differ significantly; the holo-form exhibits greater resistance to chemical and physical stress compared to the apo-form. This dual functionality and structural adaptability make ovotransferrin an important protein for both biological and industrial applications (Wu & Acero-Lopez, 2012).

Ovomucin is a highly viscous sulfated glycoprotein characterized by both soluble and insoluble components (Omana et al., 2010). The soluble component has a molecular weight of 8.3 kDa, while the insoluble fraction ranges between 220 and 270. It is a high molecular weight protein to which carbohydrates are covalently attached (Hiidenhovi, 2007). On average, carbohydrates

constitute approximately 33% of ovomucin, including galactose, galactosamine, and sialic acid (Hiidenhovi, 2007).

Ovomucin plays a critical role in maintaining the gel-like structure of egg white, which is essential for its stability during storage (Hiidenhovi, 2007; Toussant & Latshaw, 1999). The structural of the ovomucin is closely linked to the egg white's viscoelastic properties. In solution, ovomucin exhibits remarkable heat stability. Within the pH range of 7.1 to 9.4, ovomucin maintains its viscosity and light transmittance, even after being subjected to 90°C for 2 hours (Hiidenhovi, 2007).

Lysozyme, a crucial antibacterial protein, that is widely distributed in nature. The form of lysozyme found in eggs is particularly notable for its high solubility and stability (Lesnierowski & Kijowski, 2007). Lysozyme has a molecular weight of 14.4 kDa and consists of a single polypeptide chain with 129 amino acids (Lesnierowski & Kijowski, 2007). Lysozyme is heat stable due to the presence of four disulfide bridges in its structure. One of the main lysozyme characteristics is that it can hydrolyzing N-acetylneuraminic acid and the  $\beta(1-4)$  glycosidic bond between N-acetylglucosamine units in bacterial cell walls (Chipman & Sharon, 1969).

Early studies suggested the presence of six globulin fractions in egg white, identified as macroglobulin, ovoglobulin G1, G2, G3, and two additional globulins Ahmed et al., 2007). Later on research reclassified these proteins, identifying the two additional globulins as ovoinhibitors, and ovoglobulin G1 as lysozyme (M. et al., 2013; Stevens, 1991). Currently, the term ovoglobulin specifically refers to G2 and G3 ovoglobulins, with molecular weights of 36 kDa and 45 kDa, respectively (M. et al., 2013). While the precise biological functions of these proteins remain unclear, they are believed to contribute significantly to the foaming properties of egg white (Stevens, 1991).

Ovomacroglobulin is the second-largest glycoprotein in egg white, following ovomucin, with a molecular weight ranging between 760 and 900 kDa. Similar to ovomucin, ovomacroglobulin has the ability to inhibit hemagglutination (Mann, 2007).

Ovoflavoprotein has a molecular weight of 32–36 kDa, containing a carbohydrate component comprising mannose, galactose, and glucosamines, as well as 7–8 phosphate groups and 8 disulfide bonds. It is also known as riboflavin-binding protein due to its ability to bind riboflavin in a 1:1 molar ratio (Chang et al., 2018). This binding is disrupted at the protein's isoelectric pH of 4.2.

The protein's antimicrobial activity is attributed to its capacity to bind to riboflavin, depriving microorganisms of this essential nutrient (Croguennec et al., 2007).

Avidin is a basic glycoprotein, and a tetrameric protein composed of identical subunits, each with a molecular weight of 15.6 kDa and consisting of 128 amino acids (Green, 1975). These four polypeptide subunits are connected via disulfide bonds. Although avidin constitutes only a trace component of egg white, it has been extensively studied due to its remarkable ability to bind biotin with high specificity and affinity, rendering biotin inaccessible to bacteria (Livnah et al., 1993). Avidin is irreversibly denatured at 70°C when unbound, yet the avidin-biotin complex remains stable even at 100°C (Livnah et al., 1993).

Cystatin is a small, heat-stable proteinase inhibitor protein with a molecular weight of 12.7 kDa. It has earned significant attention due to its broad potential applications in medical treatments, as documented in the scientific literature (Barrett, 1981).

### 2.2.2 Egg yolk proteins

The egg yolk, which constitutes approximately 30% of the egg's total content, is rich in lipid-based substances. Simple fats, primarily triglycerides, account for approximately 66% of its lipid content, while the remaining fraction primarily consists of phospholipids and cholesterol (Anton, 2013).

Carotenoids are responsible for the characteristic golden-yellow color of the egg yolk, which can range from pale yellow to deep red depending on their concentration and composition. They are known for their antioxidant properties, and anti-tumor activity by inhibiting the growth of cancerous cells (L. De Souza et al., 2019). In egg yolks, the carotenoids lutein and zeaxanthin play a crucial role in egg healthy benefits. They have anti-aging effects and are pivotal in preventing macular degeneration, a leading cause of vision loss (Huang & Ahn, 2019).

Lecithin, a major component of the granular fraction of egg yolk, accounts for approximately 70% of its lipid content (Palacios & Wang, 2005). Lecithin is crucial not only for its role in emulsion formation and stability in food technology but also for its biological significance as a key component of cell membranes. It also plays a role in human brain and nerve tissues functions (Palacios & Wang, 2005).

The carbohydrate content of egg yolk is minimal, comprising only about 0.7–1%. In contrast, its protein content is approximately 16%, with globular proteins such as livetins and phosphovitin being particularly prominent, although other proteins are also present (Yamamoto et al., 2018). The water-soluble livetins in the yolk plasma consist of 14% serum albumin, 41% glycoprotein,

and 45% immunoglobulin, reflecting a diverse protein composition. In terms of mineral content, the egg yolk contains significant amounts of calcium, phosphorus, and iron, which are present in much higher concentrations compared to the egg white (Mine et al., 2023). These essential minerals underscore the nutritional value of the yolk, making its consumption beneficial. Regarding vitamins, egg yolk is particularly rich in vitamins A, D, B1, and B5, contributing to its status as a nutrient-dense food source (Mine et al., 2023).

Cholesterol has been a focal point of criticism in discussions surrounding the consumption of egg yolks or whole eggs. Cholesterol is a complex compound, essential for various biological functions but often criticized due to its association with health risks (Brownawell & Falk, 2010). Consequently, evolving dietary trends have frequently advocated for the exclusion of egg yolks to mitigate cholesterol intake (Brownawell & Falk, 2010). Efforts to reduce the cholesterol content of eggs have led to the development of innovative techniques. For example, a 2010 study explored the fractionation of egg yolk into three distinct components: granules, lipid paste, and an aqueous fraction. The granules exhibited functional properties, such as superior emulsification and gel-forming capabilities, and contained significantly lower cholesterol levels than whole egg yolk. This fractionation process not only addresses dietary concerns but also adds value to egg-derived ingredients in food technology (Laca et al., 2010).

Moreover, emerging research has challenged the direct association between egg consumption and cholesterol-related diseases. Studies have suggested that conditions such as heart disease and arteriosclerosis are not primarily attributable to dietary cholesterol from eggs, highlighting the need for a nuanced understanding of the relationship between egg intake and health outcomes (Carter et al., 2023).

### 2.3 Egg products history and manufacturing

The history of egg products reflects the evolution of food processing and preservation technologies, with roots in ancient practices and significant advances in the 20th century. Initially, eggs were consumed fresh, and preservation methods like drying and salting were used on a small scale in various cultures (Lechevalier et al., 2011). However, the industrialization of egg products began in the early 1900s, driven by the need to stabilize eggs for transportation, storage, and military use (Wu, 2014).

Egg processing expanded into commercial food production, with products such as liquid, frozen, and powdered eggs becoming staples in the food industry (Gautron et al., 2022). These forms

allowed for easier handling, measurement, and incorporation into recipes compared to shell eggs, supporting the growth of baking, confectionery, and prepared food industries (Gautron et al., 2022). In the mid-20th century pasteurization was used in egg products industry to improve the safety of egg products, to eliminate concerns about Salmonella and other pathogens, this development led to the widespread adoption of liquid egg products in commercial kitchens and by consumers seeking convenience and safety (Wu, 2014).

### 2.3.1 Liquid egg production

Liquid whole eggs are widely used in the food industry for their versatility and functionality (Wu, 2014). They are manufactured to meet stringent food safety and quality standards, ensuring a product that retains the natural properties of fresh eggs while offering longer shelf life, convenience, and consistency (Haas, 2015). The production process can be divided into the following key stages (Haas, 2015):

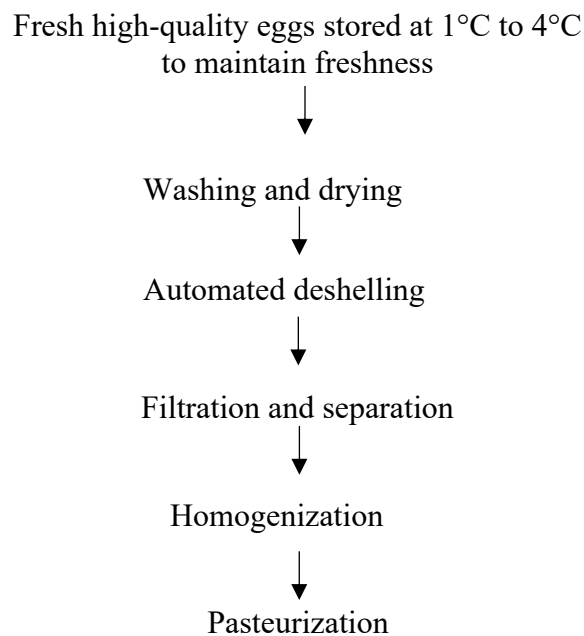


Figure 3: The production process of liquid egg products

### 2.3.2 Powdered egg production

The production of powdered whole egg is a well-established process in the food industry, designed to extend the shelf life of eggs while maintaining their nutritional value, functionality, and microbiological safety (Haas, 2015). The process involves several critical steps, including raw material selection, liquid egg preparation, pasteurization, drying (typically spray drying), and final

packaging. Each stage plays a vital role in ensuring the quality, solubility, and rehydration properties of the final powdered product, making it suitable for various applications in bakery, confectionery, processed foods, and industrial food formulations (Lechevalier et al., 2013).

The production process begins with the selection of high-quality whole eggs, then the eggs undergo washing and sanitization to remove external dirt and microbial contaminants before being cracked using automated egg-breaking machines (Lechevalier et al., 2013). The liquid whole egg (a homogenous mixture of egg white and yolk) is then collected and filtered to remove shell fragments and chalazae (Koç et al., 2011). This filtration step ensures a smooth consistency and prevents unwanted particles from interfering with processing efficiency (Haas, 2015). The next stage is pasteurization, where the liquid egg is heat-treated at approximately 60–64°C for 3–5 minutes. The pasteurization step is to assure the safety of the liquid whole eggs, without significantly denaturing egg proteins, thereby preserving its functional properties such as emulsification, foaming, and gelation (Lechevalier et al., 2017).

Once pasteurized, the liquid egg is concentrated using vacuum evaporation to remove excess water and improve the efficiency of the drying process (Lechevalier et al., 2017). The most widely used technique for converting liquid whole egg into powder is spray drying, due to its ability to rapidly dehydrate the liquid while preserving protein functionality (Stadelman, 1994). In this step, the concentrated liquid egg is fed into a spray dryer equipped with an atomizer, which disperses the liquid into fine droplets inside a heated drying chamber. The inlet temperature typically ranges between 160–180°C, while the outlet temperature remains between 70–90°C to ensure efficient moisture removal while preventing excessive protein denaturation (Stadelman, 1994). The hot air rapidly evaporates the moisture, leaving behind fine, free-flowing powdered whole egg particles that are collected at the base of the drying chamber (Lechevalier et al., 2013).

The final step in production involves cooling, and packaging. The dried egg powder is cooled to prevent post-processing moisture condensation, which could lead to clumping and reduced shelf stability (Lechevalier et al., 2013). The powder is then sieved to ensure uniform particle size and packaged in airtight, moisture-resistant containers or vacuum-sealed bags to prevent oxidation, microbial contamination, and nutrient degradation (Lechevalier et al., 2013). Some powdered whole egg formulations may include anti-caking agents, such as tri-calcium phosphate or silicon dioxide, to improve flowability and prevent lump formation during storage. The final product

typically contains 3–4% moisture and can have a shelf life of 12–24 months under proper storage conditions (Lechevalier et al., 2013).

The resulting powdered whole egg is highly valued for its long shelf life, ease of transportation, and versatility in food applications. It can be reconstituted with water at a standard ratio of 1 part powder to 3 parts of water, making it a practical alternative to fresh eggs in industrial food production (Pirkwieser et al., 2022).

### 2.3.3 Liquid whole egg rheological properties

Rheology properties are used to understand the response of a certain food toward stress and forces offering useful information regarding its structure and interaction with other components (Day & Golding, 2016). The flow behavior of fluid can vary depending on many factors such as the fluid type; whether its laminar or turbulent, temperature, and fluid viscosity (McKenna & Lyng, 2003). Fluid foods are subjected to different temperatures during manufacturing, starting from the processing stage, storage, transportation or consumption, for that reason the viscosity of these foods is studied in different temperature settings (Day & Golding, 2016).

Several studies found that the rheological characteristics of eggs are leaning toward a Newtonian as well as time dependent non-Newtonian shear thinning behavior (Atılğan & Unlutürk, 2008; P. M. De Souza & Fernández, 2013; Jaekel & Ternes, 2009; Kumbár, Nedomová, et al., 2015; Kumbár et al., 2021). A study investigated the rheological behavior of egg white, both thick and thin portions, on different temperatures found that viscosity of egg white decreases with increase of temperature (Lang & Rha, 1982). Different rheological behavior was observed for the thin and thick portion of egg white at 5 °C, thin portion showed no decrease in viscosity with shearing time at constant shear rates, while thick portion showed a decrease within the first 6 mins of shearing then remained constant (Lang & Rha, 1982).

More studies investigating liquid egg rheological properties found that liquid egg white and liquid whole eggs rheological behavior are more heat sensitive than liquid egg yolk, liquid whole egg showed a fluctuation in apparent viscosity around pasteurization temperature leaning toward thixotropic behavior in comparison to liquid egg white which was more stable (Kumbár, Nedomová, et al., 2015). On the hand apparent viscosity of liquid egg white and liquid whole egg at 4°C was constant over the given time showing time-independent behavior (Kumbár, Nedomová, et al., 2015). In a study conducted by Atılğan and Unlutürk (2008), the rheological properties of raw liquid whole egg were examined under three temperature conditions: refrigerated (4 °C), room

temperature (25 °C), and pasteurization temperatures (60 °C for liquid whole egg and liquid egg yolk, and 55.6 °C for liquid egg white). The results indicated that all liquid egg products exhibited slight pseudoplastic behavior (Atılgan & Unluturk, 2008). To analyze the data, the Herschel–Bulkley model was applied to liquid whole egg and liquid egg white samples, while the power law model was used for liquid egg yolk (Kumbár, Strnková, et al., 2015; Lovato et al., 2022). At pasteurization temperatures, both liquid whole egg and liquid egg white demonstrated thixotropic and time-dependent behavior. Additionally, liquid egg yolk exhibited thixotropic behavior under refrigerated conditions (Kumbár, Strnková, et al., 2015). Another study investigated the viscosity of egg liquids from three different species: chicken, Japanese duck, and goose, at pasteurization temperatures, found that all three types of liquid eggs exhibited a pseudoplastic behavior (Kumbár et al., 2021). Among the species, the yolk exhibited the most significant differences, with goose egg yolk displaying the highest apparent viscosity values, while Japanese duck yolk showed the lowest. These differences are likely attributable to variations in dry matter and lipid content. The experimental data were analyzed using the Herschel–Bulkley and power-law models (Kumbár et al., 2021).

#### 2.4 Nutritional Enhancement on Egg Products

Nutritional enhancement of egg products has gained traction in recent years as consumers increasingly seek functional foods that provide health benefits beyond basic nutrition. Fortified and enriched egg products are now widely available, addressing specific dietary needs or boosting overall nutritional profiles (Siró et al., 2008). For example, omega-3 enriched eggs are produced by feeding hens diets high in omega-3 fatty acids from sources like flaxseed or fish oil, resulting in eggs with significantly higher levels of this essential fatty acid, which supports heart and brain health (Yalçın & Ünal, 2010). Similarly, vitamin D-enriched eggs are created by supplementing poultry diets with vitamin D-rich ingredients, helping consumers meet their daily requirements for this critical nutrient, particularly in regions with limited sunlight exposure (Barnkob et al., 2020). Eggs enriched with selenium or lutein are also available, targeting antioxidant benefits and improved eye health, respectively (Kralik et al., 2023).

Additionally, fortified egg products extend beyond whole eggs; for instance, liquid egg whites and powdered egg products are often enhanced with additional protein or fiber to be promoted to athletes, bodybuilders, and individuals seeking weight management solutions (Martinez et al., 2019). Processed egg products like scrambled egg mixes and ready-to-drink egg-based protein

shakes are also being enhanced with added vitamins, minerals, and amino acids to increase their functional appeal (Martinez et al., 2019). These innovations not only improve the nutritional value of egg products but also align with modern dietary trends, such as ketogenic diets, gluten-free lifestyles, and clean eating movements (Henchion et al., 2021). Nutritional enhancement of eggs and egg-derived products illustrates the industry's ability to combine natural foods with advanced nutritional science, ensuring that eggs remain a cornerstone of healthy diets while addressing specific health concerns and preferences of diverse consumer groups (Henchion et al., 2021).

#### 2.4.1 Protein enrichment and enhancement

Protein is an essential macronutrient of a healthy diet. Consumers believe that adding high amounts of protein to their dietary intake has multiple benefits such as weight management, satiety and weight loss (Rovai et al., 2024). The high-protein food trend has gained significant momentum worldwide, driven by growing awareness of the role of protein in supporting muscle growth, weight management, and overall health (Vaccaro et al., 2024). This trend is fueled by the increasing demand for fitness-oriented diets, such as keto, paleo, and high-protein meal plans, which emphasize protein as a key nutrient for energy and satiety (Dixon et al., 2023). Globally, the market for high-protein foods spans diverse categories, including protein bars, shakes, dairy products, plant-based alternatives, and fortified snacks (Vaccaro et al., 2024). The rise of plant-based diets has further expanded the scope, with innovative sources like pea protein, soy, and chickpea protein catering to both health-conscious consumers and those seeking sustainable food options (Jafarzadeh et al., 2024). Additionally, advances in food technology have enabled the production of protein-rich products with improved taste, texture, and nutritional profiles, appealing to a broader audience (Jafarzadeh et al., 2024). This growing emphasis on high-protein foods reflects a collective shift toward proactive health management and the pursuit of balanced nutrition (Vaccaro et al., 2024).

Protein makes up a small and relatively constant proportion of total energy intake in adults, typically ranging from 14% to 18% (Berryman et al., 2018). There is ongoing debate within the scientific community regarding the role of protein intake in weight management (Leidy et al., 2015). Some researchers propose that dietary protein plays a vital role in driving food intake, as it is essential for key physiological functions such as growth, tissue repair, enzyme activity, and hormone production (Carbone & Pasiakos, 2019). In the context of weight control, if an individual chooses high-carbohydrate and/or high-fat foods with little protein content, the individual will

need to consume a larger volume of food to obtain the necessary amount of protein. Studies have shown that diets with high protein content, exceeding 25% of total energy intake, can suppress overall energy consumption in both humans and animals. In humans, the consumption of high-protein foods has been observed to acutely reduce subsequent energy intake when compared to low-protein alternatives, supporting the potential role of protein in regulating appetite and energy balance (Delimaris, 2013; Leidy, 2014).

Consumer attitudes toward protein-enriched foods have evolved significantly in recent years, driven by increasing awareness of the health benefits associated with dietary protein. Many consumers view protein as a vital nutrient for aiding in weight management, which has led to growing demand for protein-enriched products. This trend is particularly strong among health-conscious individuals and fitness enthusiasts (Akinmeye et al., 2024).

The perception of protein-enriched foods varies across demographics and regions. Younger consumers and urban populations tend to embrace these products more readily, associating them with modern, healthy lifestyles. Plant-based protein alternatives have gained traction among environmentally conscious consumers and those seeking sustainable or vegan-friendly options. However, barriers such as cost, taste preferences, and limited awareness in certain populations can impact the adoption of these products (Hartmann & Siegrist, 2017).

Brands that effectively communicate the nutritional benefits, quality, and sustainability of their protein-enriched offerings are more likely to resonate with consumers. Innovations in flavor, texture, and product variety, such as protein-fortified snacks, beverages, and dairy alternatives, further appeal to a diverse audience (Hartmann & Siegrist, 2017). Overall, the growing focus on health, fitness, and sustainability continues to shape positive consumer attitudes toward protein-enriched foods globally.

#### 2.4.1.1 Whey proteins

Whey protein, a high-quality protein derived from milk during the cheese-making process, is widely recognized for its superior amino acid profile and bioavailability (Ramos et al., 2016). It is a complete protein, containing all nine essential amino acids, with a particularly high concentration of branched-chain amino acids (BCAAs) such as leucine, which play an important role in muscle protein synthesis and recovery (Madureira et al., 2007). Whey protein has high solubility across a wide pH range, making it a great choice in various food formulations (Ramos et al., 2016). It is also characterized by its rapid digestibility and high biological value, allowing efficient absorption

and utilization by the body (Foegeding et al., 2002). Functional properties such as emulsification, foaming, gelling, and water-binding capacity make whey protein an invaluable ingredient in food processing. It also has a clean, neutral flavor profile, which facilitates its use in diverse food products without altering the taste (Foegeding et al., 2002).

Whey protein is available in various forms, including concentrate, isolate, and hydrolysate, each differing in protein content and processing methods (Minj & Anand, 2020). Its rapid digestion and absorption make it a preferred choice for athletes and individuals seeking to enhance muscle growth, improve exercise performance, or aid in post-exercise recovery (L. Wang et al., 2024). Due to its versatility and nutritional value, whey protein is extensively used in dietary supplements, protein bars, functional foods, and beverages, catering to a growing global demand for high-protein, health-oriented products (Yiğit et al., 2023). In the dairy sector, whey protein enhances the texture and nutritional value of yogurts, ice creams, and cheeses. Its emulsifying and gelling properties make it ideal for bakery products, confectionery, and processed meats, improving structure and shelf life (Królczyk et al., 2016). Whey protein is also used in infant formula to mimic the protein composition of breast milk and in medical nutrition products for patients requiring high-protein diets. The clean-label and health-focused consumer trends have further popularized whey protein as a source of functional and nutritional enhancement across various food categories (Martin et al., 2016).

#### 2.4.1.2 Egg white proteins in industry

In the food industry, egg white protein is widely used as a functional and nutritional ingredient (Guha et al., 2019). Its foaming properties are integral to bakery products such as meringues, soufflés, and angel food cakes, providing volume and texture (Guha et al., 2019). Its emulsifying capabilities make it a key component in dressings, sauces, and mayonnaise (Tian et al., 2024). The gelling properties of egg white protein are harnessed in processed meats and desserts to improve texture and stability (Tian et al., 2024). It is also used in confectionery for products like marshmallows and nougat. Due to its high protein content and minimal allergens compared to other sources, egg white protein is incorporated into protein powders, bars, and ready-to-drink beverages for consumers and athletes (Lotfian et al., 2019). Its role in extending shelf life and enhancing product quality further solidifies its importance in food processing and innovation (Lechevalier et al., 2011). Egg white protein composition and functional properties are described in detail in chapter 2.3.1 egg white proteins

#### 2.4.2 Essential oils

Essential oil (EO) is a term used to describe natural, complex, volatile compounds or combinations of secondary metabolites from plant liquids which consist of terpenes and phenylpropenes. Essential oils are highly concentrated volatile compounds extracted from plants, have found extensive applications in science, food preservation, and flavoring due to their natural bioactive properties. Essential oils can be extracted from plant organs buds, bark, seeds, leaves, fruits, twigs, wood, roots, herbs and flowers (e.g. basil, thyme, oregano, cinnamon, clove, and rosemary, tea, sage, mint, ginger, marjoram, and caraway), fruits (e.g. grapes, pomegranate, and date), vegetables, (e.g. broccoli, potato, drumstick, pumpkin, curry, nettle and bulbs of garlic and onion) and naturally occurring polymers (chitosan) (Eslahi et al., 2017; Manzoor et al., 2023; Mohamed & Alotaibi, 2023; Sadgrove et al., 2022).

EOs are primarily stored in specialized structures within plants, such as secretory cells, cavities, canals, and glandular trichomes (Eslahi et al., 2017). EOs exhibit a broad range of biological activities, including antimicrobial, antifungal, and antiviral properties, making them effective in inhibiting the growth of yeast, molds, and various pathogens (Tanasă et al., 2024). They also function as antiparasitic agents, antiseptics, and sensory preservatives in various formulations. For example, oils like tea tree, oregano, and thyme have shown antimicrobial effects, while lavender and chamomile are widely used in aromatherapy for stress relief and relaxation (Tanasă et al., 2024).

In the food industry, essential oils serve as natural preservatives, offering an alternative to synthetic additives. Their antimicrobial properties help extend the shelf life of different foods by combating spoilage-causing microorganisms. Essential oils from clove, cinnamon, and rosemary are particularly effective in preventing oxidative damage and microbial contamination in meats, dairy, and baked goods (Konfo et al., 2023; Pateiro et al., 2021). Additionally, they are used to create edible coatings for fruits and vegetables, providing a protective barrier that reduces moisture loss and microbial growth. These natural solutions align with the growing consumer demand for clean-label and chemical-free food products (Gupta et al., 2024).

EOs also play a significant role in flavoring, enhancing the sensory attributes of a wide variety of foods and beverages. Citrus oils like orange, lemon, and lime are commonly used in candies, baked goods, and beverages for their refreshing taste, while spice-derived oils such as cinnamon, nutmeg, and clove contribute to warm, aromatic flavors in savory and sweet dishes alike (Ameh &

Obodozie-Ofoegbu, 2016; Smelcerovic et al., 2013). Furthermore, essential oils offer health benefits beyond flavor, with compounds like eugenol in clove oil and limonene in citrus oils exhibiting antioxidant and anti-inflammatory effects. Their dual functionality as flavoring agents and health-promoting additives has made them indispensable in both traditional culinary practices and modern food science (Alagawany et al., 2021).

#### 2.4.2.1 Basil oil

The essential oil of basil, obtained through steam distillation of its leaves and flowering tops, is a complex mixture of bioactive compounds, including pinene, methyl chavicol, d-camphor, cineol, and ocimene (Avetisyan et al., 2017). These components contribute to the oil's unique aroma and flavor profile, making it a popular ingredient in the food industry. Basil essential oil is extensively used as a flavoring agent in a variety of food products, such as spiced meats, sauces, confectionery, ice creams, and puddings. Beyond its culinary uses, basil essential oil is gaining recognition for its potent antioxidant activity, attributed to the presence of phenolic compounds and terpenoids. Antioxidants in basil essential oil, such as eugenol and linalool, scavenge free radicals and reduce oxidative stress, which is a major contributor to chronic diseases (Zagoto et al., 2021).

Research also highlights basil essential oil's potential anticancer properties. Studies indicate that the bioactive compounds in the oil can inhibit the proliferation of cancer cells and induce apoptosis (programmed cell death) in various cancer models. In addition to its health benefits, basil essential oil exhibits antimicrobial properties, effectively inhibiting the growth of pathogens such as *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. These antimicrobial activities make it a valuable natural preservative in food products, contributing to extended shelf life and improved food safety. The oil's insecticidal properties have also been explored, showing efficacy in repelling or controlling agricultural pests and vectors of diseases, such as mosquitoes (Swamy et al., 2016).

#### 2.4.2.2 Garlic oil

Garlic oil, derived from the bulbs of *Allium sativum*, is widely recognized for its rich history in traditional medicine and its indispensable role in global cuisines. With its distinctive aroma and robust flavor, garlic oil has become a valued ingredient in the culinary world. Beyond its culinary significance, garlic oil is celebrated for its remarkable bioactive properties, which have positioned it as a subject of interest in food science, medicine, and health (Okoro et al., 2023; Shang et al., 2019). Garlic oil has garnered attention for its powerful antioxidant properties. Allicin, a key

compound in garlic oil, exhibits strong free radical-scavenging activity, reducing oxidative stress and mitigating damage caused by reactive oxygen species (Tesfaye, 2021). These antioxidant effects are associated with a range of health benefits, including cardiovascular protection.

Garlic oil's antimicrobial activity is one of its most notable attributes. It has been shown to effectively inhibit the growth of a broad spectrum of pathogens, including bacteria such as *Escherichia coli* and *Staphylococcus aureus*, and some fungi. This antimicrobial efficacy makes garlic oil a valuable natural preservative for food products, enhancing shelf life and safety without the need for synthetic additives (Bhatwalkar et al., 2021).

#### 2.4.2.3 Rosemary oil

Rosemary oil is a rich source of phytochemicals, including cineole, camphor,  $\alpha$ -pinene, borneol, and rosmarinic acid, which contribute to its unique sensory profile and biological activity (Christopoulou et al., 2021). In the culinary, rosemary oil is valued as a flavoring agent for meats, soups, stews, and baked goods, adding an herbaceous and slightly woody note that enhances the depth of dishes. Its natural antioxidant properties also make it an ideal preservative, preventing oxidative rancidity in high-fat food products such as oils, dressings, and processed meats (Nieto et al., 2018). Beyond its culinary significance, rosemary oil is prized for its potent antioxidant properties, primarily attributed to rosmarinic acid, carnosic acid, and other polyphenolic compounds (Boutekedjiret et al., 2003). These antioxidants neutralize free radicals, reduce oxidative stress, and protect against cellular damage (Erkan et al., 2008).

Another compelling attribute of rosemary oil is its antimicrobial activity. It has been shown to effectively inhibit the growth of bacteria, including *Escherichia coli* and *Staphylococcus aureus*. This antimicrobial efficacy makes rosemary oil a valuable natural preservative for food and cosmetics, ensuring extended shelf life and enhanced safety (Kačániová et al., 2023; Stojiljkovic, 2018).

#### 2.4.3 Vegetable oils in the food preparing

Oils are important ingredients in the food industry due to their functional properties, ability to enhance flavor, influence on texture and shelf life in wide range of food products. They serve multiple roles, including cooking mediums, flavor carriers, preservatives, and emulsifiers (Marcus, 2013). Common vegetable oils like olive oil, palm oil, sunflower oil, and coconut oil each offer distinct characteristics that make them ideal for specific culinary applications (Abrante-Pascual et al., 2024). Furthermore, oils enhance the texture, flavor, and mouthfeel of food, making dishes

more satisfying and enjoyable (Marcus, 2013). In cooking, oils act as effective heat transfer agents, enabling even cooking and preventing food from burning or sticking (Kaur et al., 2014). They also aid in the formation of emulsions, such as in salad dressings or mayonnaise, where they stabilize mixtures of water and fat-based ingredients (Marcus, 2013).

Oils significantly influence the properties of food in multiple ways, impacting texture, flavor, moisture retention, nutritional content, and overall sensory qualities. In terms of texture, oils play an important role in altering the consistency of food, as they contribute to tenderness in baked goods by coating flour particles and reducing gluten formation, which results in a softer, moister product (Abrante-Pascual et al., 2024). In frying, oils help create a crispy crust by forming a barrier that prevents moisture loss while facilitating even heat distribution (Abrante-Pascual et al., 2024). Oils also enhance the flavor of food by acting as carriers for fat-soluble compounds such as spices and herbs, thereby improving the overall flavor profile. Furthermore, oils help retain moisture in foods like baked goods and meats, preventing dryness and ensuring juiciness (Shahidi & Hossain, 2022). Oils also aid in the emulsification process, where they help combine water and fat-based ingredients, forming smooth, stable mixtures in products like mayonnaise and salad dressings (Marcus, 2013). Additionally, the heat stability of oils affects their performance during cooking, oils such as sunflower and avocado oil are ideal for high-heat applications like frying due to their higher smoke points, which prevent the production of harmful compounds (Abrante-Pascual et al., 2024). Oils can also act as preservatives by providing a barrier against moisture and oxygen, extending the shelf life of products by reducing oxidation (Olvera-Aguirre et al., 2023). The use of oils thus not only enhances the texture, flavor, and appearance of food but also contributes to the nutritional value and longevity of food products (Olvera-Aguirre et al., 2023).

#### 2.4.3.1 Olive oil

Olive oil, a fundamental component of the Mediterranean diet, is widely recognized for its unique character and flavor, which is derived from the fruit of the olive tree and produced exclusively through mechanical or physical methods (Ray et al., 2022). Currently, Greece leads the world in olive oil consumption per capita (Di Yang, 2024). However, the global market for extra virgin olive oil (EVOO) continues to grow each year, driven by increasing recognition of its exceptional organoleptic qualities and a growing awareness of its health benefits (Di Yang, 2024).

EVOO was previously not recommended for frying due to its relatively low smoke point around 205°C compared to other oils like peanut oil 225°C, sunflower oil 255°C, soybean oil 242°C, and

palm oil 227°C, under the assumption that a lower smoke point leads to faster oxidation of fats (Lozano-Castellón et al., 2022). However, recent studies have shown that the smoke point is not a reliable indicator of oil performance or stability. EVOO is now considered one of the best oils for frying, as it is high in monounsaturated fatty acids and low in polyunsaturated fatty acids. Additionally, its antioxidant compounds provide a protective effect against degradation during cooking (Lozano-Castellón et al., 2022).

#### 2.4.3.2 Sunflower oil

Sunflower oil, derived from the seeds of the sunflower plant *Helianthus annuus*, is a versatile edible oil widely used in the food industry due to its light taste, high smoke point, and rich nutritional profile (Souza et al., 2004). It serves as an ideal medium for frying, baking, and as a key ingredient in margarine, salad dressings, and processed snacks. Its high content of vitamin E also offers potent antioxidant properties, protecting cells from oxidative damage and bolstering skin health (Pal, 2011). Furthermore, it is often used in health-conscious food formulations due to its low levels of saturated fats and trans fats, making it a preferred choice for those managing cardiovascular conditions or aiming to maintain a balanced diet (Pal, 2011). Advances in food technology have also allowed for the development of high-oleic variants of sunflower oil, which enhances its thermal stability and shelf life, further solidifying its role in the culinary and processed food industries (Dichtyar et al., 2017).

#### 2.4.3.3 Palm oil

Palm oil (*Elaeis guineensis*) is the world's largest edible oil in terms of production and trade. It is widely used in the food industry due to its versatility, cost-effectiveness, and stability at high temperatures. It is derived from the fruit of the oil palm tree and is a common ingredient in a variety of processed foods, including baked goods, margarine, snack foods, and fried foods (Rey et al., 2023). Palm oil has a high resistance to oxidation and a relatively long shelf life, making it ideal for use in products that require extended storage. Its semi-solid state at room temperature gives it a desirable texture in products like spreads and margarines (Alhaji et al., 2024).

Palm oil is composed of approximately 50% saturated fatty acids, 40% monounsaturated fatty acids, and 10% polyunsaturated fatty acids and it's a cholesterol free oil. This unique composition makes palm oil, and its derivatives increasingly utilized in a variety of food products, including cooking oils, margarines, shortenings, and confectionery items. The versatility of palm oil in

different food applications can be attributed to its distinct chemical structure, which allows it to function effectively in various culinary processes (Alhaji et al., 2024).

In addition to its fatty acid profile, palm oil contains significant amounts of tocopherols and tocotrienols, forms of vitamin E with potent antioxidant properties. These compounds help protect the body from oxidative stress and reduce inflammation, potentially lowering the risk of chronic diseases such as heart disease and certain types of cancer (Zainal et al., 2022).

#### 2.4.3.4 Coconut oil

Coconut oil is a versatile edible oil derived from the meat of mature coconuts harvested from the coconut palm *Cocos nucifera*. The average annual production of coconut oil ranges between 3 and 4 million tones, with the majority originating from the Philippines, Indonesia, and India (Siriphanich et al., 2011). It is primarily composed of medium-chain triglycerides, with lauric acid being the predominant fatty acid, accounting for nearly 50% of its total fat content (Duranova et al., 2025). Other significant components include myristic acid, caprylic acid, capric acid, and palmitic acid (Duranova et al., 2025).

Coconut oil serves multiple purposes, functioning as a cooking fat, hair oil, body oil, and industrial oil. Refined coconut oil, specifically processed for industrial applications, is used in the production of biscuits, chocolates, ice creams, margarine, and various confectionery products (Lima & Block, 2019). It plays an important role in the manufacturing of paints and pharmaceutical formulations. The oil's favorable characteristics, including a low melting point, high resistance to rancidity, a pleasant flavor, and excellent digestibility, make it a valuable and versatile ingredient within the food industry (Arias et al., 2023). In the food industry, coconut oil is widely used for its stability, long shelf life, and ability to withstand high temperatures, making it ideal for frying, baking, and sautéing. Its unique flavor profile also lends itself to traditional and contemporary dishes, including desserts, curries, and confectionery. Its semi-solid state at room temperature allows it to serve as a substitute for butter and margarine in vegan and dairy-free recipes (Boateng et al., 2016).

#### 2.5 Consumer acceptance and sensory attributes of liquid egg products

Consumer acceptance is a critical determinant of the success and market viability of any food product, particularly when developing reformulated or functionally enhanced versions of familiar staples like liquid whole eggs (Baker et al., 2022). Regardless of nutritional improvements or technological advancements, products that do not align with consumer expectations in terms of taste, texture, appearance, or overall sensory appeal are unlikely to gain widespread adoption.

Sensory perception is inherently subjective and influenced by cultural, psychological, and contextual factors, making it essential to evaluate consumer preferences through structured sensory analysis. Furthermore, as food innovation increasingly intersects with health, sustainability, and functionality, ensuring that new formulations meet consumer standards without compromising traditional sensory qualities becomes essential. Incorporating consumer-driven insights during product development not only enhances acceptance but also supports successful product positioning in competitive markets (Shan et al., 2017).

Sensory attributes such as flavor, aroma, texture, color, and overall mouthfeel, play a critical role in determining consumer acceptance of liquid whole egg products. While eggs are widely appreciated for their unique functional and nutritional profile, any modification or fortification of their composition can influence their sensory perception. Studies have shown that even minor changes in ingredient composition, such as the addition of proteins, lipids, or antioxidants, may alter attributes like creaminess, egg aroma, or surface appearance (Meilgaard et al., 1999). Consumer acceptance of liquid eggs is closely linked to their familiarity with traditional sensory cues associated with fresh eggs, making it essential to maintain or improve these qualities in enhanced formulations. Sensory evaluation methods, including hedonic scaling and descriptive profiling, are thus indispensable tools in the development of fortified egg products, ensuring that technical improvements align with consumer expectations and market success (Meilgaard et al., 1999).

Two primary types of sensory panels are employed in food research: trained panels and consumer panels, each serving distinct purposes. A trained sensory panel consists of a small group of 8–15 individuals who have undergone rigorous training to identify and quantify specific sensory attributes such as texture, flavor, aroma, and appearance using descriptive analysis methods. These panelists are calibrated to use standardized terminology and scales, enabling objective and reproducible comparisons between samples. On the other hand, a consumer sensory panel involves a larger and demographically diverse group, typically ranging from 50 to several hundred untrained participants, who evaluate products based on personal preference, liking, or purchase intent using hedonic scales or ranking tests. The type of questions posed also differs: trained panels answer analytical questions like “How intense is the sulfur note?” using structured line scales, while consumers answer affective questions such as “How much do you like the taste?” using 9-point hedonic scales (Losó et al., 2012).

To evaluate newly developed nutritionally enhanced liquid whole egg products, a two-tiered sensory testing approach is recommended. Initially, trained panelists should conduct descriptive sensory analysis to generate a detailed sensory profile and detect subtle changes in attributes such as eggy aroma, creaminess, viscosity, or off-notes introduced by added proteins or oils. Following this, a consumer acceptance test can be conducted using hedonic evaluation to assess overall liking and purchase intent in the target population. This combined strategy ensures both the technical quality and market potential of the product are adequately assessed, thus facilitating product optimization and successful commercialization (Losó et al., 2012).

While numerous studies have focused on the physicochemical and techno-functional characteristics of egg-based formulations, consumer perception remains an essential criterion for successful product development and market adoption (Baba et al., 2017). Modifications in formulation such as the addition of proteins, oils, or bioactive compounds, can significantly influence sensory qualities, potentially affecting the product's appeal despite improvements in nutritional or functional value. Therefore, it is vital to assess how such enhancements alter sensory characteristics and whether these changes align with consumer preferences. Integrating sensory evaluation into the research framework not only validates the technological interventions but also ensures that the final product meets the expectations of end users, supporting the development of acceptable and marketable egg-based functional foods (Nasrabadi et al., 2021).

### 3. MATERIALS AND METHODS

#### 3.1 Materials

Pasteurized homogenized liquid egg products were obtained from a liquid egg plant (Capriovus Ltd., Szigetcsép, Hungary). Powdered egg white protein was obtained from the same company as well. All essential oils were obtained from RASP GmbH (Austria). Ascorbic acid was obtained from Chem-lab NV (Belgium), both citric acid 99% and phosphoric acid 99% were obtained from Sigma-Aldrich (Germany). Whey protein isolate (WPI90) was obtained from Buda family kft (Hungary). Olive oil was obtained from Uncle Chris company (Athena, Greece). Sunflower oil was obtained from floriol (Hungary). As for Coconut oil and Palm oil both were obtained from Szekszard (Hungary). In the preparation of Patal De Nata, a dough was purchased from “Tante Fanny, Friss Linzertészta” (Austria), 2.8% fat milk was purchased from Mizo (Hungary), corn starch was purchased from Dr.Oetker (Germany), sugar was purchased from Koronás Cukor (Hungary), liquid vanilla was purchased from Dr.Oetker (Germany).

#### 3.2 Preparation of liquid eggs samples

Fresh liquid egg products were obtained from Capriovus Ltd., Szigetcsép, Hungary and transported to the laboratories at the Department of Refrigeration and Livestock Product Technology (Faculty of Food Science, Mate University- Hungary) within 24 hours of production. It was produced from fresh, medium size, class “A”, enriched cages hens following the registration of EU. Eggs are disinfected, then the shells are removed to create the liquid eggs. In case of liquid egg yolk and white separation take a place at this step. Homogenization and pasteurization are followed, in case of liquid egg yolk pasteurization occur at 65 °C for 10 minutes, on the other hand liquid egg white is pasteurized at 56 °C for 3 minutes. As for liquid whole egg it is pasteurized at 70 °C for 3 minutes. With these rates 600 kg of liquid egg yolk per hour, while 2000 kg of liquid whole egg and liquid egg white per hour were produced. The product then is filled in 1 liter PET (Polyethylene Terephthalate) bottles and refrigerated at  $0-4 \pm 0.5$  °C. Experiments were conducted at room temperatures between 22 and 25 °C at the same day of sample arrival.

In some of the experiments shell eggs was used to create liquid whole eggs or liquid egg white in the laboratories at the Department of Refrigeration and Livestock Product Technology (Faculty of Food Science, Mate University- Hungary). In this case medium size, cage free shell eggs following the EU regulation of egg production, were obtained from Capriovus Ltd., Szigetcsép, then cracked

and homogenized at 10000 rpm for 3 minutes using IKA T-18 Ultra Turrax Digital Homogenizer (Germany), then heat treated at  $60 \pm 0.2^{\circ}\text{C}$ , with holding time of 3.5 minutes in water bath, then cooled down immediately to  $4^{\circ}\text{C} \pm 0.1$  using an ice bath.

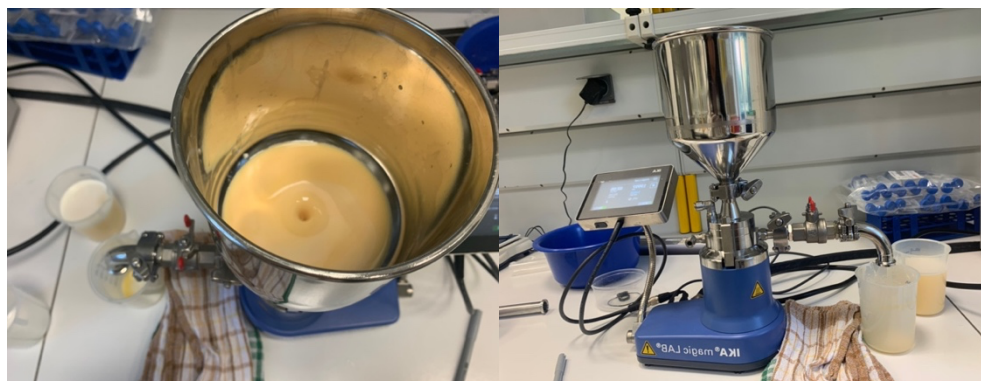


Figure 4: Liquid whole egg homogenization using IKA T-18 Ultra Turrax Digital Homogenizer (Germany).

### 3.3 Experimental design and liquid egg product treatments

Experiments were performed to enhance the nutritional value of liquid egg products by fortifying, enriching or supplementing them with different ingredient. Different proteins were chosen to increase protein content while different essential oils and vegetable oils were added to enhance bioactive benefits. Several studies have extensively analyzed the physicochemical, functional, and rheological properties of liquid eggs, focusing on their stability, viscosity, emulsification, and processing characteristics. However, limited research has been conducted on enhancing the nutritional value of liquid eggs through fortification or supplementation with additional proteins, oils, or bioactive compounds. While existing literature primarily examines the structural behavior and processing effects, there remains a gap in understanding how nutritional enrichment impacts the overall quality, sensory attributes, and functional performance of liquid egg formulations. All experiments were conducted in a fully randomized design to minimize bias. Measurements and sensory evaluations were performed in separate sessions to avoid carry-over effects. Each treatment was replicated a statistically enough times to ensure repeatability. Repeatability was verified through independent repetitions under identical conditions, and variability was monitored to confirm data reliability

### 3.3.1 Egg white proteins addition to liquid whole eggs

In the first experiment the aim was to investigate the effects of fortifying raw unpasteurized liquid whole eggs and liquid egg white with powdered egg white protein at different concentrations (0%, 3%, 5%, and 10% W/W) and subjecting them to heat treatment at various temperatures, 60°C, 65°C, and 70°C for liquid whole egg and 50°C, 55°C, and 60°C for liquid egg white, for 15 minutes in a water bath, followed by rapid cooling to 4°C using an ice bath. every 500 ml of the sample was stored in sterilized glass bottles prior to heat treatment then after cooling down samples were immediately stored at 4°C. The experiment evaluated how protein fortification and heat treatment influence the physical and chemical properties of liquid egg products. The untreated samples serve as control groups to compare the effects of these modifications. There is a limited understanding of how protein enrichment interacts with heat processing to alter the structural and functional properties of liquid egg products. While thermal treatments are commonly used for pasteurization and safety enhancement, their effects in combination with protein fortification have not been fully explored. The experiment provides insights into optimizing processing conditions for enhanced nutritional value, texture, and stability in liquid egg-based formulations.

### 3.3.2 Egg white proteins addition to liquid whole eggs with storage

In the second experiment, a storage condition of the previously made samples was addressed, samples which were made in the first experiment were stored for 21 days in 4°C condition. Measurements to check quality changes were made at the day of the production, day 7, day 14, day 21. The purpose of the experiment is to evaluate the effect of refrigerated storage 4°C over 21 days on the quality and stability of liquid egg products fortified with powdered egg white protein and subjected to different heat treatments in the fifth experiment.

### 3.3.3 Whey proteins addition to liquid whole eggs

The third experiment was designed to evaluate the effects of fortifying liquid whole eggs with whey protein at different concentrations (1%, 2%, and 3% W/W) on their physicochemical properties. The liquid whole egg samples were mixed with whey protein and homogenized using a Robot Coupe MiniMP160 mixer (France) before undergoing analytical measurements. The purpose of this experiment is to investigate how whey protein supplementation influences the functional characteristics of liquid whole eggs, such as viscosity, pH, and color. Whey protein was selected due to its high solubility, emulsification properties, and ability to enhance protein content while improving the structural and thermal stability of food products. Understanding these effects

could provide valuable insights into developing high-protein egg formulations with improved texture, nutritional value, and processing performance for use in various food applications.

#### 3.3.4 Different proteins addition to liquid whole eggs

The fourth experiment was conducted to evaluate the effects of fortifying liquid whole eggs with powdered egg white protein and whey protein at varying concentrations (3%, 5%, and 10% W/W) on their physicochemical properties. A total of 1400 g of raw liquid whole eggs were divided into seven beakers, each containing 200 g of sample. Three samples were fortified with powdered egg white protein at 3%, 5%, and 10% W/W, while another three samples were fortified with whey protein at the same concentrations. The seventh sample served as the control group. All samples were homogenized at 10,000 rpm for 3 minutes using an IKA T-18 Ultra Turrax Digital Homogenizer (Germany), followed by heat treatment at  $60 \pm 0.2^\circ\text{C}$  with a holding time of 3.5 minutes in a water bath, and then rapidly cooled to  $4^\circ\text{C}$  using an ice bath. a second batch of 1400 g of liquid whole eggs was processed in the same conditions, with protein addition occurring after heat treatment and subsequent homogenization at 10,000 rpm for 3 minutes.

Both powdered egg white protein and whey protein were selected due to their high digestibility and complete essential amino acid profile, with a protein digestibility-corrected amino acid score (PDCAAS) of 1, indicating their optimal nutritional value for human consumption. This experiment provides insights into the formulation of enriched liquid egg products for functional food applications, catering to health-conscious consumers and athletes seeking high-protein dietary options.

#### 3.3.5 Increasing the egg yolk content of liquid whole eggs

The fifth experiment was conducted to investigate the effect of varying egg yolk ratios on the physicochemical and sensory properties of liquid whole eggs and their application in a custard tart (Pastel de Nata). Five different samples were prepared, including pure egg yolk, liquid whole egg, and three samples with 20%, 50%, and 80% additional yolk content. The samples were homogenized to ensure uniformity before further analysis. Following the physicochemical analysis, the liquid egg samples were used to prepare custard tart fillings, following a standardized recipe that included sugar, milk, and other ingredients. The tarts were baked under controlled temperature conditions to ensure uniformity. The final product was then evaluated through sensory analysis. A trained sensory panel assessed the samples for appearance, color, texture, creaminess, and aftertaste using a structured sensory evaluation form in the sensory lab of the Hungarian

university of agriculture and life sciences. The results from both physicochemical and sensory analyses were statistically analyzed to determine how yolk ratio influenced the liquid egg properties and the final quality of the custard tart.

### 3.3.6 Adding essential oils to liquid whole eggs

In the sixth experiment, homogenized and pasteurized liquid whole egg samples were mixed with different percentages (1,2,3% W/W) of different essential oils (basil, garlic, rosemary) then homogenized using robot coupe MiniMP160 mixer and samples for each parameter were then placed in bakers to go through measurements, samples that didn't receive any treatment acted as reference sample. By comparing treated samples to untreated reference sample, the study aimed to assess the potential of these essential oils as natural additives to enhance nutritional value and functionality of liquid egg products. pH, color, viscosity and antioxidant activity were done to evaluate the effect.

### 3.3.7 Adding cooking oils to liquid whole eggs

The seventh experiment was designed to evaluate the effects of different types of oils (olive, sunflower, palm, and coconut oil) at varying concentrations (2.5%, 5%, and 7.5% V/V) on the physicochemical properties like pH, color, and viscosity, of homogenized and pasteurized liquid whole eggs. Sensory attributes of the cooked scrambled eggs are assessed by 12 trained panelists to determine the impact of oil addition on the taste, texture, and overall acceptability of the final product. While the functional and nutritional benefits of oils in various food systems are well-documented, there is limited research on their specific impact on the physicochemical properties and sensory qualities of liquid egg products and their cooked version. This experiment investigated how different oils, and their concentrations influence both the processing characteristics and consumer acceptability of scrambled eggs, providing insights for improving the formulation of enriched egg-based products.

## 3.4 Procedures and measurements

### 3.4.1 Measurement of pH

The pH value of liquid egg products samples was measured in different experiments, and the readings were recorded in triplicate, using a portable pH meter by immersing a pH electrode (Testo 206; Testo-AG, Germany) about 1 cm into the liquid samples.



Figure 5: pH measurement for liquid whole eggs using a portable pH (Testo 206; Testo-AG, Germany)

### 3.4.2 Color measurement

The color values of liquid whole eggs were measured using CIELAB (CIE, 1986) scoring system. The following parameters were obtained:  $L^*$  (lightness),  $a^*$  redness ( $+a$ , red;  $-a$ , green), and  $b^*$  yellowness ( $+b$ , yellow;  $-b$ , blue) by using Konica Minolta CR-400 colourimeter (Konica Minolta Sensing Inc., Japan) making sure calibration was carried out before taking a reading. Results from  $L^*$ ,  $a^*$ , and  $b^*$  were recorded as the mean of five random readings.

The CIELAB color space, also known as the CIE  $L^*a^*b^*$  system, provides a quantitative framework for characterizing colors based on three orthogonal axes. The  $L^*$  parameter represents lightness and is measured along a vertical axis ranging from 0 (black) to 100 (white). The  $a^*$  coordinate defines the red-green chromatic component, where positive values ( $+a^*$ ) indicate redness and negative values ( $-a^*$ ) indicate greenness. Similarly, the  $b^*$  coordinate represents the yellow-blue chromatic component, with positive values ( $+b^*$ ) corresponding to yellow and negative values ( $-b^*$ ) to blue. The intersection of the  $a^*$  and  $b^*$  axes provide the neutral or achromatic point. In this color space, chroma ( $C^*$ ), or the saturation of a color, is determined by the radial distance from the neutral axis, while hue ( $h^\circ$ ) is represented by the angular position on the chromaticity plane. The CIELAB system is widely used in various scientific disciplines.

The CIELAB system was developed by the Commission Internationale de l'Éclairage (CIE) and remains a standardized model for color representation in scientific and industrial applications.

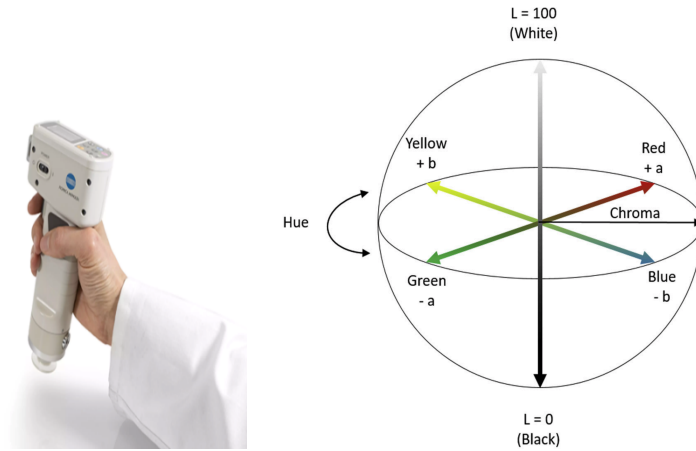


Figure 6: Konica Minolta CR-400 colorimeter and the CIELAB system is schematic representation by Ly et al., 2020

### 3.4.3 Determination of the rheological properties of liquid egg products samples

At the day of production samples were used to examine the rheological behavior of liquid whole egg, it was done using MCR 92 rheometer (Anton Paar, Les Ulis, France) in rotational mode equipped with a concentric cylinder with a concentric cylinder (cup diameter 28.920 mm, bob diameter 26.651 mm, bob length 40.003 mm, active length 120.2 mm, positioning length 72.5 mm). To control the equipment, Anton Paar RheoCompass software was used. A constant temperature of 15 °C was kept throughout the rheological measurements, shear stress was measured by logarithmically increasing and decreasing shear rate between 1 and 1000 1/s for 32 measurement points and in triplicates for each sample.

Following the literature this study chose Herschel Bulkley model to describe the rheological behavior of liquid whole egg (Atılgan & Unluturk, 2008; Kumbár, Strnková, et al., 2015; Uysal et al., 2019). Herschel Bulkley model is often chosen for liquid egg products because it exhibits a yield stress and shear thinning behavior and this model takes into consideration these factors Equation (1) was used to analyze the flow curves (shear rate-shear stress diagrams).

$$\tau = \tau_0 + K\dot{\gamma}^n \quad (1)$$

Where:

$\tau$  = shear stress (Pa)

$\tau_0$  = the yield stress (Pa)

$\dot{\gamma}$  = the shear rate (1/s)

$K$  = the consistency coefficient ( $\text{Pas}^n$ )

$n$  = is the flow behavior index. (-)



Figure 7: Measurements of rheological properties of liquid egg products samples with a rotational rheometer using MCR 92 rheometer (Anton Paar, Les Ulis, France).

#### 3.4.4 Determination of total antioxidant capacity

The total antioxidant capacity was measured using Ferric reducing antioxidant power (FRAP) method described by Benzie and Strain, 1996 (Benzie & Strain, 1996). The FRAP reagent was freshly prepared as a mixture of acetate buffer (300 mM, pH = 3.6), TPTZ (10 mM), and ferric chloride (20 mM) at a 10:1:1 (v/v/v) ratio, respectively. For sample preparation, 2 mL of homogenized sample was centrifuged at 10000 rpm for 20 mins, then the clear supernatant was used for measurements. Then, 1.5 mL of the FRAP reagent, 10  $\mu\text{L}$  of the sample and 40  $\mu\text{L}$  of distilled water, were mixed and put to rest for 5 minutes. The absorbance was determined at 593 nm using Hitachi U-2900 spectrophotometer and against a blank sample containing all the reagents. A calibration was carried out using ascorbic acid solutions between 0.01 and 0.1- mM concentrations. The results were expressed as kg ascorbic acid equivalent/ $\text{m}^3$  liquid whole egg. Measurements were made in triplicate.

#### 3.4.5 Sensory evaluation of scrambled eggs preparation

Using a non-stick pan a scrambled eggs were fried on medium heat without any extra oil addition for 3 minutes then samples were added to plates for sensory evaluation. Sensory evaluation of

scrambled liquid egg and different oils mixture samples were conducted for sensory attributes: the intensity of color, aroma, appearance, and acceptability to buy. The panel consisted of 10 researchers, teachers, and technicians of MATE University (50 % male/female, aged between 25 and 57 years) they were familiar with scrambled egg consumption. The assessment was conducted using a 9-point hedonic scale (Meilgaard et al.,1999): 1, dislike extremely; 2, dislike very much; 3, dislike moderately; 4, dislike slightly; 5, neither like nor dislike; 6, like slightly; 7, like moderately; 8, like very much; 9, like extremely. All samples were coded with 3-digit random codes and offered to the individuals in the random order. The sequence in which treatments were offered to each individual was randomized.



Figure 8: Preparation of scrambled eggs of liquid whole egg mixed with different percentages of different vegetable oils for sensory.

#### 3.4.6 Preparing Pastel De Nata

To prepare the Portuguese pastry "Pastel de Nata," the original recipe was followed for the custard filling, while the dough was purchased from "Tante Fanny, Friss Linzertészta" to optimize the crust. Each pastry was made using 25 g of dough and 30 mL of liquid custard. For a batch of 12 pieces, the custard was prepared using 120 g of liquid egg samples, combined with 280 g of milk, 100 g of sugar, 56 g of starch, and 1 g of vanilla. All ingredients were thoroughly mixed using a Robot Coupe MiniMP160 mixer (France) to achieve a smooth consistency. The dough was placed in a cupcake tray, and the custard was strained through a fine sieve in each cup cake. The pastries were then baked at 180°C for 20 minutes. After baking, the samples were left to cool in the tray for 20 minutes before being stored in a refrigerator at 4°C for sensory evaluation the following day.



Figure 9: Pastel De Nata prepared samples with different yolk percentages for sensory test.

### 3.4.7 Sensory evaluation of Pastel De Nata

On the day of the experiment, samples were transferred to the sensory lab which is designed following ISO 8589:2007 at the department of postharvest and sensory analysis, for thirteen panelists to test. Panelists were selected based on their prior experience in descriptive sensory analysis. Prior to the test day, an expert panel evaluated the products to define reference values for each attribute, after which the panel leader designed the score sheet in consultation with the panel. Sample codes and a presentation order are generated for each assessor, to provide a balanced test environment. Assessors work separately in the sensory booths. The personalized score sheets are copied to the booth PC's. Panelists evaluate the samples according to the defined terminology following ISO 6658:2017 standards. Color of surface, inner color, egg odor intensity, texture of the custard, creaminess, sweet taste intensity, and aftertaste were the attributes to be tested. Data were recorded digitally and analyzed quantitatively.





Figure 10: (a) sample preparation, (b) tray preparation, (c) placing the sample tray in the sensory booth, (d) panelist testing the samples and (e) after testing tray

#### 3.4.8 Statistical analysis

To analyze data statistically the statistical package for social science (SPSS, version 27.0, 2020, Chicago, IL) was used. A one way and two-ways analysis of variance (ANOVA) test was performed to test the difference between the treatments. Followed by mean separation using Tukey HSD Analysis. Means with different letters differ significantly at  $p < 0.05$ .

## 4. RESULTS AND DISCUSSION

### 4.1 The effect of different protein additions on liquid egg properties.

#### 4.1.1 The effect of adding egg white protein to liquid whole egg and liquid egg white properties during storage

##### 4.1.1.1 Changes in pH for liquid whole egg

The properties of liquid eggs vary due to differences in composition, processing methods, storage conditions, and external factors. Processing methods such as pasteurization and homogenization can alter protein structure, affecting texture and stability, while additives like citric acid help maintain consistency (Rossi et al., 2010). The color variation in liquid egg products across different production packs is influenced by yolk pigment content, processing conditions, oxidation, pH changes, and rheological properties. The primary factor is the natural variation in carotenoid content (lutein, zeaxanthin, beta-carotene) in egg yolks, which depends on the hens' diet; yolks from corn-fed hens tend to be more yellow- orange, while wheat-fed hens produce paler yolks (Dvořák et al., 2012). Additionally, changes in the egg white-to-yolk ratio impact color, as a higher yolk content results in a deeper yellow hue, whereas more egg white leads to a paler color (Dvořák et al., 2012). Pasteurization and heat processing cause protein denaturation and Maillard reactions, affecting both color and rheology (Wang et al., 2024). Longer or high-temperature pasteurization can darken the product due to increased protein cross-linking and interactions between amino acids and sugars, while mild heat treatment preserves a lighter color (L. Liu et al., 2025). With storage, pH changes significantly ( $p < 0.05$ ) influencing both color and rheology; fresh liquid egg has a lower pH of 7.6-8.0, maintaining a bright yellow color, but as pH increases during storage due to CO<sub>2</sub> loss, proteins rearrange, affecting light scattering, viscosity, leading to duller appearance. These pH-dependent structural changes also alter the rheological properties, impacting thickness, viscosity, and flow behavior (Panaite et al., 2019). Although all the samples that are used in the experiments are from the same source and are treated in the same way, a variation between produced liquid egg products can happen for the above- mentioned reasons, this will be seen in the variation of results of all control groups in different experiments. For that each experiment had its own control group which was produced in the same way to have more standardized experiments and better reference for comparison.

In this experiment unpasteurized liquid whole egg was fortified with egg white protein and homogenized then pasteurized at different temperatures of 60°C, 65°C, and 70°C. then it was cooled to reach 4 °C after that samples were set at room temperature to measure pH. Each group had its own control samples which were used to compare results and results were also compared between groups. It was found that pH measurements of the egg mixture following pasteurization at 60°C, 65°C, and 70°C indicate that as the percentage of egg white powder increases in liquid whole egg, the pH decreases across all pasteurization temperatures of 60°C, 65°C, and 70°C. The most significant pH drop occurs at 60°C, where the pH declines from 7.58±0.50 for the control group to 6.85±0.31 for 10% added egg white, whereas at 65°C and 70°C, the pH drop is less pronounced, with final values of 7.21 and 6.99±0.91, respectively. This trend can be explained by the impact of egg white proteins, enzymatic activity, and heat-induced protein interactions during pasteurization. Egg white naturally has a high pH due to its protein composition, including ovalbumin, conalbumin, lysozyme, and ovotransferrin. When mixed with whole egg and subjected to heating, these proteins interact with yolk phospholipids, leading to a gradual acidification of the mixture (Razi et al., 2023). As more egg white powder is added, a greater proportion of these reactive proteins contributes to the observed pH decrease. However, pasteurization temperature also plays a crucial role in pH stabilization. At 60°C, enzyme activity is still relatively high, particularly glucose oxidase, which oxidizes glucose into gluconic acid and hydrogen peroxide, both of which lower the pH significantly. In contrast and as seen in figure 11, at higher temperatures at 65°C and 70°C, heat-induced denaturation reduces enzymatic activity, leading to a more stable pH over time (Sisak et al., 2006).

Additionally, protein denaturation and interactions with egg yolk phospholipids, such as lecithin, influence the buffering capacity of the egg mixture. At higher temperatures, protein aggregation limits the availability of free amino groups, which can help stabilize pH (Palacios & Wang, 2005). This explains why at 70°C, the pH remains relatively higher despite the increasing concentration of egg white powder.

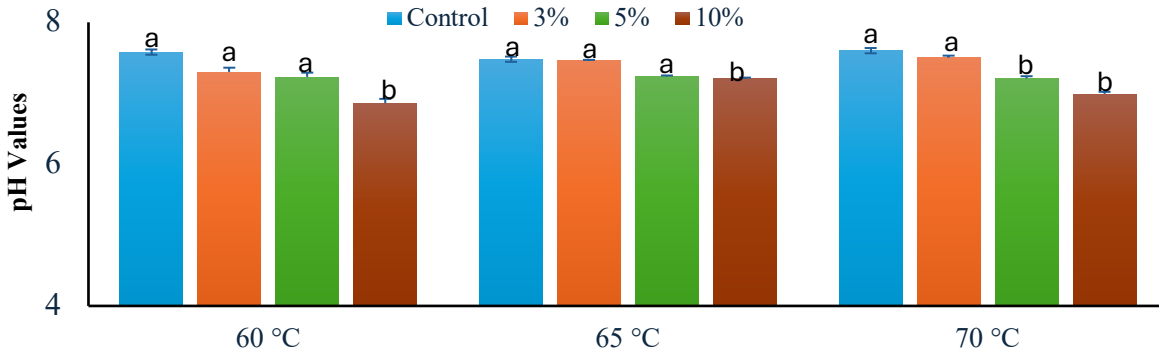


Figure 11: The effect of adding different percentage of powdered egg protein to liquid whole egg and pasteurizing at different heat treatment on pH values in comparison to the control group, different letters differ significantly, Tukey HSD  $p < 0.05$ .

As for pH values, for samples which were pasteurized at 60 and stored for 21 days, were dramatically changing. By day 7, there is a significant pH drop across all samples, particularly in the control sample which dropped to  $7.06 \pm 0.10$  and the 3% added protein sample to  $6.05 \pm 0.20$ . This decline is likely due to residual enzymatic activity, especially glucose oxidase, which catalyzes the oxidation of glucose to gluconic acid, a reaction that contributes to the acidification of the egg matrix (Sisak et al., 2006). The observed stability in pH with storage among different added egg white protein concentrations suggests that the protein buffering capacity of added egg white may have slowed acidification in some cases (Cunningham and Cottrell, 1963).

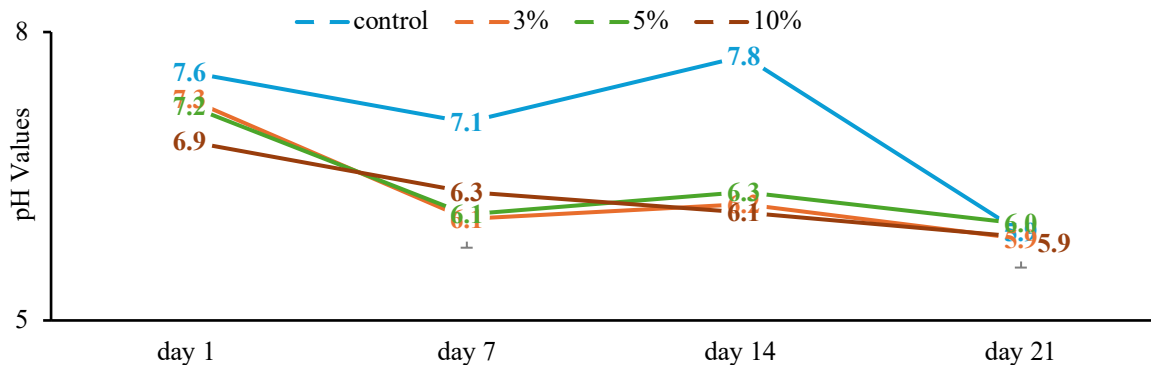


Figure 12: The trend of the effect of adding different percentages of powdered egg protein to liquid whole egg and pasteurizing at 60°C, after 21 days of storage on pH values in comparison to the control group.

On the other hand, by day 14, a slight pH increase is observed in most samples, with the control rising to  $7.75 \pm 0.50$ , while other samples also show a mild increase ranging between  $6.21 \pm 0.10$  and

6.33±0.30. This temporary pH increase could be due to protein unfolding and interactions leading to the release of basic amino groups, which may counteract acidity. Another possible explanation is the consumption of acidic metabolites by naturally occurring microorganisms, which could alter the chemical balance in the system (Fan et al., 2024). By day 21, a significant pH drop occurs in all samples, with the control reaching 5.91±0.1 and the 10% added egg white protein sample at 5.87±0.1. This final phase of acidification is likely due to the fact that prolonged storage at refrigeration temperatures can promote the slow breakdown of proteins and lipids, generating free fatty acids and amino acid-derived acidic compounds, further lowering the pH (Luo et al., 2020). Figure 12 shows the trend of pH change with storage period of 21 days. As for samples that were pasteurized at 65 °C, a slight pH drop illustrated in figure 15 below was observed across all samples by day 7, particularly in the 3% added protein reaching 6.88±0.15 and 10% added protein reaching 6.63, while the control sample pH value was 7.43±0.10 and 5% added protein sample reached 7.03±0.11 remain relatively stable.

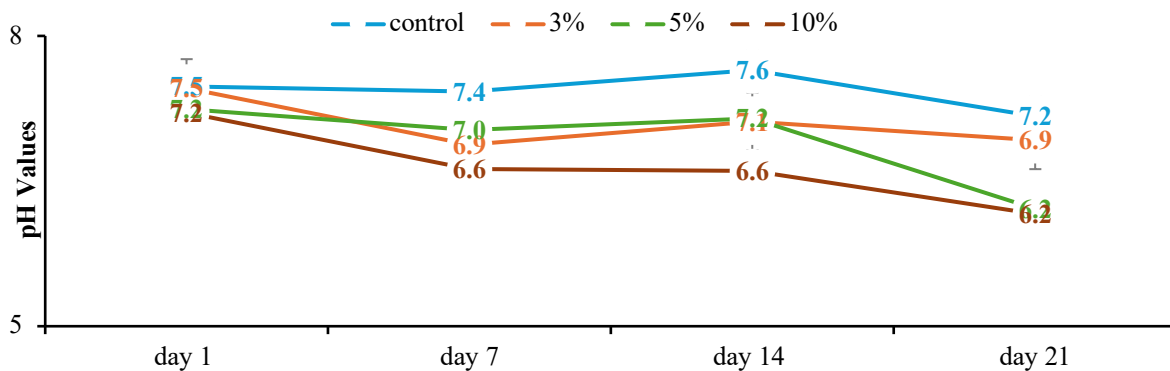


Figure 13: The trend of the effect of adding different percentages of powdered egg protein to liquid whole egg and pasteurizing at 65°C, after 21 days of storage on pH values in comparison to the control group.

This suggests that while some enzymatic activity may still be present, the higher pasteurization temperature of 65°C significantly slowed down the acidification process compared to 60°C (Sisak et al., 2006). The varying pH declines among different protein concentrations could be attributed to protein-protein interactions, where the added egg white affects the overall buffering capacity of the system (Farjami et al., 2021). By day 14, a slight increase in pH is observed, particularly in the control sample reaching 7.65±0.34 and samples with added egg white reaching 7.12±0.11 to

7.15±0.21 for 3% and 5% added protein, respectively, while the 10% protein sample remains stable at 6.61±0.21. This temporary pH increase is due to protein unfolding and rearrangement, leading to the release of basic amino groups from proteins, which slightly counteract acidity (Farjami et al., 2021). By day 21, a gradual pH decrease resumes, with the control sample dropping to 7.17±0.30, while samples with added protein decrease further reaching to 6.92±0.21, 6.21±0.11, and 6.16±0.13 for 3%, 5%, and 10% protein, respectively. This final decline is due to long-term biochemical reactions, including protein hydrolysis, Maillard reactions, and slow microbial metabolic activity despite pasteurization. While pasteurization at 65°C reduced enzymatic action, prolonged storage may have allowed heat-resistant enzymes or microbial contaminants to contribute to acidification over time. Additionally, lipid oxidation in the egg yolk may have generated free fatty acids, further contributing to the gradual pH decline (Rao et al., 2020). By day 7, the pH values show irregular fluctuations. The control sample was 7.41±0.14 and 5% added protein sample was 7.2±0.17 which means they both remain relatively stable, while the 3% added protein sample experiences a significant drop reaching 6.91±0.12, and the 10% protein sample raised to 6.61±0.13. This variability is due to protein interactions and microbial factors. At higher protein concentrations of 10%, increased protein denaturation and unfolding may have released buffering amino groups, leading to the pH increase (Farjami et al., 2021). Meanwhile, the 3% added protein sample's drop could be attributed to localized microbial activity or enzymatic changes, where breakdown products contribute to acidification (Wang et al., 2024). Compared to pasteurization at 60°C and 65°C, the higher temperature of 70°C had a more pronounced stabilizing effect, particularly on enzymatic activity, leading to more controlled pH fluctuations over time (Fan et al., 2024). However, differences in protein interactions, storage-related biochemical changes, and microbial stability influenced the pH trends among the different egg white powder concentrations. Below, figure 14 shows the pH trend during the 21 days of storage (Wibowo & Sudjatinah, 2023). By day 14, the control sample was 7.55±0.15 and 10% added protein was 7.51±0.14 which means both samples remained relatively stable, while the 3% and 5% added protein samples show mild recovery scoring a 6.96±0.12 and 7.20±0.21, respectively. This temporary pH increase in some samples could result from continued protein rearrangement, where denatured proteins expose functional groups that neutralize acidity, or from microbial shifts that consume acidic metabolites (Y.-F. Liu et al., 2017). This trend, where pH stabilizes or rises mid-storage, has also been observed in the pasteurization at 65°C

experiment, suggesting that high-temperature pasteurization influences long-term pH behavior differently compared to lower temperatures (Luo et al., 2020).

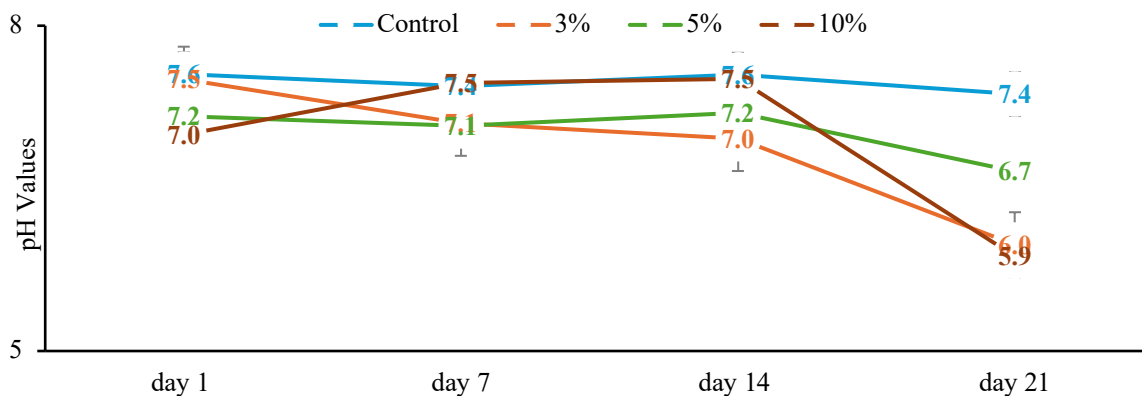


Figure 14: The trend of the effect of adding different percentage of powdered egg protein to liquid whole egg and pasteurizing at 70°C, after 21 days of storage on pH values in comparison to the control group.

By day 21, the pH values drop in all samples, with the most significant decrease occurring in the 3% added protein to reach 5.98 and 10% added protein to 5.87±0.13, while the control sample scored a pH of 7.37±0.24 and 5% added protein was 6.66±0.19 decline more moderately. This final acidification phase is due to storage-induced biochemical changes, including protein degradation, microbial metabolic byproducts, and lipid oxidation. The higher egg white concentrations of 10% added egg protein led to a stronger pH drop, suggesting that protein breakdown and interaction with egg yolk components contribute to long-term acidification. Despite pasteurization at 70°C reducing enzymatic activity, heat-resistant bacterial spores or slow chemical reactions may still contribute to this gradual acidification over extended (Allai et al., 2023).

#### 4.1.1.2 Changes in color parameters for liquid whole egg

Starting with L\*value of liquid whole egg, results show that pasteurization temperature and protein concentration influence the lightness of the egg mixture, likely due to protein denaturation, Maillard reactions, and pigment dispersion (Punidadas & McKELLAR, 1999). At 60°C, the lightest sample was the 3% protein addition with L\* value of 72.18±0.24, followed by the 10% added protein sample with value of 68.42±1.21, while the control and 5% added protein sample

were relatively darker. The higher lightness of the 3% and 10% protein samples suggests that adding egg white powder increases the reflection of light due to higher protein content, which dilutes the yellow-orange pigments from the egg yolk (Khachatryan et al., 2024). However, at 5% protein, the L\* value was the lowest, indicating that an intermediate concentration may have caused protein interactions that altered color perception.

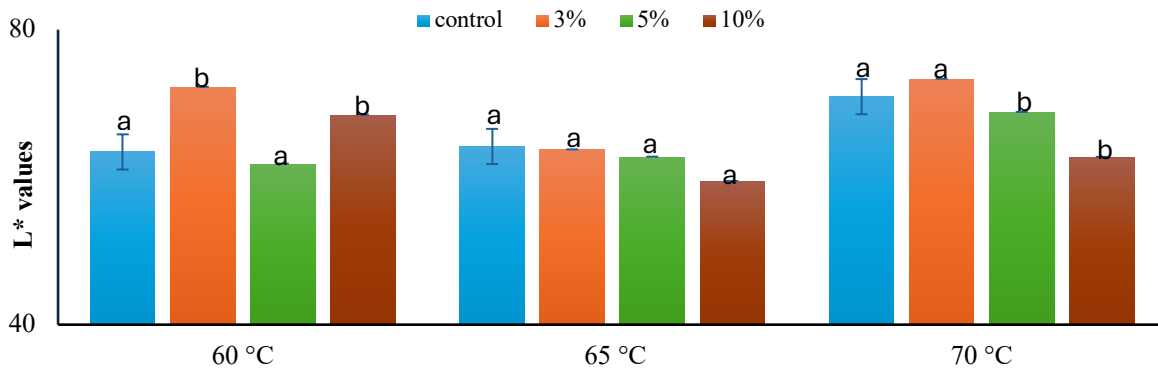


Figure 15: The effect of adding different percentages of powdered egg protein to liquid whole egg and pasteurizing at 60, 65 and 70°C and on L\* values in comparison to the control group.

At 65°C, a different trend is observed, where L\* values decrease with increasing protein concentration in comparison to the control sample which was  $64.16 \pm 3.12$  but 3%, 5%, and 10% were  $63.75 \pm 0.27$ ,  $62.76 \pm 2.14$ ,  $59.47 \pm 1.34$  respectively. This decrease in lightness with the increasing protein content suggests that pasteurization at 65°C induced structural changes in proteins that affected color reflectance. At this temperature, protein denaturation is more pronounced, leading to increased aggregation and opacity, which can scatter less light and appear darker (Ahmed et al., 2007). At 70°C, the highest lightness values were observed for 3% added protein were L\* value was  $73.31 \pm 1.24$ , while 5% added protein and 10% added protein L\* values were  $68.82 \pm 0.94$  and  $62.72 \pm 1.34$  respectively, which means they were darker. This suggests that high-temperature pasteurization causes protein denaturation and coagulation (Fan et al., 2024). The L\* value of 10% added protein sample at 70°C had a similar trend to the 10% added protein sample at 65°C, reinforcing the idea that excessive protein addition at higher temperatures leads to structural changes that reduce reflectivity and make the mixture appear darker.

Regarding a\* value for the day of production, at 60°C, the a\* values vary widely among different samples, showing both highly positive (red) and negative (greenish) values. The control sample

and 5% added protein sample show strong reddish tones with a\* value of  $8.92 \pm 1.34$  and  $9.30 \pm 0.9$  respectively, while the 3% added protein sample had a much lower red intensity with a\* value of  $1.61 \pm 1.14$  on the other hand, the 10% added protein sample was going toward greenish with a value of  $1.02 \pm 0.34$ . This variation is likely due to partial protein denaturation and pigment interactions at this lower pasteurization temperature. Since egg yolk contains xanthophylls and carotenes, which contribute to its reddish-yellow hue, the addition of egg white powder may dilute these pigments unevenly, affecting color perception (De Souza et al., 2019). The 10% protein sample's negative a value suggests excessive dilution of yolk pigments, making it appear less red or even slightly greenish due to background light scattering (Punidades & McKELLAR, 1999).

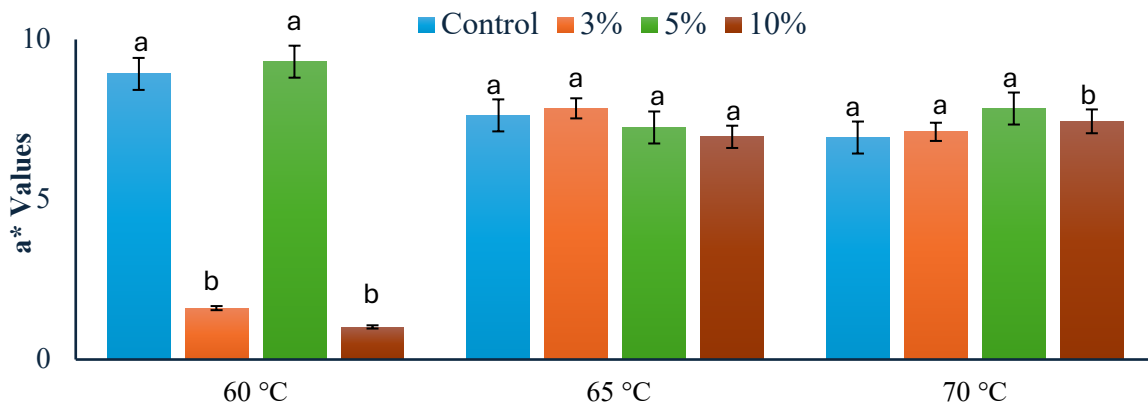


Figure 16: The effect of adding different percentages of powdered egg protein to liquid whole egg and pasteurizing at 60, 65 and 70°C and on a\* values in comparison to the control group.

At 65°C, the a\* values become more consistent and uniformly red across all protein concentrations starting from control sample and across all percentages reaching  $7.63 \pm 0.94$ ,  $7.848 \pm 0.89$ ,  $7.25 \pm 1.14$ , and  $6.958 \pm 0.44$  respectively. Compared to 60°C, the redness is lower in the control sample but more balanced across different protein concentrations. This could be due to increased protein denaturation and homogenization of yolk pigments, preventing the extreme shifts observed at 60°C (Bermudez-Aguirre & Niemira, 2023). The relatively stable a\* values suggest that moderate pasteurization helps evenly distribute yolk pigments, reducing inconsistencies caused by protein interactions (Ho et al., 2022).

At 70°C, the a\* values show a progressive increase with added protein concentration where control sample was  $6.94 \pm 0.74$ , but it increased for all added protein samples to  $7.14 \pm 0.78$ ,  $7.82 \pm 0.94$ , and  $7.44 \pm 0.88$  respectively. The general increase in redness with higher protein

concentration suggests that at higher temperatures, protein denaturation enhances the dispersion of carotenoid pigments, leading to a more uniform red appearance (Llave et al., 2018). Unlike at 60°C, where 10% added protein resulted in a greenish hue, at 70°C,  $a^*$  remained positive indicating better color retention and stabilization at higher temperatures.

As for the changes in  $b^*$  values, at 60°C the control sample and the 3% added protein exhibit the highest yellow intensity with  $b^*$  value of  $39.44 \pm 0.38$  and  $40.38 \pm 1.88$  respectively, meanwhile the 5% added protein sample had a  $b^*$  value of 38.56 which is slightly lower. As for the 10% added protein samples  $b^*$  dropped significantly ( $p < 0.05$ ) reaching  $30.62 \pm 1.67$ . The general decrease in  $b^*$  value with increasing protein concentration suggests that egg white powder addition dilutes the natural yellow pigments from the egg yolk (Vargas-del-Río et al., 2022). The sharp decline in the 10% sample indicates that excessive egg white protein addition significantly reduces yellow intensity, likely due to increased light scattering from denatured proteins and changes in pigment dispersion.

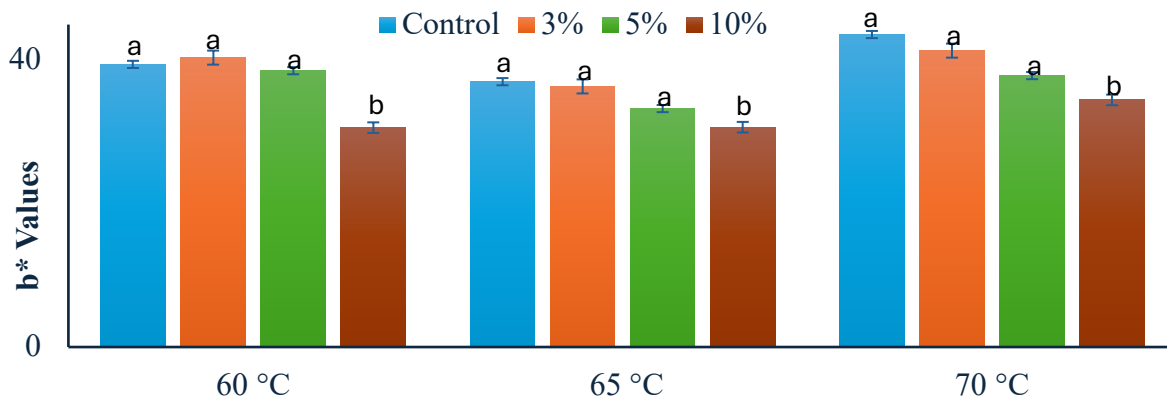


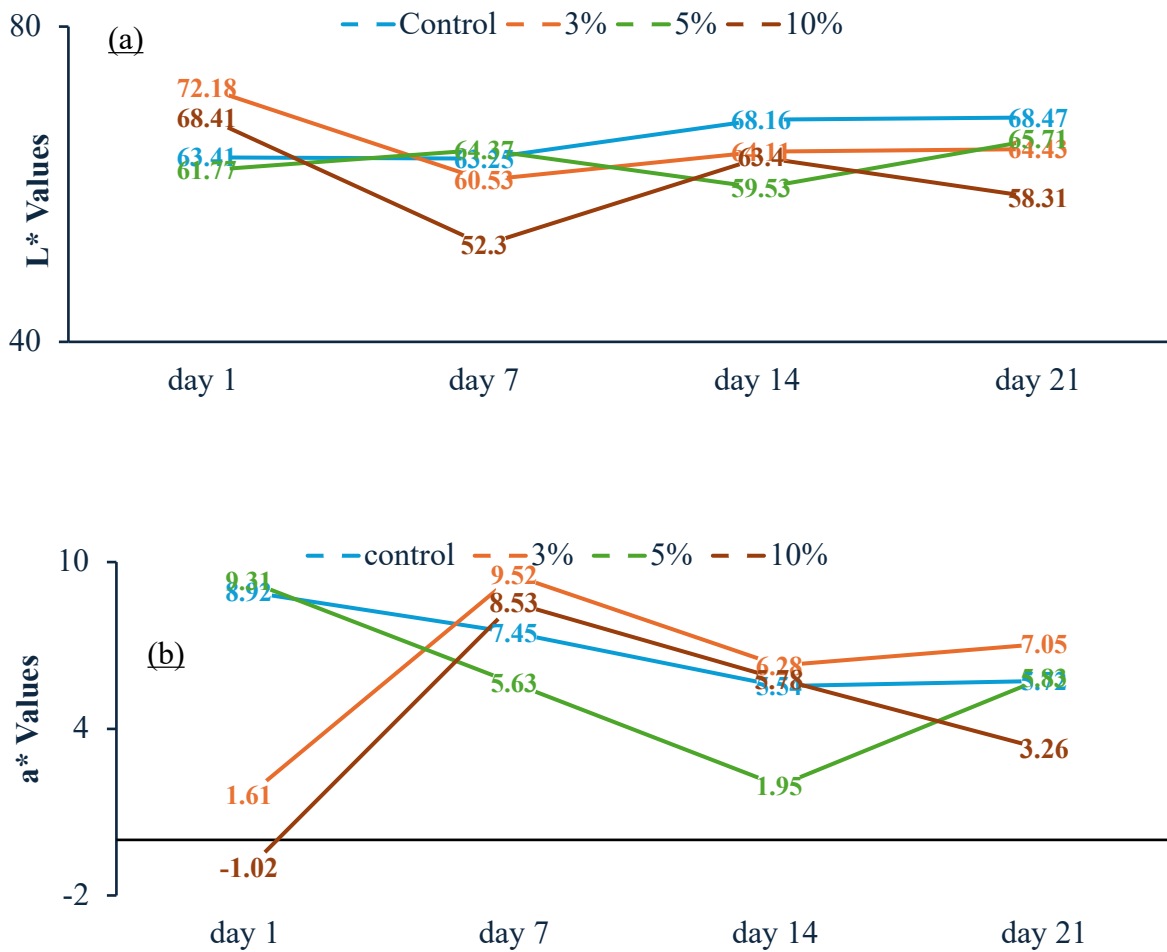
Figure 17: The effect of adding different percentages of powdered egg protein to liquid whole egg and pasteurizing at 60, 65 and 70°C and on  $b^*$  values in comparison to the control group.

At 65°C, the  $b^*$  values follow a similar trend of 60°C. The control sample and 3% added protein sample exhibit the strongest yellow tones with  $b^*$  value of  $37.03 \pm 1.34$  and  $36.37 \pm 1.28$  respectively, whereas the 5% added protein and 10% added protein samples show a gradual reduction in yellowness with  $b^*$  value of  $33.28 \pm 1.48$  and  $30.67 \pm 1.23$  respectively. Compared to 60°C, the moderate decline at in  $b^*$  values with added protein concentrations further confirms that excessive egg white addition reduces yolk pigment intensity although this applies for all the temperatures applied the effect of each temperature on the  $b^*$  is different due to protein rearrangement and denaturation.

At 70°C, a notable shift in  $b^*$  values occurred, with the control sample showing the highest yellow intensity of  $43.61 \pm 1.58$ , followed by 3% added protein of  $41.36 \pm 0.68$ , 5% added protein of  $37.88 \pm 0.28$ , and 10% added protein of  $34.48 \pm 1.68$ . Compared to 65°C, there is an increase in  $b^*$  values at 70°C for most samples, suggesting that higher temperatures promote structural changes in proteins that enhance yellow reflectance. One possible explanation is that at 70°C, protein denaturation is more complete, allowing for better pigment retention and uniform light reflection, increasing the perceived yellow color. However, as egg white concentration increases, the dilution effect of yolk pigments becomes stronger (Vargas-del-Río et al., 2022), leading to a drop in  $b^*$  value at 10% added protein sample to be  $34.48 \pm 1.48$ . As for storage effect on fortified samples over a period of 21 days, it was found that when pasteurized at 60 °C and by day 7, all samples exhibited reduced lightness, declining redness, and lower yellow intensity, suggesting protein aggregation and enzymatic activity affecting pigment visibility (Llave et al., 2018). At day 14,  $L^*$  partially recovered, but  $a^*$  and  $b^*$  continued to decrease, indicating progressive pigment degradation and Maillard reactions (Luo et al., 2020). By day 21, lightness increased, but redness and yellowness further declined, confirming long-term carotenoid degradation and pigment redistribution. These changes were influenced by protein denaturation, pigment dispersion, and storage-induced modifications in yolk protein interactions and Maillard reactions, which gradually affected color stability (Ho et al., 2022).

As for samples which were pasteurized at 65 °C, by day 7,  $L^*$  values slightly decrease across all samples, suggesting early-stage protein interactions leading to a darker appearance (Eisen et al., 1962). The control sample remains the lightest, while the 10% protein sample is the darkest. The  $a^*$  values remain stable, with slight fluctuations, implying that yolk pigment oxidation is minimal at this stage. The  $b^*$  values decrease slightly in all samples, indicating progressive carotenoid degradation or pigment redistribution due to protein aggregation (Liu et al., 2025). As illustrated in the figure below the trend of color parameters vary during storage period. By day 14, there is a significant shift in all color parameters. The  $L^*$  values increase in all samples, particularly in the control sample and 10% added protein sample, suggesting that protein rearrangement is allowing better light reflection, leading to a lighter appearance. The  $a^*$  values declined slightly, which may be attributed to carotenoid oxidation affecting the red component (Bermudez-Aguirre & Niemira, 2023). The  $b^*$  values also increase slightly, possibly due to storage-induced changes in pigment-protein interactions that enhance yellow reflectance. By

the end day 21, L\* values rise significantly, with the control sample reaching  $69.49 \pm 1.18$  and 3% added protein at  $67.65 \pm 1.38$ , suggesting further structural modifications leading to increased lightness. This may be due to protein denaturation, aggregation, and pigment redistribution. The a\* values decrease significantly, with the control dropping to  $4.91 \pm 0.92$ , confirming progressive pigment oxidation effects reducing red intensity. The b\* values remain relatively stable, indicating that while some carotenoid degradation occurs, the yellow hue persists due to bound pigments and structural changes in protein matrices (Bermudez-Aguirre & Niemira, 2023).



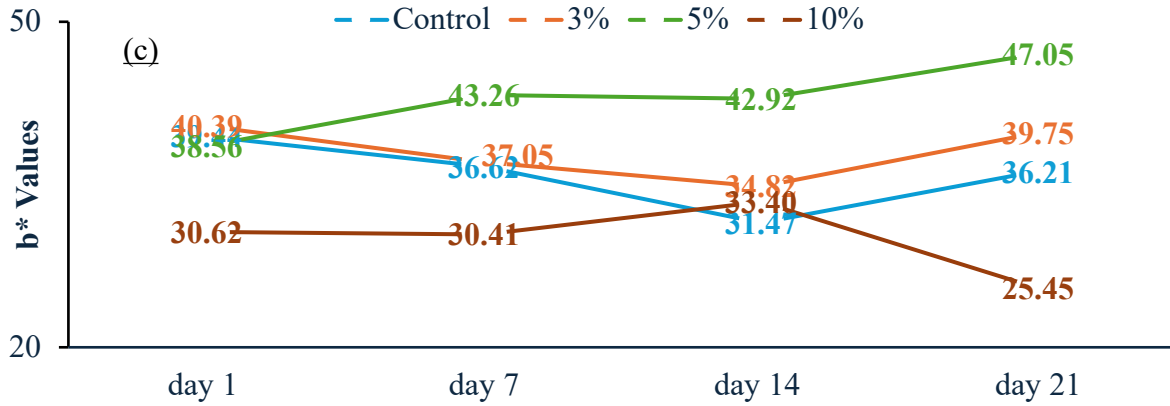
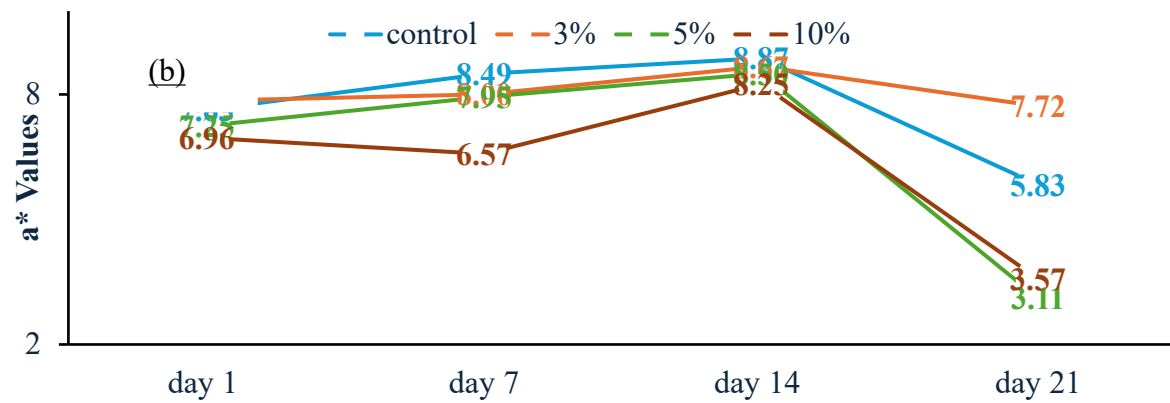
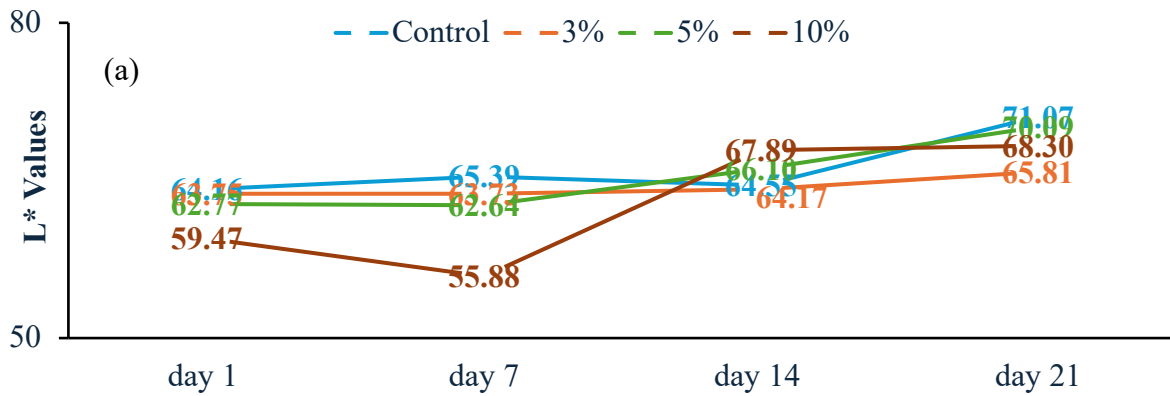


Figure 18: The effect of adding different percentage of powdered egg protein to liquid whole egg and pasteurizing at 60 °C and on: (a) L\* values, (b) a\* values, (c) b\* values in comparison to the control group and for a storage period of 21 days.



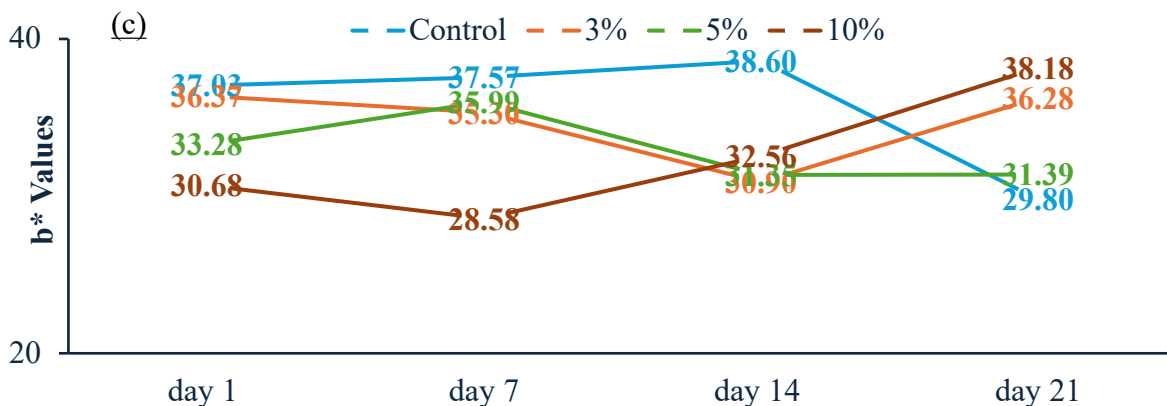


Figure 19: The effect of adding different percentages of powdered egg protein to liquid whole egg and pasteurizing at 65 °C and on: (a) L\* values, (b) a\* values, (c) b\* values in comparison to the control group and for a storage period of 21 days.

As for the results of samples that were pasteurized at 70 °C, by day 7, the L\* values increased in the control and 3% added protein sample, indicating protein restructuring and enhanced light reflection, while the 5% and 10% added protein samples remained almost the same, suggesting stabilization of proteins structure. As for a\* it declined slightly, likely due to oxidative degradation of yolk pigments, while b\* dropped significantly in the control from  $43.61 \pm 0.98$  to  $32.49 \pm 1.21$  pointing to carotenoid degradation, whereas all protein-rich samples retained more yellow pigments (Bermudez-Aguirre & Niemira, 2023). By day 14, L\* values stabilized across all samples, as protein aggregation and pigment redistribution created a uniform light-scattering effect, while a\* continued to decline due to carotenoid oxidation. b\* slightly increased in the 3% and 5% added protein samples, suggesting better pigment retention compared to the control, which lacked protein buffering (Liu et al., 2025). By day 21, L\* values slightly declined for the control and 3% added protein at, while 5% and 10% added protein samples stabilized, suggesting a slowing in structural changes. a\* significantly dropped, confirming continuous pigment oxidation, while b\* declined across all samples, with protein-rich samples maintaining slightly higher in yellow intensity than control group, implying that proteins helped slow carotenoid degradation (Liu et al., 2025).

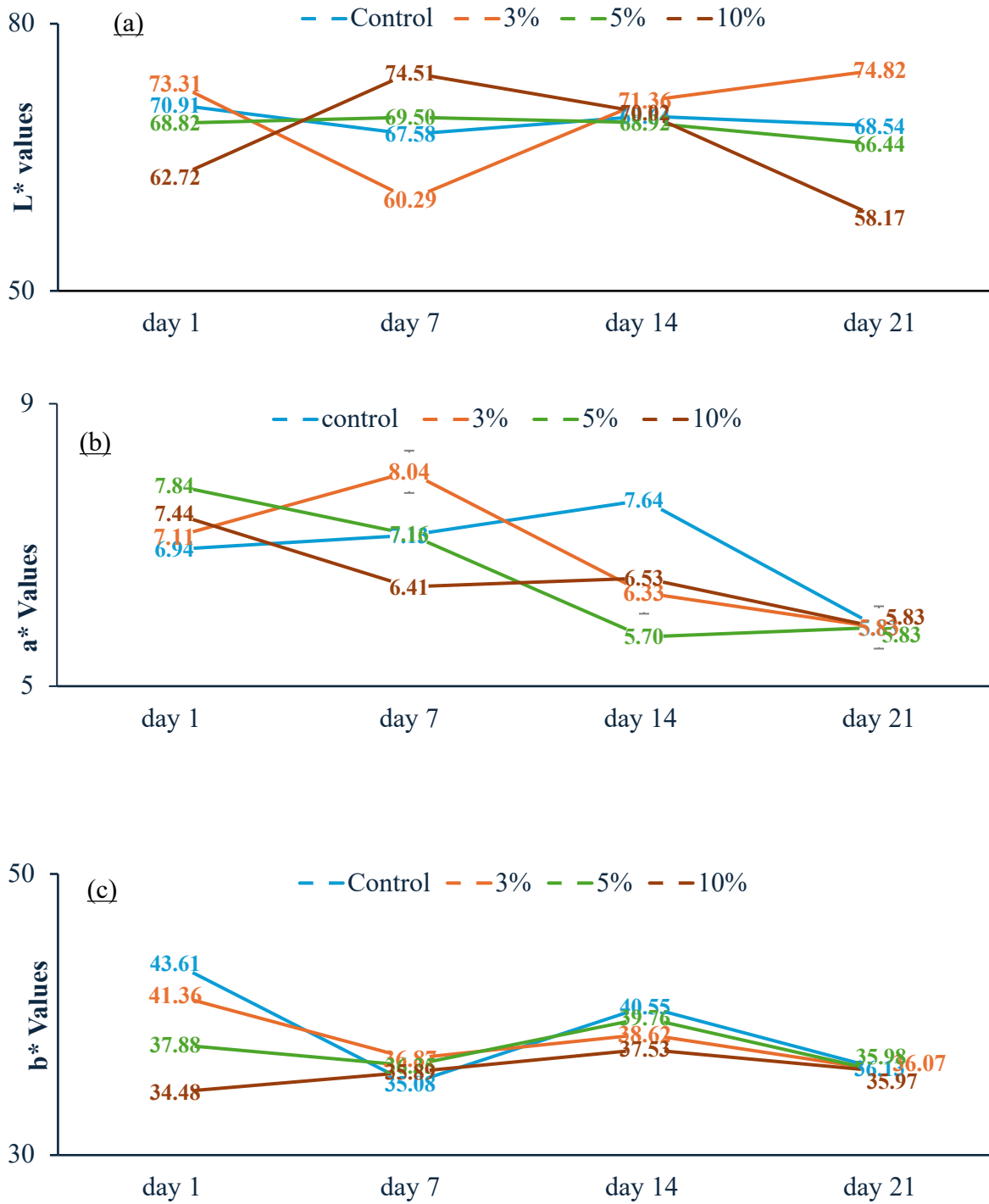


Figure 20: The effect of adding different percentages of powdered egg protein to liquid whole egg and pasteurizing at 70 °C and on: (a) L\* values, (b) a\* values, (c) b\* values in comparison to the control group and for a storage period of 21 days.

#### 4.1.1.3 Changes in rheological properties for liquid whole egg

The rheological parameters ( $\tau_0$ , K, and n) of liquid whole egg samples fortified with egg white powder (3%, 5%, and 10%), pasteurized at 60, 65 and 70°C, and stored at 4°C for 21 days, demonstrate significant changes over time. These parameters provide insight into the flow behavior, viscosity, and consistency of the samples, revealing how protein interactions, structural modifications, and storage effects influence the rheological properties.

At 60°C and on day 1, the control sample exhibits minimal yield stress of  $0.002 \pm 0.001$  Pa, K of  $0.017 \pm 0.001$  Pa·s<sup>n</sup>, and a n of  $0.986 \pm 0.001$  which are close to Newtonian behavior, suggesting a nearly fluid-like consistency with minimal resistance to flow. However, as egg white concentration increases,  $\tau_0$  rises significantly to  $0.127 \pm 0.001$  Pa in 3%,  $0.695 \pm 0.001$  Pa in 5%, and  $0.699 \pm 0.001$  Pa in 10%, indicating that higher protein content contributes to increased structural integrity and initial resistance to flow. As for K results it increases with protein concentration, particularly in the 10% sample where K was  $0.209 \pm 0.002$  Pa·s<sup>n</sup>, reflecting a thicker and more structured liquid due to protein network formation. Regarding n it was decreased with higher protein concentrations, indicating shear-thinning behavior, where the fluid becomes less viscous under shear (Kumbár, Nedomová, et al., 2015).

By day 7, the control sample shows a noticeable increase in  $\tau_0$  reaching  $0.112 \pm 0.001$  Pa, a drop in K to  $0.004 \pm 0.001$  Pa·s<sup>n</sup>, and an increase in n to  $1.141 \pm 0.001$ , suggesting a shift toward more Newtonian behavior, likely due to protein relaxation and structural breakdown over time. Meanwhile, 3% and 5% samples exhibit moderate increases in  $\tau_0$  to reach  $0.224 \pm 0.001$  Pa and  $0.468 \pm 0.001$  Pa, respectively, along with slight reductions in n, maintaining their shear-thinning characteristics. As for the 10% added protein sample, it showed a huge increase in  $\tau_0$  to  $2.068 \pm 0.701$  Pa, indicating a significant structural development and gel-like consistency, due to protein aggregation and interactions over storage (Deng et al., 2025). This suggests that higher egg white concentrations lead to stronger protein networks that become more pronounced during storage (Lee et al., 2024).

By day 14, there are more observed shifts in rheological properties. The control sample experiences a significant reduction in  $\tau_0$  to  $0.039 \pm 0.001$  Pa and an increase in K to  $0.092 \pm 0.001$  Pa·s<sup>n</sup>, with a lower n value of  $0.708 \pm 0.041$ , suggesting that storage induces some degree of structural changes even in the absence of added egg white. In contrast, the 10% added protein sample exhibits the highest  $\tau_0$  of  $4.210 \pm 0.051$  Pa, indicating strong gel-like behavior, while its n

value was 1.081 suggests a transition towards a more solid-like or non-Newtonian behavior, possibly due to continued protein-protein interactions forming a more extensive network structure (Kumbár, Nedomová, et al., 2015, Lee et al., 2024). The 5% added protein sample also shows an increase in K reaching  $0.141 \pm 0.011 \text{ Pa} \cdot \text{s}^n$  and a significant reduction in n value to  $0.732 \pm 0.077$ , suggesting it retains a thickened consistency but with shear-thinning characteristics.

By day 21, the control sample has further decreased in  $\tau_0$  to  $0.026 \pm 0.011 \text{ Pa}$  and K to  $0.008 \pm 0.001 \text{ Pa} \cdot \text{s}^n$ , but n remained above 1, suggesting a return to Newtonian-like behavior, due to protein breakdown over time. The 3% and 5% added protein samples maintained a moderate structural stability where  $\tau_0$  was  $0.248 \pm 0.001 \text{ Pa}$  and  $0.431 \pm 0.031 \text{ Pa}$ , respectively, while their n values remained below 1, preserving shear-thinning behavior. On the other hand, the 10% sample shows a significant drop in  $\tau_0$  to  $0.276 \pm 0.001 \text{ Pa}$ , K reaching  $0.043 \pm 0.0001 \text{ Pa} \cdot \text{s}^n$ , and n value of  $0.931 \pm 0.911$ , suggesting a breakdown or rearrangement of the previously formed protein structure. This could indicate structural weakening, possibly due to protein interactions reaching a dynamic equilibrium, leading to partial breakdown of the gel-like network observed earlier (Lv et al., 2022). During experiment and for samples that are pasteurized at  $65 \text{ }^\circ\text{C}$ , it was found that with the increase of storage time and protein percentage, viscosity was decreasing for all samples in comparison with control group for the first 2 weeks (Atilgan & Unluturk, 2008; Jaekel & Ternes, 2009; Kumbár, Nedomová, et al., 2015; Kumbár et al., 2021). Within groups and with the increase of storage time the viscosity was decreasing for samples with 3% added egg white protein powder and increasing with storage for the 5% and 10% added percentages. The viscosity of control group was also decreased with storage time. Both decrease and increase in viscosity at all measured days was significant. “n” values went down from  $0.910 \pm 0.030$  to  $0.901 \pm 0.010$ ,  $0.856 \pm 0.015$  and  $0.654 \pm 0.021$  for 3, 5 and 10% respectively at day 1 of measurement. Table 3 displays the values for ( $\tau_0$ ), (K), and (n).

At day of experiment control samples exhibited the highest  $\tau_0$  and low K values, indicating moderate resistance to flow and low viscosity, with a n value of  $0.910 \pm 0.030$  which shows a near-Newtonian behavior. The addition of 3% and 5% reduced  $\tau_0$  significantly, particularly for 5%, where  $\tau_0$  dropped to near-zero with a corresponding increase in k, this change shifted the flow behavior toward shear-thinning behavior where n was  $0.901 \pm 0.010$  and  $0.856 \pm 0.021$  respectively for 3% and 5%. For 10% additive,  $\tau_0$  remained near zero, but then value indicated shear-thickening properties. By day 7 control samples exhibited a decrease in  $\tau_0$  and n, but exhibit

a slight increase in  $K$  all changes were significant in comparison to the first day of experiment suggesting that viscosity increases with storage time (Panaite et al., 2019).

Table 3: The effect of adding egg white protein on actual and measured results of Herschel-Bulkley model in comparison to control at 60 °C, different letters differ significantly in comparison to control groups (Tukey's  $p < 0.05$ ).

Sample	$\tau_0$ (Pa)	$K(\text{Pa}\cdot\text{s}^n)$	$n$ (-)
control day 1	0.002±0.001	0.017±0.001	0.986±0.021
3%day1	0.127±0.001	0.016±0.001	1.003±0.211
5%day1	0.695±0.021	0.072±0.001 a	0.832±0.061 a
10%day1	0.699±0.071 a	0.209±0.301 a	0.755±0.011 a
control day 7	0.112±0.021a	0.004±0.001	1.141±0.011
3%day7	0.224±0.011 a	0.011±0.001	1.032±0.781
5%day7	0.468±0.021 a	0.033±0.001	0.935±0.341
10%day7	2.068±0.701b	0.064±0.001 a	0.902±0.331
control day 14	0.039±0.001 a	0.092±0.021 a	0.708±0.331 a
3%day14	0.249±0.001 a	0.019±0.011	0.963±0.001
5%day14	0.347±0.001 a	0.141±0.011 a	0.732±0.077 a
10%day14	4.210±0.051 b	0.072±0.031 a	1.081±0.331
control day 21	0.026±0.011	0.008±0.001	1.043±0.001
3% day 21	0.248±0.001a	0.007±0.001	1.080±0.401
5% day 21	0.431±0.001 a	0.023±0.001	0.965±0.021
10% day 21	0.276±0.001 a	0.043±0.031	0.931±0.911

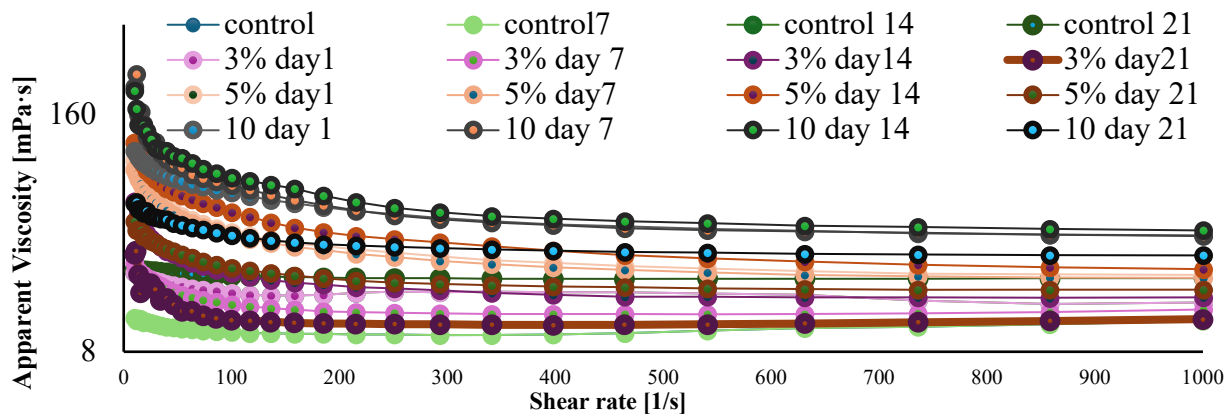


Figure 21: The viscosity curve (shear rate vs. viscosity) of added different concentration of egg white protein to liquid whole egg sample at 60 °C and stored for 21 days.

In contrast, samples with 3, 5, and 10 added proteins showed a shear thickening behavior in comparison to control group and same percentages at day 1 of experiment. Viscosity curve below represents the rheological attitude for all samples during storage period. At day 14 control sample

$\tau_0$  and  $n$  decreased while  $K$  increased which indicates an increase in viscosity and rigidity in comparison to previous results. On the other hand, all added percentages exhibited an increase in  $\tau_0$  and  $K$  and decrease in  $n$  indicating a strong shear thinning behavior with the increase of storage time and added protein percentages. In case of 10 % added protein,  $\tau_0$  was near zero while  $K$  was at the highest level for the past 14 days signifying a highly viscous behavior. The values of  $n$  showed a strong shear thinning tendencies with the increase in added protein percentage at day 14.

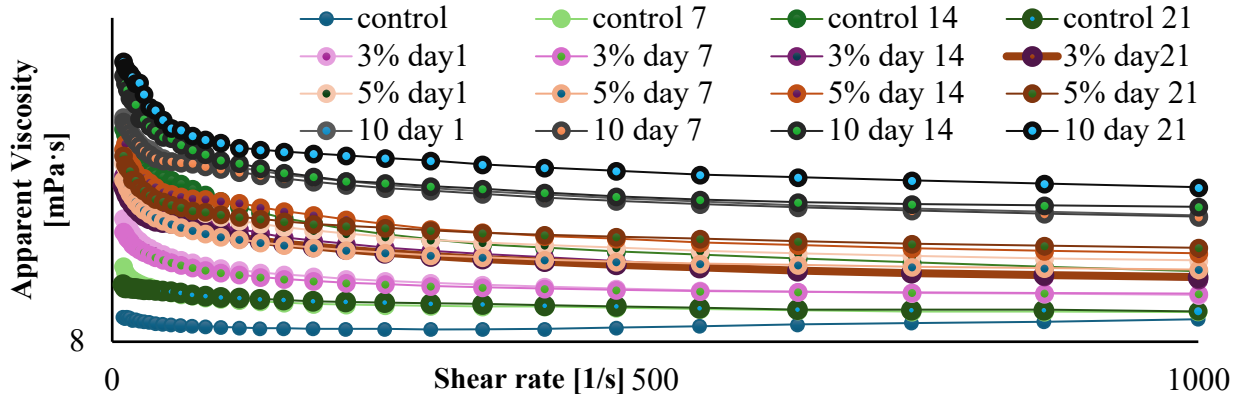


Figure 22: The viscosity curve (shear rate vs. viscosity) of added different concentration of egg white protein to liquid whole egg sample at 65 °C and stored for 21 days.

Table 4: the effect of adding egg white protein on actual and measured results of Herschel-Bulkley model in comparison to control at 65° different letters differ significantly in comparison to control groups (Tukey's  $p < 0.05$ ).

Sample	$\tau_0$ (Pa)	$K(\text{Pa} \cdot \text{s}^n)$	$n$ (-)
control day 1	0.404±0.033a	0.028±0.011a	0.910±0.030a
3%day1	0.265±0.027b	0.033 ±0.001	0.901±0.010b
5%day1	0.003± 0.020c	0.345 ±0.002b	0.856±0.015b
10%day1	0.001±0.003c	0.005±0.001	0.654±0.021b
control day 7	0.092±0.024c	0.050±0.001	0.885±0.010b
3%day7	0.377±0.016b	0.097±0.001b	0.799±0.014b
5%day7	0.001±0.003c	0.289±0.002	0.669±0.027b
10%day7	0.001±0.015c	0.758±0.001b	0.661±0.024b
control day 14	0.026±0.007c	0.092±0.001	0.708±0.011b
3%day14	0.431±0.023b	0.606±0.001b	0.693±0.028b
5%day14	0.031±0.024c	0.114±0.002b	0.659±0.015b
10%day14	0.001±0.020c	0.891±0.001b	0.688±0.023b
control day 21	0.026±0.006c	0.008±0.004	0.693±0.025b
3% day 21	1.512±0.017d	0.664±0.001b	0.648±0.016b
5% day 21	0.431±0.001b	0.023±0.001	0.965±0.021
10% day 21	0.276±0.001b	0.043±0.031	0.931±0.911

By day 21 control sample showed a similar behavior to day 14 with a slight decrease in  $n$  value showing a shear thinning behavior. All protein added samples 3% had the highest  $\tau_0$  with significantly increase  $k$  value, meanwhile  $n$  value was significantly decreased indicating a strong shear thinning behavior. Similarly, the 5% additive sample showed a significant increase in  $\tau_0$ , kind decrease in  $n$  when compared to all previous measurements. The 10% additive sample continued to show elevated yield stress and the highest consistency index, reflecting a highly structured and viscous system with pronounced shear-thinning.

On regard of samples that were pasteurized at 70 °C on day 1, the control sample exhibited a moderate  $\tau_0$  of  $0.467 \pm 0.013$  Pa, low  $K$  of  $0.059 \pm 0.012$ , and shear-thinning behavior where  $n$  was  $0.811 \pm 0.088$ , indicating a fluid-like, non-Newtonian nature with limited structural resistance to flow. As for 3% and 5% added protein samples it had a much lower  $\tau_0$  of  $0.040 \pm 0.013$  and  $0.195 \pm 0.043$ , respectively, with slightly higher  $K$  values of  $0.206 \pm 0.013$  and  $0.270 \pm 0.031$ , showing increased viscosity and minor structural thickening. As for the 10% added protein sample, it exhibited a significant increase in  $K$  to reach  $2.773 \pm 0.124$ , with a lower  $n$  value of  $0.548 \pm 0.078$ , suggesting significant thickening and structural resistance due to high protein concentration. This indicates that higher egg white concentrations enhanced intermolecular interactions, leading to increased viscosity and a more structured network (Lee et al., 2024).

By day 7, the control sample showed an increase in  $\tau_0$  to  $0.840 \pm 0.023$ , indicating a slight strengthening of the internal protein matrix, likely due to ongoing structural rearrangements. As for 3% added protein sample, it maintained a low  $\tau_0$  of  $0.058 \pm 0.041$  and exhibited an  $n$  value closer to Newtonian behavior of  $0.921 \pm 0.103$ , suggesting it remained highly fluid-like with minimal structural development. The 5% and 10% added protein samples showed an increased in  $\tau_0$  to  $0.300 \pm 0.011$  and  $0.230 \pm 0.009$ , respectively, with the 10% sample having an extreme increase in  $K$  to  $8.773 \pm 0.933$  accompanied with a very low  $n$  of  $0.432 \pm 0.093$ , indicating the formation of a highly viscous, gel-like structure. This significant viscosity increases in the 10% added protein sample suggests strong protein-protein interactions, aggregation, and potential gelation over time, leading to a dense, less flowable liquid matrix.

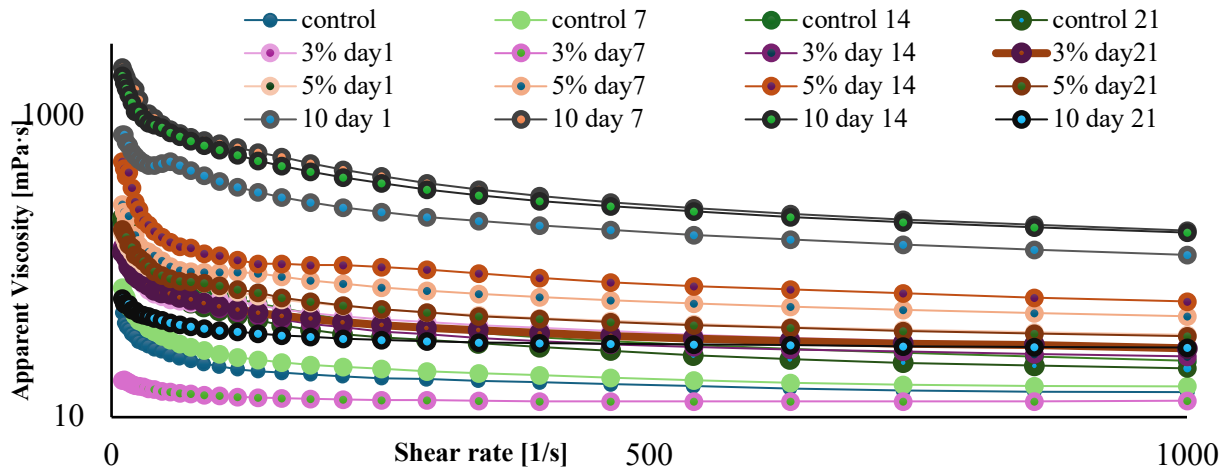


Figure 23: The viscosity curve (shear rate vs. viscosity) of added different concentration of egg white protein to liquid whole egg sample at 70 °C and stored for 21 days.

Table 5: The effect of adding egg white protein to liquid whole egg on actual and measured results of Herschel-Bulkley model in comparison to control at 70 °C different letters differ significantly in comparison to control groups (Tukey's  $p < 0.05$ ).

Sample	$\tau_0$ (Pa)	$K(\text{Pa} \cdot \text{s}^n)$	$n$ (-)
control day 1	$0.467 \pm 0.013$ a	$0.059 \pm 0.012$ a	$0.811 \pm 0.088$ a
3%day1	$0.040 \pm 0.013$ a	$0.206 \pm 0.013$ b	$0.716 \pm 0.055$ b
5%day1	$0.195 \pm 0.043$ a	$0.270 \pm 0.031$ b	$0.711 \pm 0.087$ b
10%day1	$0.222 \pm 0.001$ a	$2.773 \pm 0.124$ c	$0.548 \pm 0.078$ b
control day 7	$0.840 \pm 0.023$ a	$0.081 \pm 0.001$ a	$0.778 \pm 0.066$ b
3%day7	$0.058 \pm 0.041$ a	$0.027 \pm 0.042$ a	$0.921 \pm 0.103$ b
5%day7	$0.300 \pm 0.011$ a	$0.466 \pm 0.012$ b	$0.669 \pm 0.001$ b
10%day7	$0.230 \pm 0.009$ a	$8.773 \pm 0.933$ d	$0.432 \pm 0.093$ c
control day 14	$0.901 \pm 0.063$ a	$2.996 \pm 0.041$ c	$1.847 \pm 0.013$ d
3%day14	$0.201 \pm 0.075$ a	$1.750 \pm 0.833$ b	$1.411 \pm 0.893$ d
5%day14	$0.955 \pm 0.023$ a	$1.516 \pm 0.533$ b	$1.592 \pm 0.573$ d
10%day14	$1.371 \pm 0.056$ b	$0.013 \pm 0.001$ a	$2.190 \pm 0.872$ e
control day 21	$0.916 \pm 0.098$ a	$0.223 \pm 0.001$ b	$0.672 \pm 0.021$ b
3% day 21	$0.337 \pm 0.001$ a	$0.160 \pm 0.001$ b	$0.749 \pm 0.071$ a
5% day 21	$0.381 \pm 0.001$ a	$0.195 \pm 0.034$ b	$0.778 \pm 0.055$ a
10% day 21	$0.238 \pm 0.001$ ab	$0.063 \pm 0.001$ a	$0.896 \pm 0.021$ a

The viscosity behavior is represented in figure 23 showing the trend of change for the whole 21 days. By day 14, the control sample exhibited a significant increase in both  $\tau_0$  and  $K$  reaching  $0.901 \pm 0.063$  and  $2.996 \pm 0.041$  respectively, with  $n$  value of  $1.847 \pm 0.013$  nearing Newtonian behavior, indicating thickening and structural modifications over storage. The 3% protein sample had an extreme increase in  $K$  reaching  $1.750 \pm 0.833$  and increased  $n$  to  $1.411 \pm 0.893$ , suggesting it had undergone major structural changes, forming a semi-gel-like consistency. The 5% sample also

demonstrated high viscosity where  $K$  was  $1.516 \pm 0.533$  and non-Newtonian behavior where  $n$  was  $1.592 \pm 0.573$ , indicating stronger protein network formation. As for, the 10% added protein sample it exhibited an extremely low  $K$  value of  $0.013 \pm 0.001$ , but the highest  $n$  of  $2.190 \pm 0.872$ , suggesting a significant phase separation or protein destabilization, potentially due to over-aggregation and syneresis.

By day 21, the control sample showed a reduction in  $K$  value to  $0.223 \pm 0.001$  and a lower  $n$  value reaching  $0.672 \pm 0.021$ , suggesting a gradual breakdown in viscosity and a shift towards non-Newtonian flow. The 3% and 5% added protein samples maintained moderate consistency  $K$  of  $0.160 \pm 0.001$  and  $0.195 \pm 0.034$ , respectively, with flow  $n$  value of  $0.749 \pm 0.071$  and  $0.778 \pm 0.055$  respectively that suggest partial stabilization. As for the 10% added sample, however, exhibited a significant decrease in both  $\tau_0$  to  $0.238 \pm 0.001$  and  $K$  to  $0.063 \pm 0.001$ , with an  $n$  value of  $0.896 \pm 0.021$  that implies a shift back toward a more flowable state, due to protein network breakdown and weakened interactions over prolonged storage.

#### 4.1.1.4 Changes in pH for liquid egg white

The experiment investigated how adding varying percentages of egg white protein (3%, 5%, 10%) affects the pH of liquid egg white after pasteurization at  $50^\circ\text{C}$ ,  $55^\circ\text{C}$ , and  $60^\circ\text{C}$ , with storage monitored over 21 days at  $4^\circ\text{C}$ . Initially, the control samples consistently showed the highest pH, reflecting the natural alkalinity of fresh egg whites. At  $50^\circ\text{C}$ , 3% protein slightly increased pH due to buffering, while 5% protein led to stabilization. However, 10% protein caused significant pH reduction to  $8.5 \pm 0.3$  due to protein-protein interactions, unfolding, and exposure of acidic groups (Cunningham and Cottrell, 1963). A similar trend was observed at  $55^\circ\text{C}$ , though pH values were slightly lower, suggesting more pronounced denaturation and acidification (Liu et al., 2017). At  $60^\circ\text{C}$ , initial pH values were highest, but higher protein concentrations still resulted in greater acidification, with the 10% protein sample dropping to  $8.8 \pm 0.2$ .

During storage pH increases in control and low-protein samples, while higher protein samples showed continuous pH declines. At  $50^\circ\text{C}$  and  $55^\circ\text{C}$ , the 10% protein samples exhibited the most significant pH drops down to  $7.3 \pm 0.5$  by Day 21, likely due to ongoing protein degradation and exposure of acidic residues (Zhang et al., 2023). At  $60^\circ\text{C}$ , while the control and 3% protein samples maintained high pH levels, the 10% sample showed notable acidification but some stabilization after Day 14. Overall, increased protein concentration combined with higher pasteurization

temperatures intensified protein denaturation, hydrolysis, and exposure of acidic groups, leading to lower pH, particularly in the higher protein treatments over time (Punidades & McKELLAR, 1999).

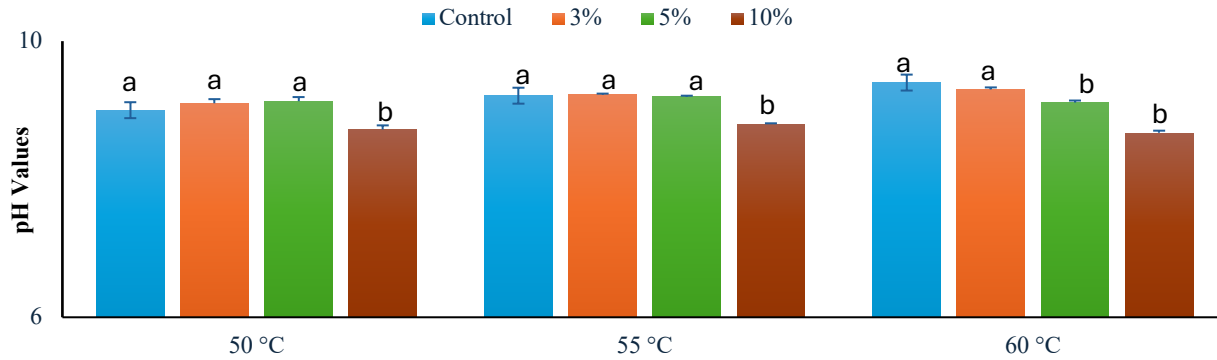


Figure 24: The effect of adding different percentages of powdered egg protein to liquid egg white and pasteurizing at different heat treatment in comparison to the control group, different letters differ significantly, Tukey HSD  $p < 0.05$ .

When liquid egg white with added egg white protein was pasteurized at 60°C, the pH values showed a continued trend of gradual acidification as temperature increased. The control sample had a slightly higher pH of  $9.4 \pm 0.5$ , suggesting that pasteurization at 60°C may have led to CO<sub>2</sub> release, which is known to slightly increase pH in egg white (Lechevalier et al., 2017). The 3% and 5% added protein samples exhibited minor pH reductions to  $9.3 \pm 0.1$  and  $9.2 \pm 0.9$ , respectively, indicating that the increased protein concentration slightly enhanced buffering capacity, preventing a sharp drop in pH. However, at 10% added protein, the pH dropped more significantly ( $p < 0.05$ ) to  $8.8 \pm 0.6$ , following the same pattern observed at 50°C and 55°C, where higher protein concentrations led to greater protein-protein interactions, partial denaturation, and exposure of acidic functional groups. The progressively lower pH values at 60°C compared to 55°C and 50°C suggest that increased pasteurization temperature intensifies protein unfolding, hydrolysis, and molecular interactions (Punidades & McKELLAR, 1999). This confirms that as pasteurization temperature rises, particularly at higher protein concentrations, induce structural modifications in egg white proteins contribute to measurable decreases in pH due to increased exposure of acidic residues.

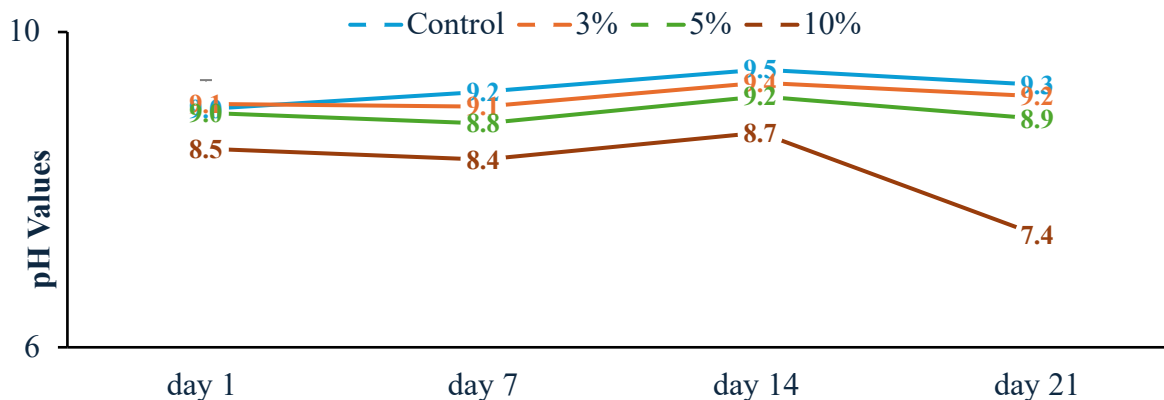


Figure 25: The trend of the effect of adding different percentages of powdered egg protein to liquid egg white and pasteurizing at 50°C, after 21 days of storage on pH values in comparison to the control group.

Over 21 days of storage at 4°C, the pH of liquid egg white pasteurized at 50°C exhibited different trends depending on the percentage of added egg white protein. The control sample showed a gradual increase in pH from 9.0±0.01 on Day 1 to 9.3±0.5 on Day 21, likely due to CO<sub>2</sub> loss, which reduces carbonic acid concentration and increases alkalinity (Lechevalier et al., 2017). The 3% added protein sample initially decreased slightly from 9.0±0.5 to 9.0±0.1 by Day 7 and remained stable until Day 14 before rising to 9.2±0.2 by Day 21, indicating a buffering effect from added proteins, delaying alkalization. Similarly, the 5% added protein sample showed a minor decrease from 8.98±0.6 on day 1 to 8.8±0.3 on day 7 and 14, before slightly increasing to 8.9±0.4 on day 21, suggesting that moderate protein levels help stabilize pH over time. However, the 10% protein sample exhibited a significant and continuous pH decline from 8.5±0.5 on Day 1 to 7.4±0.6 on Day 21, indicating potential protein degradation and enzymatic activity, which may have led to the release of acidic amino acids (Fan et al., 2024).

The pH trend of liquid egg white pasteurized at 55°C over 21 days of storage shows distinct variations based on protein concentration. Initially, on Day 1, the control and 3% added protein samples had the highest pH of 9.2±0.1, while 5% added protein had a slightly lower pH of 9.1, and the 10% added protein sample had the lowest pH of 8.7±0.4. Over the first 7 days, the control and 3% added protein samples showed a slight decline reaching to 9.143 and 8.923 respectively, likely due to the same previously mentioned reasons. The 5% added protein sample remained relatively stable at 8.8±0.5, whereas the 10% added sample dropped slightly to 8.4±0.7, suggesting a gradual acidification process.

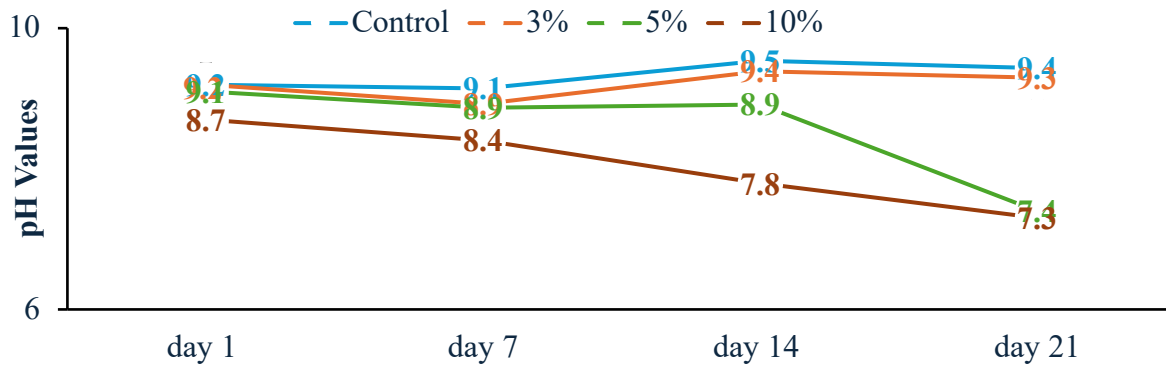


Figure 26: The trend of the effect of adding different percentage of powdered egg protein to liquid egg white and pasteurizing at 55°C, after 21 days of storage on pH values in comparison to the control group.

By Day 14, the control was  $9.5 \pm 0.7$  and 3% added protein was  $9.4 \pm 0.4$  both exhibited an increase in pH, which aligns with the expected  $\text{CO}_2$  loss and progressive alkalization during refrigerated storage (Lechevalier et al., 2017). However, the 5% added protein sample remained stable at  $8.9 \pm 0.7$ , and the 10% protein sample experienced a noticeable drop to  $7.8 \pm 0.4$ , indicating protein hydrolysis or enzymatic degradation effects leading to acidification. By Day 21, a clear trend emerged: the control sample maintained a high pH of  $9.4 \pm 0.5$ , and the 3% added protein sample followed closely by  $9.3 \pm 0.3$  confirming that lower protein concentrations buffer pH effectively. However, the 5% added protein sample dropped significantly ( $p < 0.05$ ) to  $7.4 \pm 0.3$ , and the 10% protein sample reached its lowest pH of  $7.3 \pm 0.3$ , suggesting intensified protein degradation and increased exposure of acidic residues (Fan et al., 2024).

The pH trend of liquid egg white pasteurized at 60°C over 21 days reveals how protein concentration and heat treatment affect pH stability during storage. On Day 1, the control sample and 3% added protein sample had the highest pH of  $9.4 \pm 0.1$  and  $9.3 \pm 0.7$  respectively, while 5% added protein was slightly lower at  $9.2 \pm 0.6$ , and 10% protein had the lowest pH of  $8.8 \pm 0.7$ . This initial trend suggests that higher protein concentrations lead to increased buffering interactions, slightly reducing alkalinity. By day 7, there was a noticeable pH decline across all samples, with the control dropping to  $9.1 \pm 0.6$  and the 3% added protein sample to  $9.03 \pm 0.4$ , which explained above and for the exact same reasons (Lechevalier et al., 2017). The 5% sample declined to  $8.9 \pm 0.4$ , and the 10% sample dropped further to  $8.4 \pm 0.4$ , showing that higher protein concentrations undergo faster acidification during early storage. On day 14, the control and 3% added protein samples showed a pH rebound to  $9.5 \pm 0.7$  and  $9.4 \pm 0.6$ , respectively, due to  $\text{CO}_2$  loss, which naturally increases alkalinity over time.

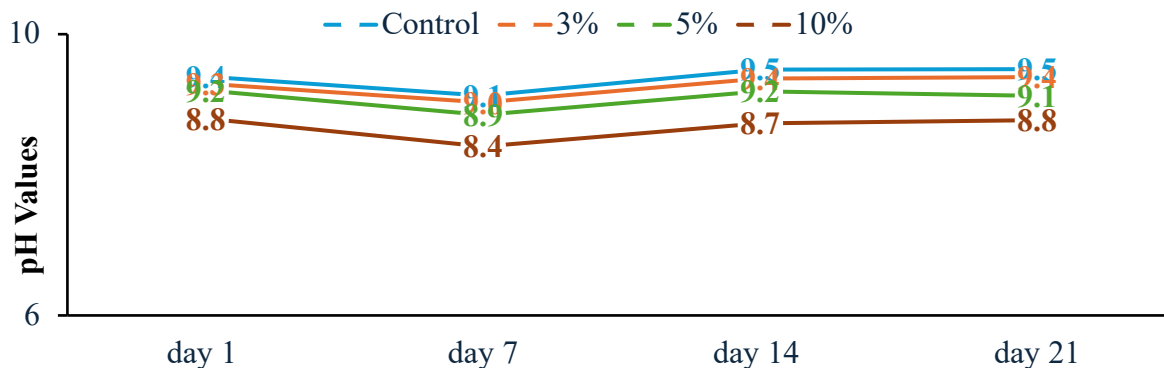


Figure 27: The trend of the effect of adding different percentage of powdered egg protein to liquid egg white and pasteurizing at 60°C, after 21 days of storage on pH values in comparison to the control group.

The 5% sample also recovered slightly to  $9.2 \pm 0.6$ , but the 10% sample remained lower at  $8.7 \pm 0.3$ , indicating a weaker buffering effect and potential ongoing protein hydrolysis (Lechevalier et al., 2017). By day 21, the control sample and 3% added protein sample remained stable at  $9.5 \pm 0.5$  and  $9.4 \pm 0.3$  respectively, confirming that lower protein concentrations buffer pH effectively. The 5% added protein sample slightly decreased to  $9.1 \pm 0.3$ , while the 10% sample remained the lowest at  $8.8 \pm 0.7$ , but with minor stabilization compared to Day 14. Overall, pasteurization at 60°C promotes initial acidification, followed by gradual stabilization, with the control and 3% protein samples maintaining higher pH, while 5% and 10% protein samples exhibit a more pronounced decline due to increased protein interactions and degradation.

#### 4.1.1.5 Changes in color parameters for liquid egg white

The  $L^*$  value is an important parameter in evaluating the color stability of liquid egg white after pasteurization at 50°C, 55°C, and 60°C with varying concentrations of added egg white powder (0%, 3%, 5%, 10%). The results indicate that pasteurization temperature and protein concentration significantly affect  $L^*$ , with trends suggesting structural and chemical modifications in the egg white matrix.

The  $L^*$  values of liquid egg white with added egg white powder were affected by pasteurization at 50°C, 55°C, and 60°C, showing distinct trends based on temperature and protein concentration. At 50°C, the control sample retained the highest  $L^*$  of  $83.74 \pm 0.31$ , while 3% protein, 5%, and 10% added protein samples showed a decrease reaching  $81.70 \pm 0.62$ ,  $76.33 \pm 0.32$ ,  $79.99 \pm 0.33$  respectively, which indicates that higher protein concentrations reduced light reflection due to increased turbidity and molecular interactions (De La Fuente et al., 2002). At 55°C, the control

sample remained the brightest, but the 5% added protein sample had a slight increase in  $L^*$ , suggesting that moderate heating stabilized protein dispersion. However, the 10% added protein sample dropped significantly to  $76.31 \pm 0.12$ , showing that high protein concentrations promote cloudiness due to aggregation. At  $60^\circ\text{C}$ ,  $L^*$  values declined further across all samples, with the control dropping to 82.66, 3% to 80.38, 5% to  $77.29 \pm 0.12$ , and 10% to the lowest to  $72.99 \pm 1.60$ , showing that increased pasteurization temperature accelerates protein unfolding, aggregation, and opacity, leading to reduced brightness (Bermudez-Aguirre & Niemira, 2023). The overall trend suggests that higher protein concentrations and elevated pasteurization temperatures cause structural modifications in egg white proteins, increasing turbidity and light scattering, ultimately reducing  $L$  values and altering visual appearance (Fan et al., 2024).

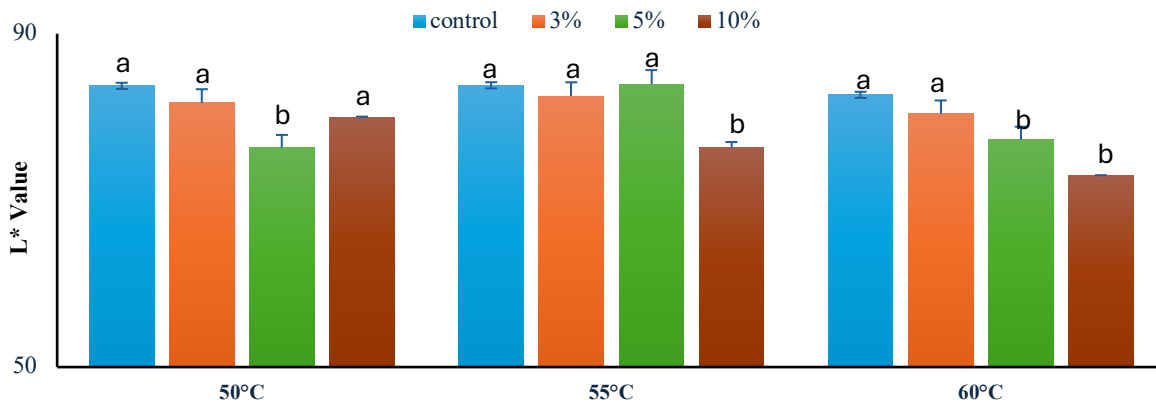


Figure 28: The effect of adding different percentages of powdered egg protein to liquid egg white and pasteurizing at 50, 55 and  $60^\circ\text{C}$  and on  $L^*$  values in comparison to the control group.

On regards of  $a^*$  values of liquid egg white with added egg white powder, were influenced by pasteurization at  $50^\circ\text{C}$ ,  $55^\circ\text{C}$ , and  $60^\circ\text{C}$ , showing variations in redness/greenness due to protein interactions and color stability. Negative  $a^*$  values indicate a greenish tint, while higher values suggest a shift toward neutral or reddish tones. At  $50^\circ\text{C}$ , the control sample showed a mild green tint with  $a^*$  value of  $-0.95 \pm 0.43$ , while 3% and 5% protein samples shifted slightly toward neutral  $a^*$  value of  $-0.70 \pm 0.12$  and  $-0.68 \pm 0.12$  respectively, suggesting that small protein additions help maintain color stability by reducing color deviations. However, the 10% protein sample exhibited a stronger green shift reaching  $-1.30 \pm 0.21$ .

At  $55^\circ\text{C}$ , the control sample remained close to its  $50^\circ\text{C}$  value with  $a^*$  value of  $-0.91 \pm 0.21$ , suggesting minimal impact of pasteurization at this temperature. However, higher protein concentrations resulted in progressively lower  $a^*$  value, with the 3% at  $-0.89 \pm 0.11$  and 5% at -

1.03±0.17 samples showing increased greenness. The 10% sample exhibited the strongest green shift with  $a^*$  of  $-1.56±0.21$ , indicating again that the idea that higher protein content leads to increased protein interactions and molecular rearrangements affecting light absorption and scattering. At 60°C, a significant variation in  $a^*$  values was observed, with the control at  $-1.12±0.21$ , 3% at  $-0.75±0.31$ , and 5% at  $-0.83±0.53$  samples showing moderate shifts, but the 10% protein sample exhibiting a huge drop to  $-5.34±0.23$ , indicating an extreme green shift. This change occurred because of the same reasons that effected  $L^*$ . The increased green hue in the 10% sample at 60°C is also result from altered light reflection due to the formation of protein complexes, oxidation reactions (Bermudez-Aguirre & Niemira, 2023).

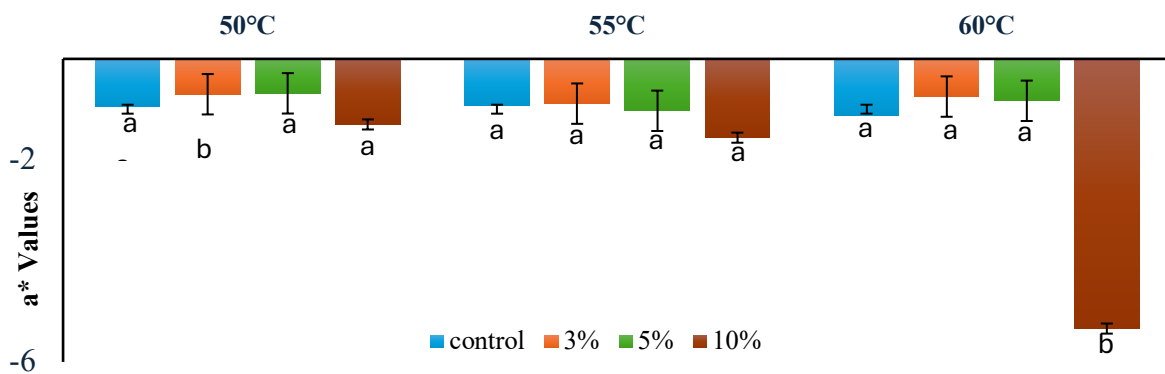


Figure 29: The effect of adding different percentages of powdered egg protein to liquid egg white and pasteurizing at 50, 55 and 60°C and on  $a^*$  values in comparison to the control group.

The  $b^*$  values, showed a significant variation in yellowness due to protein concentration and heat-induced structural changes (Fan et al., 2024b). At 50°C, the control sample retained a mild yellow tint with  $b^*$  value of  $3.44±0.32$ , while 3% and 5% protein samples exhibited slightly reduced yellowness, likely due to stabilized protein interactions minimizing color deviations. However, the 10% protein sample showed a strong increase in  $b^*$  reaching  $7.64±0.66$ , suggesting that higher protein concentrations increased light scattering, molecular interactions, or early-stage Maillard reactions, leading to enhanced yellow tones (Bermudez-Aguirre & Niemira, 2023). At 55°C, a general increase in  $b^*$  values were observed, with the control reaching  $3.60±0.32$  and 3% protein at  $3.21±0.64$  remaining close to their 50°C values, indicating that moderate heat treatment had a limited effect on color in lower protein concentrations. However, the 5% and 10% protein samples showed a more noticeable rise in  $b^*$  reaching  $4.00±0.37$  and  $5.60±0.21$  respectively, suggesting increased protein denaturation and light absorption changes with higher protein levels. At 60°C, all samples exhibited a strong yellow shift, with the control to 4.27 increasing

moderately, while 3%, 5% and 10% protein samples showed substantial increases in  $b^*$  reaching  $7.10 \pm 0.22$ ,  $6.56 \pm 0.45$  and  $7.90 \pm 0.62$  respectively, confirming that higher temperatures cause protein unfolding, aggregation, and potential non-enzymatic browning (Bermudez-Aguirre & Niemira, 2023).

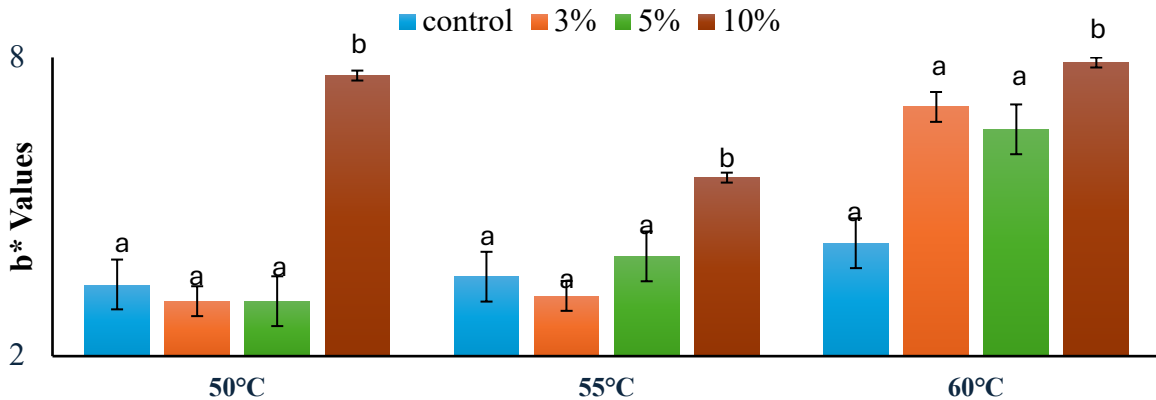


Figure 30: The effect of adding different percentage of powdered egg protein to liquid egg white and pasteurizing at 50, 55 and 60°C and on  $b^*$  values in comparison to the control group.

During four weeks of storage at 50°C, the color parameters ( $L^*$ ,  $a^*$ ,  $b^*$ ) of liquid egg white with added egg white powder changed significantly, particularly in samples with higher protein concentrations. Initially, all samples had high  $L^*$  values, indicating brightness, with minor variations in  $a^*$  and  $b^*$  values. However, by Day 7,  $L^*$  values decreased noticeably, especially in higher protein samples, due to protein aggregation and increased opacity reducing light reflectance. Simultaneously,  $a^*$  values became more negative, shifting the color towards green, likely due to oxidative reactions, with the 10% protein sample showing the most pronounced change. The  $b^*$  values, indicating yellowness, increased in some samples, suggesting protein modifications and oxidation (Llave et al., 2018). By Day 14, darkening and green shifts intensified, particularly in higher protein samples, while  $b^*$  values showed variability, reflecting a balance between oxidation and browning (Bermudez-Aguirre & Niemira, 2023). By Day 21, lower protein samples showed slight recovery in  $L^*$  values, whereas higher protein concentrations remained darker due to irreversible aggregation. Overall, higher protein levels resulted in more pronounced color changes, driven by protein-protein interactions, oxidation, and structural modifications during storage.

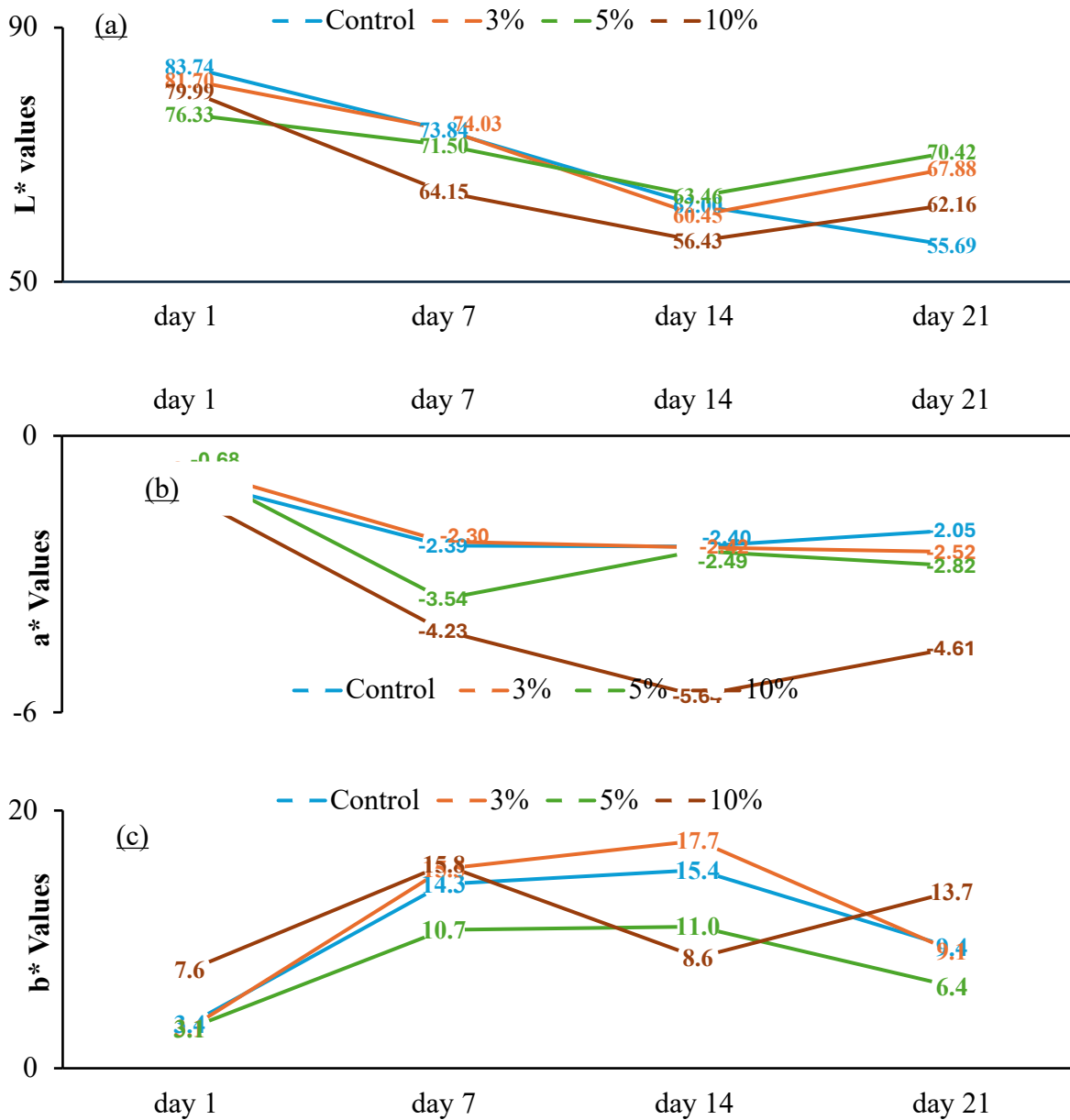
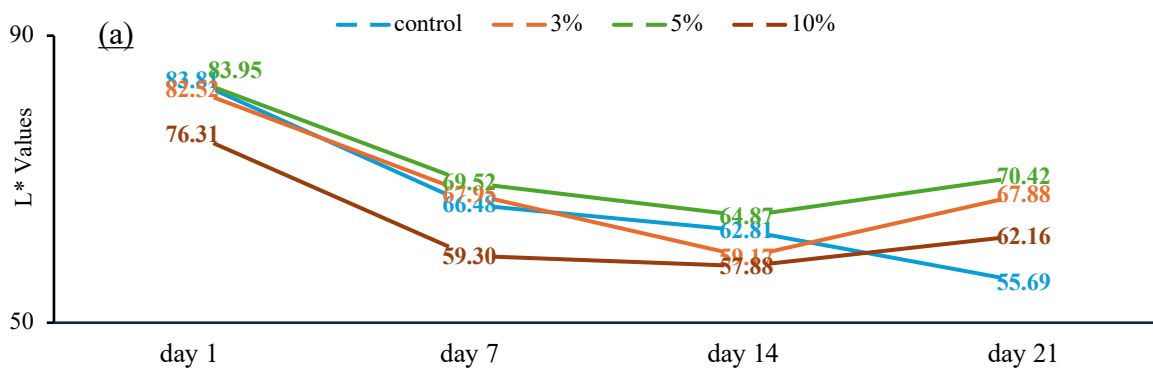


Figure 31: The effect of adding different percentages of powdered egg protein to liquid egg white and pasteurizing at 50 °C and on: (a) L\* values, (b) a\* values, (c) b\* values in comparison to the control group and for a storage period of 21 days.

During four weeks of storage at 55°C the results demonstrate that protein concentration, storage time, and protein-related biochemical reactions significantly influenced the color of liquid egg white. On the day of production, all samples showed high L\* and slight green -a\* and yellow tones, with higher protein concentrations leading to reduced lightness and increased opacity due to protein aggregation. Over storage, L\* values consistently declined, particularly in the 10% protein

sample, reflecting progressive darkening and protein structural changes.  $a^*$  values became more negative across all samples, indicating a green shift likely due to oxidation.  $b^*$  values increased, especially in higher protein concentrations, suggesting enhanced yellowness from protein degradation and possible Maillard reactions. By Day 21, while some stabilization occurred, the highest protein samples continued to exhibit pronounced darkening, green shifts, and yellow color persistence, confirming that higher protein levels accelerate structural and oxidative changes during storage.

At the beginning, the control sample displayed high brightness with a slight green tint and mild yellowness, typical for pasteurized egg white. Increased protein concentrations reduced lightness, especially in the 10% protein sample where  $L^*$  value was  $72.99 \pm 0.64$ , accompanied by a strong green shift with  $a^*$  of  $-5.34 \pm 0.62$  and higher  $b^*$  of  $7.90 \pm 0.23$ , due to protein aggregation and early Maillard reactions. By Day 7, all samples showed a significant drop in  $L^*$ , reflecting increased opacity from protein denaturation. The 10% sample's  $a^*$  further decreased to  $-6.37 \pm 0.24$ , indicating intensified oxidation, while  $b^*$  values fluctuated, suggesting interactions affecting yellow color formation. On Day 14,  $L^*$  values slightly recovered, but green shifts and elevated  $b^*$  values persisted, particularly in higher protein samples, highlighting ongoing oxidation and browning reactions. By Day 21,  $L^*$  values increased further, suggesting stabilized protein interactions, while  $a^*$  and  $b^*$  values plateaued, indicating that oxidative changes and color development had reached equilibrium.



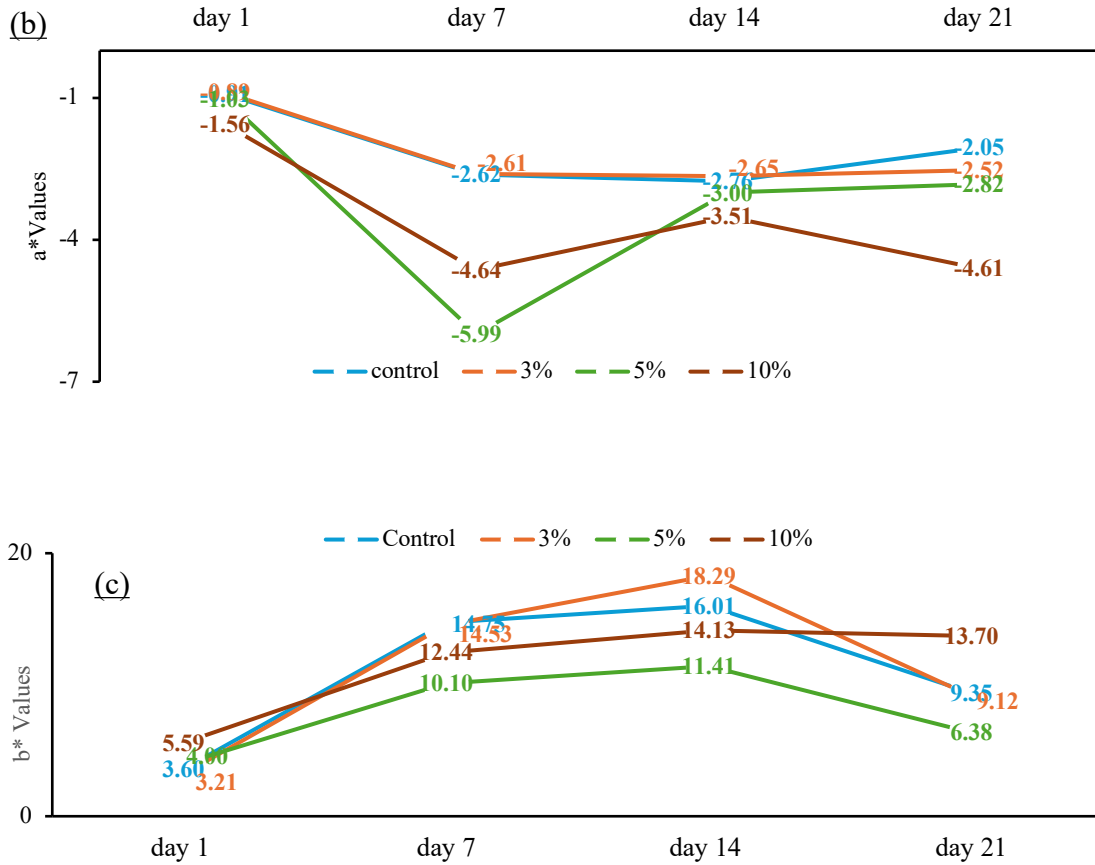


Figure 32: The effect of adding different percentages of powdered egg protein to liquid egg white and pasteurizing at 55 °C and on: (a) L\* values, (b) a\* values, (c) b\* values in comparison to the control group and for a storage period of 21 days.

At the beginning, the control sample displayed high brightness with a slight green tint and mild yellowness, typical for pasteurized egg white. Increased protein concentrations reduced lightness, especially in the 10% protein sample where L\* value was  $72.99 \pm 0.64$ , accompanied by a strong green shift with a\* of  $-5.34 \pm 0.62$  and higher b\* of  $7.90 \pm 0.23$ , due to protein aggregation and early Maillard reactions. By Day 7, all samples showed a significant drop in L\*, reflecting increased opacity from protein denaturation. The 10% sample's a\* further decreased to  $-6.37 \pm 0.24$ , indicating intensified oxidation, while b\* values fluctuated, suggesting interactions affecting yellow color formation (Llave et al., 2018). On Day 14, L\* values slightly recovered, but green shifts and elevated b\* values persisted, particularly in higher protein samples, highlighting ongoing oxidation and browning reactions. By Day 21, L\* values increased further, suggesting stabilized protein interactions, while a\* and b\* values plateaued, indicating that oxidative changes and color development had reached equilibrium (Llave et al., 2018)

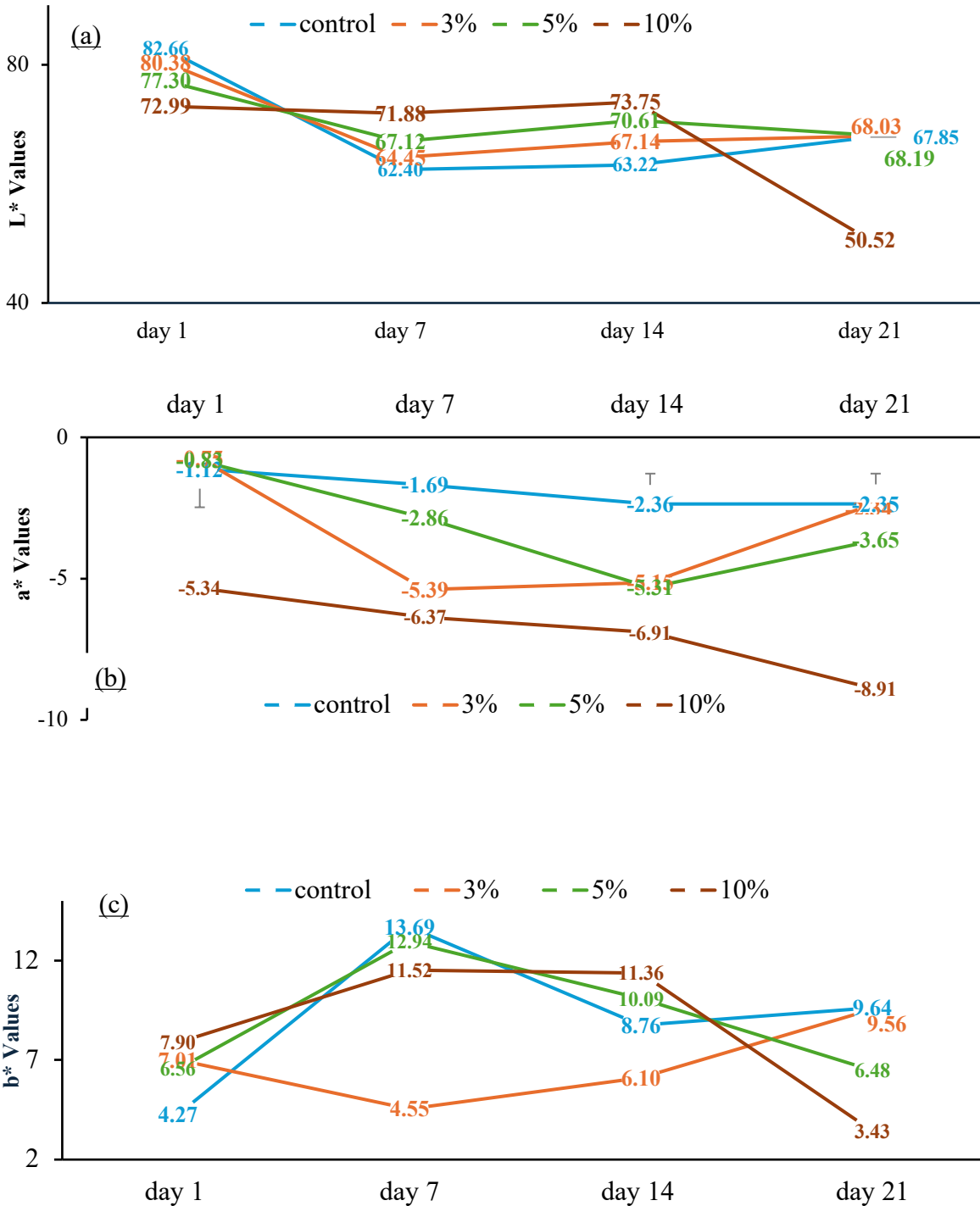


Figure 33: The effect of adding different percentage of powdered egg protein to liquid egg white and pasteurizing at 60 °C and on: (a) L\* values, (b) a\* values, (c) b\* values in comparison to the control group and for a storage period of 21 days

#### 4.1.1.6 Changes rheological properties for liquid egg white

The rheological properties of liquid egg white pasteurized at 50°C and stored for 21 days are shown in table 6 and demonstrated progressive changes in viscosity, flow behavior, and structural stability, influenced by protein concentration and storage duration. Initially, on Day 1, the control sample exhibited low  $\tau_0$  of  $0.011 \pm 0.001$  Pa, moderate K of  $0.424 \pm 0.021$ , and shear-thinning behavior where n was  $0.509 \pm 0.011$ . The 3% protein sample showed slightly higher yield stress of  $\tau_0$   $0.133 \pm 0.012$  but lower K of  $0.103 \pm 0.001$ , suggesting minor structural modifications without significant thickening. In contrast, the 5% and 10% protein samples displayed a substantial increase in K reaching  $2.294 \pm 0.234$  and  $2.476 \pm 0.545$  respectively with stronger shear-thinning, indicating increased protein interactions. By Day 7, the control sample developed a higher  $\tau_0$  reaching  $0.083 \pm 0.001$  and became more Newtonian-like where n is  $0.937 \pm 0.341$ , suggesting some stabilization in structure. The 3% protein sample exhibited a significant increase in n reaching  $1.332 \pm 0.231$ , indicating structural rearrangements that reduced viscosity, while the 5% and 10% protein samples continued showing strong shear-thinning behavior with n values of  $0.631 \pm 0.011$  and  $0.457 \pm 0.023$  respectively, indicating enhanced protein aggregation. By Day 14, the control remained stable with n of  $0.925 \pm 0.431$ , while the 5% and 10% samples reached their peak structural stability, with high yield stress of  $\tau_0 = 0.993 \pm 0.001$  in 5% and increased K value of  $1.492 \pm 0.403$  and  $1.321 \pm 0.245$  respectively, confirming stronger protein-protein interactions (Ahmed et al., 2007). However, by Day 21, the control sample exhibited a decrease in yield stress and viscosity, indicating partial structural breakdown over prolonged storage. The 3% sample retained its Newtonian-like properties where n  $1.257 \pm 0.651$ , while the 5% and 10% samples displayed a minor decrease in viscosity where K was  $1.590 \pm 0.672$  –  $2.057 \pm 0.891$  respectively. These results suggest that higher protein concentrations enhance viscosity and structural stability initially but may lead to partial destabilization over extended storage. The observed trends confirm that protein aggregation and molecular interactions significantly impact the flow behavior of stored liquid egg white (Kumbár, Nedomová, et al., 2015).

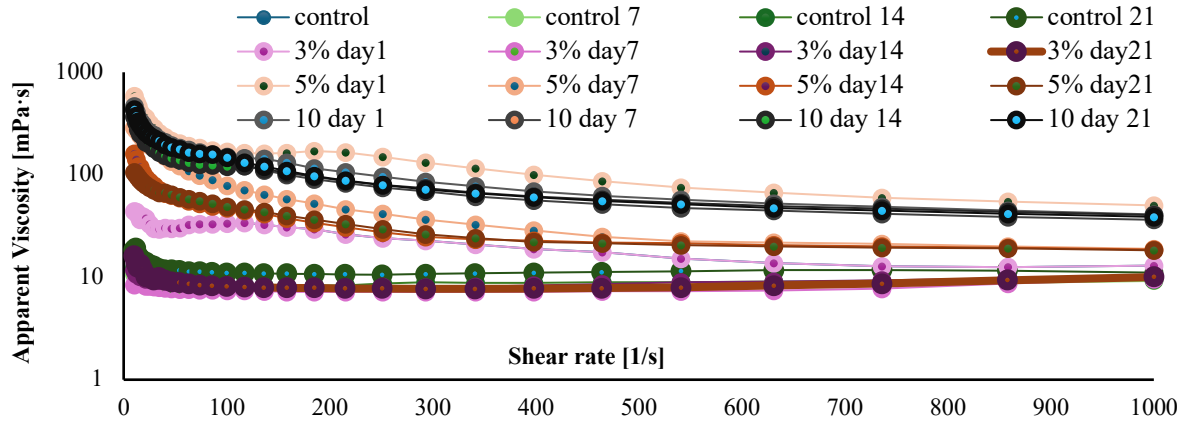


Figure 34: The viscosity curve (shear rate vs. apparent viscosity) of added different concentration of egg white protein to liquid egg white sample at 50 °C and stored for 21 days.

Table 6: The effect of adding egg white protein to liquid egg white on actual and measured results of Herschel-Bulkley model in comparison to control at 50 °C different letters differ significantly in comparison to control groups (Tukey's  $p < 0.05$ ).

Sample	$\tau_0$ (Pa)	$K(\text{Pa} \cdot \text{s}^n)$	$n$ (-)
control day 1	0.011±0.001	0.424±0.021	0.509±0.011
3%day1	0.133±0.012	0.103±0.011	0.782±0.001
5%day1	0.012±0.001	2.294±0.234	0.349±0.001
10%day1	0.001±0.001	2.476±0.545	0.422±0.001
control day 7	0.083±0.001	0.107±0.021	0.937±0.341
3%day7	0.220±0.001	0.001±0.001	1.332±0.231
5%day7	0.401±0.001	0.393±0.011	0.631±0.001
10%day7	0.001±0.001	1.669±0.789	0.457±0.023
control day 14	0.085±0.001	0.044±0.011	0.925±0.431
3%day14	0.194±0.001	0.003±0.001	1.183±0.512
5%day14	0.993±0.301	1.492±0.403	0.492±0.043
10%day14	0.001±0.001	1.321±0.245	0.509±0.019
control day 21	0.022±0.001	0.400±0.001	0.738±0.013
3% day 21	0.154±0.001	0.002±0.001	1.257±0.651
5% day 21	0.327±0.001	1.590±0.672	0.421±0.001
10% day 21	0.001±0.001	2.057±0.891	0.434±0.001

Table 7 shows the rheological properties of liquid egg white pasteurized at 55°C and stored for 21 days which exhibited distinct differences compared to samples pasteurized at 50°C, in  $\tau_0$ ,  $K$ , and flow behavior index. On day 1, the control sample at 55°C exhibit a  $\tau_0$  of 0.011±0.001,  $K$  of 0.597±0.001,  $n$  of 0.439±0.001 had slightly higher viscosity but a lower flow index compared to the 50°C control, suggesting stronger shear-thinning behavior. The 3% added protein sample at 55°C exhibited a higher yield stress than at 50°C, indicating enhanced protein interactions due to

increased pasteurization temperature. The 5% and 10% added protein samples at 55°C displayed moderate viscosity where K was  $0.738\pm 0.021$  and  $0.453\pm 0.002$  respectively, with lower n values of  $0.658\pm 0.002$  and  $0.620\pm 0.001$  respectively. By Day 7, both 5% and 10% added protein samples at 55°C experienced a dramatic increase in  $\tau_0$  reaching  $2.101\pm 0.501$  and  $2.201\pm 0.301$  respectively, which was higher than at 50°C, confirming that pasteurization at a higher temperature led to more rapid network formation (Abbasnezhad et al., 2014). The control sample at 55°C and the 3% sample maintained more Newtonian-like properties with n values of  $0.965\pm 0.051$  and  $1.204\pm 0.022$  respectively, while the 10% sample exhibited strong gel-like behavior, unlike at 50°C where structural formation was more gradual. By Day 14, the 5% protein sample at 55°C began to show network breakdown, suggesting that while the higher pasteurization temperature promoted early structural formation, it also led to earlier partial destabilization. Meanwhile, the 10% sample at 55°C retained its structural integrity but showed lower viscosity than at 50°C, indicating that pasteurization at 55°C modified protein interactions differently over time (Atilgan & Unluturk, 2008). By day 21, the control and 3% protein samples retained Newtonian-like behavior, whereas the 5% sample showed reduced yield stress where gel strength persisted longer (Lee et al., 2024). The 10% sample at 55°C exhibited an increase in viscosity compared to earlier days, indicating stronger resistance to breakdown than the 50°C counterpart. These results suggest that pasteurization at 55°C accelerates protein network formation, resulting in earlier gelation, by day 7, whereas at 50°C, structural changes occur more gradually, leading to continued aggregation up to day 14 before eventual stabilization. However, while higher pasteurization temperatures induce faster gel-like behavior, they also appear to cause partial breakdown of the network in mid-storage, particularly in the 5% protein sample. Overall, 50°C pasteurization promotes a slower, more progressive structural evolution, while 55°C causes rapid network formation that stabilizes sooner but exhibits different breakdown trends over time (Deng et al., 2025; Kumbár, Nedomová, et al., 2015).

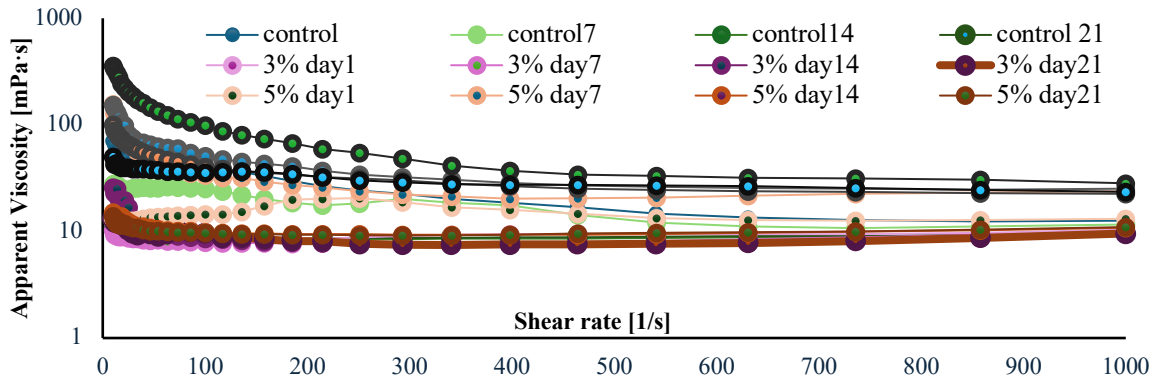


Figure 35: The viscosity curve (shear rate vs. apparent viscosity) of added different concentration of egg white protein to liquid egg white sample at 55 °C and stored for 21 days.

Table 7: The effect of adding egg white protein to liquid egg white on actual and measured results of Herschel-Bulkley model in comparison to control at 55 °C different letters differ significantly in comparison to control groups (Tukey's  $p < 0.05$ ).

Sample	$\tau_0$ (Pa)	$K(\text{Pa}\cdot\text{s}^n)$	$n$ (-)
control day 1	0.011±0.001	0.597±0.001	0.439±0.001
3%day1	0.315±0.001	0.004±0.001	1.124±0.001
5%day1	0.012±0.002	0.738±0.021	0.658±0.002
10%day1	0.461±0.001	0.453±0.001	0.620±0.001
control day 7	0.102±0.001	0.040±0.001	0.965±0.051
3%day7	0.144±0.001	0.003±0.001	1.204±0.022
5%day7	2.101±0.501	0.009±0.001	1.162±0.041
10%day7	2.201±0.301	0.274±0.001	0.700±0.001
control day 14	0.171±0.002	0.014±0.001	1.006±0.432
3%day14	0.231±0.004	0.002±0.001	1.222±0.361
5%day14	0.072±0.001	0.024±0.001	0.964±0.044
10%day14	1.252±0.082	0.214±0.001	0.721±0.012
control day 21	0.176±0.001	0.011±0.001	1.003±0.043
3%day 21	0.240±0.001	0.002±0.001	1.255±0.103
5%day 21	0.079±0.001	0.008±0.001	1.075±0.071
10% day 21	0.248±0.001	0.631±0.201	0.614±0.001

The rheological properties of liquid egg white with added egg white protein, pasteurized at 60°C and stored for 21 days is shown in table 8, it shown that samples exhibited a significant difference compared to 50°C and 55°C pasteurized samples. The higher pasteurization temperature led to stronger and faster protein network formation, especially in higher protein concentrations of 5% and 10%, where aggregation was more pronounced. On day 1, the control sample at 60°C exhibit a  $\tau_0$  of 0.001±0.001, K of 1.774±0.045, and n of 0.356±0.025 which is a higher viscosity and stronger shear-thinning behavior compared to the 50°C and 55°C

samples. The 3% protein sample had a higher yield stress than at 50°C and 55°C, indicating stronger initial structural formation.

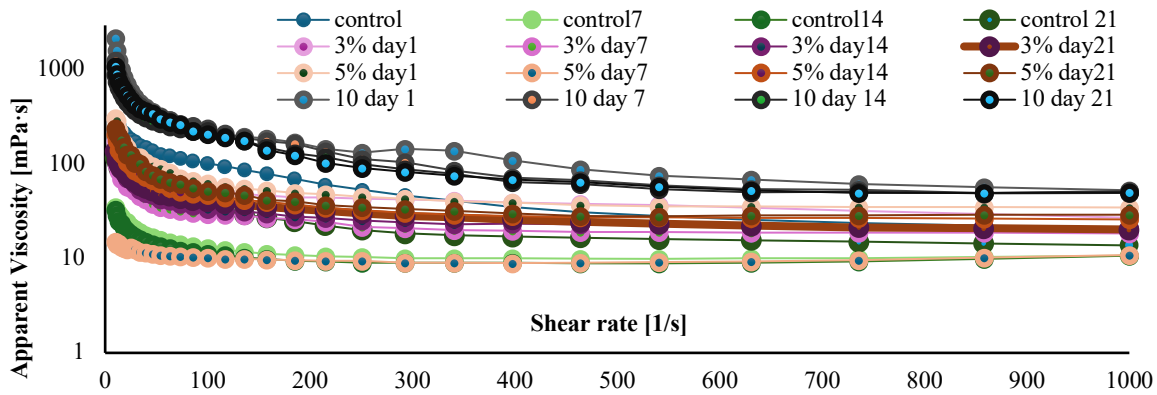


Figure 36: The viscosity curve (shear rate vs. apparent viscosity) of added different concentration of egg white protein to liquid egg white sample at 60 °C and stored for 21 days.

Similarly, the 5% sample at 60°C with  $\tau_0$  of  $1.864 \pm 0.198$ , had significantly higher yield stress than at 50°C and 55°C, showing that pasteurization at 60°C facilitated early gelation (Abbasnezhad et al., 2014). The 10% sample at 60°C had a  $\tau_0$  of  $5.899 \pm 0.651$ , K of  $3.560 \pm 0.712$ , and n of  $0.368 \pm 0.007$ , exhibited exceptionally high yield stress and viscosity, much greater than at 50°C and 55°C, indicating rapid protein aggregation. By day 7, the 10% protein sample at 60°C showed a substantial decrease in yield stress and an increase in viscosity, indicating early gel restructuring, unlike at 50°C and 55°C, where yield stress continued to increase. By day 14, the control sample at 60°C remained stable, while the 10% protein sample had a  $\tau_0$  of  $6.510 \pm 0.959$ , K of  $1.658 \pm 0.769$ , and n of  $0.474 \pm 0.001$ , which is a very high yield stress, significantly higher than at 50°C and 55°C, confirming that higher pasteurization temperatures resulted in more rigid protein structures. By day 21, the 10% protein sample at 60°C displayed the highest yield stress across all temperatures, indicating that the protein network was highly resistant to breakdown. The 5% sample at 60°C had a  $\tau_0$  of  $2.270 \pm 0.771$  which is higher yield stress than at 55°C and 50°C, showing that protein network formation was stronger at 60°C. Compared to 50°C and 55°C pasteurization, 60°C led to faster and stronger gelation, particularly in 5% and 10% protein samples, where structural changes occurred earlier and stabilized into a highly rigid gel by day 21. Unlike at 50°C and 55°C, where protein aggregation was more gradual and exhibited partial breakdown over time, the 60°C samples maintained their strong protein network, suggesting that higher temperatures enhance cross-linking and aggregation.

Table 8: The effect of adding egg white protein to liquid egg white on actual and measured results of Herschel-Bulkley model in comparison to control at 60 °C different letters differ significantly in comparison to control groups (Tukey's  $p < 0.05$ ).

Sample	$\tau_0$ (Pa)	$K(\text{Pa}\cdot\text{s}^n)$	$n$ (-)
control day 1	0.001±0.001a	1.774±0.045a	0.356±0.025a
3%day1	0.916±0.001b	0.146±0.001	0.787±0.091b
5%day1	1.864±0.198b	0.087±0.001	0.899±0.031b
10%day1	5.899±0.213c	3.560±0.712d	0.368±0.007
control day 7	0.613±0.111	0.220±0.001	0.855±0.013
3%day7	1.122±0.301b	0.015±0.001	1.041±0.051c
5%day7	0.309±0.001	0.003±0.001	1.195±0.027c
10%day7	0.086±0.001	6.608±0.389c	0.276±0.008
control day 14	0.834±0.001	0.043±0.001	0.956±0.014
3%day14	1.280±0.063b	0.029±0.001	0.968±0.001
5%day14	1.953±0.001b	0.031±0.001	0.964±0.023
10%day14	6.510±0.959	1.658±0.769b	0.474±0.001
control day 21	0.365±0.001	1.333±0.460b	0.476±0.001
3%day 21	0.777±0.001b	0.104±0.001	0.777±0.021
5%day 21	2.270±0.771c	0.014±0.001	1.079±0.033c
10%day 21	11.081±1.285d	0.240±0.001	0.808±0.034

#### 4.1.2 The effect of adding whey isolate protein on liquid whole egg properties

##### 4.1.2.1 change in pH values

This is a pilot study to evaluate whether the addition of whey protein could improve the functional properties of liquid whole eggs. Based on the findings of this experiment which provided insights into the potential benefits of fortification, subsequent trials were conducted using higher whey protein levels (3%, 5%, and 10%) to achieve a greater degree of protein enrichment and to further enhance the targeted techno-functional properties. The pH values of liquid whole egg with added whey protein show a decrease as the whey protein concentration increases, indicating an acidifying effect on the system. The control sample, without whey protein, had the highest pH of  $7.5 \pm 0.1$ , while samples with 1%, 2%, and 3% whey protein exhibited decreasing pH values of  $7.4 \pm 0.1$ ,  $7.2 \pm 0.2$ , and  $7.2 \pm 0.2$ , respectively. This trend suggests that whey protein addition disrupts the natural buffering capacity of egg proteins, leading to a shift toward lower pH values. Although the reduction was not big, still it was statistically significant in all the added protein samples. The acidic nature of whey protein itself plays a major role in this decrease, as whey proteins contain residual lactic acid and lactose from milk processing, both of which contribute to an overall reduction in pH (Chandrapala et al., 2015). Additionally, whey proteins introduce amino acids and peptides that interact with the egg's natural buffering system, altering its ionic

equilibrium and further lowering the pH. The effect becomes more pronounced at higher whey protein concentrations, where greater amounts of acidic amino acids, such as glutamic and aspartic acid, release hydrogen ions into the solution, intensifying the pH reduction (Chandrapala et al., 2015).

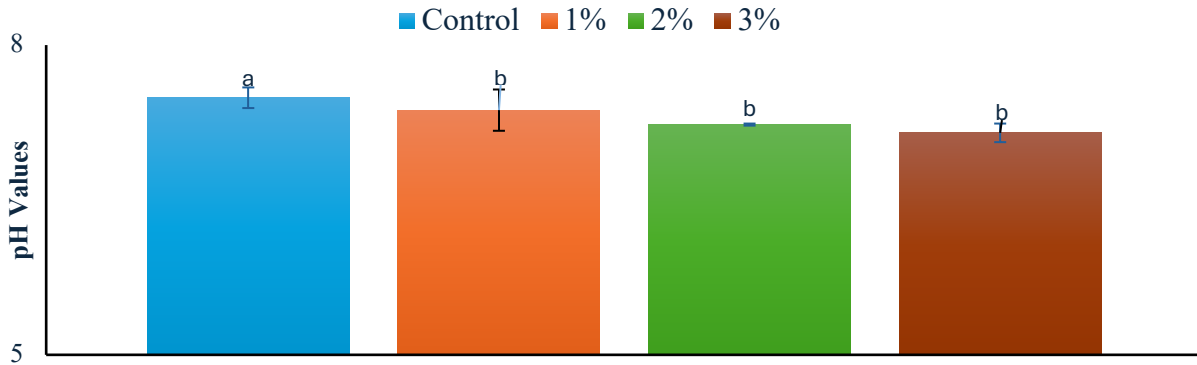


Figure 37: The effect of adding different percentage of powdered whey egg protein to liquid egg whole in comparison to the control group, different letters differ significantly in comparison to the control group, Tukey HSD  $p < 0.05$ .

#### 4.1.2.2 Change in color parameters

$L^*$ ,  $a^*$ ,  $b^*$  of liquid whole egg with added whey protein indicates significant changes in lightness, red-green balance, and yellow-blue intensity as whey protein concentration increases. The control sample scored a  $L^*$  of  $64.60 \pm 32$ ,  $a^*$  of  $-0.88 \pm 0.01$ , and  $b^*$  of  $20.12 \pm 0.02$ , and exhibited the highest  $L^*$ , a nearly neutral red-green balance, and a moderate yellow intensity. However, as whey protein was added,  $L^*$  decreased, indicating a darkening effect, with the 3% whey protein sample, with  $L^*$  of  $57.61 \pm 0.34$ , being the darkest. This decrease in  $L^*$  can be attributed to the increased protein interactions, which affect light scattering, causing reduced reflectance and darker appearance. The  $a^*$  values increased from slightly negative in the control to positive values, with the 3% whey sample showing the strongest red shift where  $a^*$  was  $2.02 \pm 0.32$ . This suggests that whey protein addition introduced color changes that enhanced the red hue, possibly due to the influence of residual milk proteins or Maillard reaction interacting with egg components. The  $b^*$  values increased initially but then slightly decreased at higher concentrations. This indicates that whey protein initially enhanced yellow intensity but may have altered pigment dispersion at higher concentrations, reducing the intensity of yellow tones (Lonchamp et al., 2022).

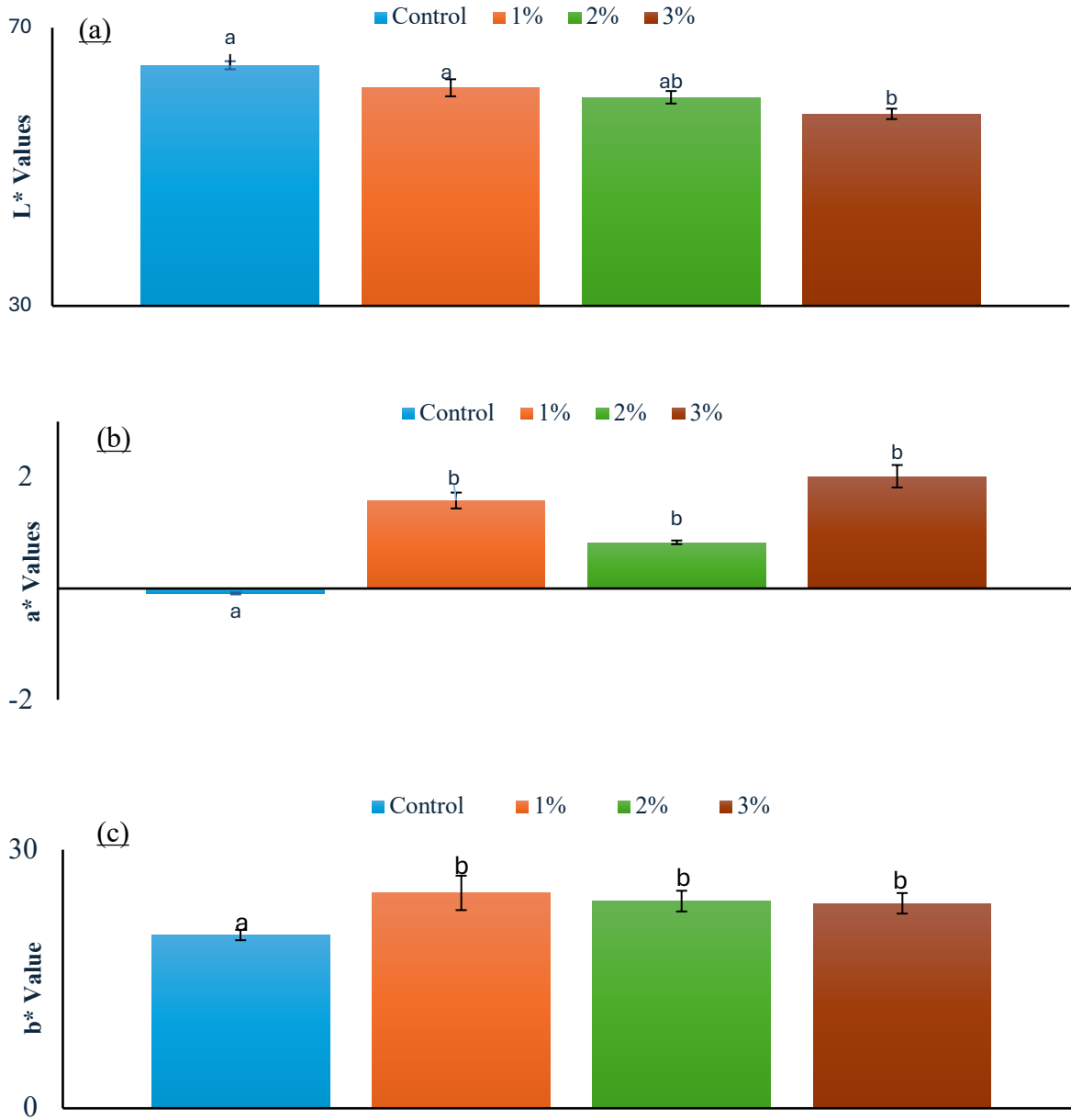


Figure 38: The effect of adding different percentage of powdered whey protein to liquid whole egg on: (a) L\* values, (b) a\* values, (c) b\* values in comparison to the control group.

These trends are likely due to changes in protein aggregation, light absorption, and molecular interactions between whey and egg proteins, which influence how color is perceived (Razi et al., 2023). The overall findings suggest that adding whey protein significantly alters the color characteristics of liquid whole egg, with darker, redder, and slightly more yellow hues developing as whey concentration increases.

#### 4.1.2.3 Change in rheological properties

The rheological properties of liquid whole egg with added whey protein were analyzed in terms of  $\tau_0$ , K, and n in table 9. The results indicate variations in viscosity and flow behavior as whey protein concentration increases. The control sample exhibit a  $\tau_0$  of  $0.109 \pm 0.001$ , K of  $0.007 \pm 0.001$ , and n of  $1.049 \pm 0.022$  had the highest yield stress and a slightly shear-thinning behavior ( $n > 1$ ), indicating a relatively stable protein network that resists flow. However, as whey protein was added,  $\tau_0$  fluctuated, with whey 1% decreased to  $0.063 \pm 0.002$ , suggesting weaker structural integrity compared to the control. The whey 2% sample had a  $\tau_0$  of  $0.097 \pm 0.001$ , and had a yield stress closer to the control, while whey 3% had a  $\tau_0$  of  $0.075 \pm 0.007$  with a slight reduction again, indicating possible disruptions in protein interactions due to increasing whey concentrations. All reductions were statistically significant in comparison to the control group. K, on the other hand, increased progressively from  $0.007 \pm 0.001$  in the control group, to  $0.013 \pm 0.001$  in the sample of 3% added whey protein, indicating that whey protein contributes to thickening. This suggests that higher whey protein concentrations increase molecular interactions, resulting in more structured, viscous liquid (Quevedo et al., 2021).

Table 9: The effect of adding whey protein to liquid whole egg on actual and measured results of Herschel-Bulkley model in comparison to control, different letters differ significantly in comparison to control group (Tukey's  $p < 0.05$ ).

Sample	$\tau_0$ (Pa)	K(Pa·s <sup>n</sup> )	n (-)
control	$0.109 \pm 0.001$ a	$0.007 \pm 0.001$ a	$1.049 \pm 0.033$ a
Whey 1%	$0.063 \pm 0.002$ b	$0.008 \pm 0.002$ a	$1.040 \pm 0.022$
Whey 2%	$0.097 \pm 0.001$ c	$0.011 \pm 0.0018$ b	$1.017 \pm 0.021$ b
Whey 3%	$0.075 \pm 0.007$ b	$0.013 \pm 0.001$ b	$1.012 \pm 0.012$ b

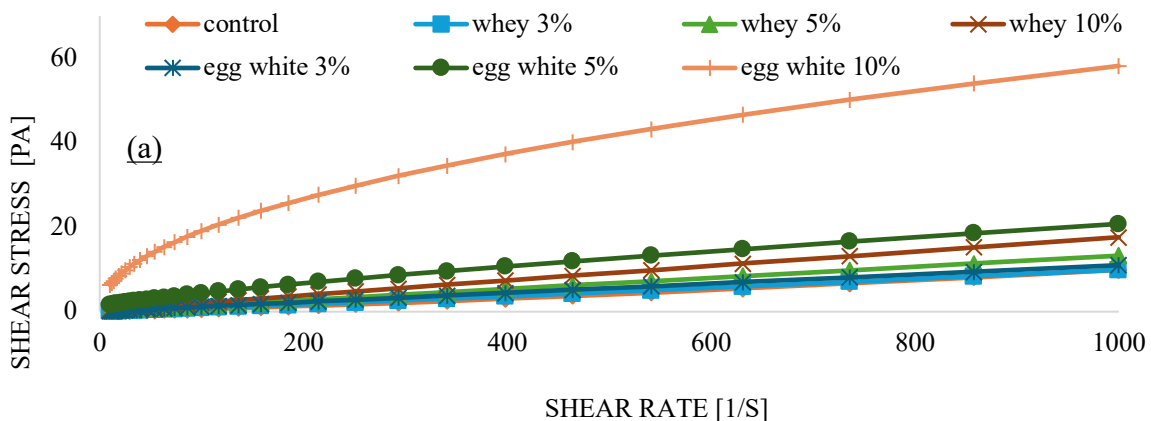
The increase in K value was significant in all groups. The flow behavior index (n) remained close to 1 in all samples, indicating that the samples exhibit nearly Newtonian behavior, meaning their viscosity remains relatively stable under shear. However, the control sample, with n of 1.049, showed slightly more shear-thinning properties than the whey samples, suggesting that the original egg protein matrix was more structured and resistant to shear compared to samples with whey protein. The changes in n were significant only in case of 2 and 3% added whey protein (Elayan et al., 2025).

#### 4.1.3 A comparison between the effect of adding whey protein and egg white protein to liquid whole egg rheological properties

Rheological properties are essential for understanding high-protein liquid whole egg behavior, because they directly influence critical aspects of food processing and product development. In processes like extrusion, rheology affects fluid flow, pump selection, equipment design, and the adaptation of raw materials to meet specific processing and product design requirements (McKenna & Lyng, 2003). Proteins, which exhibit both elastic and viscous behavior, play an important role in determining the texture, mouthfeel, and structural integrity of foods. By examining rheological behavior, researchers can better customize protein-based ingredients for improved performance and sensory attributes in food products (Ahmed et al., 2007).

Flow properties of protein added liquid whole eggs were shown to exhibit a pseudoplastic behavior, Figures 39 (a and b) shows the flow behavior for samples that were heat treated after adding proteins and those which were heat treated before respectively. The figures show the relationship between shear stress (Pa) on the y-axis and shear rate (1/s) on the x-axis.

Figure 39a shows that adding whey and egg white protein then heat treat the samples increases the shear stress which indicates that liquid whole egg is becoming thicker. At equivalent concentrations, egg white protein enriched samples show a higher shear stress than whey fortified samples this shows that egg white protein has a higher influence on the overall flow behavior of liquid whole egg making it more viscous and resistant to flow. Meanwhile, figure 39b shows that heat treatment prior to protein addition of liquid whole eggs with added proteins showed a Newtonian-like behavior, meaning that viscosity of the samples remains constant despite of the applied shear rate.



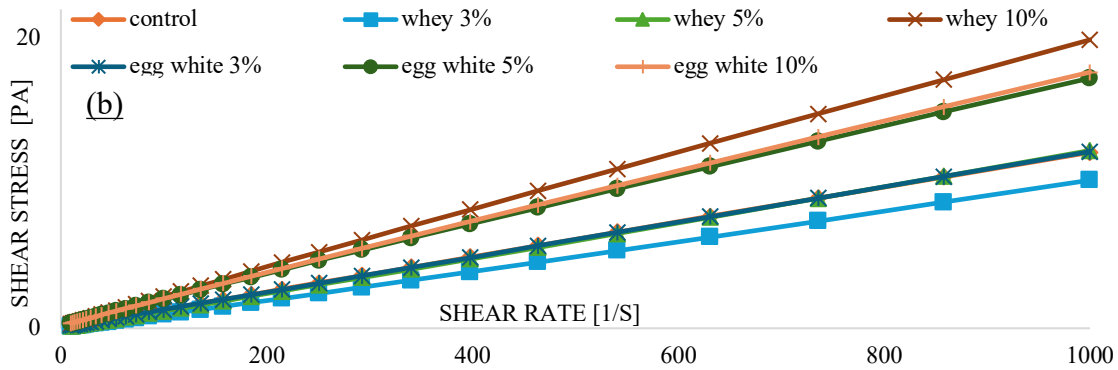


Figure 39: The flow curves of different concentrations of added whey and egg white protein to liquid whole egg: (a) pasteurizing after adding proteins at  $60 \pm 0.2^\circ\text{C}$  with 3.5 minutes of holding time; (b) pasteurizing before adding proteins at  $60 \pm 0.2^\circ\text{C}$  with 3.5 minutes of holding time.

Figure 40 (a and b) viscosity ( $\text{mPa}\cdot\text{s}$ ) on the y-axis against shear rate ( $1/\text{s}$ ) on the x-axis for samples that were heat treated after adding proteins and those which were heat treated before respectively. Figure 40a shows that samples viscosity decreases with increasing shear rate for all samples indicating a shear thinning behavior when proteins were added before heat treatment. Although all samples show a shear thinning behavior, the highest shear thinning behavior was seen in added 5% and 10% of egg white protein which starts with the highest viscosity at low shear rates and decreases significantly as the shear rate increases. As for figure 40b, it is clearly seen that whey protein samples exhibit almost a Newtonian behavior where viscosity remind constant with the increase of shear rate. Meanwhile egg white protein samples showed a thinning behavior which was more obvious with the increase in egg white protein concentrations and almost identical to the control group behavior in sample with added 3% of egg white protein.

The flow behavior and viscosity data collected experimentally align well with the calculated values using the Herschel-Bulkley model. This alignment confirms that the samples exhibit the predicted flow behavior characteristics, validating the Herschel-Bulkley model's accuracy in describing these non-Newtonian properties.

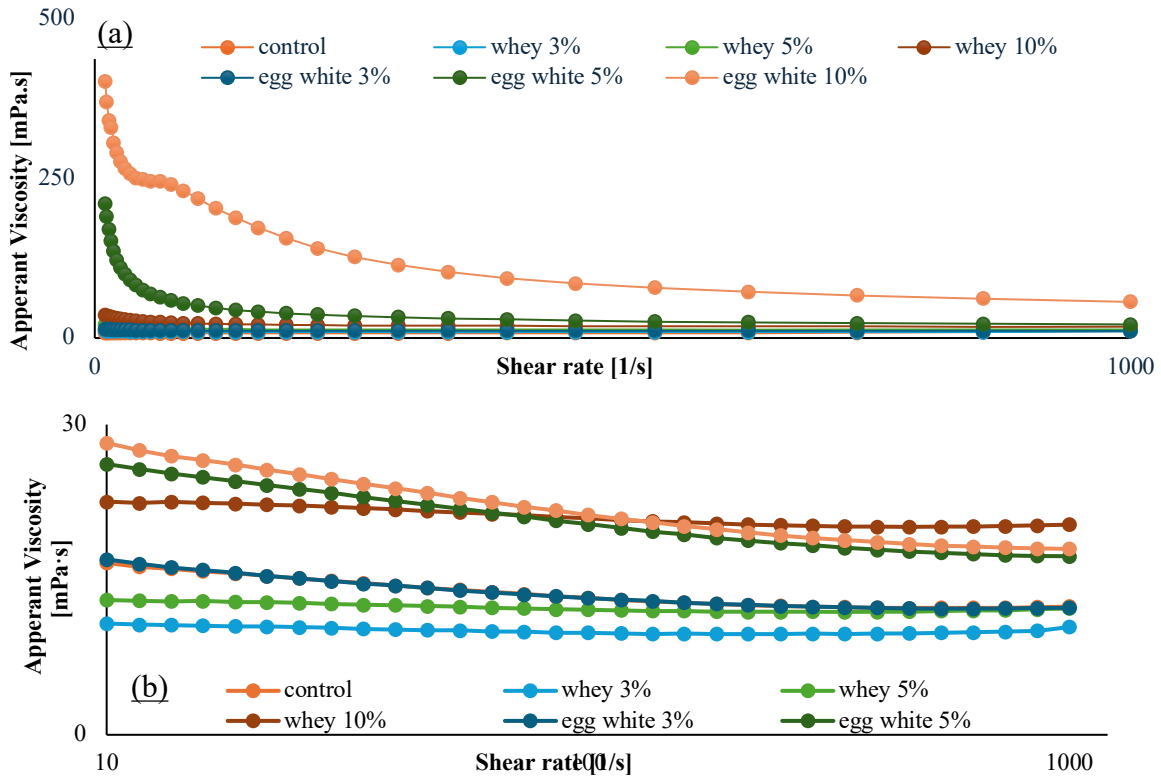


Figure 40: The viscosity curves (shear rate vs. apparent viscosity) of different concentrations of added whey and egg white protein to liquid whole egg: (a) pasteurizing after adding proteins at  $60 \pm 0.2^\circ\text{C}$  with 3.5 minutes of holding time; (b) pasteurizing before adding proteins at  $60 \pm 0.2^\circ\text{C}$  with 3.5 minutes of holding time.

Table 10 shows the Herschel-Bulkley model parameters describing the flow behavior of samples that pasteurized after adding the proteins at  $60 \pm 0.2^\circ\text{C}$  with 3.5 minutes of holding time.  $\tau_0$ ,  $K$ , and  $n$  for all samples were calculated to evaluate the flow behavior in reference to a control group with no added proteins.  $\tau_0$  for control group was  $0.108 \pm 0.044$  which is the lowest amount of stress needed to start flowing,  $\tau_0$  started to increase with the increase of added powered proteins percentages whether it was whey protein or egg white protein.  $\tau_0$  shifted from  $0.108 \pm 0.044$  for the control group to  $0.083 \pm 0.062$ ,  $0.134 \pm 0.008$  and  $0.204 \pm 0.006$  respectively for 3, 5, 10% added whey protein samples, all these changes were insignificant ( $p > 0.05$ ) in comparison to control group. on the other hand,  $\tau_0$  shifted to  $0.061 \pm 0.076$ ,  $1.088 \pm 0.068$  and  $1.937 \pm 0.057$  respectively for 3, 5, 10% added egg white protein samples, the increment was significant when it comes to 5, and 10% added egg white protein but insignificant ( $p > 0.05$ ) decrement was observed at 3% added egg white protein. Regarding consistency index ( $K$ ) which reflexes thickness or viscosity of a fluid, it moved from  $0.001 \pm 0.001$  to  $0.004 \pm 0.001$ ,  $0.013 \pm 0.002$ ,  $0.022 \pm 0.005$  Pa.s $n$  respectively

for 3, 5, 10% added whey protein samples and  $0.011 \pm 0.002$ ,  $0.097 \pm 0.045$ ,  $2.064 \pm 0.002$  Pa.s<sup>n</sup> respectively for 3, 5, 10% added egg white protein samples, this shift in K was only significant in case of 5% and 10% added egg white protein. This change indicates that with the addition of both proteins increased the viscosity of liquid whole eggs. As for the flow behavior index (n), it shifted from  $1.296 \pm 0.054$  for the control group, which shows a shear thickening behavior, to  $1.117 \pm 0.031$ ,  $0.997 \pm 0.016$ ,  $0.969 \pm 0.029$  respectively for 3, 5, 10% added whey protein showing a more of a pseudoplastic behavior, this decrease was significant in case of 10% but insignificant ( $p > 0.05$ ) for 5% and 3% added whey protein. On the other hand, calculated n value for 3, 5, 10% added egg white dropped significantly to  $0.994 \pm 0.023$ ,  $0.783 \pm 0.084$ ,  $0.483 \pm 0.021$  which shows a strong shear thinning behavior.

Table 10: Measured results of Herschel-Bulkley model at different concentrations of added whey and egg white protein after pasteurizing at  $60 \pm 0.2^\circ\text{C}$  with 3.5 minutes of holding time in comparison to the liquid whole egg control group, different letters differ significantly in comparison to control group (Tukey's  $p < 0.05$ ).

Sample	$\tau_0$ (Pa)	K(Pa·s <sup>n</sup> )	n (-)
control	$0.108 \pm 0.044a$	$0.001 \pm 0.001a$	$1.296 \pm 0.054a$
3% whey protein	$0.083 \pm 0.062a$	$0.004 \pm 0.001a$	$1.117 \pm 0.031a$
5% whey protein	$0.134 \pm 0.008a$	$0.013 \pm 0.002a$	$0.997 \pm 0.016$
10% whey protein	$0.204 \pm 0.006a$	$0.022 \pm 0.005a$	$0.969 \pm 0.029b$
3% egg white protein	$0.061 \pm 0.076a$	$0.011 \pm 0.002a$	$0.994 \pm 0.023b$
5% egg white protein	$1.088 \pm 0.068b$	$0.097 \pm 0.045b$	$0.783 \pm 0.084c$
10% egg white protein	$1.937 \pm 0.057c$	$2.064 \pm 0.002c$	$0.483 \pm 0.021c$

Table 11 shows the measured results of Herschel-Bulkley model at different concentrations of added whey and egg white protein to pasteurized liquid whole egg. Liquid whole egg was pasteurized at  $60 \pm 0.2^\circ\text{C}$  with 3.5 minutes of holding time then cooled down in an ice bath to  $4^\circ\text{C}$  after that left to rest in room temperature where proteins were added then measurement were made in comparison to the liquid whole egg control group.  $\tau_0$  started to increase with the increase of added powered proteins percentages whether it was whey protein or egg white protein. No significant difference was observed on  $\tau_0$  in comparison to the control group for 3, 5, 10% added whey protein samples. Meanwhile  $\tau_0$  changed significantly for 3, 5, 10% added egg white protein samples in comparison to control group.

As for K it was increasing for all added proteins percentages this increase was significant for 10% added whey protein, 3%,5% and 10% added egg white protein in comparison to the control group indicating that the mixture was tending to get more viscous with the increase of protein percentage.

On the other hand, n values showed an increasing trend in all added concentration except for 10% of added egg white protein, n slightly decreased. The changes in n values were minimal, with all samples exhibiting near-Newtonian behavior.

Table 11: Measured results of Herschel-Bulkley model at different concentrations of added whey and egg white protein to pasteurized liquid whole in comparison to the liquid whole egg control group, different letters differ significantly in comparison to control group (Tukey's  $p < 0.05$ ).

Sample	$\tau_0$ (Pa)	$K(\text{Pa}\cdot\text{s}^n)$	n (-)
control	0.031±0.035a	0.002 ±0.001a	0.986±0.022
3% whey protein	0.043±0.040a	0.007±0.001a	1.006±0.020
5% whey protein	0.053±0.003a	0.010±0.002a	1.030±0.010
10% whey protein	0.055±0.048a	0.026±0.013b	1.105±0.068
3% egg white protein	0.086 ± 0.006b	0.012±0.002b	1.008±0.013
5% egg white protein	0.114 ± 0.008b	0.023±0.001b	1.003±0.014
10%egg white protein	0.127± 0.004b	0.024±0.002b	0.960±0.001

The viscosity of liquids in general is influenced by several factors, including temperature, molecular structure, and the interactions between molecules (Ahmed et al., 2007). Usually as temperature increases the viscosity of a liquid decreases due to the fact that molecules line up easier allowing it to flow easier (Wenhao, 2021). Molecular structure also has a huge effect on viscosity, the more complex the structure is the more it resists to flow. This resistance can happen due to the strong intermolecular bond between the liquid molecules which increases the overall viscosity (McKenna & Lyng, 2003).

In the case of liquid whole egg, viscosity is primarily affected by the protein content and how these proteins interact with each other (Varga-Tóth et al., 2023). Proteins in the egg, such as ovalbumin and ovotransferrin, can form networks that trap water and increase resistance to flow, especially when subjected to heat (Wu & Acero-Lopez, 2012). This explanation is clearly seen in the results of  $\tau_0$ , it increased significantly with the increase of added proteins percentages and drastically increased when heat was applied after adding the protein. Adding different percentages of whey or egg white powdered proteins influences liquid whole egg viscosity. With the increase of protein content the viscosity increases, this is due to the several types of interactions of proteins that occur in the solution (Guha et al., 2019).

Whey protein solution shows near Newtonian behavior at low concentration when no heat treatment is applied, but tend to show shear thinning behavior at high concentration or when heat treatment near whey protein denaturation temperature is applied which ranges from 65 to above

70 °C. In this experiment whey protein was dissolved in liquid whole eggs then heated to 60°C, this temperature is a relatively mild heat treatment for whey protein. At this temperature setting, ovalbumin in egg whites and  $\beta$ -lactoglobulin in whey, may start to partially unfold leading to expose hydrophobic regions and reactive sites on the proteins, this could facilitate hydrophobic interactions and some degree of aggregation between whey and egg proteins, but the extent of bonding is limited at this temperature(Lonchamp et al., 2022).Another interaction that may affect the mixture properties is disulfide bonds, both egg and whey proteins contain sulfhydryl group, although that 60°C is not the optimal temperature for a disulfide cross-links to happen but a weak interaction can occur(Van Der Plancken et al., 2005).

The previous explaining and linkages occur also in this egg white protein added solution, but more factors are involved which causes a more severe results. Shifting from a shear thickening to a strong shear thinning behavior when adding egg white protein to liquid whole egg can be caused by crowding effect of the increase in total protein concentration (Yang & Foegeding, 2011). Although this crowding applies for samples with whey protein as well, but its effect is more significant in egg white protein case due to its high-water holding capacity of egg white proteins (Yang & Foegeding, 2011). When egg white protein powder is dissolved in liquid egg and heat-treated at 60°C, partial denaturation occurs, exposing polar regions that strongly bind water molecules. The reduction of free water in the mixture affects its flowing behavior and increases its viscosity (J. Li et al., 2020). Meanwhile as the concentration of egg white protein in liquid whole egg increases, and when combined with moderate heating, a weak gel-like structure may form. Although full gelation of egg proteins typically requires higher temperatures, partial aggregation and mild cross-linking of proteins can occur at these conditions (Gharbi & Labbafi, 2018). This results in a semi-structured network that is not a fully solid gel but behaves as a viscous, cohesive matrix, which resists flow and increases the overall thickness of the mixture. The shear thinning behavior which was clearly seen when high concentrations of egg white protein were added, is due to the denser and more interconnected protein network. As shear rate increases, the protein network aligns in the direction of flow, leading to a reduction in viscosity.

In regards of samples that were not heat treated, since no heat treatment was applied, the added proteins remained in their native form. In this state, protein-protein interactions were limited, as the hydrophobic and reactive regions of the proteins remained unexposed, preventing significant intermolecular interactions (Farjami et al., 2021; H. Li et al., 2021). Additionally, whey protein's

high solubility and low intrinsic viscosity in solution contribute to its minimal impact on viscosity. Although egg white protein is less soluble than whey protein, the concentrations used were insufficient to reach saturation or to induce notable changes in the viscosity profile of the mixture.

#### 4.1.4 The effect of adding different percentages of liquid egg yolk to liquid whole egg

##### 4.1.4.1 Change in pH

The pH values observed indicate that as the percentage of added liquid egg yolk increases, the overall pH of the liquid whole egg decreases progressively. The liquid whole egg without additional yolk, has a pH of  $7.43 \pm 0.19$ . This sample naturally contains both egg white and yolk in their standard ratio, with egg white being more alkaline and yolk slightly more acidic. When 20% more liquid egg yolk is added, the pH decreases to  $7.10 \pm 0.20$ , highlighting the acidic influence of the yolk diluting the alkalinity of the egg white present in the whole egg (Razi et al., 2023). As the yolk content increases to 50%, the pH drops further to  $7.05 \pm 0.23$ , showing a continued shift toward acidity. At 80% added yolk, the pH reaches  $6.70 \pm 0.13$ , approaching the pH of pure egg yolk, which is measured at  $6.40 \pm 0.23$ . This trend occurs because egg yolk naturally contains phospholipids, free fatty acids, and proteins with acidic amino acid residues, all of which contribute to its lower pH compared to the egg white (Su et al., 2015). The increasing concentration of these components leads to enhanced buffering against the alkalinity of the egg white, pulling the overall pH downward (Razi et al., 2023). Comparatively, the whole egg sample maintains the highest pH due to the dominance of egg white proteins, particularly albumen, which has a naturally higher pH. The consistent, stepwise decline in pH as more yolk is added is therefore a direct result of the acidic nature of the yolk progressively outweighing the alkaline components in the whole egg. The pronounced drop indicates that the structural and compositional differences between yolk and white, particularly the presence of lipids and acidic proteins in yolk, play a major role in determining the final pH of the mixture (Oladimeji & Gebhardt, 2023).

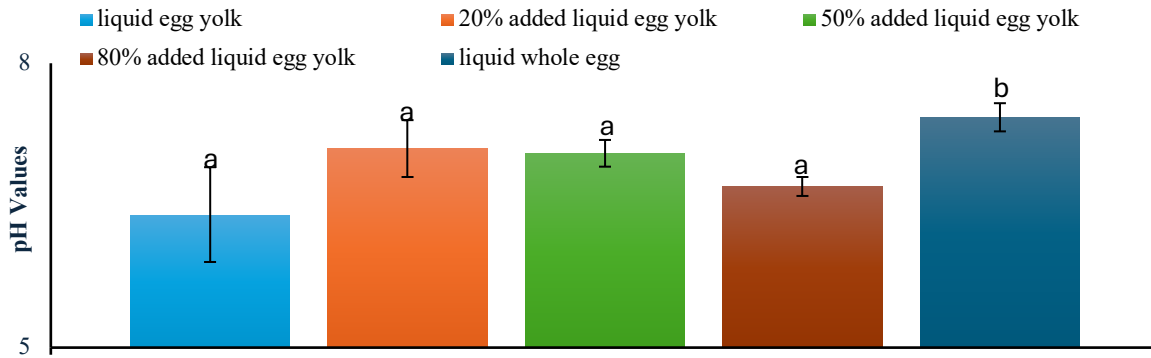


Figure 41: The effect of adding different percentage of liquid egg yolk to liquid egg whole pH in comparison to the control group, different letters differ significantly in comparison to control group, Tukey HSD  $p < 0.05$ .

#### 4.1.4.2 Change in color parameters

The color measurements  $L^*$ ,  $a^*$ ,  $b^*$  demonstrate noticeable changes as the percentage of added egg yolk decreases, with the 100% yolk sample serving as the control group. The control yolk sample shows an  $L^*$  value of  $63.0 \pm 1.03$ , indicating moderate brightness, an  $a^*$  value of  $3.2 \pm 0.03$ , and a  $b^*$  value of  $49.9 \pm 1.23$ , reflecting the strong yellow-orange color characteristic of egg yolk due to its high content of carotenoids and lipids.

When liquid whole egg is mixed with lower percentages of yolk, the color parameters shift (Szabó, & Kerti, 2007). At 80% yolk, the  $L^*$  value slightly decreases to  $68.9 \pm 0.99$ , showing increased brightness compared to the control, possibly due to dilution of the dense pigments and lipids by egg white components (L. De Souza et al., 2019). The  $a^*$  value drops to  $2.8 \pm 0.17$ , indicating a reduction in red tones, while the  $b^*$  value decreases slightly to  $47.6 \pm 1.25$ , showing a minor reduction in yellowness.

As added yolk percentage continues to decrease to 50% and 20%, these trends become more pronounced. The  $L^*$  values increase further to  $70.6 \pm 1.01$  and  $72.0 \pm 0.61$ , respectively, indicating greater lightness as more egg white is present, which has a higher reflectance. The  $a^*$  values reduce to  $2.4 \pm 0.16$  at 50% and  $1.5 \pm 0.21$  at 20%, moving further from red toward neutral. Similarly, the  $b^*$  values drop to  $46.7 \pm 0.73$  and  $41.6 \pm 1.12$ , reflecting a significant decrease in yellow intensity due to dilution of yolk pigments. The liquid whole egg sample, which has the least yolk, shows the lowest  $L^*$  value of  $61.2 \pm 0.12$ , the most negative  $a^*$  value of  $-2.7 \pm 0.23$  shifting to green and a  $b^*$  value of  $31.4 \pm 1.01$ , the lowest yellow intensity. This clearly indicates that as the yolk

concentration decreases, the samples become lighter, less red, and less yellow. In comparison to the yolk control, the changes are significant. The reduction in yolk percentage leads to a consistent decrease in redness and yellowness, directly related to the dilution of yolk pigments and lipids. Additionally, the increased lightness in the intermediate samples (especially 20% and 50% yolk) reflects the influence of egg white proteins and water content, which scatter more light and reduce the visual impact of yolk color compounds. Therefore, the decrease in yolk concentration significantly alters the color profile, making the samples lighter, less red, and less yellow compared to the control yolk sample.

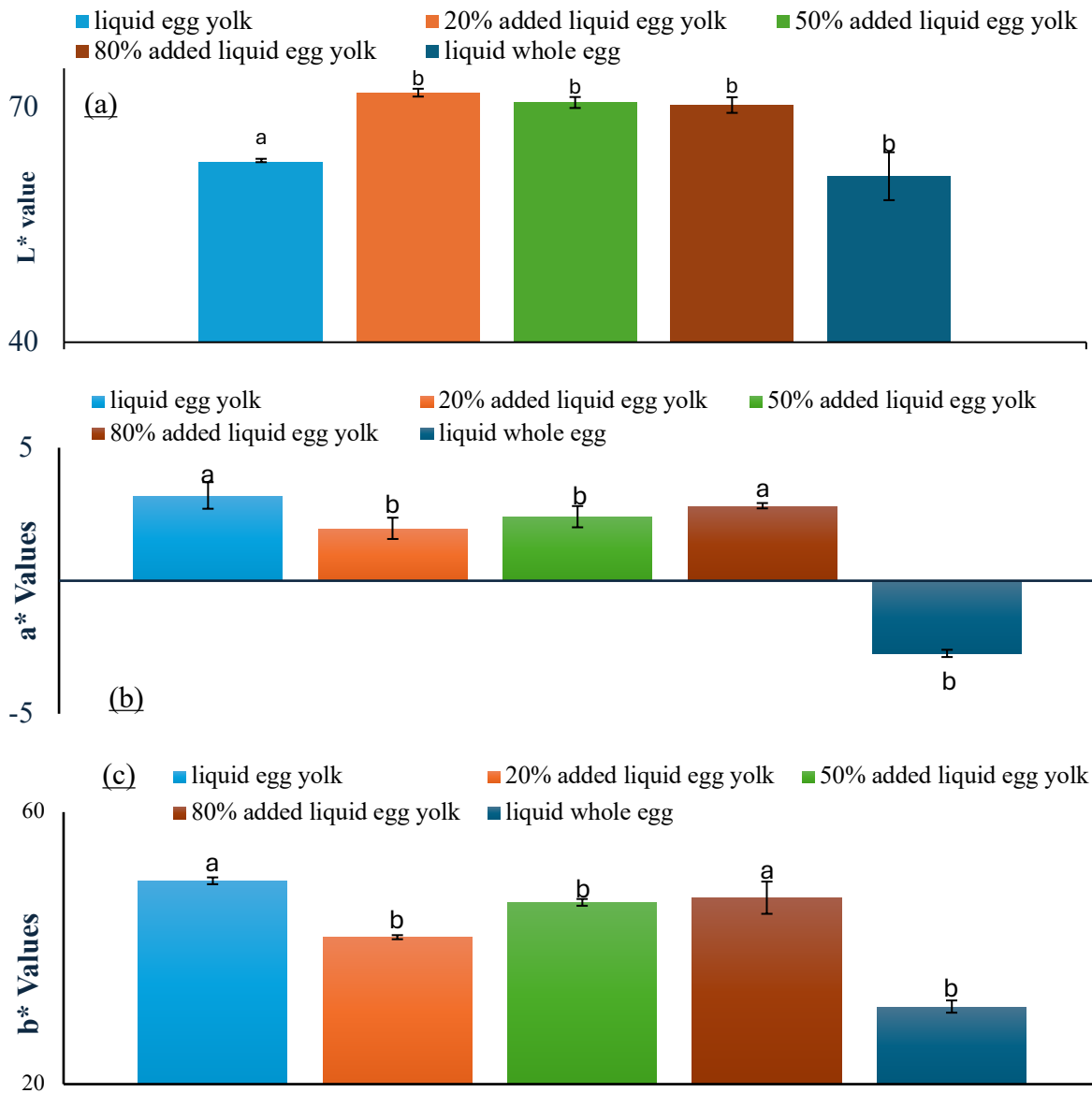


Figure 42: The effect of adding different percentage of added liquid egg yolk to liquid egg whole in comparison to the control group on: (a) L\* values, (b) a\* values, (c) b\* values in comparison to the control group, Tukey HSD  $p < 0.05$ .

#### 4.1.4.3 Change in rheological properties

The rheological data reveals how the flow behavior of the liquid egg mixtures changes with varying yolk concentrations, using yolk as the control group. The control yolk sample has a very low yield stress of  $0.001\pm 0.001$  Pa, a high K of  $0.176\pm 0.002$ , and n of  $0.915\pm 0.053$ , indicating a pseudoplastic behavior with moderate resistance to flow and significant viscosity. This is expected due to the high lipid and protein content in egg yolk, which contributes to its thicker, more structured consistency.

As yolk concentration decreases with the addition of whole egg, there is a marked increase in the yield stress values which is clearly seen in table 12. This suggests that the mixtures require more force to initiate flow compared to pure yolk. The reason lies in the interaction between egg white proteins and yolk components, which likely enhances the formation of a weak protein network, contributing to a higher initial resistance to flow (Atilgan & Unluturk, 2008). K values decrease significantly in the diluted samples, dropping from  $0.176\pm 0.002$  in pure yolk to  $0.015\pm 0.001$  and  $0.018\pm 0.002$  in the 20 and 80% yolk samples. This reduction reflects a lower apparent viscosity, as the whole egg dilutes the rich lipid-protein matrix of the yolk, making the mixture thinner and easier to flow once the yield stress is overcome (Kumbár et al., 2021).

The flow behavior index also shifts closer to 1 in the mixed samples ranging from  $0.995\pm 0.203$  to  $1.007\pm 0.023$ , indicating a transition toward Newtonian behavior, where the viscosity remains relatively constant regardless of shear rate. In contrast, the control yolk sample, with an n value of  $0.915\pm 0.051$ , exhibits stronger pseudoplasticity, meaning its viscosity decreases more significantly under shear. This shift is attributed to the higher water and lower fat content in the mixed samples, which weakens shear-thinning properties (Jaekel & Ternes, 2009).

The liquid whole egg sample, which contains the least yolk, shows similar trends, with a  $\tau_0$  of  $0.094\pm 0.001$  Pa, a lower K value of  $0.012\pm 0.003$ , and an n value of  $1.007\pm 0.026$ , highlighting the dominance of egg white behavior. In comparison to the 100% yolk control, the changes are substantial. The addition of egg white reduces viscosity, diminishes pseudoplastic behavior, and increases yield stress, indicating that the rheological properties of the mixture become more fluid-like and less structured as yolk concentration decreases (Atilgan & Unluturk, 2008). This is primarily due to dilution of yolk's lipid-protein matrix and the higher water content of egg white, leading to a more homogeneous and less resistant flow profile (Atilgan & Unluturk, 2008).

Table 12: Measured results of Herschel-Bulkley model at different concentrations of added liquid egg yolk to liquid egg whole in comparison to the control. different letters differ significantly in comparison to control group (Tukey's  $p < 0.05$ ).

Sample	$\tau_0$ (Pa)	$K(\text{Pa}\cdot\text{s}^n)$	$n$ (-)
Liquid egg yolk	0.001±0.001a	0.176±0.002a	0.915±0.051a
20% liquid egg yolk	0.095±0.001b	0.015±0.001b	1.002±0.130b
50% liquid egg yolk	0.107±0.003b	0.020±0.002b	0.995±0.203b
80% liquid egg yolk	0.104±0.007b	0.018±0.004b	0.999±0.013b
Liquid whole egg	0.094±0.001b	0.012±0.003b	1.007±0.026b

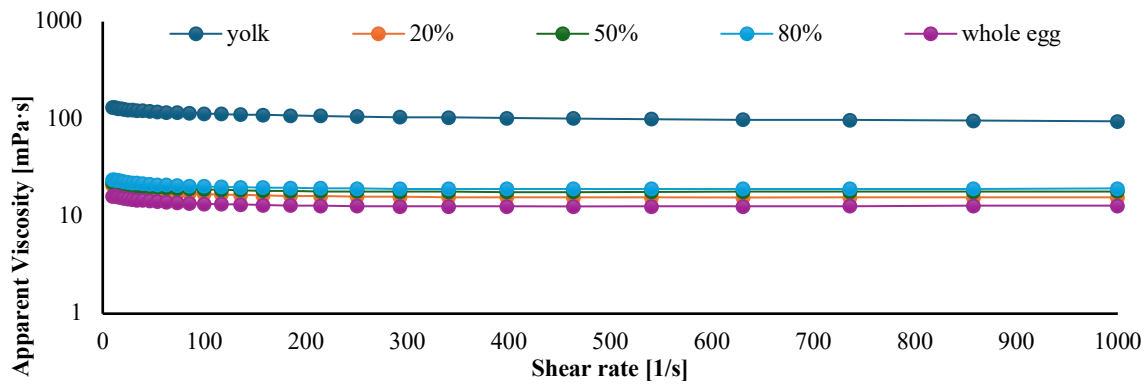


Figure 43: The viscosity curves (shear rate vs. apparent viscosity) of added different concentration of egg yolk to liquid whole egg samples.

#### 4.1.4.4 Change in sensorial attributes of Pastel De Nata with the change of yolk percentages

In a comprehensive sensory evaluation of *pastel de nata* custard reformulated with varying egg yolk concentrations, a series of perceptual and physicochemical changes were observed across seven key attributes when compared to a 100% yolk control. The surface color, a critical visual for quality and consumer appeal, showed a marked reduction in yellowness in samples containing whole eggs or lower yolk ratios (20% and 50%). These variations were statistically significant ( $p < 0.01$ ) when compared to the control, highlighting the importance of yolk-derived xanthophylls in imparting the characteristic golden hue (Mine et al., 2023). Conversely, the 80% yolk formulation did not differ significantly from the control, indicating that a threshold near this concentration is sufficient to preserve the desired surface appearance. A similar trend was observed for inner custard coloration, where whole egg and 20% yolk samples exhibited a noticeably paler, whitish tone, while the 80% yolk sample approximated the control's intensity without statistical deviation, reinforcing the influence of yolk pigmentation on both surface and internal chromatic

attributes. Odor analysis revealed that whole egg and 20% yolk samples emitted a significantly more intense egg odor than the yolk control ( $p < 0.05$ ), which was attributed to the presence of sulfur-containing proteins predominantly found in egg whites. However, the 50% and 80% yolk samples did not differ significantly from the control, suggesting that minimizing albumen content can effectively moderate off-odors associated with cooked egg volatiles (Sun et al., 2025).

The assessment of custard texture further underscored the structural role of yolk; the yolk control was rated as the firmest sample, while whole egg and particularly the 20% yolk samples exhibited significantly softer textures ( $p < 0.01$ ), likely due to the increased water content and reduced lipid-protein network formation from diluted yolk presence (Deleu et al., 2017).

Creaminess, a fundamental attribute in custard quality perception, showed the most profound divergence from the control. All reduced-yolk samples, particularly the 20% and 50% formulations, were significantly less creamy ( $p < 0.01$ ), implicating the role of yolk lipids and lecithin in establishing emulsification and mouth-coating sensations (Suhag, 2024). Only the 80% yolk sample approached the sensory richness of the control, with a mild but significant reduction ( $p < 0.05$ ), suggesting a near-complete retention of yolk functionality at this concentration. Regarding sweetness, it remained statistically unaffected across most comparisons, with the exception of a slight increase in perceived sweetness in the whole egg sample ( $p < 0.01$ ), potentially due to reduced fat content leading to diminished flavor masking (Pedersen et al., 2023). This aligns with established sensory literature indicating that lipid matrices can suppress the intensity of sweet perception via fat-taste interaction pathways (Pedersen et al., 2023).

Finally, aftertaste duration, an attribute often linked to flavor retention and overall satisfaction, was longest in the yolk control and only marginally diminished in the 80% yolk sample ( $p < 0.05$ ). All other formulations did not differ significantly, suggesting that fat content plays a moderate but relevant role in sustaining flavor persistence post-ingestion. Collectively, these findings demonstrate that the yolk component in *pastel de nata* custard is instrumental not only in visual and flavor attributes but also in structural integrity and overall sensory satisfaction. The 80% yolk formulation emerges as an optimal compromise, preserving most desirable qualities while enabling partial yolk reduction, which could be advantageous for product reformulation efforts aimed at cost, sustainability, or nutritional improvements without compromising traditional sensory expectations.

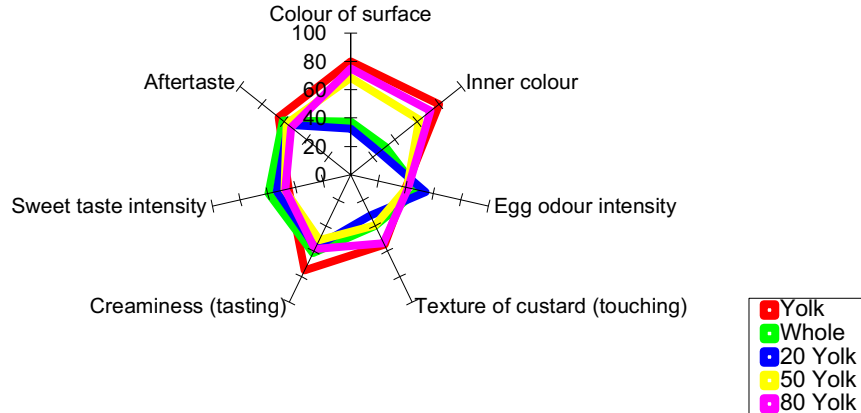


Figure 44: The effect of egg yolk different ration on the sensory attributes of Pastal de Nata.

The sensory results align well with the measured previous results, as the egg yolk sample and egg yolk sample with 80% yolk should the highest intensity of color in both instrumental measurement and panelist opinions. It also showed that with the increase of yolk percentages, the viscosity increased, and this also aligns to the mouthful feeling of the custard that the panelist felt with the increase of yolk percentage

#### 4.2 The effect of different oil additions on liquid egg properties

##### 4.2.1 Effect of Essential oils on liquid whole eggs properties and antioxidant activity

###### 4.2.1.1 Changes in pH

The pH of the whole liquid egg for this experiment was  $6.9 \pm 0.06$  at the beginning but the readings were decreasing with the increase of the added oils percentage in the three different oils, this decrease was insignificant ( $p < 0.05$ ) when compared to the control group.

The slight decrease in pH observed with the addition of basil, rosemary, and garlic oils can be attributed to several factors related to their unique chemical composition. Unlike neutral edible oils, these essential oils contain bioactive compounds such as phenolic acids, flavonoids, and sulfur-containing compounds, which introduce mild acidity into the liquid whole egg system (Azizah et al., 2023; Christopoulou et al., 2021; Shang et al., 2019). Basil and rosemary oils contain rosmarinic acid, carnosic acid, and caffeic acid, while garlic oil includes allicin and diallyl disulfide, all of which can contribute to slight acidification (Azizah et al., 2023; Christopoulou et al., 2021; Shang et al., 2019). Their strong antimicrobial properties also allow them to interact with

egg proteins, potentially disrupting the ionic balance of the mixture. Since eggs are a water-rich system, the hydrophobic nature of essential oils can lead to slight phase separation, influencing the ionic balance and pH stability. This effect is similar to how emulsification occurs but may also lead to minor shifts in pH. However, the pH decrease remains statistically insignificant ( $p > 0.05$ ) due to the strong buffering capacity of egg proteins and the relatively small quantity of oil added.

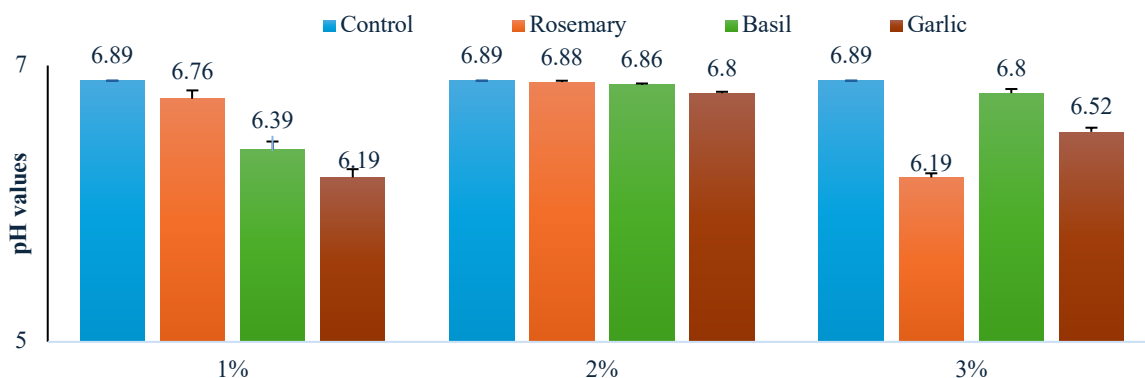


Figure 45: The effect of adding different percentages of different essential oils on pH value of liquid whole eggs in comparison to control group, Tukey HSD  $p < 0.05$ .

#### 4.2.1.2 Changes in color parameters

Color properties of liquid whole egg samples are shown in figure, figure 16, 17, and 18. According to the results the color of the whole egg changed but the change was insignificant compared to the control sample ( $p < 0.05$ ).  $L^*$ ,  $a^*$ , and  $b^*$  values showed a decreasing trend with the increase in the concentration for all oils. By naked eye a change is seen due to few oil globules, liquid whole egg samples that are fortified with oils are having more intense color in comparison to the control group, this change is not seen statistically.

The insignificant change in color observed when basil, rosemary, and garlic essential oils were added to liquid whole eggs can be attributed to several factors. First, the low concentration of essential oils 1-3% results in a dilution effect, meaning that any natural pigments present in these oils are too weak to significantly alter the overall color of the egg matrix (Dvořák et al., 2012). Unlike ingredients rich in carotenoids or other strong pigments, basil, rosemary, and garlic oils do not contain high levels of color-altering compounds, limiting their impact on measurable  $L^*$ ,  $a^*$  and  $b^*$  values. Additionally, the natural yellow to orange color of liquid whole eggs is primarily influenced by egg yolk pigmentation, which contains lutein and zeaxanthin, highly stable compounds that dominate the overall appearance (De Souza et al., 2019). Since the inherent

pigmentation of the yolk is already intense, adding small amounts of essential oils does not override its strong color presence.

The intensity and rich color of liquid whole eggs is not easily affected, but in this case it was enhanced, many studies indicate that more yellowish to orange whole egg color means more customer acceptance(De Souza & Fernández, 2012) , as seen in figure for b\* values they are increasing toward yellowish color for both basil and rosemary oils, on the other hand L\* values in figure were increasing for garlic oil at 2 and 3% which indicates a more light yellow color for samples with garlic oil in comparison to the control group of liquid whole eggs.

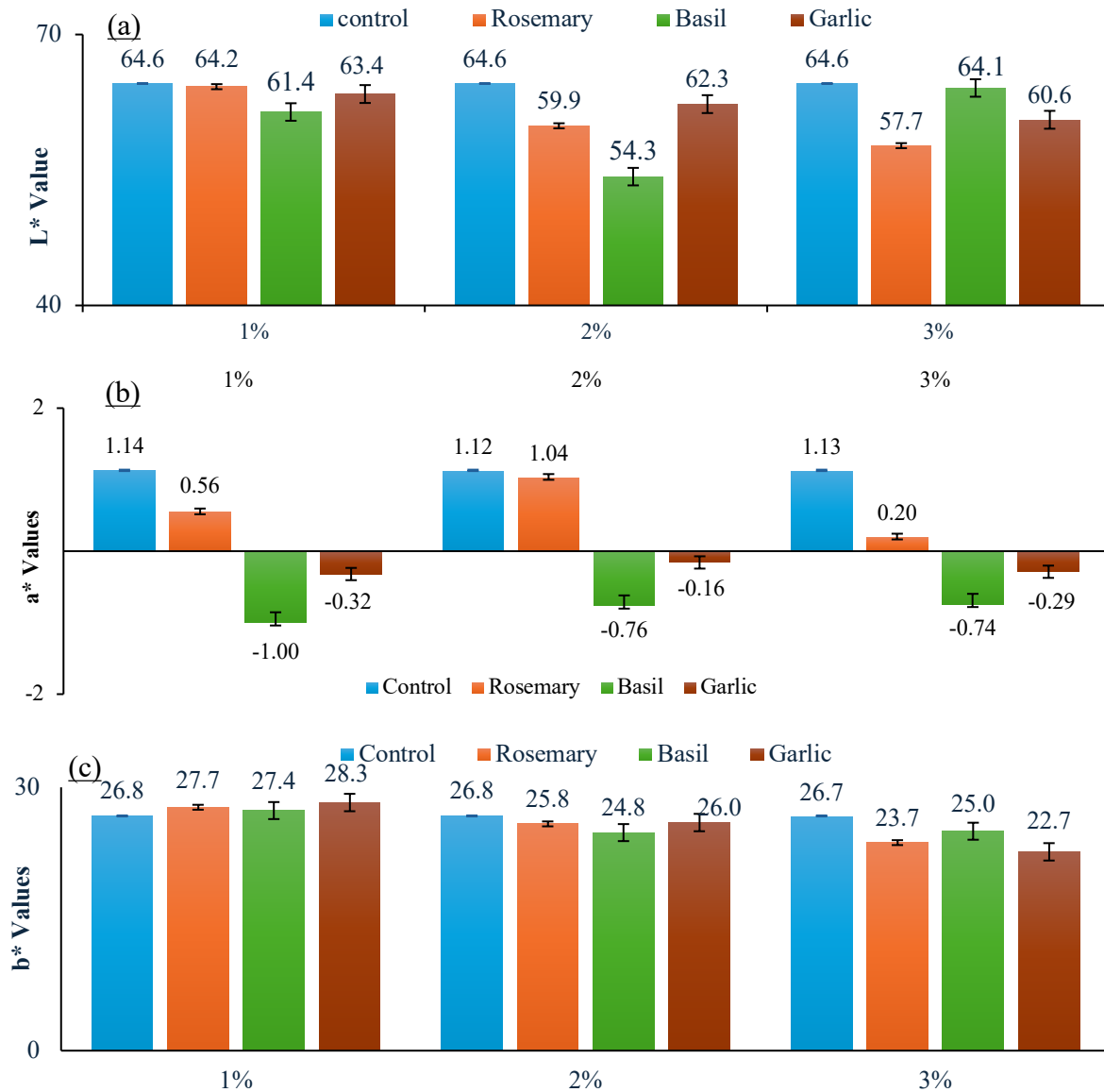


Figure 46: The effect of adding different percentages of different essential oils on: (a) L\* values, (b) a\* values, (c) b\* values in comparison to the control group, Tukey HSD  $p < 0.05$ .

#### 4.2.1.3 Changes in rheological properties

Liquid egg products are known as non-Newtonian fluids with shear-thinning characteristics, the viscosity of liquid whole egg is typically dependent on the shear rate. It's known that liquid whole eggs viscosity changes with temperature, studies found that liquid whole eggs are time dependent around their pasteurization temperature and time independent at lower temperatures. As illustrated in figures 19 shear stress of samples was observed to be almost the same as control sample for all oils and at all concentrations (Atilgan & Unluturk, 2008).

For the control sample and as shown in table 13, the yield stress was  $0.104 \pm 0.001$ , representing the baseline resistance to flow. With rosemary oil,  $\tau_0$  values remained consistent at  $0.102 \pm 0.012$  across all concentrations (1%, 2%, and 3%), indicating that rosemary oil had no significant effect on the liquid egg's ability to resist flow. On the other hand, basil oil led to an increase in  $\tau_0$ , from  $0.111 \pm 0.023$  in 1% and 2% to  $0.127 \pm 0.012$  at 3%, suggesting enhanced resistance to flow due to stronger protein-oil interactions at higher concentrations (Atilgan & Unluturk, 2008). Garlic oil showed the most significant effect, with  $\tau_0$  reaching  $0.110 \pm 0.011$  at 2% and  $0.136 \pm 0.004$  at 3%, indicating a strengthening of the protein network. The consistency coefficient (K) measures the viscosity of the liquid at a given shear rate. For the control sample, K was  $0.006 \pm 0.001$ , in rosemary oil samples K remained stable at  $0.005 \pm 0.001$  across all concentrations. It means that rosemary oil had no notable effect on viscosity. As wise basil oil slightly decreased K to  $0.004 \pm 0.002$  at 1% and 2%, but it returned to  $0.005 \pm 0.001$  at 3%, indicating that basil oil may initially reduce viscosity but did not significantly alter it at higher levels. Garlic oil maintained a consistent K value of  $0.004 \pm 0.002$  at all concentrations, showing that garlic oil had minimal influence on the viscosity of the liquid egg despite its other rheological effects. The flow behavior index (n) determines whether the fluid exhibits Newtonian, shear-thinning, or shear-thickening behavior. For the control sample, n was  $1.083 \pm 0.014$ , indicating slightly shear-thickening behavior. With rosemary oil, n increased marginally from  $1.091 \pm 0.015$  at 1% to  $1.107 \pm 0.024$  at 3%, showing minimal impact and maintaining near-Newtonian to weakly shear-thickening behavior. Basil oil showed higher n values at lower concentrations,  $1.134 \pm 0.021$  at 1% and  $1.130 \pm 0.023$  at 2%, but decreased to  $1.108 \pm 0.022$  at 3%, indicating a shift toward reduced shear-thickening behavior at higher concentrations. Garlic oil caused the most significant increase in n, rising from  $1.110 \pm 0.021$  at 1% to  $1.142 \pm 0.011$  at 2%, and remaining stable at  $1.141 \pm 0.012$  at 3%. This stronger shear-thickening behavior at higher garlic oil concentrations suggests a denser

and more interconnected protein network under shear stress. The rheological behavior of liquid whole eggs enriched with essential oils is influenced by the chemical composition and interaction potential of the oils with the protein-water matrix (Dang et al., 2025). Rosemary oil, which contains phenolic compounds like rosmarinic acid and carnosic acid, has low reactivity with proteins and limited water-binding properties. As a result, rosemary oil does not significantly interact with egg proteins or alter the matrix structure, leading to minimal changes in  $\tau_0$ , K, and n (Christopoulou et al., 2021). In case of basil oil, it contains bioactive compounds such as linalool and eugenol, that has moderate hydrophilic and hydrophobic properties which can interact with egg proteins. These interactions slightly alter protein structure and increase water-binding capacity, resulting in a modest increase in  $\tau_0$  and n at higher concentrations, due to mild protein aggregation and structure formation (Avetisyan et al., 2017). Garlic oil is rich in sulfur-containing compounds like allicin and diallyl disulfide, exhibits the most significant effects (Shang et al., 2019). These compounds are highly reactive with egg proteins, forming disulfide bonds or temporary cross-links, especially under mechanical stress. At moderate concentrations, 2% and 3%, garlic oil strengthens the protein network, significantly increasing  $\tau_0$  and inducing strong shear-thickening behavior. Egg white proteins, such as ovalbumin and ovotransferrin, are particularly sensitive to hydrophobic and reactive compounds, they partially unfold to expose hydrophobic regions that enhance protein-oil interactions (Giansanti et al., 2012; Hsien & Regenstein, 1992).

Table 13: The effect of adding rosemary, basil, and garlic essential oils on actual and measured results of Herschel-Bulkley model and FRAP values in comparison to control at different percentages 1, 2 and 3%. different letters differ significantly in comparison to control group (Tukey's  $p < 0.05$ ).

Sample	$\tau_0$ (Pa)	K(Pa·s <sup>n</sup> )	n (-)	FRAP value kg/m <sup>3</sup>
Control	0.104±0.001a	0.006±0.001	1.083±0.014a	19.3±0.9a
Rosemary 1%	0.102±0.012	0.005±0.001	1.091±0.015	23.4±0.4 b
Rosemary 2%	0.102±0.001	0.005±0.001	1.100±0.001	24.1±0.1 b
Rosemary 3%	0.102±0.021	0.005±0.001	1.107±0.024	24.4±0.2 b
Basil 1%	0.111±0.023	0.004±0.002	1.134±0.021b	21.8±1.1 b
Basil 2%	0.111±0.022	0.004±0.001	1.130±0.023b	22.3±0.6 b
Basil 3%	0.127±0.012b	0.005±0.001	1.108±0.022	23.6±0.8 b
Garlic 1%	0.100±0.0131	0.005±0.012	1.110±0.021	21.6±0.3 b
Garlic 2%	0.110±0.011b	0.004±0.002	1.142±0.011b	22.1±0.5 b
Garlic 3%	0.136±0.004b	0.004±0.0021	1.141±0.012b	22.7±0.2 b

Additionally, the water-binding capacity and emulsification properties of the oils vary. Rosemary oil's limited water-binding properties lead to negligible changes, while basil oil's moderate capacity induces slight structural changes, and garlic oil's strong water-binding capacity enhances protein interactions and matrix density, particularly at 3% (Jackson-Davis et al., 2023). At low concentrations, the oils are diluted, resulting in minimal impact on rheological properties, while at moderate concentrations 2% and 3%, optimal interactions occur, particularly for garlic oil, which strengthens the matrix. Figures 19 and 20 show these effects on liquid whole egg flow and viscosity behaviors

Previous studies found that essential oils, in specific rosemary oil, can reduce the viscosity and product absorption of sunflower oil while frying, this was explained due to the polymerization, oxidation, hydrolysis and isomerization effect of those essential oils while heating (Tokur et al., 2021; F. Wang et al., 2023), although no heating occurred in this experiment, but the insignificant effect was seen.

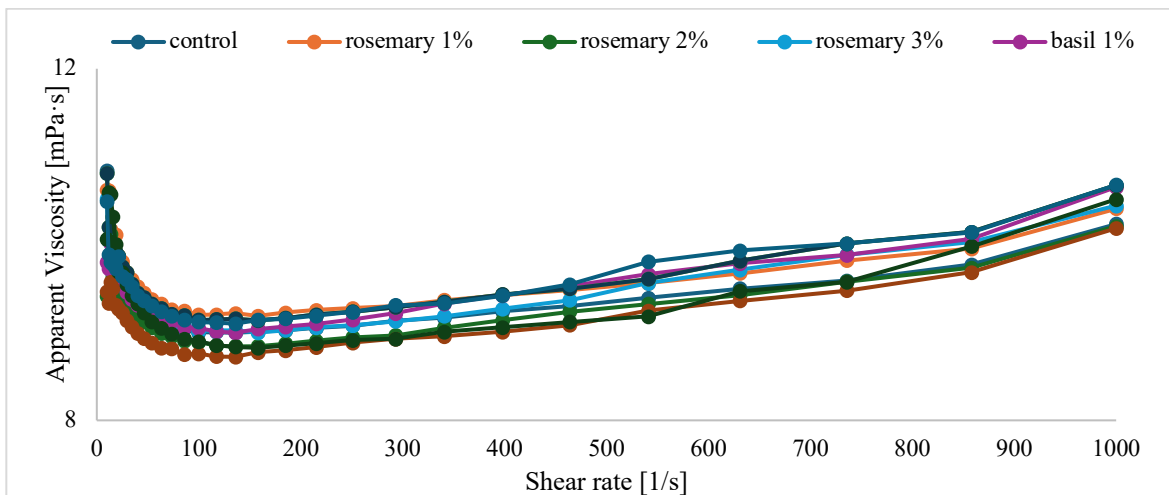


Figure 47: The viscosity curve (shear rate vs. apparent viscosity) of added different concentration of essential oils to liquid whole egg sample

#### 4.2.1.4 Change in antioxidant activity

The FRAP values (mentioned in table 13) indicate the antioxidant capacity of liquid whole eggs mixed with different essential oils. The control sample, without any essential oil, had the lowest FRAP value of  $19.3 \pm 0.9$  kg/m<sup>3</sup>, suggesting minimal baseline antioxidant activity. Among the tested oils, rosemary exhibited the highest antioxidant potential, with FRAP values increasing from  $23.4 \pm 0.4$  at 1% concentration to  $24.4 \pm 0.2$  at 3%, due to its rich phenolic content, including carnosic acid and rosmarinic acid (Nieto et al., 2018). Basil also enhanced antioxidant activity,

with FRAP values ranging from  $21.8 \pm 1.1$  at 1% to  $23.6 \pm 0.8$  at 3%, attributed to its flavonoids and polyphenols, though its effect was slightly lower than rosemary. Garlic showed the least increase in FRAP values, from  $21.6 \pm 0.3$  at 1% to  $22.7 \pm 0.2$  at 3%, possibly due to its sulfur-containing compounds like allicin and diallyl sulfides, which, while antioxidant, are less effective in ferric reduction than rosemary's phenolics (Shang et al., 2019). Overall, rosemary demonstrated the strongest antioxidant effect, followed by basil and then garlic, with higher essential oil concentrations leading to greater antioxidant capacity in a dose-dependent manner.

#### 4.2.2 Effect of adding different cooking oils on liquid whole eggs properties

##### 4.2.2.1 Changes in pH

Adding different oils to liquid whole eggs effect its pH values, the results indicate a slight increase in pH with the addition of oils, with the control sample having an average pH of  $5.93 \pm 0.1$ , while samples containing olive, sunflower, coconut, and palm oils exhibited incremental increases in pH depending on the concentration. Notably, higher oil concentrations consistently resulted in a greater pH shift, with Palm 7.5% showing the highest pH value of  $6.16 \pm 0.1$ , compared to the control. These variations suggest that the type and concentration of added oils influence the buffering capacity and ionic equilibrium of whole egg proteins.

The observed pH increase can be attributed to several key mechanisms. Firstly, the incorporation of lipids may have diluted the acidic components present in whole eggs, leading to a relative increase in pH (Sunwoo & Gujral, 2015). Moreover, some oils, particularly those rich in free fatty acids or unsaponifiable fractions, may altered the protein-lipid interactions, affecting the ionization of amino acid residues in egg proteins. The impact of different oils also varies, as seen in figure 48 with palm oil exhibiting the greatest pH elevation, due to its higher saturated fat content and interaction with egg proteins, altering their charge balance (Alhaji et al., 2024). Coconut oil, rich in saturated fats, showed a similar effect, though slightly lower than palm oil (Arias et al., 2023). Meanwhile, olive and sunflower oils, which contain higher levels of monounsaturated and polyunsaturated fatty acids, resulted in relatively moderate pH increases (Dichtyar et al., 2017; Varzakas, 2021). The significance of these pH shifts lies in their potential effects on protein functionality, stability, and emulsification (Zhang et al., 2023). A higher pH can lead to reduced protein coagulation, which may impact the foaming and gelation properties of eggs (Zhang et al., 2023). These changes could also influence microbial stability, as the pH of eggs is a critical factor

in their shelf-life and susceptibility to bacterial growth (Mafe et al., 2024). The findings suggest that oil addition, particularly at higher concentrations, could modify the physicochemical behavior of whole egg systems, which is relevant for applications in food processing, emulsified products, and egg-based formulations.

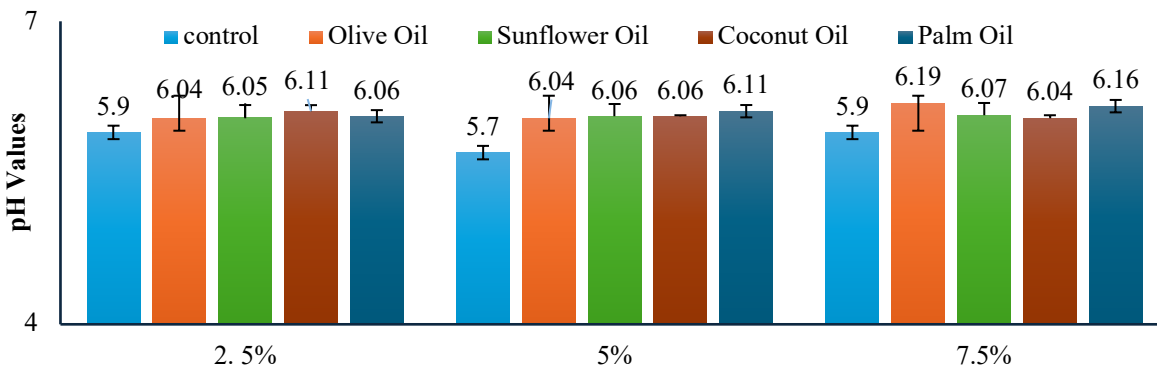


Figure 48: The effect of adding different percentages of different oils on pH value of liquid whole eggs in comparison to control group, Tukey HSD  $p < 0.05$ .

#### 4.2.2.2 Changes in color parameters

The  $L^*$  value in the CIELAB color space represents lightness, where higher values indicate a lighter appearance, and lower values suggest darker or more coloration. The results indicate that the addition of oils to liquid whole eggs significantly influenced their  $L^*$  values, with all oil-treated samples exhibiting an increase in lightness compared to the control. This suggests that lipid incorporation affects the optical properties of egg components, likely through interactions between fat droplets and protein matrices, leading to increased light scattering and a shift in visual perception (Tian et al., 2024). The most pronounced increases in  $L^*$  values were observed in samples containing higher concentrations of oil, particularly at 7.5%, reinforcing the role of oil concentration in modifying egg appearance.

Among the tested oils and as seen in figure 22, palm oil showed the highest  $L^*$  value at 7.5% reaching  $63.16 \pm 0.09$ , followed by coconut oil reaching  $61.29 \pm 0.01$  and olive oil reaching  $67.122 \pm 0.08$  at the same concentration, whereas sunflower oil exhibited more variable effects with a slightly lower  $L^*$  value at 7.5% reaching  $50.2 \pm 0.01$  compared to mid-range concentrations. The variations among different oils can be attributed to differences in their fatty acid composition, saturation levels, and interaction with egg proteins (Tian et al., 2024). Palm and coconut oils, which are rich in saturated fats, likely formed a more homogenous dispersion in the egg matrix enhancing light scattering, thus resulting in higher  $L^*$  values (Arias et al., 2023). Meanwhile, olive

and sunflower oils, which contain more unsaturated fats, may have exhibited phase separation tendencies at higher concentrations, influencing the uniformity of light dispersion and causing more fluctuations in  $L^*$  values.

The  $a^*$  value results indicate that the addition of oils to liquid whole eggs significantly influenced  $a^*$  value, with noticeable reductions in the intensity of green coloration compared to the control. The control sample had the most negative  $a^*$  value, indicating a stronger green hue, whereas most oil-treated samples exhibited shifts toward a more neutral (less green) or slightly reddish tone. This suggests that lipid incorporation alters the optical properties of the egg matrix, by influencing pigment dispersion, light absorption, and protein-fat interactions, which in turn affect color perception (Tian et al., 2024).

The extent of change varied depending on oil type and concentration. Palm oil at 5% was the only sample to exhibit a positive  $a^*$  value, suggesting a slight shift toward red, while other oil-treated samples remained in the negative, but with reduced green intensity compared to the control. As seen in figure 48 sunflower oil at 2.5% and 7.5% showed the least green tint, indicating a significant neutralization of the original egg color. Coconut and olive oil-treated samples also exhibited a shift toward less negative  $a^*$  values, but the magnitude of change depended on concentration. The highest deviation from the control occurred at mid-range concentrations of 5% for most oils, suggesting optimal dispersion and interaction at this level.

The observed changes in  $a^*$  values can be explained by several physicochemical factors. The incorporation of oils likely reduced the visibility of egg pigments, such as riboflavin and xanthophylls, which contribute to the natural greenish tint of liquid whole eggs (Lokaewmanee et al., 2010). The presence of lipid droplets within the protein matrix may have altered light scattering and absorption, thereby shifting the overall color perception. The higher shift toward red tones in palm oil-treated samples, particularly at 5%, may be attributed to the natural carotenoid content in palm oil, which introduces a slight reddish hue into the mixture (Rey et al., 2023). Meanwhile, sunflower and coconut oils, which are more translucent and neutral in color, led to the most substantial neutralization of green intensity, possibly due to better homogenization within the egg matrix and light diffusion effects.

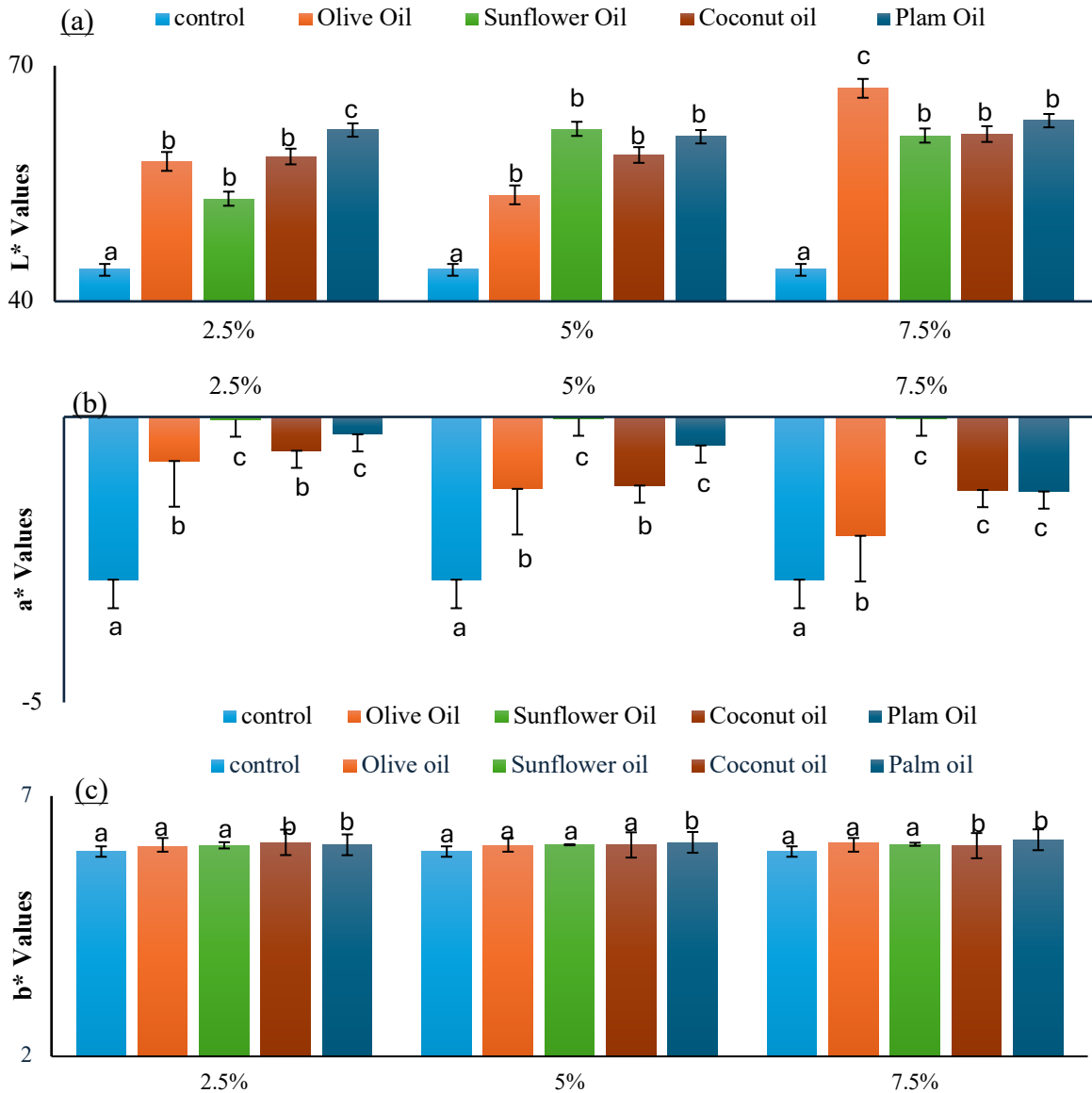


Figure 49: The effect of adding different percentages of different oils: (a) L\* values, (b) a\* values, (c) b\* values in comparison to the control group, Tukey HSD  $p < 0.05$ .

The addition of oils to liquid whole eggs significantly influenced b\* values, indicating a stronger yellow hue. The control sample had a b\* value of  $12.41 \pm 0.56$ , while oil-treated samples exhibited increased yellowness, with palm oil at 5% to  $24.376 \pm 0.41$  and coconut oil at 2.5% to  $24.10 \pm 0.41$  showing the highest increases. This suggests that lipid incorporation enhances pigment visibility and light scattering within the egg matrix, intensifying the perception of yellow coloration. The presence of fat droplets may have altered pigment dispersion, allowing xanthophylls and riboflavin to be more pronounced, particularly in saturated fat-rich oils like palm and coconut oils, which naturally contain carotenoids that contribute to the enhanced yellowness (Lokaewmanee et al.,

2010). In contrast, olive oil at 7.5% exhibited a decrease in  $b^*$  value reaching  $10.17 \pm 0.29$ , indicating a shift toward a more neutral or slightly blue hue, this can be due to olive oil's own greenish tint, which may have counteracted the natural yellow color of the egg mixture at higher concentrations.

These shifts in  $a^*$  and  $L^*$  values have significant implications for food applications where color perception is critical for consumer acceptance. A reduction in greenish hues and a shift toward neutral or red as well as, an increase in  $L^*$  can enhance the visual appeal of egg-based formulations, particularly in bakery, sauces, and emulsified products, where an overly green tint or darker appearance may be undesirable. The type and concentration of oil influence the final color outcome, meaning that oil selection can be optimized to achieve specific visual and functional properties in processed egg products. The nonlinear trends observed across different oil concentrations suggest that emulsification efficiency, light scattering, and pigment interactions all contribute to final color characteristics, which are essential for product consistency and appearance in commercial formulations. The addition of palm oil led to a shift toward a reddish hue, while sunflower and coconut oils effectively neutralized the natural green tint, making them ideal for applications requiring enhanced yellowness or improved color uniformity. Furthermore, the increase in  $L^*$  with higher oil concentrations, particularly in palm and coconut oils, suggests modifications in protein-lipid interactions, which could impact emulsification, heat stability, and gelation properties. These findings provide valuable insights for food processing applications, where color and appearance modifications are critical for product quality and consumer perception, ensuring optimal formulation strategies for oil-enriched egg-based products.

#### 4.2.2.3 Changes in rheological properties

The addition of oils to liquid whole eggs significantly altered their rheological behavior, as reflected in changes in  $\tau_0$ ,  $K$ , and  $n$ . The control sample exhibited a yield stress of  $0.073 \pm 0.001$  Pa, indicating a relatively low resistance to flow, with a moderate consistency index where  $K = 0.028 \pm 0.001$  Pa·s <sup>$n$</sup>  and  $n = 0.968 \pm 0.021$ , suggesting a near Newtonian behavior. The impact of different oils varied based on fat type and concentration, demonstrating distinct structural interactions between the lipid phase and egg proteins. Table 14 shows the results of Herschel-Bulkley model for all sample in comparison to control group.

Olive oil treated samples showed a gradual increase in  $\tau_0$  with increasing concentration, reaching  $0.124 \pm 0.001$  Pa at 5%, before slightly decreasing at 7.5% to  $0.083 \pm 0.001$  Pa. The flow behavior index  $n$  increased above 1, indicating a shift toward shear-thickening behavior, which suggests that the presence of olive oil may have influenced molecular interactions, leading to structural modifications that increase viscosity under applied stress. However,  $K$  was between  $0.003 \pm 0.001$  to  $0.004 \pm 0.001$  Pa·s<sup>n</sup> across all olive oil concentrations suggests that the oil phase did not significantly contribute to thickening the mixture, possibly due to incomplete emulsification or phase separation at higher oil levels.

Sunflower oil exhibited a similar trend, with an initial increase in yield stress at lower concentrations reaching to  $0.140 \pm 0.001$  Pa at 2.5% but a decrease at 7.5% to  $0.079 \pm 0.011$  Pa.  $n$  increased from  $1.184 \pm 0.011$  at 2.5% to  $1.197 \pm 0.021$  at 7.5%, indicating a shear-thickening behavior, which suggests that sunflower oil may have altered the egg protein matrix, making it more resistant to flow under higher stress.  $K$  remained constant at  $0.003 \pm 0.001$  Pa·s<sup>n</sup> across sunflower oil samples indicates that, like olive oil, it did not significantly contribute to increasing viscosity, likely due to weaker emulsification properties or insufficient interaction with egg proteins (Abker et al., 2023).

Table 14: The effect of adding different oils on actual and measured results of Herschel-Bulkley model. different letters differ significantly in comparison to control group (Tukey's  $p < 0.05$ ).

Sample	$\tau_0$ (Pa)	$K$ (Pa·s <sup>n</sup> )	$n$ (-)
Control	$0.073 \pm 0.001$ a	$0.028 \pm 0.001$ a	$0.968 \pm 0.021$ a
Olive oil 2.5%	$0.086 \pm 0.001$	$0.003 \pm 0.001$	$1.144 \pm 0.021$
Olive oil 5%	$0.124 \pm 0.001$	$0.003 \pm 0.001$	$1.154 \pm 0.041$
Olive oil 7.5%	$0.083 \pm 0.001$	$0.004 \pm 0.001$	$1.167 \pm 0.051$
Sunflower oil 2.5%	$0.140 \pm 0.001$ b	$0.003 \pm 0.002$	$1.184 \pm 0.033$ b
Sunflower oil 5%	$0.100 \pm 0.031$	$0.003 \pm 0.001$	$1.192 \pm 0.031$ b
Sunflower oil 7.5%	$0.079 \pm 0.011$	$0.003 \pm 0.001$	$1.197 \pm 0.076$ b
Coconut 2.5%	$4.843 \pm 0.091$ c	$2.836 \pm 0.061$ b	$0.319 \pm 0.001$ c
Coconut 5%	$6.612 \pm 0.321$ d	$0.176 \pm 0.031$	$1.095 \pm 0.081$
Coconut 7.5 %	$20.994 \pm 0.901$ e	$2.074 \pm 0.301$ b	$0.320 \pm 0.003$ c
Palm 2.5%	$0.229 \pm 0.071$ b	$0.012 \pm 0.001$	$0.978 \pm 0.021$
Palm 5%	$0.186 \pm 0.061$ b	$0.019 \pm 0.001$	$0.948 \pm 0.011$
Palm 7.5%	$0.560 \pm 0.001$ b	$0.010 \pm 0.001$	$1.015 \pm 0.041$

Coconut oil treated samples demonstrated a totally different rheological behavior, exhibiting the highest yield stress and viscosity values. At 2.5% coconut oil,  $\tau_0$  was  $4.843 \pm 0.091$  Pa, and  $K$

was  $2.836 \pm 0.061 \text{ Pa} \cdot \text{s}^n$ , while at 7.5%,  $\tau_0$  reached  $20.994 \pm 0.901 \text{ Pa}$ , with  $K$  reached  $2.074 \pm 0.301 \text{ Pa} \cdot \text{s}^n$ , indicating an extreme increase in structural resistance and thickness.  $n$  dropped significantly to  $0.319 \pm 0.001$  and  $0.320 \pm 0.003$  at 2.5% and 7.5% respectively, meaning coconut oil induced a strong shear-thinning effect, making the system highly viscous at rest but more fluid under shear stress. This behavior is likely due to the high saturation level of coconut oil, leading to stronger fat-protein interactions, enhanced aggregation, and the formation of a more structured, gel-like network that significantly increased viscosity (Arias et al., 2023). Additionally, coconut oil's solidifying properties at lower temperatures may have contributed to this behavior, forming a partially structured phase within the liquid egg matrix (Lima & Block, 2019). Palm oil treated samples also exhibited higher yield stress and consistency than the control, but to an extent than coconut oil.  $\tau_0$  increased gradually with concentration, reaching  $0.560 \pm 0.021 \text{ Pa}$  at 7.5%, while  $K$  remained relatively low for all concentrations reaching  $0.012 \pm 0.001$ ,  $0.019 \pm 0.001$ ,  $0.010 \pm 0.002 \text{ Pa} \cdot \text{s}^n$  for 2.5, 5 and 7.5 respectively. As for  $n$ , it fluctuated around 1, indicating a balance between Newtonian and slightly shear-thickening behavior. Palm oil, being semi-solid at room temperature and high in saturated fats, likely formed a more stable dispersion within the egg matrix, enhancing its structural properties without causing extreme thickening. The moderate increase in viscosity suggests that palm oil contributed to slight structural reinforcement, likely due to partial fat crystallization and protein interactions (Dzindziora et al., 2024). Palm oil treated samples also exhibited higher yield stress and consistency than the control, but to an extent than coconut oil.  $\tau_0$  increased gradually with concentration, reaching  $0.560 \pm 0.021 \text{ Pa}$  at 7.5%, while  $K$  remained relatively low for all concentrations reaching  $0.012 \pm 0.001$ ,  $0.019 \pm 0.001$ ,  $0.010 \pm 0.002 \text{ Pa} \cdot \text{s}^n$  for 2.5, 5 and 7.5 respectively. As for  $n$ , it fluctuated around 1, indicating a balance between Newtonian and slightly shear-thickening behavior. Palm oil, being semi-solid at room temperature and high in saturated fats, likely formed a more stable dispersion within the egg matrix, enhancing its structural properties without causing extreme thickening. The moderate increase in viscosity suggests that palm oil contributed to slight structural reinforcement, likely due to partial fat crystallization and protein interactions (Dzindziora et al., 2024).

#### 4.2.2.4 Changes in sensory attributes of scrambled eggs cooked with different oils

The sensory evaluation of scrambled eggs enriched with varying concentrations (2.5%, 5%, and 7.5% V/V) of olive oil, sunflower oil, palm oil, and coconut oil revealed significant differences

in color, taste, texture, and aroma as perceived by 10 trained panelists. Among the samples, sunflower oil at 5% and coconut oil at 2.5% were rated the highest in overall sensory attributes, indicating that these formulations provided an optimal balance in color intensity, flavor enhancement, and textural properties. Sunflower oil at 5% scored the highest in terms of color and texture, this may be attributed to its higher content of unsaturated fats, which likely contributed to a smoother, softer texture in the scrambled eggs due to improved lipid-protein interactions (Beitia et al., 2025). Sunflower oil is also known for its mild flavor profile, which has helped in enhancing natural egg flavors without overpowering the sensory perception (Temelkowska et al., 2023). The coconut oil at 2.5%, on the other hand, may have improved the mouthfeel and taste perception due to its medium-chain triglyceride composition, which contributes to a rich, creamy texture and a subtle buttery aroma, enhancing consumer appeal (Duranova et al., 2025). Figure 50 shows the effect of different oils on the sensory attributes of scrambled eggs cooked with different oils.

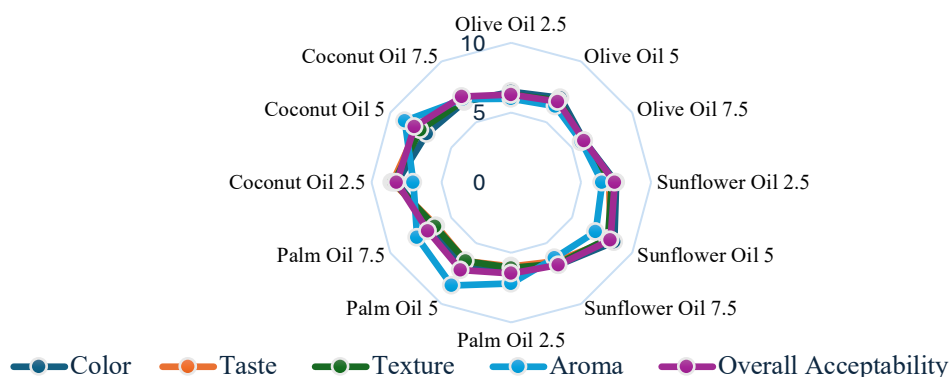


Figure 50: The effect of different oils on the sensory attributes of scrambled eggs cooked with different oils.

Regarding aroma, coconut oil at 5% and palm oil at 5% were rated as having the most desirable smell. Coconut oil is known for its distinctive aromatic profile, which contains volatile compounds such as lactones that impart a pleasant sweet and nutty fragrance (Suryani et al., 2020). The increase in oil concentration to 5% may have further amplified these volatile compounds. Similarly, palm oil at 5% was rated highly for aroma, likely due to the presence of naturally occurring carotenoids and minor volatile compounds that can enhance the cooked egg aroma.

Overall, the selection of oil type and concentration plays an important role in modifying the sensory characteristics of scrambled eggs, influencing color, texture, taste, and aroma. The optimal acceptance of sunflower oil at 5% and coconut oil at 2.5% suggests that moderate levels of

unsaturated or medium-chain fats enhance texture without negatively affecting structural integrity or mouthfeel. Meanwhile, the enhanced aroma in coconut oil 5% and palm oil 5% suggests that specific lipid-derived volatiles significantly influence the sensory perception of cooked eggs.

## 5. CONCLUSIONS AND RECOMMENDATIONS

This study investigated the effects of fortifying liquid whole eggs with functional ingredients such as egg white powder, whey protein isolate, essential oils, and different vegetable oils, on their techno-functional, rheological, and sensorial properties. The findings provide valuable insights into how these modifications influence product quality, stability, and consumer acceptability, with implications for both industrial processing and functional food development.

The incorporation of egg white powder or whey isolate at concentrations of 3%, 5%, and 10%, combined with pasteurization at 60°C, 65°C, and 70°C, revealed that both protein content and thermal treatment played significant roles in determining the rheological behavior of liquid egg products over a 21-day refrigerated storage period. Higher protein concentrations led to increased yield stress and consistency index while reducing the flow behavior index, indicating a shift toward a more structured and viscous system. This effect was particularly evident in the 10% protein sample, which demonstrated gel-like behavior by Day 7. However, prolonged storage and elevated pasteurization temperatures accelerated protein aggregation. These results underscore the importance of optimizing both formulation and processing parameters to achieve stability in protein-enriched egg products.

The addition of 20% liquid whole to liquid egg yolk did not affect the techno-functional and sensory characteristics when compared to those of samples containing 100% yolk. This similarity was observed not only in instrumental analyses but also in sensory evaluations, where trained panelists reported minimal perceptible differences in texture, taste, and overall acceptability between the two formulations. These findings carry significant implications for the egg processing industry. By utilizing formulations with 80% yolk instead of 100%, manufacturers have the potential to reduce production costs, optimize ingredient utilization, and improve sustainability without compromising product quality or consumer satisfaction. Such an approach can be especially valuable in large-scale production. However, further studies should assess the long-term stability, and nutritional impact of this change.

The addition of essential oils primarily enhanced antioxidant activity, contributing to the product's functional value without negatively affecting other physicochemical or rheological properties. This selective enhancement suggests essential oils may serve as natural antioxidants that extend

shelf life and health appeal without compromising structural integrity or negatively effecting the sensory attributes.

The incorporation of different vegetable oils had diverse impacts on the rheological properties of liquid eggs. Coconut oil induced the most pronounced changes, significantly increasing yield stress and consistency due to strong fat-protein interactions and network formation, while also producing a strong shear-thinning behavior. In contrast, olive and sunflower oils caused mild shear-thickening effects, with minimal contributions to consistency, likely due to limited emulsification or weaker interactions with egg proteins. Palm oil exhibited a more balanced profile, modestly increasing viscosity and displaying near-Newtonian flow characteristics.

Sensorial evaluations of cooked scrambled egg formulations enriched with these oils revealed that sunflower oil at 5% and coconut oil at 2.5% provided the most favorable sensory profiles, particularly in terms of color, texture, and overall acceptability. These effects were attributed to the fatty acid composition and aromatic profiles of the oils, which improved mouthfeel, flavor balance, and aroma. Additionally, coconut and palm oils at 5% enhanced aroma due to their volatile compound profiles. While this study primarily focused on rheological, techno-functional, and sensory attributes, it is critical to conduct microbiological testing and shelf-life assessments, as the incorporation of proteins, changing yolk percentages in liquid whole egg, adding essential oils, and various types of oils along with different pasteurization treatments, can significantly influence the microbiological safety and stability of the product. Evaluating microbial load over time under various storage conditions will ensure consumer safety and regulatory compliance. Expanding research to include different storage conditions and packaging technologies, such as modified atmosphere packaging or room temperature storage, will help determine product stability in varying distribution environments, thereby supporting commercial scalability and extended shelf life. Additionally, the antioxidant benefits observed from essential oil inclusion suggest potential for further exploration of natural bioactive compounds or dietary fibers to enhance nutritional value. In summary, the fortification of liquid whole eggs with carefully selected proteins, essential oils, and lipid sources presents a promising strategy to develop nutritionally enhanced, functionally stable, and sensorially appealing egg-based products. These results emphasize the necessity of understanding ingredient-function interactions and highlight the potential of customized formulations to meet consumer demand for high-quality, functional food products.

## 6. NEW SCIENTIFIC RESULTS

1. I found that 5 and 10 percentages (w/w) of both egg white and whey isolate proteins combined with pasteurization temperature of 65°C and 70°C in liquid whole egg, and 55°C and 60 °C in liquid egg white, increased protein aggregation and accelerated early gelation. With the 10-percentage added proteins, sample showed the most significant viscosity changes, including potential destabilization with storage time, in case of liquid whole egg, and mid-storage breakdown in case of liquid egg white at 55 °C.
2. I found that 21-day storage at 4°C affected the stability of liquid whole egg and liquid egg white, especially with 10% W/W added egg and whey proteins and at pasteurization temperature of 60–70°C. In liquid whole egg, 10% W/W egg white protein with 70°C pasteurization increased viscosity early on but showed gel weakening and phase separation after 14 days. Liquid egg white was more stable when pasteurized at 60°C for both proteins, meanwhile at 55°C it showed some degradation after two weeks. liquid egg white with 5%W/W egg white protein pasteurized at 60°C retained structural over the full storage period, indicating it as the most favorable condition.
3. I found that the addition of whey isolates and egg white proteins into liquid whole egg after pasteurization on  $60 \pm 0.2^\circ\text{C}$  with 3.5 minutes of holding time, showed minimal impact on liquid whole egg viscosity parameters. This indicates that these protein sources do not significantly disrupt the rheological behavior of liquid whole eggs.
4. I found that across all measured parameters, the percentages of yolk were the primary contributor to techno-functional properties. At measured parameters and sensory testing, samples with 80% (w/w) yolk scored close results to those with 100% yolk. This finding suggests that manufacturers can utilize formulations with 80% yolk to reduce production costs without causing noticeable changes in product quality.
5. I found that the addition of any percentage of essential oils significantly increased antioxidant activity in liquid whole egg samples but did not affect other rheological or structural parameters. Meanwhile, the addition of oils significantly affected the rheology of liquid whole eggs. coconut oil caused the greatest increase in yield stress with strong shear-thinning behavior at all added concentrations, while olive and sunflower oils induced mild shear-thickening behavior, and palm oil moderately enhanced viscosity with near-

Newtonian flow. The increase in antioxidant activity with the addition of essential oils suggests that they can be incorporated as natural functional additives to improve nutritional value without compromising processing characteristics.

## 7. SUMMARY

This thesis explored the impact of fortifying liquid whole eggs with various functional ingredients like egg white powder, whey protein isolate, essential oils, and different types of vegetable oils, on their technological, functional, rheological, and sensory properties. The study also examined the influence of different pasteurization temperatures and storage duration on product quality and stability. The incorporation of proteins at levels of 3, 5, and 10 percent, in combination with pasteurization at 60, 65, and 70 °C for liquid whole eggs, demonstrated that both the amount of protein and the applied thermal treatment significantly affected the structural development and viscosity of liquid whole egg products during a refrigerated storage period of 21 days. Higher protein concentrations, at 10 percent, and elevated pasteurization temperatures led to increased protein aggregation and gel-like behavior, especially evident after 7 days of storage. However, prolonged storage resulted in destabilization, including gel weakening and phase separation, especially in samples treated at 70 °C. In contrast, liquid egg white with added proteins at levels of 3, 5, and 10 percent, in combination with pasteurization at 50, 55, and 60 °C samples exhibited greater structural stability, particularly when treated at 60 °C with 5 percent added protein, which retained its structural integrity and foaming properties over the full storage period. Pasteurizing at 55 °C led to early gelation but was associated with mid-storage breakdown, indicating that 60 °C was the optimal thermal treatment for maintaining long-term stability in liquid egg white formulations.

Further investigation revealed that the addition of whey protein isolate or egg white powder after pasteurization at 60 °C with a holding time of 3.5 minutes caused minimal changes in the viscosity of liquid whole egg, indicating that post-thermal protein enrichment does not significantly interfere with rheological behavior.

Reducing the yolk content to 80 percent by partially substituting with liquid whole egg showed no significant difference in techno-functional or sensory properties compared to samples with 100 percent yolk. This finding, confirmed by both instrumental measurements and sensory panel evaluations, suggests that such formulations can lower production costs without affecting consumer acceptability, offering a sustainable alternative for industrial production. The inclusion of essential oils contributed to increased antioxidant activity in the egg matrix without altering the

rheological or structural characteristics, making them suitable as natural additives for enhancing nutritional quality while maintaining product integrity.

Vegetable oils exerted diverse effects on the rheology of liquid whole eggs. Coconut oil, in particular, significantly increased viscosity and yield stress and exhibited strong shear-thinning behavior, suggesting intense interactions between fat and proteins. Palm oil led to moderate thickening and displayed near-Newtonian flow behavior, while olive and sunflower oils caused mild shear-thickening effects with minimal impact on viscosity. Sensory evaluations of cooked egg formulations enriched with these oils showed that sunflower oil at 5 % and coconut oil at 2.5 % produced the most favorable outcomes in terms of flavor, texture, and overall acceptability, which was attributed to their specific fatty acid profiles and aromatic compounds. These results demonstrate that the type and concentration of oil play a crucial role in optimizing sensory performance in fortified egg products.

Although this study focused primarily on functional, rheological, and sensory properties, it is essential to highlight that all processing and formulation treatments, such as protein enrichment, yolk modification, essential oil incorporation, and oil type, may affect the microbiological stability of the product. Therefore, a microbiological challenge test is necessary as the next phase of this research to evaluate microbial safety during extended storage. This step will help ensure compliance with food safety regulations and support the commercialization of these functional liquid egg formulations.

This thesis underscores the potential of customizing liquid whole egg formulations through strategic fortification and thermal processing to meet functional, economic, and sensory goals. The findings contribute to the growing field of functional foods and provide a foundation for industrial-scale innovation in egg-based product development.

## 8. APPENDICES

### *Annex 1*

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