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ANALYSIS OF THE QUALITY CHARACTERESTICS OF DIFFERENT PRODUCTS CONTAINING ENZYME-TREATED EGGS

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1. INTRODUCTION

Eggs are a plentiful and versatile source of nutrition, valued for their use in both food preparation and nutrition. The structural complexity and chemical composition of eggs make them an intriguing subject for food engineers and researchers to investigate. Their proteins, lipids, and various bioactive compounds not only contribute to their distinct flavour and texture, but they are also essential ingredients in a wide variety of food creations. The utility of eggs in various food applications, on the other hand, can be greatly increased through innovative techniques, with enzymatic hydrolysis, specifically the use of food-grade aminopeptidase, being one such promising avenue.

Frozen egg products represent a transformative solution in food science, combining simplicity and healthy quality. As a response to contemporary lifestyle demands, these products, which include whole eggs as well as specific components such as yolks or whites, provide an appropriate and adaptable component that transcends traditional food boundaries. Even after extended storage, the freezing process maintains the nutritional integrity of eggs by retaining essential proteins, lipids, and bioactive compounds.

However, freezing liquid egg products presents several challenges that must be carefully considered. The formation of crystals during freezing can affect the texture and integrity of the product. When ice crystals form haphazardly, they can disrupt the protein matrix, resulting in a less desirable texture when thawed. Furthermore, the freezing process can interfere with the functionality of various bioactive compounds found in eggs, potentially reducing their nutritional value.

As liquid eggs freeze, the proteins within undergo complex changes, resulting in the formation of a gel-like structure. This process is not only scientifically fascinating, but it is also critical in determining the quality and usability of frozen egg products. Innovative methods, such as enzymatic hydrolysis, are emerging as promising methods for increasing the usefulness of frozen egg products. The dictated break down of proteins by enzymatic action can alleviate gelation and texture problems potentially offering an approach to the issues that have been posed by freezing.

In the following chapters, we will delve into the methodologies, findings, and implications of our research, ultimately providing a deeper understanding of the intriguing interplay between enzymatic treatment and the quality of egg-derived culinary delights.

2. AIM AND OBJECTIVES

This research aims to give a better explanation on the effect of applying enzymatic hydrolysis (Food grade aminopeptidase) treatment on the quality attributes of eggs and products made from them like mayonnaise and sponge cake.

The impact of enzymatic hydrolysis treatment on the gelation characteristics of eggs which are formed due to freezing is the second goal for this research.

The following perspectives are the core objectives of this research:

a) Evaluation of changes in physic-chemical properties of fresh and frozen-thawed enzyme treated liquid egg yolk and liquid whole egg, including pH, colour, and turbidity measurements.
b) Comparing the quality properties of products made with the enzyme treated eggs to the ones made from the untreated eggs by performing colour measurements and texture analysis on the mayonnaise and the sponge cake. Additionally, the effect of freezing on mayonnaise and sponge cake made from both enzyme-treated and untreated liquid egg products was investigated.

3. LITERATURE OVERVIEW

3.1.Egg Structure

A laid avian egg has the potential to provide life for the next generation of birds (Yamamoto et al., 2018). Since ancient times, humans have considered hen eggs to be one of the best sources of nutritional food (Wiley et al., 2008).

Eggs consist of three main parts (Figure 1) which are eggshell with the eggshell membrane, albumen (egg white), and yolk. Yolk is covered with an albumen layer which is enclosed by the eggshell membrane and a hard eggshell as the outer final covering layer (Wiley et al., 2008).

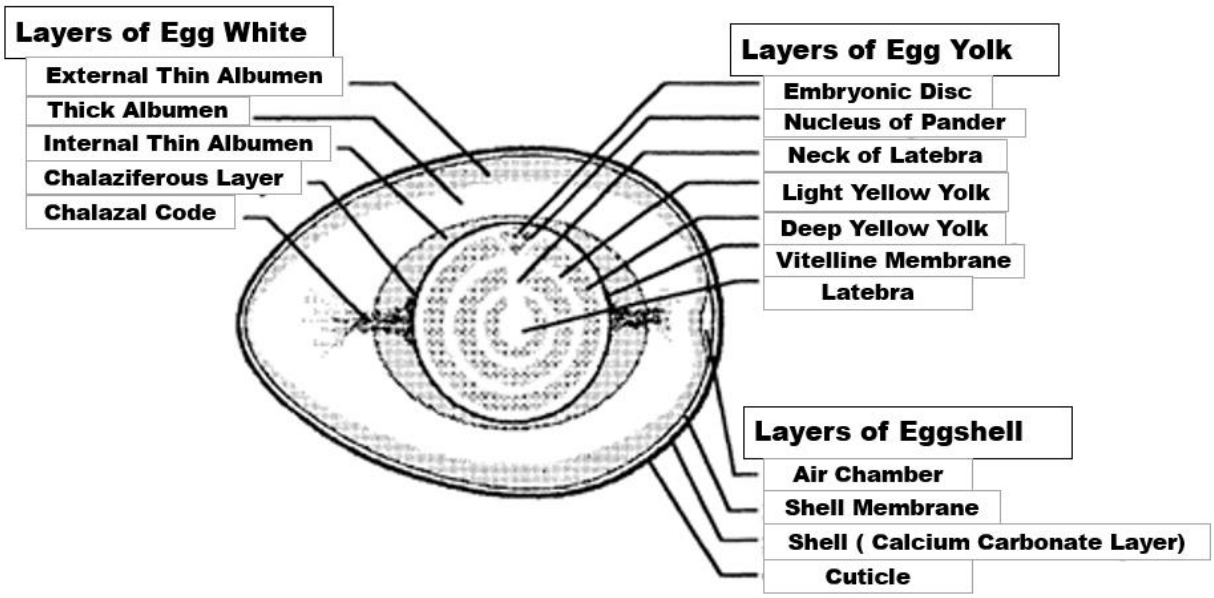

Figure 1. Egg Structure (Yamamoto et al., 2018).

3.1.1. Eggshell

The eggshell is a complex structure composed of several layers, each serving specific functions. It consists of a thin layer of cuticle, a layer of calcium carbonate, and two shell membranes (Yamamoto et al., 2018). This external shell exhibits remarkable features essential to the egg's protection and gas exchange. On its surface, there are approximately 10,000 pore canals, each with a diameter ranging from 10 to 30 µm, facilitating gas exchange (Yamamoto et al., 2018).

The cuticle, a water-insoluble layer, acts as the outermost protective barrier for the egg (Yamamoto et al., 2018). It covers the pore canals and, to some extent, shields the egg from moisture and microbial invasion. Despite its protective role, the cuticle still allows for gas exchange within the egg. Comprising proteins, carbohydrates, and lipids, the cuticle consists

of two layers: an inner mineralized foamy layer and an outer compact layer composed solely of an organic matrix (Dennis et al., 1996).

Beneath the cuticle lies the Shell Matrix, primarily composed of calcium carbonate (Yamamoto et al., 2018). This layer exhibits a layered structure, consisting of a vertical crystal layer, palisade layer, and mammillary knob layer (Yamamoto et al., 2018). Calcium carbonate, in the form of calcite, forms elongated structures known as palisades (Arias et al., 1993). The interaction between calcium carbonate minerals and organic matrix molecules influences the size, shape, and orientation of the calcite crystals within the hen eggshell (Hincke et al., 1999). Figure 2 provides a schematic illustration of the Shell Matrix's structural layers.

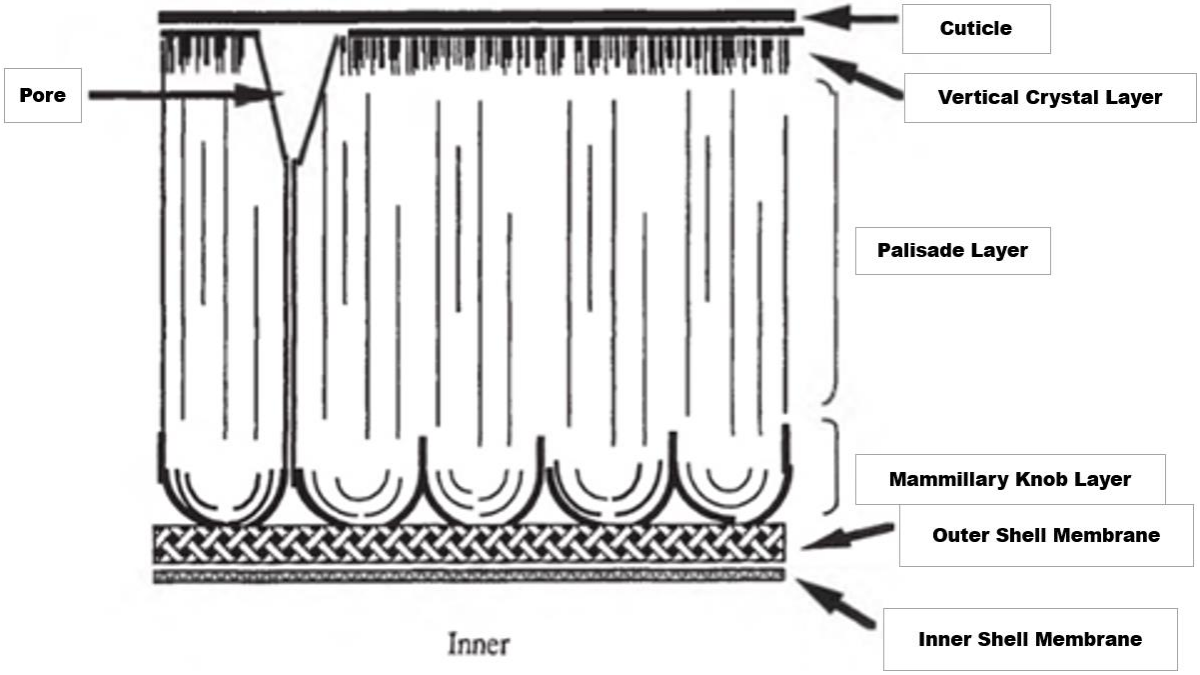

Figure 2. A schematic illustration of hen eggshell (Yamamoto et al., 2018).

The eggshell is further reinforced by the presence of inner and outer membranes (Yamamoto et al., 2018). These membranes form a meshwork-like structure, serving as an effective defense against invading microorganisms. The outer membrane, approximately 50 µm thick, and the inner membrane, approximately 15 µm thick, consist of 70% organic matter, 10% inorganic matter, and 20% moisture. The primary organic constituent is protein, with minor amounts of lipids and carbohydrates contributing to the composition. This lamellar structure of the shell membrane consists of a thin, insoluble fibrous protein layer intertwined with numerous meshworks (Yamamoto et al., 2018) (see Figure 3 for a visual representation of the membrane structure).

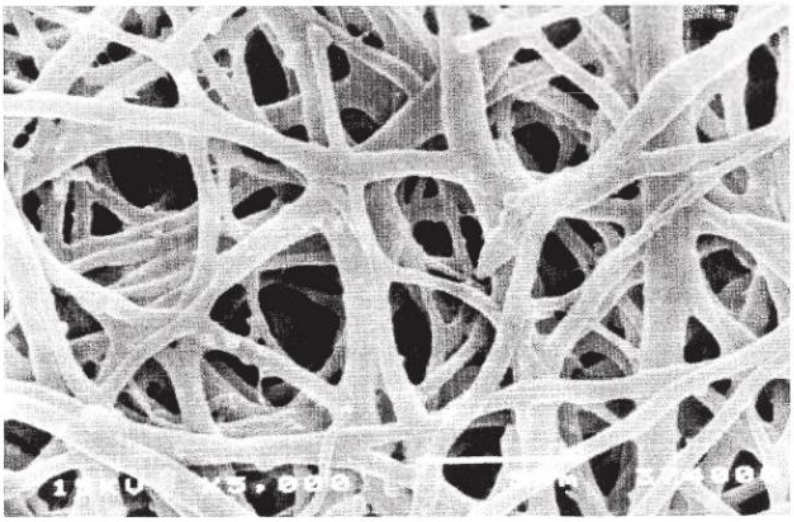

Figure 3. Photograph of a shell membrane taken with a scanning electron microscope (Yamamoto et al., 2018).

3.1.2. Egg Albumin

The transparent cytoplasmic liquid contained within an egg is known as egg white or albumen, and it plays a crucial role in the overall structure and composition of the egg. Albumen is a pseudoplastic fluid, and its viscosity is notably influenced by shearing force (Belitz et al., 2008). Enclosed by the protective shell membranes, albumen surrounds and safeguards the egg yolk, acting as an elastic and shock-absorbing viscous material with a relatively high water content (Rahman, 2014).

Albumen can be divided into four distinct layers: an outer thin white layer adjacent to the shell membrane, a viscous or outer thick white layer, an inner thin white layer, and a chalaziferous or inner thick layer. These layers have approximate content ratios of 23.3%, 57.3%, 16.8%, and 2.7%, respectively, though these ratios may vary depending on factors such as the hen's breed, environmental conditions, egg size, and production frequency (Wiley et al., 2008).

One of the critical factors contributing to the differing viscosity of albumen layers is the presence of ovomucin, a heat-stable protein found in egg white (Wiley et al., 2008). Ovomucin not only affects the viscosity of albumin but also plays a crucial role in thinning the egg white during storage (Belitz et al., 2008).

The egg yolk is centrally located within the egg and is surrounded by the thick albumen layer, which covers the inner thin albumen and the chalaziferous layer (see Figure 4). This thick albumen, as mentioned earlier, exhibits higher viscosity compared to the thin albumen due to the higher concentration of ovomucin (Yamamoto et al., 2018).

Figure 4. Internal Demonstration of Egg Structure (J. Spiegle B.S & Y. Morishita, 2022).

The chalaziferous layer is a fibrous and unique component that entirely enshrouds the egg yolk. This layer bends at both sides of the yolk membrane, aligning longitudinally within the egg to form a thick, rope-like structure known as the chalazae cord (Yamamoto et al., 2018). The cord wraps clockwise at one end of the egg and counterclockwise at the other. Comprising twisted ropes of protein fibres, the chalazae are firmly attached to both the egg's vitelline membrane (VM) on the inside and the eggshell on the outside (Rahman, 2014).

3.1.3. Egg Yolk

The egg yolk, constituting approximately 36% of the weight of a fresh whole hen egg (Huopalahti et al., 2007), is a spherical structure encompassing a germinal disc and enclosed by a thin, transparent vitelline membrane. Suspended within the egg white by chalazae (Rahman et al., 2007), the yolk is a complex system characterized by particles suspended in a clear yellow plasma containing proteins. These particles can be categorized into three types: spheres, profiles, and granules (Huopalahti et al., 2007). White yolk, comprising less than 2% of the total egg yolk, originates from the maturing white follicle in the ovary (Wiley et al., 2008).

The vitelline membrane, as examined by Bellairs et al. (1963) and Rahman et al. (2009) through electron microscopy, comprises two primary layers: an inner layer in contact with the yolk and an outer layer in contact with the egg white, with a "continuous membrane" perpetually positioned between them. The vitelline membrane consists of three distinct layers—outer, continuous, and inner—with a total thickness of about 10 mm. The outer layer, approximately 5 mm thick, features a rigid structure with fibre layers, while the continuous layer is a thin film

measuring 0.1 to 0.2 mm in thickness. The inner layer, adhering closely to the continuous layer, exhibits a more compact structure with thick net-like fibres (MINEKI & KOBAYASHI, 1997).

The vitelline membrane, weighing an average of 51 mg per egg, contains a solid matter content ranging from 20% to 30%. This solid matter consists predominantly of protein (87%), with smaller proportions of lipids (3%) and carbohydrates (10%). Additionally, it contains DNA and RNA. The protein composition of the vitelline membrane does not fall into traditional categories such as collagen, keratin, or elastin due to its unique amino acid composition (Yamamoto et al., 2018).

Yellow yolk exhibits two distinct types of lipoprotein emulsion: deep yellow yolk and light-yellow yolk. These variations in yolk coloration arise from diurnal fluctuations in protein concentration in the blood serum. Deep yellow yolk forms during the day, while light-yellow yolk forms at night, resulting in the characteristic alternating and circular appearance of these yolk layers (Yamamoto et al., 2018; Romanoff & Romanoff, 1949).

3.2.Egg Chemical Composition

The primary chemical constituents of hen eggs are 12% proteins, 12% lipids, and the remaining amount (75%) is water and small amounts of minerals and carbohydrates.

Eggs contain a variety of minerals that are required for the egg hatches most minerals are conjugated, with only a small portion present as inorganic compounds or ions.

Egg yolk presents a homogeneous liquid with its predominant component being lipoproteins(Figure 5), a blend of plasma (supernatant upon centrifugation) and granules (precipitate upon centrifugation). A comprehensive insight into egg yolk protein chemistry and localization is detailed in Table 1.

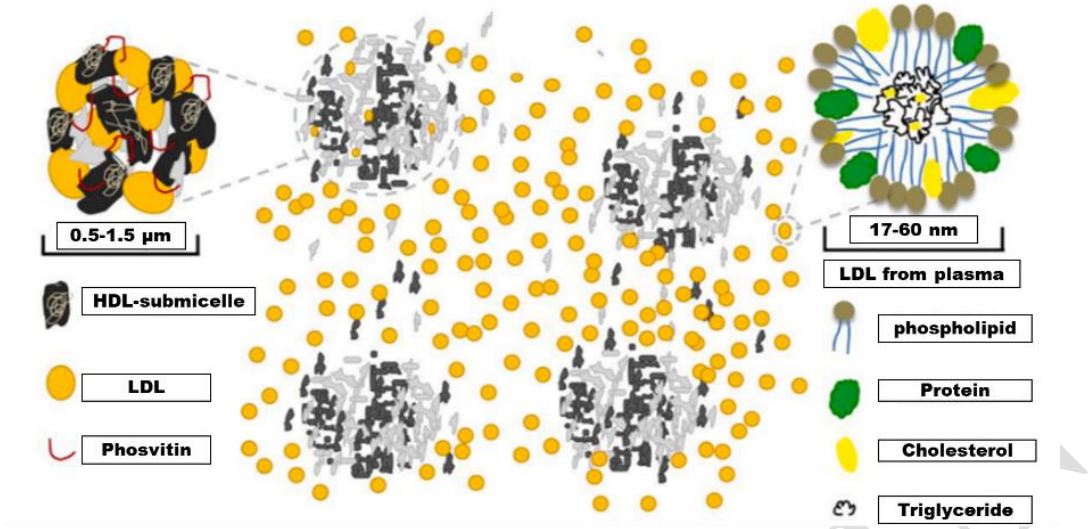

Figure 5. Lipoprotein sapromolecule structure in egg yolk (Zhao et al., 2021).

Table 1. Distribution of proteins in egg yolk (Yamamoto et al., 2018).

Fraction	***Plasma***	***Granule***
Protein	72 % (100)	22% (100)
High Density Lipoprotein (HDL) α -Lipovitellin β -Lipovitellin	 - -	 (41) (29)
Low Density Lipoprotein (LDL)	(87)	(12)
Phosvitin	-	(17)
Livetin	(13)	-

Egg yolk boasts a modest carbohydrate content, accounting for approximately 1.0% of its composition. Among these carbohydrates, 0.7% is composed of protein-bound oligosaccharides, primarily consisting of mannose and glucosamine, while the remaining 0.3% consists of free carbohydrates such as glucose. Within the realm of mineral composition, phosphorus reigns supreme in egg yolk, constituting 1% of the total mineral content. A significant proportion of this phosphorus is found in phospholipids, surpassing 61% of the total phosphorus content within egg yolk.

Egg whites, comprising approximately 11% protein, encompass a diverse array of over 40 distinct protein types, with many remaining unidentified due to their relatively low concentration. The primary egg white protein, ovalbumin, dominates at around 54% of the total egg white protein content (Table 2). Other significant proteins include ovomucoid

(approximately 12%) and ovotransferrin (conalbumin, approximately 11%). Additional proteins present in smaller quantities include lysozyme E, ovomucin, ovomacroglobulin, ovoglobulin, ovoglycoprotein, ovoinhibitor, avidin, cystatin, and flavoprotein.

Table 2. Proteins in egg white (Yamamoto et al., 2018).

Proteins	***Content % of total Protein***
Ovalbumin	54
Ovotransferrin	12
Ovomucoid	11
Ovomucin	3.5
Ovoglobulin G2	4
Ovoglobulin G3	4
Lysozyme	3.4
Ovomacroglobulin	0.5
Ovoglycoprotein	1
Flavoprotein	0.8
Ovoinhibitor	1.5
Cystatin	0.05
Avidin	0.5

While fresh egg whites exhibit minimal lipid content (about 0.02%), the extended storage of eggs may lead to lipid migration. Triglycerides and cholesterol esters are believed to diffuse into the egg white over time, potentially altering its foaming properties due to the yolk membrane's weakening.

Egg white contains carbohydrates in both free (0.4% of egg white) and bound (0.5% of egg white) forms, primarily in the glycoprotein configuration. Glucose prevails as the predominant free form (approximately 98%). Minor traces of mannose, fructose, ribose, arabinose, and xylose are also present. These reducing sugars contribute to aminocarbonyl reactions, which can induce the browning process observed in powdered whole egg or egg white products.

The major inorganic components of egg white encompass potassium, sulphur, chlorine, and sodium, while iron is present in trace amounts. Following these elements in importance are calcium, phosphorus, and magnesium (Yamamoto et al., 2018).

3.3.Egg Nutritive Value

Hen eggs represent a significant dietary source, with approximately 74.57% of their composition consisting of water. Despite their high-water content, eggs are rich in essential nutritional components, including proteins (12.14%) and lipids (11.15%). The nutritional value of egg proteins has been extensively investigated, revealing a perfect balance of nutritionally

essential amino acids. Thus, eggs can effectively supplement amino acid-deficient foods. Additionally, eggs are a noteworthy source of omega-3 fatty acids. Due to their high nutritional value, low caloric content, mild flavour, and ease of digestion, eggs are a suitable dietary option for individuals of all ages, including both the young and the elderly, as well as those in good health or convalescence (Yamamoto et al., 2018).

Egg proteins, present in both the yolk and albumen, are regarded as complete proteins, characterized by an exceptional amino acid profile. Egg yolk contains most lipids in eggs, making it a significant potential energy source, constituting more than 65% of its dry weight. The lipid composition includes triglycerides, phospholipids, and sterols. Notably, egg yolks are rich in linoleic acid, an essential component of human nutrition. The egg albumen contains most egg carbohydrates, although they constitute only about 1.0% of the total egg composition, rendering them an insignificant energy source. The yolk contains most of the vitamins found in eggs, particularly the fat-soluble vitamins. Except for vitamin C, hen eggs are considered a source of most vitamins required for human nutrition. One egg contains nearly 12% vitamin A, over 6% vitamin D, 9% riboflavin, and 8% panthotenic acid (Yamamoto et al., 2018).

Eggs serve as a valuable source of essential minerals, including iron, phosphorus, zinc, copper, and various trace minerals. (Yamamoto et al., 2018). Numerous factors directly influence nutrient concentrations in eggs. These factors encompass the age, species, and strain of hens, individual variations in egg production, the nutritional and environmental conditions of hens, egg storage conditions and duration, as well as the manufacturing, processing, and cooking techniques applied (Stadelman & Cotterill, 1995).

3.4.Liquid egg products processing

Egg products are products derived from eggs, their components, or mixtures of them, leaving out the shell and membranes, that are intended for human consumption (Anton et al., 2016). A broad range of egg products are produced for many different purposes, and depicting these various types is provided in Table 3 (Forsythe, 1970).

Table 3. Types of egg products.

Types of Products	***Source of Products***
Liquid Egg Products	From: • White Egg • Egg Yolk • Whole Egg
Frozen Egg Products	From: • Whole Egg • Plain Yolk • White Egg • Fortified Whole Egg / Yolk • Salted Whole Egg • Sugared Egg Yolk

Egg products are divided into two distinct groups: egg products after primary processing (whole egg, egg yolk, or egg white presented in various forms for use as techno-functional ingredients) (Figure 6), and egg products after secondary processing (industrial-scale preparation of eggs based on classic methods of preparation, such as cooked or precooked products) (Anton et al., 2016).

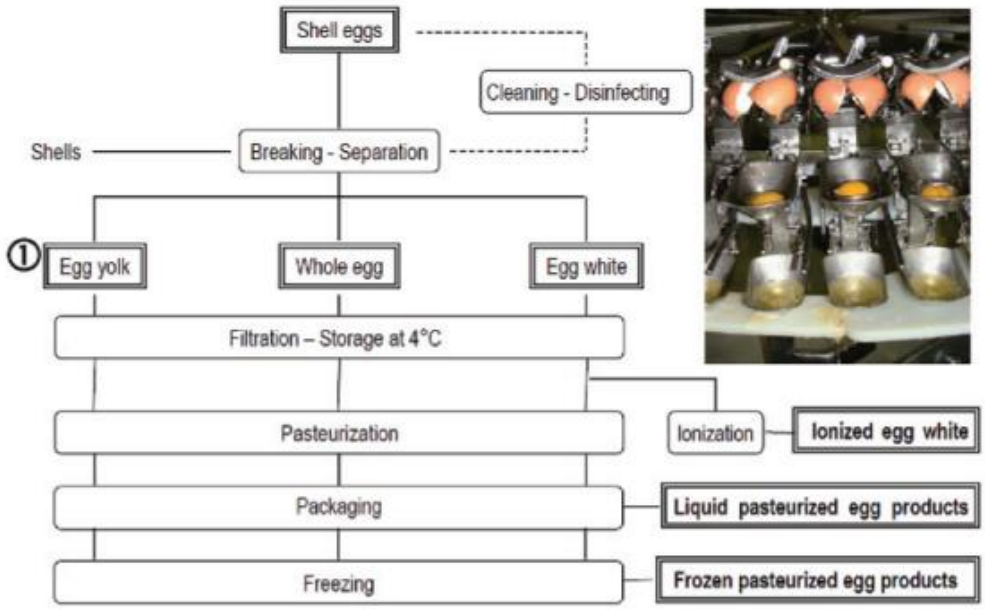

Figure 6. Diagram of the primary processing of egg products (Anton et al., 2016).

3.5.Freezing of Food

Freezing is a vital method for protecting product quality and is commonly utilised in the food processing sector. Frozen foods are thought to represent the next era of food for convenience

(Zhao & Takhar, 2017). Freezing inhibits microbial growth, reduces the activity of water, and slows chemical and enzymatic processes.as a result, frozen products have an extended shelf life and can be shipped across lengthy journeys (Sun, 2012).

Because egg juice lacks a physical cell structure and tissue structure, the effect of freezing on the quality of egg products differs from that of meat and vegetables. Because eggs contain higher concentrations of soluble than most liquid foods, their freezing behaviour differs from that of milk and fruit juices (Dawson, 2019). Frozen egg products have a longer shelf life than shell eggs and egg juices because freezing reduces the number of bacteria in egg juice. When whole egg juice and yolks are frozen at -6°C or lower to temperatures below or equal to freezing, an irreversible change occurs due to gelatinisation of the yolk. When thawed, these products do not have the same properties as shell eggs or fresh egg products (Hidas, 2022).

Freezing causes significant modifications to texture in certain products made from eggs, as well as a significant decrease in the number of bacteria. Even though the functional characteristics are only impacted slightly. Viscosity increases and gelation takes place during freezing and preservation. All flexibility is eventually vanished. As a result, this gelled product is unable to be processed in the same way that other liquids can. It is difficult to combine with other components and has an unappealing physical appearance. (Stadelman & Cotterill, 1995).

3.6.Effect of freezing on the egg products

Food freezing has negative aspects and restrictions as well. Denaturation of proteins and accumulation, for example, may occur in protein-rich foods. The formation of ice frequently results in a change in the configuration of the hydrophobic amino acids part because of ice crystal interaction. Furthermore, a rise in concentration of solutes caused by freezing may deteriorate proteins. This phenomenon is linked with a shift in the pH and denaturation of proteins, possibly aggregation (Hidas, 2022).

While freezing leads to only slight modifications in egg whites, such as diluting of the thick protein according to Stadelman and Cotterill (1995), it causes permanent changes in yolk mobility when the yolk is cooled to 6°C or below (Meyer & Woodburn, 1965). As a result, the yolk develops a paste-like arrangement, making it hard to handle and combine while decreasing its utility. The simplest explanation for egg yolk gel formation throughout freezing is that the ice particles created concentrate the yolk's parts, leading to the formation of lipoproteins with a low density in the yolk's plasma fractions (Hidas, 2022). The process of LDL aggregation has received a great deal of attention.

According to Nicoletti and Kieckbusch (1997), protein dehydration on the surface of LDL micelles causes LDL aggregation after LDL micelles rupture. Jun-ichi Kurisaki et al. (1980) said that exterior components of LDL are released throughout the freezing and thawing processes, resulting in an accumulation of freshly subjected sites. But according to WAKAMATU et al. (1982) LDL aggregation is caused by conformational changes rather than the release of LDL components. According to the research (Wang et al., 2010), protein aggregation could be the cause of this type of egg yolk gel that results. Particularly, the bonding of ice crystals and proteins, as well as the reduction of steric barriers in protein-protein interactions, facilitate protein aggregation.

Because of their inherent and high aggregation tendency during various manufacturing processes and storage, proteins have been identified as a major challenge in the development and commercialization of food products. Protein aggregates have the potential to significantly impact product quality, safety, and/or efficacy (Wang et al., 2020b).

According to the research of Wang and his co-workers (2020b), as the freezing time increased, the ability of the yolk to adhere to the walls increased. When the egg yolk in the centrifuge tube was frozen for more than 7 hours, it lost its fluidity as you can see in Figure 7. The structure of the yolk proteins was destroyed, and the hydrophobic sites of the proteins were exposed in frozen-thawed egg yolk, resulting in an increase in disulfide and hydrogen bond formation and a decrease in sulfhydryl group content. This improved interactions between protein molecules as well as between proteins and lipids. Through hydrophobic interactions and disulfide bonds, frozen-thawed egg yolk gels formed dense gel network structures. As a result, his findings revealed that the characteristic gel of freeze-thaw egg yolks was caused by multiple molecular interactions between proteins.

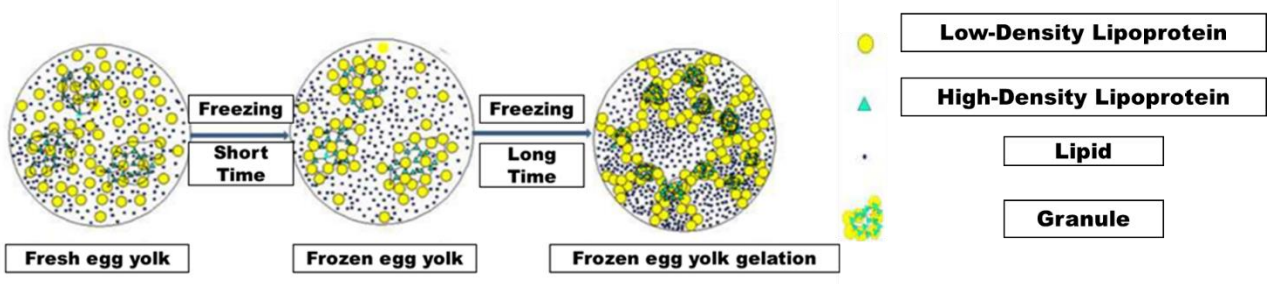

Figure 7. Egg yolk changes during freezing (Wang et al., 2020b).

3.7.Methods to prevent gelation during freezing.

In the past few years, researchers have used many kinds of physical and chemical techniques to avoid or minimise the degree to which gelation occurs (Stadelman & Cotterill, 1995).

The use of cryoprotective substances for this purpose is one heavily researched topic. Cryoprotectants are compounds that improve the performance and shelf life of frozen foods. Any compound that aids in the prevention of freezing damage in foods is referred to as a cryoprotectant. These substances may be added to foods during processing and manufacturing, or they may be produced naturally in the living organism from which the food is derived (MacDonald & Lanier, 1997). Glycerol, dimethyl sulfoxide, and ethylene glycol are examples of intracellular cryoprotective substances, while low molecular weight sugars and their derivatives, high molecular weight polymers, albumin, starch, polyvinylpyrrolidone, and polyethylene glycol are examples of extracellular protective agents (Hidas, 2022).

Moran (1925) was the first to figure out that sucrose inhibited the gelatinization of egg yolks during freezing. He discovered that when 10 m/m% sucrose was added to egg yolks, freezing to -11°C and thawing caused no significant change in yolk fluidity. LOPEZ and co-workers (1954) also observed an effective gelatinization inhibition influence with 10 m/m% arabinose and galactose to the yolks of eggs, but no inhibitory impact was noticed with the same concentration of different sugars (maltose, lactose, raffinose, cellobiose).

Furthermore, the improvement of both the freezing and thawing processes was examined, as proved the impact of the addition of proteolytic enzymes by using 0.05 wt.% papain (Lopez et al., 1955). However, there is not much data accessible regarding the scope of modifications to liquid whole egg, and no treatment has been developed to avoid them and enhance the utility of frozen-thawed specimens (Hidas, 2022).

According to Lopez et al. (1955), experiments on the process for action of enzymes in preventing yolk gel formation showed that some enzymes' gelation-inhibiting action can be triggered by:

a) The deterioration of the substances that cause coagulation in yolk and the resulting creation of derivatives products that do not have the capability to cause gelation.
b) The generation of a blocking substance that avoids gel formation as the consequence of the enzyme's activity on yolk parts.
c) The enzyme itself, as it might possess gelation-inhibitory characteristics.

In light of the absence of up-to-date measurement methods, Ma et al. (2021) observed that neutral protease significantly lowered gelatine formation of egg yolk during freeze-thawing in their recent study.

3.8.Rheological behaviours of liquid products.

Rheology is a branch of physics that studies how materials deform or flow when they are subjected to pressure or stresses. Rheological properties are the characteristics of materials that govern their deformation or flow behaviour. Temperature has a significant impact on these properties (Hidas, 2022).

Various fluid types exhibit distinct rheological behaviours in the study of dynamic viscosity, each defined by its response to shear rate. Understanding fluid classifications is critical for simplifying the equations that govern fluid mechanical problems and tailoring solutions to specific applications. The following are the primary fluid categories based on dynamic viscosity behaviour:

- Newtonian fluids, this is true viscous flow, in which shear rate is proportional to shear stress. It only occurs in laminar flow range and viscosity remains constant in the laminar flow range. The fluid will lose its Newtonian behaviour when turbulent flow begins. The slope of the shear stress and shear rate curve determines viscosity. Water, tea, coffee, beer, carbonated beverages, sugar syrups, most honeys, edible oils, filtered juices, and milk are all Newtonian fluids. Newtonian fluids have simple flow properties that can be described by the equation $\eta = \sigma/\gamma$,where η represents viscosity, σ is shear stress and γ ,the shear stress.
- Bingham fluids, a type of non-Newtonian fluid, introduce the concept of yield stress, which is a critical shear stress that must be exceeded before flow can begin. This distinct behaviour can be found in a variety of food products, including mayonnaise, whipped cream, whipped egg white, and margarine. A shear stress-shear rate curve illustrating a linear relationship after the yield stress is exceeded graphically, represents the flow properties of Bingham fluids. However, complexities arise as a result of potential curvature at low shear rates and various manifestations of plastic flow, such as dilatant and pseudoplastic behaviours. Those fluids are described by Herschel-Bulkley equation.
- Pseudo-plastic Fluids (Shear-Thinning) are observed when shear stress increases at a decreasing rate as shear rate increases. The flow curve has a convex profile, and the

slope decreases as shear rate increases, indicating that viscosity decreases. This property of pseudoplastic fluids involves an apparent decrease in flow resistance (shear stress) as shear rates increase. This behaviour can be both reversible and irreversible.

- Dilatant flow behaviour occurs when shear stress increases faster than shear rate. The flow curve has a convex profile, indicating that the slope becomes more inclined as shear rate increases. As a result, viscosity rises in parallel with the increase in shear rate. It is important to understand that dilatant flow behaviour can be reversible or irreversible (Bourne, 1982).

These fluid behaviour classifications aid in understanding material responses in a variety of engineering and scientific applications, facilitating the analysis and design of fluid flow and deformation systems (Rapp, 2017).

The egg industry procedures egg yolk at a variety of the temperatures and unit processes such as the pumping process, pasteurisation, freezing, and drying by spray. Understanding of the rheological behaviour of egg yolk influenced by the temperature is essential for appropriate process planning, operations, and authority (Telis-Romero et al., 2006).

According to Atılgan and Unluturk (2008) and Telis-Romero et al. (2006) we can determine that the rheological behaviour of an egg yolk has been shown to be pseudoplastic and influenced by temperature.

4. MATERIALS AND METHODS

4.1. Materials

4.1.1. Pasteurized Egg Products

Two bottles of liquid egg yolk – liquid whole egg each bottle one litre, were provided by CAPRIOVUS Ltd. company one day before the experiment and were stored and cooled in a fridge at 3°C.

Pasteurized liquid egg yolk was produced under factory conditions by separation, homogenization, and pasteurization at 65°C for 10 minutes. One kg is formed from 63 eggs. On the other hand, pasteurized liquid whole egg containing both egg yolk and egg white in natural proportions was also produced under the same factory conditions but undergoes pasteurization at 70°C for 3 minutes. One kg of it is made from 22 eggs.

4.1.2. Enzyme Preparation

A commercial aminopeptidase Flavorpro™ 750MDP (F750MDP) brown powder enzyme preparation of optimum pH range 5.5 ~ 7.5 and optimum temperature range 45-55°C was used for an enzyme treatment process. This Enzyme preparation was processed using *Aspergillus spp.* microorganism in a fermentation process. It was bought from the BIOCATALYSTS company.

4.2.Experimental Design

For both the liquid egg yolk and the liquid whole egg, four different samples of 500 g each were prepared. Three of the samples were treated using different concentrations of enzyme (0.1, 0.2, 0.3 w/w%), the control sample - without enzyme. The weight of the enzymes was measured by analytical balance, then dissolved in 3 ml of water and stirred with a spoon till they were homogenised.

20 ml of each sample was taken in a centrifuge tube. Then, each sample was divided into 250-250 grams in plastic bags (polyamide-polyethylene 90 µm) which were then sealed. The samples were labelled and put in a water bath (except for the four-control sample) at a temperature of 40°C for 2 hours.

After two hours, the samples were taken out of the water bath. Samples were cooled in melting ice. Half of the samples were put in a freezer at -24°for 14 days, and the other half undergo some of the measurements.

The thawing process was done before using the frozen egg samples, they were put in a thermal bath for 2 hours to reach the temperature of 4°C to thaw so that they can be used for further measurements in the experiment.

The measurements before and after freezing were the followings:

- Colour measurements
- pH measurements
- Measurement of emulsion stability (Turbidity)

Finished products like mayonnaise and sponge cake were made from both enzyme-treated and untreated liquid egg products. Colour measurements and texture analysis were performed on the finished products to investigate the freezing effect and check the quality characteristics. A schematic summary of the experimental design is shown in Figure 8.

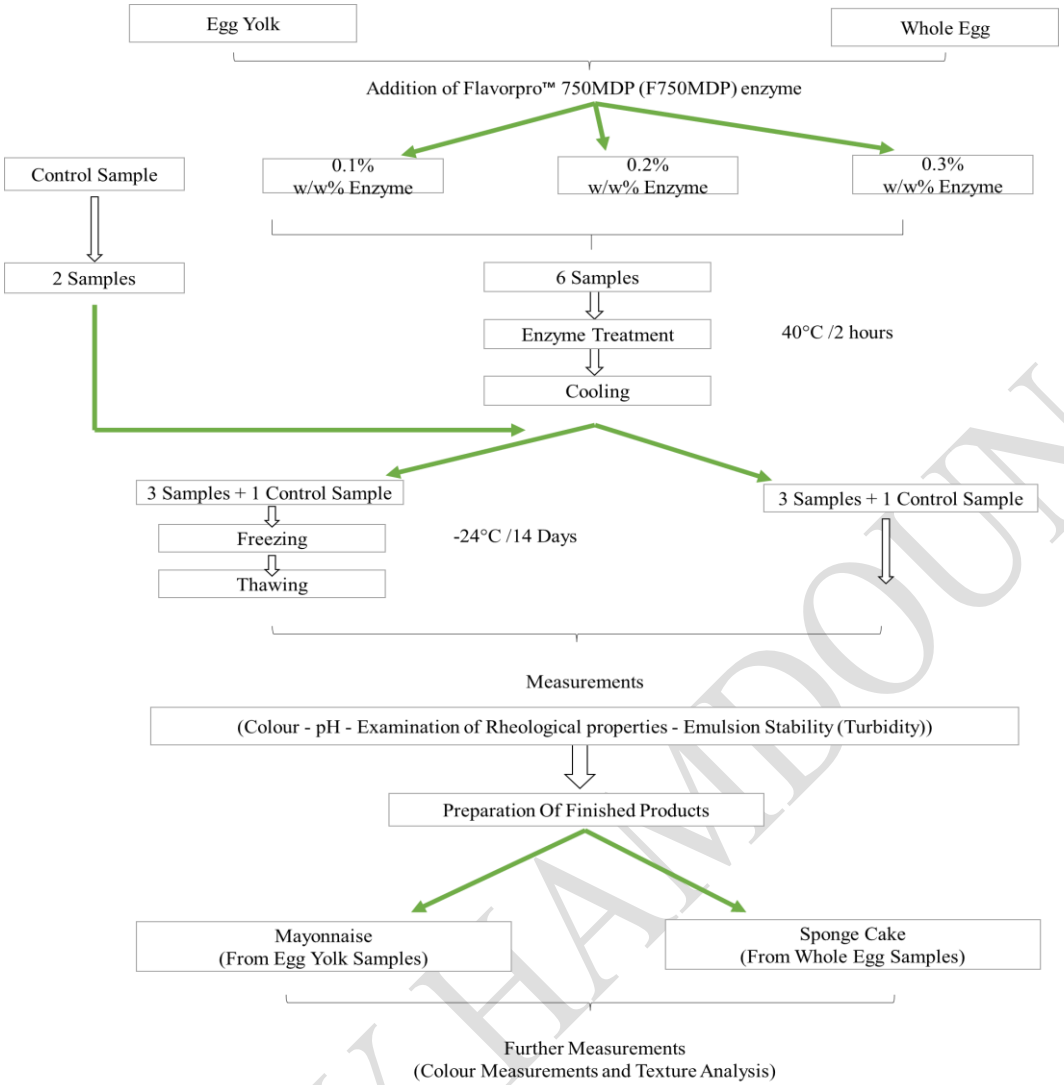

Figure 8. Schematic Diagram of the experiment procedures.

4.3.Preparation of The Finished Products

4.3.1. Mayonnaise Preparation.

Before and after freezing with the use of the enzyme-treated and control samples, 250 grams of Mayonnaise products were made based on the method of Huang and his co-workers (2016) with some modifications.

Table 4. Recipe used for making mayonnaise.

Ingredients	***Quantity (g/100 g)***
Vinegar	3.1
Sugar	2.3
Salt	1.2
Liquid Egg Yolk	18.9
Oil	74.5

Based on the recipe in table 4, a cylinder was used to make mayonnaise in it to get better emulsion capacity. Sugar and salt were added to the egg yolk in the cylinder and started mixing using a hand mixer (Serie HM04, TEFAL, ~200W) on maximum 5 speed for 5 minutes. The oil was added to the emulsion during mixing in a slow pouring flow rate for three and half minutes and during the last minute of the mixing process the vinegar was added.

After the different mayonnaise products were ready, they were poured into small glass containers and covered from the top using foil and put in the fridge to cool.

During making the mayonnaise using the frozen-thawed egg yolk samples, the entire process took 6 minutes instead of 5 minutes because the mayonnaise emulsion was resistant to the oil during pouring it.

4.3.2. Sponge Cake Preparation.

Before and after freezing with the use of the enzyme-treated and control samples, 250 grams of sponge cake samples were baked using certain amounts of ingredients which are:

Table 5. Ingredients used in the sponge cake recipe.

Ingredients	***Quantity (g/100 g)***
Whole Liquid Egg	30
Sugar	35
Flour	35

Sugar was added to the liquid whole egg in a plastic bowl and were mixed using hand mixer (Serie HM04, TEFAL, ~200W) on maximum 5 speed for one minute then half the amount of flour was added to the mixture and mixed with a hand blender till they were blended. At the end of the mixing process the rest of the flour was added, and a spatula was used to perform the final mix. After the mixture was ready, it was put in paper pie forms of 18 cm diameter, labelled, and baked in a LAINOX (VE051P) Oven~400V~50Hz at 180°C for 16 minutes.

The cake samples were left to cool down at room temperature. The crust of cake samples was removed by a knife and sponge cake samples with a height and diameter of 2 cm were cut out from the midsection of the cakes.

4.4.Measurements Methods

4.4.1. Determination of pH

Temperature and pH measurements were done on the fresh and frozen-thawed egg yolk and whole egg samples. The measurement was done using TESTO 206-pH1 pH measuring device (Figure 9). Before using the pH meter, it was calibrated using two buffer solutions 4 and 7. The measurement was carried out in triplicates.

Figure 9. TESTO 206-pH1 pH meter (Internet 1).

4.4.2. Measurement of Colour

Three parallel colour measurements were performed on the finished products (mayonnaise and sponge cake) and the fresh-thawed samples using a chromameter (CR-400 Chroma Meter) shown in Figure 10. Three parallel measurements were performed. The colour system parameters were measured (L*, a*and b*). Those parameters represent the colour's quantitative relationship on three axes as shown in Figure 11. L* value is on a vertical axis and refers to the brightness while a* and b* are chromaticity coordinates, a* value indicates red-green component of a colour and b* represents the yellow-blue components of the colour (Ly et al., 2020).

Figure 10.CR-400 Chromameter (Internet 2).

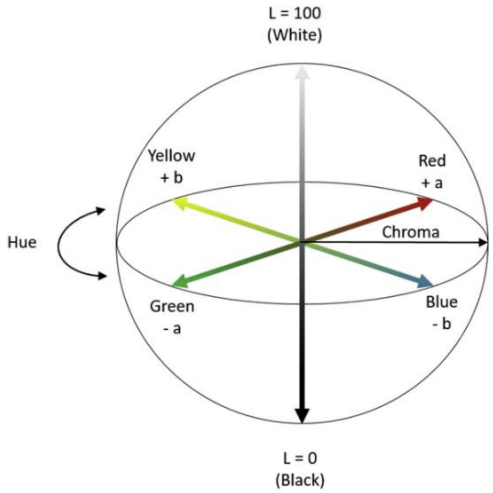

Figure 11. The CIELAB colour space diagram (Ly et al., 2020).

4.4.3. Measurement of Emulsion Stability (Turbidity)

Egg yolk fresh and frozen-thawed samples were used for the turbidity measurements. One gram of each sample was dispersed in 10% NaCl solution (1:100 Dilution). (Wang et al., 2020). The measurement was performed using a U-2900 spectrophotometer (Figure 12) at a 660 nm wavelength. Three replicates of each sample were measured.

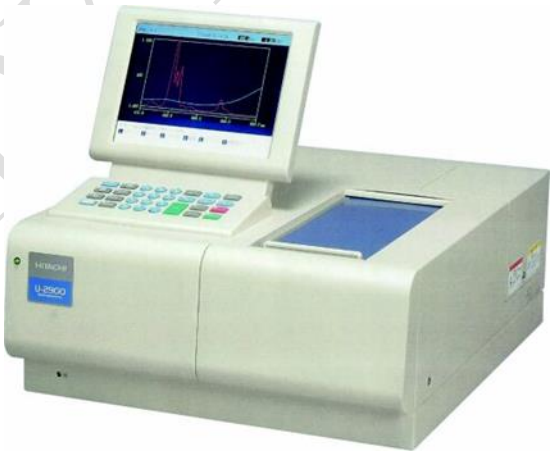

Figure 12. U-2900 Spectrophotometer (Internet 3).

4.4.4. Sponge Cake Texture Analysis

For the sponge cakes, a different test probe was used to perform the texture profile analysis. A cylindrical probe was used using 5 kg load cell (Figure 13). The distance was set to 10mm with a penetration speed of 1mm/s. The chosen probe is commonly used with bakery products. (Liu et al. (2019)).

We used sponge cake samples of 2 cm height and 2 cm diameter that were cut from the midsection of the cake. A texture analyser ((TA-XT Plus, Stable Micro Systems Ltd., Surrey, UK) with a 35 mm diameter cylindrical probe, 50% compressing and a test speed of 1.0 mm s^{-1}.Other parameters were defined as well like, pre-test speed 2.0 mm s^{-1}, post-test speed 2.0 mm s^{-1} and trigger force 5 g (Salehi & Kashaninejad, 2018).

The device squeezes bite-sized pieces of food two times and creates a force-time curve, that then serves to determine a couple of the textural attributes (Figure 14) (Rahman, 2005). Using the force-time curve (Figure 14) recorded and evaluated with Texture Exponent 32 software,

the following parameters were determined by reading:

- Areas A1 and A2: Area under the force-time curve of the first and second compression cycles of the measurement from the start of the measurement to the maximum force value and from the start of the second compression to the second compression the maximum force measured in the second cycle, for time deformation due to uniform motion can be converted and the area values can be matched with the first and second "chewing" on the sample compression work on the sample.
- Hardness (F1, [N]): The maximum deformation force measured during the first bite cycle.
- Cohesiveness (A2/A1): A dimensionless parameter, the measure of the shape stability.
- Springiness (S, [mm]): Deformation measured during the second compression.
- Gumminess (G, [g]): product of hardness and cohesion: G = F1 · C (Karina, 2022).

Minimum of ten replicates were measured for every sample.

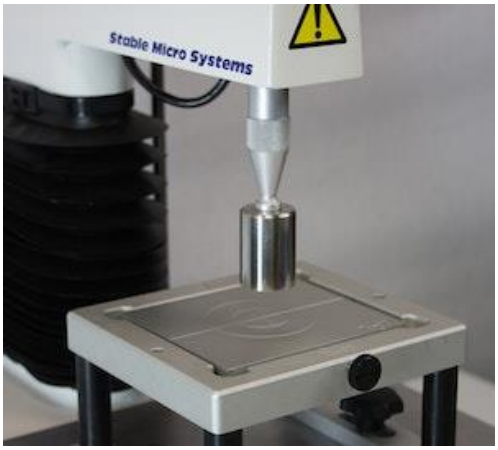

Figure 13. Cylindrical probe (Internet 4).

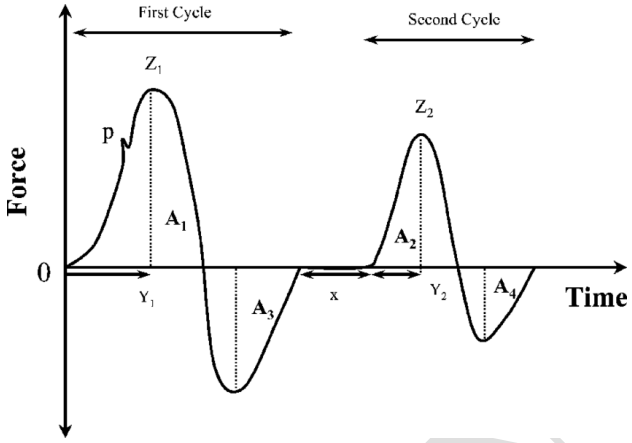

Figure 14. An analysis of instrumental texture profile (TPA) graph (Rahman, 2005).

4.4.5. Mayonnaise Texture Analysis

TA-XT texture analyser device was used to perform texture profile analysis and extrusion test. (Figure 15). The measurement was done on the finished products: Mayonnaise and Sponge cake using two different test probes.

Figure 15. TA-XT Texture Analyser Device (Internet 5).

In Mayonnaise, the samples had a temperature of 15°C. A conical probe was used (Back Extrusion Cell) with 35mm disc and extension bar using 5 kg load cell. (Figure 16). The distance was set and calibrated to 65mm with a penetrating rate of 1 mm/s. This probe was chosen because of its suitability for performing extrusion tests on fluids and semifluid's. At least six replicates were measured for each sample (Liu et al. (2019)).

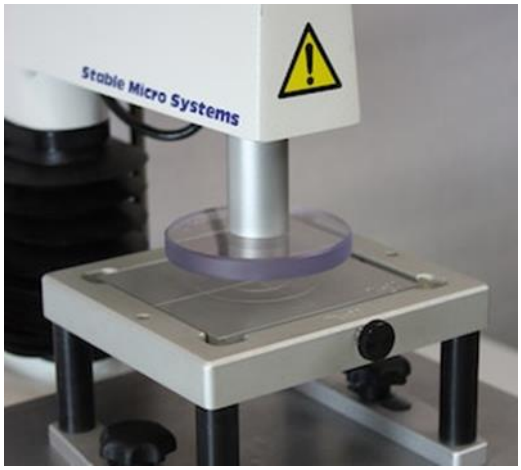

Figure 16. Back Extrusion Cell Probe (Internet 6).

A typical diagram to evaluate back extrusion force is depicted in Figure 17 showing the parameters that were recorded and measured during the analysis; Firmness (with a maximum compressing force), Adhesiveness (Consistency), Minimum Retracting Force (Index of Viscosity), and Cohesiveness.

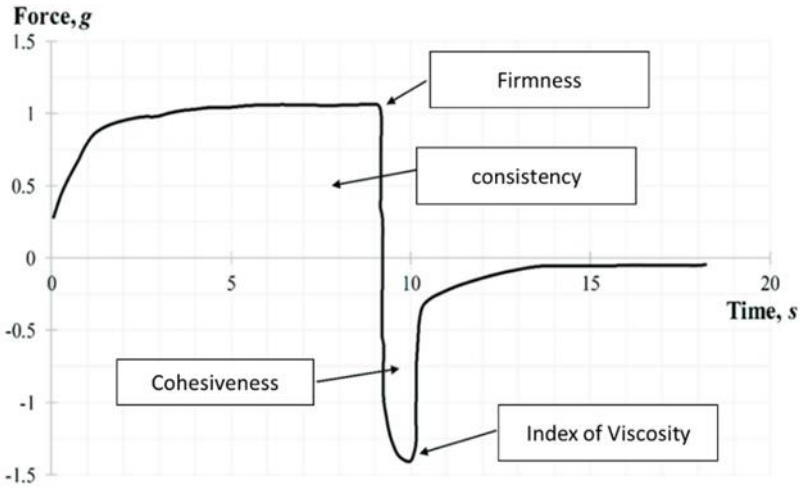

Figure 17. Graph for Back Extrusion During Texture Analysing (Maslii et al., 2020).

The value of the maximum force applied at the maximum penetration depth gives the strength of the sample (g), while the area under the curve up to this point is the consistency (g·s). When the gauge head returns to its initial position, the diagram shows a negative force value. The maximum force measured during the backward movement is the cohesion (g) and the area under the curve corresponding to this section is the viscosity index (g·s) (Hidas, 2022).

4.4.6. Statistical analysis

Each measurement method was performed three times for each sample and thus average and standard deviation were calculated to evaluate the final data.

The measured data were statistically evaluated using one-way ANOVA (IBM Statistics 24 software) at 5% significance level ($p<0.05$). The Shapiro-Wilk test was used to determine the normality of error terms, and Levene's test was used to determine the uniformity of variance. If the ANOVA was significant, Tukey's HSD test was applied to distinguish between the various groups if the homogeneity of variance state was met, and Games-Howell's test if it was not (Hidas, 2022).

Bar charts have been used to illustrate the mean values. Error bars, which represent standard deviations, have been included to depict the variability around the mean, providing a comprehensive understanding of both central tendencies and data distribution. Different small letters on the bar charts indicate significantly distinct groups ($p<0.05$).

5. RESULTS AND DISCUSSION

5.1.The effect of the enzymatic treatment and freezing on the liquid egg products

5.1.1. pH changes during the freezing procedure

The pH levels of both egg yolk and whole egg samples were measured both prior to and after freezing. Figure 18 and Figure 19 depict the results of pH measurements performed on the control samples as well as the three different concentrations (0.1, 0.2, 0.3 w/w%) of Flavorpro™ 750MDP enzyme-treated eggs.

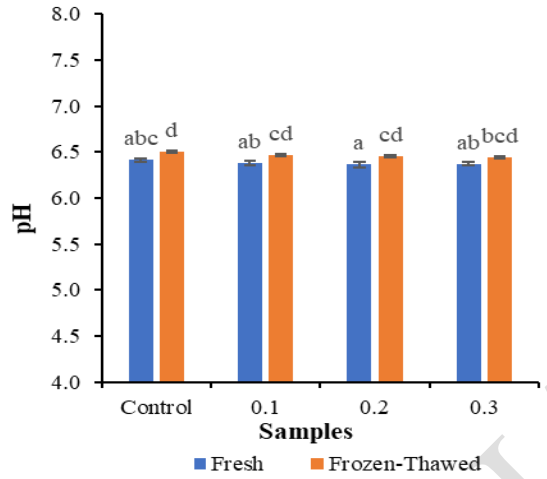

Figure 18. pH Results in Egg Yolk Samples.

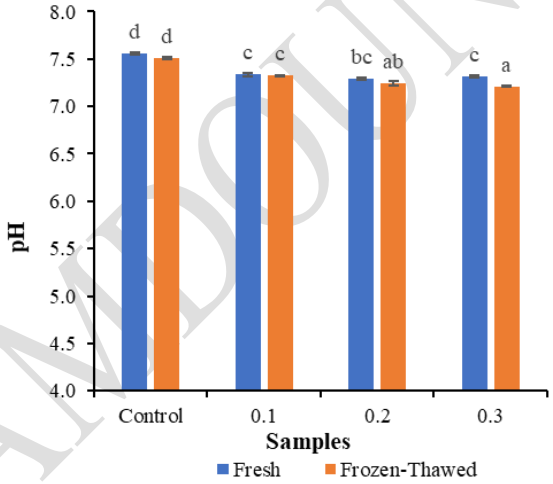

Figure 19. pH Results in Whole Egg Samples.

Several observations and conclusions can be drawn from the data presented in the column charts for pH measurements of fresh and frozen-thawed egg yolk and whole egg samples under various conditions:

By Comparing both Egg yolk and Whole Egg samples together, we can see that in the:

Fresh Control Samples: The pH difference between these two control samples is substantial. Whole eggs have a higher pH (are more alkaline) than egg yolks. This is due to the presence of proteins like albumin and ovalbumin in the egg white, which makes up most of the whole egg (Yamamoto et al., 2018).

Fresh vs. Frozen-Thawed Control Samples: In the Egg Yolk Samples, the frozen control sample had a slightly higher pH value than the fresh counterpart and they are statistically different. This pH unit difference indicates a minor increase in acidity upon freezing while in the whole

Egg Samples The frozen-thawed sample showed slightly lower pH value compared to the fresh one which indicates a minor decrease in pH upon freezing and there is no significant difference.

Effect of Enzyme Treatment: For egg yolk samples, when fresh enzyme-treated samples (0.1, 0.2, and 0.3 w/w% concentrations) are compared to their frozen counterparts, it is observed that the fresh samples have slightly lower pH values than the frozen counterparts in most cases. For example, the pH of the fresh 0.1 w/w% enzyme-treated egg yolk sample was 6.38, while the pH of the frozen counterpart was 6.46. This pattern suggests that the enzyme treatment may have a minor alkalizing effect on the samples as the enzyme may have contributed to protein hydrolysis resulting in releasing of amino acids where some of them can be basic in nature causing increase in pH.

Influence of Concentration: There is no consistent trend in pH changes for either fresh or frozen samples as the enzyme concentration increases from 0.1 to 0.3 w/w%. It is worth noting, however, that the pH differences between fresh and frozen samples remained relatively small across all enzyme concentrations.

5.1.2. Alterations in colorimetric parameters during the freezing process

The aim of this study is to shed light on the colour profiles of both egg yolks and whole eggs. Colour measurements on eggs are critical because they directly influence consumer preferences.

Our investigation begins with a focus on the L* graph, a critical aspect of colour measurement that quantifies brightness from total darkness (L* = 0) to absolute brightness (L* = 100).

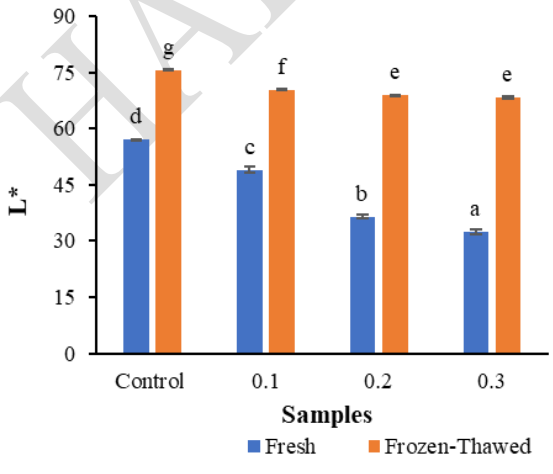

Figure 20. L* measurement results on Egg Yolk Samples

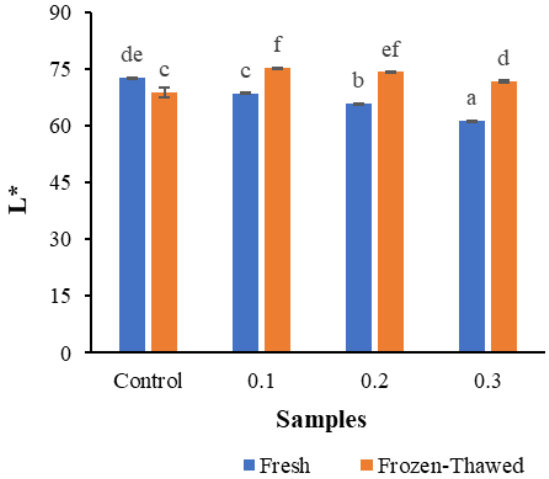

Figure 21. L* measurement results on Whole Egg Samples

Some conclusions can be drawn from the provided data in Figure 20 and Figure 21 based on the L* results of the colour measurements on the egg samples:

By comparing both egg yolk and whole egg samples together, we can see that in the:

Freezing Effect on Control Samples: freezing had a more significant impact on the control egg yolk samples than the whole egg samples. This is due to the composition of whole eggs, which contain both the egg white and yolk. Because it contains mostly of 74.57% water (Yamamoto et al., 2018), it may undergo minor colour changes when frozen. The egg yolk specifically as well, contains fats and lipoproteins that can crystallise and denature due to freezing as proposed by Wang and his co-workers (2020b) in section 3.6. resulting in a more significant change in colour, manifested as an increase in L* value.

Enzyme Treatment Effects on Fresh Samples: Higher enzyme concentrations in both whole eggs and egg yolks resulted in a darker colour due to enzymatic reactions altering the composition and colour properties. In addition to light absorbance due to protein denaturation, pigments such as xanthophylls and carotenoids can be responsible for the colour of egg yolks as well (Scott et al., 1968). Enzymes may change the colour of the egg components by degrading or modifying those pigments.

Effect of Freezing on Colour (Control Samples): In case of the Egg Yolk samples, the fresh control sample had a L* value of 57.07, indicating a moderate brightness. The frozen-thawed control sample, on the other hand, had a L* value of 75.6, which was significantly different and higher than the fresh control. This significant increase in L* value indicates that freezing has a significant effect on the brightness of egg yolks, making them appear noticeably lighter. While in case of Whole Egg samples, the control fresh sample had a L* value of 72.58, indicating that it was relatively bright. The frozen-thawed control sample, on the other hand, had a L* value of 68.7, which was slightly lower than the fresh control as shown in Figure 22.

(A) (B) (A) (B)

Egg Yolk Control Samples Whole Egg Control Samples

Figure 22. Colour Changes in Control Samples.

Effect of Enzyme Treatment on Colour (Fresh Samples): As the enzyme concentration increased, the L* values of the enzyme-treated samples (0.1, 0.2, and 0.3 w/w%) decreased. The 0.3 w/w% enzyme-treated fresh sample had the lowest L* value of 32.39, indicating a darker colour than the control fresh sample. This suggests that higher concentrations of enzyme treatment tend to darken the colour of the egg yolk as seen in figure 23.

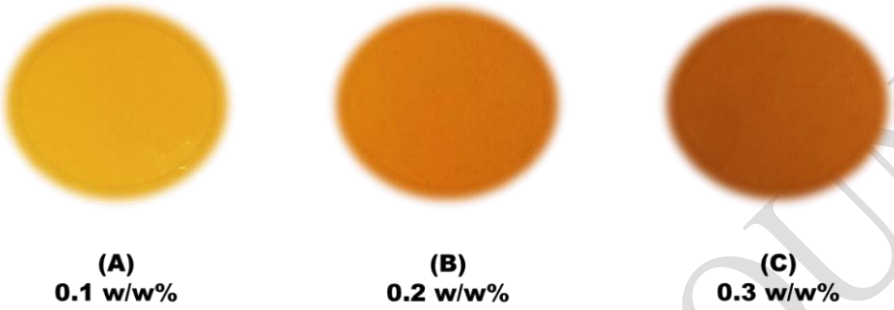

Figure 23. Colour Changes in different enzyme concentrations.

Effect of Freezing on Enzyme-Treated Samples: When we investigated the impact of freezing on enzyme-treated samples, we discovered that freezing consistently resulted in a significant increase in L* values, causing the samples to become noticeably lighter in colour. For example, in the egg yolk, the L* value of the 0.3 w/w% enzyme-treated frozen sample was 68.29, which was significantly higher than the L* value of the fresh counterpart (L* = 32.39). This pattern indicates that freezing has a consistent effect on all enzyme-treated samples, resulting in increased brightness.

The study continues with a close look at the a* graph, a parameter in colour measurement that represents the spectrum from green (-a*) to red (+a*).

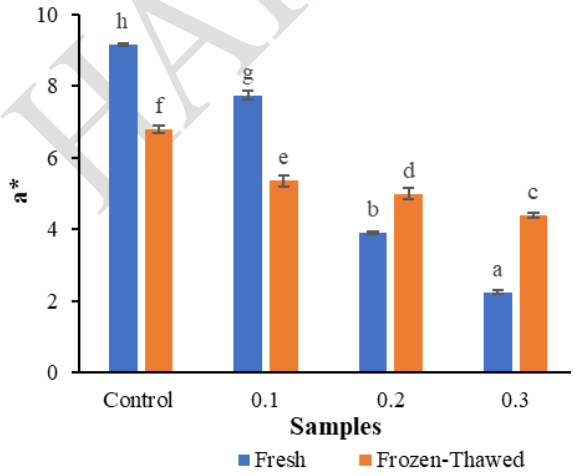

Figure 24. a* measurement results on Egg Yolk Samples.

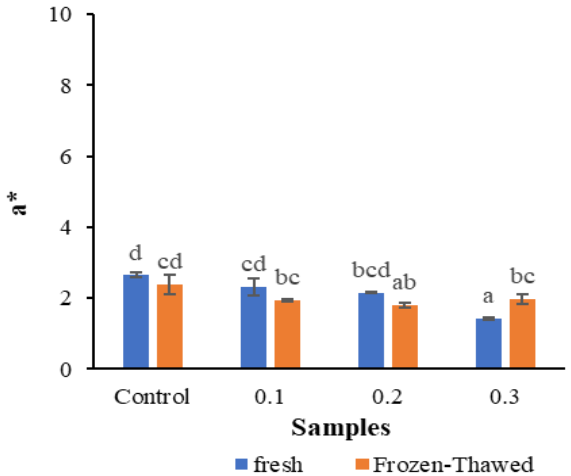

Figure 25. a* measurement results on Whole Egg Samples.

As shown in Figure 24 and Figure 25, the following conclusions can be detected:

Effect of Freezing on Colour (Control Samples): In case of the egg yolk samples, we can see that the control fresh egg yolk sample exhibited an a* value of 9.16, indicating a positive red hue. While the frozen control sample showed an a* value of 6.8, which is lower than the fresh control, suggesting a shift towards a less intense red hue. Based on that we may say that the colour change of egg products during freezing is connected to the denaturation undergoing in the freezing process.

In case of the whole egg sample, freezing tends to have a minor, not significant effect on the colour of whole egg samples resulting in a slight decrease in the positive a* value, which represents a less intense red hue.

Effect of Enzyme Treatment on Colour (Fresh Samples): From the results we can see that the 0.3 w/w% enzyme-treated fresh sample had an a* value of 2.24, indicating a substantial reduction in redness compared to both the control and lower enzyme concentrations. The decreasing a* values as enzyme concentration increases indicate a shift towards a less intense red colour, and by looking at the fresh whole egg samples results we can see that, enzyme treatment appears to have a limited impact on redness (a* value). Even at the highest enzyme concentration (0.3 w/w%), the change in redness is minor when compared to the control.

Effect of Freezing on Enzyme-Treated Samples: Freezing consistently resulted in further reductions in a* values in all enzyme-treated samples, making the samples less red in colour. As an explanation to that we can say that by potentially intensifying the changes initiated by enzyme treatment, freezing can further influence the colour attributes. Egg yolk samples showed a significant difference in the reduction of the a* value while in contrast to egg yolk samples, freezing appears to have a relatively minor effect on the colour of enzyme-treated whole egg samples, as it showed no significant difference between the samples.

The study finishes with a close look at the b* graph, that represents the extent of blue (-b*) to yellow (+b*) hues in the analysed samples. A critical parameter in egg colour measurement because consumers connect yellow colour with better quality.

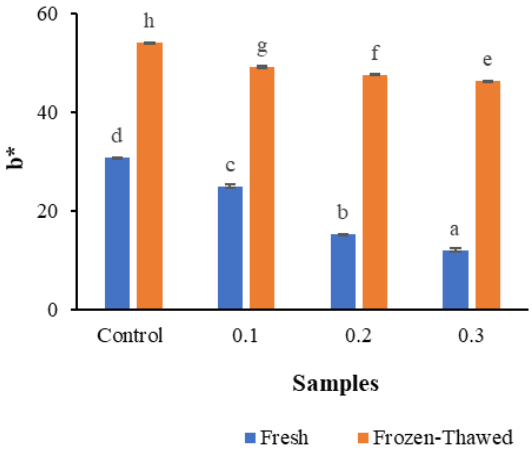

Figure 26. b* measurement results on Egg Yolk Samples.

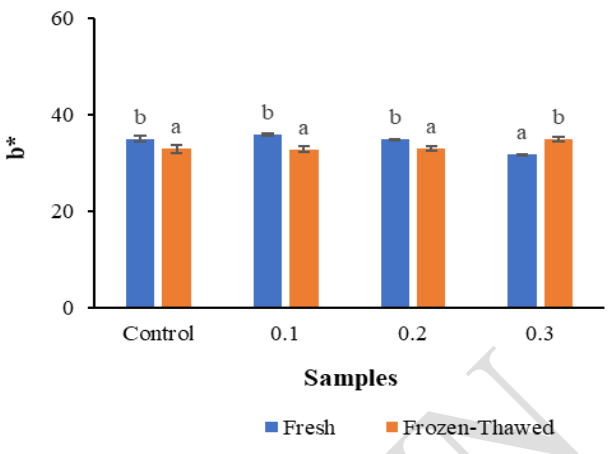

Figure 27. b* measurement results on Whole Egg Samples.

By Looking at both Figure 26 and Figure 27 we can see that:

Effect of Freezing on Colour (Control Samples): The control fresh sample exhibited a b* value of 30.90, indicating a yellowish hue. The frozen control sample showed a substantially higher b* value of 54.02, which suggests a pronounced shift towards a more intense yellow hue upon freezing. This explains that the b* values of control egg yolk samples are significantly affected by freezing, resulting in a significant increase in the positive b* value and a more intense yellow colour. This modification might have been caused by crystallisation or other chemical changes in the yolk during freezing as discussed in section 3.6. unlike the whole egg we can see that the freezing of control whole egg samples had a minor effect on the b* values, resulting in a small decrease in the positive b* value. This indicates a slight shift away from the yellow hue, but the difference is minor.

Effect of Enzyme Treatment on Colour (Fresh Samples): By looking at the enzyme treated egg yolk samples, we can say that enzyme treatment resulted in a progressive decrease in positive b* values with a significant difference in the fresh egg yolk samples, indicating a shift towards a less intense yellow hue. This suggests that enzyme treatment has a strong effect on reducing the yellow colour of egg yolks, with higher enzyme concentrations producing more noticeable results. While in whole egg the 0.1 w/w% enzyme-treated fresh sample showed a slightly higher b* value of 35.91 compared to the control fresh sample but with no significant difference between them. The 0.2 w/w% enzyme-treated fresh sample exhibited a b* value of 34.84, which is like the control and 0.1 w/w% enzyme-treated fresh sample, indicating no significant change in yellowish colour. The 0.3 w/w% enzyme-treated fresh sample had a lower b* value of 31.83, indicating a shift away from the yellow hue compared to the control. This shows us

that enzyme treatment resulted in subtle variations in b* values in fresh whole egg samples. The effect on the yellowish hue appeared to be minor, with the direction of change determined by enzyme concentration.

Effect of Freezing on Enzyme-Treated Samples: In all enzyme-treated egg yolk samples, freezing consistently resulted decrease in b* values, making the samples appear-even less yellow in colour. On the other hand, whole egg, in all enzyme-treated samples, freezing consistently resulted in slight variations in b* values.

5.1.3. The effect of freezing and enzyme treatment on the emulsifying properties of egg yolk.

From the turbidity measurement, we can conclude on the emulsifying properties of egg yolk. Spectrophotometry, a non-destructive analytical device, was used with applying 660 nm wavelength for quantifying turbidity in egg yolk samples. Spectrophotometers enable precise evaluation of particle size distribution and concentration by analysing the scattering and absorption of light.

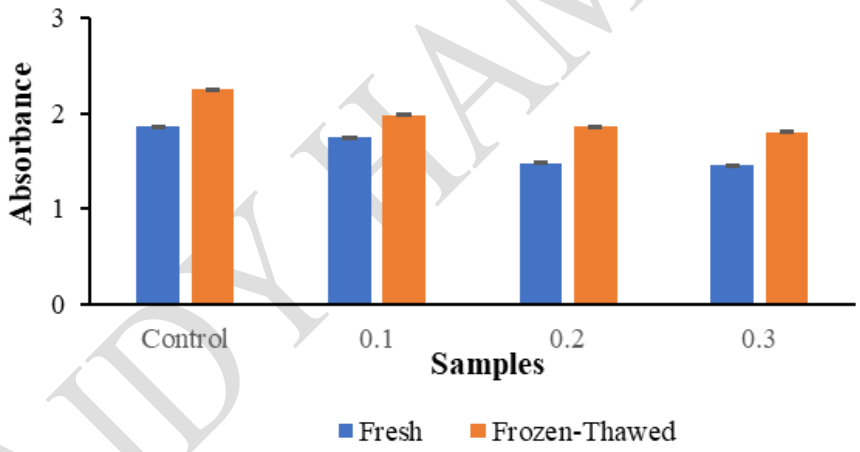

Figure 28. The effect of freezing and enzyme treatment on the emulsifying properties of egg yolk.

Figure 28, which depicts the absorbance (ABS) values for various egg yolk samples, provides useful information about their turbidity and emulsion stability. This measurement is typically used for the egg yolk, so only egg yolk samples were measured. The results show distinct trends across the samples, with differences in absorbance levels.

Fresh vs. Frozen Yolk Samples: The control fresh egg yolk sample has a lower absorbance (ABS 1.86) than the frozen sample (ABS 2.249). As a result of comparing the other samples,

the frozen yolk samples showed to have higher turbidity, as indicated by its higher absorbance value. According to Wang et al. (2020b), these results showed that freezing damage to the yolk caused molecule aggregation, resulting in larger aggregates (Figure 29). Chang and his co-authors (1977) experienced a similar trend in the turbidity of yolk solutions. He attributed this phenomenon to two factors. On the one hand, it is possible that components of the yolk such as LDL and HDL combine with each other to create large insoluble lumps through slow freezing. On the other hand, yolk aggregation appears to be caused by the buildup of ice particles, which results into the deterioration of yolk particle form and bursting of components throughout freezing and thawing.

(A) (B)

Figure 29. Aggregations caused by freezing.

Enzyme Treatment: Enzyme-treated samples (0.1, 0.2, and 0.3 w/w% enzyme concentrations) have lower absorbance than their untreated counterparts. Turbidity is reduced may be due to the enzymatic hydrolysis of phospholipids and proteins, causing breaking down of the aggregated molecules. As the concentration of enzyme increases, more of these aggregated molecules are broken down, resulting in improved emulsion stability and lower turbidity. As a result, enzyme-treated fresh and frozen samples consistently have lower ABS values than their untreated counterparts.

These factors may highlight the possibility for the enzymatic treatment to improve emulsion stability in egg yolk-based formulations, with the concentration of enzyme used playing an important role in achieving the desired level of stability.

5.2.The effect of the enzymatic treatment and freezing on the finished egg products

5.2.1. Impact of freezing on colorimetric changes in finished products

5.2.1.1.Sponge Cake

Colour measurements were carried out on a variety of sponge cake samples, and the results were evaluated as shown in the Figures 30-32.

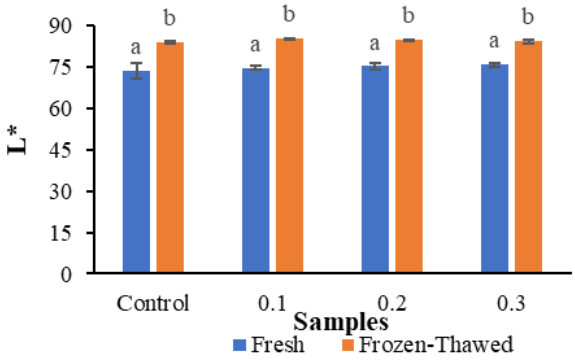

Figure 30. L* measurement results on Sponge Cake Samples

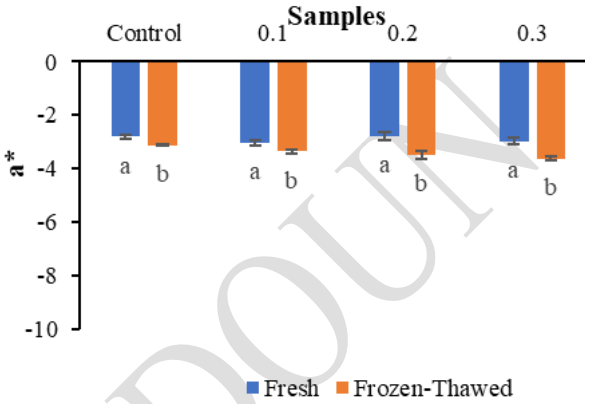

Figure 31. a* measurement results on Sponge Cake Samples.

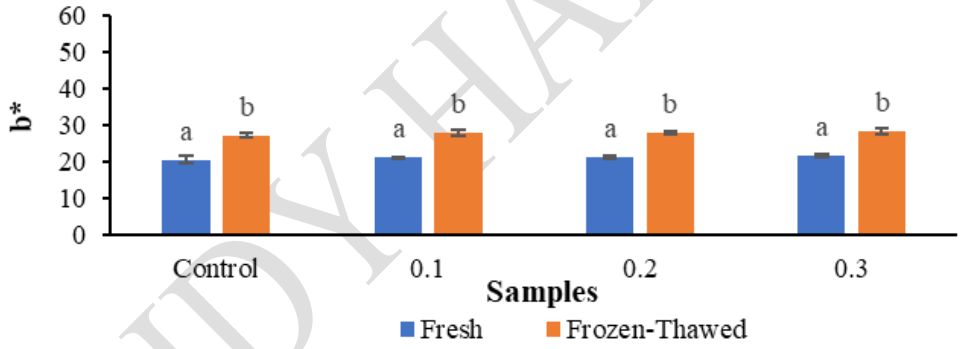

Figure 32. b* measurement results on Sponge Cake Samples.

From Figure 30 we can see that the sponge cakes made from the fresh and the frozen-thawed egg samples show a consistent trend. This trend is visible when comparing the L* values of the control samples (73.64 for fresh vs. 84.1 for frozen), and it is also evident in enzyme-treated samples. Fresh whole egg samples result in a slightly lighter sponge cake than using frozen whole egg samples. When enzyme treatment is applied to both fresh and frozen egg samples, L* values increase slightly but not significantly. With the enzyme treatment, increasing the enzyme concentration (from 0.1 to 0.3 w/w%) generally leading to higher L* values. Based on the statistical analysis, its showing that there is no significant difference between brightness among the sponge cakes made from fresh control and enzyme-treated whole egg samples and

among the ones made from the frozen-thawed control and enzyme-treated whole egg samples. When the results of whole egg samples are compared to the results of sponge cake made from whole eggs, a consistent trend emerges. Freezing has a significant impact on the brightness of both whole egg samples and the resulting sponge cake. Enzyme treatment darkens the colour of whole egg samples, which is reflected in higher L* values. When applied to sponge cake, however, enzyme treatment has no discernible effect on brightness, with no discernible difference between control and enzyme-treated samples.

Looking into Figure 31 we can clearly see that the data show a consistent trend that using fresh whole egg samples results in a slightly decrease of the absolute value for less a* value with no significant difference in the sponge cake than using frozen whole egg samples. It is also evident in enzyme-treated samples that enzyme treatment may have a minor beneficial effect on reducing redness in sponge cake colour. When the results of whole egg samples are compared to the results of sponge cake made from whole eggs, we can see that the effect of freezing and enzyme treatment on colour in whole egg samples may be transferred to sponge cake, with enzyme treatment potentially resulting in minor improvements in redness.

By examining Figure 32 we can conclude that the data show that using frozen-thawed whole egg samples results in a slightly more positive (yellowish) b* value in the sponge cake when compared to using fresh whole egg samples. This trend is visible when comparing the b* values of the control samples (fresh: 20.46 vs. frozen: 27.24), and it is also evident in enzyme-treated samples. There is no significant difference when comparing the fresh enzyme- treated sponge cake samples to the fresh control one and the same can be noticed when comparing the frozen-thawed sponge cake samples. When the results of whole egg samples are compared to the results of sponge cake made from whole eggs, we can see that the effect of freezing and enzyme treatment on colour in whole egg samples may be transferred to sponge cake with slight differences in yellowish hue.

5.2.1.2.Mayonnaise

The colour of the mayonnaise samples prepared with the addition of fresh and frozen control and enzyme-treated samples was measured. The data are shown on Figures 33-35.

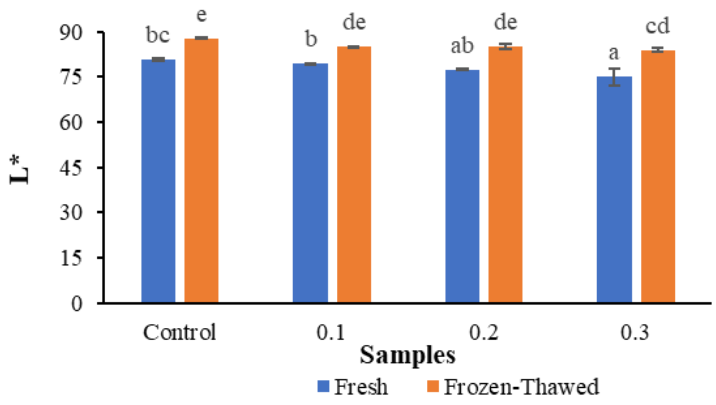

Figure 33. L* measurement results on Mayonnaise Samples.

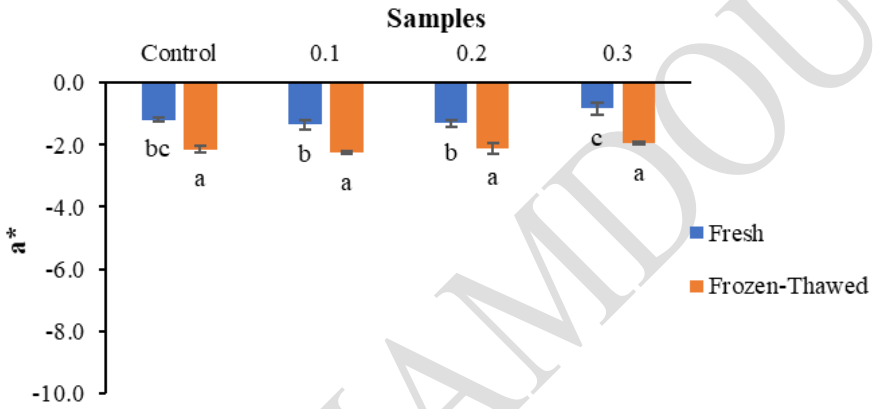

Figure 34. a* measurement results on Mayonnaise Samples.

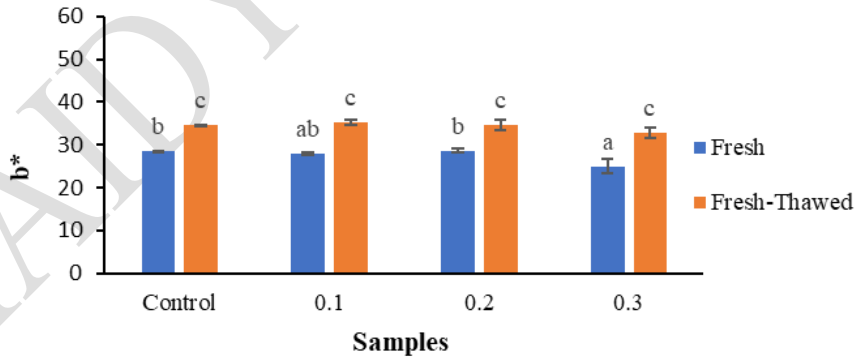

Figure 35. b* measurement results on Mayonnaise Samples.

The data on L* values from Figure 33 for various mayonnaise samples show that mayonnaise made from frozen-thawed egg yolk samples is brighter in colour than the one made from the fresh samples. By applying the enzyme treatment to the egg yolk samples, no significant difference was noticed among the fresh samples and the frozen-thawed ones. When the egg

yolk samples are compared to the mayonnaise results, it is discovered that freezing has a significant impact on the brightness (L* values) of the egg yolk samples, making them noticeably lighter. This matches the mayonnaise results, which show that mayonnaise made from frozen-thawed egg yolk samples is brighter than mayonnaise made from fresh samples. Freezing appears to contribute to increased brightness in both cases. Furthermore, enzyme treatment in egg yolk samples, particularly at higher concentrations, darkens the colour, as evidenced by lower L* values. In contrast, enzyme treatment on egg yolk samples did not result in a significant difference in brightness between fresh and frozen-thawed samples in mayonnaise.

According to the a* values in figure 34, freezing egg yolks produces mayonnaise with a stronger greenish tint than using fresh egg yolks with no significant difference among the samples. When the egg yolk samples are compared to the mayonnaise results, the impact of freezing and enzyme treatment on colour attributes is noticeably different. Freezing causes a significant reduction in the red hue (lower a* values) in egg yolk samples, indicating a shift towards a less intense red colour. In contrast, freezing egg yolks produces a stronger greenish tint in mayonnaise, indicating a different colour change trend. Furthermore, enzyme treatment reduces redness in egg yolk samples, whereas there is no significant difference in a* values between fresh and frozen mayonnaise samples.

Based on Figure 35, We can reach a conclusion about the yellow-blue colour component (b*) and its differences between control and enzyme-treated egg yolk samples made from fresh and frozen egg yolks. We can notice that the b* value of the mayonnaise control sample made from fresh egg yolks is 28.48, indicating a yellowish tint while the mayonnaise control sample made from frozen egg yolks, on the other hand, has a more pronounced yellowish tint and a higher b* value of 34.63. Mayonnaise made from fresh egg yolk samples showed significant difference among the samples while the one made from the frozen-thawed egg yolk samples showed no significant difference at all among the samples. When the yellow-blue colour component (b* values) of the egg yolk samples is compared to the mayonnaise results, there are distinct trends. Freezing significantly increases the yellow hue (higher b* values) in egg yolk samples, indicating a more intense yellow colour. Enzyme treatment in egg yolk samples, on the other hand, results in a progressive decrease in b* values, indicating a shift towards a less intense yellow hue. On the other hand, while freezing egg yolks results in a more pronounced yellow tint, which is reflected in higher b* values in mayonnaise, Enzyme

treatment in fresh egg yolk mayonnaise produces significant differences between samples, whereas frozen-thawed egg yolk samples produce no significant differences.

5.2.2. Texture analysis of finished products pre- and post-freezing.

5.2.2.1.Sponge Cake

Texture analysis was performed on sponge cake samples, and the results were analysed to determine hardness, cohesiveness, springiness, and gumminess, as shown in the figures below (36-39).

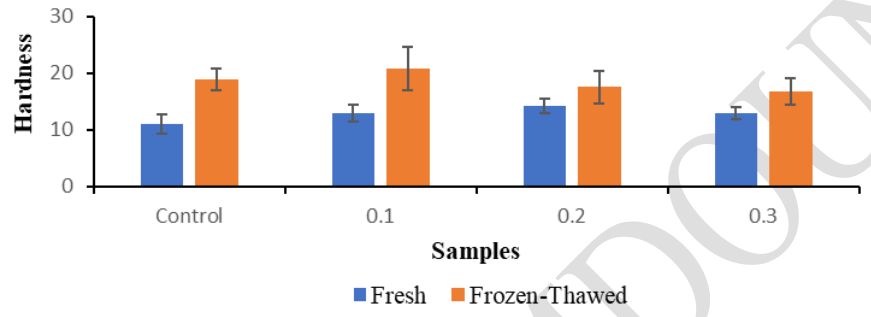

Figure 36. Variation in Cake Hardness Across Samples.

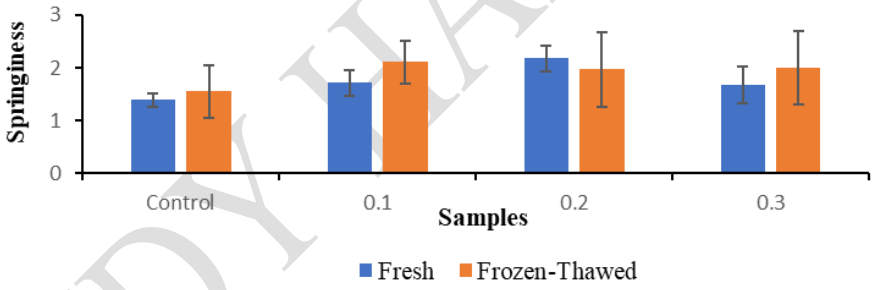

Figure 37. Variation in Cake Springiness Across Samples.

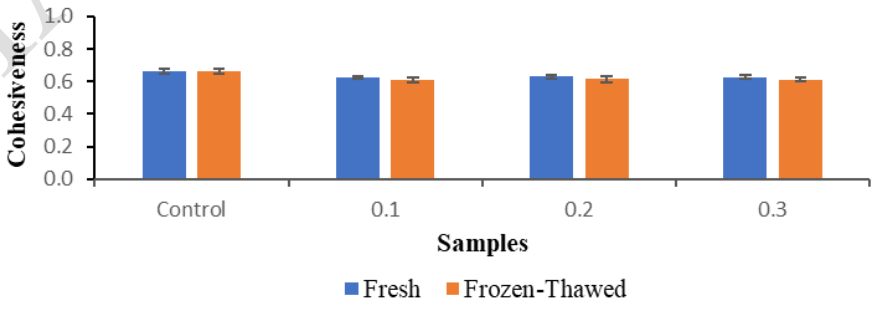

Figure 38. Variation in Cake Cohesiveness Across Samples.

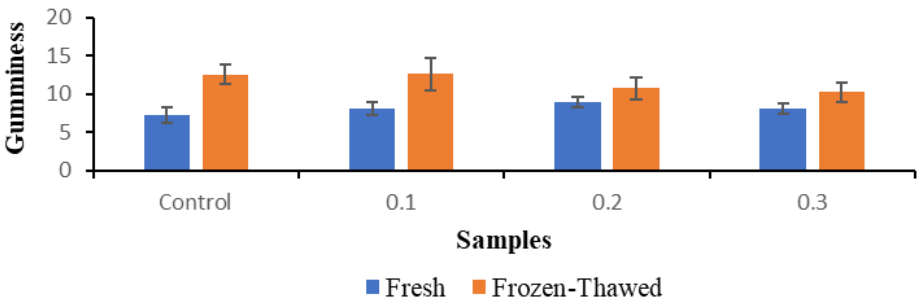

Figure 39. Variation in Cake Gumminess Across Samples.

Based on Figure 36, the data show that freezing whole eggs increased the hardness of the sponge cake significantly. Furthermore, enzyme treatment had a little impact regarding the hardness. Among the enzyme treated fresh samples, the sample of 0.3 w/w% was the most similar to the fresh control sample. Also, among the enzyme-treated frozen-thawed samples, the sample of 0.3 w/w% was the most similar to the fresh control sample.

Based on Figure 37, the data show that freezing whole eggs had little effect on springiness of the sponge cakes, making them denser and less likely to spring back when compressed. In other words, it has less elasticity and resilience. While enzyme treatment increased the springiness of the sponge cake, up to a certain enzyme concentration, indicating that the cake is more elastic and can quickly regain its shape after compression.

From Figure 38, we can see that sponge cake made from both the fresh whole egg control sample and the frozen whole egg control sample had the same cohesiveness value (0.66). This indicates that freezing the entire egg component had no effect on the cohesiveness of the sponge cake because the values remained consistent. There is little variation in cohesiveness values across all enzyme-treated samples. Fresh samples with enzyme concentrations of 0.1, 0.2, and 0.3 w/w% showed consistent cohesiveness values of 0.63, while frozen samples showed consistent cohesiveness values of 0.61. These findings suggest that cohesiveness is relatively stable and unaffected by freezing whole eggs and the use of enzymes in this context. Regardless of whether fresh or frozen eggs or enzyme treatments are used, the cake retains its ability to hold together and resist crumbling or disintegration on a consistent basis.

In Figure 39, we can clearly see that freezing the whole egg component had a significant impact on sponge cake gumminess, resulting in a chewier texture. In some cases, such as with certain candies or gum products, an increase in gumminess may be desirable. Excess gumminess, on the other hand, may not align with the desired sensory attributes in other food products, such

as cakes, and may be considered a texture defect. Furthermore, enzyme treatment had a little impact regarding the gumminess. Among the enzyme treated fresh samples, the sample of 0.1 w/w% was the most similar to the fresh control sample. While, among the enzyme-treated frozen-thawed samples, the sample of 0.3 w/w% was the most similar to the fresh control sample.

5.2.2.2.Mayonnaise

Texture analysis was performed on mayonnaise samples, and the results are shown in the figures below (40-43).

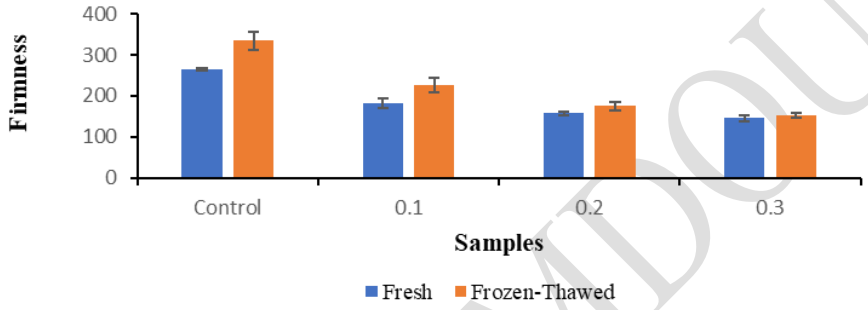

Figure 40. Variation in Mayonnaise Firmness Across Samples.

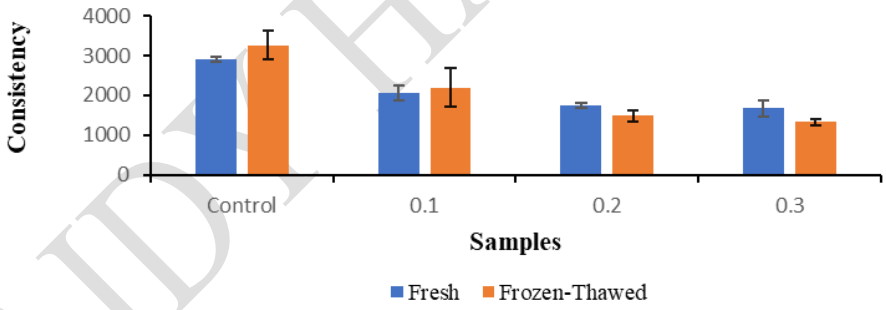

Figure 41. Variation in Mayonnaise Consistency Across Samples.

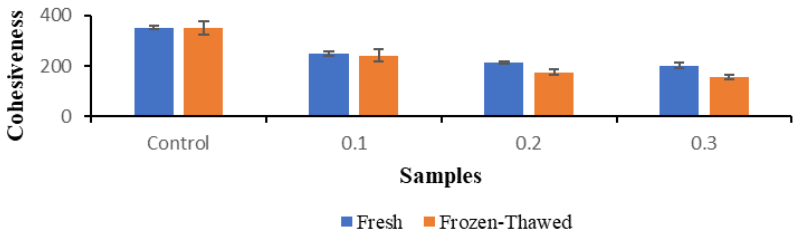

Figure 42. Variation in Mayonnaise Cohesiveness Across Samples.

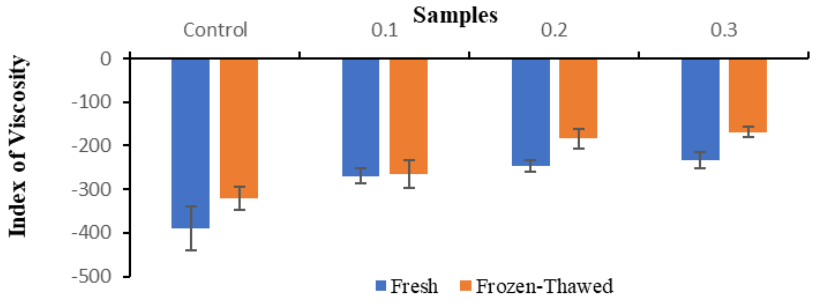

Figure 43. Variation in Mayonnaise Viscosity Indexes Across Samples.

From Figure 40 we can see that the firmness of the mayonnaise made from fresh egg yolk control sample (265.43) was lower than that of the frozen egg yolk control sample (334.31). This significant increase in firmness in the frozen egg yolk sample suggests that freezing the egg yolk component had a significant impact on mayonnaise firmness, resulting in a firmer texture. Among the enzyme-treated samples, increasing the enzyme concentration from 0.1 to 0.2 w/w% resulted in a decrease in firmness in both fresh (182.05 to 157.90) and frozen (226.49 to 175.25) samples. When the enzyme concentration was increased to 0.3 w/w%, the firmness values in both the fresh (145.31) and frozen (152.18) samples decreased.

Based on Figure 41 we can conclude that the consistency of the mayonnaise made from fresh control egg yolk sample was lower (2909.61) than the consistency of the mayonnaise made from frozen-thawed control egg yolk sample (3269.29). This increase in consistency in the frozen egg yolk sample suggests that freezing the egg yolk component had a significant impact on the mayonnaise's consistency, resulting in a thicker and more viscous texture. The reason behind those observations may be attributed to the physical changes that occur in egg yolk during freezing. The protein structure of the egg yolk can change due to freezing. When proteins are frozen and thawed, they may denature, unfold, and interact differently (Anson, 1945). These altered protein structures may have an effect on the overall texture and rheological properties of the mayonnaise, resulting in increased consistency. Among the enzyme-treated samples, increasing the enzyme concentration from 0.1 to 0.3 w/w% resulted in a decrease in consistency in both fresh and frozen samples. Aminopeptidase is an enzyme that catalyses peptide hydrolysis, breaking it down into smaller peptides and amino acids. The enzyme may be degrading proteins in the egg yolk components, lowering the overall molecular weight of the protein network. This can lead to reduced viscosity and consistency. Enzymatic hydrolysis of certain components, such as proteins or polysaccharides, may result in the release of previously bound water. This water release can dilute the mayonnaise and contribute to a loss

of thickness and consistency. Furthermore, the stability of the mayonnaise texture should be measured because based on the turbidity measurement, the turbidity of the frozen control sample was the highest among all the other samples, showing that it has worse emulsifying properties.

In Figure 42, The cohesiveness values for the fresh egg yolk control sample and the frozen egg yolk control sample are very close. This suggests that freezing the egg yolk component had no effect on the mayonnaise's cohesiveness, as the values remained relatively consistent. Among the enzyme-treated samples, increasing the enzyme concentration from 0.1 to 0.2 w/w% resulted in a decrease in cohesiveness in both fresh and frozen samples. When the enzyme concentration was increased to 0.3 w/w%, the cohesiveness values in both the fresh and frozen samples decreased.

The texture analysis of mayonnaise samples yielded viscosity index results as shown in Figure 43. The viscosity index is a useful parameter for determining the flow and thickness of mayonnaise (Taslikh et al., 2021). The fresh egg yolk control sample has a lower index of viscosity (388.99) than the frozen egg yolk control sample (320.47). This implies that freezing the egg yolk component influenced the viscosity index, with the frozen sample exhibiting a less negative value, indicating a potentially less viscous texture. In summary, when compared to using fresh egg yolk, freezing appears to increase the index of viscosity, indicating that freezing may influence the texture properties of mayonnaise. In both fresh and frozen samples, increasing enzyme concentration results in a consistent decrease in the index of viscosity. This suggests that enzymatic activity is breaking down components responsible for viscosity, resulting in a less negative viscosity index.

SUMMARY

Eggs play an important role in the human diet and are widely used as food ingredients in many industries, including pasta, pastry, bakery, and catering. To improve convenience and reduce microbiological risks, food industry manufacturers frequently choose processed egg products such as liquid eggs, egg powders, and cooked egg products over shelled eggs. However, once the eggshell is broken, the eggs' natural protection is lost, resulting in the rapid degradation of liquid egg products, posing a challenge to the food industry. Freezing emerges as a potential solution for long-term storage, but gelation during freezing prevents frozen eggs from being used optimally. To address this issue, researchers are investigating the use of enzyme-based approaches. A possible approach is to use an enzyme preparation containing aminopeptidase activity to reduce gelation and improve the rheological properties of liquid egg products.

To achieve the research goals, eight samples of liquid egg products, derived from egg yolk and whole egg, were experimentally examined. Six samples underwent treatment with varying concentrations of the aminopeptidase enzyme Flavorpro™ 750MDP (0.1%, 0.2%, 0.3% w/w), while two served as control without enzyme. The enzymatic treatment involved a 2-hour water bath at 40°C, except for the control. Subsequently, half of the samples were frozen at -24°C for 14 days, followed by thawing in a 4°C thermal bath for 2 hours. The treated and untreated samples were then used to produce finished products, such as sponge cake and mayonnaise, based on specific recipes, allowing for a comparison of characteristics and quality.

Evaluations were done on both egg yolk and whole egg samples such as, pH and colour measurements while turbidity measurements were only performed on egg yolk samples to check the emulsifying properties. On the other hand, regarding the finished products, colour measurements and texture analysis were performed on both mayonnaise and sponge cake.

Results showed that in the pH analysis of enzyme-treated egg yolk samples, fresh samples exhibited a lower pH than frozen ones, suggesting a minor alkalizing effect from enzyme treatment, likely linked to protein hydrolysis releasing basic amino acids. Regarding colour parameters, freezing had a greater impact on control egg yolk than whole egg, attributed to the latter's higher water content. Enzyme-treated samples darkened with increasing enzyme concentration. Freezing consistently reduced redness (a* values) in all enzyme-treated samples, more pronounced in egg yolk than whole egg. Enzyme treatment of fresh egg yolk samples reduced the intense yellow hue (b*) significantly, with higher concentrations producing more noticeable effects. Freezing consistently reduced the yellow hue in all enzyme-treated egg yolk

samples. In emulsifying properties, enzyme-treated samples consistently showed lower absorbance, indicating improved emulsion stability. This is associated with enzymatic hydrolysis breaking down aggregated molecules formed during freezing. Both fresh and frozen enzyme-treated samples consistently outperformed untreated ones in terms of lower absorbance values, underscoring the potential of enzymatic treatment to enhance emulsion stability in egg yolk-based formulations.

Sponge cake texture analysis revealed that freezing increases hardness significantly, while enzyme treatment has minimal impact. The 0.3 % w/w enzyme concentration in both fresh and frozen samples closely resembles the respective controls. Freezing reduces springiness, making the cake denser, while enzyme treatment enhances springiness up to a certain concentration. Freezing elevates gumminess significantly, creating a chewier texture, whereas enzyme treatment has limited effect. In mayonnaise texture analysis, freezing was found to boost firmness, while enzyme treatment decreases it, especially at higher concentrations. Consistency increases in frozen-thawed egg yolk mayonnaise, indicating a thicker texture. Enzyme treatment reduces consistency due to aminopeptidase activity, lowering molecular weight and viscosity. Cohesiveness is minimally affected by freezing but decreases with higher enzyme concentrations. The viscosity index shows frozen egg yolk mayonnaise is more viscous, while increasing enzyme concentration consistently reduces the index.

Eventually, the study demonstrated discernible effects on liquid egg yolk and whole egg, emphasising changes in pH, colour, and gelation properties caused by freezing. Freezing caused significant changes in texture attributes in sponge cake, whereas enzyme treatment had concentration-dependent effects. Lower concentrations (for example, 0.1 % w/w) may preserve a texture similar to the fresh control, whereas higher concentrations (for example, 0.3 % w/w) may yield a softer texture with promising springiness. In case of mayonnaise, both freezing and enzyme treatment had a significant impact on firmness, consistency, cohesiveness, and viscosity index. Lower enzyme concentrations (0.1 % w/w) reduced firmness and consistency, while higher concentrations (0.3 % w/w) reduced these attributes even further. Cohesiveness decreased as enzyme concentration increased, and the viscosity index decreased consistently at higher concentrations. Choosing the optimal enzyme concentration should correspond to the desired sensory and textural properties of the product. However, in future experiments, higher enzyme concentrations and longer freezing times can be applied to see how they affect liquid egg yolk and whole egg quality. Additionally, combined enzyme treatments that use multiple enzyme types can be a way to find potential synergies for improved product characteristics.

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