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THE EFFECT OF STORAGE ON PROTEIN FORTIFIED LIQUID WHOLE EGG RHEOLOGICAL PROPERTIES

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Abstract: Liquid egg products are globally recognized and increasingly used in large-scale industries due to their microbiological stability and ease of handling. A 100 g of liquid whole egg contains 76.15 g of water, 9.5 g of fat, and 12.56 g of protein. Egg proteins are high quality proteins, characterized by an amino acid score of 100 and the highest net protein utilization rate among dietary proteins. Many studies have examined the characteristics and health benefits of egg white proteins, revealing that their consumption can promote muscle mass and strength, reduce visceral fat, and lower blood cholesterol levels. Given these health advantages, this study aimed to enhance the nutritional value of liquid whole egg by fortifying it with powdered egg white protein. The impact of this addition was assessed through the evaluation of the rheological properties of liquid whole egg. Powdered egg white protein was added in varying concentrations (3%, 5%, and 10%) to 200 g of raw, homogenized liquid whole egg. The mixtures were then subjected to heat treatment at 65 °C for 15 minutes in a water bath and subsequently stored at 4 °C for 21 days. Rheological measurements, specifically viscosity, were performed on days 1, 7, 14, and 21 using an MCR 92 rheometer at 15 °C. The results demonstrated that viscosity was influenced by both the concentration of egg white protein and the duration of storage, exhibiting notable changes throughout the experimental period.

Keywords: liquid whole eggs, egg protein, rheological properties.

1. Introduction

Eggs are common food item consumed worldwide, known for their high amount of protein, nutritional values, and culinary uses. They contain all the essential nutrient for human life except for vitamin C and dietary fibers. Although eggs has a high nutritional values, they have been linked with salmonellosis breakout for years, to overcome such an issue pasteurized egg products were created. Egg products are defined as eggs which has been processed, modified, or transformed from their original state (Gautron et al. 2002). Liquid eggs, egg powders, liquid egg yolks and egg whites emerged as convenient alternatives for food manufacturers, offering extended shelf life and ease of handling compared to shelled whole eggs (Lechevalier et al. 2017). Due to pasteurizing process egg products are microbiologically safe products which makes it the most preferred option by large scale manufactures (Amiali et al. 2007). Egg proteins offer exceptional functionality and health benefits, making them valuable in various food applications. Their properties, such as emulsification, coagulation, foaming, and binding, are widely utilized in producing baked goods, sauces, dressings, and confectionery items. Adding egg proteins also enhances the color, texture, flavor, and structure of a broad range of food products. Egg proteins are highly digestible and bioavailable, providing all essential amino acids along with key nutrients like vitamins A, D, E, and B12, as well as iron, zinc, and selenium. These qualities make egg proteins an excellent choice for athletes, fitness enthusiasts, and individuals aiming to build or maintain muscle mass.

Research suggests that regular consumption of eggs, as part of a balanced diet, may promote satiety, aid in weight management, and contribute to overall health. Moreover, the nutritional benefits of eggs have made them indispensable in the production of protein supplements and fortification of foods with essential nutrients (Réhault-Godbert et al. 2019). Beyond their culinary and nutritional importance, egg products have also found significant applications in fields such as the pharmaceutical industry, particularly in vaccine production. Egg proteins, such as phosvitin, are notable for their potent antioxidant properties due to their high phosphoserine content. Additionally, ovotransferrin contributes to antioxidant activity through its ability to bind iron. Ovalbumin also enhances antioxidant activity by interacting with polysaccharides, further broadening the functional applications of egg-derived proteins (Jung et al. 2012, Rakonjac et al. 2014). These proteins play a significant role in reducing lipid oxidation by binding to metal or scavenging free radical, this antioxidant activity of eggs can be used well in food and cosmetics industry. Egg white proteins have a significant and important role in food industry for example, lysozyme is well known for its capability to control the growth of foodborne microorganisms such as listeria monocytes and control the toxin formation of clostridium botulinum especially in fish, chickens, and some vegetables. Due to its strong antimicrobial properties, lysozyme was allowed by the world health organization to be use as a preservative in many food items such as sushi, kimchi pickles and wine production. Similar to lysozyme, ovotransferrin has a strong antimicrobial activity a study showed that it can increase the activity of piperacillin-tazobactam against *E.coli* using its iron chelating activity (Babini & Livermore 2000). A recent a study found that not only ovotraferrin but also its derived peptides have the ability to kill some kinds of bacteria by damaging their cell membrane (Ibrahim et al. 2000). Additionally, to their uses in food industry egg white protein are used in supplementation industry, ovotransferrin can bind to iron at pH <4.5 making it a great source of iron supplements for human (Abeyrathne et al. 2013).This study investigate storage effect on liquid whole eggs that are fortified with different percentages of powdered egg white protein rheological properties.

2. Materials and methods

2.1. Materials

A quantity of two kilogram of raw homogenized liquid whole egg was acquired from a liquid egg plant operated by Capriovus Ltd., located in Szigetcsép, Hungary. Powdered egg white protein was procured from the same company.

2.1.1. Sample preparation

Two kilogram of liquid whole eggs was divided into 5 different beakers with 400g each, then fortified with powdered egg white protein with different percentages 0, 3, 5 and 10 % W/W after that heat treated at 65°C for 15 minutes in water bath, then cooled down immediately to 4°C using an ice bath. Liquid whole that wasn't fortified served as the control. Each sample was divided into 4 bottles of 100 g of the samples and stored at 4°C for Continuously until the day of measurement.

2.1.2. Examination of Rheological Properties

At the day of production, day 7, day 14, and day 21 a bottle of 100 g of sample was used to examine the rheological behavior of liquid whole egg, it was done by 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. The Herschel—Bulkley model (Equation (1)) was used to analyze the flow curves (shear rate-shear stress diagrams).

***Eguation 1.*:**

$$\tau = \tau 0 + K\dot{\gamma} n$$

Where:
τ = shear stress (Pa)
τ0 = ithe yield stress (Pa)
γ˙= the shear rate (1/s)
K = the consistency coefficient (Pa·sn)
n = is the flow behavior index.

2.2. Statistical Analysis

To analyze data statistically using the statistical package for social science (SPSS, version 27.0, 2020, Chicago, IL) was used. A two-way 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 superscripts a, b, c and d differ significantly at $p < 0.05$.

3. Result

During experiment 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. 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 to 0.901, 0.656 and 0.654 for 3, 5 and 10 % respectively at day 1 of measurement. Table 1 displays the values for Yield stress (Tau0), Consistency Index (K), and Flow Behavior Index (n). Tau0 represents the minimum stress required for the material to start flowing, K measures the change in viscosity when shear rate is changing, meanwhile n describes the kind material flow attitude.

Table1: The effect of adding egg white protein on results of Herschel-Bulkley model values in comparison to control at different percentage 3, 5 and 10%. * Is for significantly different groups (Tukey's $p<0.05$).

Sample	τ0 (Pa)	K (Pa·sn)	N
control day 1	0.404±0.033	0.028±0.011	0.910±0.030
3% day 1	0.265±0.027*	0.033 ±0.001	0.901±0.010*
5% day 1	0.003± 0.020*	0.345 ±0.002*	0.856±0.021*
10% day 1	0.001±0.003*	0.005±0.001	1.11±0.020*
control day 7	0.092±0.024*	0.050±0.001*	0.885±0.010*
3% day 7	0.377±0.016*	0.097±0.001*	0.799±0.014*
5% day 7	0.001±0.003*	0.289±0.002	0.669±0.027*
10% day 7	0.001±0.015*	0.758±0.001*	0.661±0.024*
control day 14	0.026±0.007*	0.092±0.001	0.708±0.011*
3% day 14	0.431±0.023*	0.606±0.001*	0.693±0.028*
5% day 14	0.031±0.024	0.114±0.002*	0.659±0.015*
10% day 14	0.001±0.020	0.891±0.001*	0.688±0.023*
control day 21	0.026±0.006	0.008±0.004	0.693±0.025*
3% day 21	1.512±0.017*	0.664±0.001*	0.648±0.016*
5% day 21	1.430±0.028*	0.199±0.002*	0.626±0.081*
10% day 21	1.231±0.029*	0.948±0.003*	0.608±0.019*

At day of experiment control samples exhibited the highest $\tau0$ and low K values, indicating moderate resistance to flow and low viscosity, with a n value of 0.910±0.030 which shows a near-Newtonian behavior. The addition of 3% and 5% reduced $\tau0$ significantly, particularly for 5%, where $\tau0$ dropped to near-zero with a corresponding increase in K this changed shifted the flow behavior toward and shear-thinning behavior where n was 0.901±0.010 and 0.856±0.021 respectively for 3% and 5%. For 10% additive, $\tau0$ remained near zero, but the n value indicated shear-thickening properties. By day 7 control samples exhibited a decrease in $\tau0$ and n, but exabit a slight increase in K all changes were significant in comparison to the first day of experiment suggesting that viscosity increases with storage time. 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. At day 14 control sample $\tau0$ and n decreased while K increased which indicates an increase in viscosity and rigidity in comparison to pervious results. On the other hand, all added percentages exhibited an increase in $\tau0$ 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, $\tau0$ 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. 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 $\tau0$ 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 $\tau0$, k and 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.

4. Discussion

Whole eggs have a complex rheological property due to their unique composition, which includes water, proteins, fats, and other components. Whole liquid eggs typically exhibit shear-thinning behavior (Atılgan & Unluturk 2008). Shear-thinning fluids, also known as pseudoplastic fluids, become less viscous as shear rate increases. Adding powdered egg white protein to liquid whole eggs and heat treat them at 65°C can cause changes in structure and physicochemical properties of liquid whole egg. The rheological changes in the samples with increasing storage time and protein percentage are attributable to several physicochemical and structural mechanisms associated with protein interactions, molecular rearrangements, and aging effects (Hong et al. 2018).

Several studies found that flow behavior index (n) decrease with the increase of storage time combined with a change in the flow behavior and viscosity of liquid whole egg (Kumbár et al. 2015, Singh et al. 2014). This aligns with the rheological behavior which was found in this experiment for the control group, the group with 3 fortified percentages and for the first and day 7 measurement for both 5 and 10 percentages. Increasing the percentage of protein in a sample typically leads to a corresponding increase in viscosity as more protein molecules are added, there are more opportunities for protein-protein interactions to occur, leading to the formation of larger and more extensive networks. These protein networks contribute to the resistance to flow, resulting in higher viscosity (Anson & Mirsky 1932). The increased number of protein molecules enhances cross-linking, resulting in a denser network that resists deformation, leading to increased yield

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stress τ and viscosity. With the increase of storage time, protein network strengthens which contributes to the observed structural reinforcement and a more obvious shear-thinning behavior.

The observed changes in viscosity of liquid whole egg with added egg white protein with storage, can be explained by the denaturation of proteins, specifically conalbumin and low-density lipoproteins (LDL), which are present in both egg white and yolk. Heating egg white causes the denaturation of conalbumin, a major egg white protein, at around 60°C. Conalbumin denaturation leads to changes in protein structure, resulting in the unfolding and exposure of hydrophobic regions. This unfolding disrupts the weak linkages between proteins, leading to increased protein-protein interactions and the formation of a protein network. This network contributes to an increase in viscosity, as the fluid becomes more structured and resistant to flow (Hsieh & Regenstein 1992). The viscosity of liquid whole egg is influenced by changes in both yolk and white properties upon heating. In addition to conalbumin denaturation, heating can also lead to the denaturation of LDL present in the yolk. LDL denaturation similarly results in protein unfolding and changes in protein-protein interactions, contributing to viscosity changes in the liquid whole egg. The unfolding of conalbumin in the egg white further adds to the overall viscosity increase (Jaekel & Ternes 2009). The change in viscosity with the increase in both protein percentage and storage time is clearly seen through viscosity curves in *Figure 1, 2, 3* and *4*.

Figure 1.: The viscosity curves (shear rate vs. viscosity) of different concentrations of added egg white protein to liquid whole egg then pasteurized at 65 ± 0.2°C, at day 1 of storage.

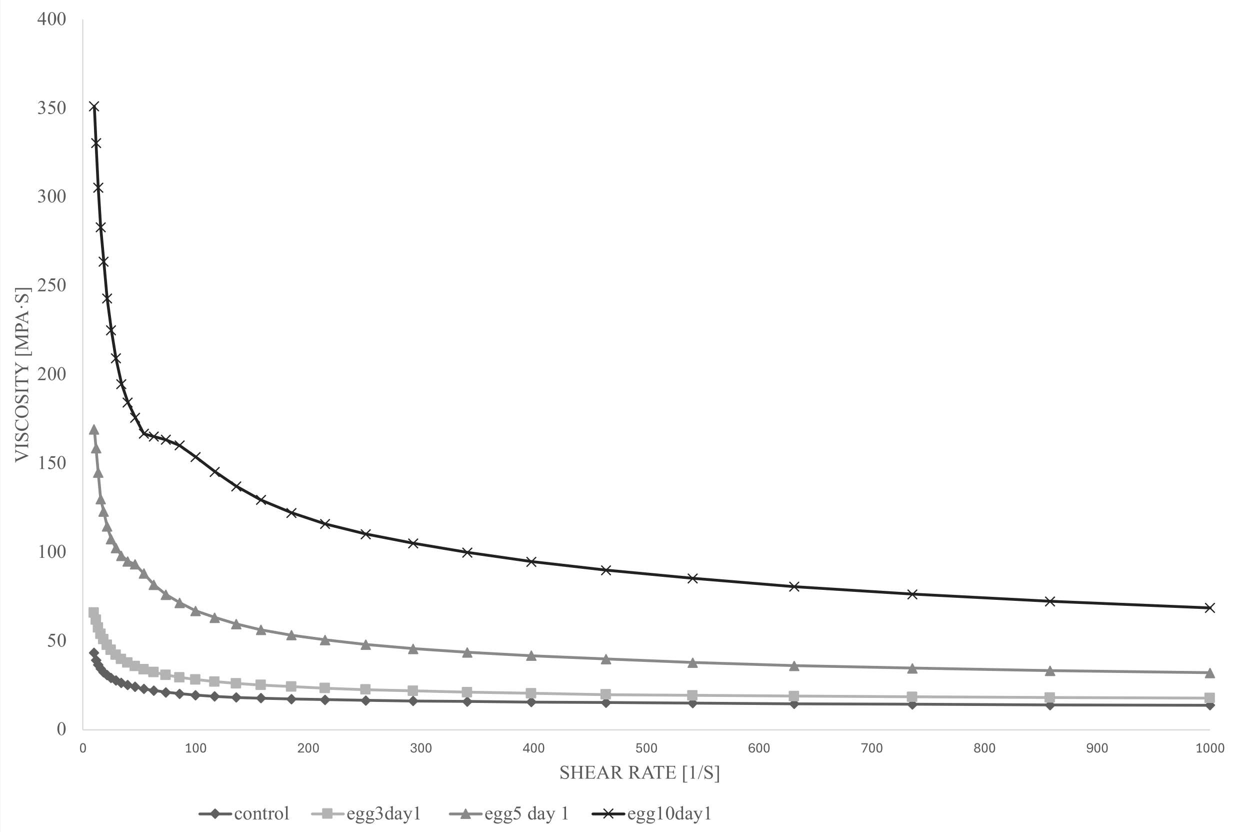

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***Figure 2.*:** The viscosity curves (shear rate vs. viscosity) of different concentrations of added egg white protein to liquid whole egg then pasteurized at 65 ± 0.2°C, at day 7 of storage.

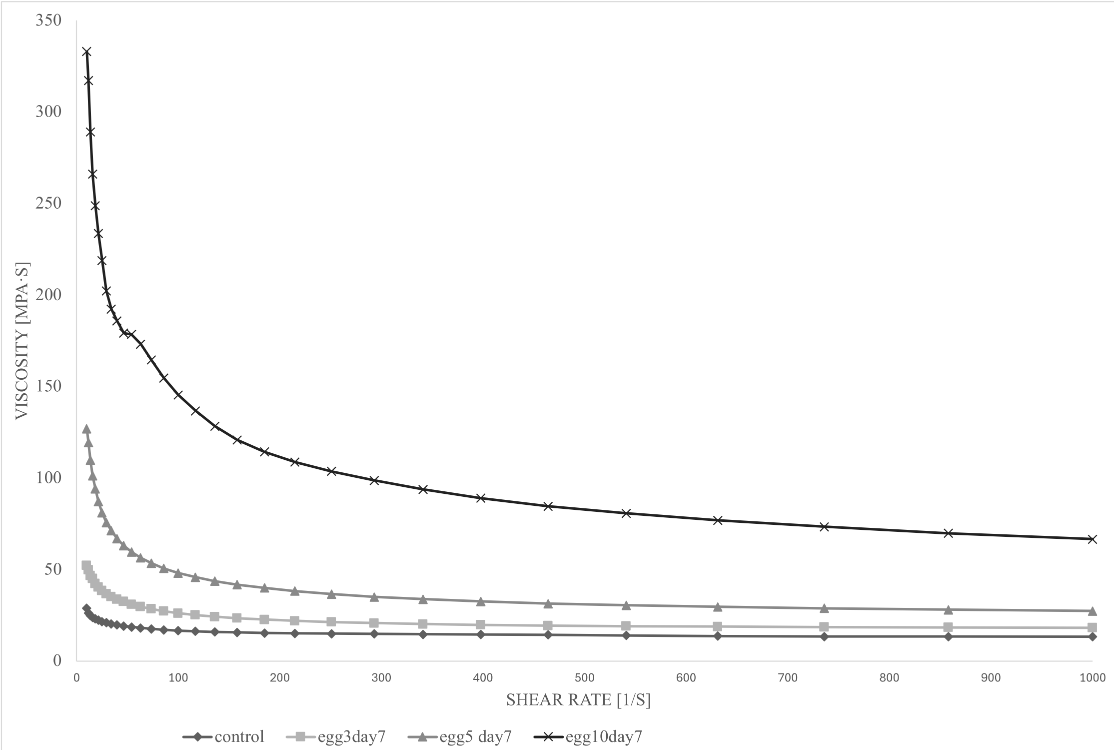


***Figure 3.*:** The viscosity curves (shear rate vs. viscosity) of different concentrations of added egg white protein to liquid whole egg then pasteurized at 65 ± 0.2°C, at day 14 of storage.

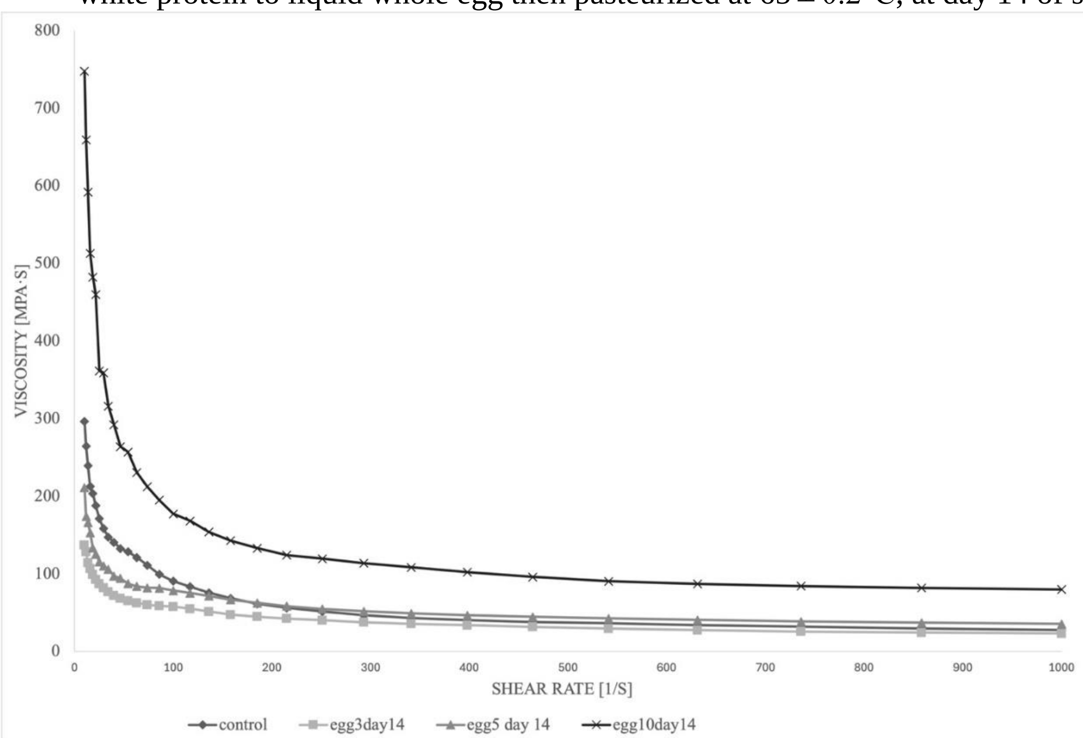
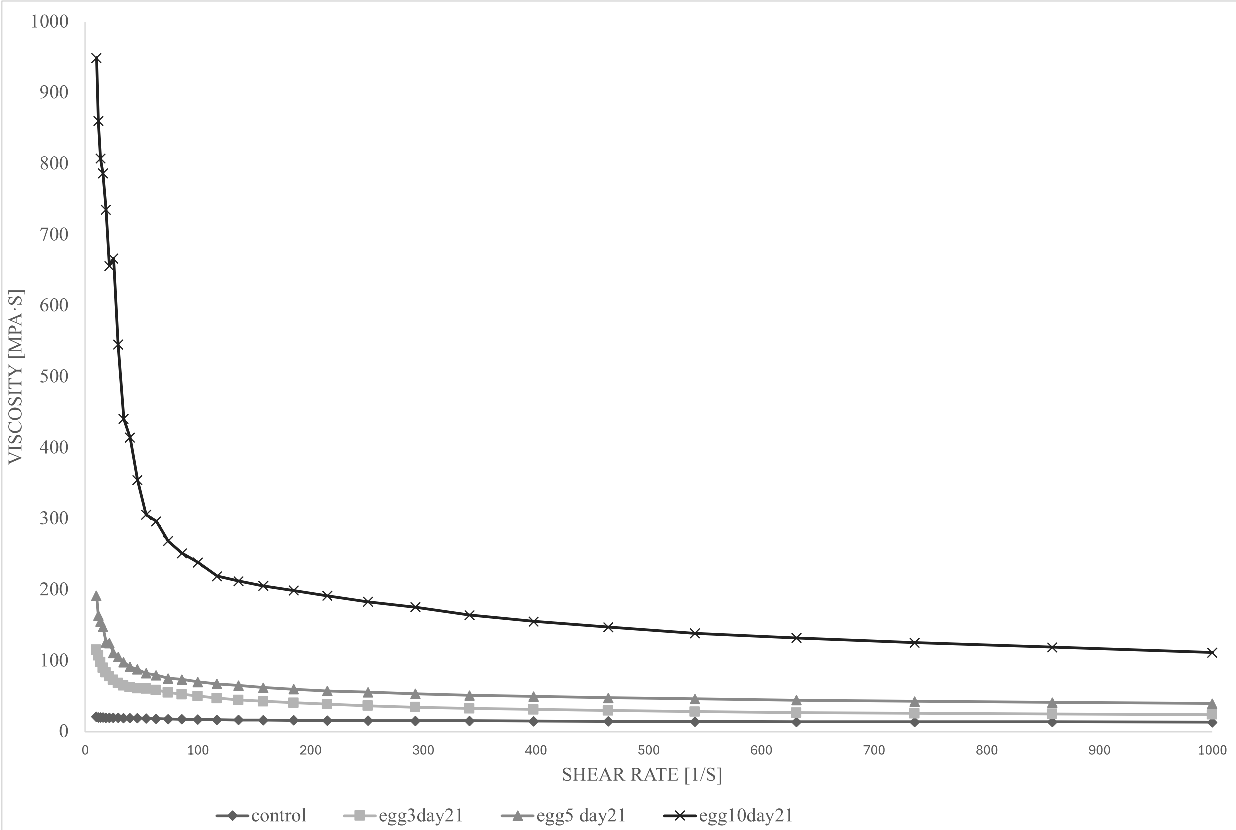


***Figure 4.*:** The viscosity curves (shear rate vs. viscosity) of different concentrations of added egg white protein to liquid whole egg then pasteurized at 65 ± 0.2°C, at day 21of storage.



During storage, proteins tend to aggregate due to continued interactions and rearrangements, leading to the formation of larger structural formations. Over time, proteins have the ability to bind or release water, leading to modifications in the hydration layers surrounding the protein molecules (Singh et al. 2014). This interaction directly influences viscosity by altering the effective forces between protein molecules and the surrounding fluid. In case of liquid whole eggs, the increase in egg white protein concentration enhances its ability to bind free water molecules. Egg white proteins form a stronger network, which binds free water, reducing the amount of water available for free movement. This water-binding property leads to a denser, more viscous system (Haug & Lantz 2011).

At low protein concentrations (3%), the network formed within the liquid whole egg matrix is weak, resulting in minimal resistance to flow and weaker viscosity change. Although storage induces some degree of structural rearrangement, the impact remains minimal due to the limited avalibility of cross-linking sites . With higher protein concentrations, more cross-linking and aggregate formation occure, leading to significantly effect on structural rigidity, increasing yield stress, and elevated viscosity (Zhao et al. 2021). The denser network created by the increased protein interactions exhibits greater sensitivity to shear, contributing to stronger shear-thinning behavior. The fortified liquid whole egg matrix demonstrates resistance to flow at low shear rates, but under higher shear, it begins to deform and align, resulting in a significant reduction in viscosity. This behavior becomes increasingly clear with denser networks formed at higher protein levels or after extended storage periods. Over time, aging further enhances network structuring, making the system more organized but also more prone to shear-induced alignment, which contributes to a progressive decline in viscosity under shear (Wang et al. 2020).

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