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The dynamic oscillatory rheological properties of hen's egg white proteins using various enzymes

Muhammed Yüceer

Department of Food Processing, Canakkale Onsekiz Mart University, 017020-Canakkale, Turkey. E-mail: myuceer@comu.edu.tr

ABSTRACT. This study evaluated the effect of different commercial enzymes on the rheological behavior of the treated hen's egg white protein (HEWP). The enzymes of phospholipase A_2, lipase, and protease were used in the research. These research findings indicate that all treated and non-treated HEWP samples exhibited non-newtonian behavior. It was observed that enzyme-hydrolyzed HEWP flow behavior was similar to that of the control HEWP samples where viscosity decreased with an increase in the shear rate. The lower storage modulus was obtained by phospholipase A_2 and protease-treated samples. The protease enzyme treatment leads to a decrease in viscous modulus and an increase in storage modulus values. The overall observation confirmed that the HEWP loses its liquidity after 64°C.

Keywords: processed egg; enzymatic hydrolysis; protein modification; rheological characterization.

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Introduction

Eggs are an excellent source of the highest quality major nutrients such as complete protein, fats and minerals. Egg white is used as a critical ingredient in prepared food systems such as angel food cakes, egg white cookies, meringues and noodles, due to its exceptional functional characteristics of foaming, binding, and gelling (Campbell et al., 2003). Chicken-hen egg white protein (HEWP) manufactured through the processing of fresh shell eggs is a promising vital foaming characteristic used in food manufacturing (Gharbi & Labbafi, 2019).

Enzymes have been recognized as promising processing agents in the food industry. Enzymes are used in food applications and they can stabilize the structure and modify the rheology and texture of aqueous food systems such as the treatment of liquid egg products. Different commercial enzymes (phospholipase A_2, lipases, and proteases) are used for treatment of the liquid egg products to improve the functionality of processed eggs and in the preparation of final products such as mayonnaise, meringue, and salad dressing (Daimer & Kulozik, 2008; Zhao et al., 2010).

Phospholipase A_2 is a complex of proteins that includes crucially important lipolytic enzymes that catalyze the hydrolysis of the ester bond of phospholipids to free fatty acids, choline phosphate, di-acylglycerols, lyso-phospholipids and phosphatides (De Maria et al., 2007; Hui, 2012). The phospholipase A_2 used in HEWP to improve the functionality and maintains the structural and physicochemical quality attributes during the storage period. The proteolytic enzyme (protease) is used in HEWP to enhance emulsifying properties by hydrolyzing the peptide bonds present in the secondary structure of egg white proteins (Ai et al., 2019).

Lipase is mainly used in HEWP to degrade the lipids of egg yolk diffused (during extended storage) or contaminated to egg white during breaking, which affects the physical, technological and functional characteristics of albumen (Yüceer & Asik, 2020; Macherey et al., 2011). A preliminary investigations conducted to investigate the physicochemical effect of the various enzymes on HEWP (Yüceer & Caner, 2024). However, the detailed characterization of enzymes on the rheological behavior of HEWP remains unknown.

To our knowledge, there are extensive studies about the effect of various enzymes such as phospholipase A_2 with 0.1, 0.2 and 0.3% -v/v, lipase with 0.01, 0.02 and 0.03% -w/v, and protease with 0.5, 1, and 1.5% - w/v (Yüceer & Caner, 2024). However, there are limited research has been done on comparative studies of the quality properties and storage stability in bakery products in terms of optimum enzymatic treatments during storage conducted by our research group (Yuceer & Caner, 2022). Therefore, the goal of the present research was to understand the behavior of changes in the functionality of treated and untreated HEWP.

In this investigation, the rheological characteristics of the various types of commercial enzymes treated (phospholipase A_2-0.3%, lipase-0.03% and protease-0.5%) HEWP samples were evaluated for an extended storage period (27 days in raw egg) at 4°C. Thus, the purpose of our study was to consider the enhancement of various enzymes and find the best enzymatic hydrolysis pre-treatments on the rheological behavior (viscosity with the shear rate, dynamic viscoelastic moduli, deformation and temperature ramp) during storage.

Material and methods

Liquid egg white

Hen egg-white protein (HEWP) samples were provided from Keskinoglu Tavukculuk (Manisa, Turkey). The samples were treated with various microbial enzymes; Phospholipase A_2 enzyme (Maxapal A_2™, DSM B.V., The Netherlands); Lipase (Lipomod 34P™, Biocatalysts Ltd, UK) and Protease (Promod 194SP™, Biocatalysts Ltd. UK).

Enzymatic hydrolysis

The HEWP samples were treated using phospholipase A_2. The optimum concentration of Phospholipase A_2 enzymatic treatment (0.3% -v/v at 45°C±0.2°C for 3h) was selected based on the results obtained through previous studies (Yuceer & Caner, 2022).

The HEWP samples were hydrolyzed using lipase. The pH of HEWP samples was adjusted to 5.0 using citric acid (10% w/w) to obtain optimum enzymatic reaction rates. The optimum treatment concentration (0.03% - w/v at 50°C±0.2°C for 3h) was selected through the previous study (Yüceer & Asik, 2020).

The HEWP was treated using protease. The pH of HEWP samples was adjusted to 7.0 using citric acid to obtain optimum enzymatic reaction rates (0.5% - w/v, at 50±0.2°C for 3h) was determined through the previous study (Yüceer & Caner, 2024).

The enzyme treatments of albumen were performed at pH 9.1, 5.0, 7.0 phospholipase A_2, lipase and protease, respectively. The pH of 9.1, 7.0 and 5.0 control samples was analyzed, and no significant difference was observed in all parameters ($p > 005$). Therefore, the neutral pH (9.1) of HEWP was used as the control group. The HEWP samples were divided into four experimental groups: a) control (untreated HEWP), b) phospholipase A_2 (0.3%), c) lipase (0.03%), and d) protease (0.5%). All enzyme-treated and control groups were packaged in 100 mL of the bag in box (Makplast A.S., Istanbul, Turkey) and then measured during refrigerated storage (4°C initial day, and day 27). A headspace of 2 cm was left for each package before tightly closing with caps and seal.

Dynamic rheological analysis

The rheological behavior of HEWP as affected by various enzymes was conducted out the oscillatory and shear test using a rheometer (DHR-2, TA Instruments, DE, USA) using a 40 mm, plate-plate probe (gap:1 mm). The dynamic rheological test was measured at 25°C between 0.01-100 s^{-1} of shear rate for 150 s. After the oscillation amplitude analysis, the angular frequency analysis was performed at a frequency of a 20 rad s^{-1}, and between 0.01-100% was used. The oscillation frequency was measured at 0.01 - 10 Hz frequency using the strain (%). In the experiment, the temperature ramp was analyzed at 40 - 70°C at a rate of heating 1°C $min.^{-1}$ under a frequency of 20 rad s^{-1}. Rheological parameters (flow behavior index, yield stress) were calculated using Herschel-Bulkley equation 1.

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

wherein γ is the shear rate (1/s), τ is shear stress (Pa), K is consistency index (Pa·s), n is the flow behavior index and τ_0 is yield stress (Pa).

Statistical Analysis

Significant differences in HEWP samples with different types of enzyme application were analyzed statistically. The results were subjected to analysis by LSM-PROG GLM using the statistical program (SAS Institute, Cary, NC, USA). The experiment was replicated three times for each treatment and average values were reported. The data was calculated using a two-way analysis of variance (ANOVA, enzyme type x storage time) model with Tukey post-hoc comparison test carried out in comparing treated and untreated (significance: $p < 0.05$).

Results

The rheological properties of HEWP are presented in Figure 1a. It was observed that enzyme-hydrolyzed HEWP flow behavior was similar to the control HEWP samples by decreases in the viscosity with increasing the shear rate. In general, all experimental egg samples exhibited clear shear-thinning fluid behavior (low ''n'' values) over a wide range of shear rates of 0.01 to 100 s^{-1} because the structure of HEWP deformed and disrupted with an increase in flow yield. The viscosity decreased upon increasing the shear rate. The viscosity curves of hydrolyzed HEWP matched with the Herschel-Bulkley model. The nonlinearity of the shear stress contrasts shear rate values demonstrating a pseudoplastic, non-newtonian behavior. A minor change can be observed in protein structure in pH-adjusted samples by adding citric acid (Ayari et al., 2019). Figure 1b presents oscillation frequency results obtained by frequency sweep tests carried out on HEWP samples. The storage modulus shows the ability to retain elastic deformability, which is related to the elastic solid properties, whereas G'' is related to the viscous liquid behavior of HEWP (Mu et al., 2020). The proper stress value in the LVE region was carried out and G'- G'' was used in the measurement. The rheological behavior of treated HEWP was measured by conducting the frequency test in the linear region. The higher storage modulus value indicates the stiffness of HEWP and the larger LVE region indicates a stable HEWP within increasing shear stress without structural deformation. The decrease in loss modulus results in the enhancement of ductility and an increment leads to decrement in the rigidity of HEWP samples. The research revealed that viscous modulus is much higher than G', and enzyme-treated HEWP samples formed semi-liquid complexes (Figure 1b). The protease enzyme treatment leads to a decrease in viscous modulus and an increase in storage modulus values (G'>G'') while the HEWP solution acts as a viscoelastic semi-solid. The lover modulus values were obtained in phospholipase A_2 treated egg samples.

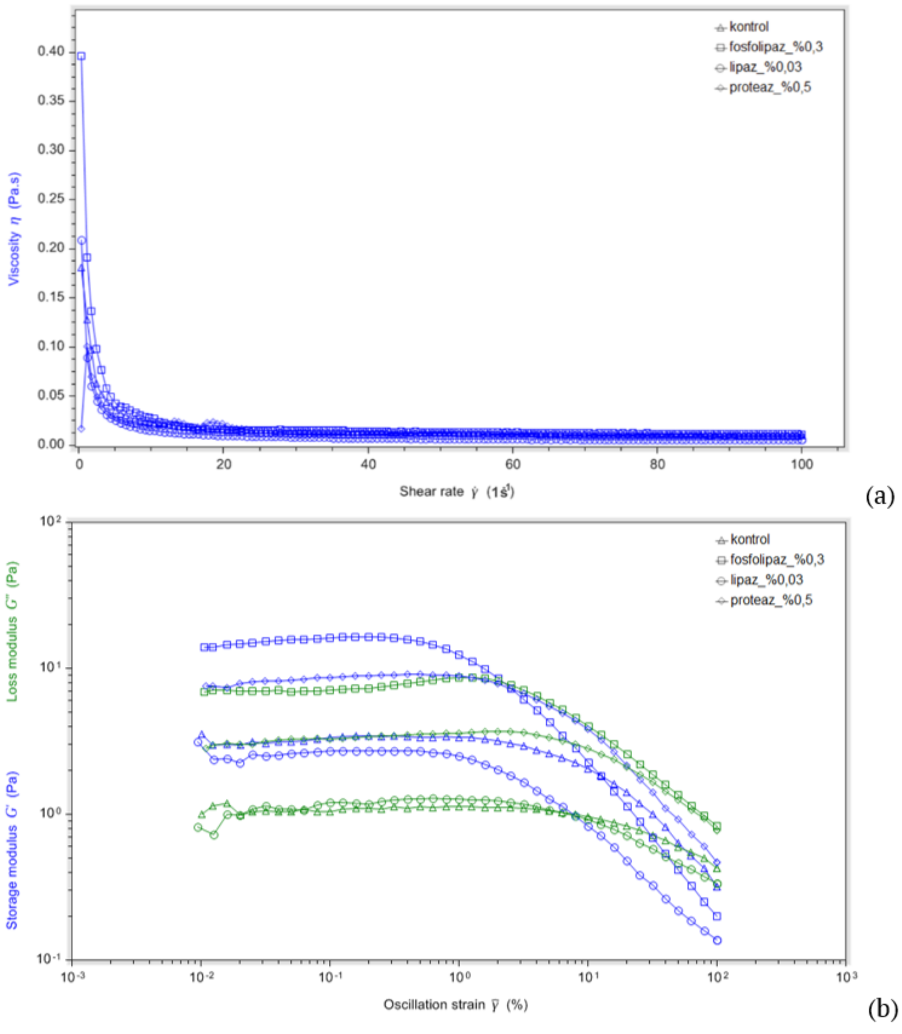

Figure 1. Effect of phospholipase A_2, lipase and protease enzymes treatments on HEWP's; a) viscosity curve b) deformation curve. CNT: Control, PLA: Phospholipase A_2

The frequency sweep graph is presented in Figure 2a. Lower storage modulus obtained by phospholipase A_2 and protease-treated samples. Our findings indicated that with enzymatic hydrolysis similar rheological behaviors were observed by (Yüceer & Asik, 2020).

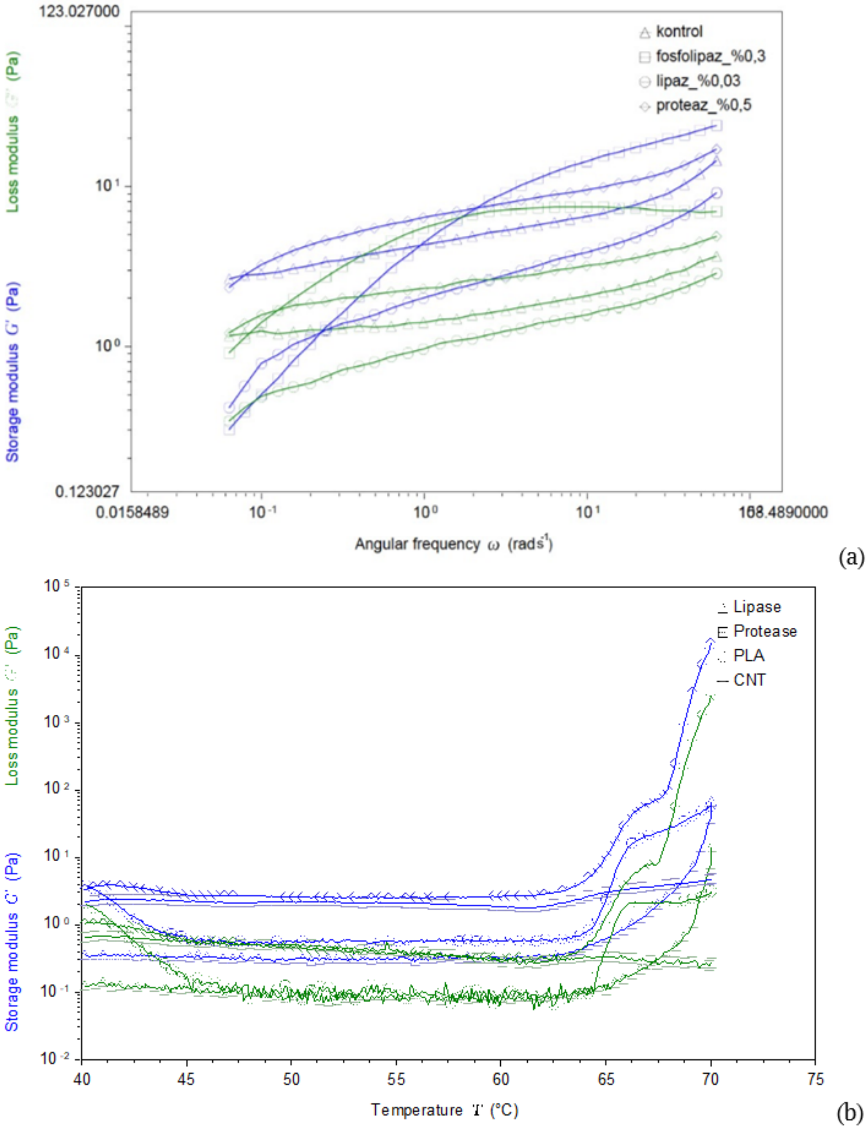

Figure 2. Effect of phospholipase A_2, lipase and protease enzymes treatments on HEWP's; a) elastic modulus and viscous modulus as a function of frequency, b) temperature scanning curve. CNT: Control, PLA: Phospholipase A_2

The structure of egg protein's denaturation and aggregation was evaluated during the oscillation temperature ramp test. The HEWP samples are heated and gellable aggregates are obtained. The temperature ramp test graphic is presented in Figure 2b and the coagulation temperature was calculated. The highest storage modulus values obtained in the control treatment indicate coagulation levels (Hsieh et al., 1993). The overall observation confirmed that the HEWP loses its liquidity after 64°C. The findings claimed that the denaturing temperature was 65°C in all trials. However, the gelling aggregates (coagulation) from denaturing of proteins were observed at 60 to 65°C to denaturation of ovotransferrin (Croguennec et al., 2002). Nyemb-Diop et al. (2016) pointed out that the thermogram of HEWP is explained by elastic modulus according to (Yüceer & Asik 2020). The study's findings regarding thermal denaturation and coagulation behaviors provide valuable insights into optimizing processing conditions for enzyme-treated HEWP. For instance, understanding that HEWP loses liquidity at 64°C can inform pasteurization protocols, ensuring food safety while maintaining functional properties. This knowledge is particularly relevant for heat-sensitive products like custards and sauces. Lipase-treated HEWP, with its minimal structural alterations, could be applied in products requiring extended storage stability while retaining original textural properties. The findings also suggest potential for enzyme-treated HEWP in reducing waste by improving the usability of egg whites contaminated with yolk, which often impacts functional quality. Furthermore, the development of enzyme modified egg products and other functional egg-based ingredient with improved profiles is another area of potential future research. In conclusion, utilizing these functional egg-based ingredients in the baking and food industries will preserve the nutritional efficacy and health benefits of egg-containing foods for the continually growing population. The modifications observed in enzyme-treated HEWP suggest potential for creating specialized functional foods. For example, protease-treated HEWP, with its improved gelling and emulsifying properties, could be used in low-fat or high-protein formulations, catering to health-conscious consumers. Moreover, enzyme-treated HEWP may be suitable for applications in hypoallergenic or tailored protein products, depending on its structural alterations. Also, developing tailored enzymatic treatments for specific functional needs—such as foaming in aerated desserts or thickening in soups—would expand the utility of HEWP across a broader range of applications.

Conclusion

This study provides valuable insights into the enzymatic modification of hen egg white proteins with significant practical implications for the food industry. Enzymatic treatments, such as those with phospholipase A2, lipase, and protease, offer a versatile approach to tailoring the rheological and functional properties of HEWP for diverse applications. For instance, the enhanced viscoelastic properties achieved through protease treatment could be utilized in bakery products to improve texture and stability. Similarly, phospholipase A2's ability to modify protein interactions can find applications in emulsion-based products like mayonnaise and dressings, where stable texture and flow properties are critical. The effects of phospholipase A_2, lipase, and protease treatments on liquid egg white rheological behavior were investigated in this study. All treated and non-treated HEWP samples exhibited non-newtonian behavior. The protease enzyme treatment leads to a decrease in viscous modulus and an increase in storage modulus values. The gelling aggregates were observed and the protease enzyme inhibited the fluidity of the egg albumen. However, lower storage modulus was obtained by phospholipase A_2 and the protease-treated samples.

Future research should delve into detailed microstructural analyses of enzyme-treated HEWP to elucidate the mechanisms driving rheological changes. Additionally, exploring synergistic effects of combining enzymes or incorporating other functional ingredients could lead to more robust modifications. Long-term studies on storage stability and sensory evaluation in real-world food systems will further validate these treatments' commercial viability.

Data availability

The datasets generated during and analyzed during the current study are available from corresponding author upon reasonable request.

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