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The enhancement of the physicochemical and functional characterisation of egg white proteins using different enzymes during storage

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ABSTRACT

In this research, the impacts of various enzymes (phospholipase-A_2 (0.3% v/v), lipase (0.03% w/v), and protease (0.5% w/v)) on the physico-chemical and functional characteristics of the treated egg white protein (EWP) were determined. The pH, turbidity, colour (L^*, a^*, b^*, and ΔE^*), gas concentrations in the package, relative foaming capacity (RFC), and foaming stability were analysed during storage at 4 °C. The protease (1,000 ± 60.82) and lipase (790 ± 41.63) increased RFC values significantly (control: 616 ± 36.05) on the initial day. The enzymes significantly decreased ($P < 0.05$) the turbidity values from 0.46 ± 0.10 (control) to −0.30 ± 0.05 (lipase) and −0.35 ± 0.03 (protease), whereas it was increased by phospholipase-A_2 (0.53 ± 0.06). This research points out the efficacy of the enzymes in improving functionality of EWP. In conclusion, treatment with protease enzyme provided the best RFC values at day 27. However, utilisation of protease led to decrease in L^* and b^* values.

KEYWORDS

enzymatic-hydrolysis, modification of egg albumen, foaming properties, physico-functional characteristics, physicochemical stability

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1. INTRODUCTION

Eggs are a very good source of the highest quality major nutrients such as whole protein, fats, and minerals. Egg white is used as a key component in the preparation of foods such as angel food cakes, meringues, egg white cookies, and noodles, due to its exceptional functional characteristics of foaming, binding, and gelling. Chicken egg white protein (EWP) manufactured through the processing of fresh shell eggs is a promising critical foaming ingredient for the food industry (Gharbi and Labbafi, 2019).

The egg market is in demand for processed egg products due to assurance of safety, innovation, enhanced product functionality, and extended shelf-life. Enzymes have been recognised as promising processing agents in the egg industry to improve functionality of egg products and enhance the textural characteristics of baked products. Commercial enzymes are used in the food applications and they can stabilise the structure and modify the rheology and texture of aqueous food systems such as liquid egg products. The globally increasing needs for food quality led industries to invest in novel methods that enhance storage stability and quality of liquid egg whites. Different commercial enzymes (phospholipases A_2, lipases, and proteases) are used in egg processing and in preparation of final products such as mayonnaise, meringue, and salad dressing (Daimer and Kulozik, 2008; Macherey et al., 2011; Yüceer, 2020a, 2020b; Yüceer and Asik, 2020; Yüceer and Caner, 2021a).

In this investigation, the physicochemical and functional characteristics of EWP samples treated by various types of enzymes (phospholipases A_2: 0.3%, lipase: 0.03%, and protease: 0.5%) were evaluated for extended storage period (27 days in raw egg) at 4 °C. Thus, the purpose of our study was to find the best enzymatic hydrolysis pre-treatments to enhance physicochemical (pH, turbidity, gas concentrations in the package headspace, and colour parameters (L^*, a^*, b^* and ΔE^*)) and functional properties (relative foaming capacity and foam stability) of EWP during storage.

2. MATERIALS AND METHODS

2.1. Materials

Hen egg white protein (EWP) samples were supplied by Keskinoglu Co. (Manisa, Turkey). The protein concentrations of samples were about 12%, and the total soluble dry matter values were 11% on wet basis. The samples were treated with various microbial enzymes: phospholipase A_2 (Maxapal A_2^{TM}, enzyme activity-CPU: 12.622 U mL^{-1}, DSM B.V., The Netherlands), lipase (Lipomod 34P^{TM}, enzyme activity: 115,000 U g^{-1}, Biocatalysts Limited, UK), and protease (Promod 194SP^{TM}, enzyme activity: 200 Casein protease U g^{-1}, Biocatalysts Limited. UK).

2.2. Enzymatic hydrolysis

The EWP samples were treated with phospholipase A_2 at the optimal concentration of 0.3% v/v at 45 °C ± 0.2 °C, natural egg pH of 9.00 for 3 h based on previous results (Yüceer, 2020a; 2020b). The optimal lipase concentration (0.03% w/v at 50 °C ± 0.2 °C, pH of 5.00 for 3 h) was selected according to the results of a previous study (Yüceer and Asik, 2020). The optimal protease reaction rates (0.5% w/v, at 50 ± 0.2 °C, pH of 7.00 for 3 h) were determined based on a previous study (Yüceer and Caner, 2021b). The egg white protein samples pH values were adjusted using citric acid to obtain optimum enzyme reaction rate.

The EWP samples were divided into four experimental groups: a) control (untreated EWP), b) phospholipase A_2 (0.3%), c) lipase (0.03%), and d) protease (0.5%). All enzyme-treated and control groups were placed into 100 mL of bag in box packages and stored at 4 °C. The samples were measured on the initial day and on day 27.

2.3. pH measurement

The pH values of hydrolysed and unhydrolysed eggs were measured potentiometrically using a pH meter (3,100 model Ohaus Corporation, NJ, USA) for 27 days (Yüceer et al., 2016).

2.4. Colour analysis

The colour values of hydrolysed and unhydrolysed samples were measured using a colorimeter (CR-400, Konica Minolta, Osaka, Japan), and colour parameters were calculated according to Yüceer (2020b).

2.5. Turbidity measurement

The turbidity analyses of samples were performed after each enzymatic treatment using a Spectrophotometer (UV-1240, Shimadzu Co, Kyoto, Japan). The absorbance was used to indicate the turbidity of EWP. The turbidity was measured at day 27 as the transmittance (T%) at a specific wavelength (600 nm) and calculated according to Wu et al. (2016).

2.6. Headspace (O_2 and CO_2) gas analysis

The changes of O_2 and CO_2 gas concentrations in the bag-in-box (BB) package (PA/EVOH/PE) headspace were measured using a gas analyser (Oxybaby, HTK, Hamburg, Germany) just before opening the bag on the initial day and on day 27. All gas analyses were conducted just before opening the bag, at ambient temperature, in four replicates, and gas concentration results were expressed as v/v percentage. The evaluation of gas atmosphere concentration inside the packages were measured by injecting the needle of gas analyser into the BB package through an adhesive plastic rubber in order to prevent gas escaping from the BB, and each package was used in a single measurement. Following the headspace gas measurement, the EWP in the BB packages were used for quality testing. Three BB packages per enzymatic treatment were used at each sampling point. The concentrations of gases in the headspace were determined only on the initial day and after 27 days of storage.

2.7. Relative foaming capacity (RFC) measurement

The RFC was measured by whipping 100 mL of EWP using a Hobart mixer (N50CE, Hobart Foster A/S, Denmark) at 580 r.p.m. for 3 min and calculated according to Yüceer (2020b). The RFC analysis was performed at 20 °C, and 1,000 mL graduated transparent cylinder was used to measure the foam.

2.8. Foam stability measurement

The stability of prepared foam was determined by measuring the liquid drainage of foam capacity and carried out by analysing the EWP drainage after fixed time (1 h) of holding the foam

in a cylinder at room temperature and calculated according to Yüceer (2020b) and defined as percentage.

2.9. Statistical analysis

The results were subjected to analysis by LSM-PROG GLM using the SAS software (SAS, 2003). The study was repeated twice, and the data was calculated using a two-way analysis of variance (ANOVA, enzyme type x storage time) with Tukey's post-hoc comparison test to compare treated and untreated samples (significance: $P < 0.05$).

3. RESULTS AND DISCUSSION

Enzymes have certain characteristics in hydrolysing substrates, which can affect the nutrient composition of EWP, so they can affect their physical and chemical properties. The pH level of the EWP primarily affects shelf life, microbial load, functional and technological qualities of eggs and egg-based ingredients, in preparation of baked products quality attributes such as whipping, gelling, coagulation, and emulsifying activity. Also, the pH of protein dispersions in terms of isoelectric point is critical in the food processing by minimising electrostatic interactions in egg protein molecules. Different enzymes have lowered the pH values with increasing storage time. Since liquid egg ages during storage, the microbial load of egg product increases, consequently the changes in pH lead to deterioration in functional and technological properties (Necidova et al., 2019). The pH values of EWP significantly decreased during storage from the initial day to day 27 ($P < 0.05$). During the study, pH values ranged from 5.97 to 9.19 on the initial day to 5.49 and 9.08 on day 27 of storage depending on treatment (Fig. 1). The difference was observed between the samples treated with protease and lipase enzymes and those samples that pH adjusted prior to enzymatic treatment. Yüceer (2020b) studied the use of phospholipase A_2 enzyme (0.1, 0.2, and 0.3%) on EWP and observed the decrease in pH values during storage periods. The pH values of EWP samples were 9.07 ± 0.01 at the initial day (day 0) and ranged from 9.07 to 9.12 with phospholipase A_2 enzyme treatment as a processing agent. There was no significant change in the pH of the control group and phospholipase enzyme treated groups

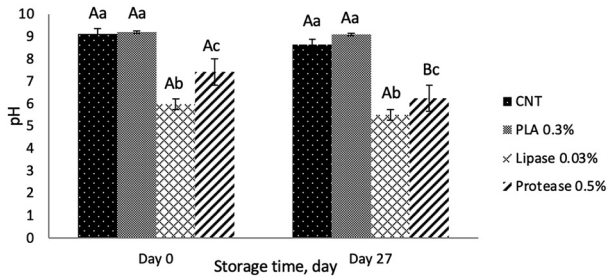

Fig. 1. Effect of phospholipase A_2, lipase, and protease enzyme treatments on pH values of liquid egg white on day 0 and day 27 of storage at 4 °C. CNT: control; PLA: phospholipase A_2. A–B: Different capital letters mean significant differences ($P < 0.05$) between day 0 and day 27 of storage. a–b: Different lowercase letters mean significant differences ($P < 0.05$) between treatment groups

(Yüceer, 2020b; Yüceer and Asik, 2020). The differences in the hydrolysis characteristics of the EWP component led to different pH values during storage. Marked differences at the end of storage in EWP samples of treated and untreated were observed in pH values. Yüceer (2020b) also found a lower pH of 7.82 ± 0.11 in the control samples (untreated) compared to phospholipase A_2 treated eggs. The study demonstrated that an increase in phospholipase A_2 enzyme concentration enhances the pH values obtained leading to longer shelf life. According to the study of Necidova et al. (2019), the total plate count value of control group whole eggs was observed 5.67 log CFU g^{-1} with a pH of 7.48 at the end of storage.

The colour parameters (L^*, a^*, b^*, and ΔE^*) of enzyme-treated and untreated EWP were analysed on the initial day and on day 27 of storage period at 4 °C (Table 1). The L^* parameters of control, phospholipase A_2, lipase, and protease treatments were 36.77 ± 0.43, 36.02 ± 1.06, 35.58 ± 1.17, and 35.94 ± 1.12, respectively, on the initial day, the differences between treatments were not significant ($P > 0.05$). However, the L^* parameters of the protease treated samples were significantly different ($P < 0.05$) from the control on day 27. The protease enzyme treatment led to a decrease in L^* and b^* values. This might be due to the hydrolysis of the amino acid chains that modify the protein structure in EWP. For a^* values, the differences between the enzyme-treated groups and the control on the initial day and day 27 were not significant ($P > 0.05$). ΔE^* colour parameters among the EWP samples were also calculated. Consequently, the highest ΔE^* value (4.39) was obtained for the lipase treated group on the initial day, whereas during storage ΔE^* decreased to 2.18. It was not easy to spot the difference between the ΔE^* values of phospholipase A_2 treatment group and control on the initial day, however, for the phospholipase A_2 treatment group ΔE^* increased to 5.56 on day 27. The colour difference could be explained by the different pH values used during the enzyme modification process and protein solubility of EWP.

Turbidity measurement was used to analyse the macroscopic changes that depend on protein value and pH value (Ball and Winn, 1982; Wu et al., 2016). Also, transmittance was measured to obtain transparency and to characterise the formation of aggregates of EWP during enzymatic processes based on the study of Yüceer (2020b). The absorbance of the EWP was measured via an UV-spectrophotometer. The turbidity values increased significantly ($P < 0.05$) with phospholipase A_2 treatment (0.53 ± 0.06) compared to untreated samples (0.46 ± 0.10). However, a decrease was observed for lipase (–0.30 ± 0.05) and protease treatment (–0.35 ± 0.03) of EWP (Table 2). The particle turbidity values increased significantly in all experiment groups during storage ($P < 0.05$). The increase in turbidity values are probably caused by the decreased solubility of EWP (Min et al., 2012). The alkalinity of EWP leads to lower turbidity values compared to an acidic environment (Ai et al., 2019). Turbidity is a good way to quantify improvements in protein solubility (Kulchaiyawat, 2015). Our findings indicate that the lower pH of EWP after enzymatic treatment leads to a decrease in protein solubility and formation of aggregate substances that increase the particle turbidity of EWP. Similar results were obtained in a study conducted by Kulchaiyawat (2015), Yüceer (2020b), and Yüceer and Caner (2021a).

The gaseous atmosphere in the headspace of the packages was evaluated to determine the shelf life of liquid eggs with characterising spoilage odours produced by bacterial growth. A gas analyser device was used as a rapid, inexpensive, easy, and simple identification method compared to gas chromatography. In our study, CO_2 and O_2 gas compositions inside the packages of EWP were measured on day 27 (Table 3). As expected, O_2 levels in the BB packages decreased and CO_2 levels increased in control samples. At the end of storage, the O_2 levels

Table 1. Effect of phospholipase A_2, lipase, and protease enzyme treatments on the changes of liquid egg white's L^*, a^*, b^*, and ΔE^* colour parameters on day 0 and day 27 of storage at 4 °C

Treatments	Storage period (day)/liquid egg albumen colour parameters							
	Day 0				Day 27			
	L^*	a^*	b^*	ΔE^*	L^*	a^*	b^*	ΔE^*
CNT	36.77 ± 0.43^{Aa}	-1.21 ± 0.07^{Aa}	10.66 ± 0.90^{Aa}	0	35.06 ± 0.67^{Aa}	-0.89 ± 0.04^{Ba}	3.75 ± 0.68^{Ba}	0
PLA 0.3%	36.02 ± 1.06^{Aa}	-1.12 ± 0.15^{Aa}	9.15 ± 0.63^{Aa}	1.69	34.80 ± 0.76^{Ba}	-1.11 ± 0.08^{Ba}	9.30 ± 0.38^{Ab}	5.56
Lipase 0.03%	35.58 ± 1.17^{Ab}	-1.09 ± 0.09^{Aa}	6.44 ± 1.07^{Ac}	4.39	34.11 ± 1.45^{Aa}	-0.99 ± 0.16^{Aa}	5.71 ± 0.91^{Ad}	2.18
Protease 0.5%	35.94 ± 1.12^{Ab}	-1.08 ± 0.08^{Aa}	8.44 ± 0.69^{Ab}	2.37	31.15 ± 0.86^{Bb}	-0.98 ± 0.03^{Aa}	7.58 ± 0.57^{Ac}	5.47

CNT: control, PLA: phospholipase A_2.
$^{A-B}$: Means in the same row with different capital letters are significantly different ($P < 0.05$).
$^{a-d}$: Means in the same column with different lowercase letters are significantly different ($P < 0.05$).

Table 2. Effect of phospholipase A_2, lipase, and protease enzyme treatments on liquid egg white's turbidity (OD_{600}) values on day 0 and day 27 of storage at 4 °C

	Liquid egg white turbidity (OD_{600})	
Treatments	Day 0	Day 27
CNT	0.46 ± 0.10^{Aa}	0.56 ± 0.14^{Aa}
PLA 0.3%	0.53 ± 0.06^{Ab}	0.73 ± 0.03^{Bb}
Lipase 0.03%	-0.30 ± 0.05^{Ac}	-0.10 ± 0.04^{Bc}
Protease 0.5%	-0.35 ± 0.03^{Ac}	-0.09 ± 0.02^{Bc}

CNT: control; PLA: phospholipase A_2.
$^{A-B}$: Means in the same row with different capital letters are significantly different ($P < 0.05$).
$^{a-d}$: Means in the same column with different lowercase letters are significantly different ($P < 0.05$).

Table 3. Effect of phospholipase A_2, lipase, and protease enzyme treatments on liquid egg white's O_2 and CO_2 concentration values (%) of package headspace at day 27 of storage at 4 °C

	Gases concentration values (%)	
Treatments	O_2	CO_2
CNT	14.75 ± 0.85^{a}	2.23 ± 0.81^{a}
PLA 0.3%	14.50 ± 1.03^{a}	0.87 ± 0.34^{b}
Lipase 0.03%	14.52 ± 0.72^{a}	0.84 ± 0.27^{b}
Protease 0.5%	14.36 ± 0.93^{a}	0.22 ± 0.16^{c}

CNT: control; PLA: phospholipase A_2.
$^{a-c}$: Means in the same column with different lowercase letters are significantly different ($P < 0.05$).

decreased to 14.75 ± 0.85 for control and 14.36–14.52% for other treatments. Also, the CO_2 levels increased to 2.23 ± 0.8 for control and ranged from 0.22 to 0.87 for the enzyme treated groups. No significant differences ($P > 0.05$) could be detected among treatments for O_2 concentrations except for protease treatment. Yüceer (2020b) evaluated the package headspace gas concentrations in egg products, where increase in O_2 concentration with a decrease in CO_2 concentration during storage were observed due to the presence of active bacterial community. Also, a decrease in pH levels corresponds to shorter shelf-life of whole eggs (Necidova et al., 2019). The O_2 level of all treated and non-treated samples were significantly similar ($P > 0.05$). However, the CO_2 levels significantly different between treated and control samples at the end of storage at 4 °C: 2.23% ± 0.81 (control), 0.87% ± 0.34 (PLA), 0.84% ± 0.27 (lipase) and 0.22% ± 0.16 (protease). Enzymatic treatments led to formation of volatiles due to oxidative changes in EWP. The decrease in CO_2 concentration variation may be due to dissolution in the egg albumen fraction by an aggregate of bicarbonate and other forms. However, the phenomenon of the package headspace O_2 and CO_2 gas concentration in liquid eggs is still unknown.

Foam capacity acts as an indicator of foaming property. The foaming ability is affected mainly by surface hydrophobicity, charge density and distribution, flexibility of the protein, and the nature of food. Foams are present in many baked foods such as meringues, cakes, cookies, nougats, etc. and play an important role in incorporating air while structuring of dough made of beaten egg white and sugar (Pernell et al., 2000). An increase in egg white aqueous foams was

observed for all enzymatic treatments. A main finding of the present study is that with protease treatment significantly ($P < 0.05$) higher RFC values were obtained (Fig. 2A), and these values remained the highest among the treatment groups by the end of storage. The addition of phospholipase A_2 enzyme showed no statistically significant differences in the RFC values ($P > 0.05$). Our results agree with previous research conducted by Yüceer (2020b) to compare different phospholipase A_2 enzyme concentrations (0.1, 0.2, and 0.3%) as the similar RFC values obtained treatments. The present study results of relative foaming capacity support the earlier studies (Macherey et al., 2011; Yüceer and Asik, 2020; Yüceer, 2020a). The high correlation between the pH and the stability of EWP foam (drained liquid volume) was reported by Lomakina and Mikova (2006). Fig. 2B presents the effects of various enzyme treatments on the EWP foam stability during the storage period at 4 °C calculated from liquid drainage. The foaming stability of lipase treated EWP was the highest on the initial day. The foam stability of control samples increased from 79.16 (day 0) to 88.00 (day 27) during storage. Similarly, foam stability values of phospholipase A_2 treated samples increased from 75.83 to 83.66. Similar results were presented by Yüceer (2020b). However, foam stability values of lipase and protease

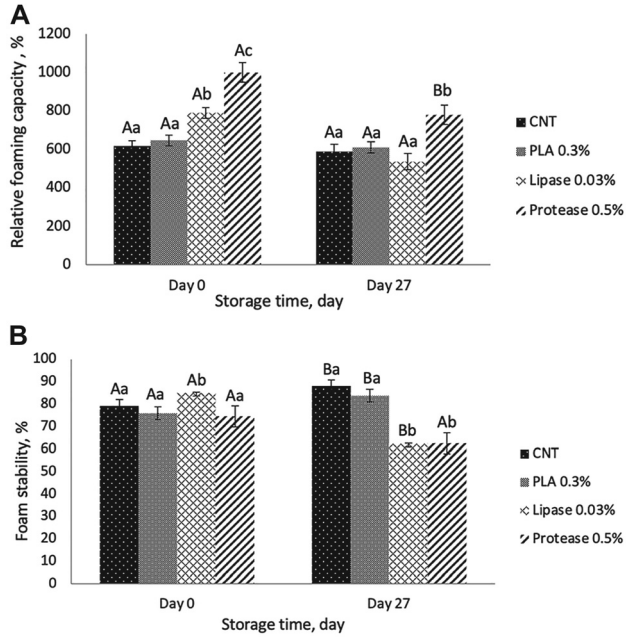

Fig. 2. A) Effect of phospholipase A_2, lipase, and protease enzyme treatments on relative foaming capacity of liquid egg white on day 0 and day 27 of storage at 4 °C. CNT: control; PLA: phospholipase A_2. A–B: Different capital letters mean significant differences ($P < 0.05$) between day 0 and day 27 of storage. a–b: Different lowercase letters mean significant differences ($P < 0.05$) between treatment groups. B) Effect of phospholipase A_2, lipase, and protease enzyme treatments on foam stability of liquid egg white on day 0 and day 27 of storage at 4 °C. CNT: control; PLA: phospholipase A_2. A–B: Different capital letters mean significant differences ($P < 0.05$) between day 0 and day 27 of storage. a–b: Different lowercase letters mean significant differences ($P < 0.05$) between treatment groups

treated EWP samples decreased from 85 to 74.5 to 62.33–62.5, respectively. The differences observed between the foam stability values at the beginning and end of storage were significant ($P < 0.05$) except for protease treated samples. The reason for this may be related to the EWP proteins presenting better stability at near-neutral pH levels and forming stable networks of proteins.

4. CONCLUSION

The effects of various enzymes (phospholipase A_2, lipase, and protease) treatments on EWP samples were analysed in the study. The research compared optimal concentrations of the different types of enzymes and also demonstrated a great potential of hydrolysis by PLA acting as foaming agent, lipase as stabilising agent, and protease as texturing agent of egg products. Though enzymes can exhibit an improvement in functional and technological characteristics of egg albumen, the physicochemical characteristics of EWP are also affected by them. This study concluded that protease treated EWP provided better technological properties than non-treated EWP while preserving physicochemical properties. Also, protease was suitable for improving the functionality of EWP. Protease and phospholipase A_2 enzymes have the future perspective to bring breakthrough innovation in the egg processing industry in preserving egg protein deformation and increasing functionality while enhancing storage stability. The gelling aggregates observed and protease enzyme inhibited the fluidity of egg.

The findings are interesting and valuable for the liquid egg industry that supplies ready to use egg-based products for bakers. Also, measurement of the gas composition (O_2 and CO_2) in the headspace of the packages has a potential in evaluation of shelf-life as a new non-destructive technique to determine chemical, biochemical changes and microbiological loads in egg products.

Further experiments are needed to address the mechanisms of action behind each enzyme in detail. Also, structural changes and interfacial properties of proteins are needed to be studied to interpret the observed physical properties and functionalities.

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