



Effectiveness of enzymes on structural, functional and creep-recovery behavior of freshly prepared meringue's batter using liquid egg albumen

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Abstract In this research, the impacts of varying concentration of phospholipase A₂ (PLA), protease and lipase enzyme-treated liquid egg white (LEW) were prepared using meringue batters. The gelling physico-chemical, and rheological properties such as rheological creep-recovery compliance behavior, color value (L^* , a^* , b^* , ΔE^* , Chroma, Hue and WI), gelling strength, pH value, gelling index as TPA parameter and specific density of freshly prepared meringue batter were analyzed. The specific density of meringue batter of control (0.49 ± 0.01) was significantly decreased ($P < 0.05$) after enzyme treatments of PLA (0.39 ± 0.01), lipase (0.33 ± 0.01), and protease (0.37 ± 0.01). Also, the enzymatic treatment significantly decreased the b^* values from -0.08 ± 0.07 to -0.76 ± 0.04 , -0.70 ± 0.06 , and -0.73 ± 0.03 , respectively. The Burgers model was used to characterize rheological behavior of enzyme treated and freshly prepared meringue samples. Creep-recovery responses of samples were satisfactory ($R^2 > 0.99$) for evaluation of creep-recovery behavior. This research points out the efficacy of lipase pre-treated LEW in meringue preparation on improving functionality such as batter density and gelling properties.

Keywords Egg albumen · Enzymes · Meringue · Techno-functional characterization · Foammability · Structural modification · Batter rheology

Introduction

Eggs offer a moderate calorie source with protein of excellent quality and low economic cost which make eggs within reach to most of the population. Liquid egg white (LEW) contains the highest quality proteins with multi-functional characteristics such as foaming, binding, and gelling ability (Yuceer and Caner 2014). The best whipping capacity as an foaming agent of LEW popular because of its aerating characteristics (protein/sugar preparation) in solidified foamed baking products such as meringue, that consist of foam from air bubbles and made from a soft-thick dough (Schuck 2013; Vega and Sanghvi 2012). Traditional meringues made by whipping LEW with sugar until stiff and stabilizing them in the oven.

In recent years, there has been an increasing demand for novel sources of valuable egg ingredients in the food industry. The enzyme-treated liquid egg market is raising popularity, and this can be associated with the fastest growing in industrially processed egg processing industry, last decade. Different commercial enzymes (lipases, phospholipases A₂-PLA, and proteases) as a processing aid are used in the treatment of egg products to enhance the functionality (Yuceer 2020b; Yuceer and Caner 2018a). Yuceer and Asik (2020) stated that the treatment of LEW using PLA improves the functional characteristics while stabilizing the structural and physical attributes of meringues produced. The proteolytic enzyme (protease) enhancing the emulsifying properties of egg white by hydrolysate the peptide bonds present in secondary

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structure of biologically active egg proteins (Ai et al. 2019). Also, enzymatic hydrolysis of protein improves their solubility in the product. The lipase enzyme used in albumen to degrade the residue of lipids migrated (during storage) or contaminated (in egg breaking and separating process) from yolk to albumen, which decreases the functionality of egg albumen (Yüceer and Asik 2020). Enzymatic treatment of egg proteins resulted in an improvement in technological and functional properties depending on enzyme type, pH, temperature, degree of hydrolysis, enzyme concentration, and reaction/hydrolysis time of egg protein etc. (Horimoto and Lim 2017). The consumer trends to get an innovative food product of bakery production such as meringue boost a rapid growth in the world. It is essential that, enzymes are needed to enhance the functionality in egg and egg-based applications. Recently, there are numerous studies on the characteristics of egg yolk, but there are limited publications which have addressed the use of optimum concentration of various enzymes in LEW (Daimer and Kulozik 2008, 2009; Jaekel and Ternes 2009; Kim et al. 2009; Macherey et al. 2011; Yüceer 2020a, b; Yüceer and Asik 2020). The influences of enzymes on LEW as a processing aid in meringue processing was evaluated to obtain an optimum enzyme for a better functional characteristic.

In this research the impact of PLA (0.3%), lipase (0.03%) and protease (0.5%) treatment of LEW on structural, functional and creep-recovery behavior of freshly prepared meringue's batter samples were investigated. Thus, the primary purpose of our research, therefore, was to examine the improvement of enzymatic treatments (PLA, lipase and protease) on the physico-functional attributes of freshly prepared meringue batters from LEW such as pH, color parameters (L^* , a^* , b^* , ΔE^* , C^* , $^{\circ}h$ and WI), specific density, gelling properties and rheological compliance behavior (creep-recovery).

Materials and methods

Materials

Chicken liquid egg white (LEW) were supplied from Keskinoglu Tavukculuk (Manisa, Turkey). Then, the LEW's were arranged to four groups for experimental design: (a) control (untreated), (b) 0.3% PLA enzyme treated sample, (c) 0.03% lipase enzyme treated sample, and (d) 0.5% protease treated sample. All samples were used in preapration of meringue dough production using powdered sugar that obtained from local market.

Enzymatic treatment

The LEW was treated with phospholipase A₂ enzyme, 9650 CPU/g enzyme activity (Maxapal A₂TM, DSM-Sanovo, Denmark) at concentration of 0.3% (v/v) at 45 °C for 90 min (Yüceer 2020a, b).

The LEW was treated with lipase enzyme (LipomodTM 34P, Biocatalysts Ltd., UK), 115.000 lipase U/g activity. The concentration of 0.03% (w/v) applied in the experiment. The LEW samples pH were adjusted to optimum enzyme reaction rate to 5.00 using citric acid (Tekkim Chem., Istanbul, Turkey) at 50 °C for 90 min (Yüceer and Asik 2020).

The LEW samples were treated with protease enzyme, obtained from microbial fermentation of *Aspergillus oryzae* (PromodTM 194SP, Wales, UK) with the activity of 200 Casein protease u/g. The pH were adjusted to optimum enzyme reaction rate to 7.00 using citric acid and the enzyme concentration was 0.5% (w/v) at 50 °C for 90 min (Yüceer and Caner 2018b). All treated/untreated (control) groups were used in the preparation of meringue batter.

Meringue batter preparation

Meringue is a cookie prepared from whipped LEW and powdered sugar. In the study, 0.2 L of LEW mixed with 350 g of powdered sugar was used as a simplest form of meringue cookies recipe to prepare a batter of meringue at room temperature. The Hobart N50CE (Hobart Foster Scandinavia A/S, Denmark) was used to mix sugar and LEW blend at 580 rpm for 90 s. to obtain soft egg white foams in first stage and mixing ended until a soft-whipped meringue batter occurs (Yüceer 2020a).

Methods

pH measurements of of meringur batter

The pH value of all prepared meringue batter samples were analysed using Ohaus Starter pH meter (Ohaus Corporation, USA) (Yüceer 2020b). The analysis was performed in triplicate. The measurement was conducted at 20 ± 1 °C.

Color measurements of of meringur batter

The surface color values of meringue batter samples were analyzed using a colorimeter (CR-400, Konica Minolta, Japan) at 20 ± 1 °C. The reflectance colorimetric measurement values were presented to identifying surface color differences as color space CIE LAB L^* , a^* , b^* parameters (Yüceer 2020b). In addition, Metric Hue Angle (h^*), Metric Chroma (magnitude- C^*), WI (Whiteness Index), and total

color difference (ΔE) were calculated according to Yüceer (2020a) based on color space CIELAB color difference metric (L , a , b , Delta E, Chroma, and Hue) values at following equations (Eqs. 1, 2, 3, 4):

$$\Delta E_{ab}^* = \sqrt{(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2} \quad (1)$$

$$\text{Chroma} = C_{ab}^* = [a^{*2} + b^{*2}]^{1/2} \quad (2)$$

$$\text{Hue} = h_{ab}^* = \arctan(b^*/a^*) \quad (3)$$

$$\text{WI} = 100 - \left[(100 - L^*)^2 + (a^{*2} + b^{*2}) \right]^{1/2} \quad (4)$$

Meringue batter density analysis

The density analysis of freshly whipped meringue batter obtained by measuring a volume of solids to weight as known a specific gravity. The volume was measured in cm^3 and presented at 20 ± 1 °C. The weight per volume (specific density) was calculated according to Eq. 5 and expressed as g/cm^3 . All measurements were conducted with three parallels.

$$\text{Batter Density} = \frac{\text{weight of whipped batter in fixed container}}{\text{weight of distilled water in a fixed container}} \quad (5)$$

Rheological measurements of meringur batter

The rheological characteristics of the meringue batter (LEW-sugar mixture foam) measurements were analyzed using a Discovery Hybrid Rheometer (DHR-2, TA Instruments, New Castle, USA) and data obtained by software (Trios Software, TA) equipped a 60 mm aluminum P-P probe geometry with 1 mm of gap. The standard procedure of creep-recovery measurements were performed according to Yüceer (2020a). In the study, the stiff peak of egg-sugar mixture foam (batter) was used in the measurement (Guadarrama-Lezama et al. 2016). The rheological behavior of meringue batter was analyzed using creep-recovery test, strain-relaxation behavior (strain sweep) and step creep (transient) phase was analysed at constant strain $\sigma_0 = 5$ Pa during 180 s in the first step to obtain deformation under constant stress/force, while and recovery phase was maintained $\sigma = 0$ Pa during 360 s of recovery curve after removal the load/force at 25 °C and the stress relaxation of the sample was measured (Yüceer 2020a; Yüceer and Asik 2020). The rheological behavior results were presented in terms of creep compliance, $J(t)$ (Pa^{-1}) = γ/σ , where σ represents the constant stress during a creep test. The best fitted data was used as a criterion to

obtain the high yield of coefficient determination R^2 was 0.99 for treatments.

Gelling properties measurements

The thermal gelation of LEW is based on textural properties of protein denaturation. The LEW-gel were prepared by heating the LEW at 45 for 45 min using a dry bath incubator (FOUR E's Scientific Co, Ltd, Guangzhou, China). A texture profile analysis (TPA) of LEW gels were measured and presented as hardness value for gelling index. The TPA analysis was performed using a texture analyzer (TA.XT Plus Texture Analyzer, Stable Micro Systems, Surrey, England), equipped with 5 kg of load. The gelling strength measurement carried out using a cylindrical probe with TA-18, 1/2" dia ball, ss at room temperature with the following parameters: test speed of 1.0 mm/s, post-test speed of 5.0 mm/s, compression distance of 10 mm, recoverable time of 5 s, and trigger force load of 5.0 g (Yüceer 2020a).

Statistical analysis

Significant differences in samples as a result of various enzyme treatments were analyzed statistically. The obtained measurement values were analyzed by LSM-PROG GLM using a SAS statistical software (SAS 2003). Three replicates for each sample were analyzed and mean values plus standard deviations were reported. The data was calculated using a one-way analysis of variance (ANOVA) model with Tukey post-hoc comparison test was performed in comparing differences in treated/untreated samples and between groups. The differences in statistical analysis were considered as significance and defined $P < 0.05$ in the study.

Results and discussion

pH measurements

The pH analysis of LEW-sugar mixture (batter) was measured after whipping stage. The pH values of meringue's batter prepared from phospholipase A₂ (PLA), lipase and protease enzyme treated and untreated liquid egg white were presented in Table 1. The pH decrease prior to enzymatic modification in lipase and protease helped to stabilizing the foam by decreasing the pH level in the meringue foam. During the study pH values ranged between 6.10 and 9.14. The expected difference was observed between the EWP samples treated with protease and lipase enzyme and those that not have the pH adjusted prior to treatment. The low pH can aid foam formation and

Table 1 The pH values of meringue's batter prepared from phospholipase A₂ (PLA), lipase and protease enzyme treated and untreated liquid egg white

Treatments	pH value
CNT*	9.14 ± 0.19 ^a
PLA	9.03 ± 0.12 ^b
Protease	6.10 ± 0.20 ^d
Lipase	7.79 ± 0.21 ^c

*CNT control, PLA phospholipase A₂

Results were presented as means ± standard deviations of triplicate measurements ($n = 3$)

^{a–d}Means in the same column with different lowercase letters are significantly different ($P < 0.05$)

stability in meringue production (Goldfarb 2016). According to results, the pH values of control, PLA, lipase and protease were 9.14 ± 0.19 ; 9.03 ± 0.12 ; 6.10 ± 0.20 , and 7.79 ± 0.21 , respectively. Yüceer (2020b) examined the use of 0.1; 0.2 and 0.3% by v/v concentrations of PLA enzyme on EWP and observed a decrease in pH values with increasing storage periods (day 45) in all treatments. The pH values of EWP samples were 9.07 ± 0.01 at the initial day (day 0) and ranged between 9.07 and 9.12 with PLA enzyme treatment as a processing aid agent. The marked differences at the end of storage in EWP samples of treated and untreated were observed in pH values. Yüceer (2020b) also found lower pH of 7.82 ± 0.11 in control samples (untreated) comparing to PLA treated eggs.

Color analysis

The color parameters (L^* , a^* , b^* , ΔE , Chroma, and Hue) of enzymes-treated and untreated freshly prepared meringue batter samples were analyzed (Table 2). According to results, the L^* parameters of control, PLA, lipase and protease were 90.09 ± 0.22 ; 91.60 ± 0.26 ; 91.76 ± 0.35 ,

and 91.94 ± 0.68 , respectively. There was no significant difference ($p > 0.05$) was observed in the L^* measurements. (Yüceer 2020a) has investigated the color parameters of meringue-batter samples treated with PLA and observed no significant difference between treated (0.1, 0.2 and 0.3%) and untreated LEW samples. The enzyme treatments led to an increase in b^* parameters. This may be related to batter density (Table 2) and the air retaining capacity that is incorporated in the batter structure (Xing et al. 2015). The lower b^* (3.86 ± 0.44) observed in protease treated samples. This may be related to hydrolyzation of proteins in batter system (Rao et al. 2012). Also, The differences between the treated groups L^* and a^* values were statistically not significant. The ΔE , chroma, and hue angle color parameters among the meringue batter samples were also calculated. Consequently, the ΔE color parameters were low and difficult to observe with human eye detecting points. The enzymatic treatments did not significantly affect the hue angles values of freshly prepared meringue samples. The protease treated samples had the lowest, where PLA treated samples had the highest whiteness index (WI).

Meringue batter density

The batter specific gravity (batter density-BD), is the typical ratio of the batter weight to water weight, reflects a specific volume of final products after baking (Xing et al. 2015). The BD values of meringue's prepared from PLA, lipase and protease enzyme treated/untreated LEW presented in Table 3. The BD of meringue batter of control (0.49 ± 0.01) was decreased significantly after treatments of PLA (0.39 ± 0.01), protease (0.37 ± 0.01) and lipase (0.33 ± 0.01). Meringue batters made using lipase enzyme were extremely lightweight. In the control group, it is observed that all enzymes used in the study decreased the BD of the batter, which may be related to foaming ability and the level of air phase that absorbs as a gas to the egg

Table 2 The effect of phospholipase A₂ (PLA), lipase and protease enzyme treated and untreated liquid egg albumen on batter prepared meringue's color value changes

Treatments	Meringue batter color values						
	L^*	a^*	b^*	ΔE	Chroma	Hue	WI
CNT*	91.09 ± 0.22 ^a	− 0.08 ± 0.07 ^a	4.48 ± 0.30 ^a	0	4.56	− 5.58	94.23
PLA	91.60 ± 0.26 ^a	− 0.76 ± 0.04 ^b	4.38 ± 0.15 ^a	0.79	4.45	− 5.73	95.51
Protease	91.94 ± 0.68 ^a	− 0.73 ± 0.03 ^b	3.86 ± 0.44 ^b	0.28	3.93	− 5.25	92.62
Lipase	91.76 ± 0.35 ^a	− 0.70 ± 0.06 ^b	4.05 ± 0.11 ^a	0.19	4.11	− 5.78	93.33

*CNT control, PLA phospholipase A₂

Results were presented as means ± standard deviations of triplicate measurements ($n = 3$)

^{a–b}Means in the same column with different lowercase letters are significantly different in each color parameter ($P < 0.05$)

Means with the same letters are not significantly different at ($P < 0.05$)

Table 3 The batter density values of meringue's batter prepared from phospholipase A₂ (PLA), lipase and protease enzyme treated and untreated liquid egg white

Treatments	Batter density values of meringues (g/cm ³)
CNT*	0.49 ± 0.01 ^a
PLA	0.39 ± 0.01 ^b
Protease	0.37 ± 0.01 ^c
Lipase	0.33 ± 0.01 ^d

*CNT control, PLA phospholipase A₂

Results were presented as means ± standard deviations of triplicate measurements (n = 3)

^{a-d}Means in the same column with different lowercase letters are significantly different ($P < 0.05$)

Means with the same letters are not significantly different at ($P < 0.05$)

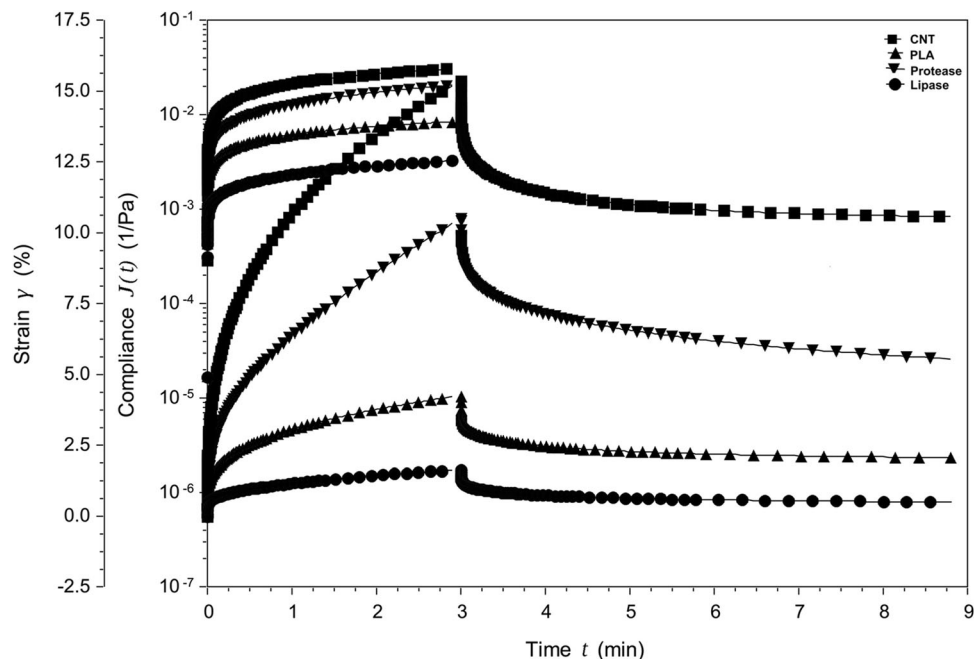
protein system (Raikos et al. 2007). Meringue is a forming of egg white protein foam (Murray 2020). Larger air bubbles in the egg protein system results in a decrease of the BD and increase in aeration of yield of foaming volume (Xing et al. 2015). The stability of meringue also shows the meringue's ability to volume and mass (Goldfarb 2016). The lipase treated samples showed higher overrun comparing other groups. According to the result of BD, it is possible to restore the physico-functional properties of meringue with the treatment of lipase enzymes and modification of the foam structure. The control samples showed runny and flat batter structure with high density foams comparing to enzyme treated samples. Enzyme treated batters resulted a light and crisp meringue cookie (Yüceer 2020a; Yüceer and Asik 2020). This result suggested that lipase could be used in LEW to decrease the typical BD, to lighten (low density foams) the batter and increase physical stability.

Rheological behavior

Foam is a dispersion of a gas phase as bubbles within continuous media in liquid or solid state (Nicorescu et al. 2011). A typical creep-recovery curve of meringue's batter samples (whipped LEW/sugar mixture) prepared from different enzymes (PLA, lipase and protease) treated and non-treated liquid egg white presented in Fig. 1. The effect of enzymes on creep-recovery characteristics of meringue batter samples were best fitted to Burgers model. According to the results, it was found that lipase enzyme treated meringue batter samples exhibited the lowest creep compliance values among the enzyme treatments. The lipase treated samples exhibited an improvement in characteristics of foam aeration. All batter samples exhibited

viscoelastic behavior. However, the incorporation of various enzymes significantly influenced creep-recovery behavior of meringue batters. Lipase treated meringue batter samples showed greater resistance to deformation with smaller compliance compared to PLA, protease and control samples, where PLA batter samples showed the lowest resistance to deformation among the treated samples. Curves from analysing data, plotted in terms of compliance vs. time, overlapped for stresses < 150 (Pa), indicating the linear viscoelastic region. The instantaneous compliance value was highest in the control sample with low stability and adoption to collapse. Protease treated samples showed the highest recovery value compared to lipase and PLA samples. The R² values of the Burgers model for the freshly prepared meringue batter using enzyme treated-LEW samples was > 0.99. The Burger model coefficient is also the common model used in soft materials such as batters and deformation structure (Das and Ghosh 2006). This indicated the used model was satisfactory describing the stress relaxation characteristic of meringue batter samples. Also this mention the stability of meringue's draining over time at room temperature (Goldfarb 2016). It can be suggested that lipase treated samples showed lowest creep-recovery behavior which resulted in a higher technological and production yield in cookies technological properties during cooking and storage. Lipase treated meringue batter samples exhibited a rheopectic behavior and showed an improvement in rheological behavior with a stronger structure and smaller creep strain than soft batter-foam samples (control). The stability is increased by reducing the bubble size in the foam structure (Goldfarb 2016). The influence of the lipase of the LEW on creep behavior observed a decrease in recoverable compliance and meringue batter strength, which is related to a smaller creep strain Culetu, Stoica-Guzun, and Duta (2020). Highest compliance values were observed in non-treated samples, which is like an ideal to solid-like formation. Similar results found by Alavi et al. (2020) in the treatment of egg white with transglutaminase enzyme application. Also, the application of minimal constant shear stress (5 Pa) and removed to zero (0 Pa), resulted in a rearrangement of the hydrogen bonds during coagulation of protein aggregates in whipping (Chang et al. 1999). The lipase provided a meringue batter with superior whipability and stability by making the foam less adoption to collapse during processing (Fig. 1). The protease treated samples showed lower compliance than control samples with a low weakening may be caused by strengthened gel network of protease (Quan and Benjakul 2019). The study results are found to be in good agreement with previous studies conducted o creep-recovery compliance on batter-dough structure (Alavi et al. 2020; Culetu et al. 2020; Mizu

Fig. 1 Typical creep-recovery tests of meringue's batters prepared from different enzyme treated and non-treated liquid egg white. *CNT control, PLA phospholipase A₂



and Nagao 2010; O'Charoen et al. 2014; Stevenson et al. 2007; Yüceer 2020a; Yüceer and Asik 2020).

Gelling properties

Liquid egg white as gelling agents using in many bakery products, such as angel cake, cookies, meringues, confectionery etc. The textural characteristics of LEW gels are shown in Fig. 2. The gelling index values of LEW gels prepared from PLA, lipase and protease enzyme treated and untreated LEW are presented in Table 4. After heat treatment of LEW the samples turned into an elastic gel. Also a solidification occurred in LEW proteins due to

Table 4 The gelling index values of meringue's batter prepared from phospholipase A₂ (PLA), lipase and protease enzyme treated and untreated liquid egg white

Treatments	Gelling index
CNT*	41.2 ± 1.19 ^a
PLA	34.3 ± 1.32 ^b
Protease	25.1 ± 1.29 ^c
Lipase	23.3 ± 1.51 ^d

*CNT control, PLA phospholipase A₂

Results were presented as means ± standard deviations of triplicate measurements (n = 3)

^{a-d}Means in the same column with different lowercase letters are significantly different ($P < 0.05$)

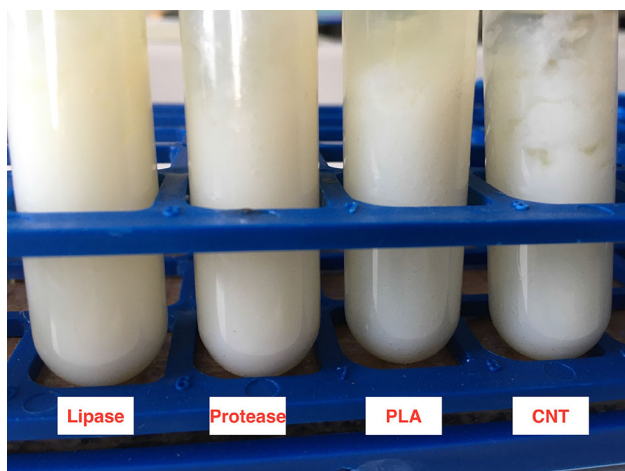


Fig. 2 Visual image of meringue's batters prepared from different enzyme treated and non-treated liquid egg white. *CNT control, PLA phospholipase A₂

thermal denaturation (Table 4). The hardness value represented to characterising the gelling index which is relate to the the maximum force required to compress the LEW-gel sample during the first compression. The gelling index of control (41.2 ± 1.19) was decreased significantly after treatments of PLA (34.3 ± 1.32), protease (25.1 ± 1.29) and lipase (23.3 ± 1.51). The enzymatic treatments effected the mechanical and textural properties of LEW-gel structure which is related to changing the surface hydrophobicity of albumen proteins (Wang 2009). The gelling index and BD values significantly decreased after enzymatic treatment of LEW and the dramatic decrease observed in lipase enzyme.

Conclusion

This study presents the results of the application and effectiveness of three enzymes (PLA, lipase and protease) treatment on meringue batter samples. Functional, textural and physicochemical challenges were analyzed in the study. The batter density of meringue was decreased while whippability increased in all-enzyme treatments which is essential for higher volume with a crispy structure of meringue cookies. The lowest BD value obtained in lipase treated while high BD values obtained in control samples. Protease enzyme has potential to become significant breakthrough in the egg industry to preserving egg proteins deformation. The presented study highlights that, determining the effect of hydrolysis by PLA (foaming agent), lipase (stabilizing agent), protease (texture profiler) enzyme-treated LEW. Also, the results of study provide practical information to the baking industry for optimal processing conditions of meringue-based foamed applications, variety of cake decoration, mousses, pastry, wafers, dessert, masking cakes and aerated confectionery products. This study concluded that, according to overall acceptability in physico-functional properties and whipping quality, lipase treated samples is suitable for improving the stability, batter yield, functionality and rheological behavior of meringue batter with a unique structure. The enzyme treatments of LEW provide as a novel processing approach for culinary concept innovations especially in meringue production. Further studies should be conducted on inhibitory activity of bioactive substances of LEW such as ovomucoid.

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Authors contribution MY conceived the designed study and project admin, MY and CC organized the whole research outlined. Manuscript drafting wrote by MY and received the final reviewed and edited manuscript with CC.

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Code availability Not applicable.

Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical approval Ethics approval was not required for this research. This study does not involve any human or animal testing.

Consent to participate Not applicable.

Consent for publication The Authors transfers to Springer the non-exclusive publication rights and they warrant that their contributions are original and that he has full power to make this grant.

Availability of data and material Data available on request from the author(s). The datasets generated during the experiment that support the findings of this study are available from the corresponding author upon reasonable request.

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