Food Sci. Biotechnol. Vol. 17, No. 6, pp. 1289 ~ 1293 (2008)

Food Science and Biotechnology

Functional Properties of Enzymatically Modified Egg Yolk Powder Produced by Phospholipase A_2 Treatment

Mi-Ra Kim, Jae-Yong Shim [1], Ki-Hwan Park[2], and Jee-Young Imm*

Department of Foods and Nutrition, Kookmin University, Seoul 136-702, Korea

[1]Department of Food and Biotechnology and Food and Bio-Industrial Research Center, Hankyong National University, Anseong, Gyeonggi 456-749, Korea

[2]Department of Food Science and Technology, Chung-Ang University, Anseong, Gyeonggi 456-756, Korea

Abstract Fresh egg yolk (EY) was enzymatically modified using phospholipase A_2 (PLA_2) to produce an enzymatically modified-egg yolk powder (EM-EYP). The EM-EYP offered significantly higher emulsifying activity, emulsion stability, protein solubility, and mayonnaise stability than the control EYP. By employing PLA_2 in the enzymatic modification process, structural changes occurred in the phospholipids and lipoproteins of the yolk, and cleavage of apo-high density lipoprotein (HDL) components (Mw 105 kDa) was detected by sodium dodecyl sulfate-polyaerylamide gel electrophoresis (SDS-PAGE). Based on its functional properties, EM-EYP has great potential as a replacement for fresh EY in the production of processed food products such as mayonnaise.

Keywords: egg yolk powder, enzymatic modification, phospholipase A_2, functional property

Introduction

Hen's egg yolk (EY) is widely used in various food emulsion products due to its flavor, color, and excellent emulsifying properties. Both the composition and structure of EY are considered complicated. Fresh EY contains approximately 48% dry matter; of which, 32% is protein and 64% is lipid. Within the plasma there are dispersed granules (20%, w/w). These granules are composed of 70% high density lipoproteins (HDL), 16% phosvitin, a phosphoglycoprotein, and 12% low density lipoproteins (LDL). The plasma is composed of 85% LDL and 15% livetin, a globular protein. In terms of lipid composition, it is composed of 62% triacylglycerides, 33% phospholipids, and 5% cholesterol. Structurally, it is noncovalently bound to proteins and forms large lipoprotein complexes (1,2). Thus, functionality of EY is dependent upon the type of supermolecular structure such as micelles and granules and structural alterations caused by pasteurization, freezing, and lipid extraction critically affect functionality (3).

EY has only a limited shelf-life. Therefore, it is frequently used either in frozen or powdered forms. For the preparation of frozen EY about 10% salt usually added to prevent gelation since irreversible gelation of EY greatly reduces its usefulness (4). The utilization of egg powder in products has steadily increased, particularly in advanced countries. The increasing popularity of dried egg products is probably due to their convenience, extended shelf-life, and product uniformity (5). Although spray-drying is essential in the production of egg yolk powder, it also decreases the emulsifying capacity (6).

*Corresponding author: Tel: +82-2-910-4772. Fax: +82-2-910-5249
E-mail: jyimm@kookmin.ac.kr
Received June 1, 2008; Revised July 10, 2008;
Accepted July 15, 2008

LDL has been reported as a major contributor to the excellent emulsifying properties of EY (7,8). Although the exact role of phospholipid in EY emulsion formation is not fully understood, phospholipid modification probably affects EY functionality by changing the structure of the lipid fraction that is specifically located within the lipid-water interface. Phospholipase A_2 (PLA_2) is an enzyme that hydrolyzes the ester bond at the sn-2 position of phospholipids. Phospholipid hydrolysis can improve the functionality of EY since fatty acid removal results in increased hydrophilic balance. Mine (9) reported that the emulsifying properties and heat stability of EY emulsions were substantially improved through the formation of a complex between phospholipase-hydrolyzed lecithin (lysolecithin), free fatty acids, and proteins.

Because processing reduces the functionality of EY, this work examined the effectiveness of PLA_2-induced EY modification for the production of a more functional EY powder. Previously, the authors examined the modification of EY with PLA_2 in mayonnaise production, and suggested the optimum conditions for modification (10). In this study, enzymatically modified-EY powder (EM-EYP) was prepared with the predetermined optimized conditions and its functionality was evaluated.

Materials and Methods

Materials Fresh eggs were kindly provided by Join Poultry Farm (Yongin, Korea). The EYs were carefully separated from the albumen, and the yolk membranes were punctured and filtered through 4 layers of cheese cloth (Daehan Wejae, Chungjoo, Korea). The EYs were gently stirred to obtain a homogeneous mixture. Lecitase 10 L (PLA_2), was purchased from Novo Nordisk (Bagsvaerd, Denmark). The ingredients used for mayonnaise preparation, including sugar, vinegar, salt, and soybean oil, were

purchased from a local supermarket (Seoul, Korea). All other chemicals were of analytical grade and obtained from Sigma-Aldrich (St. Louis, MO, USA).

Production of EM-EYP The fresh EY was diluted with deionized water at a ratio of 7:3 (w/w) to facilitate the enzyme reaction. The enzymatic modification of the EY using lecitase (PLA_2) was carried out in a 55°C water bath (Vision Scientific, Bucheon, Korea). Immediately after completing the enzymatic reaction at the predetermined optimized conditions (enzyme concentration of 7362 LEU, reaction time of 73.1 min, and 11.4% salt), the EM-EYP was produced using a spray dryer (Samjin Engineering, Seoul, Korea) with an inlet chamber temperatures of 140°C and an outlet air temperature of 85°C. The control EYP was produced using the same procedure, but without the enzymatic modification step.

Emulsifying activity and emulsion stability of EM-EYP The EM-EYP (0.5%, w/v) was dissolved in sodium phosphate buffer (100 mM, pH 7.0, 7 mL) containing 100 mM NaCl. Corn oil (Sigma-Aldrich, 1.4 mL, oil volume fraction= 0.167) was added to the sample and an oil in water emulsion was prepared using a PH 91 homogenizer (Eyela, Tokyo, Japan) by homogenizing for 1 min at a speed of 18,000 rpm. The emulsifying activity of the sample was determined by the turbidimetric method of Pearce and Kinsella (11). Immediately after the emulsion was formed, an aliquot (100 μL) was taken and diluted 1:500 with double distilled water containing 0.1% of sodium dodecyl sulfate (SDS). The absorbance of the diluted emulsion was measured at 500 nm against a blank. The emulsion stability of samples was estimated by the time-dependent changes in absorbance readings. The times (min) required to reach 3/4 and 1/2 of the initial absorbance were taken as indices of the emulsion stability.

Protein solubility of EM-EYP The protein solubility was determined using the modified method of Guilmineau and Kulozik (12). Samples (1%, w/v) were suspended at pH 4.0 and 6.5 in the presence of 2 different salt concentrations, 0.15 and 0.55 M NaCl, respectively. These conditions were selected based on the pH and ionic strength commonly found in food emulsions. The samples were placed under mild agitation at 20°C for 1 hr and then aliquots of the samples were saved to determine total protein contents. After centrifugation at 19,000×g for 20 min followed by filtration (Whatman No. 5, Whatman International Ltd., Maidstone, England), the supernatant was collected. Protein contents before and after centrifugation was determined by the modified Lowry assay of Markwell *et al.* (13). The solubility was calculated as follows:

$$\text{Solubility (\%)} = Ps/Pt \times 100$$

Ps: protein contents in the supernatant, Pt: total protein contents.

SDS-polyacrylamide gel eletrophoresis (PAGE) SDS-PAGE was carried out on 11% separating and 4% stacking gels, using a mini-protein II cell (Bio-Rad Laboratories, Hercules, CA, USA) (14). The gels were stained with 0.1%(w/v) Coomassie brilliant blue R 250 in 45%(v/v) methanol containing 10% glacial acetic acid. The gels were destained with 10% glacial acetic acid.

Table 1. Formula used for the preparation of mayonnaise

Ingredient	Concentration of egg yolk (%)		
	6	7	8
Egg yolk	6	7	8
Soybean oil	78	78	78
Vinegar	3	3	3
Salt	1.5	1.5	1.5
Sugar	1	1	1
Water	10.5	9.5	8.5
Total	100	100	100

Preparation of mayonnaise and stability test The mayonnaise was prepared using the recipe shown in Table 1. First, the water phase was mixed, where all of the ingredients except for the oil were added. The oil was incorporated at a constant rate by the aid of a peristaltic pump (Vision Scientific) under constant stirring by a mechanical overhead stirrer. The stirring speed of the homogenizer (Ika, Staufen, Germany) was steadily increased for 4 min from 500 to 10,000 rpm. The stability of the mayonnaise was determined after heating at 95°C for 30 min. The heated products were centrifuged for 30 min at 15,000×g and 4°C, and the separated oil was carefully collected using a Pasteur pipet. The stability of the mayonnaise was calculated as follows:

Mayonnaise stability (%)
=weight of mayonnaise after oil separation (g)/
initial weight of mayonnaise (g)×100

Statistical analysis All analytical measurements were done in triplicate, and the data were analyzed by analysis of variance (ANOVA) using Minitab Ver. 13.1 (Minitab Inc., University Park, PA, USA) software. Tukey's test was used for multiple comparisons of the treatment means at a significance level of $p<0.05$.

Results and Discussion

Emulsifying activity and emulsion stability of EM-EYP The EM-EYP was prepared using predetermined optimized conditions (10), and its emulsifying activity was compared to fresh EY and control EYP. In the methodology for determining emulsifying activity, higher turbidity means greater emulsifying properties due to the linear relationship between the turbidity (absorbance at 500 nm) and interfacial area of the emulsion (11). As shown in Fig. 1, spray-drying of the EY resulted in decreased emulsifying activity, which is consistent with previous reports (15). However, emulsifying activity was significantly improved when the EY was modified by PLA_2 (EM-EY), and the powdered form, EM-EYP, had emulsifying activity that was comparable to fresh EY. This result clearly suggests that enzymatic modification improved the emulsifying properties of the EY.

The emulsifying activity of the EM-EY may have been

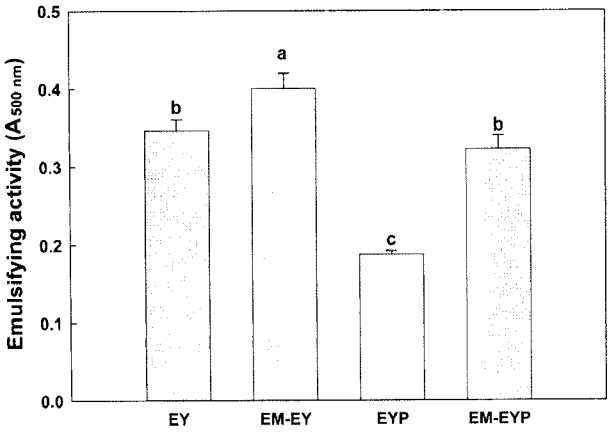

Fig. 1. Changes in emulsifying activity of emulsions prepared from fresh egg yolk, enzymatically modified-egg yolk, and their powders. EY, Fresh egg yolk; EM-EY, enzymatically modified-egg yolk; EYP, egg yolk powder; EM-EYP, enzymatically modified-egg yolk powder. [a-c]Means with different letters are significantly different at $p<0.05$.

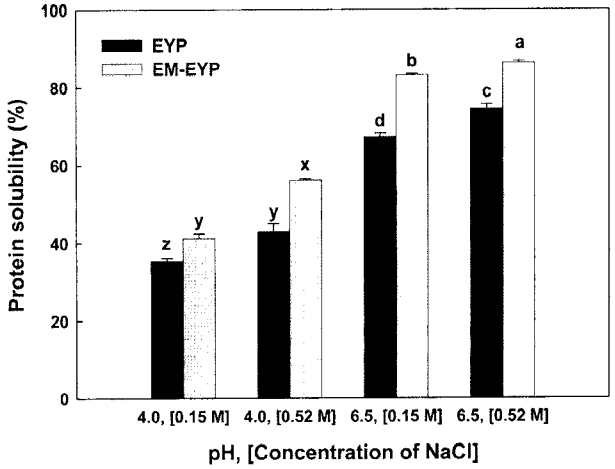

Fig. 2. Protein solubility of egg yolk powders. EYP, Egg yolk powder; EM-EYP, enzymatically modified-egg yolk powder. [a-d]Means with different letters at pH 6.5 are significantly different at the level of $p<0.05$. [x-z]Means with different letters at pH 4.0 are significantly different at the level of $p<0.05$.

enhanced by various means. For example, PLA_2 would cause the liberation of fatty acids from phospholipids, increasing polar characteristics as well as lowering the affinity for protein components. Also, with lysophospholipid generation, PLA_2 may have altered the structure of LDL. These changes could potentially increase the molecular flexibility of LDL apoproteins, allowing for efficient absorption at the oil/water interface. Similarly, heat-induced dissociation of the LDL structure can result in increased availability of the LDL constituents for the oil/water interface (16).

It was reported that not all phospholipids are able to adsorb to the oil/water interface due to competition from other components such as yolk proteins. The phospholipids can reduce interfacial tension to a greater extent than the EY, which contains phospholipids bound in the lipoprotein structure (17). Therefore, it is probable that the modification of EY by PLA_2 effectively enhanced the emulsifying properties of EY.

In stable emulsions, the interfacial area does not change with time, and such emulsions have a constant turbidity. In this study, emulsion stability was estimated by measuring the times required to reach 3/4 and 1/2 of the initial absorbance readings; thus, higher values indicate a more stable emulsion. As Table 2 shows, the emulsion stabilities of the fresh EY and EM-EY were not significantly different. Spray drying, however, reduced the emulsion stability of both the EY and EM-EY, and this decrease in emulsion stability was much more pronounced for EYP than EM-EYP.

Coalescence and flocculation irreversibly reduce the interfacial area and decrease the absorbance of samples. Mine (9) reported that the stabilization of emulsions against coalescence/flocculation depends greatly on the electrostatic repulsions between adsorbed proteins on the interfacial protein film. During the adsorption process by homogenization, LDL can be disrupted, and subsequently, apoproteins and phospholipids are liberated (18). Mine (19) verified this mechanism by showing that phosphatidyl choline/phosphatidyl ethanolamine ratios in adsorbed LDL were different from those in the original LDL. Recently, Nikiforidis and Kisseoglou (20) demonstrated that hydrophilic surfactant molecules such as Tween effectively shielded the hydrophobic patches of both adsorbed and unadsorbed yolk lipoproteins, and also prevented droplet aggregation and coalescence in the emulsion. The hydrophilic lysophospholipids that are liberated as a result of phospholipid hydrolysis may play a similar role, improving the stability of emulsions.

Protein solubility of EM-EYP The solubility of the EY protein was determined at pH levels and ionic strengths commonly found in food emulsions. Regardless of the pH or ionic strength, the EM-EYP showed significantly higher solubility than its EYP counterpart (Fig. 2). And the protein solubilities at pH 6 were greater than those at pH 3. According to a report by Le Denmat *et al.* (8), proteins in plasma were completely soluble at pH 3 and 7, even with

Table 2. Stability of emulsions prepared from fresh egg yolk, enzymatically modified-egg yolk, and their powders

Sample	$T_{0.75}$[1)] (min)	$T_{0.5}$[2)] (min)
Fresh egg yolk (EY)	22.3±1.23[a]	>60
Enzymatically modified-egg yolk (EM-EY)	26.8±3.81[a]	>60
Egg yolk powder (EYP)	7.60±0.38[b]	19.0±4.48
Enzymatically modified-egg yolk powder (EM-EYP)	15.68±1.77[c]	>60

[1)]Time to reach 3/4 of initial Abs; [a-c]means with different superscripts in the same column are significantly different at $p<0.05$.
[2)]Time to reach 1/2 of initial Abs.

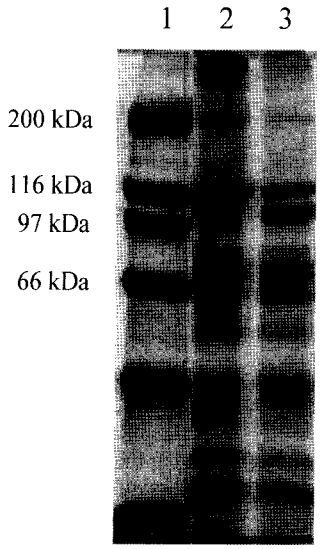

Fig. 3. SDS-PAGE profiles of egg yolk powders. Lane 1, molecular weight standard; lane 2, egg yolk powder; lane 3, enzymatically modified-egg yolk powder.

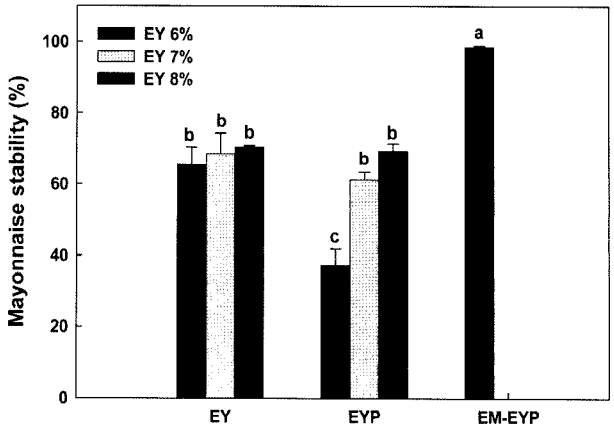

Fig. 4. Emulsion stability of mayonnaise prepared from fresh egg yolk (EY), egg yolk powder (EYP), and enzymatically modified-egg yolk powder (EM-EYP). [a-c]Means with different letters are significantly different at $p<0.05$.

0.17 M NaCl. On the other hand, granules were insoluble at pH 3, and solubility increased at pH 7 with a high salt condition (0.55 M). Anton and Gandemer (21) reported that a high sodium concentration induced the disruption of phosphocalcic bridges between HDL and phosvitin, allowing for the dissociation of granules. This made the proteins contained in HDL more soluble. The solubility of whole egg yolk protein would be between those found in plasma and granules. The improved solubility of the EM-EYP at both pH levels and salt concentrations could reflect the disorganization of the granule molecular structure.

SDS-PAGE of EM-EYP The SDS-PAGE profiles of the EYP and EM-EYP proteins were compared. As shown in Fig. 3, enzymatic modification resulted in the cleavage of an approximate 110 kDa band into low molecular weight bands. Based on a previous report (8), the band was postulated to be an apo-HDL component (Mw 105 kDa). Abousalham and Verger (22) reported that EY lipoproteins can be substrates for lipase. This data suggests that PLA_2 causes HDL apoprotein hydrolysis. Native granules tend to be less efficient at decreasing interfacial tension because large particles can not spread over the interface like individual proteins can (23). In the case of a disrupted state, the granule components could provide wider interface area coverage.

Stability of mayonnaise prepared from EM-EYP Different mayonnaise samples were prepared using the fresh EY, EYP, and EM-EYP, respectively, and then their emulsion stabilities were evaluated. The mayonnaise prepared from EM-EYP (6% EY, w/w) had the highest emulsion stability, which was even greater than that of the mayonnaise prepared using 8% fresh EY (Fig. 4). The emulsion stability of the mayonnaise prepared from EYP significantly increased as the amount of EY in the formulation increased ($p<0.05$). In the mayonnaise prepared from fresh EY, emulsion stability ranged from 65 to 70% in samples, but there were no significant differences between them.

The hydrophilic lysophospholipids that were liberated as a result of phospholipid hydrolysis would play an important role in the stability of the mayonnaise emulsions. In addition, the structural alterations caused by PLA_2 would have increased the availability of EY proteins in the oil/water interface. Micelle-forming lysophospholipids are probably able to form restructured protein-surfactant complexes (24). These complexes would improve emulsion stability because more rigid and structured layers tend to inhibit coalescence.

The data from this study suggest that EM-EYP has improved functional properties, as well as great potential as a replacement for fresh EY in the production of processed food products such as mayonnaise. Considering that dried products provide advantages over their liquid forms in terms of convenience, safety, and product uniformity, the usefulness of EM-EYP will steadily increase in the future.

Acknowledgments

This work was supported by a grant (203018) from the Agricultural Research Promotion Center, Ministry of Agriculture and Forestry, Korea, and from the Join Co., Ltd. This work was also supported by a grant from Kookmin University in 2006. The authors are thankful for the financial support.

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