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Evaluation of functional properties of egg white obtained from pasteurized shell egg as ingredient in angel food cake

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Abstract: Pasteurized shell eggs are eggs that have been thermally treated to eliminate harmful bacteria, however the treatment may also denature some of the egg white proteins. In this study the degree of denaturation and functional properties (emulsifying, foaming, and gelling properties) of egg white obtained from pasteurized eggs (EWP) were compared with those of unpasteurized eggs (EWUP). Data from differential scanning calorimeter showed that the EWP (ovotransferin, lysozyme, and ovalbumin) denatured at lower temperatures and required lower denaturation enthalpies than EWUP, indicating a partial loss of protein structure during the pasteurization process in the pasteurized eggs. The emulsion and foam stability formed from EWP were significantly ($P < 0.05$) lower than those of EWUP, however the EWP formed stronger gels than EWUP. To assess suitability of EWP as a cake ingredient, angel food cake was prepared using both egg whites. As compared to EWUP-cake, EWP-cake was significantly ($P < 0.05$) lower in volume, cohesiveness and springiness values, but significantly ($P < 0.05$) higher in hardness, gumminess and chewiness. Overall, the sensory panelists gave significantly ($P < 0.05$) higher scores for angel food cake prepared with EWUP. The differences in functional properties of egg white proteins and the quality of cake were due mainly to the higher levels of denaturation attained by EWP as a result of the pasteurization process.

Keywords: Pasteurized shell egg, protein functionality, denaturation, angel food cake

Introduction

Egg is a highly nutritious food as it contains high quality proteins and a large variety of vitamins and minerals. The egg proteins have just the right mixture of essential amino acids needed by humans to build a strong body. The egg white, also known as the albumen, comprises approximately 58 % of the weight of an egg. The egg white consists of concentric layers; two thick whites separated by inner and outer thin whites. The chalazae are located within these layers of the albumen and are continuous with the vitelline membrane that surrounds the yolk. Over half of the protein in whites is ovalbumin, although conalbumin, ovomucid, and globulins (including lysozyme, which is able to lyse some bacteria) contribute lesser percentages of protein in the egg whites (Goetz and Koehler, 2005). Whites provide more protein than the yolk and are often cooked and eaten alone or incorporated into a recipe. The addition of egg whites in place of an entire egg adds protein while limiting fat and cholesterol (Vaclavik and Charistian, 2008).

Pasteurized eggs are fresh shell eggs that are heated at a temperature sufficient to kill and reduce the bacteria and viruses that might be present in both inside and outside of the eggs, but without cooking the eggs. Treating the eggs with US standard pasteurization of 60 °C for 3.5 min results in 5 - 9 log reduction in viable *Salmonella enteritidis* and *Salmonella typhimurium*, the most frequent *Salmonella* serotypes found inside shell eggs that causes food poisoning (Whiting and Buchanan, 1997). The use of pasteurized eggs as a substitute for unpasteurized eggs is recommended and beneficial in several products that require the use of raw egg such as cold soufflés, ice cream, eggnog, Caesar salad dressing and mayonnaise (Thorne, 1991). Another application is for high-risk age groups with low immunity, such as children and elderly people. Thus, this substitution can help in minimizing the possibility of food poisoning by reducing the chances of consuming contaminated eggs.

The heat treatment during pasteurization could cause quality changes to the egg components. As some of proteins in the egg white undergo denaturation, the functional properties of the protein (coagulation, foaming, and emulsifying) are known to be affected (Hou *et al*., 1996). These adverse changes could lead to a stage where the pasteurized eggs are no longer suitable for use as ingredient in certain food products such as cake. Even though the pasteurized eggs are meant for specific applications, some consumers may still treat them as conventional eggs and use them as an ingredient for making domestic products such as cake. There is no report on the assessment of functional properties of egg white obtained from commercial pasteurised shell eggs and their uses for

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making cake.

The objectives of this study were to compare functional properties of normal egg white versus the egg white obtained from pasteurized shell egg and to compare physical and sensory qualities of angel cake made from these two types of egg. Angel food cake is an excellent system for the study of protein functionality because the final structure is dependent on thermal expansion of a protein foam and formation of protein and starch networks (Song *et al*., 2009).

Materials and Methods

Materials

Three batches of each pasteurized (Safegg™, Safe Food Corporation, Malaysia) and unpasteurized shell eggs (NutriPlus™, Lay Hong Berhad, Malaysia) were purchased from Jusco supermarket, Penang, Malaysia. The average weight of the egg was ~ 50 g per piece. Eggs were broken manually and the albumen was separated from the egg yolk. The cake ingredients including cake flour, sugar, salt, cream of tartar, and vanilla extract were purchased from the local market. All chemicals (analytical grade) used for egg white and angel cake analysis were obtained from Sigma-Aldrich and Fluka.

Thermal denaturation

Egg white was separated from the egg yolk and the chalazae were removed. The protein content of the egg white was determined using Kjeldahl method (AOAC, 1990). Egg white solutions (75% v/v) were prepared in distilled water. Solutions were stirred with magnetic stirrer for 2 min to obtain homogenous solutions prior to differential scanning calorimeter (DSC) analysis. Thermal denaturation of egg white samples was analysed using TA Instruments DSC Q200 (Newcastle DE, USA). Calibration was done with indium prior to analysis. Small amount (20 µL) of egg white solutions were hermetically sealed in hermetic aluminium pans. During analysis, samples were scanned at a heating rate of 10°C/min from 40 to 100°C. A sealed empty pan was used as the reference and for baseline corrections. The denaturation enthalpy (J/g) was estimated as the area under the heat flow line using TA Instruments Universal Analysis 2000 software. All samples were analysed in triplicate.

Foaming Properties

Foaming properties were determined by using the method described by Song *et al*. (2009) with some modifications. Egg white from pasteurized eggs (EWP) or egg white from unpasteurized eggs (EWUP) was diluted to 1:1 (v/v) with distilled water. A volume of 30 mL solution was placed into a 100 mL cylinder and whipped for 1 min with a homogenizer (IKA, Ultra-Turrax T25 digital, Germany) at 12,000 rpm at 25°C. Foam stability was expressed as percent liquid drainage in relation to initial liquid volume as a function of standing time for 30 min. Foaming stability was calculated using the following equation:

$$\%\ \text{Volume} = \frac{\text{Volume of prepared foam - Volume of liquid drainage)}}{\text{Original volume of the liquid}} \times 100\% \quad (1)$$

Emulsification properties

The emulsification properties of the egg white were determined by the method described by Pearce and Kinsella (1978). The emulsion was prepared by transferring 1.0 mL of palm oil into 3.0 mL of 0.1% w/v egg white solution in 100 mM of sodium phosphate buffer at pH 7.4. The solution was homogenized in an Ultra-Turrax T25 digital at 12,000 rpm for 1 min at 25°C. Aliquots of the emulsion (100 µL) were taken from the bottom of the test tube at 0, 1, 2, 3, 5, 10, and 20 min. Then it was serially diluted with the 5 mL of 0.1% sodium dodecyl sulfate solution. The absorbance of the diluted emulsion was measured at 500 nm for determining emulsifying properties.

Gelling properties

EWP and EWUP samples (50 g) were poured into a 50 mL beaker, that were covered with aluminum foil. Samples were placed into a preheated water bath at 85°C for 30 min. Samples were then removed from the water bath and cooled to 20°C. Gel samples were removed from the beakers and cut into 2.5 cm^3. The gel cubes were subjected to a double-compression test using TA-XT Plus Texture Analyzer (Stable Micro Systems, Surrey, England) with a 5 kg load cell and a cylindrical 25 mm diameter probe. Measurements were conducted with a pre-test speed of 1.0 mm/s, a test speed of 5.0 mm/s, and a post-test speed of 5.0 mm/s. This test comprised the texture profile analysis (TPA) described by Woodward and Cotterill (1986). Hardness, springiness and cohesiveness were computed from the TPA curves generated for each gel sample.

Cake preparation

Angel food cake was prepared by using the ingredients and procedure described by Williams (2003). EWUP or EWP (33 g) was beaten at high speed until it started to foam (about 7 min) using a mixer (KPM5, KitchenAid, USA). A mixture of sugar (231 g) and cream of tartar (3.5 g) were gently added with continue beating at high speed. When the egg whites started to increase in volume and

became firm, the vanilla extract was added and mix was beaten until stiff peaks were formed (about 15 min). A dry-blended mixture containing 90 g of cake flour and 0.8 g of salt was gently incorporated into the whipped batter. The batter was then transferred into an ungreased pan (20 cm x 5 cm) and baked in an electric oven (Moffat, Bakbar Turbofan E32, Australia) for 40 min at 160°C.

Volume measurement

Cake volume was measured by using the procedure described by Akesowan (2007). The test cake was weighed and placed inside a box of known volume, followed by the addition of rapeseeds, which were leveled across the top with a spatula. The displacement of the seeds was measured in a graduated cylinder and used to express the volume of the cakes.

Texture profile analysis

The texture of the cakes was determined according to the procedure described by Morr *et al*. (2003) using TA-XT Plus Texture Analyzer equipped with a 25 mm diameter cylindrical probe and 5 kg load cell. A 20 mm thick cross section of the whole bottom of the cakes was sliced to be used for the analysis. The centre of the cross section was compressed perpendicularly to its freshly cut surface to a depth of 8 mm. Measurements were conducted with TPA mode at a rate of 1 mm/s and the 2 cycle program with 5 s delay between cycles. Texture attributes (hardness, cohesiveness, gumminess, springiness and chewiness) were computed from the TPA curves generated for each cake sample.

Sensory evaluation

Hedonic sensory evaluations were conducted according to Gómeza *et al*. (2007) by 30 untrained panelists consisting of Food Technology Division staff and students. The panelists were asked to evaluate the hedonicity of appearance, odour, flavour, texture, and overall acceptability of the cakes on a 9-point hedonic scale test (from 1 (most disliked) to 9 (most liked)).

Statistical analysis

Data analysis was performed using SPSS version 11.5 (SPSS Inc., Chicago, USA). Paired-samples T-Test was used to evaluate the significant differences between sample means, with significance level at $\alpha = 0.05$. All data presented are mean values of triplicates, unless otherwise stated.

Results and Discussion

Heat treatment on shell egg can lead to major protein denaturation. The degree of denaturation depends on the severity of the treatment and this can be observed on the thermograms obtained from the differential scanning where the denaturation enthalpy is paralleled with the remaining content of ordered secondary structure of a protein (Ma and Harwalker, 1991). Typical thermograms obtained by differential scanning of the egg white solutions are shown in Figure 1. The left-hand peak corresponds to the denaturation of ovotransferin and lysozyme, while the right-hand peak corresponds to that of ovalbumin. The thermograms obtained in this study were similar to those reported by Donovan *et al*. (1975) and Van der Plancken *et al*. (2006). It is evident that EWP (ovotransferin, lysozyme, and ovalbumin) denatured at lower temperatures and required lower denaturation enthalpy than EWUP. The lower denaturation enthalpy in EWP is an indication of a partial loss of protein structure as a result of pasteurization. These results were in agreement with Van der Plancken *et al*. (2006), who reported the decrease on the denaturation enthalpy on egg white underwent heat treatment, even at heating temperature as low as 50°C. Thus, the egg white proteins from the two types of egg were in fact different and it is envisaged that their functional properties to be different as well. The DSC data obtained from this study also reveals the possibility of using the denaturation property of egg white proteins as a method to authenticate the pasteurized egg, and quantitatively confirm the extent of heat treatment applied. This idea needs further testing.

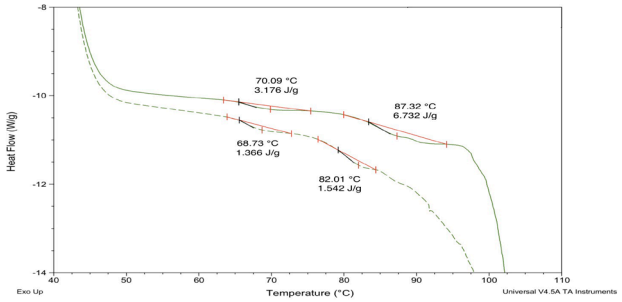

Figure 1. Denaturation temperature peak (°C) and denaturation enthalpy (J/g) of EWUP (solid line and EWP (dotted line).

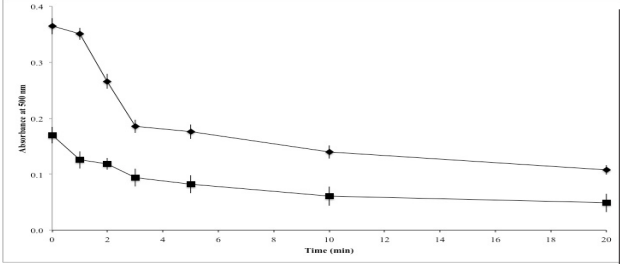

Figure 2. Emulsifying properties of EWUP (◆) and EWP (■).

Changes in structural properties of egg white protein may lead to changes in foaming, emulsifying and gelling abilities (Mine, 1996; Campbell *et al*., 2005; Song *et al*., 2009). These functional properties play an important role in the manufacture of bakery products. The foaming ability is related to the rate at which the surface tension of the air/water interface decreases. The foaming stability data of both egg white is shown in Table 1. EWP has significantly ($P < 0.05$) lower foaming stability than EWUP, and this is likely to be due to the pasteurisation process. The inferior foaming stability of EWP implies that it may not be suitable for use in bakery products as the amount of foam and its stability influence the volume and appearance of cake.

Table 1. Foaming stability of EWUP and EWP.

Sample	Foaming stability (%)
EWUP	18.23 ± 0.12^{a}
EWP	16.69 ± 0.07^{b}

Data are expressed as mean ± standard deviation, n = 3. Means within the same column with different superscripts (a-b) are significantly ($P < 0.05$) different.

To investigate emulsifying abilitty, both EWP and EWUP were mixed with palm oil. Then emulsion was taken and mixed with 0.1% SDS for 0, 1, 2, 3, 5, 10 and 20 min. The dilution of emulsions with 0.1% SDS solution produced turbid dispersion. The concentration of the diluted egg white emulsion is proportional to the absorbance up to about 0.4 (Fig. 2) (Pearce and Kinsella, 1978). The turbidity of egg white emulsion decreased from 0 min to 20 min standing. At all standing times the turbidity of EWUP emulsion was higher than that of EWP, indicating a lower emulsifying ability of the EWP compared to EWUP. Low emulsifying ability of EWP could pose negative impact on the overall flavor of bakery products.

Gel formation is an important functional property of egg white protein. Texture profile analysis (Table 2) was carried out by measuring three primary textural parameters (hardness, springiness, and cohesiveness), which are related to the mechanical properties of the egg white gel. The springiness and cohesiveness values of both gels did not differ significantly ($P > 0.05$), however EWP had significantly higher hardness ($P < 0.05$) than EWUP. Gelation of globular protein involves process of denaturation and aggregation. As a portion of protein in EWP had already been denatured, more side chains on the molecules of protein were available for the protein-protein interactions responsible for enhancing hardness of the gels. Such an attribute may be undesirable in cake since hard gels are high in density and low in volume.

Table 2. Textural properties of egg white gels prepared from EWUP and EWP.

Sample	Hardness (N)	Springiness (mm)	Cohesiveness
EWUP	915.61 ± 1.35^{a}	0.966 ± 0.01^{a}	0.68 ± 0.03^{a}
EWP	1346.29 ± 3.11^{b}	0.956 ± 0.008^{a}	0.72 ± 0.04^{a}

Data are expressed as mean ± standard deviation, n = 3. Means within the same column with different superscripts (a-b) are significantly ($P < 0.05$) different.

Angel food cake was used to assess the suitability of EWP in a bakery product. A high-quality cake is produced when the dynamic events occurring in the system yield a high volume, low density cake with fine, relatively uniform crumb structure (Pernell *et al*., 2002). Several properties (such as cake volume, texture profile and sensory qualities) of angel cakes were determined for comparison and an angel food cake made with EWUP was used as control. The volume of EWUP-cake was found to be significantly ($P < 0.05$) higher than that of EWP-cake (Table 3). This is expected since EWP had inferior foaming stability as compared to EWUP (Table 1), thus affecting the volume of cake prepared. This correlation is consistent with the finding of Song *et al*. (2009), who reported the foaming ability of egg white protein was directly proportional to cake volume.

Table 3. Cake volume of angel cake made from EWUP and EWP.

Sample	Volume (mL)
EWUP	1468.23 ± 1.66^{a}
EWP	923.60 ± 2.42^{b}

Data are expressed as mean ± standard deviation, n = 3. Means within the same column with different superscripts (a-b) are significantly ($P < 0.05$) different.

The angel food cakes were subjected to texture analysis, and the mean values of texture profile parameters (hardness, cohesiveness, gumminess, springiness, and chewiness) were compared (Table 4). All texture parameters were significantly ($P < 0.05$) different between EWP-cake and EWUP-cake. EWP-cake was higher in hardness, gumminess and chewiness, but lower in cohesiveness and springiness. The TPA results are supported by the sensory evaluation data (Table 5). Compared to EWUP-cake, the EWP-cake was significantly ($P < 0.05$) lower in the sensory scores of appearance, odour, flavour, texture and overall acceptability. The lower score for texture for EWP-cake might be due to the lower preference for harder and less springy angel cake by the panelist. Thus, the EWUP-cake, with softer and springier texture, had better appreciation by the panelists than that of EWP-cake.

Table 4. Textural properties of angel cake made from EWUP and EWP

Sample	Hardness (N)	Cohesiveness	Gumminess (N)	Springiness (mm)	Chewiness (Nmm)
EWUP	225.07 ± 0.25[a]	0.82 ± 0.001[a]	183.77 ± 0.25[a]	1.08 ± 0.01[a]	184.10 ± 0.20[a]
EWP	376.37 ± 0.60[b]	0.77 ± 0.002[b]	296.30 ± 0.75[b]	0.93 ± 0.02[b]	273.93 ± 0.31[b]

Data are expressed as mean ± standard deviation, n = 3. Means within the same column with different superscripts (a-b) are significantly (P < 0.05) different.

Table 5. Sensory evaluation of angel cake made from EWUP and EWP

Sample	Appearance	Odour	Flavour	Texture	Overall Acceptability
EWUP	8.16 ± 0.17[a]	7.37 ± 0.09[a]	7.78 ± 0.07[a]	7.30 ± 0.04[a]	6.97 ± 0.15[a]
EWP	7.54 ± 0.12[b]	6.67 ± 0.08[b]	6.93 ± 0.15[b]	5.28 ± 0.16[b]	5.31 ± 0.10[b]

Data are expressed as mean ± standard deviation, n = 30. Means within the same column with different superscripts (a-b) are significantly (P < 0.05) different.

The inferior sensory qualities of EWP-cake could be related to the reduction in foaming and emulsifying ability and to the enhancement of the gelling ability of the egg white protein (Table 1 and Table 2). During whipping, air bubbles are trapped in liquid albumen. After baking, the air bubbles expand and egg white proteins coagulate around them, giving permanence to the foam structure. In the case of EWP-cake the partially denatured egg white was underfoamed, thus the volume and texture of the finished product were less than desired. The egg white proteins of the EWP-cake had undergone more aggregation and gel formation that yielded a cake with hard texture, but low in volume. Other primary and secondary texture parameters could have also been affected by the pasteurization steps.

Conclusion

Compared to EWUP, EWP had lower emulsifying and foaming ability, but higher in gel forming ability. These differences are due to the changes on the protein structure during the pasteurization of the shell egg. Pasteurized shell eggs may not be suitable for use as ingredient in bakery products such as the angle food cake.

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