Formation of lysinoalanine in egg white under alkali treatment

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ABSTRACT To investigate the formation mechanism of lysinoalanine (LAL) in eggs during the alkali treatment process, NaOH was used for the direct alkali treatment of egg white, ovalbumin, and amino acids; in addition, the amount of LAL formed during the alkali treatment process was measured. The results showed that the alkali treatment resulted in the formation of LAL in the egg white. The LAL content increased with increasing pH and temperature, with the LAL content first increasing and then leveling off with increasing time. The amount of LAL formed in the ovalbumin under the alkali treatment condition accounted for approximately 50.51% to 58.68% of the amount of LAL formed in the egg white. Thus, the LAL formed in the ovalbumin was the main source for the LAL in the egg white during the alkali treatment process. Under the alkali treatment condition, free L-serine, L-cysteine, and L-cystine reacted with L-lysine to form LAL; therefore, they are the precursor amino acids of LAL formed in eggs during the alkali treatment process.

Key words: lysinoalanine, formation mechanism, egg white, ovalbumin, amino acid, alkali treatment

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INTRODUCTION

Lysinoalanine [N(6)-(2-amino-2-carboxyethyl)-l-lysine] is a compound formed by cross-linking lysine and other amino acids in protein molecules during an alkali or heat-treatment process. LAL was first discovered in bovine pancreatic ribonuclease A (RNase A) under alkaline conditions (pH = 13) by Bohak in 1964 (Bohak, 1964). Subsequently, LAL was also discovered in dairy and egg products with high protein contents (Friedman et al., 1984; Friedman, 1999b; Gilani et al., 2012). The formation of LAL is accompanied by the reduction and racemization of essential amino acids in food, which reduces the nutritional value of food proteins (Friedman, 1999a,b; Gilani et al., 2005). In addition, LAL can even form chelates with metalloenzymes, which specifically induce nephrocalcinosis and produce giant renal cells and necrosis of renal tubular cells in mice (Hayashi, 1982; Pearce and Friedman, 1988; Jonker et al., 1996; Somoza et al., 2006). LAL content has been used as a quality index in infant formula foods to prevent illness in infants (Cattaneo et al., 2008; Cattaneo et al., 2009).

The production of preserved eggs has a long history in China. Preserved eggs are produced by curing fresh eggs in an alkaline solution (about 4 to 5% NaOH) for a long period of time (e.g., approximately 30 to 40 d; Ma, 2007). The alkali treatment of proteins can result in isomerization of many amino acids or changes to other structures that can reduce the nutritional value of the proteins and form compounds that are potentially hazardous to human health, such as LAL, dehydroalanine (DHA), and alkylated alanine (Finley and Kohler, 1979; Maga, 1984). The strong alkali used in the curing process of preserved eggs can induce proteins to form an elastic gel, but it also provides a relatively good condition for the formation of LAL. A relatively high LAL content also had been discovered in preserved eggs (Chang et al., 1999b).

Preserved eggs are a traditional egg product that originated in China. Preserved eggs are known for their beautiful color, translucency, nutritional value, and unique flavor. In addition, according to such medical books as *Yi Lin Zuan Yao* (literally, Essential Compilation in the Field of Medicine), preserved eggs have cooling, eyesight-improving, and liver-calming effects. Therefore, preserved eggs are sought by numerous consumers in China and elsewhere. Currently, 20% of fresh duck eggs are processed into preserved eggs that are consumed by many people. Thus, the safety of preserved eggs is of great importance. However, current studies on preserved eggs are focused on processing technologies without heavy metals, as well as on quality control, to reduce and control the effects of heavy metal elements, such as lead and copper, on the safety of preserved eggs (Ma et al., 2001; Ganasen and Benjakul,

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2010; Ganesan and Benjakul, 2010; Tu et al., 2013). However, the mechanisms and control technology of the formation of LAL in preserved eggs during the production process have not been studied.

Eggs are rich in proteins. The curing process of preserved eggs is relatively long and requires 30 to 35 d to complete at 25°C. Therefore, to simplify the study, the egg white, main proteins, and amino acids in the egg white were used as the study objects in the present study. In addition, NaOH was directly used for the curing process, and the formation pattern and mechanism of LAL was investigated.

MATERIALS AND METHODS

Materials

LAL was obtained from Bachem (Bubendorf, Switzerland). DL-2, 6-diaminopimelic acid (DPA), triethylamine (TEA), N-methyl-N-(tert-butyldimethylsilyl) trifluoroacetamide (MTBSTFA), N, N-dimethylformamide (DMF), ovalbumin and amino acid were purchased from Sigma-Aldrich (St. Louis, MO). Analytical grade of hydrochloric acid (HCl), sodium hydroxide (NaOH) were purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). High purity water for the dilution of concentrated hydrochloric acid was obtained from a Mill-Q purification system (Millipore, Bedford, MA).

Fresh duck eggs laid within 5 days (65 to 70 g) were purchased from a farm in Nanchang country, Jiangxi Province, China.

Methods

Preparation of Egg White Ten fresh duck eggs were selected. After the egg yolk and egg white were separated, the egg white was homogenized (IKA T18 Ultra-Turrax, Staufen, Germany), and upper foam was removed. Subsequently, the egg white was stored in a 4°C refrigerator and used on the same day.

Alkali Treatment of Egg White

Treatment of Egg White in Alkaline Solutions With Different Alkali Concentrations. 20 mL of ultrapure water was added to 20 mL of egg white (as the control group), and 20 mL of 0.02, 0.05, 0.1, 0.2, 0.4, or 1 N NaOH solution was added to 20 mL of egg white (as the experimental group). After the samples were rapidly mixed for 30 s using a glass rod, the pH of each sample was measured, and the samples were then placed in a 25°C incubator for treatment for 8 h, after which the pH of each sample was measured again. Subsequently, 1 mL of 12 N concentrated hydrochloric acid (HCl) was added to 1 g of each treated sample to prevent further alkaline reaction. The samples to be tested for LAL contents were then stored in a −20°C refrigerator.

Alkali Treatment of Egg White at Different Temperatures. 20 mL of NaOH solution (0.2 N) was mixed rapidly with the same volume of egg white, and the homogeneous mixture was subsequently divided into multiple samples. The samples were then treated at different constant temperatures (25, 35, 45, 55, 65, 75, and 85°C) for 4 h, and then the pH and LAL contents of each sample were measured.

Alkali Treatment of Egg White for Different Times. A NaOH solution (0.2 N) was mixed with the same volume of egg white. The mixture was treated at 25°C for 0 to up to 256 h, and the pH and LAL contents of the sample were measured at specific time intervals (0, 1, 2, 4, 8, 16, 32, 64, 128, and 256 h).

Analysis of Amino Acids Egg white was mixed with the same volume of a 0.2 mol/L NaOH solution. The mixture was placed in a 25°C environment, a sample was collected from the mixture at a specific time interval, and the amino acid contents in the sample were determined. Amino acids were determined using the procedure described by Tu et al. (2009) with some modifications. The egg white samples (220 mg each) that were treated under alkaline conditions were accurately weighed. Each of the measured samples was transported to a 15 mL hydrolysis tube, into which 10 mL of 6 N HCl was added for hydrolysis. The hydrolysis tubes were then frozen using ice for 3 to 5 min, connected to a vacuum pipe and vacuum-pumped, and then the hydrolysis tubes were filled with high-purity nitrogen. Subsequently, the hydrolysis tubes were vacuum-pumped and filled with nitrogen again. The hydrolysis tubes were then sealed under a nitrogen atmosphere and placed in a 110°C incubator to allow the egg white to hydrolyze for 23 h. After hydrolysis, each hydrolyzed sample was transferred onto an evaporating dish, and each hydrolysis tube was rinsed with ultrapure water multiple times, with the rinses transferred onto corresponding evaporating dishes. The evaporating dishes were then placed in an 80°C water bath to evaporate and concentrate the samples. Subsequently, each concentrated solution was transferred to a 10 mL volumetric flask. Each evaporating dish was rinsed with ultrapure water multiple times, and the rinses were transferred to corresponding volumetric flasks. The constant volume of each solution was set to 10 mL. A Hitachi L8900 Automatic Amino Acid Analyzer (Tokyo, Japan) was used to determine the amino acid compositions. In addition, the reduction rate of the amino acid content within a specific time period was calculated.

Alkali Treatment of Ovalbumin Approximately 2 g of ovalbumin was weighed, 20 mL of ultrapure water was then added to the ovalbumin, and the mixture was stirred to allow the ovalbumin to dissolve. Afterwards, 20 mL of a 0.6 N NaOH solution was added to the ovalbumin/water solution, the mixture was stirred again, and the pH of the system was measured (pH = approximately 12.4). The system was placed in a 25°C incubator for treatment for 0, 1, 2, 4, 8, 16, 32, 64, 128, and 256 h. At specific time intervals, the pH of the

system was measured; 1 g of the sample was collected, and 1 mL of 12 N concentrated HCl was immediately added to the collected sample to prevent further alkaline reaction. The samples to be tested for LAL contents were then stored in a −20°C refrigerator.

Alkali Treatment of Amino Acids Various amino acids of different masses were weighed: L-cystine (3.61 mg), L-cysteine (3.64 mg), L-serine (3.16 mg), L-O-phosphoserine (5.56 mg), L-threonine (3.58 mg) and L-arginine (5.22 mg). Each of these amino acids was mixed with 4.39 mg of L-lysine, and 9 mL of a 0.2 N NaOH solution was added to each mixture. The mixtures were then placed in a 25°C incubator for alkali treatment for 0, 1, 2, 4, 8, 16, 32, 64, 128, and 256 h. At specific time intervals, 0.3 mL of each sample was collected for subsequent testing.

Determination of LAL The analysis method established by Luo et al. (2013) was used for the determination of LAL. The hydrolytic operation was excluded from the determination of LAL in the amino acid samples that were treated under alkaline conditions. Instead, 0.2 mL of 0.37 mmol/L (DPA)-HCl solution was directly added to each amino acid sample. A nitrogen sample concentrator was used to blow dry the samples, after which 90 μL of DMF, 10 μL of TEA, and 100 μL of MTBSTFA were added to the samples. After the samples were derivatized, a gas chromatography flame ionization detector (GC-FID) was used to determine the LAL content in each sample. The LAL contents in the egg white and ovalbumin that were treated under the alkaline conditions were recorded in units of mg/kg (fresh sample).

Statistical Analysis

The software Origin 8.5 was used to process the research results, which were expressed in the form of "Mean ± Standard Deviation" (n = 3). The Statistical Package for the Social Sciences (SPSS) Statistics 19 software was used to conduct Duncan's multiple range tests in a one-way analysis of variance. A value of $P < 0.05$ indicated significant differences.

RESULTS AND DISCUSSION

Effect of the Alkali Treatment Condition on the Formation of LAL in Egg White

Effect of the Alkali Concentration on the Formation of LAL in Egg White Figure 1a shows that after the various alkali concentrations or ultrapure water were mixed with the egg white, the pH of the systems were relatively evenly distributed between 9 and 14; the pH of the mixture with the same volumes of egg white and ultrapure water was approximately 9.12; and the pH of the egg white that was treated with 0.2 N NaOH was approximately 12.35, which is close to the pH of the egg white of preserved eggs that have been cured for 30 d (Yang et al., 2012). After 8 h of alkali treatment, the pH of all the systems slightly decreased ($P < 0.05$), which might have resulted from the alkali reacting with the proteins or amino acids in the egg white, which consumed a certain amount of alkali.

Figure 1b shows that after 8 h in a 25°C incubator, no LAL was formed in the egg white that had not been treated with alkali. However, LAL was formed in the egg white that had been treated under alkaline conditions, and the amount of LAL varied according to the alkali concentration (or pH). At 25°C, 71.10 ± 1.36 mg/kg of LAL was formed in the egg white that was treated with 0.02 N NaOH for 8 h. With increasing concentrations of NaOH, the amount of LAL gradually increased, and when the concentration of NaOH was 1 N, the amount of LAL formed in the egg white reached 1487.75 ± 53.39 mg/kg. Furthermore, higher concentrations of alkali added to the system produced higher

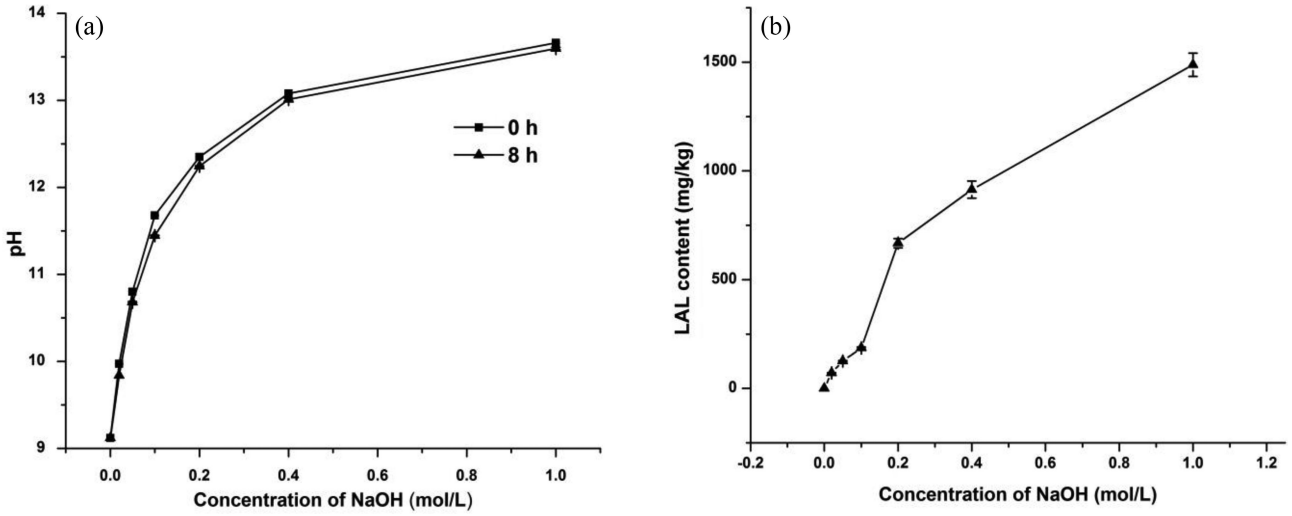

Figure 1. Effect of alkali concentration on the pH and the formation of LAL in egg white (a: Effect of alkali concentration on the pH; b: Effect of alkali concentration on LAL content, 25°C for 8 h).

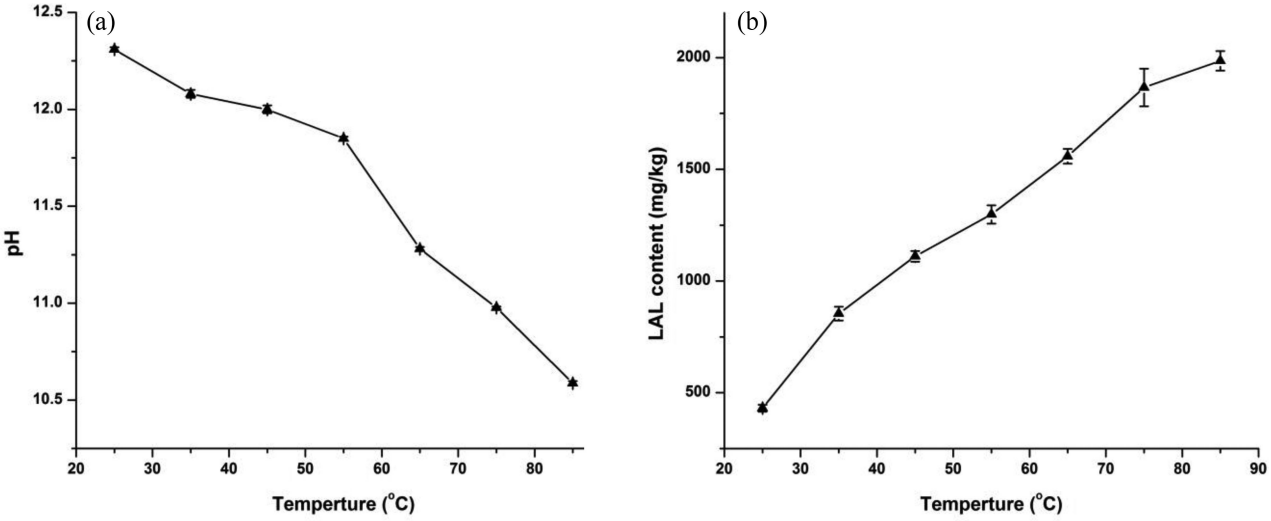

Figure 2. Effect of alkali treatment temperature on the pH and the formation of LAL in egg white (a: Effect of treatment temperature on the pH; b: Effect of treatment temperature on LAL content; 0.2 N NaOH for 4 h).

pH values. Thus, we know that alkali can result in the formation of LAL in egg white, and the amount of LAL formed in the egg white is affected by the pH of the egg white. LAL was only formed in the egg white when the pH was sufficiently high (> 9.12), and higher pH resulted in greater amounts of LAL.

Effect of Alkali Treatment Temperature on the Formation of LAL in Egg White Figure 2a shows that after 4 h of alkali treatment at different temperatures, the pH of the egg white samples all decreased. In addition, higher temperatures produced a greater decrease of pH; therefore, a greater amount of NaOH was consumed, which might have been caused by the intensification of the reaction between the alkali and egg white caused by the effect of temperature. The pH variations directly reflected the rate of the alkali treatment of egg white and indirectly reflected the formation of LAL in egg white.

Figure 2b shows the effects of temperature on the amount of LAL formed in egg white that was treated in a 0.2 N NaOH solution for 4 h. At 25°C, 430.80 ± 15.56 mg/kg of LAL was formed in the egg white treated in 0.2 N NaOH; if the treatment temperature increased continuously, the amount of LAL rapidly increased. When the treatment temperature reached 85°C, the amount of LAL formed in the egg white was as high as 1985.67 ± 44.00 mg/kg. Combined with the information in Figure 2a (the pH of the egg white treated under alkali conditions at each temperature, particularly high temperatures, decreased after 4 h of treatment), we know that high temperatures promote the effects of alkali in egg white, increase the formation efficiency of LAL, and result in the formation of more LAL in egg white.

Effect of Alkali Treatment Time on the Formation of LAL in Egg White Figure 3a shows that during the alkali treatment process, the pH of the sample decreased almost linearly; after 256 h, the pH of the system decreased to 9.77, which might have been related to the increasing impact of alkali on the egg white with increasing time, and thus, more alkali was consumed. It can be inferred from the variation of the sample's pH that the formation of LAL in egg white could also be affected by the alkali treatment time.

Figure 3b shows that the amount of LAL formed in egg white treated in a 0.2 N NaOH solution at 25°C changed constantly with time. When alkali was first added to the egg white, LAL was not formed immediately in the egg white; however, 1 h later, 151.77 ± 6.52 mg/kg of LAL was formed, and when the treatment time was increased to 16 h, the amount of LAL increased rapidly to 1131.76 ± 39.12 mg/kg. When the treatment time continued to increase to 128 h, the amount of LAL in the egg white increased to 1962.89 ± 61.4 mg/kg, and when the treatment time increased to 256 h, there was no significant change in the amount of LAL ($P > 0.05$; 1976.87 ± 76.35 mg/kg). At room temperature, the formation of LAL in egg white under the alkali treatment condition is a relatively slow process. Within a certain range of increasing treatment time, the amount of LAL formed in the system continuously increased. When the treatment time was too long, there was no significant change in the amount of LAL, which might have indicated that the process had reached an equilibrium state of LAL formation and decomposition.

Change of Amino Acid Contents in Egg White During the Alkali Treatment Process Table 1 lists the reduction rate results and shows that after the egg white was treated under alkali conditions, no significant changes were observed in the contents of aspartic acid, glutamate, proline, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, and histidine; however, the contents of threonine, serine,

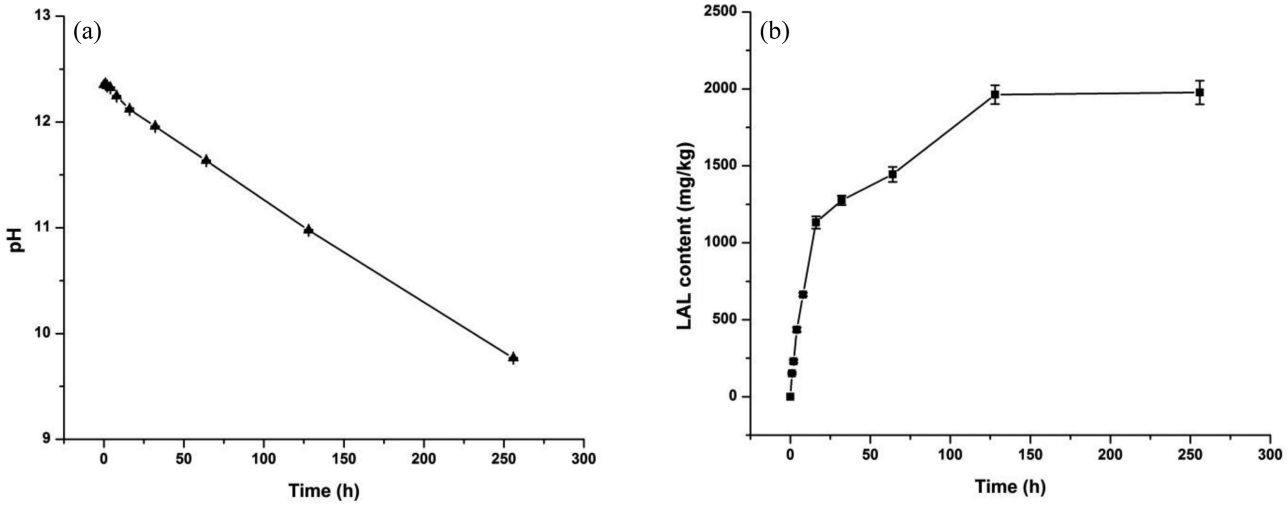

Figure 3. Effect of alkali treatment time on the pH and the formation of LAL in egg white (a: Effect of alkali treatment time on the pH; b: Effect of alkali treatment time on LAL content; 0.2 N NaOH at 25°C).

Table 1. Variations of the amino acid contents in egg white during the alkali treatment process (0.2 N NaOH at 25°C).

	Content (mg/g)			Rate of change (%)	
Amino acid	0h	64h	128h	64h	128h
Asp	1.46	1.44	1.43	1.59	1.75
Thr	1.49	1.38	1.28	7.03	13.74
Ser	2.66	2.56	1.92	3.80	28.09
Glu	9.03	8.95	8.91	0.92	1.35
Pro	7.30	7.22	7.15	1.10	1.96
Gly	0.87	0.86	0.88	0.10	−1.67
Ala	1.38	1.39	1.37	−0.53	0.94
Cys	0.88	0.33	0.16	63.25	82.04
Val	3.94	4.03	3.87	−2.34	1.63
Met	3.78	3.84	3.72	−1.76	1.52
Ile	2.50	2.48	2.45	0.84	1.90
Leu	4.92	4.88	4.79	0.81	2.84
Tyr	2.80	2.85	2.77	−1.7	1.23
Phe	4.45	4.51	4.36	−1.43	2.01
Lys	3.95	3.34	2.17	15.38	44.98
His	1.23	1.21	1.21	1.11	1.12
Arg	2.69	2.39	1.61	11.28	40.22

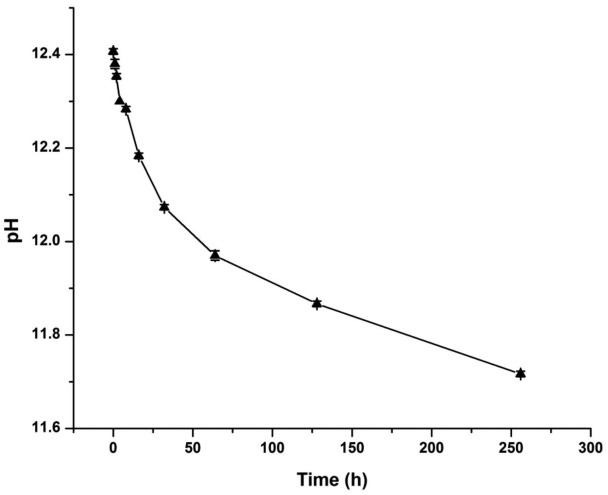

Figure 4. Change of the pH of the ovalbumin that was treated under alkaline conditions over time (0.6 N NaOH at 25°C).

cysteine, lysine, and arginine significantly decreased with increasing time, with values of 7.03, 3.80, 63.25, 15.38, and 11.28%, respectively, after 64 h of alkali treatment and 13.74, 28.09, 82.04, 44.98, and 40.22%, respectively, after 128 h of alkali treatment. Cysteine in egg white presented the most significant effects from the alkali treatment, followed by lysine and arginine; these results were caused by certain amino acids (particularly cysteine) becoming unstable during the alkali treatment process; therefore, the β-elimination reaction of these amino acids occurs easily and results in the formation of DHA and other products, with DHA the substrate of the formation of LAL (Friedman, 1999a; Nashef et al., 1977). Although the reduction of amino acids resulted from their poor alkali resistance and easy decomposition to other matter, the reduction of amino acids might have been caused by a reaction between amino acids that resulted in the formation of new products, such as the cross-linked amino acid LAL.

Effect of Alkali Treatment on the Formation of LAL in Ovalbumin

Ovalbumin accounts for approximately 54% of the proteins in egg white, and the proteins account for approximately 11% of the mass of egg white (Kovacs-Nolan et al., 2005); therefore, ovalbumin accounts for approximately 6.5% of the mass of egg white. Based on the contents of ovalbumin and water in egg white, ultrapure water was added to ovalbumin to simulate the environment of ovalbumin in egg white. In addition, a 0.6 N NaOH solution of the same volume was added to the ovalbumin/water system. The pH of the system was approximately 12.40, which was close to the pH of the egg white system that was treated in a 0.2 N NaOH solution as well as the pH of preserved eggs.

Figure 4 shows that the pH of the system gradually decreased throughout the entire process, and after 256 h, the pH of the system was 11.72. This

Table 2. Comparison between the amounts of LAL formed in the ovalbumin and egg white that were treated under alkaline conditions.

Time (h)	Content of LAL (mg/kg) Ovalbumin (Treated with 0.6 N NaOH at 25°C)	Egg wihte (Treated with 0.2 N NaOH at 25°C)	Rate(%)
1	0	151.77 ± 6.52	0
2	116.05 ± 2.95	229.96 ± 9.22	50.51 ± 2.10
4	240.35 ± 6.00	435.05 ± 13.56	55.29 ± 2.55
8	389.85 ± 11.28	664.33 ± 11.23	58.68 ± 1.17
16	612.92 ± 15.30	1131.76 ± 39.12	54.17 ± 0.87
32	683.17 ± 19.17	1276.17 ± 31.17	53.55 ± 1.77
64	768.09 ± 21.20	1444.79 ± 48.58	53.21 ± 2.54
128	1015.52 ± 27.72	1962.89 ± 61.40	51.76 ± 1.90
256	1003.90 ± 25.38	1976.87 ± 76.36	50.80 ± 0.88

phenomenon is similar to the variation trend of the pH in egg white treated under alkaline conditions; however, the pH of ovalbumin treated under alkaline conditions decreased more slowly at 16 h, which might be related to the relatively small designed protein amount in the ovalbumin system under the alkaline treatments.

Table 2 presents a comparison between the ovalbumin and egg white that were treated under alkaline conditions, and the 256 h treatment shows that the variation patterns of the LAL amount were extremely similar in these two systems; with increasing treatment time, the amounts of LAL formed in both systems continuously increased and then remained generally stable. The comparison between the amounts of LAL formed in the two systems for each same time period shows that the amount of LAL formed in the ovalbumin that was treated under alkaline conditions was significantly ($P < 0.05$) lower than that formed in the egg white by approximately 50.51 to 58.68%. This ratio suggests that ovalbumin is the main protein that participates in the formation of LAL in egg white (or the egg white of preserved eggs) during alkali treatment processes. In addition, ovalbumin accounts for approximately 54% of the protein content in egg white; therefore, ovalbumin contributes to the formation of LAL in egg white at a matching level.

Effect of Alkali Treatment on the Formation of LAL in the Amino Acid System

Threonine, serine, cysteine, cystine, and arginine (Table 1) decreased significantly in the egg white treated under the alkaline condition; cystine and L-O-phosphoserine, which were mentioned in the literature (Friedman, 1999a), were mixed with lysine, the mixtures were then treated under alkaline conditions for 0∼256 h, and the amounts of LAL formed during the alkali treatment process were measured at specific intervals (Table 3). The results show that under alkaline conditions, cross-linking reactions occurred between free amino acids, resulting in the formation of LAL. For instance, L-serine, L-cysteine and L-cystine were mixed with L-lysine and formed LAL; the formation of LAL in the mixture of L-serine and L-lysine was faster, with the amount of LAL formed in each mixture first increasing and then decreasing with increasing time, which resulted from the instability of LAL under strong alkaline conditions. The formation rate of LAL was faster than the decomposition rate of LAL earlier in the process, whereas the formation rate of LAL was slower than the decomposition rate of LAL later in the process. In addition, no LAL was detected in L-threonine/L-lysine, L-arginine/L-lysine and L-O-phosphoserine/L-lysine mixtures, which was most likely because the β-carbon atoms in serine, cysteine and cystine contain –OH, –SH or –S-S- functional groups. Thus, –OH or –SH are easily removed and –S-S- is easily heterolyzed under OH^-, resulting in the occurrence of a β-elimination reaction and the formation of DHA that contains a conjugated double bond. A nucleophilic addition reaction occurs between the double bond of DHA and ε-NH_2 of lysine, resulting in the formation of LAL. The formation rate of LAL is determined by the formation rate of DHA. In addition, the formation rate of LAL is also related to the activation energy of the β-elimination reaction of each amino acid under alkaline conditions (Maga, 1984; Friedman, 1999a). The β-carbon atom in threonine contains an –OH functional group; thus, the β-elimination reaction can occur under OH^-, although dehydrobutyrine (DHB) is formed from this β-elimination reaction. Arginine does not contain

Table 3. Amount of LAL formed in the amino acid system under alkali treatment (0.2 N NaOH at 25°C).

Time (h)	Content of LAL (μg) L-serine and L-lysine	L-cysteine and L-lysine	L-threonine and L-lysine	L-arginine and L-lysine	L-cystine and Lysine	L-O-phosphoserine and Lysine
0	0	0	0	0	0	0
1	32.28 ± 1.29	38.84 ± 1.36	0	0	27.45 ± 1.13	0
2	88.72 ± 2.92	69.19 ± 2.51	0	0	35.24 ± 0.79	0
4	494.22 ± 20.77	131.05 ± 3.07	0	0	52.50 ± 1.44	0
8	901.18 ± 29.89	255.60 ± 11.19	0	0	116.90 ± 3.97	0
16	90.58 ± 2.78	475.00 ± 2.07	0	0	221.47 ± 5.90	0
32	17.02 ± 0.72	734.79 ± 17.71	0	0	491.97 ± 19.98	0
64	0	772.88 ± 13.85	0	0	874.25 ± 17.59	0
128	0	172.63 ± 5.70	0	0	895.84 ± 18.47	0
256	0	15.98 ± 0.49	0	0	87.04 ± 3.67	0

a functional group that can be easily removed; thus, the β-elimination reaction cannot occur. The phosphate group of L-O-phosphoserine may be even more difficult to remove than the –OH group of serine; thus, the β-elimination reaction is difficult. Therefore, serine, cysteine, cystine, and lysine can be considered the precursor amino acids that participate in the formation of LAL in preserved eggs, whereas threonine, arginine, and L-O-phosphoserine are not precursor amino acids, which is inconsistent with previously proposals. For example, Chang et al. (1999a) concluded that threonine and serine participated in the formation of LAL in poultry eggs during the alkali treatment process, and Friedman (1999a) included phosphoserine as a precursor amino acid in his summary of possible formation mechanisms of LAL. LAL can be formed in the ovalbumin of the egg white of poultry eggs during the alkali treatment process because an ovalbumin molecule contains 385 amino acid residues that contain 20 lysines, 6 cysteines, and 38 serines (Nisbet et al., 1981).

In addition to the free amino acids that occur in egg white, free amino acids are also produced during the alkali treatment process because of the degradation of proteins. The free cysteine, cystine, and serine in a poultry egg may directly cross-link with lysine under alkali treatment conditions and form free LAL, or the proteins that contain these types of amino acid residue may form LAL that is bound to the proteins under alkaline conditions through a two-step reaction mechanism. However, because of the long alkali treatment process of poultry eggs, free LAL formed during the process is easily decomposed. Therefore, LAL in preserved eggs basically occurs in a protein-binding form. In addition, the ovalbumin in egg white is the main source of the proteins that participate in the formation of LAL. The contents of proteins such as ovotransferrin, ovomucoid and lysozyme are also relatively high in egg white, and these proteins also contain precursor amino acid residues that participate in the formation of LAL; therefore, these proteins may also participate in the formation of LAL. In addition, it can be inferred that egg yolk contains the proteins of the aforementioned precursor amino acids, which also participate in the formation of LAL in egg yolk. However, further studies are required to understand the specific contributions from such proteins as high-density lipoprotein, low-density lipoprotein, and levitin.

CONCLUSIONS

Alkali was directly used to treat egg white to result in the formation of LAL. The amount of formed LAL increased with increasing pH and temperature, and it first increased and then remained stable with increasing treatment time. Ovalbumin was the primary protein that participated in the formation of LAL in egg white during the alkali treatment process. Serine, cysteine, cystine, and lysine were the precursor amino acids that participated in the formation of LAL. The present study consisted of a preliminary investigation of the formation mechanism of LAL, and further investigations should study the effects of the protein amino acid sequences, effective binding sites, and cross-linked amino acid conformations on the formation of LAL and should monitor DHA intermediates.

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