

Physicochemical and nutritional characteristics of preserved duck egg white

Yan Zhao,^{*†‡} Yonggang Tu,^{*1} Mingsheng Xu,^{*} Jianke Li,^{†‡} and Huaying Du^{*}

**Jiangxi Key Laboratory of Natural Products and Functional Food, Jiangxi Agricultural University, Nanchang 330045, China; †State Key Laboratory of Food Science and Technology, and ‡Engineering Research Center of Biomass Conversion, Ministry of Education, Nanchang University, Nanchang 330047, China*

ABSTRACT In this paper, the physicochemical and nutritional characteristics of preserved duck egg white were analyzed and compared with fresh egg and hard-cooked egg white ($n = 3$). The data obtained showed that the preserved egg white was rich in essential amino acids and minerals, such as Ca, Mg, Fe, Zn, Cu, K, and Na. After fresh duck eggs were processed into preserved eggs, contents of moisture, CP, amino acid, and water-soluble vitamin of egg white significantly decreased ($P < 0.05$); however, pH, free amino acid content, and most inorganic elemental contents of egg white significantly increased ($P < 0.05$). The preserved egg white

had higher a^* (redness/greenness) and b^* values (yellowness/blueness; $P < 0.05$) and lower L^* value (lightness; $P < 0.05$) than hard-cooked egg white. The gel hardness of preserved egg white was approximately 50% of hard-cooked egg white; however, its springiness and cohesiveness were approximately 1.5 times of hard-cooked egg white. The results indicated that pickling with alkaline and other additives can significantly change physical properties and chemical composition of duck egg white, which make preserved egg white with characteristics of rich elements, brown color, and high springiness, but low vitamin.

Key words: preserved egg white, physicochemical characteristic, nutritional characteristic

2014 Poultry Science 93:3130–3137
<http://dx.doi.org/10.3382/ps.2013-03823>

INTRODUCTION

Preserved egg, also known as pidan, century egg, hundred-year egg, thousand-year egg, or thousand-year-old egg, is a unique Chinese delicacy used in many traditional dishes. Preserved egg is highly popular in China and some other South East Asian countries, such as Thailand and Malaysia. In China, about 1.5 million tons of fresh duck eggs are processed into preserved eggs every year. The preparation method of preserved eggs is believed to date back to 600 yr earlier. Besides fresh duck eggs, fresh chicken eggs or quail eggs can become preserved eggs after 4 to 5 wk of preserving in a mixture of alkali, salt, black tea, and metal ions at room temperature (Su and Lin, 1993; Wang and Fung, 1996). The formation of preserved eggs is caused by the penetration of strong alkali through the eggshell and eggshell membrane, resulting in physical and chemical changes in the egg white and yolk. Under strong alkali treatment, egg white and yolk gradually solidify, harden, and color in terms of physical appearance, and from a chemical composition aspect, proteins and fats

of egg break into a variety of peptides, amino acids, and volatile compounds (Zhao et al., 2010).

Egg white, also known as albumen, one of main constituent parts of whole egg, contains about 84 to 89% moisture from the outermost to innermost layer, 10 to 11% protein, approximately 0.2% fat, and 0.8% ash on a wet basis (Mine, 2008). On a dry basis, protein is the major component of egg white; many types of proteins are found in egg white, and the most abundant proteins in egg white are ovalbumin, conalbumin, ovomucoid, and lysozyme (Mine, 2008). Some of these proteins could denature and form gelation under the condition of heating, extreme pH, and metal ions (Handa et al., 1998; Croguennec et al., 2002; Campbell et al., 2003). Alkali treatment is an uncommon processing method for food; preserved egg is the typical food product of the alkali process. During the process of pickling preserved egg, egg white gradually formed a dark brown, transparent gel with light flavor from original translucent liquid. The transforming agents in the preserved egg are the alkaline compounds, which gradually increase the pH of the egg to approximately 12 or higher (Eiser et al., 2009). The prolonged treatment of alkaline and other addition may alter the nutrients found in egg white. Therefore, in the present paper, various kinds of modern instruments and analysis techniques were used to analyze the physicochemical and nutritional charac-

©2014 Poultry Science Association Inc.

Received December 11, 2013.

Accepted September 7, 2014.

¹Corresponding author: tygzy1212@aliyun.com

teristics of preserved egg white, and hard-cooked egg white was used as the control.

MATERIALS AND METHODS

Materials

High-performance liquid chromatography-grade methanol (Merck, Darmstadt, Germany) was used in the experiments. All of the standard compounds were purchased from Sigma Chemical Co. (St. Louis, MO). All of the other reagents used were of analytical grade and purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Pure water was obtained using a Milli-Q purification system (Millipore, Billerica, MA).

Methods

Preparation of Preserved Eggs and Hard-Cooked Eggs. Fresh duck eggs (65 g to 70 g) were obtained from a farm in Nanchang County, Jiangxi Province, China. The duck eggs were cleaned with tap water and checked for any crack before pickling. Clean duck eggs were soaked in pickling solution containing NaOH (4.5%, m/V), NaCl (3%, m/V), CuSO₄ (0.4%, m/V), and Chinese black tea (3.5%, m/V) at 25°C for 30 d, the obtained preserved eggs were then cleaned with cold boiled water. The clean fresh duck eggs were boiled in boiling water for 15 min to obtain hard-cooked eggs. Egg shell, egg white, and egg yolk of the preserved eggs and hard-cooked eggs were separated carefully.

Determination of Moisture, CP, Ash, and pH. Moisture content was obtained by drying the samples in a hot-air oven at 100 ± 5°C until constant weight (Chinese standard GB 5009.3-2010; China, 2010b). The Kjeldahl method was used to determine total nitrogen content; the nitrogen content was then multiplied by a factor 6.25 to obtain CP content (Chinese standard GB 5009.5-2010; China, 2010c). Ash content was determined by gravimetric measurement of the sample residue after ignition in an oven at 550 to 600°C to a constant weight (Chinese standard GB 5009.4-2010; China, 2010a). The pH of egg white was measured in accordance with the method of Chinese standard GB/T 5009.47-2003 (China, 2003c).

Color Measurement. The color of the preserved egg white and hard-cooked egg white was measured with a fully automatic whiteness test meter (ADCL-60-W, Cengtaike Instrument Technology Co. Ltd., Beijing, China) and expressed as L* (lightness), a* (redness/greenness), and b* (yellowness/blueness).

Texture Profile Analysis. Texture profile analysis was performed using a TEE32 texture analyzer (Stable Micro Systems, Surrey, UK). Both preserved egg white and hard-cooked egg white were cut into a piece of 10 mm × 10 mm × 10 mm. The samples were compressed twice to 70% of their original height with a compression cylindrical aluminum probe (P/50). Tex-

tural analyses were performed at room temperature. Pretest speed was 1 mm/s, test speed was 2 mm/s, and posttest speed was 2 mm/s. Hardness (*g*), springiness, and cohesiveness were evaluated. These parameters were measured using the Micro Stable software (Stable Micro Systems).

Microstructure Analysis. Microstructures of preserved egg white and hard-cooked egg white were examined using environment scanning electron microscopy (ESEM; Quanta-200F, FEI Ltd., Eindhoven, the Netherlands) according to the method of Croguennec et al. (2002) with modification. Test samples (3 mm × 3 mm × 1 mm) were cut from the center of each gel, and then fixed overnight at room temperature in 2.5% glutaraldehyde (0.1 M phosphate buffer, pH 7.2). Fixed samples were rinsed with 0.1 M phosphate buffer (pH 7.2) 3 times, followed by fixing with 0.1% osmium solution for 2 h at room temperature. The samples were dehydrated in graded series of ethanol solutions (50, 70, 80, 90, and 100%). The samples were freeze-dried (-20°C) with a freeze-dryer (Alpha1-2, Martin Christ, Osterode, Germany), mounted using a conductive adhesive, and then observed under ESEM (low vacuum mode) at 10 kV.

Determination of Amino Acids and Free Amino Acids. Amino acids were examined using the modified procedure described by Yang et al. (2007). A sample (100 mg) was heated at 110°C in 12 mL of 6 M HCl for 24 h in an evacuated, sealed tube. The hydrolysate was evaporated to dry in vacuum and dissolved in 25 mL of boracic acid buffer (pH 2.2). A series of standard stock solutions of amino acids at concentrations ranging from 0.5 to 100 µg/mL were prepared with distilled water. Then 200 µL of standard stock solution, 0.1 mol/L of 2,4-dinitro-1-fluorobenzene (DNFB, 120 µL), 0.1 mol/L borate (50 µL), and distilled water (630 µL) were added into 1.5-mL centrifuge tube and mixed well. The derivatization was performed at 60°C for 60 min in the dark. After passing through a 0.45 µm polyvinylidene difluoride microporous membrane, 20-µL samples were then injected into the HPLC (Waters Corporation, Milford, MA). An HPLC analysis was conducted using a C18 column (5 µm × 250 mm × 4.6 mm) at 26°C at a flow rate of 0.8 mL/min. The mobile phase A was 50 mmol/L of sodium acetate buffer (1% of *N,N*-dimethylformamide, pH 6.8). The mobile phase B was acetonitrile:double-distilled water, 50:50 (vol/vol). Gradient elution program of mobile phase B was 0 to 0.3 min, 16%; 0.3 to 4 min, 16 to 31%; 4 to 9.5 min, 31 to 40%; 9.5 to 17 min, 40 to 50%; 17 to 28 min, 50 to 60%; 28 to 34 min, 60 to 95%; and 34 to 36 min, 95 to 16%.

Free amino acid was extracted following the method of Cordoba et al. (1994) with some modifications. A sample (5 g) was homogenized in a glass homogenizer with 20 mL of ultrapure water. A total of 20 mL of 10% sulfosalicylic was then added into the homogenate for deproteinization, which was maintained at 4°C for 17 h. The homogenate was then centrifuged at 15,000 × *g*

for 10 min at 4°C and filtered through qualitative filter paper-medium speed. The pH of the filtrate was adjusted to 6 using 4 *N* NaOH. After derivatization with DNFB, the solution was filtered through microporous membrane and injected into the HPLC system.

Determination of Minerals. Minerals were determined using inductively coupled plasma atomic emission spectroscopy (ICP-AES) as the previously reported procedure (Tu et al., 2009). For the sample preparation for ICP-AES (OPTIMA 5300DV, PerkinElmer, Waltham, MA), 400 mg of sample was weighed into a beaker, digested in 4 mL of HNO₃-HClO₄ (4:1), and the mixture was heated to near dryness. After cooling, the residue was treated with 0.1 *N* HNO₃ and diluted to 25 mL using double-distilled water. Certified standard solutions of the elements were used for the preparation of the working standard for the elements.

Determination of Vitamins. Thiamin and riboflavin contents were measured by fluorometric method following the Chinese standard GB/T 9695.27-2008 (China, 2008a) and GB/T 9695.28-2008 (China, 2008b), respectively. Niacin and pyridoxine were examined by microbiological method following the Chinese standard GB/T 5009.89-2003 (China, 2003a) and GB/T 5009.154-2003 (China, 2003b), respectively.

Statistical Analysis. All of the experiments were performed in triplicates, and the results are expressed as means ± SD. Statistical analyses were performed using the Student's *t*-test or 1-way analysis of Duncan using SPSS 11.5 (SPSS Inc., Chicago, IL). Differences were considered significant at *P* < 0.05.

RESULTS AND DISCUSSION

Proximate Compositions and pH Analyses

The proximate compositions and pH of fresh egg white, preserved egg white, and hard-cooked egg white are presented in Table 1. Moisture analysis showed that preserved egg white contained 82.08 ± 0.43 g of water/100 g, which is significantly lower than that of fresh egg white (86.24 ± 0.03 g of water/100 g, *P* < 0.05). The moisture content of hard-cooked egg white was lower than that of fresh egg white, but higher than that of the preserved egg (*P* < 0.05). Thus, alkali and heat treatments reduce the moisture in egg white. Alkali and heat treatments all can induce the denaturation of egg white protein into gels, which may

be the cause of moisture loss. Preserved eggs was obtained by pickling in a mixture of alkali, salt, black tea, and metal ions, the difference of osmotic pressure may be also another reason for leading to moisture transfer from egg white to the pickling solution. Preserved egg white contained 12.12 ± 0.14 g of CP/100 g (Table 1), which is sufficiently lower than fresh and hard-cooked egg whites. This result may be due to the degradation of proteins into amino acids, which then form volatile compounds under alkali condition. Some amino acids also maybe transfer into pickling solutions. The ash analysis showed that preserved egg white contained 3.23 ± 0.07 g of ash/100 g, which is approximately 4× that of fresh egg white. The pickling solution used for preparing preserved eggs consisted of water, NaOH, Chinese black tea, NaCl, and metallic compounds. The water used in processing preserved egg contains many kinds of inorganic elements (Tuzen and Soylak, 2006). Sodium hydroxide and NaCl contain sodium ion. Chinese black tea is also rich in various kinds of minerals (Matsuura et al., 2001; Salahinejad and Aflaki, 2010). In the pickling process, these elements can permeate into all of the parts of duck egg, thereby increasing the inorganic element content of egg white while decreasing the proportion of moisture and proteins in egg white. In addition, no marked change in pH was noticeable between hard-cooked egg white and fresh egg white, but the alkaline treatment significantly increased pH of egg white (*P* < 0.05). Pickling solution contains 4.5% NaOH, which permeates gradually into the egg during the pickling process, thereby increasing the pH of egg white from 9.4 to 11.3.

Color of Preserved Egg White

After prolonged pickling in the mixed solution, the egg white became dark-brown gel. In this paper, the color of preserved egg white and hard-cooked egg white was determined using a whiteness test meter. The results are shown in Table 2. The L* value of preserved egg white was 30.48 ± 5.42, which is significantly lower than that of hard-cooked egg white. However, preserved egg presented higher a* and b* values (*P* < 0.05) than those of hard-cooked egg, probably because of the increased content of yellow or brown pigments. Higher a* value was more likely owing to the formation of brown pigments, which may be derived from the Maillard reaction in egg white. Although there is a tiny amount of

Table 1. The results of proximate and pH analysis of fresh egg white, preserved egg white, and hard-cooked egg white (mean ± SD, n = 3)

Item	Fresh egg	Preserved egg	Hard-cooked egg
Moisture (g/100 g)	86.24 ± 0.03 ^a	82.08 ± 0.43 ^c	85.73 ± 0.27 ^b
CP (g/100 g)	13.56 ± 0.52 ^a	12.12 ± 0.14 ^b	13.22 ± 0.29 ^a
Ash (g/100 g)	0.86 ± 0.01 ^b	3.23 ± 0.07 ^a	0.82 ± 0.07 ^b
pH	9.4 ± 0.1 ^b	11.3 ± 0.2 ^a	9.2 ± 0.2 ^b

^{a-c}Different superscripts in the same row indicate significant differences (*P* < 0.05).

Table 2. Lightness (L^*), redness/greenness (a^*), and yellowness/blueness (b^*) values of preserved egg white and hard-cooked egg white (mean \pm SD, $n = 3$)

Item	L^*	a^*	b^*
Preserved egg	30.48 \pm 5.42 ^b	9.67 \pm 1.13 ^a	10.43 \pm 1.36 ^a
Hard-cooked egg	85.88 \pm 9.86 ^a	-3.85 \pm 0.11 ^b	-1.14 \pm 0.26 ^b

^{a,b}Different superscripts in the same column indicate significant differences ($P < 0.05$).

carbohydrates in egg white, the aldehyde group of the reducing sugars can combine with the amino group of amino acids from protein degradation during the alkali pickling process. The Maillard reaction maybe gives a brown color to preserved egg white (Wang and Fung, 1996). Pigment of the Chinese black tea used in the pickling solution may also contribute to the development of the brown color under alkali condition. Higher b^* value was found in preserved egg white, which may be due to the oxidation of Chinese black tea components at significantly high pH or the color of amino acids and metal ion complexes. Higher a^* and b^* values corresponded with the lower L^* value of preserved egg white.

Texture of Preserved Egg White

Egg white is rich in proteins, such as ovalbumin, ovotransferrin, ovomucoid, and lysozyme. Some of these proteins serve as efficient raw material for the formation of egg white gel (Mine, 2008). Strong alkali treatment is a very unique food processing method, which can make preserved egg white proteins form an elastic gel. The texture properties of preserved egg white and hard-cooked egg were examined with a texture analyzer. The results are shown in Figure 1. We found that the gel hardness of preserved egg white was approximately 50% of the heat-induced gel (hard-cooked egg white); however, its springiness and cohesiveness were approximately 1.5 times of hard-cooked egg white. Lower value

of hardness may be due to less covalent cross-linking in aggregation of preserved egg white proteins. Cohesiveness is often used as an indicator of the ability of gels to maintain an intact network structure (Fernandez-Lopez et al., 2006). Therefore, higher value of cohesiveness indicates the strength of intact network structures of preserved egg gel. Higher value of springiness is probably due to the extensive formation of preserved egg white gel network structure by electrostatic repulsive forces. The results indicated that the form mechanism might be different between preserved egg white gel induced by alkali and hard-cooked egg white gel induced by heat. In heat processing, egg white gel is characterized by opaque and firm coagulation. Albumin, ovotransferrin, and lysozyme are the components cross bonding in the heat-induced formation of egg white gel (Zhao et al., 2009). The molecular conformation of heat-induced egg white proteins changes with thermal denaturation, aggregation, and molecular interactions involved in gel network formation. Some of these molecular interactions include hydrophobic interactions and sulfhydryl-disulfide reactions (Mine, 2002). However, the forming mechanism of preserved egg white gelation has not been fully elucidated and needs to be studied further.

ESEM of Preserved Egg White

Protein gelation is a continuous network of aggregation of denatured protein molecules at a certain degree (Wong, 1989). With regard to appearance, the protein gel can be divided into 2 types, namely, transparent gel and opaque gel (Tani et al., 1993; Hatanaka et al., 2009). Protein gels are also classified according to their corresponding inducing factor, such as heat-induced, high pressure-induced, ion-induced, acid-induced, and enzyme-induced protein gels (Totosa et al., 2002). We found that preserved egg white was a brown translucent gel; however, the hard-cooked egg was a typically milky opaque gel. Both gel microstructures were performed by ESEM. From Figure 2, significant differences were found between the microstructures of both gels. The gel network of preserved egg white has uniform, loose, and fine filamentous structure with many regular voids, whereas the hard-cooked egg gel has highly dense, stacked compact granular structure without voids. The differences in microstructures resulted in the difference in texture between the preserved egg white gel and hard-cooked egg white gel. Therefore, the protein aggregation mechanisms of the 2 gels are different, which need further studies.

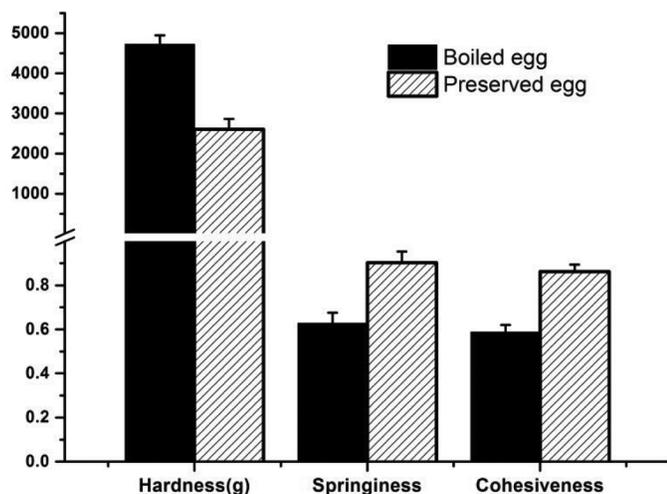
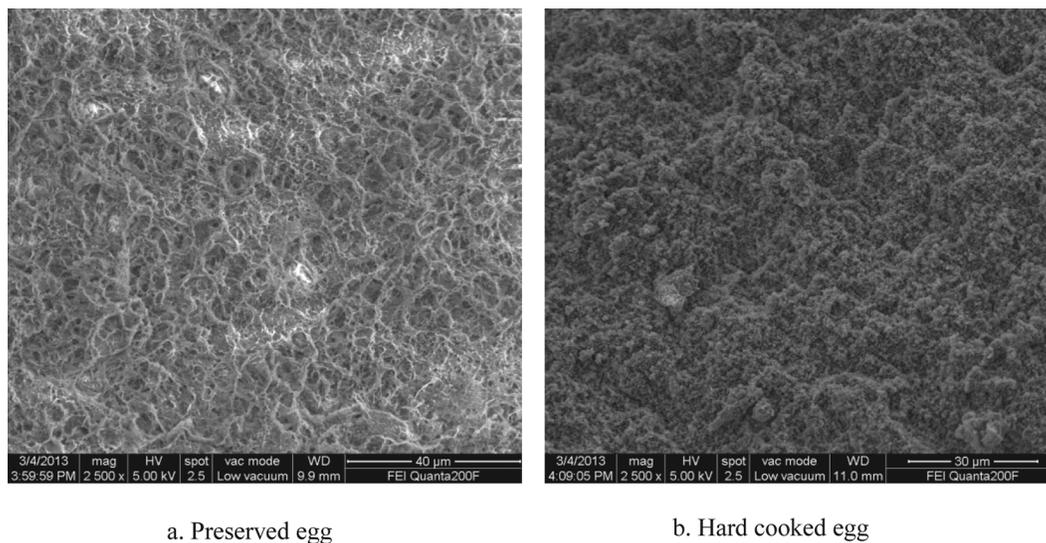


Figure 1. The texture properties of preserved egg white and hard-cooked egg white.



a. Preserved egg

b. Hard cooked egg

Figure 2. Environmental scanning electron microscopy of preserved egg white and hard-cooked egg white.

Amino Acid and Free Amino Acid Profile of Preserved Egg White

Egg white has high content of various proteins (Mine, 2008). Fresh duck egg white contains 17 amino acids and is rich in Ile, Gly, Phe, Met, and Glu (Table 3). There is no significant change in the contents of 17 amino acids between the fresh egg white and hard-cooked egg white. However, after fresh eggs were processed into preserved eggs, most of amino acids were noticeably decreased ($P < 0.05$), except for the level of Gly, which was significantly increased ($P < 0.05$). Arginine and Cys were not detected in preserved egg white.

The free amino acid analysis showed that fresh egg white, preserved egg white, and hard-cooked egg white all contained free Ala, Asp, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, and Val. There is no remarkable difference in the levels of free amino acids by comparison between fresh egg white and hard-cooked egg white. However, all of the amino acids levels in the preserved egg white were higher than those in the fresh egg white ($P < 0.05$).

These results indicated that short-time heat treatment has not affected the composition of amino acids and free amino acids of egg white. However, alkali treatment can degrade proteins into free amino acids.

Table 3. The amino acid and free amino acid profile of fresh egg white, preserved egg white, and hard-cooked egg white (g/100 g, mean \pm SD, $n = 3$)

Amino acid	Amino acid			Free amino acid		
	Fresh egg	Preserved egg	Hard-cooked egg	Fresh egg	Preserved egg	Hard-cooked egg
Ala	2.47 \pm 0.59 ^a	1.39 \pm 0.29 ^b	2.35 \pm 0.41 ^a	0.32 \pm 0.10 ^b	1.21 \pm 0.10 ^a	0.36 \pm 0.11 ^b
Arg	1.24 \pm 0.20 ^a	ND ¹	1.28 \pm 0.11 ^a	ND	ND	ND
Asp	7.92 \pm 0.51 ^a	6.42 \pm 0.36 ^b	7.16 \pm 0.72 ^a	0.43 \pm 0.05 ^b	1.64 \pm 0.12 ^a	0.39 \pm 0.09 ^b
Cys	0.90 \pm 0.20 ^a	ND	0.88 \pm 0.16 ^a	ND	ND	ND
Glu	12.93 \pm 0.62 ^a	11.48 \pm 0.78 ^b	12.88 \pm 0.32 ^a	1.12 \pm 0.08 ^b	3.06 \pm 0.26 ^a	1.14 \pm 0.06 ^b
Gly	9.05 \pm 0.39 ^b	15.32 \pm 1.10 ^a	9.35 \pm 0.69 ^b	0.98 \pm 0.04 ^b	3.05 \pm 0.21 ^a	0.91 \pm 0.05 ^b
His	4.82 \pm 0.40 ^a	3.64 \pm 0.32 ^b	4.66 \pm 0.34 ^a	1.06 \pm 0.15 ^b	2.51 \pm 0.06 ^a	1.11 \pm 0.16 ^b
Ile	8.64 \pm 0.43 ^a	6.92 \pm 0.14 ^b	8.59 \pm 0.36 ^a	1.23 \pm 0.11 ^b	2.94 \pm 0.10 ^a	1.18 \pm 0.08 ^b
Leu	6.78 \pm 0.48 ^a	5.31 \pm 0.29 ^b	6.77 \pm 0.58 ^a	1.39 \pm 0.07 ^b	2.92 \pm 0.14 ^a	1.41 \pm 0.08 ^b
Lys	5.73 \pm 0.69 ^a	4.33 \pm 0.30 ^b	5.69 \pm 0.44 ^a	0.72 \pm 0.02 ^b	2.20 \pm 0.07 ^a	0.73 \pm 0.04 ^b
Met	9.00 \pm 0.76 ^a	7.74 \pm 0.72 ^b	9.11 \pm 0.75 ^a	3.70 \pm 0.34 ^b	5.77 \pm 0.22 ^a	3.71 \pm 0.38 ^b
Phe	10.51 \pm 0.48 ^a	9.42 \pm 1.05 ^b	10.38 \pm 0.29 ^a	0.94 \pm 0.05 ^b	2.70 \pm 0.10 ^a	0.89 \pm 0.11 ^b
Pro	3.40 \pm 0.17 ^a	2.99 \pm 0.30 ^b	3.38 \pm 0.16 ^a	1.52 \pm 0.15 ^b	2.92 \pm 0.06 ^a	1.48 \pm 0.10 ^b
Ser	7.60 \pm 0.65 ^a	6.41 \pm 0.29 ^b	7.58 \pm 0.38 ^a	0.53 \pm 0.01 ^b	2.24 \pm 0.22 ^a	0.55 \pm 0.08 ^b
Thr	4.46 \pm 0.32 ^a	3.64 \pm 0.21 ^b	4.57 \pm 0.39 ^a	1.11 \pm 0.15 ^b	2.73 \pm 0.11 ^a	1.08 \pm 0.12 ^b
Trp	ND	ND	ND	0.54 \pm 0.02 ^b	2.43 \pm 0.22 ^a	0.55 \pm 0.04 ^b
Tyr	8.28 \pm 0.76 ^a	6.52 \pm 0.20 ^b	8.37 \pm 0.66 ^a	ND	ND	ND
Val	6.36 \pm 0.37 ^a	5.43 \pm 0.18 ^b	6.15 \pm 0.21 ^a	0.74 \pm 0.02 ^b	1.50 \pm 0.06 ^a	0.69 \pm 0.05 ^b
Essential AA	51.49 \pm 3.54 ^a	42.79 \pm 2.87 ^b	51.44 \pm 3.02 ^a	10.37 \pm 0.79 ^b	23.19 \pm 1.02 ^a	10.24 \pm 0.90 ^b
Total	107.64 \pm 7.46 ^a	95.56 \pm 6.22 ^b	109.33 \pm 6.97 ^a	16.33 \pm 1.38 ^b	39.82 \pm 2.04 ^a	16.18 \pm 1.55 ^b

^{a,b}Different superscripts in the same row indicate significant differences ($P < 0.05$).

¹ND = not detected.

Some amino acids can cross-link each other, resulting in the formation of cross-linked amino acids such as lysinoalanine during the alkali process (Chang et al., 1999a,b), and some amino acids may have produced the flavor compounds of preserved egg white. Therefore, most of the amino acids in egg white were decreased after alkali treatment.

Amino acids are not only important as nutrients, but they are also crucial in establishing food flavor. Free amino acids are taste compounds, and they can also cooperate and balance with each other (Nishimura and Kato, 1988; Nelson et al., 2002). Therefore, they are important factors in food flavor. Moreover, amino acids can also serve as flavor precursors; they can form volatile flavor substances via different kinds of reactions, such as deamination, transamination, decarboxylation, Maillard reaction with reducing sugars, interaction with lipids, and the Strecker degradation reaction (Cheng, 2010; Jelen, 2012; Trinh et al., 2012). Therefore, the decrease in amino acids and the increase in free amino acids may be vitally important to the specific flavor of preserved egg white.

Elemental Contents of Preserved Egg White

The contents of Al, As, Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, P, Pb, and Zn in preserved egg white were determined simultaneously by ICP-AES. Table 4 lists the amounts of the 14 minerals in the fresh egg white, preserved egg white, and hard-cooked egg white.

Mineral elements are essential to human health; they are one of the 7 kinds of nutrients that the body requires. The ICP-AES results showed that various inorganic elements are found in preserved egg white, which are particularly rich in Ca, Mg, Fe, Zn, Cu, K, and Na. With regard to the contents of essential major and trace minerals, nutrition of preserved egg white was positive. The results also presented that the contents of most of the inorganic elements, such as Al, Ca, Co,

Cr, Cu, Fe, K, Mg, Mn, Na, P, and Zn, in preserved egg white are higher than those in fresh egg white and hard-cooked egg white. The total element contents in preserved egg white were increased to approximately 190% compared with those in fresh egg, which is coincidental with the results of ash. Among the examined elements, Na content was increased to approximately 350%, which is mainly due to the NaOH and NaCl in pickling solution.

The Cu content in preserved egg reached as high as 29.11 ± 0.45 $\mu\text{g/g}$, which was significantly higher than that in fresh egg, 0.43 ± 0.02 $\mu\text{g/g}$. Preserved eggs are mainly pickled by alkaline. To prevent excessive alkali from injuring products during postpickling, appropriate amount of metal ions are added into the pickling liquid to regulate the infiltration of the alkali. In our experiment, 0.4% of CuSO_4 was added. The CuSO_4 permeates into an egg and forms an insoluble compound during pickling (i.e., CuS), which can plug shell and the membrane pores, mesh, and corrosion hole generated by the alkali processing (Zhao et al., 2010). Therefore, excessive infiltration of alkali is regulated. At the same time, the addition of CuSO_4 increases Cu content in preserved egg white. Although Cu is an essential and important nutrient, excessive levels can cause harm to the body. Excess Cu intake causes stomach upset, nausea, as well as diarrhea, and may also lead to chronic Cu toxicity, including liver cirrhosis, Indian childhood cirrhosis, and Tyrolean infantile cirrhosis (Uauy et al., 2008). The recommended daily allowance of Cu, which is established by the Food and Nutrition Board of the Institute of Medicine, National Academy of Sciences, is 0.9 mg/d for adult. A total daily Cu intake of >10 mg is considered unsafe. Preserved egg white prepared with CuSO_4 contained approximately 1 mg of Cu, which exceeds the daily adult demand. Therefore, new pickling technologies without the addition of metals for preserved egg production should be developed.

Table 4. The contents of elements in fresh egg white, preserved egg white, and hard-cooked egg white (mean \pm SD, $n = 3$)

Element ($\mu\text{g/g}$)	Fresh egg	Preserved egg	Hard-cooked egg
Al	0.58 ± 0.03^b	3.99 ± 0.18^a	0.56 ± 0.05^b
As	0.10 ± 0.01^a	0.11 ± 0.01^a	0.10 ± 0.01^a
Ca	49.35 ± 1.35^a	47.41 ± 2.79^a	52.18 ± 3.31^a
Co	ND ¹	0.01 ± 0.01	ND
Cr	ND	0.02 ± 0.01	ND
Cu	0.43 ± 0.02^a	29.11 ± 0.45^b	0.47 ± 0.03^a
Fe	1.19 ± 0.05^b	5.88 ± 0.49^a	1.11 ± 0.08^b
K	$1,499.33 \pm 90.39^a$	$1,410.73 \pm 69.33^a$	$1,559.98 \pm 45.55^a$
Mg	189.69 ± 9.36^b	221.32 ± 6.34^a	177.63 ± 13.52^b
Mn	0.03 ± 0.01^b	0.17 ± 0.03^a	0.03 ± 0.01^b
Na	$1,446.18 \pm 88.21^b$	$6,588.36 \pm 349.67^a$	$1,411.02 \pm 98.44^b$
P	128.38 ± 8.05^b	$1,355.33 \pm 76.38^a$	119.83 ± 11.46^b
Pb	0.04 ± 0.01^a	0.04 ± 0.01^a	0.03 ± 0.01^a
Zn	0.66 ± 0.04^b	2.51 ± 0.15^a	0.69 ± 0.05^b
Total	$3,315.96 \pm 197.53$	$9,664.99 \pm 505.85$	$3,323.63 \pm 172.52$

^{a,b}Different superscripts in the same row indicate significant differences ($P < 0.05$).

¹ND = not detected.

Table 5. The contents of vitamins in fresh egg white, preserved egg white, and hard-cooked egg white (mean \pm SD, n = 3)

Vitamin (mg/g)	Fresh egg	Preserved egg	Hard-cooked egg
Thiamine	0.004 \pm 0.001 ^a	ND ¹	0.003 \pm 0.001 ^a
Riboflavin	0.667 \pm 0.048 ^a	ND	0.623 \pm 0.069 ^a
Niacin	0.092 \pm 0.011 ^a	0.008 \pm 0.003 ^b	0.082 \pm 0.012 ^a
Pyridoxine	0.005 \pm 0.001 ^a	ND	0.003 \pm 0.001 ^b

^{a,b}Different superscripts in the same row indicate significant differences ($P < 0.05$).

¹ND = not detected.

Vitamin Content in Preserved Egg White

Egg white does not contain fat-soluble vitamins, but it does contain some water-soluble vitamins, including thiamine, riboflavin, niacin, and pyridoxine (Mine, 2008). In the current study, 4 kinds of water-soluble vitamins in fresh egg white, preserved egg white, and hard-cooked egg white were determined according to the Chinese national standards. The results in Table 5 showed that the thiamine, riboflavin, and niacin contents in hard-cooked egg white were similar to those in fresh egg, but the pyridoxine content was lower than that in fresh egg. Thiamine, riboflavin, and pyridoxine were not detected in preserved egg. The niacin content in preserved egg white was approximately 9% that of fresh egg. Above-mentioned results are related to the thermal stability and pH stability of these water-soluble vitamins. These results indicated that short heat treatment affected only the pyridoxine content, but alkaline treatment destroyed most of the examined water-soluble vitamins.

Conclusion

Preserved egg is produced by pickling with alkali for a long time. According to the results stated above, it could be concluded that preserved egg white is rich in a variety of nutrients such as protein and essential minerals. However, alkali treatment destroys some amino acids and vitamins in preserved egg white. At the same time, alkali treatment make fresh egg white become a gel with strong springiness and cohesiveness, and higher a^* , b^* values and lower L^* value. In the future, the forming mechanism of specific characteristics of preserved egg white should be studied, and some new technology should be built to control nutrient loss and safety of preserved egg in the pickling process.

ACKNOWLEDGMENTS

This study was financially supported by the Program of National Natural Science Foundation of China (no. 31101293, 31101321, 31360398, and 31460400) and the Open Project Program of State Key Laboratory of Food Science and Technology, Nanchang University (no. SKLF-KF-201008).

REFERENCES

- Campbell, L., V. Raikos, and S. R. Euston. 2003. Modification of functional properties of egg-white proteins. *Nahrung* 47:369–376.
- Chang, H.-M., C.-F. Tsai, and C.-F. Li. 1999a. Changes of amino acid composition and lysinoalanine formation in alkali-pickled duck eggs. *J. Agric. Food Chem.* 47:1495–1500.
- Chang, H.-M., C.-F. Tsai, and C.-F. Li. 1999b. Inhibition of lysinoalanine formation in alkali-pickled duck egg (Pidan). *Food Res. Int.* 32:559–563.
- Cheng, H. 2010. Volatile flavor compounds in yogurt: A review. *Crit. Rev. Food Sci. Nutr.* 50:938–950.
- China. 2010a. Chinese standard GB 5009.4–2010. Determination of ash in foods. The Standard Press of PR China, Beijing, China.
- China. 2010b. Chinese standard GB 5009.3–2010. Determination of moisture in foods. The Standard Press of PR China, Beijing, China.
- China. 2010c. Chinese standard GB 5009.5–2010. Determination of protein in foods. The Standard Press of PR China, Beijing, China.
- China. 2003a. Chinese standard GB/T 5009.89–2003. Determination of niacin in foods. The Standard Press of PR China, Beijing, China.
- China. 2003b. Chinese standard GB/T 5009.154–2003. Determination of vitamin B6 in foods. The Standard Press of PR China, Beijing, China.
- China. 2003c. Chinese standard GB/T 5009.47–2003. Method for analysis of hygienic standard of egg and egg products. The Standard Press of PR China, Beijing, China.
- China. 2008a. Chinese standard GB/T 9695.28–2008. Determination of riboflavin in foods. The Standard Press of PR China, Beijing, China.
- China. 2008b. Chinese standard GB/T 9695.27–2008. Determination of thiamine (vitamin B₁) in foods. The Standard Press of PR China, Beijing, China.
- Cordoba, J. J., T. Antequera, C. García, J. Ventanas, C. Lopez Bote, and M. A. Asensio. 1994. Evolution of free amino acids and amines during ripening of Iberian cured ham. *J. Agric. Food Chem.* 42:2296–2301.
- Croguennec, T., F. Nau, and G. Brule. 2002. Influence of pH and salts on egg white gelation. *J. Food Sci.* 67:608–614.
- Eiser, E., C. S. Miles, N. Geerts, P. Verschuren, and C. E. MacPhee. 2009. Molecular cooking: Physical transformations in Chinese century eggs. *Soft Matter* 5:2725–2730.
- Fernandez-lopez, J., A. Martinez, J. M. Fernandez-gines, E. Sayas-barbera, E. Sendra, and J. A. Perez-alvarez. 2006. Gelling and color properties of ostrich (*Struthio camelus*) egg white. *J. Food Qual.* 29:171–183.
- Handa, A., K. Takahashi, N. Kuroda, and G. W. Froning. 1998. Heat-induced egg white gels as affected by pH. *J. Food Sci.* 63:403–407.
- Hatanaka, Y., A. Yamauchi, O. Kobayashi, and T. Muro. 2009. Electron microscopic analysis of the effects of tea extract on strength improvement of egg white gels. *Food Sci. Technol. Res.* 15:5–10.
- Jelen, H. 2012. Food flavors: Chemical, sensory and technological properties. The Chemical Rubber Company Press, Boca Raton, FL.
- Matsuura, H., A. Hokura, F. Katsuki, A. Itoh, and H. Haraguchi. 2001. Multielement determination and speciation of major-to-

- trace elements in black tea leaves by ICP-AES and ICP-MS with the aid of size exclusion chromatography. *Anal. Sci.* 17:391–398.
- Mine, Y. 2002. Recent advances in egg protein functionality in the food system. *World's Poult. Sci. J.* 58:31–39.
- Mine, Y. 2008. *Egg Bioscience and Biotechnology*. John Wiley & Sons, Hoboken, NJ.
- Nelson, G., J. Chandrashekar, M. A. Hoon, L. Feng, G. Zhao, N. J. P. Ryba, and C. S. Zuker. 2002. An amino-acid taste receptor. *Nature* 416:199–202.
- Nishimura, T., and H. Kato. 1988. Taste of free amino acids and peptides. *Food Rev. Int.* 4:175–194.
- Salahinejad, M., and F. Aflaki. 2010. Toxic and essential mineral elements content of black tea leaves and their tea infusions consumed in Iran. *Biol. Trace Elem. Res.* 134:109–117.
- Su, H. P., and C. W. Lin. 1993. A new process for preparing transparent alkalised duck egg and its quality. *J. Sci. Food Agric.* 61:117–120.
- Tani, F., M. Murata, T. Higasa, M. Goto, N. Kitabatake, and E. Doi. 1993. Heat-induced transparent gel from hen egg lysozyme by a two-step heating method. *Biosci. Biotechnol. Biochem.* 57:209–214.
- Totosaus, A., J. G. Montejano, J. A. Salazar, and I. Guerrero. 2002. A review of physical and chemical protein-gel induction. *Int. J. Food Sci. Technol.* 37:589–601.
- Trinh, T.-T.-T., B. Yu, P. Curran, and S.-Q. Liu. 2012. Formation of aroma compounds during Longan juice fermentation by *Wil-*
liopsis saturnus var. *saturnus* with the addition of selected amino acids. *J. Food Process. Preserv.* 36:198–206.
- Tu, Y., Y. Sun, Y. Tian, M. Xie, and J. Chen. 2009. Physicochemical characterisation and antioxidant activity of melanin from the muscles of Taihe Black-bone silky fowl (*Gallus gallus domesticus* *Brisson*). *Food Chem.* 114:1345–1350.
- Tuzen, M., and M. Soylak. 2006. Evaluation of metal levels of drinking waters from the Tokat-Black sea region of Turkey. *Pol. J. Environ. Stud.* 15:915–919.
- Uauy, R., A. Maass, and M. Araya. 2008. Estimating risk from copper excess in human populations. *Am. J. Clin. Nutr.* 88:867S–871S.
- Wang, J., and D. Y. C. Fung. 1996. Alkaline-fermented foods: A review with emphasis on pidan fermentation. *Crit. Rev. Microbiol.* 22:101–138.
- Wong, D. W. S. 1989. *Mechanism and theory in food chemistry*. Avi/Van Nostrand Reinhold, New York, NY.
- Yang, X., D. Guo, J. Zhang, and M. Wu. 2007. Characterization and anti-tumor activity of pollen polysaccharide. *Int. Immunopharmacol.* 7:401–408.
- Zhao, W., R. Yang, Y. Tang, W. Zhang, and X. Hua. 2009. Investigation of the protein-protein aggregation of egg white proteins under pulsed electric fields. *J. Agric. Food Chem.* 57:3571–3577.
- Zhao, Y., M. S. Xu, and Y. G. Tu. 2010. Research progress in mechanisms of preserved egg processing. *Food Sci. (Chinese)* 17:472–475.