Effect of different alkali treatments on the chemical composition, physical properties, and microstructure of pidan white

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Abstract Changes in chemical composition, physical property and microstructure of pidan white treated with 4.5 % NaOH or 5.5 % KOH were monitored during pickling up to 4 weeks, and followed by aging for another 2 weeks. As the pickling and ageing times increased, moisture content of pidan white decreased and salt content increased for both (4.5 % NaOH and 5.5 % KOH) treatments ($P < 0.05$). Free alkalinity and pH of pidan white treated with 4.5 % NaOH increased as pickling proceeded, but decreased during ageing for both pickling treatments ($P < 0.05$). At week 4 of pickling, pidan white treated with 5.5 % KOH had higher hardness, cohesiveness, gumminess and chewiness than those treated with 4.5 % NaOH. After ageing, higher springiness, elastic modulus (G') and viscous modulus (G'') were generally found in pidan white treated with 5.5 % KOH ($P < 0.05$). As the pickling time increased, lower L^* , b^* values and higher a^* value were observed in pidan white from both treatments ($P < 0.05$). As visualized by scanning electron microscope, the aggregation of egg proteins took place in pidan white gels, irrespective of pickling treatments used. Nevertheless, closer and more orderly protein aggregates with denser network were founded in pidan white treated with 5.5 % KOH.

Keywords Egg · Pidán white · Pickling treatment · Textural property · Microstructure

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Introduction

Pickling is one of the oldest techniques in the food preservation as it not only extends the shelf life but also enhances the flavor and acceptability of the product (Ganasen and Benjakul 2011a). Pidán is usually made by the method of alkali-treated pickling of eggs with an extremely long shelf life (Wang and Fung 1996). Being one of the most traditional and popular pickled egg products, pidán is also known as alkalized egg, preserved egg, century egg and thousand-year egg in China and other South East Asian countries. Because of its special flavour and taste, pidán is adopted by most Chinese. Generally, pidán can be made of pickling duck or chicken eggs in NaOH solution at ambient temperature for a variable number of days (Chang et al. 1972; Su and Lin 1994; Chang et al. 1999; Chen and Su 2004; Zhang et al. 2011a). During pickling, the alkali penetrates the egg shell and membrane to induce chemical changes of the egg components. Blunt and Wang (1916) reported that both the white and yolk gradually became solidified and hardened during pidán process, on the other hand, the white turned brown, more or less like coffee jelly, and the yolk was changed to a greenish gray with concentric rings of different shades of gray. All changes occurring during the pickling of pidán possibly determine the preferential characteristics of pidán (Ganasen and Benjakul 2010). Usually, approximately 2 weeks is necessary for aging of pidán at room temperature after the pickling process.

NaOH is normally used for processing pidán during traditional techniques which causes the presence of a higher content of sodium in the final products. Ma and Tang (1999) reported that NaOH would increase sodium content of pidán, leading to an imbalance of dietary potassium and sodium content, which is one of the principal causes of hypertension. To explore the better benefit for consumers, the development of a new process using an alternative alkali is necessary in

order to replace the NaOH, in which traditional, safe pidan can be produced and are marketable. NaOH and KOH are the easily soluble alkalis in water, and they have similar chemical properties to make egg white form a gel. Na^+ and K^+ supplied by NaOH and KOH solution respectively could vary the action of gel formation via salt-mediated interactions of the egg white proteins. Different alkalis such as KOH could be used in the pickling and produce varying characteristics of the pidan white (Ma and Tang 1999). Addition of KOH at an appropriate level could be an alternative process to make pidan resulting in a reduction in the sodium content. It would also serve as an additional source of potassium for pidan. Information concerning pickling solution including KOH to process pidan, has rarely been reported (Ma and Tang 1999; Zhang et al. 2011b).

In general, the properties of pidan white including elastic texture with amber/brown color are used by consumers to evaluate the product. However, little information has been reported, including regarding changes in composition as well as characteristic of the pidan white, particularly during the pickled in the presence of KOH during the pickling and ageing process. The objective of this study was to investigate the changes in chemical composition, physical properties and microstructure of pidan white obtained with the aid of KOH during pickling and ageing for up to 6 weeks as compared with the pidan white treated with NaOH.

Materials and methods

Chicken egg collection

With the weight range of 55 to 60 g, fresh chicken eggs were obtained within 1 day of laying from a farm in Guangzhou, Guangdong province, China. The eggs were used within 3 days after laying and cleaned with tap water and checked for any crack prior to pickling.

Chemicals

Sodium hydroxide, potassium hydroxide, nitric acid and sodium chloride were all purchased from Guanghua Co., Ltd. (Guangzhou, China). Glutaraldehyde, ethanol, and silver nitrate were obtained from Merck (Darmstadt, Germany). Potassium thiocyanate was purchased from Sigma (St. Louis, Mo, USA).

Preparation of alkali-pickled chicken egg

Alkali-pickled chicken eggs were prepared by following the method of Zhang et al. (2011b). Fresh chicken eggs were separated into 2 groups. The first group was pickled in potassium hydroxide solution, a mixture of potassium hydroxide (5.5 %, w/v), salt (3.5 %, w/v), and Chinese tea (2.0 %, w/v). The second group was immersed in sodium

hydroxide solution, including sodium hydroxide (4.5 %, w/v), salt (3.5 %, w/v), and Chinese tea (2.0 %, w/v). For each treatment, additives including 0.1 % CuSO_4 , 0.1 % ZnSO_4 and 0.1 % FeSO_4 were added to the pickle solution. Chicken eggs of both groups were treated at the ambient temperature (24–26 °C) for 4 weeks. Pickled eggs were removed manually and washed with tap water until the shell was clean, then coated with liquid paraffin. Coated eggs were left at room temperature (24–26 °C) for another 2 weeks for ageing. During pickling and ageing, five egg whites were carefully randomly selected and pooled as the composite samples, which were then subjected to analyses.

Determination of moisture and NaCl contents of pidan white

Moisture content in pidan white samples was determined according to AOAC (2000). NaCl content in pidan white samples was measured by the method of AOAC (2000). These samples were combined with 20 mL of 0.1 mol/L AgNO_3 and 10 mL of HNO_3 . The mixture was boiled gently on a hot plate until all solids except AgCl_2 were dissolved (10 min). The mixture was cooled at ambient temperature (24–26 °C). Five millilitre of 5 % ferric alum indicator ($\text{FeNH}_4(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$) were then added. The mixture was titrated with the standardized 0.1 mol/L KSCN until the solution became permanently light brown. The percentage of NaCl was then calculated as follows:

$$\text{NaCl content (\%)} = 5.8 \times [(V_1 \times N_1) - (V_2 \times N_2)] / W$$

Where, V_1 =volume of AgNO_3 (mL); N_1 =concentration of AgNO_3 (mol/L); V_2 =volume of KSCN (mL); N_2 =concentration of KSCN (mol/L); and W=weight of sample (g).

Determination of pH and free alkalinity of pidan white

Pidan white samples with different treatments were analyzed for pH according to the method of Benjakul et al. (1997). To determine free alkalinity, samples were homogenized in 10 volumes water (w/v), and their pH values were immediately reduced with 0.1 mol/L hydrochloric acid to the neutral pH 7.0.

Determination of color of pidan white

The color of pidan white samples with different treatments was measured using a X-Rite SP62 colorimeter (X-Rite Inc., Glanville, Michigan, USA) and expressed as L^* (lightness), a^* (redness/greenness) and b^* (yellowness/blueness).

Determination of texture profile analysis (TPA) of pidan white

Pidan white samples with different treatments were subjected to TPA by the method of Kaewmanee et al. (2011) with a slight modification. TPA was performed as described by Bourne

(1978) with a TA-XT Plus texture analyzer (Stable Micro Systems, Surrey, England). The pidan white at the narrow side was elliptical in shape and was manually cut into a cube of $1.5 \times 1.5 \times 1.5 \text{ cm}^3$ and subjected to TPA analysis at room temperature ($24 \sim 26^\circ \text{C}$). Prepared samples were compressed twice to 50 % of their original height with a compression cylindrical aluminum probe (36 mm diameter). Pre- and post-test speeds were 1 and 5 mm/s respectively. The time between the 2 compression cycles was 5 s. Force-distance deformation curves were recorded at a cross head speed of 5 mm/s. Parameters including hardness, springiness, cohesiveness, gumminess and chewiness were obtained by using the Micro Stable software (Stable Micro Systems, Surrey, England).

Determination of rheological property of pidan white

The rheological measurements of pidan white samples with different treatments were performed by small-deformation rheology using a rheometer MCR101 (Anton Parr, Austria). A 50 mm stainless steel parallel plate geometry with a 1 mm gap was used. Frequency sweeps were carried out from 0.1 to 100 rad/s at a constant oscillatory strain of 0.5 %, which was within the linear region. The elastic modulus (G') and viscous modulus (G'') were recorded for samples in triplicate.

Determination of microstructure of pidan white

Microstructures of pidan white were analyzed using a scanning electron microscope following the slightly modified method of Kaewmanee et al. (2011). The pidan white was cut into a small piece ($0.3 \times 0.3 \times 0.3 \text{ cm}^3$). Samples were fixed at the ambient temperature ($24 \sim 26^\circ \text{C}$) in 2.5 % glutaraldehyde in 0.1 mol/L phosphate buffer (pH 7.2) for 4 h. Fixed samples were rinsed with 0.1 mol/L phosphate buffer (pH 7.2) for 20 min and with distilled water for 20 min. Prepared samples were dehydrated in graded series of ethanol (30 %, 50 %, 70 %, 90 %, and 100 %, v/v) for 10 min each. All specimens were coated with gold. The microstructure was visualized using a scanning electron microscope (XL-30-ESEM, Amsterdam, Holland).

Statistical analysis

The experiments were run in triplicate using 3 different lots of eggs. For each analysis, it was conducted in triplicate. Data were presented as mean $\pm$ standard deviation. One-way analysis of variance (ANOVA) was carried out and means comparisons were performed by Duncan's multiple range tests. Statistical analyses were done with the statistical program (SPSS 18.0 for Windows, SPSS Inc., Chicago, U.S.A.).

Results and discussion

Changes in chemical composition of pidan white during pickling and aging

Changes in moisture, salt content, pH and free alkalinity of pidan white obtained from NaOH and KOH during pickling and aging were determined (Table 1). It was found that the moisture content of both NaOH and KOH treated egg white decreased up to 6 weeks of the pickling and ageing times, and the salt content increased ($P < 0.05$). The moisture content of pidan white treated with KOH decreased noticeably. At 4 weeks of pickling, moisture content decreased from 87.32 to 84.59 % and 83.62 % for pidan white obtained from NaOH and KOH, respectively. The greater reduction of water from the pidan white was most possibly caused by osmosis process, since water could migrate from the egg white to egg yolk and the pickling solution (Chi and Tseng 1998). During ageing, the moisture content decreased slightly, regardless of pickling treatments. Nevertheless, the lowest moisture content was found in pidan white treated with NaOH and KOH at week 6 (82.82 % and 81.88 %, respectively). For pidan white, the moisture content of egg white from KOH treatment was slightly lower than that from NaOH treatment at the same pickling and ageing times. During pickling, the solidification of pidan white was due to the dehydration induced by series of alkalis. It was inferred that the lower moisture content found in the pidan white treated with KOH was probably associated with higher cross-linking of egg white proteins.

However, the pidan white had a remarkable increase in salt content during pickling, regardless of treatments. No change in salt content of pidan white treated with NaOH or KOH at the same week 4 was observed ($P > 0.05$). As compared with pickling period, the lower change in salt was obtained during aging. No difference in salt content was observed in pidan white treated with KOH ($P > 0.05$) at week 4 and 6, and increase in salt content was obtained in pidan white from NaOH treatment ($P < 0.05$). After ageing, the higher salt content was found in pidan white obtained from NaOH treatment than that treated with KOH ($P < 0.05$). The salt might be associated with the formation of gel-like structure of egg white proteins resulting from destabilizing of the protein molecules in egg white or inducing the coagulation of egg white proteins, especially in an alkaline solution. Disordered network in the presence of high salt could not maintain water effectively, indicating the dehydration of pidan white was accompanied with more concentrated salt content in pidan white. Consequently, gelation could be hardened by the increased concentrations of proteins resulted from the reduction of moisture content of pidan white during pickling and ageing.

It was also found that the pH and free alkalinity of egg white increased with increasing pickling time, then decreased

Table 1 Moisture content, salt content, pH and free alkalinity of pidan white treated with NaOH and KOH during pickling and aging

Pickling treatments	Pickling/Aging time (week)	Parameters			
		Moisture content (%)	Salt content (%)	pH	Free alkalinity (mg/100 g)
No pickling (fresh egg)	0	87.32±0.02 ^a	0.05±0.01 ^f	9.34±0.03 ^d	63.33±3.66 ^e
NaOH	2	85.65±0.04 ^b	0.20±0.01 ^e	11.15±0.03 ^b	402.53±9.78 ^c
	4	84.59±0.09 ^d	0.40±0.01 ^c	11.25±0.02 ^a	420.51±14.20 ^a
	6	82.82±0.15 ^f	0.45±0.03 ^a	10.68±0.03 ^c	241.37±7.40 ^d
KOH	2	84.77±0.05 ^c	0.26±0.01 ^d	11.13±0.03 ^b	405.77±7.19 ^{ab}
	4	83.62±0.02 ^e	0.40±0.02 ^{bc}	11.16±0.01 ^b	406.58±12.68 ^{bc}
	6	81.88±0.09 ^g	0.42±0.01 ^b	10.65±0.01 ^c	237.81±2.47 ^d

^a Different superscripts in the same column indicate significant differences ($P<0.05$)

Values are mean ± standard deviation ($n=3$)

noticeably during ageing, regardless of pickling treatments (Table 1). Free alkalinity and pH of pidan white increased up to 2 weeks of pickling, regardless of treatments ($P<0.05$). No difference in pH and free alkalinity was found in pidan white obtained from KOH treatment ($P>0.05$) at week 2 and 4, while the increase in pH and free alkalinity was observed in pidan white treated with NaOH ($P<0.05$). At the same pickling or aging time (week 2 or 6), there was no difference in pH of pidan white obtained from both treatments ($P>0.05$). Remarkable change in pH and free alkalinity was observed in pidan white obtained from both treatments ($P<0.05$) at the same week 4 of pickling, but pidan white treated with KOH had the lower pH and free alkalinity. The pH of pidan white obtained from KOH treatment after 4 weeks of pickling was 11.16, and a final pH 11.12 of pidan white pickled for 20 days was reported by Chang et al. (1999). At an alkaline pH, protein molecules have negative net charges on their surfaces with repulsive forces that increase, decreasing the protein-protein interactions and increasing the protein-water interactions (Ganasen and Benjakul 2011a). Consequently, alkali might have a significant effect on the aggregation of egg white proteins, resulting in the formation of a gel-like structure.

Changes in textural properties of pidan white during pickling and aging

Textural properties of pidan white obtained from both treatments during pickling and ageing were shown in Fig. 1. Since it could not withstand the compression of analyzer, TPA of pidan white could not be detected at week 1 due to weak aggregates of egg white.

Hardness of pidan white obtained from both treatments increased continuously and reached the maximum at week 6 ($P<0.05$) (Fig. 1a), which indicated that the gel strength of pidan white of both treatments became more resistant to compression, most possibly due to the continually enhanced

aggregation of egg white proteins along with alkali penetration and salt migration. At the same pickling or ageing time (week 2 or 6), no difference in hardness was found between pidan white obtained from both pickling treatments ($P>0.05$). However, during week 3 to 5 of pickling and ageing, the hardness of pidan white obtained from KOH treatment was higher than that found in NaOH treatment. The result showed that KOH treatment tended to be more effective in protein aggregation than the NaOH treatment. No difference in hardness was observed in pidan white treated with KOH ($P>0.05$) during ageing, while the increase in hardness was obtained in pidan white from the NaOH treatment ($P<0.05$). The result was likely owing to the decrease in pH of egg white, which might lower repulsive force between protein molecules and strengthen the protein gel network previously formed to lead to a fine structure of protein aggregates.

Springiness and cohesiveness reflected the development of internal bonding in 3-dimensional egg white gels network. For springiness, no difference in pidan white was obtained from both treatments at week 2 to 5 of the pickling and ageing ($P>0.05$), and no difference was obtained between pidan white from both treatments ($P>0.05$) at the same pickling and ageing times (week 2 to 5) (Fig. 1b). During ageing, no change in springiness was found in pidan white treated with KOH ($P>0.05$), while the slightly decrease in springiness was obtained in pidan white from the NaOH treatment ($P<0.05$), and the springiness of pidan white from KOH treatment was a little higher than that from NaOH treatment ($P<0.05$). Cohesiveness of pidan white increased up to 2 week of pickling, regardless of treatments ($P<0.05$) (Fig. 1c), in accordance with the change in hardness of pidan white. During ageing, the change in cohesiveness was comparable with the change in springiness of pidan white, but no difference in cohesiveness was found in pidan white from both treatments at the same ageing times ($P>0.05$). The result was in good agreement with Ganasen and Benjakul (2011a)

Fig. 1 Hardness (a), springiness (b), cohesiveness (c), gumminess (d) and chewiness (e) of pidan white obtained from NaOH and KOH treatments during pickling and ageing. Bars represent the standard deviation ($n=3$). Different letters on the bar indicate significant differences ($P<0.05$)

who reported that cohesiveness of pidan white was slightly decreased during ageing.

Gumminess and chewiness of pidan white from both treatments increased up to 5 weeks of pickling and ageing ($P<0.05$) (Fig. 1d and e). No difference in gumminess and chewiness was observed in pidan white obtained from KOH treatment ($P>0.05$) during ageing, while an increase in gumminess and chewiness was obtained in pidan white from the NaOH treatment ($P<0.05$). At the same pickling or ageing time (week 2, 3 or 6), no difference in gumminess was found between pidan white obtained from both pickling treatments ($P>0.05$). However, the chewiness of pidan white from KOH treatment was slightly higher than that from NaOH treatment during ageing ($P<0.05$), indicating the stronger gel-like structure of egg white proteins. This result might be attributed to the lowered moisture content of pidan white.

Changes in rheological property of pidan white

Parameters obtained from rheological measurements were the storage or elastic modulus G' , which was a measure of the amount of energy that was stored during a periodic application of stress or strain, the loss or viscous modulus G'' , which was a measure of the energy loss (Foegeding et al. 2003; Montesinos-Herrero et al. 2006). Rheological properties of

pidan white obtained from both treatments during pickling and ageing were shown in Fig. 2. G' and G'' of pidan white obtained from both treatments were increased continuously and reached the maximum at week 6 ($P<0.05$), which was comparable with the change in hardness of pidan white. This result indicated the gel-like structure of egg white was becoming stiffened and more compact along with increasingly pickling and ageing times. Weijers et al. (2002) reported that a high concentration of protein was needed to form a gel, and once a gel was formed an increase in gel concentration resulted in a large increase in G' . No difference in G' was observed in pidan white obtained from NaOH treatment ($P>0.05$) during week 4 to 5 of pickling and ageing. At the same pickling (week 1 to 4), no change in G' and G'' was found in pidan white obtained from both treatments ($P>0.05$), while the G' and G'' of pidan white from KOH treatment was a little higher than that from NaOH treatment ($P<0.05$) at the same ageing (week 5 to 6). This result indicated pidan white treated with KOH might form a stronger three-dimensional protein network.

Changes in microstructure of pidan white after pickling

Microstructures of pidan white obtained from both treatments after pickling for 4 weeks visualized by SEM were illustrated in Fig. 3. Aggregation of egg white protein molecules was

Fig. 2 Elastic modulus (G') and viscous modulus (G'') of pidan white obtained from NaOH (**a** and **b**) and KOH (**c** and **d**) treatments during pickling and ageing

noticeable in pidan white treated with NaOH and KOH (Fig. 3). Pidán white treated with KOH showed closer aggregates with a denser network, while the pidán white obtained from NaOH treatment exhibited a little looser network with many small irregularly shaped voids. As compared with those of pidán white treated with KOH, less uniformity was observed in pidán white obtained from NaOH treatment. The lower hardness might be reflected by less ordered network. With the more compact structure of protein

aggregates, pidán white from KOH treatment might be more stable under the increasing alkaline condition, especially as the pickling or aging time increased.

Changes in the color of pidán white during pickling and aging

The color of pidán white obtained from both treatments during pickling and aging was shown in Table 2. L^* value of pidán white decreased continuously as the pickling and aging times

Fig. 3 Scanning electron microscopic photograph of pidán white obtained from NaOH (**a**) and KOH (**b**) treatments at week 4 of pickling. Magnification: 10000 $\times$. Scale bar=5 μ m; P = protein aggregate; arrow head = voids

Table 2 Color of pidan white obtained from NaOH and KOH treatments during pickling and aging

Pickling treatments	Pickling/Aging time (week)	Color		
		L^*	a^*	b^*
No pickling (fresh egg)	0	25.65±0.18 ^a	−0.53±0.10 ^d	3.98±0.35 ^a
NaOH	2	20.99±0.32 ^c	0.32±0.07 ^c	3.59±0.33 ^b
	4	19.63±0.06 ^c	2.71±0.27 ^a	1.26±0.19 ^c
	6	19.04±0.16 ^f	0.15±0.02 ^c	−0.42±0.07 ^d
KOH	2	22.30±0.39 ^b	1.68±0.17 ^b	3.43±0.34 ^b
	4	20.10±0.11 ^d	2.85±0.15 ^a	1.44±0.21 ^c
	6	19.12±0.33 ^f	0.23±0.03 ^c	−0.48±0.07 ^d

^a Different superscripts in the same column indicate significant differences ($P<0.05$)

Values are mean ± standard deviation ($n=4$)

increased up to week 6, regardless of treatments ($P<0.05$). This result was in good agreement with Chen and Su (2004) who reported that lightness decreased along with the increasing pickling and ageing times. During pickling, L^* value of pidan white treated with KOH was higher than that of pidan white obtained from NaOH treatment ($P<0.05$). This was most likely owing to the higher aggregation of proteins, resulting in the formation of larger coagulate with more surface area which exhibited higher light-scattering effect (Kaewmanee et al. 2011; Ganasen and Benjakul 2011a). However, no difference in L^* value of pidan white obtained from both treatments was observed ($P>0.05$) during ageing. With the significant amount of glucose naturally presented in the egg white proteins, Maillard reaction had remarkable impact on color of pidan white (Ganasen and Benjakul 2011c). Increase of a^* value was found in pidan white from both treatments during pickling ($P<0.05$), most likely due to the formation of brown pigments, which might derive from the Maillard reaction between the glucose and amino acid in egg white (Wang and Fung 1996; Li and Hsieh 2004). This was in agreement with Ganasen and Benjakul (2011a) who reported the browning intensity in pidan white increased with increasing pickling times. At week 6, the lower a^* value was found in pidan white ($P<0.05$), irrespective of treatments. This was most likely due to more cross-linking of protein molecules reflected by the higher hardness of pidan white, hence amino groups were less available for Maillard reaction. Color of b^* value in pidan white from both treatments decreased with increasing pickling and ageing times ($P<0.05$), possibly owing to the formation of green or brown pigments. Furthermore, black tea in pickling solution might enhance the brown color in the pidan white due to oxidation of flavonols in the alkaline environment (Ganasen and Benjakul 2011b). The changes in the color of pidan white from both treatments during pickling and ageing might be related to moisture loss, because the lowered moisture was associated with the increased concentration of the pigments (Kaewmanee et al. 2011). No change in a^* and b^* value was found in pidan white obtained from both treatments ($P>0.05$) at the same pickling or ageing time (week 4 or 6), which was illustrated by

the similar appearance observed between pidan white obtained from both pickling treatments.

Conclusion

Characteristics of egg white were remarkably affected by alkalinizing. Different alkali treatments had varying influence on texture properties and color of the pidan white to some degree. Pidant white treated with KOH showed the more stabilizing effect on gel formation during pickling than that treated with NaOH, which reflected from the higher hardness, cohesiveness, gumminess and chewiness of pidan white. Furthermore, the closer and more ordered proteins aggregates with denser network were more pronounced in pidan white treated with KOH. However, it was noted that the similar appearance was observed between pidan white obtained from both pickling treatments after ageing. Therefore, the use of other alkalis such as KOH might be an alternative alkali for pidan production.

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