Optimization of Process Parameters in Two-Stage Brining of Salted Eggs with Low NaCl Content

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***Abstract*—Salted duck egg is one of the most traditional and popular preserved egg products in Asian countries. In this article, the processing technology of salted eggs with low NaCl content was studied, the study include the effects of chlorine dioxide pretreatment on the microbial of eggshell, and a two-stage immersion process on salt content in egg white and quality of salted eggs. The optimized process included the following steps: eggs were washed with water, disinfected with 30.0–50.0 mg/L stabilized chlorine dioxide for 3–5 min, salted in 16.0%–20.0% salinity first brine solution for 10 days, then the eggs were transferred to the second brine solution containing 4.0%–6.0% salt, 0.05%–0.10% anise and Chinese prickly for 20–25 days. Our optimized procedure could produce salted duck eggs of high quality with low salt content in the egg white as well as high oil content in the egg yolk.**

***Index Terms*—chlorine dioxide pretreatment, two-stage brining, salted eggs, low NaCl content**

I. Introduction

As one of the most traditional and popular preserved egg products, salted eggs contain abundant nutrients, including proteins, amino acids, lipids, vitamins, and many trace elements [1], [2]. Conventionally, salted eggs are prepared from duck eggs because they have more desirable characteristics than hen eggs [3]. The salting process removes moisture and allows for the diffusion of salt into the egg yolk and white [4], this decreases the viscosity of the egg white, reducing whipping capability and making the egg watery. An orange color, oil exudation and gritty texture are desirable characteristics of the salted egg yolk [5].

Salting methods impact the characteristics and properties of egg white and yolk, particularly after cooking [4]. Generally, salted duck eggs can be processed using two methods. One method involves brining eggs in saline for approximately 15–30 days, and another method involves coating the eggs with soil paste-like mixture consisting of red soil, salt and water for the same amount of time [6], [7]. Some researchers have reported the effects of coating or one-stage immersion methods on chemical composition and textural properties [8]. Compared to the coating method, the immersion method is faster and more convenient for the production of salted eggs [9]. Some new technology such as pulsed pressure has been employed to enhance the mass transfer of osmotic dehydration in the immersion method [5]. Two immersion methods have been reported for producing salted eggs, which involve either one or two stages. One-stage immersion is a traditional salting process, in which duck eggs are directly salted in high salt liquid. Two-stage immersion involves sequential salting of the duck egg at two concentrations of salt liquids. Compared to one-stage immersion, the final NaCl content of the egg albumen and yolk in two-stage immersion can be better controlled, and the problems associated with the uneven distribution of salt in egg albumen and yolk may be resolved. Two-stage immersion may also decrease the amount of salt, and the salt solution can be recycled for next use. Our goal was to optimize a process to produce low NaCl salted eggs containing low NaCl content in the egg white, making the eggs taste better and healthier compared to traditional salted eggs, because high intake of salt have been linked to high blood pressure and heart disease [10]. Therefore, a two-stage immersion method was developed in this study.

The surface of fresh duck eggs always stuck with contaminations such as feathers, blood, feces and soil, and amount of microorganism on egg's surface was very high, therefore, immersion method may lead to the accumulation of a large number of microorganisms, even pathogens such as Salmonella Enteritidis in the brine solution, thus contaminating the salted eggs [11], [12]. A previous study showed that in each egg, the amount of microbiota on the surface could be 107-109 colony-forming units (CFU). Furthermore, the microorganisms residing on the surface of the egg shells can form biofilms, protecting the bacteria from disinfection and allowing the salmonellae to recover and grow during salting [13], [14]. Therefore, the surface of fresh duck eggs should be cleaned and sanitized to ensure food safety and quality. Acetic acid, protease and sodium dodecyl sulfate were reported for the pretreatment of eggs before salting [9], [15]. In addition to these agents, chlorine dioxide (ClO_2) is a powerful oxidizing agent that is 2.5-fold more effective than chlorine as an antimicrobial and is less corrosive, thus has been widely used in the food industry such as drinking water treatment, food processing and preservation [16], [17]. Studies showed that chlorine dioxide could inactivated pathogens on the surface of eggshells and did not damage to the surface of eggshells (cuticle layer), thus did not promote microbial penetration through the eggshell [18],

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Therefore, chlorine dioxide was employed to pretreat duck eggs in our research.

Therefore, the aims of this work were to optimize the pretreatment procedure, using chlorine dioxide and to optimize a two-stage immersion procedure, focusing on the effect of the process parameters on NaCl content in egg white and oil content in egg yolk, to produce salted egg with low NaCl concentration.

II. Materials and Methods

A. Materials and Chemicals

Fresh duck eggs (*Anas plotyrhyncus*) with weight range of 65–75 g were obtained from a local producer in Tonglu, Zhejiang Province, China. $AgNO_3$, $FeNH_4(SO_4)_2 \cdot 12H_2O$, HNO3 and KSCN were purchased from Huadong Medicine Co. Ltd. (Hangzhou, China). Anise, Chinese prickly ash, buckwheat liquor and NaCl were purchased from local food market.

B. Analysis of Salt Content

Salt content in egg white was measured by the method of AOAC [19]. Briefly, sample (1 g) was treated with 20 ml of 0.1 N $AgNO_3$ and 10 ml of HNO_3. The mixture was boiled gently on a hot plate until all solids except $AgCl_2$ were dissolved (usually 10 min). The mixture was cooled using running water. Five ml of 5% ferric alum indicator ($FeNH_4(SO_4)_2 \cdot 12H_2O$) were added. The mixture was titrated with the standardised 0.1 N KSCN until the solution became permanently light brown. The percentage of salt was then calculated as (1).

$$\text{Salt content}(\%)=5.8\times[(V_1\times N_1)-(V_2\times N_2)]/W \quad (1)$$

where V_1 is the volume of $AgNO_3$ (ml); N_1 is the concentration of $AgNO_3$ (N); V_2 is the volume of KSCN (ml); N_2 is the concentration of KSCN (N); and W is the weight of sample (g).

C. Yolk Index (YI)

YI were measured by the method of Wang with some modifications [20], briefly, break eggshell of salted egg carefully, gently poured the contents on a horizontal glass plane, after 2 min, measure the height (H_1) and diameter (H_2) of egg yolk of egg yolk by calipers, YI was calculated as (2).

$$YI = H_2/H_1\times 100\% \quad (2)$$

D. Preparation of Salted Duck Eggs with Low NaCl Content

Fresh duck eggs were washed with water, and pretreated with 10.0, 20.0, 30.0, 40.0 or 50.0 mg/L chlorine dioxide solutions for 1, 2, 3, 4 or 5 min, respectively. Then duck eggs were prepared using the two-stage immersion method. In the first stage, duck eggs were immersed in the first brine solution with high salt concentration (14.0, 16.0, 18.0, 20.0 or 22.0%,w/w) for less than 10 days, and then the duck eggs were moved to the second brine solution with low salt concentration (4.0, 5.0, 6.0, 7.0 or 8.0%,w/w) for another 20-25 days. The second brine solution was diluted with the first brine solution as a stock solution, thus the salting solution could be reused and could reduce waste emissions. In some cases, 0.1, 0.2, 0.3, 0.4 or 0.5%（w/w） of buckwheat liquor (alcohol concentration was 52%,v/v) and two spices, anise and Chinese prickly ash, were added to the second brine solution. The eggs were salted at 15 ℃, 25 ℃ and 35 ℃ during the whole immersion, respectively.The salt content in the egg white was measured every 5 days. The experiments were conducted in triplicate.

E. Preparation of Second Brine Solution with Spice

Two spices, anise and Chinese prickly ash, were packed in a gauze bag and placed in a pot with salt and water. The proportion of the two spices and water were both 0.05%-0.10%（w/w）. After the pot was boiled for 3 min, the gauze bag was removed and cooled to room temperature. The soup will be used as the first and second brine solution with spice.

III. Results and Discussion

A. Pretreatment of Eggs

Fresh duck eggs are often contaminated with contaminations such as feathers, blood, feces and soil. In order to reduce costs, some enterprises employ the traditional salting process, called one stage immersing. Using this method, duck eggs were directly salted in saturated brine solution, however, the quality was not good, and salt content in egg white was too high. The desirable NaCl content in the egg albumen and yolk of the final product should be approximately 4.0% and below 1.5%, respectively, and the egg yolk should have high oil exudation (OE) and gritty texture [9], it is difficult to meet these requirement using one stage immersion. Additionally, the brine liquid could not be reused, as the content of organic matter in brine solution was very high, and the liquid would even always stinking. Thus, in order to clean eggs and reduce microbial content on the egg surface, one of the key technology of our two-stage egg salting process is cleaning the surface of the eggs before salting. Stabilized chlorine dioxide is a highly effective disinfectant because it is broad-spectrum and has no by-products and residue, making it widely used to disinfection in water, food and hospitals.

Fresh duck eggs were washed with water to wash away feathers and feces, then the total number of colonies on the egg surface was measured. After washing, the total number of colonies was decreased from 9.23 ± 0.96 to 6.02 ± 0.72 lg(CFU/egg), and the microorganisms on the surface of egg were decreased by 34.8%.

Next, the cleaned eggs were further sterilized with stabilized chlorine dioxide, which was dissolved into water to get 10.0, 20.0, 30.0, 40.0 and 50.0 mg/L disinfectant solution, and the eggs were added to the solution for 1, 2, 3, 4 and 5 min, respectively. The number of colonies was measured (Fig. 1). The total number of colonies on the egg surface decreased after treatment with stabilized chlorine dioxide, and the inhibition rate enhanced with increasing of stabilized chlorine dioxide concentration. For example, when the eggs were treated with 10.0 and 20.0 mg/L stabilized chlorine dioxide for 5 min, the inhibition rates were 17.9 and 39.6%, respectively, and the inhibition effect was not obvious. However, when the concentrations of stabilized chlorine dioxide were 30.0-50.0 mg/L, a strong antibacterial effect was observed even after 3 and 5 min treatment, the total number of colonies was decreased to less than 102 and 101 CFU/egg, respectively. Thus, the optimal procedures for pretreatment included washing and disinfecting the eggs with 30.0-50.0 mg/L stabilized chlorine dioxide for 3-5 min.

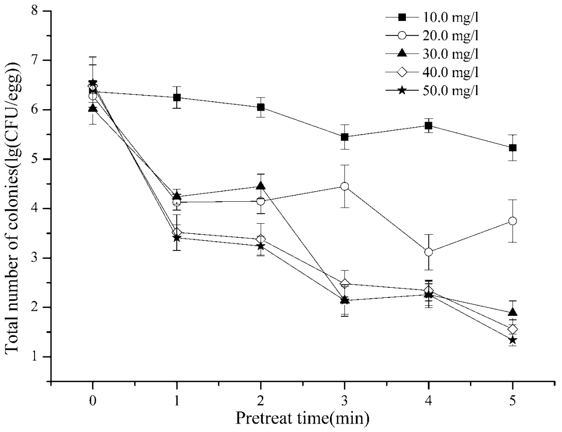

Figure 1. Changes of total number of colonies on eggs surface after pretreatment by ClO_2. Bars represent standard deviation from triplicate determinations.

B. *Effect of Salt Concentration in the First Brine Solution on Salt Content in Egg White*

Eggs were cleaned and disinfected, salted in the first brine solutions with salt concentrations of 14.0, 16.0, 18.0, 20.0 and 22.0%, respectively, and then 0.1% buckwheat liquor (alcohol concentration was 52%) was added to the surface of the liquid to seal the pot. The salting temperature was 25 ℃. The salt content in duck white was measured in every 5 days (Fig. 2)

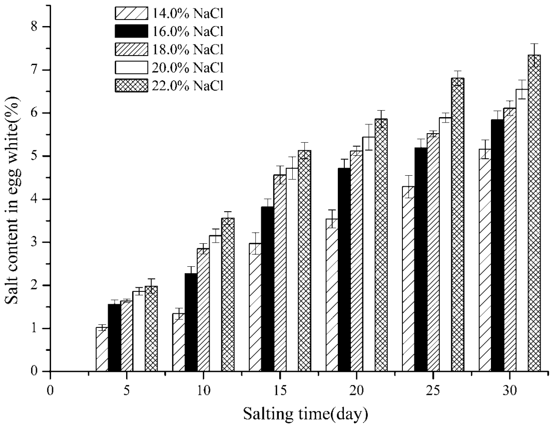

Figure 2. The effect of NaCl concentration in the first brine solution on the salt content of egg white. Bars represent standard deviation from triplicate determinations.

Before replace by the second brine solution, the optimal salt content in the egg white should be 2.0%-3.0%. The salting process can remove moisture from the egg yolk and salt diffuses into the egg white and yolk [6], thus the salt content in the egg white was increased with increasing salting time, and higher the salinity in the first brine resulted in a faster increase in the salt content in egg white. When the salt content of the first brine solution was 14.0%, the eggs should be salted for 15 days, greatly prolonging the processing cycle and reducing production efficiency. The salt content in egg white was 2.0%-3.0% after salting in the first brine solutions at salinities of 16.0, 18.0 and 20.0% for 10 days. Using this salt content, the first brine solution could be replaced with the second brine solution. After salting in the salinities of 22.0% for 10 d, the salt content in egg white was 3.56%, higher than 3.0%. Therefore, the selected salt content of the first liquid was 16.0%-20.0%, which was suitable for large-scale production process.

C. *Effects of Salt Concentration in the Second Brine Solution on Salt Content of Egg White and Quality of Salted Eggs*

After salting in the first brine solution at salinity of 18.0% for 10 days, the eggs were moved to a new jar for the second salting step. In this step, the salt concentration of the solution in each jar was 4.0, 5.0, 6.0, 7.0 and 8.0%, respectively, and the salt content in egg white was measured every 5 days. The result was depicted in Figure 3. As expected, the salt content in egg white increased with increasing salting time, and higher salt concentration in the immersion solution resulted in a higher penetration rate of salt into egg white.

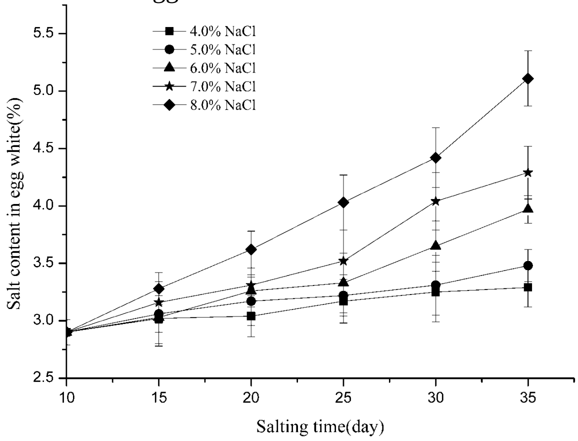

Figure 3. The effect of NaCl concentration in second brine solution on the salt content of egg white. Bars represent standard deviation from triplicate determinations.

To prepare salted eggs with low NaCl content, salt content in the egg white should not exceed 4.0%. We found that when eggs were salted in 4.0% second brine solution for 35 days, the salt content in egg white was 3.29%. When salted in 5.0% and 6.0% brine solutions, salt content increased to 3.45% and 3.97%, respectively. When eggs were salted in 7.0% second brine solution for 25 days, the salt content in egg white was 3.52%. After salting in 8.0% solution for 20 days, salt content in the egg white was 3.65% (Fig. 3). Therefore, increasing the salt concentration of the second brine solution could shorten the salting time. However, in such short time, it is difficult to form oil exudation and oily egg yolk. Oil exudation is generally one of desirable characteristics of salted eggs, which is an important index of salted duck egg quality [21]. Moreover, higher salt concentration could increase the salt usage amount, and increase the cost of production as well. If the salt concentration in brine solution was less than 4.0%, the salting time could be extend, but longer salting time could reduce the production efficiency, and more importantly, duck eggs would prone to become black and rotten during the second brine procedure when the salt concentration in brine solution was less than 4.0%, and yolk oil could not formed as well. Thus, the salt concentration in the second brine solution should be 4.0%-8.0%.

A high oil-off ratio of yolk occurred after 4-6 weeks of brining, indicating that the salted duck eggs were high quality. This might be related to the solidification of yolk protein with low NaCl content and low moisture [6], [7]. To examine the effect of salt concentration of the second brine solution and salting time on the quality of duck eggs, YI and oil content in the egg yolk were analyzed. YI is an indicator of the maturity of salted duck eggs. A lower YI value indicates a more flat egg yolk in the horizontal plane, suggesting lower maturity of salted duck eggs. In contrast, a higher YI represents a more spherical egg yolk and higher maturity of salted duck eggs. When the YI is close to 1, it indicates that the salted duck egg was ripe [20]. Table I shows that although the technical requirements could be met when eggs were salted in 7.0% and 8.0% brine solution for 25 or 20 days, and salting time was short, the quality of salted duck eggs in terms of yolk index and oil content was lower compared to other procedure. With extended salting time, the salt concentration in egg white increased, and thus the salt concentration of the second brine solution should be 4.0%-6.0%. When salted in the second brine solution of 4.0%, the second stage salting time should be 25 days. When the salt concentration of the second brine solution was 5.0% -6.0%, the second stage salting time should be decreased to 20 days.

TABLE I. THE EFFECT OF SALT CONCENTRATION AND PICKLE TIME ON THE QUALITY OF SALTED DUCK EGGS IN THE SECOND BRINING PROCEDURE

Salt concentration (%)	Salting time(day)	Salt content in egg white (%)	Yolk index	Oil (%)
4.0	35	3.29±0.16	0.81±0.03	33.5±1.8
5.0	30	3.31±0.14	0.83±0.02	33.9±2.1
5.0	35	3.48±0.15	0.88±0.01	35.4±1.9
6.0	30	3.65±0.18	0.86±0.02	34.8±1.4
6.0	35	3.97±0.12	0.89±0.02	36.6±2.3
7.0	25	3.52±0.18	0.78±0.03	31.3±1.8
8.0	20	3.65±0.21	0.78±0.01	31.8±1.6

Data were presented as the mean ± standard deviation (SD) of triplicate experiments.

D. *Effect of Salting Temperature on the Quality of Salted Duck Eggs*

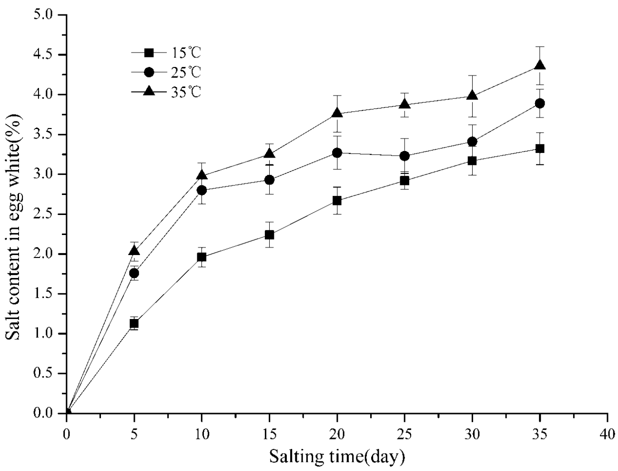

Figure 4. The effect of salting temperature on the salt content of egg white. Bars represent standard deviation from triplicate determinations.

After salting in the first brine solution with 18.0% salinity for 10 days, eggs were salted in the second brine solution at 5.0% salt concentration. The temperature varies in different season, to examine the effect of salting temperature on the quality of duck eggs, the eggs were salted at 15 ℃, 25 ℃ and 35 ℃, respectively, and the salt content in egg white was detected every 5 days. At each salting temperature, salt content in the egg white was increased (Fig. 4). In the experimental temperature range, with increase temperature, the salt content in the egg white increased. When the eggs were salted at 15 ℃ for 35 days, the salt content in egg white was 3.32%; at 25 ℃ and 35 ℃ for 35 days, the salt contents were 3.89% and 4.36%, respectively. We also found that when the duck eggs were salted at 35 ℃ for 30 days, the egg white salt content was 3.98%. Thus, if the salting temperature was 15 ℃ or 25 ℃, the total salting time (including the first and second stage) should not exceed 35 days; if the salting temperature was 35 ℃, the duck eggs should not salted for more than 30 days.

E. *Effect of Alcohol Content on the Quality of Salted Duck Eggs*

After salting in in the first brine solution (salt content was 18.0%) for 10 days, the eggs were transferred to the second brine solution with a salt concentration of 5.0% at 25 ℃. In the second brine step, buckwheat liquor (alcohol concentration was 52%) was added to each brine solution to the final concentration of 0.0, 0.1, 0.2, 0.3, 0.4 and 0.5%, respectively. To study the effect of alcohol concentration on the quality of salted duck eggs, the salt concentration in egg white, yolk index and oil content at yolk were measured in 30 and 35 days (Table II).

The results in Table II show that adding alcohol to the brine solution increased salt content in egg white, possibly alcohol may have increased the osmotic pressure in the brine solution. Additionally, alcohol increased the oil content in the yolk, but there was no clear effect on yolk index. Adding alcohol to the surface also inhibited the growth of microorganisms. Thus, adding alcohol to the liquid surface might decrease the amount of salt, increasing the quality of salted duck eggs. The amount of alcohol used depends on the salt concentration. When the salt concentration in the second brine solution was 4.0%, we could add as much as 0.5% alcohol to the liquid, but higher addition might cause salted duck eggs to have a bitter taste and increase the cost of production. If the salt concentration in the second brine solution was 6.0%, the amount of alcohol should be less than 0.2%, otherwise, excessive salt content in egg white may be observed.

TABLE II. THE EFFECT OF ALCOHOL ON THE QUALITY OF SALTED DUCK EGGS IN THE SECOND BRINING PROCEDURE

The dosage of alcohol	Salt concentration in egg white (%)		Yolk index		Oil content in yolk	
	30 days	35 days	30 days	35 days	30 days	35 days
0.0%	3.05±0.12	3.11±0.12	0.83±0.02	0.85±0.02	30.5±1.5	31.5±1.4
0.1%	3.28±0.17	3.34±0.18	0.84±0.01	0.85±0.01	31.8±1.7	32.3±1.6
0.2%	3.36±0.19	3.48±0.11	0.84±0.01	0.86±0.02	32.1±1.7	34.5±1.7
0.3%	3.41±0.15	3.48±0.17	0.83±0.02	0.85±0.02	32.2±1.8	35.1±1.5
0.4%	3.65±0.12	3.78±0.12	0.85±0.01	0.86±0.02	33.5±1.6	34.6±1.8
0.5%	3.71±0.19	3.76±0.14	0.84±0.02	0.86±0.01	33.6±1.3	35.4±1.5

Data were presented as the mean ±standard deviation (SD) of triplicate experiments.

Therefore, the addition of alcohol to the surface of the second brine solution improved the quality of salted duck eggs. However, the amount used depends on the salt content in the second brine solution, and the total amount should be controlled within 0.1%-0.5%.

F. Effect of Spice on the Quality of Salted Duck Eggs

During the salting procedure, spices were added to the first and second brine solutions. The amount of anise and Chinese prickly were 0.05%-0.10%. After salting for 30 days, the salt content in the egg white was measured (Table III).

Salt content in the egg white was increased when spices were added to the brine solution (Table III). Some components in the star anise and Chinese prickly ash might have increased osmosis, thus, spices might promote the maturation of salted duck eggs and shorten the salting period. The addition of spices had no obvious effect on yolk index, but improved the quality of salted duck eggs. As shown in Table III, compared with the salted duck eggs without spices, the addition of anise and Chinese prickly ash removed the fishy smell of duck eggs, increase the fragrance quality. However, the color of the egg white and yolk was slightly browner, which was conducive to improving the taste of salted duck eggs. If the color was too deep, the appearance of salted duck eggs would be poor. Therefore, the amount of spice should be controlled, with the optimal amount within 0.05%-0.10%.

TABLE III. THE EFFECT OF SPICE ON THE QUALITY OF SALTED DUCK EGGS

Dosage of spice	Salt in egg white (%)	Yolk index	Quality of salted duck eggs[a]
Not added	3.38±0.13	0.84±0.01	/
anise 0.05% Chinese prickly ash 0.05%	3.42±0.15	0.83±0.01	fishy smell (-), fragrance(+), color of egg white(+), color of yolk(+)
anise 0.06% Chinese prickly ash 0.06%	3.48±0.12	0.84±0.02	fishy smell(-), fragrance(+), color of egg white(+), color of yolk(+)
anise 0.07% Chinese prickly ash 0.07%	3.48±0.17	0.84±0.01	fishy smell(-), fragrance(+), color of egg white(++), color of yolk(+)
anise 0.08% Chinese prickly ash 0.08%	3.56±0.16	0.84±0.01	fishy smell(--), fragrance(++), color of egg white(++), color of yolk(++)
anise 0.09% Chinese prickly ash 0.09%	3.61±0.16	0.84±0.02	fishy smell(--), fragrance(++), color of egg white(++), color of yolk(++)
anise 0.10% Chinese prickly ash 0.10%	3.65±0.15	0.84±0.01	fishy smell(--), fragrance(+++), color of egg white(++), color of yolk(++)

Data were presented as the mean ±standard deviation (SD) of triplicate experiments.
a:Compared with salted duck eggs that not added spice and -: the same as; --: less than; +: better than, the number of + indicate the level of degree.

After salting using the optimized procedure, the salt content in our low NaCl salted duck eggs varied from 3.0% to 4.0%, which is lower than many reports previously [7], [8], and the salted duck eggs had round and oily yolks, indicating that the salted duck eggs were high quality.

IV. CONCLUSION

This study reported a processing technology for the production of high quality, low NaCl salted duck eggs. Our method includes chlorine dioxide pretreatment and a two-stage immersion method. The second brine solution was diluted with the first brine solution as a stock solution; spices and the buckwheat liquor were added to the second brine solution. The salt in this study was recycled, and thus the salting solution could be reused to reduce waste. The salted duck eggs contained low NaCl content in the egg white, and the quality of our salted duck eggs was good.

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