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Use of Incinerated Eggshells to Produce Pidan

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Abstract: Preserved eggs (pidans) are used in traditional Chinese cuisines. However, the alkaline conditions and metal ions generated during its preparation have caused some concerns. This study developed an innovative process for pidan processing using incinerated eggshell powder, an abundant by-product that can generate a highly alkaline solution and provide calcium ions (Ca^{2+}). Either 0.5, 3, or 5% of the eggshell powder solution was used for basic pickling. Different combinations of $ZnSO_4$ (0.175%), $MgCl_2$ (0.08%), and $CuSO_4$ (0.16%) were added. Duck eggs were pickled for 25 days at 25–27 °C, followed by 14 days of ripening. The pidan processed in 5% eggshell powder containing 0.175% $ZnSO_4$ demonstrated the closest physiochemical and sensory characteristics to commercial pidans. Thus, the results offer a new technique to manufacture pidans and reduce the harmful impact of metal ions on human health and the environment.

Keywords: incinerated eggshell powder; pidan; preserved egg; metal ions; by-product

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1. Introduction

Preserved eggs (pidans) are derived from traditional Chinese culinary cultures. Pidan has a long shelf life and a flavorful aroma that is usually consumed by individuals who have an acquired taste for it [1]. There is an alkaline environment composed of metal ion salts. The solution permeates the eggshell and egg membrane, leading to egg protein chemical changes resulting in gelation [2–4]. The classic recipe for pidan involves covering the egg with rice bran, clay, and wood ash only. Currently, the commercial production of pidan primarily employs pickling solutions in complex combinations of 5–6% sodium hydroxide (NaOH) with 4–5% salt and metal ions [5]. Wang, et al. [6] developed the heavy metal-free preserved eggs in a solution of NaOH (6, 0.3, and 0.1% w/v respectively) and NaCl (4.0%, w/v) in three stages. NaOH gradually penetrates through the duck egg during processing, denatures the albumen, and reacts with metal ions to form a gel [7–9]. When albumen is adjusted by heat or pH, the denatured proteins aggregate into a three-dimensional network structure and form gel [10–13]. These metal ions, especially divalent ones, promote the penetration of alkaline substances to stabilize the coagulation of denatured proteins and egg white [14]. However, the study by Zhao, et al. [15] showed that strong alkali environments cause lysine and alanine formation in pidan, which may reduce the nutritional value of the proteins and pose as a potential health hazard. In addition, hydrogen sulfide (H_2S) reactions occur when metal ions react with alkaline-degraded proteins to form insoluble compounds that block eggshell pores and membranes to regulate the alkali concentration in the egg [16]. Uncontrolled doses of metal ions used to minimize pickling and maturation times increase the heavy metal residues in pidan, which influences the food's safety and quality. The previous process used lead (Pb^{2+}) as the main addition of divalent metal ions until the lead was reported as hazardous to human health [17,18]. Many results indicated that preserved eggs formed with PbO, $CuSO_4$, $FeCl_3$, $ZnSO_4$, and $CuSO_4/ZnSO_4$ preparation are potentially dangerous to human health. However, lead-free pidan has been an issue of concern in recent years [1,19–24]. An additional study related to dietary health found that the pidan diet might have an anti-inflammatory effect by modulating specific intestinal bacteria to alter the ratio of short-chain fatty acids in the intestine of rats [25]. In recent decades, global egg production has reached 73 million tons [26]. Eggs are one of the most consumed food products. The primary waste from egg processing is eggshells. The following processes have been widely available for the incineration of eggshells. The major component of eggshells is calcium carbonate ($CaCO_3$), which is transformed into calcium oxide by incineration at high temperatures (600–900 °C). When $CaCO_3$ reacts with water, it forms calcium hydroxyl groups ($CaOH_2$), creating a highly alkaline solution [27]. Studies have reported that using highly alkaline solutions consisting of powdered incinerated oyster shells, which also consist of $CaCO_3$, effectively reduced bacterial counts due to the presence of potential ingredients for food cleansers, preservatives, or hand sanitizers [28,29]. In contrast, a mixture of 0.2% oyster shells and 0.3% eggshells increased the organoleptic quality of cooked pork, which proved to be an alternative to artificially-synthesized phosphate salts [30]. In the study, we evaluated the possibility of making pidans through a novel process. According to the literature and preliminary experiments, the incinerated eggshell powder can provide an alkaline environment and supply calcium ions (Ca^{2+}), sufficient to improve the current commercial process.

2. Materials and Methods

2.1. Materials

Duck eggs were purchased from Songgao egg enterprise (Tainan City, Taiwan), and all eggs were laid within three days before the experiment. The average weight of the eggs was 50–60 g. Ripe pidan was purchased from the same company and used as a positive control. Eggshells were provided by Fu-Che Frozen Foods Co. (Kaohsiung City, Taiwan). All chemicals used in the current study are analytical-grade reagents.

2.2. Preparation of Incinerated Eggshell Powder

The incinerated eggshell powder was prepared following the methods described by Tangboriboon, et al. [31] and Bwc, et al. [32]. After washing with tap water to remove surface debris, clean eggshells were incinerated for 12 h at 900 °C. The calcium carbonate in the eggshell decomposes into calcium oxide (CaO) and carbon dioxide [33]. Following incineration, the eggshells were ground into a powder, filtered through a sieve (Retsch Test Sieve No. 170, 90 µm, Haan, Germany), and stored in a desiccator until further use.

2.3. Pickling and Ripening Process

The above prepared CaO mixed with H_2O to form a $Ca(OH)_2$ solution, followed by sodium carbonate (Na_2CO_3) to the obtained natural alkaline solution [34]. Equal molar ratios of eggshell powder and Na_2CO_3 were added to water to increase the solution's pH (over 13.0). The pH value was determined using a pH meter (SP 2500, Suntex Ins, New Taipei City, Taiwan). After stirring for 30 min, the solution was placed at room temperature (25 ± 2 °C) for 24 h and then filtered using a 5A filter paper (ADVANTEC, Tokyo, Japan). The pickling solutions were prepared according to different conditions: (i) negative control: 4.2% NaOH + 5% NaCl [9]; (ii) A group: with 5% incinerated eggshell powder, 0.175% $ZnSO_4 \cdot H_2O$, and 5% NaCl; (iii) B group: 0.175% $ZnSO_4 \cdot H_2O$; (iv) C group: with 0.5% incinerated eggshell powder, 0.175% $ZnSO_4 \cdot H_2O$, 0.1% $MgCl_2$, and 0.165% $CaCl_2 \cdot 2H_2O$; (v) D group: with 3% incinerated eggshell powder and 0.175% $ZnSO_4 \cdot H_2O$. In addition, the same components as the negative control were added to groups B, C, and D. The positive control was commercially available pidan. Twelve duck eggs were pickled in 1 L of solution at 25 ± 2 °C for 25, 30, and 40 days with 14 days ripening (in the incubator at 25 ± 2 °C). Each time, three duck eggs were taken randomly for follow-up analysis. Physicochemical analysis was performed on random samples (n = 3) from each group after rinsing with tap water.

2.4. Hardness Ratio of Yolk

According to the method mentioned in Ganasen and Benjakul [14] study, as shown in the formula below, the hardness ratio of the yolk is calculated only for the weight ratio of solidified yolk and whole yolk, without counting the unconsolidated part.

$$\text{Hardness ratio of yolk (\%)} = \text{weight of solidified yolk/weight of the whole yolk} \quad (1)$$

2.5. Texture Analysis

Following the method described by Zhao, et al. [16], a texture analyzer (Brookfield CT3-1000, Middleboro, MA, USA) investigated texture characteristics such as hardness, elasticity, cohesiveness, gel strength, and chewiness of the albumen and yolk. Moreover, the appearance of the albumen and yolk was observed and recorded comprehensively. A cube with dimensions of 15 mm (L × W × H) was cut from the albumen of the pidan. The samples were compressed to 50% of their original height using a cylindrical plunger (36 mm diameter) at a pressing speed of 1 mm/s and a 5.5 mm/s retrieval speed. This process was performed twice for chewiness purposes with an interval of 5 s between the compressions.

2.6. Color Characteristics

Color changes of semi-products and products during the pickling and ripening processes were monitored for Hunter's L (brightness), a (redness–greenness), and b (yellowness-blueness) values were determined using a colorimeter (Nippon Denshoku, SA2000, Tokyo, Japan).

2.7. Sensory Evaluation

The development of sensory characteristics, including appearance, flavor, taste, texture, and overall acceptance, was based on Drake and Delahunty [35] evaluation for cheese sensory characteristics, with modifications. Then the sensory evaluation was evaluated by 20 trained panelists. A pidan was sliced equally into four parts, labeled randomly. Each element was assessed on a scale of 1 to 9 (1 = extremely dislike, 9 = extremely like). The panelists tasted one slice from each group and used gargling with drinking water twice between each sample. Commercial pidan was used as a positive control and served as the training standard for panelists. The room temperature was maintained at 25 ± 2 °C throughout. Notably, the appearance and texture of albumen and yolk were evaluated separately because the different focuses of the sensory evaluation were assessed independently.

2.8. Mineral Analysis

Mineral analysis of optimal manufacturing conditions for pidan products was performed. The samples were sent to an independent laboratory for mineral analysis to guarantee objective analytical results. The method used was described by Kelleher, et al. [36], Eric, et al. [37], outlined in the requirements prescribed by AOAC 984.27.

2.9. Statistical Analysis

All measurements were performed in triplicates, and values in the tables are expressed as means (standard deviation of triplicate measurements). Statistical analysis was performed using SPSS (version 12.0; International Business Machines Corporation, Armonk, NY, USA). The statistical significance of differences among means was evaluated using one-way analysis of variance and Duncan's multiple range tests at a significance level of p = 0.05.

3. Results and Discussion

3.1. Confirmation of the Availability of the Pidan Ripening Process

According to the composition of the incinerated eggshell powder, it should be released calcium ions. To determine the optimal process, we assessed the effect of different chemicals on the pickling and ripening process in different pidans. The 39-day pickled pidan showed choking behavior, i.e., difficulty swallowing due to taste (data not shown). Research by Zhang, et al. [13] also mentioned that the unique odors and flavors resulting from alkaline treatment need to be researched further. Hence, we refrained from the condition of only 39 days pickled within the pidan production for this study. After pickling for 25 days and ripening for 14 days; therefore, it proved to be the most suitable process for this study. Figure 1 shows that the appearance of groups A–D was well-formed in all cases except the negative control, thus demonstrating that the formation of ovalbumin in group B was under-formed, which is related to the poor elasticity and cohesiveness of the texture characteristics. As shown in Figure 1, the cohesiveness effect using a single metal ion (group B) was less effective than the other groups using more than two types of metal ions (A, C, and D). Meanwhile, the texture characteristics, gel strength, and chewiness were improved to the desired level after the ripening process. Collectively, the available evidence showed that incinerated eggshell powder is an optimal source of metal ions (Ca^{2+}), and it can be used to generate other metal ions (Zn^{2+} or Mg^{2+}) as well. In addition, the number of metal ions could be reduced by using incinerated eggshell powder. The composition of eggshells in group A was to check whether an increase in the amount of incinerated eggshell powder could reduce the number of metal ions and create a strongly alkaline solution.

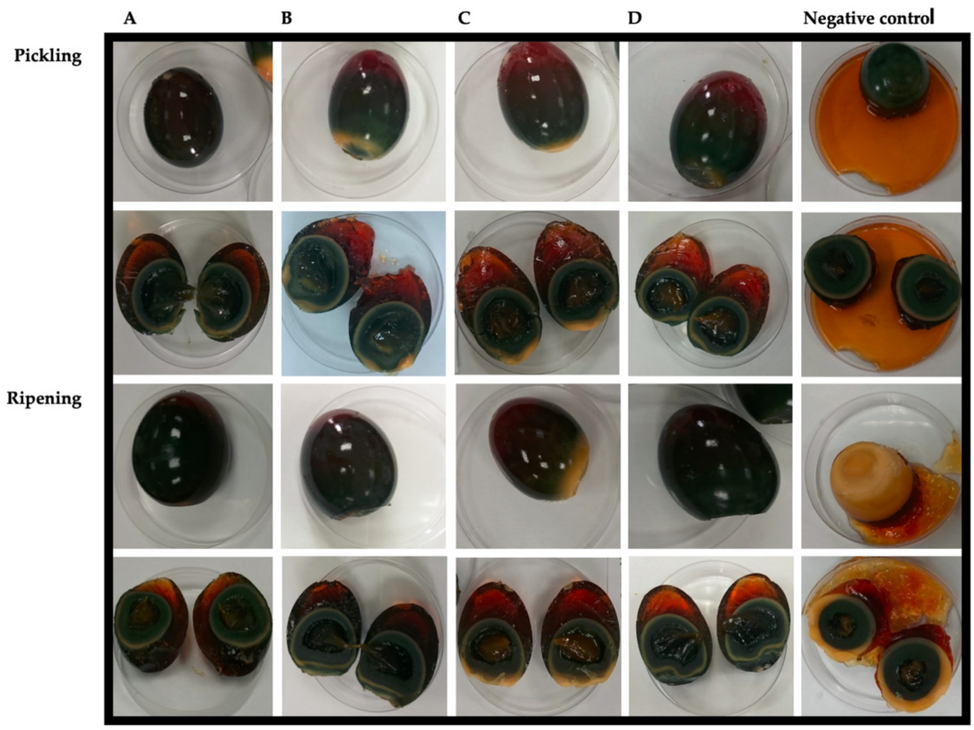

Figure 1. Images of pidan in different condictiones during pickling and ripening process. A group: with 5% incinerated eggshell powder + 0.175% $ZnSO_4{\cdot}H_2O$ + 5% NaCl; B group: 0.175% $ZnSO_4{\cdot}H_2O$; C group: with 0.5% incinerated eggshell powder + 0.175% $ZnSO_4{\cdot}H_2O$ + 0.1% $MgCl_2$ + 0.165% $CaCl_2{\cdot}2H_2O$; D group: with 3% incinerated eggshell powder + 0.175% $ZnSO_4{\cdot}H_2O$; Negative control: 4.2% NaOH + 5% NaCl (Groups B, C and D have also been added).

3.2. The Hardening Percentages and Texture Characteristics

In Table 1, almost all values of the A-D group showed an increase in yolk hardening rate, hardness, gel strength, and chewiness after ripening compared to pickling. The elasticity and cohesion of groups A–D did not change significantly after the ripening and pickling treatments. As shown in Table 1, there were no significant differences in the yolk hardening rate between all groups, including the positive control group, after pickling or ripening. However, the negative control was excluded due to conformability for determination. The yolk hardening rate during ripening was significantly higher than the rate during pickling. However, the yolk hardening rates did not differ with various pickling conditions. Interestingly, group A (81.59 ± 2.26) was the highest in the ripening process, with significant differences from the other treatment groups. Moreover, group A was higher than those of the standard value of the positive control. Especially the two aspects are the gel strength (84.90 ± 9.44) and chewiness (0.93 ± 0.03) in group A. Unfortunately, the cohesiveness in groups B (0.85 ± 0.14–0.87 ± 0.05) and D (0.94 ± 0.03–0.90 ± 0.12) was lower than that in the positive control (0.90–0.95 and 0.93–0.97) in terms of pickling and ripening; thus, a significant improvement in gel strength and chewiness for each group was observed during the ripening process and better than the positive control (Table 1). Overall, there was no significant difference in the hardening percentages and texture characteristics between the treatment and the positive control groups.

Table 1. The hardening percentages and texture characteristics of albumen and yolk in different concentrations of metal ions and the incinerated eggshell powder during the pickling and ripening process.

	Yolk Hardening Rate (%)		Hardness (g)		Elasticity		Cohesiveness		Gel Strength		Chewiness (mJ)	
Group	**Pickling**	**Ripening**	**Pickling**	**Ripening**	**Pickling**	**Ripening**	**Pickling**	**Ripening**	**Pickling**	**Ripening**	**Pickling**	**Ripening**
A	72.34 ± 7.39 abA	81.59 ± 2.26 bcB	63.50 ± 6.98 ab	91.20 ± 9.62 c	0.78 ± 0.02 b	0.76 ± 0.07 abc	0.93 ± 0.03 ab	0.93 ± 0.03 ab	58.88 ± 7.26 b	84.90 ± 9.44 d	3.11 ± 0.31 c	3.97 ± 0.40 d
B	73.07 ± 4.72 abA	81.33 ± 9.31 bcB	59.67 ± 13.53 ab	92.33 ± 11.79 c	0.61 ± 0.21 a	0.67 ± 0.02 a	0.85 ± 0.14 a	0.87 ± 0.05 a	49.13 ± 2.37 ab	80.67 ± 11.32 d	2.39 ± 0.18 a	3.98 ± 0.74 cd
C	76.34 ± 11.33 abA	78.78 ± 2.02 bcB	57.00 ± 1.80 ab	78.30 ± 3.47 b	0.82 ± 0.03 b	0.78 ± 0.06 bcd	0.94 ± 0.01 ab	0.93 ± 0.04 ab	53.83 ± 2.05 ab	72.68 ± 3.90 c	2.65 ± 0.14 b	3.31 ± 0.25 cd
D	69.7 ± 2.75 abA	80.47 ± 8.92 bcB	52.10 ± 4.55 a	83.13 ± 7.41 bc	0.80 ± 0.02 b	0.73 ± 0.09 ab	0.94 ± 0.03 ab	0.90 ± 0.12 ab	48.88 ± 4.53 a	74.38 ± 10.22 cd	2.46 ± 0.26 a	3.53 ± 0.40 cd
Negative control	89.03 ± 0.0 cA	86.53 ± 4.91 bB	–	–	–	–	–	–	–	–	–	–
Positive control #	65–80	70–85	45–65	50–70	0.70–0.85	0.75–0.88	0.90–0.95	0.93–0.97	40–60	45–65	1.90–2.80	2.00–2.85

–: Non-detection; the gelatinization of albumen is incomplete and cannot be detected. #: Those were the value obtained by purchasing commercial products for analysis. As standard values for this study, so no statistics were conducted. Data are presented as average ± standard deviation. Uppercase (horizontal axis) and lowercase (vertical axis) averages with different letters in the same column are significantly different ($p < 0.05$). A group: with 5% incinerated eggshell powder + 0.175% $ZnSO_4 \cdot H_2O$ + 5% NaCl; B group: 0.175% $ZnSO_4 \cdot H_2O$; C group: with 0.5% incinerated eggshell powder + 0.175% $ZnSO_4 \cdot H_2O$ + 0.1% $MgCl_2$ + 0.165% $CaCl_2 \cdot 2H_2O$; D group: with 3% incinerated eggshell powder + 0.175% $ZnSO_4 \cdot H_2O$; Negative control: 4.2% NaOH + 5% NaCl (Groups B, C and D have also been added).

Since earlier studies have shown that the optimal physicochemical characteristics were obtained from pickling for 25 days and ripening for 14 days, the current findings are comparable with similar to those of Zhang, et al. [38] at 24–26 °C and 28 days of pickling with 14 days of ripening. Group A had the best pickling and ripening characteristics; groups C and D were ranked second. The study also showed that free metal ions were released during the pickling process from the incinerated eggshell powder. The results indicated that although a complete pidan was not formed, adding the incinerated eggshell powder resulted in gel formation. Ganasen and Benjakul [14] and Zhao, et al. [16] found that added metal ions and a salt bridge promote the binding of Cu^{2+}, Zn^{2+}, and Pb^{2+} with negatively charged protein molecules, facilitating gel formation [12].

3.3. Color Characteristics of Pidan

Both brown albumen and black-green yolk are typical characteristics of pidan [6,39]. Therefore, good color and appearance are essential aspects of commercialization. For the colors, three parameters were evaluated: L, a, and b-values. From the evaluation above (as shown in Figure 1), the brightness (18.99–23.62, in Table 2) of egg yolk in all groups decreased during processing, and the yellow color gradually disappeared and became darker. Measurements of the pidan samples showed that the albumen was progressively converted to translucent or opaque (Figure 1). There were no significant differences in Hunter whiteness (Wh) values between all treated samples and the positive control (Table 2). The color found in group C (80.45) was closest to the commercial product (81.05) within this category.

Table 2. Colorimetric results and sensory evaluation of pidan in different concentrations of metal ions and the incinerated eggshell powder during pickling and ripening process.

Group	Wh	*L*	*a*	*b*	Appearances				Mouthfeel		
					Color of Albumins	Soft-Boiled of Yolk	Flavor	Taste	Elasticity of Egg White	Texture of Egg Yolk	Overall
A	77.11 ab	18.99 ± 1.53 ab	2.40 ± 0.46 a	1.34 ± 0.15 b	6.97 a	6.47 a	6.44 a	6.35 a	6.62 a	6.65 a	6.56 a
B	76.64 b	23.62 ± 0.91 b	6.21 ± 0.76 d	1.17 ± 0.35 b	6.37 b	6.00 b	4.53 c	4.77 c	5.23 b	5.19 b	4.67 b
C	80.45 a	19.60 ± 1.38 ab	3.66 ± 0.44 b	0.67 ± 0.05 a	6.82 ab	6.59 a	5.94 ab	5.68 b	6.35 a	6.26 a	6.38 a
D	78.17 ab	21.97 ± 1.69 ab	4.44 ± 1.06 bc	1.11 ± 0.35 ab	4.70 c	4.91 c	4.98 c	4.91 c	5.23 b	4.72 c	4.86 b
Positive control *	81.05 a	18.99 ± 1.53 ab	2.40 ± 0.46 a	1.34 ± 0.15 b	6.15 b	6.03 b	5.56 b	5.91 ab	6.59 a	6.32 a	6.29 a

* Positive control was commercial products. Data are presented as average ± standard deviation. Averages with different letters in the same column are significant different ($p < 0.05$). Wh: Hunter whiteness = $100 - [(100 - L)^2 + (a^2 + b^2)]1/2$. A group: with 5% incinerated eggshell powder + 0.175% $ZnSO_4{\cdot}H_2O$+ 5% NaCl; B group: 0.175% $ZnSO_4{\cdot}H_2O$; C group: with 0.5% incinerated eggshell powder + 0.175% $ZnSO_4{\cdot}H_2O$ + 0.1% $MgCl_2$ + 0.165% $CaCl_2{\cdot}2H_2O$; D group: with 3% incinerated eggshell powder + 0.175% $ZnSO_4{\cdot}H_2O$; Negative control: 4.2% NaOH + 5% NaCl (Groups B, C and D have also been added).

The primary purpose of pidan browning and gel formation during processing is the reaction of egg proteins by alkaline degradation to peptides, which react with metal ions in a stable metal ion-peptide complex. In addition, some amino acids are degraded to produce H_2S and NH_3 (sulfhydryl (SH) oxidation and SH-disulfide bonds (SS)), which react with other organic substances and are responsible for the unique aroma of pidan [12,40,41]. The lipid compounds of eggs were also solidified in high pH and metal ions, whose procedures were similar to those reported by Zhao, et al. [16]. In this study, a high pH environment was produced by all treatment groups, which was suitable for the processing of pidan. Hence, this solution of incinerated eggshell powder is a potential candidate for partial replacement of NaOH and less additive metal ions when making pickling solutions for pidan.

3.4. Sensory Evaluation of Pidan

The sensory evaluations showed that groups A and C were in the high score group with similar values to the positive control group; however, groups B and D were close to each other with low scores (Table 2). Overall, sensory performance decreased in the following order: A > C > positive control > D > B group. In terms of overall performance, groups A and B had the highest and lowest scores among all treatment groups. Groups A and C were organoleptically evaluated for the second time, and still, there was no significant difference in appearance and taste. Thus, the overall acceptability for pidans in both groups was similar to that of their commercial counterparts. Other evaluation indexes showed identical results. Nevertheless, the overall scores of group C (6.38) were closer to the positive control (6.29) than group A (6.56). Therefore, the incinerated eggshell powder can be an adequate replacement method for pidan processing.

3.5. Mineral Analysis

For the above-mentioned overall evaluation scores, A and C are the closest to the commercially available pidan. Hence, these two groups will be investigated for metal content to prepare for commercialization—mineral analysis of optimal conditions for pidan products. The maximum value of calcium capacity in yolk during the process was approximately 133 mg/100 g in groups A and C, which is worthy of further investigation (Table 3). Studies by Tu, et al. [42] also showed that Na, K, P, Ca, Mg, Fe, Cu, and Zn are present and that the yolk contains more inorganic elements than albumin. Interestingly, Zn is an essential trace element that significantly enhances immunity [43]. Zn has been shown to modulate antiviral and antibacterial immunity and to regulate inflammatory responses [44]. Furthermore, it is notable that Zn (categorized according to Zn cut-off: 80 µg/dL, uptake 150 mg) has been shown to inhibit a specific protease of the COVID-19 virus, and supplementation in appropriate amounts may improve patient survival [45]. The applied value of food ingredients as dietary supplements highlights pidan production in our study.

Table 3. Minerals analysis of the optimal pidan products. Unit: mg/100 g dry weight basis.

Group	Sampling Area	Calcium	Magnesium	Zinc
A	albumen	6 b	N.D.	0.3 b
	yolk	133 a	N.D.	2.21 a
C	albumen	4 b	26.3 a	0.3 b
	yolk	133 a	3.48 b	2.21 a

N.D.: No detection. Averages with different letters in the same column are significant different ($p < 0.05$). A group: with 5% incinerated eggshell powder + 0.175% $ZnSO_4 \cdot H_2O$ + 5% NaCl; C group: with 0.5% incinerated eggshell powder + 0.175% $ZnSO_4 \cdot H_2O$ + 0.1% $MgCl_2$ + 0.165% $CaCl_2 \cdot 2H_2O$.

4. Conclusions

The 25-day pickling with 14-day ripening was optimal for producing a good quality pidan. The sensory evaluation results showed that group A (5% incinerated eggshell powder in a basic solution containing 0.175% $ZnSO_4$) had the closest characteristics to commercial products. The study successfully used a significant waste of the egg industry, eggshells, to produce high-quality pidan, with minimal use of metal ions. The study aims to develop and utilize new applications for the by-products (eggshell), which have been proven for the pidan processing application and might be investigated for other purposes in the future. Thus, this method could be helpful for pidan production, and it could decrease the potentially negative impact of metal ions on health and the environment.

Author Contributions: C.-M.L. and P.-H.H. wrote the manuscript. Y.-L.H. and C.-Y.H. carried out the experiment, analyzed and interpreted the data. C.-M.L., C.-Y.H. and Y.-L.H. designed the conception of this study. M.-K.S. and C.-W.H. help interpret the data and participate in writing the manuscript. All authors have read and agreed to the published version of the manuscript.

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