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(Research Article)

Optimizing Acetic Acid Pretreatment and Pickling Duration to Reduce Cholesterol, Trimethylamine, and Malondialdehyde While Enhancing Physicochemical Quality of Pidan Duck Eggs

Rut Listyadi [1, *], Tuti Suryati [2] and Zakiah Wulandari [2]

[1] Departement of Bachelor in Animal Husbandry, Faculty of Animal Husbandry, Udayana University, Jalan Kampus Bukit Jimbaran Kuta, Bali, 80361, Indonesia.

[2] Departement of Animal Production and Technology, Faculty of Animal Science, IPB University, Jalan Agatis, IPB Dramaga Bogor, 16680, West Java, Indonesia.

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Abstract

Pidan eggs, a traditional alkaline-preserved food product widely consumed in Indonesia, require process optimization to improve safety and quality attributes. This study evaluated the impact of 5% acetic acid pretreatment combined with varying pickling durations (1 to 4 weeks) on the physicochemical and biochemical characteristics of pidan duck eggs (*Anas platyrhynchos*), with a specific focus on cholesterol, malondialdehyde (MDA), and trimethylamine (TMA) levels. Utilizing a factorial completely randomized design, 300 eggs with an initial cholesterol concentration of 333.25 mg/100 g were subjected to pretreatment followed by immersion in an alkaline pickling solution containing 4.2% NaOH, 0.2% $ZnCl_2$, 5% black tea extract, and 5% NaCl. The results indicated that a 30-minute pretreatment significantly influenced yolk and albumen properties, including enhanced brown color intensity, improved textural firmness, reduced water activity (a_w), and stabilized pH values. Importantly, pretreatment effectively decreased TMA and cholesterol concentrations, markers associated with lipid degradation and off-flavor formation, alongside the modulation of MDA content, indicating reduced lipid oxidation. The optimal condition identified was a 30-minute pretreatment followed by three weeks of alkaline pickling, yielding samples with notably lowered cholesterol (276.2 mg/100 g), TMA (0.75 mg/100 g), MDA (0.52 mg/100 g), and acceptable a_w (0.85–0.89), indicative of enhanced safety, sensory qualities, and shelf stability. This research contributes critical insight into the biochemical and physical transformations during pidan production, offering a scientifically substantiated protocol to improve the quality and health aspects of this culturally significant preserved egg product.

Keywords: Acetic Acid; Cholesterol; MDA; Pidan; TMA.

1. Introduction

The pidan egg, also referred to as century egg or preserved egg, is a traditional Chinese food product capable of being stored for up to 200 days under suitable conditions (Wang & Fung, 1996). The earliest recorded documentation of pidan production dates back to the Ming Dynasty in 1640, as documented by Yang Yu Yue Ling. Two prevalent preservation methods are employed: traditional salting and alkaline pickling. Ganasen *et al.* (2014) demonstrated that both pickling and salting processes enhance the protein content of duck eggs. However, due to the inherently high cholesterol content in duck eggs, ongoing monitoring is critical to ensure dietary safety. Li-Yun *et al.* (2005) reported that pidan contains lower cholesterol levels compared to fresh eggs, tea eggs, salted iron eggs, and simmered eggs. During the alkaline pickling process, the strongly basic conditions induce complex chemical reactions that may result in the formation of

* Corresponding author: Rut Listyadi.

unstable compounds and random molecular bonds (Cao *et al*., 2021). Therefore, further research is necessary to optimize pidan processing techniques and thoroughly assess potential nutritional and safety implications.

Trimethylamine (TMA) and malondialdehyde (MDA) are critical chemical markers widely used to assess food quality and safety. TMA is predominantly associated with the characteristic ammonia-like odor found in fish and serves as an important freshness indicator (Suwetja, 2011). Within the context of pidan production, elucidating the relative contributions of TMA versus non-TMA compounds to the unique ammonia aroma is essential for optimizing flavor profiles. Conversely, MDA is a secondary product generated through lipid peroxidation involving unsaturated fatty acids and is commonly employed as a biomarker of oxidative stress within both food matrices and biological systems. Elevated MDA concentrations have been correlated with oxidative stress in type II diabetes (Asbaghi *et al*., 2021), exposure to environmental pollutants in fish products (Garcia *et al*., 2020), and mechanisms of protein unfolding (Zhu *et al*., 2020).

The manufacturing of pidan typically involves two principal stages: alkaline pickling and aging. The pickling process involves immersing duck eggs in an alkaline solution comprising 4.2% NaOH and 5.0% NaCl at ambient temperature for approximately 20 days, followed by coating the eggs with a mud or clay mixture and aging them for an additional 21 days (Ganasen & Benjakul, 2011b). The pickling phase primarily contributes to textural gelation and physicochemical modifications in the yolk and albumen, whereas the aging phase facilitates color maturation and mitigates the overly intense ammonia odor (Ganasen & Benjakul, 2011a).

Typically, the overall process duration ranges from 4 to 6 weeks for pickling with an additional aging period of three weeks, which is significantly longer than conventional salted eggs requiring 2–3 weeks (Sun *et al*., 2017; Bao *et al*., 2020). Several studies, including those by Surjoseputro *et al*. (1995), Ganasen & Benjakul (2011b), Kaewmanee *et al*. (2012), and Sun *et al*. (2017), have recommended incorporating a 5% acetic acid (CH_3COOH) pretreatment step to enhance eggshell permeability and facilitate chemical infiltration during subsequent pickling. Nonetheless, the direct impact of 5% acetic acid pretreatment combined with varying pickling durations on cholesterol, TMA, and MDA levels in pidan eggs has been insufficiently explored. Thus, the present study aims to systematically investigate the effects of acetic acid pretreatment and distinct pickling durations (1, 2, 3, and 4 weeks) on the physicochemical attributes and biochemical quality parameters of pidan duck eggs.

2. Materials and methods

A total of 300 fresh duck eggs from the Tegal duck breed (*Anas plathyrynchos*) were used in this study. The experiment employed a factorial completely randomized design (CRD) with two factors arranged in a 2×4 factorial scheme and three replications per treatment. The first factor was acetic acid (CH_3COOH) pretreatment, consisting of two levels: with pretreatment (P) and without pretreatment (SP), each involving 150 eggs. The second factor was pickling duration, consisting of four levels (1, 2, 3, and 4 weeks), with approximately 30 eggs assigned to each treatment group. Data were analyzed statistically using Microsoft Excel and IBM SPSS Statistics version 23. Homogeneity of variance was tested using Levene's test. When the assumption of homogeneity was met (Levene's test, $p>0.05$) and interaction effects between treatments were significant ($p<0.05$), the means were compared using the Tukey's HSD test. Conversely, when the assumption of homogeneity was violated (Levene's test, $p<0.05$) but interactions remained significant ($p<0.05$), the Games–Howell test was applied. Differences in shell thickness and eggshell membrane thickness were further analyzed using a paired *t*-test to determine statistical significance.

2.1. Pidan Making Process

The experiment began by soaking 150 duck eggs in a 5% acetic acid (CH_3COOH) solution for 30 minutes at room temperature (28–30 °C). This group was designated as the P group. After soaking, the eggs were rinsed with distilled water and air-dried for approximately 1 hour (Ganasen & Benjakul, 2011b). The control group, referred to as the SP group, did not undergo pretreatment but was similarly rinsed and air-dried.

All eggs were then immersed in a pickling solution containing 4.2% sodium hydroxide (NaOH), 5% sodium chloride (NaCl), 0.2% zinc chloride ($ZnCl_2$), and 5% black tea, with an egg-to-solution ratio of 10:1 (v/v). The pickling process was divided into four treatment durations—1, 2, 3, and 4 weeks—at room temperature (28–30 °C), with approximately 30 eggs per group. After the designated pickling period, the eggs were rinsed with distilled water and air-dried for about 1 hour.

For the aging process, the surface of each egg was coated with a mixture of red brick powder and water at a ratio of 4:1, forming a layer approximately 2–3 mm thick. The coated eggs were then allowed to dry at room temperature before being stored in plastic containers for three weeks at the same temperature conditions **(Ganasen & Benjakul, 2011b).**

2.2. Physical Properties

The physical properties of the eggshell and eggshell membrane were measured using a digital micrometer with a precision of 10^{-3} mm (Mitutoyo 293-240 Digital Micrometer). Measurements were taken at five different points on each egg to ensure accuracy. The shell membrane was carefully separated from the shell using tweezers, following the procedure described by Ganasen & Benjakul (2011b).

The texture, specifically the resilience, of pidan samples was measured using a texture analyzer (Pertent® Instrument TVT 6700, Sweden) equipped with a 673020 compression probe. The analysis was conducted under compression mode using a predefined single test code, with the instrument connected to a computer for data acquisition. This method allows for precise quantification of the sample's ability to recover its original shape after deformation, reflecting its elasticity and firmness.

Color measurements were performed with a UV-Vis spectrophotometer (Agilent 8453 UV-Visible Spectroscopy, USA) at 420 nm wavelength. Prior to measurement, each sample was diluted 20-fold with distilled water (*aquadest*) according to the method of Benjakul *et al*. (2005).

2.3. Chemical Properties

Cholesterol content was determined using the Liebermann–Burchard method (Kleiner & Dotti, 1958). Approximately 0.2 g of pidan yolk was added gradually to 12 mL of an alcohol–ether mixture (3:1, v/v) in a 15 mL centrifuge tube using a glass stirrer. The mixture was vortexed for 1 minute, allowed to stand for 30 minutes, and centrifuged for 3 minutes at low speed using a clinical centrifuge. The supernatant was carefully decanted into a 50 mL beaker and evaporated in a water bath until dryness. The residue was extracted 2–5 times with chloroform, and the final extract was adjusted to 5 mL with chloroform. To each tube, 2 mL of glacial acetic acid (CH_3COOH) and 0.1 mL of concentrated sulfuric acid (H_2SO_4) were added, shaken gently, and incubated in darkness for 15 minutes. Absorbance was measured at 420 nm using a UV-Vis spectrophotometer (Agilent 8453 UV-Visible Spectroscopy, USA).

MDA levels were determined based on Thiobarbituric Acid Reactive Substances (TBARS) as described by Li-Yun *et al*. (2005). A 3 g sample was homogenized in a Waring blender with 50 mL of distilled water. The homogenate was quantitatively transferred to a 1 L distillation flask, rinsed with 48.5 mL of distilled water, and mixed with 1.5 mL of 4 N HCl (1:2) to adjust the pH to 1.5. A few boiling stones and drops of antifoam were added before distillation to 50 mL. The distillate was stirred, filtered, and 5 mL of the filtrate was mixed with 5 mL of TBA reagent (0.02 M TBA in 90% glacial acetic acid) in a stoppered Erlenmeyer flask. The mixture was heated in boiling water for 35 minutes, cooled under running water, and the absorbance was measured at 532 nm.

TMA content was determined using the Conway micro-diffusion method (SNI 2354.8:2009). Samples were extracted with 7% trichloroacetic acid (TCA) solution, and a few drops of 37% formaldehyde were added in a Conway dish. The dish was left at room temperature overnight to allow complete reaction. The released TMA was titrated with 0.02 N HCl to determine concentration.

The pH value was determined following AOAC (2005) and Yang *et al*. (2019). The sample was homogenized with distilled water at a ratio of 2:1 (w/v). A portion equivalent to 10 g of the sample (approximately 15 g mixture) was transferred into a beaker, and distilled water was added to reach a volume of 150 mL. The mixture was filtered using double-layer Whatman filter paper, and pH was measured with a calibrated pH meter (SCHOTT® Instrument Lab 850, Germany) using standard buffer solutions.

Water activity (a_w) was measured according to Susanto (2009) using a Novasina MS-1 a_w meter. The sample was placed in the sample chamber, and the instrument was sealed and equilibrated for 3 minutes. The a_w reading was then recorded once the value stabilized.

3. Result and discussion

3.1. Physical Characteristic

3.1.1. Eggshell and Shell Membrane Measurement

Treatment with 5% acetic acid (CH_3COOH) had a statistically significant effect on the thickness of both eggshells and shell membranes ($p < 0.05$). Specifically, eggs in the P group exhibited shell thicknesses that were, on average, 0.10 mm thinner than those in the SP group. These findings are consistent with the results of Kaewmanee *et al*. (2012), who reported that duck eggs subjected to 5% CH_3COOH pretreatment for 30 minutes developed significantly thinner shells (0.37 ± 0.03 mm) compared to eggs without pretreatment (0.43 ± 0.02 mm).

Table 1 Eggshell, shell membrane thickness of duck egg treated with and without 5% CH_3COOH as pretreatment

	Without pretreatment (SP)	**Pretreatment (P)**
Eggshell (mm)	0.44±0.065	0.36±0.022
Shell membrane (mm)	0.09±0.006	0.07±0.014
Eggshell+shell membrane (mm)	0.53±0.071^{a}	0.43±0.036^{b}

Numbers followed by different superscripts in the same row are significantly different (p<0.05)

Eggshells consist of approximately 3.3% organic matter and 95.1% inorganic matter. The primary inorganic constituent is calcium carbonate ($CaCO_3$), also known as calcite, which accounts for about 90.9% of the eggshell composition **(Ajayan *et al*., 2020)**. When exposed to a 5% acetic acid solution, the calcium carbonate component undergoes dissolution through a decalcification process **(Sujoseputro *et al*., 1995; Kaewmanee *et al*., 2012)**. This chemical reaction can be represented by the following equation :

$$CaCO_3\ (s) + 2CH_3COOH\ (aq) \rightarrow Ca(COOH)_2\ (aq) + H_2O(aq) + CO_2\ (g)$$

In this reaction, calcium carbonate reacts with acetic acid to form calcium acetate, carbon dioxide, and water. This process effectively reduces eggshell thickness by dissolving the mineral matrix, which is consistent with findings that pretreatment with acetic acid results in thinner eggshells (Figure 1). Understanding this reaction is crucial for optimizing pretreatment conditions in pidan egg production, as the controlled dissolution of eggshell mineral content can influence the texture and quality of the final product.

Figure 1 Duck egg with (left) and without (right) pretreatment.

3.2. Browning Intensity and Resilience of Pidan Eggs

In Table 2, treatment with 5% acetic acid (CH_3COOH) and pickling time significantly affected the browning intensity of pidan eggs. The results show that the treatments SP4, P1, and P4 exhibited the highest browning values. Previous studies on pidan eggs have indicated that browning intensity is primarily associated with the formation of Amadori products (ketoamines) (Ganasen & Benjakul, 2010; Ganasen & Benjakul, 2011a; Zhao *et al*., 2014).

Table 2 Browning intensity of pidan eggs treated with 5% CH_3COOH as pretreatment and different pickling duration

Pickling time (weeks)	SP	P
1	0.38±0.01^{d}	1.13±0.00^{a}
2	0.50±0.00^{c}	0.97±0.00^{b}
3	0.11±0.00^{e}	0.98±0.00^{b}
4	1.12±0.00^{a}	1.11±0.01^{a}

SP = Without pretreatment, P = With pretreatment numbers followed by different superscripts in the same row are significantly different ($p<0.05$)

Based on the overall results in Table 2, samples with thinner eggshells tend to contain more melanoidin pigment than those with thicker eggshells. Some researchers have found that this phenomenon is likely due to cross-linked protein products (Alavi *et al*., 2020; Vandermoortele *et al*., 2021). The alkaline solution (pH >12) promotes numerous cross-linking reactions in proteins, which mainly lead to the formation of Maillard Reaction Products (MRPs) (Zhu *et al*., 2020). The color changes observed in Figure 2, where the albumen and yolk develop blackish-brown and brownish-green hues, can be attributed to the Maillard reaction (browning) occurring during pidan production (Zhu *et al*., 2020; Boateng & Yang, 2021).

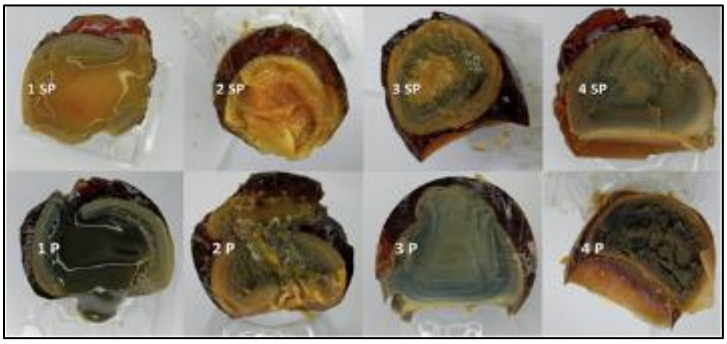

Figure 2 Peeled pidan egg (half-cutted) SP1=Pidan without preteatment in 1 w of pickling, SP2= Pidan without preteatment in 2 w of pickling, SP3= Pidan without preteatment in 3 w of pickling, SP4= Pidan without preteatment in 4 w of pickling, P1= Pidan with preteatment in 1 w of pickling, P2= Pidan with preteatment in 2 w of pickling, P3= Pidan with preteatment in 1 w of pickling, P4= Pidan with preteatment in 4 w of pickling

Resilience values represent the firmness and jelly-like texture of pidan albumen. Consumer preference tends to favor a jelly and solid texture rather than a mushy or runny consistency in either the pidan yolk or albumen. Visually, pidan exhibited a mushy and runny texture during the first and second weeks of pickling (Figure 2). According to Table 3, there was a significant interaction between 5% acetic acid (CH_3COOH) treatment and pickling time on resilience. The groups SP1, SP2, SP3, SP4, P3, and P4 showed the highest resilience values, whereas P1 and P2 had the lowest ($p < 0.05$). Additionally, no significant difference was observed between the resilience values of the SP and P groups pickled for three and four weeks.

Table 3 Resilience of pidan eggs treated with 5% CH_3COOH as pretreatment and different pickling duration

Pickling time (weeks)	No pretreatment (Pa)	Pretreatment (Pa)
1	0.39±0.22^{ab}	0.17±0.11^{b}
2	0.99±0.10^{a}	0.19±0.04^{b}
3	0.40±0.32^{ab}	0.66±0.18^{ab}
4	0.81±0.35^{ab}	0.49±0.34^{ab}

Numbers followed by different superscripts in the same row are significantly different ($p<0.05$)

Marked in Table 3, except for the runny and mushy texture of egg yolks observed in Figure 2, the optimal color and texture of pidan eggs were found in the SP4, P3, and P4 groups. The brownish-green color combined with a firm, jelly-like texture likely indicates stable protein aggregation, which may help inhibit microbial contamination. This physical stability not only enhances the sensory qualities of pidan eggs but also contributes to their safety and shelf life (Ganasen & Benjakul, 2010; Ganasen & Benjakul, 2011a; Zhao *et al*., 2014). These findings support the importance of pickling duration and pretreatment with acetic acid in achieving desirable product properties.

3.3. Chemical Characteristic

3.3.1. Cholesterol, TMA, and MDA Content in Pidan Egg

Pretreatment and pickling time significantly affected the cholesterol content of pidan egg yolks, as shown in Figure 3. In this experiment, the highest cholesterol concentration was observed in the SP2 group. In contrast, the lowest cholesterol values were found in the SP1, P2, and P3 groups. The elevated cholesterol content detected in the SP2 group is likely attributable to incomplete hydrolysis, rather than representing a true maximum value. This conclusion is supported by the physical appearance of the yolk in SP2, which exhibited no noticeable changes in color or texture when compared to the P2 group. These findings highlight the importance of both pretreatment and pickling duration in optimizing the nutritional quality of pidan eggs.

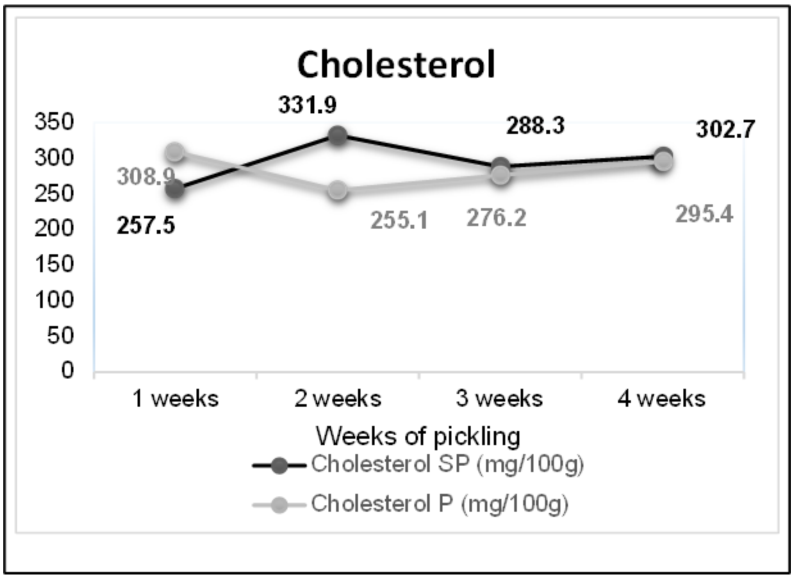

Figure 3 Pidan cholesterol chart

Alkaline conditions play a prominent role in driving unstable chemical reactions, making chemical and physical stability crucial during pidan egg processing (Cao *et al*., 2021). In this experiment, the cholesterol content in pidan eggs was lower than the average cholesterol in fresh eggs (333.25 mg/100 g), which is suspected to be due to the effect of thinner shells accelerating the cholesterol hydrolysis process. These results are consistent with Li-yun *et al*. (2005), who reported that pidan processing reduces cholesterol in duck eggs from 3800 mg/100 g to 2851 mg/100 g. The solidity observed in the pidan yolk could indicate lipid exudation, reflecting changes in lipid molecules that contribute to a firmer texture. Increased cholesterol stability (Figure 3) correlated with the solid texture of pidan (Figure 2). This study found that cholesterol stability was optimal in groups pickled for three weeks, regardless of pretreatment. Among all groups, the P3 group showed the most favorable cholesterol stability and content to date.

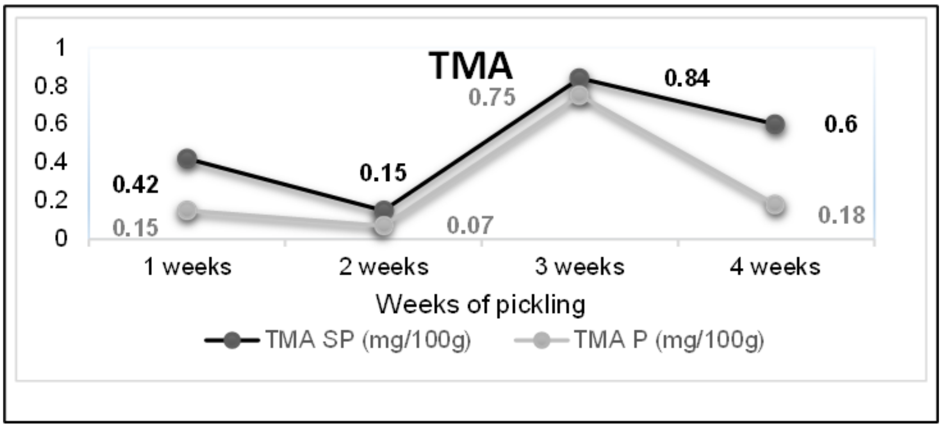

Figure 4 Pidan TMA chart.

Based on Figure 4, the SP3, SP4, and P3 groups exhibited the highest trimethylamine (TMA) content, whereas the lowest levels were observed in the P1, P2, and P4 groups. Currently, there is no official TMA standard for food safety; however, Suwetja (2011) reported that fish containing 0.40 mg/100 g TMA is considered fresh, while levels between 4–6 mg/100 g correspond to the loss of the distinctive fresh fish odor. The TMA levels found in this study fall below these freshness thresholds, indicating safety for consumption.

Regarding malondialdehyde (MDA), concentrations in pidan samples ranged from 0.5384 to 1.2343 mg/100 g (Figure 5). These values are well below the tolerance limit set by the European Food Safety Authority Scientific Committee, which recommends a maximum intake of 1500 mg/100 g per day for individuals weighing 50 kg (Ma *et al*., 2021). These findings collectively suggest that the evaluated pidan eggs are within safe limits for TMA and MDA levels, supporting their suitability for consumption.

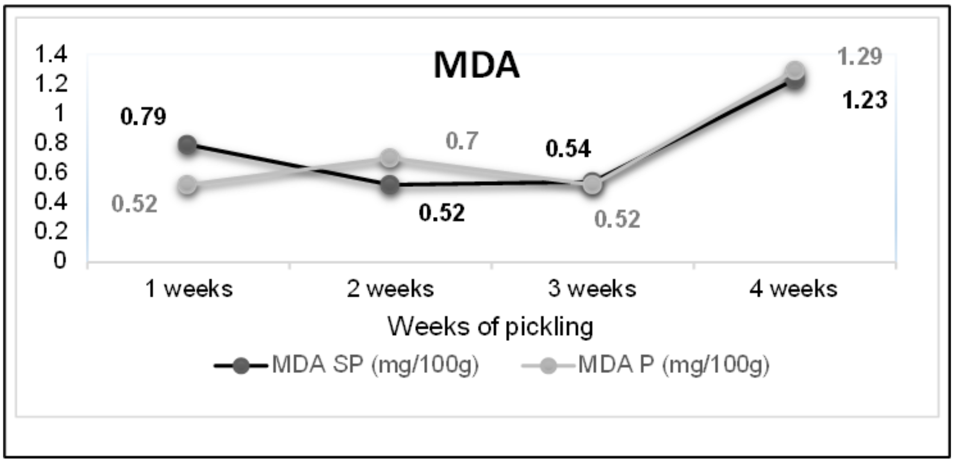

Figure 5 Pidan MDA chart.

Hydrolysis of yolk lipids in pidan eggs may occur via saponification reactions, as described by Wang and Fung (1996) and Cao *et al*. (2021). The diffusion of alkaline solutions into the yolk is evident from the measured pH values in pidan yolks after the aging process, ranging from 8.23 to 9.97 (Table 4). These strongly alkaline conditions not only facilitate protein-lipid cross-linking but also contribute to reduced levels of malondialdehyde (MDA) and cholesterol, as supported by Ma *et al*. (2021).

This study found the lowest MDA values in the P3 group, indicating that three weeks of pickling with highly alkaline solutions (pH >12.00, unpublished data) promotes oxidation reactions that increase hydrophilic and electrostatic properties in the yolk. The reactive nature of resulting lipid compounds, whether as cross-linked products or carbonylated proteins, warrants further research to fully understand their implications in pidan egg quality and safety (Wang & Fung, 1996; Ganasen & Baharin, 2014; Wang *et al*., 2019).

3.3.2. pH and Water Activity (a_w) in Pidan Eggs

The pH measurements in Table 4 show that the lowest albumen pH is on SP4 and P3 groups. In fresh duck eggs, the pH albumen is around 7.6 (Chen *et al*. 2005). This research shows a pH range from 0.82-0.91 for albumen and 0.84-0.89 for the yolk, where a pH value >7 makes pidan into the alkaline food group.

Table 4 Contents of pH, and a_w in pidan eggs treated with 5% CH_3COOH in different pickling duration

	SP groups in the weeks of-				**P groupss in the weeks of-**			
	1	2	3	4	1	2	3	4
pH yolk	9.97 ± 0.01^{a}	8.23 ± 0.04^{h}	8.54 ± 0.04^{g}	8.86 ± 0.02^{f}	$9.02\pm.02^{e}$	9.68 ± 0.02^{b}	9.4 ± 0.01^{d}	9.56 ± 0.01^{c}
pH albumen	9.18 ± 0.03^{d}	9.67 ± 0.02^{c}	9.82 ± 0.04^{b}	8.96 ± 0.02^{e}	9.93 ± 0.02^{a}	9.90 ± 0.03^{ab}	9.10 ± 0.06^{d}	9.70 ± 0.01^{c}
a_w *yolk*	0.87 ± 0.00^{e}	0.88 ± 0.00^{b}	0.87 ± 0.00^{d}	0.84 ± 0.00^{f}	0.87 ± 0.00^{c}	0.89 ± 0.00^{ab}	0.89 ± 0.00^{a}	0.84 ± 0.00^{f}
a_w albumen	0.82 ± 0.00^{f}	0.86 ± 0.00^{bc}	0.84 ± 0.00^{cd}	0.86 ± 0.00^{bc}	0.84 ± 0.00^{bcd}	0.91 ± 0.00^{a}	0.85 ± 0.01^{bcde}	0.86 ± 0.00^{bcd}

SP = Without pretreatment, P = With pretreatment Numbers followed by different superscripts in the same row are significantly different ($p<0.05$)

Pidan is an exceptional animal-derived food classified within alkaline food groups, alongside fruits, nuts, legumes, and vegetables (Hanley & Whiting, 2013). According to Wang *et al*. (2021), who investigated microbial contamination in pidan under various packaging and storage conditions, unpackaged pidan products can remain uncontaminated at room temperature for up to three months.

The water activity (a_w) of pidan eggs reached a maximum of 0.906 in P2 albumen and 0.838 in P3 yolk, both notably lower than the typical value for fresh eggs (0.998). As shown in Table 4, the a_w values for pidan albumen and yolk are below the threshold for potential contamination by *Salmonella enterica* spp. (a_w <0.93). In addition to water activity, factors such as pH, temperature, and storage conditions also play critical roles in ensuring the microbial safety of food products (Susanto, 2009).

4. Conclusion

Pidan eggs subjected to pretreatment showed significantly greater reductions in trimethylamine (TMA) and cholesterol content compared to those without pretreatment. Eggs with thinner shells allowed faster penetration of alkaline solutions than those with thicker shells. The decalcification process induced by 5% acetic acid pretreatment helped synchronize degradation reactions during alkaline pickling over a four-week period. Thinner eggshells began showing yolk color changes after one week of pickling and achieved an ideal solid-yolk texture by the third week, whereas thicker eggshells started texture changes at two weeks and color changes at three weeks. The increase in pH during pidan processing promotes lipid and protein degradation and the formation of random bonds among proteins, lipids, and carbohydrates, resulting in lowered cholesterol levels. Both thick and thin eggshell groups reached optimal cholesterol, TMA, and malondialdehyde (MDA) values, but thinner eggshell groups achieved these optimal values in a shorter pickling time. Overall, soaking eggs in 5% acetic acid for 30 minutes as pretreatment followed by three weeks of pickling is the most effective method to produce pidan eggs with desirable appearance and reduced TMA (0.75 mg/100 g), cholesterol (276.2 mg/100 g), and MDA (0.52 mg/100 g) content.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no potential conflicts of interest.

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