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Research Article

Physicochemical and microbiological characteristics of pindang eggs with variations in tannin sources from different leaf extracts

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Abstract

Eggs are a source of protein that can be processed into various food products. One of these processed egg products in Asia is pindang eggs, which undergo a tanning process using natural tannin sources. This study aims to determine the effects of egg tanning using different leaf extracts as tannin sources on the physicochemical and microbiological characteristics of pindang eggs. This experimental study used a completely randomized design with different tannin sources: P1 (*Catappa leaves*), P2 (*Moringa leaves*), and P3 (*Stevia leaves*). The physicochemical and microbiological quality analyses included texture profile, color, proximate composition, and total microbial count. The hardness values of pindang eggs were 37.01N, 42.94N, and 41.44N, respectively. The protein content of pindang eggs was 14.91%, 17.12%, and 13.12%, while the fat content was 5.67%, 5.06%, and 4.43%, respectively. The antioxidant activity of the eggs was 11.48%, 14.81%, and 33.98% for P1, P2, and P3, respectively. On day 0, total microbial counts were 6.46, 5.84, and 6.82 CFU/g, but after three days, all samples were too numerous to count (TNTC). This study concluded that egg tanning with different tannin sources from various plant leaves significantly affects the physicochemical properties of pindang eggs.

Keywords

catappa, moringa, pindang egg, stevia, tannin

Abbreviations

CFU – colony forming unit; CRD – completely randomized design; DPPH – 2,2-difenil-1-pikrilhidrazil; DMRT – duncan's multiple range test; TPA – texture profile analyzer; TNTC – too numerous to count.

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Introduction

Eggs are an essential protein source that fulfils the nutritional needs of society. They contain essential proteins that support human growth and development (Wulandari and Arief 2022). Additionally, eggs provide essential fats required by the body (Lestari et al. 2021). Some eggs contain omega-3 fatty acids, which have anti-inflammatory properties and play a preventive role against degenerative diseases (Hardisman 2015).

Commonly consumed eggs come from poultry species such as chickens, ducks, geese, and quails. These eggs can be processed into various food products using different methods. Egg processing aims to enhance nutritional value, improve sensory quality, and extend shelf life (Habiburrochman et al. 2023). Boiled eggs, a common cooking method, are highly perishable if not treated appropriately to prolong their shelf life. Improper handling can lead to microbial contamination and spoilage (Mudawaroch et al. 2022). Microbial contamination degrades macromolecules such as proteins, causing spoilage and off-odors in eggs (Habiburrochman et al. 2023).

One method to prevent egg spoilage is pindang processing. This technique involves egg tanning using tannins (Hakim et al. 2019). Tannins bind with egg proteins, resulting in a brownish color on the egg surface. The tanning process enhances egg preservation by sealing egg pores, preventing microbial infiltration. Pindang processing also increases antimicrobial compounds, extending the shelf life of eggs. Traditionally, guava leaves are used as a tannin source in pindang processing, improving the physicochemical quality and shelf life of eggs (Mudawaroch et al. 2022).

Recent studies have explored the use of natural plant extracts in traditional egg processing to enhance shelf life and microbiological safety. Hakim et al. (2019) examined the effect of guava leaf extract, shallot skin, onion, and tea dregs on the total microbial count of pindang eggs. Similarly, Lestari et al. (2022) investigated the use of tanning agents from guava (*Psidium guajava*) and teak (*Tectona grandis*) leaves in the production of pindang eggs from broiler chicken eggs, focusing on their organoleptic properties. Additionally, Habiburrochman et al. (2023) studied the shelf life and physical characteristics of quail eggs soaked in

ethanol extract of mangosteen peel. These studies demonstrate a growing interest in utilizing natural antioxidants and antimicrobials in egg-based products. However, the use of moringa, catappa, and stevia leaf extracts as tannin sources in the processing of pindang eggs has not yet been explored. According to Susanty et al. (2019) moringa (*Moringa oleifera*), catappa (*Terminalia catappa*), and stevia (*Stevia rebaudiana*) exhibit high antioxidant activity.

Antioxidants play a crucial role in food preservation by inhibiting bacterial growth, thus preventing food spoilage (Sumpono et al. 2017). Various plant sources, including leaves, contain high antioxidant levels. Previous studies indicate that moringa, stevia, and catappa leaves possess strong antioxidant properties. Moringa leaves have been extensively studied for disease prevention, while stevia contains bioactive compounds, which exhibits antibacterial properties (Hardiansyah et al. 2022; Saadh et al. 2023). Stevia is significantly sweeter than sucrose and possesses antibacterial properties (Harismah et al. 2021). Meanwhile, catappa leaves contain high levels of antioxidants, yet research on their applications remains limited. Catappa antioxidants have been found to scavenge free radicals effectively (Salimi et al. 2022). Apart from antioxidants, moringa, catappa, and stevia leaves also contain tannins, which can be utilized for pindang egg tanning.

Although several studies have explored pindang egg processing, research on the use of tannins derived from moringa, stevia, and catappa leaves remains limited. Addressing this knowledge gap, the present study investigates the effects of pindang processing using tannin extracts from moringa, catappa, and stevia leaves. The objective is to evaluate the influence of these tannin sources on the physicochemical and microbiological properties of pindang eggs.

Materials and Methods

Materials. The equipment used in this study included a laboratory drying oven (Memmert, Germany) was used to dry the materials, thermometer, Soxhlet flask, Kjeldahl flask, spectrophotometer, colony counter, incubator, muffle furnace, chromameter, and texture analyser. The materials used were duck eggs, catappa leaves

collected from the university garden in Surakarta, moringa leaves from Kartasura market Indonesia, and stevia leaves from a medicinal plant farm in Karanganyar Indonesia. Additional reagents included DPPH, distilled water, Plate Count Agar, and H₂SO₄.

Research design. This study employed a completely randomized design (CRD) with one factor: different tannin sources from leaves. The three types of leaves used as tannin sources were P1: catappa, P2: moringa, and P3: stevia leaves. A total of 30 duck eggs were used in this study, divided evenly into three treatment groups (10 eggs per group). Each group represented a different tannin source. From each group of 10 eggs, samples were randomly selected and assigned for different analyses: 2 eggs were used for texture profile analysis, 2 eggs for color measurement, 3 eggs for proximate composition (chemical composition), and 3 eggs for microbiological analysis.

Preparation of leaf extracts. The extraction process for catappa, moringa, and stevia leaves involved several steps: (1) selecting intact leaves of uniform maturity; (2) drying the leaves in an oven at 60°C for 6 h; (3) weighing 50 g of each dried leaf; (4) preparing 1 L of distilled water for extraction; (5) boiling the distilled water; (6) immersing the leaves in boiling at 100°C for 15 min; (7) filtering the extract; and (8) storing the extracts for use in pindang egg processing.

Pindang egg processing. The pindang egg preparation followed these steps: (1) selecting duck eggs of uniform grade; (2) washing the eggs to remove surface dirt; (3) boiling the eggs in distilled water over low heat (70°C) for 15 min.; (4) cooling the eggs to room temperature and peeling the shells; (5) preparing the boiling medium using catappa, moringa, or stevia leaf extracts; (6) heating the extracts to a boil; (7) immersing the peeled eggs completely in the boiling extracts; (8) simmering for three hours; (9) after the 3-hour simmering process, the eggs were drained and allowed to air-dry at 28°C for 1 h.

Texture analysis of pindang eggs. The texture analysis of pindang eggs was conducted using the texture profile analyzer (TPA). The procedure for texture analysis followed the study by (Indiarto et al. 2013). The analysis was carried out by preparing a sample of the egg white from pindang eggs with

dimensions of 3×3×3 cm; placing the egg white sample on the probe; and pressing it twice using a probe with a diameter of 6 mm. The probe speed was set to 5 mm.s⁻¹, and the sample was compressed to 30% of its initial height. The texture analysis parameters included hardness, cohesiveness, adhesiveness, and gumminess.

Proximate analysis. The moisture content of the eggs was analyzed using the gravimetric method. The fat content was determined using the Soxhlet method, while the protein content of the preserved eggs was analyzed using the Kjeldahl method.

Antioxidant activity. The procedure for analysing antioxidant activity was carried out using the DPPH measurement method. The initial step of this test involved preparing 0.1 mM DPPH solution. Next, 100 µL of the 0.1 mM DPPH solution was pipetted into 100 mL volumetric flask, and methanol was added up to the mark (0.1 mM DPPH). The maximum wavelength of 2 mL of the 0.1 mM DPPH solution was determined using a cuvette and measured at a wavelength range of 400-800 nm using a UV-Vis spectrophotometer, with the maximum wavelength observed at 517 nm. The next step was the preparation of a blank solution. Subsequently, a stock solution of the extract with a concentration of 1000 ppm was prepared by dissolving 100 mg of the sample in methanol and transferring it into a 100 mL volumetric flask, with the volume adjusted to the mark using methanol. The absorbance was then measured using a UV-Vis spectrophotometer. For the test, 2 mL of each test solution concentration was added to a test tube, followed by 2 mL of the 0.1 mM DPPH solution. The mixture was vortexed for 30 min. Next, the absorbance was measured at a wavelength of 517 nm. The final step was the determination of the percentage inhibition. The radical scavenging activity was expressed as the percentage inhibition, which was calculated using the following formula:
% Inhibition = (Abs Blank - Abs Sample) / (Abs Blank) × 100%

Total microbial count. The total microbial count was analyzed using the total plate count method (Tama et al. 2023). One gram of egg sample was aseptically collected and homogenized in 9 mL of distilled water, followed by serial dilutions up to 10⁻⁵. The diluted samples were then inoculated onto petri dishes containing nutrient agar and incubated

at 38°C for 48 h. Colony counts were determined using the standard plate count method.

Statistical analysis of data. The research data were analyzed using SPSS 20 software with ANOVA. If the ANOVA test indicated a significant effect, it was followed by Duncan's Multiple Range Test (DMRT).

Results and Discussion

Texture profile. The physical quality analysis was conducted on pindang egg samples, including hardness, cohesiveness, adhesiveness, and gumminess. The average values for texture pindang eggs are presented in Table 1.

Based on statistical analysis using ANOVA, it was found that different tannin sources did not significantly affect the hardness, cohesiveness, adhesiveness, and gumminess of pindang eggs. According to Table 1, the hardness values of pindang eggs processed with tannin sources from catappa, moringa, and stevia leaves were 37.01 N, 42.94 N, and 41.44 N, respectively. The cohesiveness values for pindang eggs processed with tannin from catappa, moringa, and stevia leaves were 0.44, 0.45, and 0.32, respectively. Table 1. also shows that the adhesiveness values for pindang eggs processed with tannin from catappa, moringa, and stevia leaves were 1.40 N.mm, 0.31 N.mm, and 0.89 N.mm, respectively. Meanwhile, the gumminess values for pindang eggs were 17.34 N, 19.97 N, and 13.37 N, respectively. Tannins

interact with proteins through hydrogen bonds and hydrophobic interactions, forming protein-tannin complexes that improve egg hardness and cohesiveness (Tsurunaga et al. 2024). Polyphenolic compounds can interact with egg yolk proteins through hydrophobic interactions, hydrogen bonds, and ionic bonds, leading to a more stable protein structure and preventing unfolding during boiling. This process improves texture and reduces oxidation reactions (Tang et al. 2025). Molecular structure and size play a crucial role in protein aggregation. Larger tannin molecules have been found to have a more significant effect on protein aggregation, thereby influencing the texture of protein-rich foods such as eggs (Ropiak et al. 2017).

Protein-tannin complexes can form a denser network, thereby increasing egg hardness. Tannin-protein interactions enhance the internal structural stability of food, improving cohesiveness (Bourne 2002). The increase in gumminess is a result of the combined effect of increased hardness and cohesiveness, contributing to a more elastic food texture (Bourne 2002).

Stevia and moringa leaves contain bioactive compounds in addition to tannins that are more effective in forming stable protein-tannin complexes, potentially increasing hardness (Goyal et al. 2010). In contrast, although catappa leaves contain high tannin levels, their interaction with egg proteins may be less optimal, resulting in lower hardness.

Table 1. Texture profile of Pindang eggs with variations in tanning leaf extracts

Tannin leaf extract	Texture profiles of Pindang eggs			
	Hardness, N	Cohesiveness	Adhesiveness, N.mm	Gumminess, N
Catappa	37.01±6.16	0.44±0.07	1.40±0.37	17.34±6.15
Moringa	42.94±1.61	0.45±0.02	0.31±0.08	19.97±1.48
Stevia	41.44±3.25	0.32±0.04	0.89±0.05	13.37±0.64

Table 2. Colour characteristics of Pindang eggs with variations in tanning leaf extracts

Tannin Leaf Extract	Colour characteristics of Pindang eggs		
	L*	a*	b*
Catappa	40.00±0.55 ^a	14.05±0.31 ^a	18.87±0.68 ^a
Moringa	44.40±0.18 ^b	11.85±0.10 ^b	23.85±0.83 ^b
Stevia	36.80±0.94 ^c	4.97±0.09 ^c	12.70±0.67 ^c

Note: The same superscript letters indicate no significant difference between treatments

**Figure 1.** Appearance of Pindang egg colour**Table 3.** Properties and antioxidant activity of Pindang eggs

Chemical composition parameter	Tannin leaf extract		
	Catappa	Moringa	Stevia
Moisture content, %	42.53±0.53 ^a	30.30±0.64 ^b	40.99±0.62 ^c
Fat content, %	5.67±0.19 ^a	5.06±0.57 ^b	4.43±1.07 ^b
Protein content, %	14.91±0.76 ^a	17.12±0.53 ^b	13.12±0.09 ^c
Water activity, %	0.95±0.00 ^a	0.94±0.00 ^a	0.98±0.00 ^b
Antioxidant activity, %	11.48±0.56 ^a	14.81±1.09 ^b	33.98±1.73 ^c

Note: The same superscript letters indicate no significant difference between treatments

Colour characteristics. The physical quality of pindang eggs was assessed through color analysis using the L*, a*, and b* parameters. The average values of L*, a*, and b* are presented in Table 2.

ANOVA results showed that the different tannin extracts from catappa, moringa, and stevia leaves significantly affected the L*, a*, and b* color values of pindang eggs. The highest L* value, indicating the brightest appearance, was observed in eggs treated with moringa leaf extract, while the lowest L* value (36.8) was found in eggs treated with stevia leaf extract.

Although moringa leaves contain tannins, they are also rich in bioactive compounds such as chlorophyll, flavonoids, and vitamin C. Chlorophyll, in particular, can influence the color of pindang eggs by giving them a bright green hue, thereby increasing the L* value (Anwar et al. 2007). According to Siagian et al. (2020), the addition of stevia leaves to a product can result in a decrease in brightness levels.

As presented in Table 2, the a* value was highest in pindang eggs treated with catappa leaf extract, reaching 14.05, compared to the other two treatments. The lowest a* value was observed in pindang eggs treated with stevia leaf extract, at 4.97.

The b* value analysis showed that pindang eggs treated with moringa leaf extract had the highest b* value of 23.85, while the lowest b* value, 12.7, was found in pindang eggs treated with stevia leaf extract.

Variations in the a* and b* values among pindang eggs processed with different tannin sources indicate differences in the resulting egg color. This is influenced by the tannin compounds present in the catappa, moringa, and stevia leaves extracts, as well as their interactions with egg components during the tanning process. Tannins in catappa leaves tend to produce a reddish-brown color due to their high phenolic compound content and the Maillard reaction during pindang processing (Herly et al. 2020). The color alterations induced by the Maillard reaction can influence the sensory acceptability of processed egg products (Al-Abbasy et al. 2024).

Based on Fig. 2, pindang eggs treated with moringa leaf extract exhibited the brightest color, followed by those treated with catappa leaf extract. In contrast, pindang eggs treated with stevia leaf extract appeared to have the darkest colour. The higher the tannin concentration, the more intense the colour change that occurs (Hakim et al. 2019).

Chemical composition and antioxidant activity.

The average moisture content, fat content, protein content, water activity, and antioxidant activity of pindang eggs processed with different tannin sources are presented in Table 3.

As shown in Table 3, different tannin sources significantly affected the chemical composition and antioxidant activity of pindang eggs. Moisture content was also significantly influenced, with the highest value observed in eggs treated with catappa leaf extract, followed by stevia, and the lowest in eggs treated with moringa leaf extract. Tannins from catappa leaves interact with egg proteins, leading to protein precipitation, which alters the structural integrity of the egg matrix, thereby reducing its ability to retain water (Adamczyk et al. 2012).

Stevia (*Stevia rebaudiana*), as a tannin source, exhibited lower moisture content than catappa but higher than moringa. This may be due to stevia's ability to modulate moisture through tannin interactions with proteins and carbohydrates. Stevia is also known to have moderate hygroscopic properties, allowing it to maintain moisture content within a specific range (Gasmalla et al. 2014). The lowest moisture content was found in pindang eggs treated with moringa leaf extract, which can be attributed to the strong ability of moringa tannins to bind water through hydrogen bond formation with hydroxyl (-OH) groups (Gopalakrishnan et al. 2016).

The fat content of pindang eggs treated with catappa leaf extract was significantly different from those treated with moringa and stevia leaf extracts, which showed no significant difference between them. Catappa leaves have a high tannin content (Salimi et al. 2022). The interaction between tannins and proteins can lead to higher fat retention in eggs boiled with Catappa extract. Additionally, differences in fat content among pindang eggs may be attributed to variations in the composition of different compounds involved in lipid degradation during the cooking process, ultimately affecting fat retention in eggs (Salimi et al. 2022).

As shown in Table 3, the protein content of pindang eggs treated with moringa leaf extract was higher than that of eggs treated with catappa and stevia leaf extracts. The average protein content of pindang eggs treated with moringa leaves was 17.12%, while the protein content of eggs treated with catappa and

stevia leaf extracts was 14.91% and 13.12%, respectively. The high protein content in moringa leaves may contribute to an increase in egg protein during the boiling process (Rossida et al. 2019). Other studies also suggest that during pindang processing, protein compounds from moringa leaf extract accumulate in the eggs (Mariati et al. 2022). The study results also indicated that there was no significant difference in the average water activity of pindang eggs treated with catappa and moringa leaf extracts. However, the water activity of pindang eggs treated with stevia leaf extract showed a significant difference compared to those treated with catappa and moringa leaf extracts.

The research findings presented in Table 3, also revealed significant differences in antioxidant activity among pindang eggs treated with different tanning leaf extracts. The study results showed that pindang eggs treated with Stevia leaf extract had the highest antioxidant activity at 33.98%, followed by eggs treated with moringa and catappa leaf extracts at 14.81% and 11.48%, respectively. The use of natural tanning agents can influence the characteristics of pindang eggs, including their antioxidant activity (Mentari et al. 2025). Stevia leaves contain various bioactive compounds, with a total phenolic content of 86.30 ± 1.14 mg GAE.g⁻¹, which correlates with the level of antioxidant activity (Frankson et al. 2024).

Total microbial count. The microbiological quality analysis of pindang eggs was conducted by measuring the total bacterial count at 0 and 3 days of storage. The Pindang eggs were stored without packaging at 30°C, 80% RH to reflect traditional practices. Microbial counts were determined on day 0 and day 3. On the third day, microbial growth was too numerous to count (TNTC), and further enumeration was discontinued. The average total microbial count of pindang eggs is presented in Table 4.

Based on the research findings presented in Table 4, the total microbial count of pindang eggs treated with tannins from catappa, moringa, and stevia leaves at 0 days of storage was 6.46 CFU.g⁻¹, 5.84 CFU.g⁻¹, and 6.82 CFU.g⁻¹, respectively. Saad et al. (2022) reported that boiling quail eggs for 5 min combined with refrigerated storage effectively eradicates *Escherichia coli* O157.

While the quality parameters of hard-boiled eggs stored at room temperature and under refrigeration appeared similar, storing them at ambient temperature increases the likelihood of microbial proliferation and product spoilage, which may compromise food safety. In contrast, refrigeration helps minimize moisture loss, thereby maintaining the eggs' sensory attributes such as texture and flavour, while also inhibiting the growth of pathogenic bacteria (Eregama et al. 2024). Storing pindang eggs at a low temperature of approximately 4°C can slow down microbial growth, thereby extending the shelf life of pindang eggs (Amalo et al. 2016).

Table 4. Total microbial count. of Pindang eggs with variations in tanning leaf extracts

Tannin Leaf Extract	Total microbial count. of Pindang eggs, CFU.g ⁻¹	
	0 day	3 days
Catappa	6.46	TNTC
Moringa	5.84	TNTC
Stevia	6.82	TNTC

Conclusions

Tannin compounds from different leaves used as tanning agents for eggs can influence the physical properties of pindang eggs, particularly texture and color, as reflected in the L*, a*, and b* values. Variations in tannin sources also affect the chemical composition of pindang eggs, including antioxidant activity. Pindang eggs treated with different tannin extracts, when stored at room temperature, experienced a decline in microbiological quality by the third day of storage. It is recommended that future studies focus on assessing the sensory quality and conducting comprehensive pathogen bacterial profiling of eggs subjected to different storage temperature.

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Author Contributions

Conceptualization, E.S.A. and K.O.K.; methodology, E.S.A., K.O.K. and I.O.O.; formal analysis E.S.A., and K.O.K.; investigation, E.S.A., I.A.E., and K.O.K.; resources, K.O.K., F.O.A., I.O.O., and E.S.A.; data curation, E.S.A., and I.A.E.; writing – original draft preparation, I.A.E., and E.S.A; writing - I.A.E., E.S.A., and K.O.K., review and editing, K.O.K., F.O.A., and I.O.O.; supervision, F.O.A., and I.O.O.; All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

The experimental protocol and treatment of the animals was in accordance with the principle of laboratory animal care (NIH Publication 85-93, revised 1985) and OAUSTECH ethical approved committee standards with reference code (OAUSTECH/ETHC–BCH/2022/05)

Informed Consent Statement

Not applicable.

Data Availability Statement

The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding authors.

Conflicts of Interest

The authors declare no conflicts of interest.

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