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Roselle powder proportion in the aging process enhances pidan's antioxidant activity and hedonic value

Siti Susanti[1], Heni Rizqiati[1], Nuryanto Nuryanto[2], Lutfi Purwitasari[1], Fahmi Arifan[3*]

[1]*Food Technology, Department of Agriculture, Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, Indonesia.*
[2]*Departement of Nutrition Science, Faculty of Medicine, Diponegoro University, Indonesia.*
[3]*Industrial Chemical Engineering Technology, Vocation School, Diponegoro University, Semarang, Indonesia.*

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*Correspondence:

Corresponding author: Fahmi Arifan
E-mail address: fahmiarifan@live.undip.ac.id

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ABSTRACT

This study was about the potential of roselle (*Hibiscus sabdariffa* Linn.) powder as a functional compound in the production of pidan eggs. The research was evaluating its antioxidant activity, chemical composition, and sensory characteristics. In this study, roselle powder was used to partially replace the red brick which traditionally included in the coating mixture, at substitution levels of 0%, 10%, 20%, 30%, 40%, and 50%, while keeping the amount of black tea constant. Antioxidant activity was determined using the DPPH radical scavenging assay, chemical composition was assessed using AOAC methods, and sensory evaluation was conducted by trained panelists using a hedonic scale. The results showed that increasing roselle concentration significantly enhanced antioxidant activity, with the highest activity recorded at the 50% substitution level (91.60 ppm). The fat and protein content remained relatively stable across treatments, ranging from 21% to 24% for fat and 16% to 18% for protein. Sensory evaluation indicated that higher Roselle levels improved color, excessive concentration negatively affected aroma and taste, and the best overall acceptability achieved at the 20% substitution level. The addition of roselle powder to production of pidan eggs effectively enhanced the antioxidant properties without substantially affecting their nutritional profiles. The best acceptability of the pidan was achieved with a 20% addition of roselle.

Introduction

Pidan commonly known as century eggs, are traditional preserved egg products originating from mainland China (Fu *et al*., 2014). Typically made using salted duck eggs, have long been consumed in countries such as China, Thailand, and Singapore (Huang *et al*., 2019). The diversification of duck eggs processing into pidan eggs remains limited in Indonesia. Unlike salted or smoked eggs, pidan eggs are not widely recognized. They are mostly found as toppings in certain Chinese dishes, such as savory rice porridge, in Chinese restaurants.

Pidan are alkaline-fermented products in which the maturation process involves high-pH substances that significantly influence the color, aroma, and texture of egg (Li *et al*., 2018). Pidan are typically characterized by a greenish-brown color, a strong sulfurous odor, and a firm, jelly-like texture (Zhang *et al*., 2015). The production process uses coating agents such as clay, black tea, and salt, along with alkaline materials such as calcium hydroxide. These ingredients enhance mineral content of egg, providing essential elements such as calcium, magnesium, iron, zinc, copper, potassium, and sodium (Zhao *et al*., 2014).

Based on Wu *et al*. (2023), the preservation of eggs using traditional alkali-pickled methods with black tea has been shown antioxidant activity, caused by the presence of superoxide dismutase (SOD) and catalase. The use of black tea as a component in the coating agent suggests that pidan eggs may be categorized as functional food with antioxidant properties. Black tea has been reported to enhance glutathione levels, activities of SOD, and catalase enzymes (Xiao *et al*., 2020). Pidan has been modified using various types of tea, including green tea, Chinese tea, and black tea (Ganasen and Benjakul, 2011; Li *et al*., 2024). However, antioxidant activity and total phenolic content of these teas remain relatively low compared to other types of tea, such as roselle (Shannon *et al*., 2017; Nguyen and Chuyen, 2020). Roselle is a flower known for its high antioxidant activity up to 64.83% and has been widely used in foods, beverages, additives, and traditional herbal products (Solangi *et al*., 2017; Miranda *et al*., 2025). Previous research reported that the addition of Roselle powder in fish sausage batter can significantly enhance antioxidant activity of the products (Imran *et al*., 2017).

In this study, roselle powder was used to partially replace red brick in the coating mixture applied during the aging process of duck eggs into pidan. Black tea was included at a constant level in all treatments. The research aimed to assess the effect of roselle substitution on the chemical characteristics and hedonic properties of the resulting pidan eggs. While Roselle replaced part of the red brick, it acted as a functional enhancer alongside black tea, with the potential to improve the antioxidant capacity and sensory acceptability of pidan eggs.

Materials and methods

Formulation and pidan egg production

Duck eggs (66 eggs), with an average weight of 70.0±5.8 grams, were obtained from a duck farm located in Banyuanyar, Semarang District, Central Java Province. Prior to treatment, all eggs were thoroughly cleaned. The eggs were then coated with ingredients formulated as shown in Table 1. The primary treatment in this study was the variation in Roselle (*Hibiscus sabdariffa* Linn.) powder concentrations, which were used to substitute part of the brick component in the coating dough. The treatment levels were 0% (control), 10, 20, 30, 40, and 50% Roselle powder. Each treatment group consisted of four replicates. Following the coating process, the eggs were subjected to an aging period of six weeks to allow fermentation. After aging, the samples were analyzed for further evaluation.

Antioxidant activity analysis

Antioxidant activity was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method (Barqi *et al*., 2017). DPPH is a stable free

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radical that able to interact with antioxidants in the sample, causing a color change that is inverse to the antioxidant activity. A 0.1 mM DPPH solution in methanol was prepared and mixed with the sample extract at varying concentrations. The mixture was then incubated in the dark at room temperature for 30 minutes. The absorbance was measured at 517 nm using a UV-Vis spectrophotometer. The scavenging activity was calculated as the percentage reduction in absorbance compared to the control.

Chemical properties analysis

The chemical properties analyzed in this study were fat and protein content. Fat content was measured using the Soxhlet extraction method (AOAC, 1995). A 1.5 g sample was weighed and put through hot solvent extraction. The extraction temperature depended on the solvent mixture used. After extraction, the solvent evaporated at room temperature for 1 hour. The sample was then dried in an oven at 105°C for 1 hour and subsequently weighed. The difference in weight before and after extraction was used to calculate the fat content.

Protein content was determined using the Kjeldahl method (AOAC, 2012), consisting of three main steps: digestion, distillation, and titration. Selenium was used as a catalyst during the digestion step. Distillation was conducted using 33% NaOH, and titration was conducted using HCl. Titration was complete when the solution turned into a stable purple color.

Hedonic evaluation

Hedonic evaluation was carried out with 25 panelists, which had familiarity with pidan eggs. The panelists were asked to assess four sensory attributes of the pidan eggs: color, taste, aroma, and overall acceptability. The hedonic evaluation was performed using a 4-point scale: 1= dislike, 2= slightly like, 3= moderately like, and 4= like. Each panelist rated the samples in a randomized order to minimize bias. Water was provided between tastings to cleanse the palate. The primary aim of the hedonic evaluation was to identify the preferred formulation of pidan eggs with varying Roselle powder substitution levels. The panelists' preferences were recorded and analyzed to determine which treatment was most acceptable.

Data analysis

The data were analyzed using one-way ANOVA followed by Duncan's multiple range test to evaluate differences in chemical characteristics. Hedonic evaluation data were analyzed using the non-parametric Kruskal–Wallis test, and significant differences were further examined using the Mann–Whitney U test.

Results

Antioxidant activity

The antioxidant activity of pidan eggs increased significantly ($p < 0.05$) with the addition of Roselle powder (Figure 1). The control group (F0), which contained no Roselle powder, exhibited the lowest antioxidant activity at 0.11 ppm. A gradual increase was observed with increasing Roselle substitution levels: F1 (10%) reached 27.44 ppm, F2 (20%) reached 35.85 ppm, and F3 (30%) showed a further increase to 46.83 ppm. A marked enhancement was seen in F4 (40%) with 62.55 ppm, and the highest antioxidant activity was found in F5 (50%), reaching 91.6 ppm. These results indicate that the level of Roselle powder substitution had a significant effect on antioxidant activity in pidan eggs ($p < 0.05$), with higher substitution levels resulting in significantly increased antioxidant activity. It is likely due to the high content of phenolic compounds and anthocyanins in Roselle, which are known for their strong antioxidant properties. These compounds can act as radical scavengers, metal chelators, and reducing agents, as a result enhancing the antioxidant potential of the product as the Roselle concentration increases.

Chemical properties

Based on Figure 2, the chemical properties of pidan eggs showed slight variations with the increasing substitution of Roselle powder. Fat

Table 1. Coating Formulation

Materials	Compositions					
	F_0	F_1	F_2	F_3	F_4	F_5
Red Brick Powder (g)	312.5	281.25	250	218.75	187.5	156.25
Mud (g)	312.5	312.5	312.5	312.5	312.5	312.5
Calcium hydroxide (g)	150	150	150	150	150	150
Charcoal (g)	25	25	25	25	25	25
Salt (g)	150	150	150	150	150	150
Black tea (g)	31.25	31.25	31.25	31.25	31.25	31.25
Roselle powder (g)	-	31.25	62.5	93.75	125	156.25
Mineral water (ml)	150	150	150	150	150	150

F_{0-5}: Treatments with Roselle powder substituting red brick powder at 0%, 10%, 20%, 30%, 40%, and 50%, respectively.

Table 2. Hedonic Evaluation Pidan Egg with The Roselle Powder Substitution in Different Proportion.

Hedonic Attribute	Treatments					
	F_0	F_1	F_2	F_3	F_4	F_5
Aroma	2.23 ± 0.10^{a}	2.43 ± 0.87^{a}	2.04 ± 0.93^{a}	2.46 ± 1.15^{a}	3.20 ± 0.97^{b}	2.00 ± 0.75^{a}
Texture	2.40±1.19	2.96±0.99	2.60±0.95	2.63±0.99	2.36±1.07	2.58±0.96
Taste	$2.62\pm0,70^{b}$	3.45 ± 0.82^{cd}	3.68 ± 0.50^{d}	3.00 ± 0.86^{c}	1.87 ± 0.70^{a}	2.24 ± 0.86^{b}
Color	$2.31\pm0,97^{a}$	2.27 ± 1.01^{a}	2.83 ± 0.78^{b}	2.92 ± 0.95^{b}	2.80 ± 0.84^{b}	2.67 ± 0.86^{b}
Overall Acceptance	2.84 ± 0.85^{b}	2.12 ± 0.93^{a}	2.36 ± 0.81^{a}	2.51 ± 1.00^{b}	2.02 ± 0.75^{a}	3.30 ± 0.81^{c}

Data was expressed as mean ± standard deviation. Different superscripts on the same row indicate a significant difference ($p<0.05$) between the treatment groups. F0-5 is Roselle Powder substitution at 10, 20, 30, 40, and 50%, respectively. Interpretation of hedonic scale of 1=dislike, 2= slightly like, 3= moderately like, and 4 = like

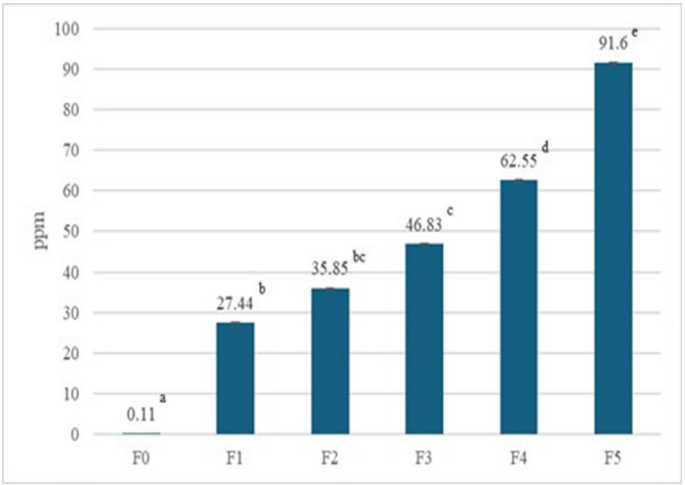

Fig 1. Antioxidant Activity of Pidan Egg with The Substitution of Roselle Powder with Different Proportions. Different superscripts in the same row indicate a significant difference ($p<0.05$) between the treatment groups. F0-5 is a proportion between roselle and red brick powder at 0, 10, 20, 30, 40, and 50%, respectively.

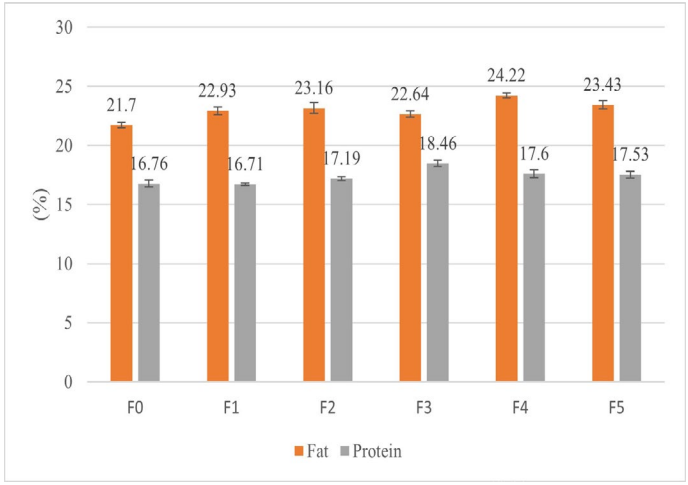

Fig 2. Chemical Properties of Pidan Egg with different Roselle powder substitution levels (F0–F5: 0%, 10%, 20%, 30%, 40%, 50%). No significant differences were observed among treatments ($p > 0.05$).

content ranged from 21.7% to 24.22%, with the control group (F0) had the lowest value (21.7%) and F4 reaching the highest fat content at 24.22%. Treatments F1 to F5 showed relatively stable fat content, ranging from 22.93% to 23.43%, indicating that Roselle substitution led to a modest increase in fat levels. Protein content also increased slightly with higher Roselle proportions, starting from 16.76% in F0 and peaking at 18.46% in F3. However, protein content remained fairly consistent across treatments F1 to F5, ranging between 16.71% and 17.53%. Based on the results of one-way ANOVA, these differences in fat and protein content were not statistically significant ($p > 0.05$), suggesting that the substitution of Roselle powder in the coating mixture did not significantly affect the chemical composition of pidan eggs.

Hedonic evaluation

The hedonic evaluation of pidan eggs with varying levels of Roselle powder substitution (F0–F5) revealed significant differences ($p < 0.05$) in aroma, taste, color, and overall acceptability, while texture remained unaffected by the treatments (Table 2). Aroma scores were highest in treatment F4 (40% Roselle), which recorded a mean score of 3.20 ± 0.9, significantly surpassing the other groups. This suggests that Roselle at this concentration effectively masked undesirable odors. In contrast, F5 (50% Roselle) showed the lowest aroma score of 2.00 ± 0.7. Texture scores did not differ significantly across treatments, ranging from 2.36 ± 1.0 to 2.96 ± 0.9, indicating that Roselle substitution had minimal influence on perceived texture. This may be because the Roselle powder was incorporated into the coating solution rather than the egg matrix itself, resulting in limited interaction with the internal protein or gel structure of the pidan, which primarily determines texture. The most favorable taste was observed in F2 (30% Roselle), with a score of 3.68 ± 0.5, significantly outperforming the control (F0), F4, and F5. Conversely, F4 had the lowest taste score at 1.87 ± 0.7, implying a potential negative impact of higher Roselle levels on flavor. For color, F3 (30%) received the highest rating at 2.92 ± 0.9, followed closely by F2 and F4, indicating that moderate Roselle additions produced an appealing mild red hue. Meanwhile, F0 and F1 had the lowest color preferences. Interestingly, despite lower aroma and taste scores, F5 achieved the highest overall acceptability score of 3.30 ± 0.8, significantly higher than the other treatments, suggesting that its antioxidant-enhanced profile and overall sensory balance were well received by panelists. The lowest overall acceptability was observed in F4 at 2.02 ± 0.7.

Discussion

The antioxidant activity showed that the antioxidant levels in pidan eggs increased with the percentage of Roselle (*Hibiscus sabdariffa* Linn.) powder used in the coating mixture. The highest antioxidant activity was observed in the pidan eggs treated with 50% Roselle powder (91.6 ppm). Based on Djaeni (2017), high antioxidant activity is generally characterized by IC_{50} values ranging from 50 to 100 ppm, it is indicating that the pidan eggs with roselle powder substitution had high antioxidant activity. This enhanced activity is likely caused by the polyphenolic compounds present in roselle, which are well known for their free radical scavenging capacity. Roselle contains approximately 51% anthocyanins and 24% total antioxidant compounds (Tsai *et al.*, 2002). This aligned with Dwiloka *et al.* (2020) resulted that higher antioxidant activity is closely associated with higher total phenolic content. Anthocyanins are phenolic compounds known for their antioxidant properties and known as part of the flavonoid group. In addition to anthocyanins, Roselle also contains other bioactive components, including organic acids, phytosterols, and polyphenols, which may contribute to its antioxidant capacity (Huang *et al.*, 2019; Bharadhi *et al.*, 2023).

Benedé and Molina (2020) reported that eggs naturally contain antioxidants in the egg whites such as ovotransferrin, ovalbumin, lysozyme, and in yolk such as phosvitin. Eggs also contained free aromatic amino acids such as tryptophan and tyrosine; lipophilic antioxidants such as vitamin E, carotenoids, selenium, and iodine (Wang *et al.*, 2019). Antioxidants of roselle not only can enhance the shelflife of food but may also offer important health benefits. Research shows that antioxidants can neutralize free radicals, which in turn may lower the risk of degenerative diseases like cancer and diabetes. Roselle reported to lowering cholesterol and blood pressure (Al-Jubouri and Algboory, 2022). The substitution of red bricks with Roselle in the pidan aging process was effective in enhancing the antioxidant activity of pidan egg, its occurrence not because of red bricks since it is not an antioxidant compound, however Roselle is rich in bioactive substances such as anthocyanins and polyphenols. These compounds were absorbed through the shell and interacted with the egg components during the alkaline ripening, increasing the total antioxidant activity. This finding supports the potential of Roselle as a natural functional additive in traditional food products to improve their health-promoting properties.

The results showed that the substitution of roselle powder in the coating mixture did not significantly affect the fat content of the pidan eggs. The fat content ranged from 21% to 24%, indicating that the variation in Roselle powder levels did not alter the lipid profile of the product. This was expected, considering that Roselle powder itself contained only about 0.1 g of fat, a negligible amount that would not substantially contribute to the overall fat content of the eggs. However, compared to salted eggs and fresh duck eggs, the pidan eggs in this research demonstrated a higher fat content. These results contrasted from the reports of

Ganesan *et al*. (2014) and Suretno *et al*. (2021), which stated that salted duck eggs contained around 16% fat or lower, fresh duck eggs contained 11–14% fat, and pidan eggs had lower fat than the others, around 10.7%. This lower fat content in pidan eggs was usually due to the saponification reaction of yolk lipids that occurred during the ripening process. The alkaline conditions caused by the presence of calcium oxide in the coating mixture may have triggered saponification, leading to the breakdown of fats (Ganesan *et al*., 2014). Saponification is a reaction in yolk triglycerides which are broken down into glycerol and fatty acid salts, leading to reduced fat content in pidan eggs during ripening.

The difference between the results of this research and previous reports could have been caused by the high fat that naturally contained in the fresh duck eggs used in this study. Different types of ducks showed different fat contents, such as Putat Atas (33%), Hambuku Raya (34%), Pekin (35.9%), and Cherry Valley (34.46%) (Bedekovic *et al*., 2020; Fajarwati *et al*., 2020). Another factor that could have affected the results was that even without intentional dehydration (as in salted eggs), some moisture loss from the yolk might have concentrated the fat slightly in pickled egg (Yang *et al*., 2019). The fat content was significantly higher in the yolk than in the egg white. According to Acosta *et al*. (2014), duck egg yolk contained approximately 32.5% fat.

The protein content of the pidan eggs was measured using the Kjeldahl method, and the results indicated no significant differences across the different levels of Roselle (*Hibiscus sabdariffa* Linn.) powder substitution. The protein content of the pidan eggs ranged from 16% to 18%, suggesting that the addition of Roselle powder in the coating formulation did not affect the protein concentration of the final product. Protein denaturation was typically influenced by temperature and pH (Evanuarini *et al*., 2017). In the case of pidan eggs, the alkaline conditions caused by the coating materials during ripening likely contributed to the denaturation of egg proteins. The increased pH may have disrupted the tertiary and quaternary structures of the proteins by breaking non-covalent interactions such as hydrogen bonds and hydrophobic interactions (Chen *et al*., 2015). However, structural changes of the egg proteins do not affect the total nitrogen content, which explains the relatively stable protein values observed. The denatured proteins in the egg white tended to aggregate and form polymerized structures under alkaline conditions, resulting in the characteristic gel-like texture and translucent brown coloration of pidan eggs (Li *et al*., 2021). These findings showed that the functional and visual transformation of pidan eggs was largely influenced by protein structural changes rather than compositional losses. Compared to pidan eggs reported by Ganasen *et al*. (2013), which had 13.1% protein content, the pidan eggs in this research showed a higher protein content. This could have contributed by the type of eggs used as raw material, the protein content of fresh duck eggs could range from lower values of 9–11% to higher values of 15.76–17.29% (Ganesan *et al*., 2014; Bedekovic *et al*., 2020). The higher protein results of the pidan eggs in this research could also have been influenced by the addition of Roselle powder, which contains a high protein content of about 5.7% to 27.06% (Salami and Afolayan, 2021).

The hedonic evaluation was conducted to analyze preferences of people or in this case panelists regarding the color, taste, aroma, and overall acceptability of the pidan eggs. The results showed that the aroma of pidan eggs increased in preferences with increasing concentrations of Roselle powder in the coating formulation, with the highest preference observed at the 40% substitution level. Duck eggs are known to have a more pronounced fishy odor compared to other poultry eggs, primarily due to their higher protein and amino acid content. One of the compounds responsible for this odor is trimethylamine (TMA), a volatile base produced through microbial degradation of trimethylamine oxide or choline, which as concentrations increase, the odor becomes more prominent and less acceptable to consumers (Li *et al*., 2018, 2019). Pidan eggs are known to emit an off-odours mainly because alkali penetration that caused higher release of free lipid caused oxidation and maillard reaction due to the alkaline pH of the egg which form aldehydes, ketones, pyrazine and other compound related to off-odor (Ganasen *et al*., 2013; Lei *et al*., 2015). Roselle powder demonstrated an ability to mask or neutralize this strong odor, likely due to its own intense sour aroma and the presence of aromatic compounds which reducing the perception of sulfurous notes, and polyphenol compounds are known to influence the aroma profile of food products and possess antimicrobial properties that inhibit spoilage microorganisms, thereby potentially reducing TMA production and extending product shelf life (Duweini and Trihaditia, 2017; Sundari *et al*., 2020).

Regarding taste, the pidan eggs with a 20% Roselle powder substitution received the highest preference score. These eggs had a subtle sourness that complemented the traditional taste of pidan without overpowering it. The moderate presence of Roselle flavor was considered acceptable by the panelists. As the concentration of Roselle powder increased above 20%, the sourness became more pronounced, and the hedonic rating for taste decreased accordingly. This trend supports previous findings indicating that excessive Roselle addition can negatively affect sensory characteristics due to its dominant sour taste (Putri *et al*., 2016). Meanwhile the preference panelist of the pidan egg texture didn't affect by the addition of roselle. The pidan eggs coated with 10% Roselle powder is most preferred by panelist which produced a slight red hue in the eggs, which enhanced the visual appeal without altering much far from the traditional appearance of pidan eggs. The anthocyanin compounds present in Roselle function as natural pigments and were responsible for giving the reddish color (Yuniati *et al*., 2021). At concentrations above 10%, the red coloration became more intense, which may have altered the visual expectations for pidan, potentially influencing panelist preferences negatively. In terms of overall acceptability, pidan eggs with 50% Roselle powder substitution received high preference scores. This result suggests that despite stronger coloration and taste changes, panelists appreciated the combined attributes at this concentration. It was different from the result observed in the addition of roselle in fish sausage, that overall acceptance began to decline at higher substitution levels, possibly due to an imbalance in flavor or changes in texture and appearance (Imran *et al*., 2017). These findings indicate that while Roselle powder substitution can enhance specific sensory attributes, its concentration must be carefully optimized to maintain a balance between traditional pidan characteristics and improved acceptability.

Conclusion

The addition of roselle powder in the aging process effectively enhances the antioxidant properties of pidan eggs without totally affecting their nutritional profile. The best acceptability of pidan product is achieved in 20% addition of that powder as part of coating agent.

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Conflict of interest

The authors have no conflict of interest to declare.

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