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Antioxidant activity of egg whites from Indonesian chicken and duck eggs with addition of pineapple extracts

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Abstract

Inherently, egg whites are known for their antioxidant properties due to the presence of various proteins, peptides, phenolic compounds, and flavonoids. Further, the antioxidant properties of egg whites are believed to be enhanced by the hydrolysis of proteases, which produces various antioxidant peptides. While numerous studies have explored the production of antioxidant peptides through the hydrolysis of egg white proteins using commercially available proteases, none have employed natural proteases derived from pineapple extract, particularly on egg whites from Indonesian poultry species. Therefore, the present work aimed to assess the antioxidant activity of egg whites from two Indonesian poultry species: Kampung chicken (KC) and Alabio duck (AD), following the addition of pineapple extract. Results revealed that the Haugh units of KC and AD were significantly different, accompanied by differences in their protein concentrations, with AD having a higher protein concentration than KC. Hydrolysis of KC and AD with bromelain-containing pineapple extracts at 0, 0.5, and 1% concentrations increased protein concentration and flavonoid content of the hydrolysates. This pattern was observed in both KC and AD, with varying magnitudes. Antioxidant activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, which demonstrated that both KC and AD effectively reduced DPPH activity, with IC_{50} values of 433.47 µg/mL (AD) and 504.82 µg/mL (KC). These findings suggested that the hydrolysis of egg whites with pineapple extract significantly enhanced the antioxidant potential of the eggs.

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Introduction

While the modern era has brought about convenience and practicality, these advancements have also led to lifestyle changes that contribute to various health issues. According to Sharifi-Rad *et al.* (2020), one such health issue related to modern lifestyles is the increased risk of oxidative stress. Lobo *et al.* (2010) define antioxidants as compounds that counteract free radicals by donating electrons with oxidising properties to cells, thereby reducing cell damage. Antioxidants play a crucial role in human health by inhibiting and neutralising oxidation reactions involving free radicals. Inhibition occurs when oxidative reactions of lipids or other molecules in the body are absorbed and neutralised by free radicals. Neutralisation is achieved by donating an electron, resulting in a more stable compound (Lü *et al.*, 2010). Akbarian *et al.* (2022) further explain that bioactive peptides serve multiple functions, including antimicrobial, anti-inflammatory, and immune system regulation. Additionally, some peptide chains in eggs can inhibit oxidative reactions, and act as antioxidants. The activity of peptides is determined by the amino acid sequences from which they are composed (Bizzotto *et al.*, 2024). Scientifically, whole proteins influence bioactivity of its peptides, and if bioactivity is low, proteins must be broken down into shorter fragments to be effective (Riyadi, 2018). Bioactive peptides can function in immune defense, bone and dental health, digestion, and weight regulation (Sitanggang *et al.*, 2018).

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Among the many bioactive peptides, antioxidant peptides show great promise as ingredients for producing antioxidant functional foods or drugs. According to Tadesse and Emire (2020), antioxidant peptides are specific protein fragments that possess antioxidant activity, and can be used to maintain human health, food safety, and quality by mitigating oxidative stress and lipid peroxidation caused by free radicals generated during oxidation reactions within the human body and in food products (Gil-Chávez *et al.*, 2013; Hema *et al.*, 2017). These peptides are derived from various food protein sources through enzymatic hydrolysis, as well as microbial fermentation.

Various food-derived protein sources have been utilised to produce bioactive peptides, including eggs (Wang *et al.*, 2018; Eckert *et al.*, 2018). Egg whites account for 56 - 60% of an egg's weight, and are rich in protein, primarily ovalbumin (54%), ovotransferrin (12%), ovomucoid (11%), ovoglobulin (8%), ovomucin (3.5%), and lysozyme (3.5%) (Abeyrathne *et al.*, 2021). These proteins serve as parent sources for the production of various bioactive peptides with diverse activities, including antioxidant properties (Liao *et al.*, 2018; de Campos Zani *et al.*, 2018; Moreno-Fernández *et al.*, 2020). As Indonesia is rich in various local egg-laying poultry breeds with different egg characteristics and protein contents (Khaerunnisa *et al.,* 2016), these eggs could be promising raw materials for the production of antioxidant bioactive peptides. The potential lies in the variation of protein sequences among the eggs, which may lead to the production of peptides with diverse sequences that modulate their biological activity. This aligns with the findings of Wijedasa *et al.* (2020), who stated that eggs from different poultry species exhibited different characteristics. Additionally, Wulandari (2021) demonstrated that lysozyme from Kampung (native) chickens, commercial chickens, and local ducks exhibited different characteristics due to sequence variations. This indicates the potential for differences in bioactive peptides with antioxidant capabilities among eggs from different local poultry breeds.

Another important factor in the production of bioactive peptides through enzymatic hydrolysis is the type of protease used. Numerous proteolytic enzymes are employed to produce bioactive peptides, with the most popular commercially available ones being Alcalase™, Protamex™, and Flavourzyme™ (Cruz-Casas *et al.*, 2021). It is also common to use enzymes with digestive activities found in the human body, such as pepsin, trypsin, and chymotrypsin (Boukil *et al.*, 2018). While many studies have focused on producing egg white hydrolysates, the use of proteases derived directly from plants remains limited. In this context, pineapple (*Ananas comosus*) extract serves as a powerful source of cysteine protease for hydrolysing precursor proteins in the production of bioactive peptides. Rowan *et al.* (1990) demonstrated that the pineapple contains at least four distinct cysteine proteinases, with bromelain being the most prominent. Mala *et al.* (2021) and Johny *et al.* (2022) have previously reported the use of bromelain-containing pineapple extract to produce egg hydrolysate with certain biological activities. This suggests the promising application of pineapple extract in hydrolysing egg whites, and producing functional bioactive peptides. Although some studies have investigated the use of bromelain from pineapple extracts, none have focused on egg white proteins from Indonesian poultry, or assessed the bioactive peptides produced through such hydrolysis.

Therefore, the present work aimed to assess the antioxidant capabilities of egg whites from two Indonesian poultry species (Kampung chicken and Alabio duck) under hydrolysis treatment with pineapple extract. The present work would provide the first evidence of the remarkable antioxidant activity of both egg whites, which is significantly enhanced through hydrolysis with pineapple extract.

Materials and methods

Pineapple extract and egg white preparation

The pineapple extract (PE) was prepared based according to Johny *et al.* (2022), with some modifications. Pineapple stems, collected from a local market in Bogor, were first pulverised using a blender in ice, followed by filtration through a mesh cloth. The filtrate was then suspended in 0.02 M phosphate buffer at pH 7.0 in a 1:1 ratio, which was prepared according to Budiman *et al.* (2011). The mixture was centrifuged at 4°C for 20 min to separate the insoluble components. The resulting supernatant, a golden-yellow liquid, was collected for further experiments. The egg whites (EW) were obtained from the eggs of two Indonesian poultry breeds: Kampung chicken (KC) and Alabio duck (AD). The KC eggs were sourced from a commercial market in Darmaga, Bogor, while the AD eggs were obtained from the Livestock Research Centre in Ciawi, Bogor.

Haugh unit (HU) value measurement

The Haugh unit (HU) of the EW was measured according to Purwati *et al.* (2015). First, the egg weight was measured using an analytical balance. The egg was then carefully cracked, and the EW was separated from the yolk. The height of the EW was measured using a tripod. The HU was calculated using Eq. 1:

$$\text{Haugh Unit} = 100 \log (H + 7.75 - 1.7W^{0.37}) \quad \text{(Eq. 1)}$$

Protein concentration measurement

Protein concentration in the EW and PE was measured using a modified Lowry method, as described by Mæhre *et al.* (2018). The assay was performed by diluting the extracts to 1 mL with H_2O, and adding 0.9 mL of solution A (2 g/L potassium sodium tartrate ($KNaC_4H_4O_6 \cdot 4H_2O$) and 100 g/L sodium carbonate (Na_2CO_3) in 0.5 M NaOH), followed by incubation for 10 min at 50°C. After cooling the samples to room temperature, 1 mL of solution B (0.2 g/L $KNaC_4H_4O_6 \cdot 4H_2O$ and 0.1 g/L copper sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$) in 0.1 M NaOH) was added, and the mixture was left for 10 min. Finally, 3 mL of solution C (Folin-Ciocalteu phenol reagent diluted in H_2O at a 1:16 v/v ratio) was added, followed by 10-min incubation at 50°C. A standard curve was prepared using bovine serum albumin (BSA) at concentrations of 0, 0.0625, 0.125, 0.25, 0.5, and 1 g/L, and absorbance was measured at 650 nm.

Egg white hydrolysis

The EW was first suspended in autoclaved distilled water at a 10-fold volume. The suspension was then mixed with PE at various concentrations (0, 0.5, and 1 mg/mL). The mixture was incubated at 4°C overnight (12 h) to allow hydrolysis to occur. The hydrolysis was then terminated by heating the mixture at 80°C for 15 min.

Total flavonoid content

The total flavonoid content of the samples was determined according to Zou *et al.* (2004) and Razak *et al.* (2021). First, 1 mL of each sample solution was mixed with 4 mL of distilled water and 0.3 mL of 10% $NaNO_2$. The mixture was incubated for 6 min, then 0.3 mL of 10% $AlCl_3$ and 4 mL of 10% NaOH were added, followed by distilled water to bring the total volume to 10 mL. The mixture was incubated for 15 min, then the absorbance of the mixture was measured at 495 nm against a blank containing all reagents except the sample. Standard flavonoid solutions were measured under the same conditions to create a calibration curve. All determinations were performed in triplicate. The total flavonoid content in the samples was expressed as grams of quercetin equivalents (QE) per 100 grams of sample (% w/w QE).

Total phenolic content

The total phenolic content of the samples was determined according to Chun *et al.* (2003) and Razak *et al.* (2021). First, 1 mL of each sample solution was mixed with 0.4 mL of Folin-Ciocalteu reagent. The mixture was then incubated for 5 min, then 4 mL of 7% Na_2CO_3 and distilled water were added to bring the total volume to 10 mL. The mixture was then incubated for 2 h. The absorbance was measured at 755 nm against a blank containing all reagents except the sample. Standard phenolic solutions were measured under the same conditions to create a calibration curve. All determinations were performed in triplicate. The total phenolic content of the samples was expressed as grams of gallic acid equivalents (GAE) per 100 g of sample (% w/w GAE).

Quantification of α-amino acids

The α-amino acid content was determined according to Benjakul and Morrissey (1997). To 125 µL of the diluted samples, 2.0 mL of 212.5 mM phosphate buffer (pH 8.2) and 1.0 mL of 0.01% TNBS solution were added. The solution was mixed thoroughly, and incubated in a 50°C water bath for 30 min in the dark. The reaction was terminated by adding 2.0 mL of 0.1 M sodium sulphite. The mixture was then cooled to room temperature for 15 min. The absorbance was measured at 420 nm, and the α-amino acid content was expressed in terms of L-leucine.

DPPH radical scavenging assay

The antioxidant activity against the DPPH radical was measured according to Kikuzaki *et al.* (2002) with slight modification. In this assay, 1 mL of each sample solution was mixed with 3 mL of ethanol (p.a.) and then with 1 mL of 0.4 mM DPPH solution. The mixture was shaken vigorously using a vortex for 1 min, and then incubated for 15 min at 25°C in the dark. The absorbance of each solution was measured at 517 nm using a spectrophotometer, with ethanol as the blank. The antioxidant activity was calculated as the IC_{50} value, which represents the

concentration of the sample (µg/mL) required to reduce 50% of the DPPH activity. The calculation was performed using a four-parameter logistic curve with GraphPad Prism (version 9.0, GraphPad Software Inc., CA, USA).

Statistical analysis

The data were expressed as mean ± standard deviation. Differences among means were determined using analysis of variance (ANOVA) followed by Tukey's *post-hoc* test, as described by Steel and Torrie (1991). All experiments were conducted with three biological replicates.

Results and discussion

The Haugh unit (HU) value was measured to assess the quality and freshness of the eggs used in the present work. The HU value is based on the condition of the EW, specifically the ratio of egg weight to EW height. Table 1 shows that the HU values for both Kampung chicken (KC) and Alabio duck (AD) EW fall within the AA category, as classified by Da Silva Pires *et al.* (2020). Eggs categorised as AA are considered fresh. Furthermore, Table 1 demonstrates that the HU values of KC EW are lower compared to AD EW. This agrees with Bondoc *et al.* (2020), who reported differences in the physicochemical properties of eggs, including HU, among various avian species and breeds. According to Bondoc *et al.* (2020), duck eggs generally tend to have higher HU values compared to chicken eggs. However, Chaiyasit *et al.* (2019) reported contrasting results, which may be attributed to differences in egg handling processes.

Table 1. HU values of KC and AD eggs.

Egg	HU	Remarks
KC	94.56 ± 0.05^{b}	AA
AD	99.88 ± 0.05^{a}	AA

Means followed by different lowercase superscripts are significantly very different ($p < 0.01$).

The HU value reflects the viscosity of the EW, with higher values indicating thicker albumin. Ovomucin, found in the albumin, is responsible for water binding, which causes the albumin to form a gel-like consistency, and results in increased thickness. As the albumin becomes thicker, the ovomucin network becomes more extensive and robust, leading to higher albumin viscosity. Therefore, a higher HU value corresponds to a greater presence of ovomucin and, thus, better internal egg quality (Jiang *et al.*, 2022). Conversely, Jones *et al.* (2010) found a correlation between the total solids (dry matter) in the EW and HU values, with eggs containing higher total solids exhibiting higher HU values. Since bioactive antioxidant components in eggs are generally localised within the solid fraction, this suggested a possible positive correlation between high HU values and a high concentration of bioactive antioxidant components in the EW. Notably, Jiang *et al.* (2022) indicated that HU values are correlated with egg freshness. A lower HU value might imply that the egg has been stored for a certain period, resulting in a decrease in albumin thickness due to evaporation.

Figure 1 shows the protein content of EW hydrolysed by PE at different concentrations. On average, the protein content in AD EW was 64.42 mg/mL, which was significantly higher compared to 52.97 mg/mL for KC EW. This agreed with Sun *et al.* (2019), who observed that duck eggs had higher protein and amino acid contents compared to Kampung chicken (KC), quail, and pigeon eggs. The protein and amino acid levels in duck eggs are comparable to those found in goose eggs. However, the protein concentrations reported in the present work were lower than those reported by Iwashita *et al.* (2015) and Guyot *et al.* (2016) in the range of 100 - 120 mg/mL. The differences may be attributed to various factors, including genetics, management practices, egg preparation methods, and analytical techniques. Guyot *et al.* (2016) and Jabalera *et al.* (2022) also reported lower EW protein concentrations, some below 100 mg/mL.

Figure 2 also demonstrates a significant increase in protein concentration with the addition of PE in each treatment ($p < 0.05$), whether in KC or AD eggs. For KC eggs, a significant increase was observed at 1% PE. Meanwhile, the protein content in AD eggs increased starting from the addition of 0.5% PE. This increase is likely attributed to the additional protein from the PE in the eggs. Generally, Nasution *et al.* (2020) stated that pineapple contains approximately 0.84% protein. Furthermore, pineapple is rich in bromelain, which is found in almost all parts of the fruit (crown, skin, flesh, and stem). In the present work, the pineapple stem was

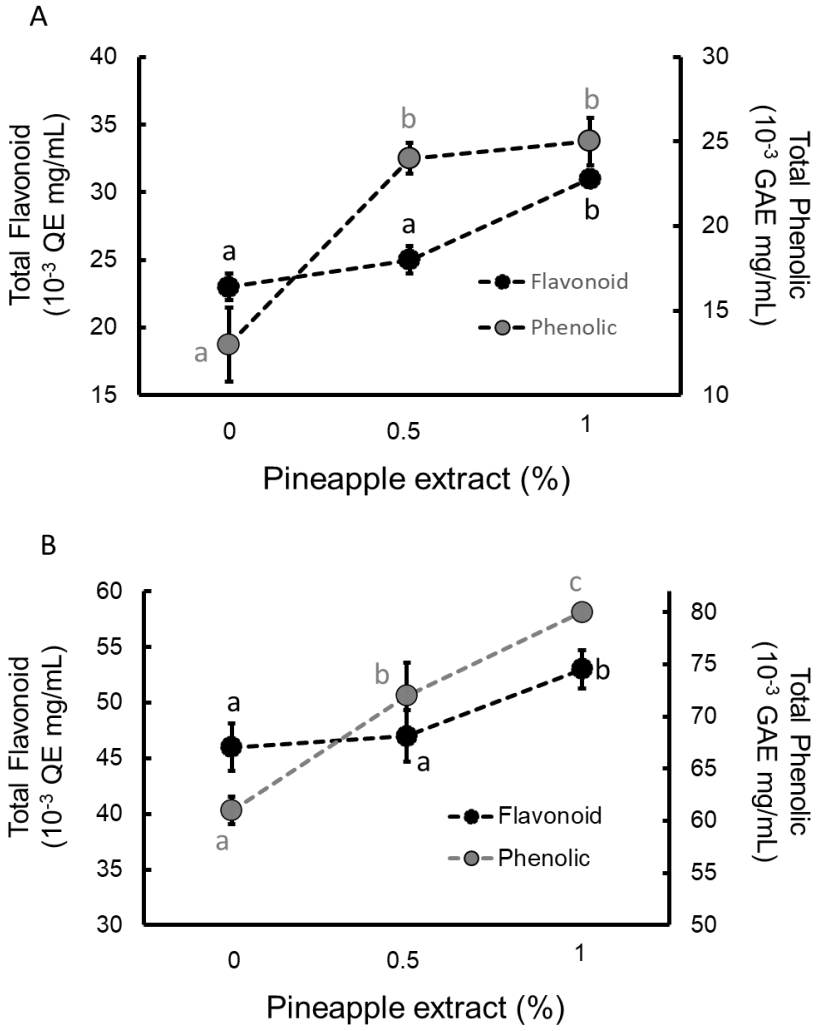

Figure 1. Total flavonoid and phenolic contents of KC **(A)** and AD **(B)** in the presence of different concentrations of pineapple extract. Different lowercase letters indicate significant difference ($p < 0.05$).

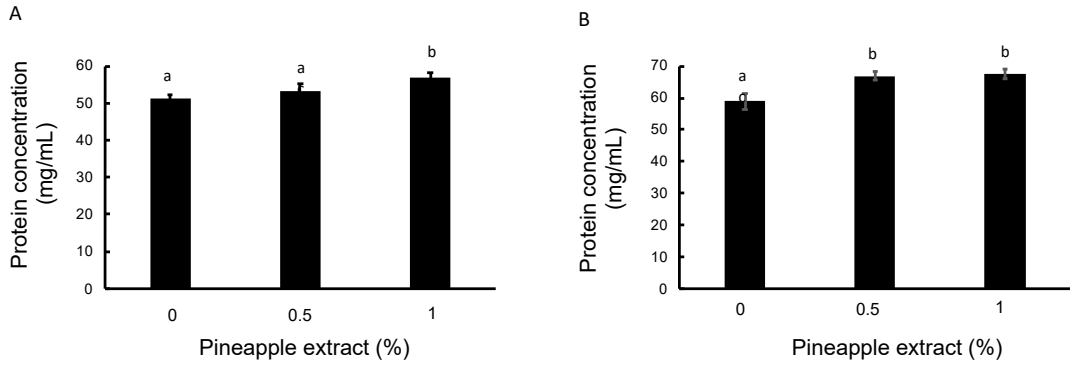

Figure 2. Protein concentration of egg whites from KC **(A)** and AD **(B)** treated by different concentrations of pineapple extract. Different lowercase letters indicate significant difference ($p > 0.05$).

used to prepare the extract. According to Nurnaningsih and Laela (2022), pineapple stem contains approximately 27.3 U/mg of bromelain. The protein and bromelain contents in pineapple were contributing factors to the increased protein content in the EW. The addition of PE containing bromelain was intended to obtain bioactive peptides through the hydrolysis of EW proteins. As noted by Utami *et al.* (2011), bromelain can break down protein molecules into short peptide fragments, some of which may exhibit antioxidant properties.

Figure 1 shows the total phenolic and flavonoid contents in KC and AD eggs treated with different concentrations of PE. The measurement of phenolic and flavonoid contents was conducted to explore other bioactive components within eggs that may have antioxidant potential. Figure 1 demonstrates an increase in flavonoid and phenolic contents in both KC and AD eggs with the addition of PE. This is because PE also contains phenolics and flavonoids, as reported by Hossain and Rahman (2011). According to their study, the phenolic and flavonoid contents in pineapples are 51.1 mg/100 g GAE and 55.2 mg/100 g GE, respectively. The higher levels of flavonoids and phenolics have the potential to enhance the antioxidant capacity of the material. This agreed with the findings of Nur *et al.* (2019), who stated that higher total phenolic and flavonoid values correspond to increased antioxidant ability due to their capacity to donate electrons, and suppress the development of free radicals.

Figure 1 also demonstrates that the total flavonoid and phenolic contents in AD eggs tend to be higher than in KC eggs ($p < 0.05$). The phenolic and flavonoid levels obtained in the present work were higher than those reported by Nahariah *et al.* (2014). Factors influencing the phenolic and flavonoid levels in eggs include the type of feed given to the poultry. According to Edi *et al.* (2018), supplementary feed ingredients are provided to support livestock performance, and as noted by Pasaribu (2019), these typically include plant bioactive compounds rich in phenols, tannins, flavonoids, essential oils, curcumin, saponins, and phytol. Most poultry feed is derived from plants, such as grains (corn, rice, legumes, millet, sorghum, black glutinous rice, and paddy). Legumes, in particular, contain condensed phenolic acids, flavonoids, and tannins. These compounds are distributed differently in seed coats (especially flavonoids) and cotyledons (which contain non-flavonoid acids such as hydroxycinnamic and hydroxybenzoic acids) (Diniyah and Lee, 2020). Phenolic and flavonoid compounds are abundant in various plant parts, including leaves, fruits, seeds, roots, and barks (Hikmah *et al.*, 2020). Plants have high levels of phenolics and flavonoids because these compounds are produced through plant metabolism, and possess antioxidant activity.

Further, the free α-amino groups in the EW with and without the addition of PE are shown in Figure 3. According to Aspmo *et al.* (2005), the amount of free α-amino groups indicates the number of peptide bonds broken during the hydrolysis reaction. As shown in Figure 3, the amount of free α-amino groups in the absence of PE (0%) was significantly lower than in the presence of PE (0.5 and 1%). The PE was found to significantly enhance ($p < 0.01$) the amount of free α-amino groups. This indicated that the presence of PE was able to hydrolyse EW proteins by breaking the peptide bonds, and releasing the free α-amino groups. The ability of PE to hydrolyse EW proteins is likely associated with its proteases, particularly the cysteine protease bromelain, which is found in the stem, fruit, crown, peel, and leaves of pineapple (Razali *et al.*, 2021; 2023; Saptarini *et al.*, 2023). Interestingly, the amount of free α-amino groups between KC and AD was found to be significantly different ($p < 0.05$), suggesting that the digestibility of KC and AD egg white proteins by PE differed. This might be due to differences in the amount, sequences, and structure of EW proteins between the two types of eggs. Notably, the amount of free α-amino groups observed in the present work differed from that reported by Cho *et al.*

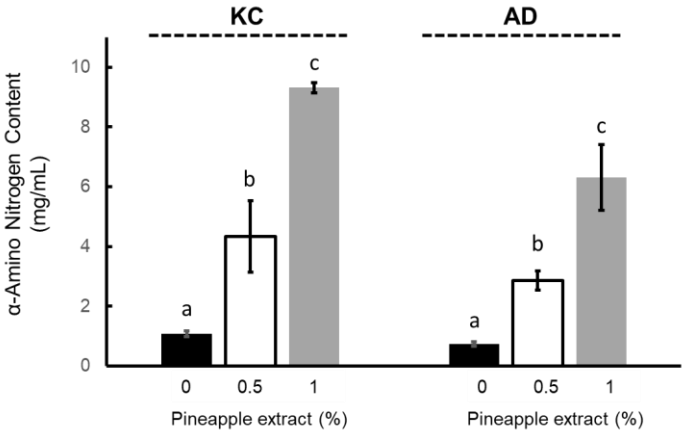

Figure 3. Free α-amino groups in KC and AD egg whites with the addition of pineapple extracts at different concentrations. Different lowercase letters indicate significant difference ($p < 0.05$).

(2014), who hydrolysed chicken EW using commercial proteases, indicating possible differences in hydrolytic activity, although this remains to be confirmed through direct experimental comparison. As a note, Saptarini *et al.* (2023) reported that the proteolytic activity of PE ranged from 38.32 to 46.78 U, suggesting its potential as a natural source of proteases.

Further, the antioxidant activities of KC and AD EW, both with and without PE, were first determined by measuring the scavenging activity against DPPH (2,2-diphenyl-1-picrylhydrazyl). DPPH is a commonly used free radical model in antioxidant testing. When exposed to antioxidants, the free radical properties of DPPH are neutralised through the stabilisation of unpaired electrons (scavenging) by antioxidants (Wulandari, 2021). Huang *et al.* (2005) explained that in the absence of antioxidants, electron transfer from DPPH occurs, resulting in a purple colour. This electron transfer can be prevented by antioxidants, thereby avoiding the formation of the purple coloration. Figure 4 illustrates the inhibition pattern of DPPH free radicals in the presence of various concentrations of KC and AD EW. Interestingly, in the absence of PE (0%), both KC and AD EW exhibited some ability to inhibit DPPH, albeit at low levels. This indicated that EW naturally possesses antioxidant capabilities, as previously reported by Nimalaratne and Wu (2015). Benede and Molina (2020) reported that some EW proteins, such as ovalbumin, ovotransferrin, and lysozyme, including their hydrolysates, have antioxidant capabilities. Furthermore, the contribution of phenolic and flavonoid components to EW antioxidants was also supported by Aryal *et al.* (2019), who reported that both compounds are important natural antioxidants. Nonetheless, the ability of both EW to inhibit DPPH was significantly enhanced by the addition of PE (Figure 4). The increase in DPPH inhibition activity observed with higher concentrations of PE was expected, as it contained a mixture of cysteine proteases, including bromelain (Nor *et al.*, 2016; Chakraborty *et al.*, 2021). These proteases can degrade EW proteins into

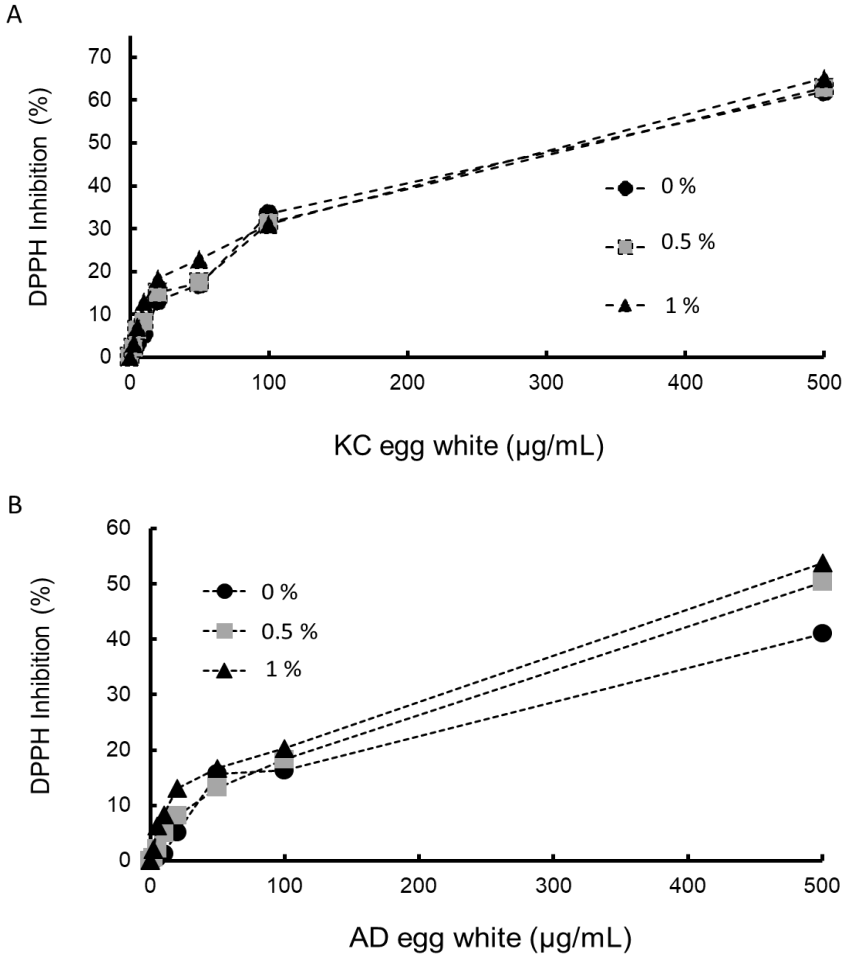

Figure 4. Inhibition of DPPH by KC and AD egg whites with pineapple extracts at different concentrations.

peptide fragments, some of which may exhibit antioxidant properties. Johny *et al.* (2022) also reported similar findings, showing that bromelain was able to produce EW hydrolysates with antioxidant properties against DPPH free radicals.

Further, the IC_{50} values of KC and AD EW against DPPH, as shown in Figure 5, indicate that the concentration of PE had significant effect on the IC_{50} values ($p < 0.05$ for KC; $p < 0.01$ for AD). For KC, 0.5% PE significantly reduced the IC_{50} value, indicating that the ability to inhibit DPPH at this concentration was considerably better than at 0%, yet comparable to that of 1% PE. On the other hand, significant differences among IC_{50} values for each concentration were observed for AD EW treated with PE. The IC_{50} value of AD EW in the presence of 1% PE was significantly lower than those at 0.5 and 0%. Meanwhile, 0.5% PE yielded a significantly lower IC_{50} than 0%. Notably, the best IC_{50} values for KC and AD were calculated to be 401.52 and 475.03 µg/mL, respectively. This indicated that the type of EW had significant effect, with KC EW inhibiting DPPH better than AD EW, as evidenced by its lower IC_{50} value. This difference might be attributed to variations in the sequence of EW proteins between the two types. Sequence differences can affect the digestibility of the proteins by proteases. For example, sequence discrepancies among chicken and duck EW proteins have been shown in lysozyme and ovomucoid, as reported by Araki and Torikata (1999) and Nurliyani *et al.* (2022). Nurliyani *et al.* (2022) also noted structural differences between duck and chicken EW proteins, although these differences are minor. Alternatively, this suggested that hydrolysis of EW proteins by PE in KC produced more antioxidant peptide fragments compared to AD.

Nevertheless, both values were much higher compared to the IC_{50} value of ascorbic acid, a well-known antioxidant, which was reported to be lower than 30 µg/mL (Jadid *et al.,* 2017; Madhvi *et al.,* 2020; Razali *et al.,* 2022). However, the IC_{50} values obtained in the present work were much better than those of EW hydrolysate against DPPH reported by other studies, including Cho *et al.* (2014), Noh and Suh (2015), and Homayouni-Tabrizi *et al.* (2015). In the present work, the primary objective was to evaluate the relative antioxidant potential between two EW sources (KC and AD) under identical experimental conditions, rather than to benchmark against a commercial or well-established antioxidant standard. The absence of a positive control, such as ascorbic acid, was intentional, as our focus was strictly comparative to determine whether antioxidant compounds were inherently present in these two EW types, and how PE concentration could modulate their activity. Including a commercial antioxidant at this exploratory stage may shift focus from the intrinsic antioxidant contribution of the biological samples under investigation. Notably, even though the IC_{50} value for both KC and AD were much lower than the well know antioxidant, the fact that hydrolysates (in the presence of 5 and 10% of PE) were able to exhibit antioxidant activity better than unhydrolysed one (0% PE) convincingly indicated that this approach could be promising to generate antioxidant peptides. Improvement of their activity is feasible by either optimising the hydrolysis process or purification, as the extracts might contain some antagonistic peptides.

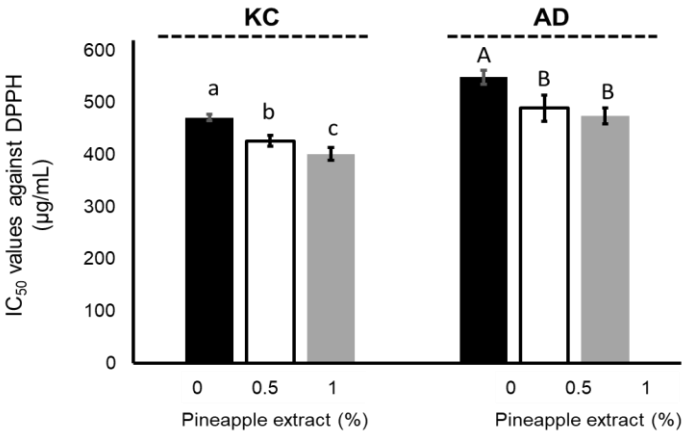

Figure 5. IC_{50} values of KC and AD egg whites hydrolysed by different concentrations of pineapple extracts. Different lowercase letters indicate significant difference ($p < 0.05$). Different uppercase letters indicate highly significant difference ($p < 0.01$).

In addition, the concentrations of parent proteins exhibiting antioxidant properties in KC, as reported by Benede and Molina (2020), were assumed to be higher than those in AD. Earlier studies by Alamprese *et al.* (2012), Liu *et al.* (2018), and Chaiyasit *et al.* (2019) indicated that the protein contents of chicken and duck EW differed, which could lead to variations in their physicochemical properties. Additionally, SDS-PAGE analysis by Chaiyasit *et al.* (2019) revealed that duck albumen contained lower levels of lysozyme and ovotransferrin, which, according to Benede and

Molina (2020), are parent proteins with antioxidant activity. Interestingly, Figure 2 shows that the protein concentration in AD was higher than in KC. However, this does not necessarily imply that a higher concentration correlates with greater antioxidant properties. The high concentration of proteins in AD might be due to non-antioxidant proteins, which would not affect the overall antioxidant activity. Moreover, a high concentration of AD proteins could negatively impact the hydrolysis rate of the proteins. Some reports have indicated that while the enzymatic hydrolysis rate initially increases with substrate concentration, it often decreases at high substrate concentrations due to negative feedback effects of the hydrolysis products (Huang and Penner, 1991; Penner and Liaw, 1994; Cheung and Anderson, 1997; Obeng *et al.,* 2017; 2018). Based on this premise, a high concentration of AD proteins might produce peptides with antagonistic effects on other antioxidant proteins or peptides, or inhibit the proteolytic activity of the PE. Indeed, some peptides have been reported to have inhibitory effects against certain proteases (Zhao *et al.*, 2014; Rudzińska *et al.*, 2021; Razali *et al.*, 2021).

Conclusion

The present work showed that EW from Kampung chicken (KC) and Alabio duck (AD) of Indonesian poultry differed in their HU, protein contents, and phenolic-flavonoid contents. Treatment with bromelain-containing PE effectively hydrolysed the proteins, as indicated by the increased release of free α-amino acids. Both EW exhibited inherent antioxidant activity against DPPH, with KC showing better antioxidant activity than AD. More interestingly, the hydrolysis of both KC and AD EW using PE significantly affected their antioxidant activity. The best IC_{50} values for KC and AD were found to be 401.52 and 475.03 µg/mL, respectively, which were obtained from the hydrolysis using 10% of PE. This indicated that the hydrolysis of EW by PE successfully increased the antioxidant capacity of the eggs.

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