Evaluation of Particle Size, Total Flavonoid, and Antioxidant Activity of Onion Peel (*Allium cepa* L.) in Different Forms as a Poultry Feed Additive

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Abstract. This study aims to evaluate the particle size, flavonoid content, and antioxidant activity of onion peel in various forms as a poultry feed additive. The material used in this study was onion peel. Particle size was analyzed using laser diffraction to obtain uniform particle size data. Flavonoid content was determined using UV-Vis spectrophotometry to obtain the total flavonoid content in onion peel. Antioxidant activity was tested using the DPPH method with a UV-Vis spectrophotometer to determine the ability of onion peel to scavenge free radicals. The results showed that the particle size of onion peel in powder form was (36.24 µm), solution form (0.98 µm), and extract form (3.47 µm). The highest flavonoid content was found in the powder form (335.97 mg QE/g), followed by the extract form (11.91 mg QE/g), and the solution form (1.66 mg QE/g). The strongest antioxidant activity was demonstrated by the IC_{50} value of the extract (1.69 µg/mL), followed by the powder (8.57 µg/mL), and the solution (458.18 µg/mL). It can be concluded that onion peel has the potential to be used as a feed additive, as indicated by its particle size, flavonoid content, and antioxidant activity.
Keywords: antioxidant activity, flavonoid content, onion peel, particle size, poultry feed additive.

1 Introduction

The broiler chicken industry is a significant contributor to meat production. This industry plays a key role in meeting human needs for animal protein. Demand for meat consumption is increasing in line with population growth, coupled with rising public awareness of the importance of consuming animal protein. Factors driving the growth in poultry demand include population growth, economic growth, and affordable meat prices. Meat production must be continuously improved to meet public demand. Chicken meat production is primarily influenced by proper husbandry management. Optimal chicken growth is supported by effective and efficient feeding, but feed costs remain the largest expense in the livestock industry. Feed costs reach 60-70% of total production costs, therefore the use of local feed ingredients, low-cost feed ingredients with high nutritional content can reduce production costs [1]. Efforts to improve feed efficiency include the use of feed additives.

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Additives in feed can be provided using phytobiotics to support the Minister of Agriculture Regulation Number 14/PERMENTAN/PK.350/5/2017 concerning the Classification of Animal Drugs which contains a prohibition on the use of antibiotics as feed additives. Phytobiotics are feed additives derived from herbal plants and are widely applied in livestock production. The addition of phytobiotics in animal feed can improve animal health and feed efficiency, as well as serve as an alternative to antibiotics in feed. Onions are one such ingredient, used both as a culinary ingredient and a medical agent. Onions are utilized not only for their bulbs but also for their outer peel. Utilizing agricultural by-products and processing waste can reduce production costs, minimize waste, and increase the economic value of raw materials. Onion peel contains high levels of bioactive compounds, such as flavonoids, particularly quercetin, and antioxidant activity, which is beneficial for livestock [2]. This study aims to evaluate particle size, flavonoid effectiveness, and antioxidant activity as effective and innovative broiler feed additives. With the increasing demand for sustainable meat products, the findings of this study are expected to provide benefits and solutions to farmers regarding their impact on broiler health and productivity.

2 Materials and methods

2.1 Materials

The main material used in this study was the peel of New Zealand onion varieties obtained from the Gadang Main Market, Malang City. The onion peel was then processed into three forms, namely powder, solution, and extract for laboratory testing. The reagents used in making the solution, making the extract, and testing the particle size were distilled water, for the total flavonoid test were ethanol P.A., quercetin standard, $AlCl_3$, CH_3COONa, and distilled water, and for the antioxidant activity test were ethanol P.A., DPPH, and ascorbic acid. The equipment used included scales, ovens, grinders, sieves, rotary evaporators, UV-Vis spectrophotometers, and particle size analyzers.

2.2 Methods

2.2.1 Making onion peel powder *(Allium cepa* L.*)*

The process of producing onion peel powder begins with collecting onion peel from Gadang Market, Malang City. The next step is sorting to separate clean peel from dirty and rotten peel. The peels are then air-dried to reduce the moisture content, followed by oven-drying at 50°C for 24 hours until the moisture content is less than 10%. The dried peels are then ground using a grinder to produce a coarse powder [3]. This powder is then sieved using a 230-mesh sieve to obtain fine powder.

2.2.2 Making onion peel solution *(Allium cepa* L.*)*

The onion peel solution begins by preparing 100 grams of onion peel powder, which is then placed in a container and added to distilled water. The ratio used is 1:10 (100 grams of sample : 1 liter of distilled water). This ensures complete immersion of the sample. The solution is stirred thoroughly and left for 24 hours at room temperature to allow the bioactive compounds to dissolve into the solvent. The solution is then filtered using filter paper to separate the residue from the filtrate. The resulting filtrate can be used as an onion peel solution.

2.2.3 Making onion peel extract *(Allium cepa* L.*)*

Making onion peel extract can be considered a follow-up step to making onion peel solution. Making onion peel extract begins by preparing 1 kg of onion peel powder, which is then placed in a container and added to distilled water. The ratio used is 1:10 (1 kg of sample: 10 liters of distilled water). This ensures complete immersion of the sample. The solution is stirred thoroughly and left for 24 hours at room temperature to allow the bioactive compounds to dissolve into the solvent. The solution is then filtered using filter paper to separate the residue and the filtrate. The resulting filtrate is then placed in a rotary evaporator for 3 hours to evaporate most of the solvent, followed by further evaporation in a water bath to obtain a thick extract [4].

2.2.4 Particle size testing of onion peel *(Allium cepa* L.*)*

Particle size tests were conducted at the Integrated Laboratory, Faculty of Agricultural Technology, Universitas Brawijaya. Particle size testing was conducted to determine the particle size by dispersing the sample in a liquid medium. This test was measured using the laser diffraction method to obtain uniform particle size with a Shimadzu SALD-7500 nano instrument. A 1 gram sample was used in powder form and dissolved in 10 mL of distilled water, while the liquid sample did not need to be dissolved. The particle size analyzer and computer were turned on, then the chamber was cleaned with distilled water. The instrument was blanked, and the sample was placed in the chamber with approximately 2 mL of water, then sonicated for 30 minutes until the sample size stabilized. The measurements were repeated twice and the particle size measurements were obtained [5].

2.2.5 Testing the total flavonoid content of onion peel *(Allium cepa* L.*)*

The total flavonoid content test was conducted at the Herbal Materia Medica Laboratory, Batu. The total flavonoid content of onion peel was analyzed using the UV-Vis spectrophotometry method [6]. The testing process began with preparing a sample of 16.61 mg, which was then dissolved in 10 mL of ethanol P.A. and homogenized using a sonicator for 1 hour. The solution was filtered and added with ethanol P.A. to the limit mark, then 0.5 mL of the sample was taken and mixed with 1.5 mL of ethanol P.A., 0.1 mL of 10% $AlCl_3$, 0.1 mL of CH_3COONa, and 2.8 mL of distilled water, then incubated at room temperature for 30 minutes. The second process is the preparation of a standard solution by dissolving 4 mg of quercetin standard with 10 mL of ethanol P.A., then making a series of dilutions with concentrations of 250, 200, 150, 100, and 50 µg/mL, then treated the same as the previous sample solution. The absorbance of the sample solution, standard solution, and blank was measured at a wavelength of 425 nm. The quercetin content was calculated using the following formula:

$$x = \frac{y-b}{a} \quad (1)$$

Description:
x = test sample concentration
y = test sample absorbance
a = slope
b = intercept

2.2.6 Testing the antioxidant activity of onion peel *(Allium cepa* L.*)*

Antioxidant activity test was conducted at the Herbal Materia Medica Laboratory, Batu. The antioxidant activity of onion peel was tested using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method with a UV-Vis spectrophotometer [7]. The testing process began with the preparation of a DPPH solution of 0.39 g dissolved in ethanol P.A. to a concentration of 0.1 mM, then incubated for 30 minutes at room temperature. The maximum wavelength was determined between 500-530 nm. The second process involved dissolving 16.61 mg of sample in 25 mL of ethanol P.A. then homogenized using a sonicator for 1 hour, then filtered and added ethanol P.A. to the limit mark. Preparation of a dilution series with concentrations of 500, 250, 125, 62.5, 31.25, and 15.625 ppm, each of which was taken 1 mL to be mixed with 1 mL of 0.1 mM DPPH solution and incubated for 30 minutes. The next process is the preparation of a reference solution by preparing 2.5 mg of ascorbic acid dissolved in 25 mL of ethanol P.A., then a dilution series was made with concentrations of 50, 25, 12.5, 6.25, 3.125, and 1.5625 ppm and then treated the same as the previous process. The final testing process is the absorbance of the sample solution, reference solution, and blank measured at the maximum wavelength to obtain the percentage of inhibition. Measurement of the IC_{50} value is determined using the following formula:

$$IC_{50} = \frac{50-b}{a} \quad (2)$$

Description:
a = slope
b = intercept

2.2.7 Observation variables

The variables observed in this study were particle size, total flavonoid content, and antioxidant activity of onion peel in three sample forms (powder, solution, and extract).

2.2.8 Data analysis

The study data were analyzed descriptively. The results were presented as averages and standard deviations, which were then interpreted to determine the potential of the tested samples.

3 Results and discussion

3.1 Particle size analysis of onion peel (*Allium cepa* L.)

Particle size testing was conducted to determine the distribution and uniformity of feed additive size, as it significantly influences the physical, chemical, and biological properties of the feed. The results of the onion peel particle size test are presented in Table 1.

Table 1. Results of particle size analysis of onion peel (*Allium cepa* L.).

Test Parameters	Sample Forms		
	Powder	**Solution**	**Extract**
Particle Size (µm)	36.24	0.98	3.47

Laboratory analysis results show that onion peel powder has a particle size of 36.24 µm, solution form 0.98 µm, and extract form 3.47 µm. The specific particle size value categories can be considered as (very fine if the size is <0.4 mm, fine if the size is 0.4–0.8 mm, medium if the size is 0.8–1.4 mm, and coarse if the size is >1.4 mm [8]. The particle size of feed additives affects the level of homogeneity in the feed mixing process. Particles that are too coarse tend to settle, making the feed additive not mixed evenly in the feed. Finer particles make the process of mixing feed additives easier and more consistent so that livestock receive the appropriate dose of additives [9]. Particle size is an important factor because the digestive process is a series of preparations for the nutrient absorption process. Choosing a balanced particle size is better because it can maintain an even mixing process and help the chicken's digestive process more optimally.

3.2 Total flavonoid content analysis of onion peel (*Allium cepa* L.)

Onion peel has the potential to be a nutraceutical that is rich in polyphenol compounds, including flavonoids and non-flavonoids. The results of the total flavonoid content analysis in onion peels are presented in Table 2.

Table 2. Results of total flavonoid content analysis of onion peel (*Allium cepa* L.).

Test Parameters	Sample Forms		
	Powder	Solution	Extract
Total Flavonoids (mg QE/g)	335.97	1.66	11.91

The laboratory analysis results above indicate that the flavonoid content in onion peel varies depending on the sample form. The powder form had the highest content of 335.97 mg QE/g, followed by the extract at 11.91 mg QE/g, and followed by the solution form which showed the lowest content of 1.66 mg QE/g. The powder form had the highest total flavonoid content, indicating that the drying and milling processes were able to retain bioactive compounds more optimally. Flavonoid compounds in the powder form were still stored throughout the plant cell tissue, so the total number of compounds measured was higher than in the solution and extract forms. This condition indicates that flavonoids in the powder form were still stored intact in the plant cell tissue, so the total flavonoid value detected was higher [10]. The high total flavonoid content in this study indicates that flavonoid compounds in the powder form are structurally present in large quantities and indicate the potential for interactions between bioactive compounds and the contribution of non-flavonoid bioactive compounds in the material composition.

The high flavonoid content in onion peels in the form of powder has the potential to be used as a feed additive for broiler chickens because flavonoids act as antioxidants that can improve health status, suppress oxidative stress, and potentially improve growth performance. Onion peels, which are considered waste, contain bioactive compounds such as flavonoids that have various pharmacological activities, including antioxidants, anti-inflammatory, antimicrobial, and anticancer. Onion peels have a higher flavonoid content than the bulb, with quercetin as the main component known to have anti-inflammatory and antioxidant effects. Flavonoids, especially quercetin, which is an active compound from the polyphenol group, play a role in improving nutrient digestibility by stabilizing the digestive system and improving intestinal function through their antioxidant, anti-inflammatory, and antimicrobial effects [11].

3.3 Antioxidant activity analysis of onion peel (*Allium cepa* L.)

Antioxidant activity analysis was conducted to determine the ability of onion peel in scavenging free radicals. Antioxidant activity is the ability of a compound to combat oxidative damage in the body caused by free radicals. Free radicals are formed by several factors, such as environmental heat stress, metabolic processes in the body, toxin contamination in feed, and intestinal parasite infections, which ultimately impact chicken health and the quality of livestock products. The results of the antioxidant activity analysis of onion peels are presented in Table 3.

Table 3. Results of antioxidant activity analysis of onion peel (*Allium cepa* L.).

Test Parameters	Sample Forms		
	Powder	Solution	Extract
Antioxidant Activity IC_{50} (µg/mL)	8.57 ± 2.00	458.18 ± 2.00	1.69 ± 2.00

Laboratory analysis results showed that onion peel powder had an IC_{50} antioxidant activity of 8.57 µg/mL, a solution value of 458.18 µg/mL, and an extract value of 1.69 µg/mL. The determination of antioxidant activity was expressed in the IC_{50} value (µg/mL) as a measure of antioxidant capacity. The IC_{50} value is defined as the concentration of the test compound capable of inhibiting free radicals by 50%. A smaller IC_{50} value indicates higher free radical scavenging. The IC_{50} value category varies widely and is distinguished as (IC_{50} < 10 µg/mL as very strong, IC_{50} between 10-50 µg/mL as strong, 50-100 µg/mL as moderate, 100-250 µg/mL as weak, and IC_{50} > 250 µg/mL as inactive) [12]. This shows that the active compounds in onion peel in the form of extracts show the strongest category of antioxidant activity. The extraction process encourages flavonoid compounds to be released from plant cell tissue so that they are more available to react in antioxidant activity tests. The extraction process also dissolves various non-flavonoid antioxidant compounds that interact synergistically with flavonoids in scavenging free radicals. Therefore, although the total flavonoid content in the extract is lower than in the powder form, the increased availability of bioactive compounds as well as the contribution and synergy between flavonoids and non-flavonoid antioxidants results in higher measured antioxidant activity. This explains the inverse relationship between total flavonoid content and antioxidant activity in this study [13].

The active compounds in onion peels consist of phenolics, flavonoids (quercetin), flavanols, anthocyanins, tannins, vanillic acid, and ferulic acid. Phenolic and flavonoid compounds, especially quercetin, in onion peels play a crucial role in antioxidant activity. Quercetin has strong antioxidant activity that can protect the body from diseases caused by oxidative stress. High phenol content can improve chicken performance, improve chicken health, increase amino acid digestibility, support intestinal health, increase absorption surface area, and increase digestive enzyme activity in the gastric mucosa [14]. Previous studies have shown that the use of onion peels in broiler feed can increase chicken antioxidant activity, which is characterized by increased endogenous enzyme activity such as superoxide dismutase (SOD) and glutathione peroxidase (GPx), increase resistance to oxidative stress, and improve meat quality. Antioxidant compounds also help prevent tissue damage by effectively scavenging free radicals, thus preventing oxidation in chicken meat [15]. This indicates that adding onion peels to feed has the potential to improve chicken health and performance, improve digestive function, and increase endogenous enzyme activity. Further research is needed to determine the optimal dosage and better understand its mechanism of action in the chicken digestive system.

4 Conclusion

These findings indicate that onion peel has good potential as a source of flavonoids and antioxidants in broiler feed. The high flavonoid content and antioxidant activity of onion peel positively impact cell protection from oxidative damage, thus supporting overall health, including performance, and the chicken's digestive tract. The results indicate that onion peel has the potential to be a safe and effective ingredient for development as a chicken feed additive.

The authors would like to express their gratitude to all parties who have provided guidance, assistance, and support during the course of this study.

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