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FLAVONOID (QUERCETIN) CONTENT, YEAST AND MOLD CONTAMINATION IN DUCK MEAT SOAKED IN SHALLOT (ALLIUM ASCALONICUM) SKIN WASTE

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ABSTRACT

Introduction: The flavonoid content in shallot skin is 2–10 g/kg higher than that in the edible portion, with < 0.03 to 1 g/kg. **Aims:** To determine flavonoid (quercetin) content and yeast and mold contamination in duck meat soaked in shallot (Allium ascalonicum) skin waste. **Methods:** The sample was shallot skin waste from 14 districts in Brebes Regency, Indonesia. Analysis was performed in several steps, including the production of shallot skin flour and measurement of flavonoid (quercetin) levels using a UV-Vis spectrophotometer. This study analyzed the amount of yeast mold growth on duck meat after soaking in shallot skin waste extract and the test data obtained will be analyzed descriptively and presented in the form of a table. **Results:** The average flavonoid (quercetin) content in the shallot skin waste was 4.33% w/w. The lowest flavonoid (quercetin) content in the Sirampog district was 4.29% w/w and the highest quercetin content was 4.40% w/w in the Wanasari district. The mold and yeast contamination levels varied among treatments, with treatment 1 having the highest contamination rate of 96 colonies/g, followed by treatments 2 and 3 with contamination rates of 20 and 15 colonies/g, respectively, and treatment 4 having contamination rates of 3 colonies per gram. **Conclusion:** The flavonoid content of shallot skin waste varied across the districts. Mold and yeast contamination levels were significantly different among the treatments, with treatment 1 showing the highest contamination rate. Treatment 4 resulted in a very rapid reduction in the number of molds and yeast (3 colonies).

Keywords: Shallot, Waste, Flavonoids, Molds, Yeast, Meat

INTRODUCTION

Shallots are food crops that produce wastes. Processing shallots creates a lot of waste, especially the skin (Osojnik Črnivec et al., 2021a; ‘Aqilah et al., 2023). Shallot production has increased over the past decade, with approximately 98 million tons being produced worldwide (Hidayah et al., 2023). The waste generated from shallot processing poses environmental challenges owing to its high organic content, which contributes to greenhouse gas emissions and soil pollution (Beesigamukama et al., 2023). Improper disposal of shallot waste can lead to water contamination and harm aquatic ecosystems. Implementing appropriate waste management practices in waste-processing facilities is crucial to mitigate these environmental impacts.

Brebes in Indonesia have the highest total production of shallot skins. This regency accounted for 18.5% of the total national shallot skin-waste production, and 57% of the total production in Central Java. According to Brebes agricultural land observation data in 2016, the availability of dried shallot waste as a whole of waste was 0.68 t/ha and the weight of dry shallot leaves reached 0.87 t/he. The amount of shallot produced by Brebes affects the quantity of shallot skin waste, which in general has an impact on environmental quality. Most shallot skin waste is not recycled but simply discarded.

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Harvesting shallots generates waste in the form of shallot skins and roots, which accounts for approximately 0.5% of the total yield (Bhosale et al., 2022).

Chemical compounds in shallot skin (Allium cepa L.), such as flavonoids, polyphenols, saponins, alkaloids, terpenoids, tannins, quercetin, and kaempferol, have a bacteriostatic (antibacterial) effect against Streptococcus mutans(Mandal, Sarkar and Chakraborty, 2023). Flavonoid chemical compounds can denature proteins such that metabolism in bacterial cells can stop (Carrillo-Martinez et al., 2024). The saponins contained in shallot skin interact with bacterial cells, causing them to rupture or lyse (Tatli Cankaya and Somuncuoglu, 2021). Tannins can bind to lipoteichoic acid on the surface of Streptococcus mutans cells (Czerkas et al., 2024). Based on the chemical compounds present in shallot skin (Allium cepa L.), it can be used as a preservative in meat and to maintain meat freshness.

The urgent need for research on alternative methods of waste disposal in the food industry is highlighted by the potential health risks associated with mold and yeast contamination of meat products. Understanding the impact of flavonoid-rich ingredients like shallot skin waste on food safety could lead to innovative solutions for reducing waste and improving product quality (Waheed Janabi et al., 2020; Chadorshabi, Hallaj-Nezhadi and Ghasempour, 2022; Levaj et al., 2023).

The seriousness of this issue is underscored by the need for sustainable practices in food production to ensure consumer safety and minimize environmental impact. Further studies on the effectiveness of utilizing shallot skin waste for meat preservation may offer valuable insights into the industry. Exploring alternative methods for utilizing shallot skin waste in meat preservation could also have economic benefits for the industry by reducing waste disposal costs and increasing product value (Karwowska, Łaba and Szczepański, 2021; Osojnik Črnivec et al., 2021b). Additionally, collaboration between researchers, food producers, and regulatory agencies is essential in implementing these innovative solutions to address food safety concerns related to mold and yeast contamination.

The growth of this research could potentially revolutionize food safety standards and practices, ultimately benefiting both consumers and the environment. Additionally, exploring the potential antimicrobial properties of shallot skin waste could provide a natural and sustainable alternative to synthetic preservatives in meat products (Yu, Chin and Paik, 2021; Teshome et al., 2022; Karnwal and Malik, 2024). By harnessing the antimicrobial properties of shallot skin waste, researchers can reduce the need for synthetic preservatives in meat products, thereby promoting a more sustainable approach to food safety. This innovative solution has the potential to not only improve food safety standards but also contribute to environmental conservation efforts. Due to its rich chemical profile, which includes flavonoids with antibacterial qualities, especially quercetin (Thuy et al., 2019; Pebiyanti, Widiantie and Prianto, 2021), studies have suggested the possibility of using shallot skin as an ecological solution. In addition to offering a sustainable way to manage agricultural waste, these compounds may act as natural preservatives in meat products, improving food safety (Thuy et al., 2019; Hakim, Sulardi and M. Wasito, 2023).

We aimed to determine the flavonoid (quercetin) content and yeast and mold contamination in duck meat soaked in shallot (Allium ascalonicum) Skin Waste. The novelty of this study lies in its use of Brebes shallot skin as the main raw material, which has rarely been explored in similar studies. The results of this study provide valuable information regarding the potential of Brebes shallot skin as a source of flavonoids and test contamination of duck meat products.

METHOD

Samples

This study covers all districts in Brebes Regency, Indonesia, which has extensive production. In Brebes, 14 districts produce shallots. Three shallot skin-waste samples were collected from each district. It is recorded that in Brebes there are 17 districts and only 14 districts produce shallot skin. We considered the total population of districts that produce shallot skin in Brebes. We chose Brebes because it has the highest total production of shallot skin in Indonesia. This regency accounted for 18.5% of the total national shallot skin-waste production and 57% of the total production in Central Java.

Location

The study was conducted in two locations: the General Soedirman University Laboratory, Purwokerto, and the Laboratory of the Center for Standardization and Industrial Pollution Prevention Services, Semarang.

Research design

The research design used in this study was qualitative experimental research. The objective of the experiment was to determine whether there are consequences from something that is applied to the object being investigated or observed; the object studied in this research was a test for mold/yeast numbers in meat soaked with onion skin waste extract for 30 min with four factors for storage time at room temperature: P1, 4 h storage; P2, 8 h storage; P3, 12 h storage; and P4, 16 h storage.

Determination of flavonoid levels

The flavonoid (quercetin) standard (10 mg) was dissolved in 10 mL hanol to achieve a concentration of 1000 parts per million (ppm). The quercetin stock solution (1 mL) was pipetted and dissolved in 10 mL of methanol to obtain a final concentration of 100 ppm. Thereforeefore, concentrations of 4, 5, 6, 7, and 8 ppm were used. For each concentration of the quercetin standard solution, 3 mL of methanol, 0.2 mL of 10% AlCl3, and 0.2 mL of 1 M potassium acetate were combined. Aquades were then added to bring the total volume to 10 ml. The mixture was then incubated at room temperature for 30 min. Absorbance was measured using UV-Vis spectrophotometry at a specific wavelength of 431 nm. The powder (25 mg) was mixed with 10 mL ethanol to achieve a concentration of 2500 ppm. One milliliter of this solution was extracted using a pipette. Subsequently, 3 mL of methanol, 0.2 mL of a 10% AlCl3 solution, 0.2 mL of a 1 M potassium acetate solution, and 10 mL of distilled water were added. The samples were incubated for 30 min at room temperature. Absorbance was quantified using UV-Vis spectrophotometry at a specific wavelength of 431 nm. Three replicates of each sample were prepared for analysis and the average absorbance value was determined. All procedures in this section have been carried out by professional staff in their fields with laboratories that have been accredited using international principles in each part of the method, especially those that should use procedures based on AOAC.

Proximate Analysis

The parameters observed in this study were the proximate content of shallot skin waste extract (Allium cepa L.), which consisted of protein, fat, water, ash, and carbohydrate. Protein content (Method Kjedhal), fat content (Method Soxhlet), water content (method oven), ash content (method tannur), and carbohydrates (method anthrone). All procedures in this section, especially those that should have used AOAC-based procedures, were carried out by qualified experts in their field in an accredited laboratory utilizing international principles.

Yeast Mold Number Test

Determination of the yeast mold that causes contamination in meat soaked with onion skin waste extract begins with a media incubation process. Incubation was carried out by dissolving the sample in a solvent, weighing 1 g, and placing it in a petri dish. The cells were then incubated for five days at room temperature (27–28°C). The number of molds and yeasts that grew was counted to determine the number of yeast molds and then referred to in accordance with the provisions of the SNI ISO 21527:2012. The procedures described in this section, particularly those requiring AOAC-based methods, were conducted by qualified experts in an accredited laboratory using international principles.

Analysis

The analysis was performed in several steps, including the production of shallot skin flour and the measurement of flavonoid (quercetin) levels using a UV-Vis spectrophotometer. In addition, this study analyzed the amount of yeast mold growth on duck meat after soaking in shallot skin waste extract, and the test data obtained will be analyzed descriptively and presented in the form of a table. Determination of proximate analysis was carried out using the AOAC 2016([AOAC] Association of Official Analytical Chemists, 2016)standard and was carried out by the General Laboratory of Soedirman University, Purwokerto, and the Center for Standardization and Industrial Pollution Prevention Services Laboratory, Semarang.

RESULTS

Table 1 shows the Brebes district that produces shallots. Ketanggungan district has the highest Shallot Productivity, with a production value of 126.90 Kw/Ha. Meanwhile, the district has the lowest Shallot Productivity in Sirampog, with a production value of only reaching 65.00 Kw/Ha. The data in Table 1 highlight the significant variation in overall productivity among Brebes districts.

Table 1. The Shallot Productivity in Brebes Regency

District	Productivity of Shallot								
	Harvest Area (Ha)			Production (Kw)			Average Production (Kw/Ha)		
	2022	2023	2024	2022	2023	2024	2022	2023	2024
Bantarkawung	166	187	173	14940	16830	15570	90	90	90
Sirampog	-	1	3	-	60	195	-	60	65
Tonjong	-	6	1	-	456	110	-	76	110
Larangan	7001	6978	7661	688450	771860	825454	98.34	110.61	107.75
Ketanggungan	1946	1769	1519	224130	207292	192764	115.17	117.18	126.90
Banjarharjo	106	109	192	11800	6722	22679	111.32	61.67	118.12

District	Productivity of Shallot								
	Harvest Area (Ha)			Production (Kw)			Average Production (Kw/Ha)		
	2022	2023	2024	2022	2023	2024	2022	2023	2024
Losari	653	703	697	67970	77023	76085	104.09	109.56	109.16
Tonjong	1581	1689	1927	148020	152660	180402	93.62	90.38	93.62
Kersana	1178	866	882	112570	44628	69790	95.56	51.53	79.13
Bulakamba	4288	4691	6304	438030	432643	615422	102.15	92.23	97.62
Wanasari	5519	5729	11385	669070	656410	1006512	121.23	114.58	88.41
Songgom	615	1319	1408	79850	147523	163939	129.84	111.84	116.43
Jatibarang	1644	1017	1241	186860	106521	133528	113.66	104.74	107.60
Brebes	4013	4087	5558	396040	408700	532661	98.69	100	95.84
Brebes Regency (Total)	28710	29151	38951	3037730	3029328	3037721	105.81	103.92	77.99

Table 2 shows that the samples contained approximately 4.33% w/w flavonoids (quercetin). The flavonoid content (quercetin) in shallot skin from the Wanasari District was the highest (4.40% w/w). Shallot skins from Brebes (4.38% w/w), Bulakamba (4.35% w/w), Losari (4.34% w/w), shallot skins from Jatibarang District (4.34% w/w), shallot skins from Songgom District (4.34% w/w), shallot skins from Tanjung District (4.34% w/w), Kersana District (4.33% w/w), Banjarharjo District (4.32% w/w), Banjarharjo District (4.31% w/w), and Larangan District (4 .31% w/w) w). Shallot skin samples from Sirampog District had the lowest flavonoid content (4.29% w/w).

Table 2. Flavonoids (quercetin) Compound Content in Shallot Skin

District	Samples	Quercetin (%w/w)	Average of Quercetin (%w/w)
Tonjong	I	4.28	4.30
	II	4.31	
	III	4.31	
Wanasari	I	4.4	4.40
	II	4.4	
	III	4.41	
Brebes	I	4.41	4.38
	II	4.35	
	III	4.38	
Larangan	I	4.32	4.31

District	Samples	Quercetin (%w/w)	Average of Quercetin (%w/w)
	II	4.31	
	III	4.31	
Bulakamba	I	4.36	4.35
	II	4.36	
	III	4.33	
Losari	I	4.33	4.34
	II	4.33	
	III	4.35	
Jatibarang	I	4.34	4.34
	II	4.36	
	III	4.32	
Songgom	I	4.33	4.34
	II	4.33	
	III	4.36	
Kersana	I	4.32	4.33
	II	4.33	
	III	4.34	
Banjarharjo	I	4.33	4.32
	II	4.3	
	III	4.32	
Ketanggungan	I	4.29	4.31
	II	4.31	
	III	4.33	
Tonjong	I	4.34	4.34
	II	4.36	
	III	4.32	
Bantarkawung	I	4.3	4.30
	II	4.3	
	III	4.31	
Sirampog	I	4.29	4.29
	II	4.28	
	III	4.31	

Table 3 shows that the proximate test results indicate that the shallot skin had a very high carbohydrate content (66.5 %). The water, ash, fat, and protein contents were only 2 %, 1.5%, 20%, and 10 %, respectively.

Table 3. Results of proximate analysis of shallot skin

Sample Type	Analysis Parameters	
Shallot Skin Waste Extract	Water content	2 %
	Ash Content	1.5 %
	Fat level	20 %
	Protein Content	10 %

Sample Type	**Analysis Parameters**	
	Carbohydrate	66.5 %

Table 4 shows that the total test for yeast mold on duck meat that has been given treatment involves soaking in waste extract from the skin of two shallots with a soaking time of 30 minutes and there are 4 storage treatments labeled P1:4 h of storage, P2:8 h of storage, P3:12 h of storage, and P4:16 h of storage. Storage was performed at room temperature, and descriptive methods were used to manage the data

Table 4. Amount of mold and yeast contamination

Sample Labels	**Parameter**	**Unit**	**Amount**
P1 (4 hours)	Mold and Yeast	Colonies/g	96
P2 (8 hours)	Mold and Yeast	Colonies/g	20
P3 (12 hours)	Mold and Yeast	Colonies/g	15
P4 (16 hours)	Mold and Yeast	Colonies/g	3

DISCUSSION

Shallot skin samples from 14 Brebes Regency districts had a flavonoid (quercetin) content of 4.33 %, with Wanasari district having the highest content at 4.40%. The other districts had similar levels, indicating that Brebes shallot skin contains flavonoids (quercetin) that can be used in many applications. The high quercetin content in shallot skin offers great potential as a supplier of active ingredients in the Indonesian industry, and the possibility of using shallot skin waste for product innovation and value addition. Quercetin, a common flavonoid, is often used as a standard for determining flavonoid levels. Quercetin belongs to a class of flavonols that is widely present in plants. Flavonols contain a keto group at C-4 and a hydroxyl group at either C-3 or C-5, which allows them to form complexes with AlCl3 (Spiegel et al., 2020; De et al., 2022).

Shallots contain significant amounts of quercetin derivatives including quercetin aglycone, quercetin monoglucosides, and quercetin diglucosides (Terao, 2023). The antioxidant activity of shallot extract is determined by its phenolic and flavonoid components. Flavonoid compounds showed stronger associations than phenols, suggesting that flavonoids have a greater impact on antioxidant activity than phenolic compounds do. Quercetin, which is mainly found in the shallot skin, is the most abundant flavonoid. These flavonoids exhibit higher antioxidant activity than other categories of flavonoids (Speisky et al., 2022; Hassanpour and Doroudi, 2023).

Many studies have confirmed that altitude affects the flavonoid content (Pandey et al., 2018; Suleiman, ALaerjani and Mohammed, 2020). The levels of quercetin, tricin, and routine were notably higher in the low-altitude populations than in the middle- and high-altitude populations. These levels were positively correlated with the amount of precipitation in natural habitats (Zhou et al.2021). Flavonoid (quercetin) levels were also affected by altitude. The factors that influence high and low flavonoid levels are the temperature and light intensity. The lower the planting area, the higher are the flavonoid levels. High levels of flavonoids result in high antioxidant activities (Shah and Smith, 2020; Hu et al., 2021).

It is known that the number of shallot skins based on shallot production in the district is highest in Kersana, with an average of 36.7 grammes of shallot skins per 1 kg of shallot. The data showed that The average amount of shallot skin waste in Brebes was 2.94 gram/kg. This is consistent with data on shallot harvest yields, which yield waste in the form of shallot skin and roots equal to approximately 1.9 x 106 kg/year or 0.5% of the overall production (Munir et al., 2018). These findings are pertinent to information regarding the possible availability of discarded dried shallots. Based on observation data from 2016 on the land of Brebes farmers, the total waste was 0.7 t/ha, and the weight of dried shallot leaves was 0.8 t/ha (Juwanda et al., 2020).

From the results of the proximate analysis carried out on the second Shallot skin waste extract, there was a very high water content where the water content in the second Shallot skin waste extract was 2%. The water content in food is related to the level of product resistance to damage, enzyme activity, and chemical activity, namely, the occurrence of non-enzymatic reactions, which can cause changes in the organoleptic properties, appearance, texture, and taste, as well as the nutritional value contained therein, as well as the acceptability, freshness, and durability of food. The ash content of the onion skin waste extract was 1.5%, which was very low. Therefore, the inorganic material content of the second onion skin waste extract is very low. The value of the fat content in the waste extract of the second skin of shallots is very low (20 %), and the fat content in food is useful as a large source of energy between proteins and carbohydrates. With low fat content, the ability of food ingredients to act as an energy source between proteins and carbohydrates does not play an optimal role. The values for protein and carbohydrate levels are 10% and 66.5%, respectively, where protein itself acts as a source of energy, carbohydrates act as a breakdown of protein levels, and carbohydrates play a role in forming color, smell, taste, and texture (Dangal et al., 2024).

In mold and yeast tests, colony planting was performed using the pour plate method. The pour plate method was used because yeast is facultative and can live under both aerobic and anaerobic conditions. In this study, the pour plate method was used to ensure that aerobic and anaerobic yeasts could grow well, and the total number of living cells per colony unit/g was calculated. After incubation for five days, the colonies growing on the medium were counted. Mold colonies were counted as filamentous cottons. Meanwhile, the yeast colonies that were counted were separate colonies that were round in shape, after which the colonies that grew were counted and analyzed to determine the total number of colonies. In the P1 sample, observations were carried out until the 5th day with a total of 96 colonies/g of mold and yeast. The P1 sample was soaked for 30 min, after which it was stored at room temperature for 4 h.

On the P2 sample, observations were carried out until the 5th day with a total of 20 colonies per gram of mold and yeast, where the P2 sample was soaked for 30 min, after which it was stored at room temperature for 8 h. Observations were made on the P3 sample until the 5th day with a total of 15 colonies/g of mold and yeast. The P3 sample was soaked for 30 min and then stored at room temperature for 12 h. On the P4 sample, observations were carried out until the 5th day with a total of three colonies/g of mold and yeast, where the P4 sample was soaked for 30 min, after which it was stored at room temperature for 16 h.

The bacterial activity that occurs when meat is soaked in shallot waste extract significantly decreases after storage at room temperature for > 12 h. Therefore, the bacteriostatic effect of shallot skin waste extract can prevent the addition of mold and yeast colonies (Khounganian et al., 2023). Duck meat can only last for a few hours at room temperature because the decay process in duck meat is so fast that in the presence of onion skin extract, which has a bacteriostatic (antibacterial) effect with its chemical content, it can stop the growth rate of mold and yeast (Kim et al., 2017).

The strength of this study is the comprehensive analysis of the proximate content of shallot skin waste extract, which provides valuable information for potential applications in the food industry. Additionally, detailed examination of mold and yeast contamination levels among different treatments highlights the importance of proper handling and storage practices to ensure product safety. A weakness of this study was the lack of information on the specific methods used for soaking and storage, which could impact the reproducibility of the results. Further research is needed to determine the potential shelf life of shallot skin waste extracts under different storage conditions.

CONCLUSION

The average concentration of flavonoids (specifically quercetin) in the shallot skin waste was 4.33% by weight. Shallot skin waste from the Wanasari district had the highest concentration of the flavonoid (quercetin) (4.40% w/w). The results of the proximate content test of the second shallot skin waste extract showed that the shallot skin waste had a very high proximate water, ash, fat, protein, and carbohydrate contents of 2 %, 1.5%, 20%, 10%, and 66.5 %, respectively. The study found that mold and yeast contamination levels varied among treatments: treatment 1 had 96 colonies/g, treatments 2 and 3 had 20 and 15 colonies/g, and treatment 4 had 3 colonies/g after soaking for 30 min and storage for 16 h.

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