Storage and Packaging Evaluation for Preserving Polyphenols in Black Garlic

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Abstract

Black garlic (*Allium sativum* L.) is produced through a controlled thermal aging process, enhancing its phenolic and flavonoid content and increasing antioxidant, anti-inflammatory, and anticancer properties. This study aimed to evaluate the effects of different storage temperatures (30°C, 35°C, and 40°C) and packaging materials (paper core–PC and aluminum foil–AF) on the stability of these bioactive compounds. Black garlic was prepared by aging single clove garlic at 74°C for 12 days using a fermenter. The aged garlic was then packaged in PC and AF and stored under specified conditions. Total phenolic content (TPC) was determined using the Folin-Ciocalteu method, while total flavonoid content (TFC) was measured with the aluminum chloride colorimetric method. Results indicated that higher storage temperatures led to an insignificant reduction in both phenolic and flavonoid contents, with AF packaging providing better retention due to its superior barrier properties (TPC = 2.62–3.51 mg GAE/g and TFC = 1.57–2.09 mg QE/g). At 35°C, PC packaging also showed comparable performance, suggesting its potential as a sustainable alternative (TPC = 2.92 mg GAE/g and TFC = 2.09 g QE/g). The study highlights the importance of optimizing storage conditions and packaging materials to preserve the functional properties of black garlic. These findings provide valuable insights for the food industry in developing effective storage strategies to enhance the benefits of black garlic.

Keywords: Bioactive Compounds, Flavonoid, Food Preservation, Phenolic.

INTRODUCTION

Black garlic (*Allium sativum* L.) has become a highly valued functional food, known for its rich content of bioactive compounds and diverse health benefits. It is produced through a thermal aging process of fresh garlic at controlled temperatures and humidity levels, which results in a distinct transformation of its chemical composition (Afzaal *et al.*, 2021). During this process, the Maillard reaction enhances the levels of phenolic compounds, flavonoids, and other antioxidants, contributing to the improved health-promoting properties of black garlic compared to its raw form (Ríos-Ríos *et al.*, 2019; Vinayagam *et al.*, 2021). Recent studies have highlighted that black garlic exhibits potent antioxidant, anti-inflammatory, and anticancer activities due to its enriched phytochemical profile (Stępień *et al.*, 2024; Matsuse *et al.*, 2024).

The stability of phenolic and flavonoid compounds in black garlic, however, can be significantly influenced by various post-processing factors such as storage temperature, duration, and packaging materials. According to Wang *et al.* (2023): high storage temperatures can accelerate the degradation of these bioactive compounds, leading to a reduction in antioxidant capacity and overall product quality. In contrast, the use of effective packaging materials can mitigate these losses by protecting the product from exposure to oxygen, moisture, and light (Cheng *et al.*, 2021). Comparative studies have shown that black garlic packaged in aluminum-PE bags retains a higher concentration of total phenolic and flavonoid contents compared to kraft paper or PET

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(Polyethylene Terephthalate) bottles, suggesting that the barrier properties of the packaging play a critical role in maintaining the stability of these compounds (Modi *et al*., 2021; Akan, 2022).

Despite the growing body of literature on black garlic, limited research has comprehensively evaluated the combined effects of storage temperature and packaging type on the retention of phenolic and flavonoid contents. A recent study conducted by Moreno-Ortega *et al.* (2020) indicated that the bioavailability of phenolic compounds in black garlic can be affected by storage conditions, which subsequently influences its health benefits. Furthermore, the bioactive content and stability are dependent on the garlic variety, processing method, and packaging environment (Sunanta *et al.*, 2023). Understanding these interactions is crucial for developing optimal storage strategies that preserve the functional properties of black garlic, ensuring its effectiveness as a functional food product.

In response to these challenges, this study aimed to investigate the effects of different storage temperatures and packaging materials on the stability of phenolic and flavonoid contents in black garlic over an extended period. The findings will provide essential insights into best practices for maintaining the bioactive profile of black garlic, supporting its use as a health-promoting food ingredient in the nutraceutical and food industries. Recent advances in sustainable packaging, such as biodegradable and paper-based options, are also explored as potential alternatives to conventional plastic-based materials, aligning with the global trend towards eco-friendly and sustainable food packaging solutions (Cheng *et al.*, 2021; Caner *et al.*, 2024). This will enable its broader application as a functional food product with enhanced health benefits, supported by a more sustainable approach to food packaging and preservation.

METHODS

The materials used in this study included single clove garlic sourced from local farmers in Lembang (West Java, Indonesia): 70% ethanol (technical grade): distilled water, 10% Folin-Ciocalteu reagent, 2% $AlCl_3$, 7.5% Na_2CO_3, gallic acid, and quercetin (Merck, Germany; Sigma-Aldrich, USA).

Black Garlic Preparation

The single clover garlic conversion into black garlic followed the combination procedure from Zhafira (2018) and Mardawati *et al.* (2024): with slight modifications. The single clove-garlic was first sorted based on size and suitability. The sorted garlic was then placed into a heating chamber NINIKU fermenter and heated at 74°C for 12 days. The resulting black garlic was peeled and separated from its skin, followed by another round of sorting based on marketability before being packaged.

Packaging and Storaging

The black garlic was packaged using two types of packaging consist of a paper core (PC) and aluminum foil (AF). The PC packaging has two layers, with the primary packaging being a clear single-use plastic bottle (75 mL) that comes into direct contact with the product. This bottle is placed inside the secondary packaging, which is a PC type that is tightly sealed with aluminum foil and a cap. Additionally, the AF packaging is made of metalized paper, measuring 10 × 12 cm with a thickness of 80 µm, and is equipped with a zipper or plastic clip for sealing. The storage conditions and duration was followed the study of Priadi *et al.* (2019) for the black garlic were set in an incubator, and adjusted to the specified temperatures of 30°C, 35°C, and 40°C that stored for 28 days and testing every 7 days (specifically on days 0, 7, 14, 21, and 28).

Extraction of Stored Black Garlic

The preparation of the maceration samples for black garlic was based on the procedure by Putranti et al. (2019): with slight modifications. Ten grams of black garlic were weighed, crushed, and placed into a glass bottle, then 75 mL of 70% ethanol (1:7.5 ratio) was added and tightly sealed. The maceration process was carried out for 24 hours at room temperature and then filtered using coarse filter paper. The obtained filtrate was subsequently evaporated using a rotary evaporator B-One RE-1000VN at 40°C with a rotation speed of 100 rpm for 30 minutes, or until a thick extract was achieved (Yudhayanti *et al.*, 2020).

Total Phenolic Content (TPC)

The procedure was followed by Nofita *et al.* (2020) with slight modification. A 1 mL sample of the black garlic extract was taken and diluted with distilled water to a final volume of 10 mL. Subsequently, 0.3 mL of this diluted sample was pipetted and combined with 1.5 mL of 10% Folin-Ciocalteu reagent, shaken, and allowed to stand for 3 minutes. Then, 1.2 mL of 7.5% Na_2CO_3 was added, and the mixture was kept in the dark at room temperature for 120 minutes. Finally, the absorbance was measured using a UV-Vis spectrophotometer DLAB® SP-UV1000 at a wavelength (λ) of 765 nm.

Total Flavonoid Content (TFC)

The procedure was followed by Dewantoro *et al.* (2022) with slight modifications. A 1 mL sample of the extract was taken and diluted with distilled water to a final volume of 10 mL. From this diluted solution, 0.5 mL was pipetted and combined with 0.5 mL of 10% $AlCl_3$. The mixture was allowed to stand at room temperature for 120 minutes, after which the absorbance was measured at a wavelength (λ) of 420 nm [21].

Data Analysis

The collected data was analyzed using analysis of variance (ANOVA) towards the differences on types of packaging, storage temperatures, and storage duration, then followed by the Duncan Multiple Range Test (DMRT) for variables with significant effects to the responses, which was included in IBM® SPPS Statistic 26.

RESULTS AND DISCUSSION

Initial Bioactive Compounds of Black Garlic

The total phenolic and flavonoid contents of black garlic were significantly higher than those of single-clove garlic, demonstrating the positive impact of the black garlic conversion process on the enrichment of these bioactive compounds, as shown in Figure 1. Specifically, the total phenolic content in black garlic reached 4.43±0.10 mg GAE/g, which is 55% higher compared to 2.86±0.14 mg GAE/g found in single clove garlic (Mardawati *et al.*, 2024). The total flavonoid content also exhibited a marked increase, reaching 2.50±0.26 mg QE/g in black garlic compared to 1.35±0.11 mg QE/g in single clove garlic. This enhancement can be attributed to the Maillard reaction, enzymatic ctivities, and non-enzymatic browning during the aging process (Ríos-Ríos *et al.*, 2019). According to Krisnawan *et al.* (2022): the thermal treatment and moisture reduction that occur during black garlic production lead to the formation of novel phenolic derivatives and increased flavonoid synthesis, thereby enriching the antioxidant profile of black garlic.

The increase in phenolic and flavonoid contents post-conversion also suggests an improvement in the functional properties of black garlic compared to its raw form. Phenolic compounds are known for their ability to scavenge free radicals and prevent oxidative damage, while flavonoids contribute to anti-inflammatory and anticarcinogenic activities (Kaurinovic & Vastag, 2019). This enrichment aligns with previous studies, that reported the fermentation and thermal processing of garlic lead to increased bioactive compound concentration, enhancing its overall health benefits (Najman *et al.*, 2020). Thus, the data from this study confirm that the aging process is not only a method for enhancing the flavor and texture of garlic but also an effective means of improving its nutraceutical properties.

Changes in TPC during Storage

Based on the results of the 4-week test, it was observed that the TPC in each packaging and at different temperatures showed a significant decrease compared to the initial TPC values. As shown in Figure 2, the TPC values at week 4 ranged from 2.60 to 3.50 mg GAE/g. This aligns with the findings that TPC tends to leach into the environment over time, leading to degradation, oxidation, and hydrolysis (Vy Do *et al.*, 2024). However, in weeks 3 and 4, the TPC stored at 40°C in both PC and AF packaging showed an increase, which may be attributed to the hydrolysis of compounds that release sugars, particularly glucose and gallic acid. These compounds, when reacting with the Folin-Ciocalteu reagent, could enhance phenolic content (Kim *et al.*, 2007; Castro-López *et al.*, 2016).

According to Sharma *et al.* (2018): TPC values are influenced by storage temperature, where higher storage temperatures tend to cause a decrease in TPC. In this study, TPC values were not significantly affected by different storage temperatures (p = 0.30). The storage at moderate temperatures (30-45°C) specifically promotes bioactive

Figure 1. This study contains black garlic conversion, storage stages, and initial total polyphenols content.

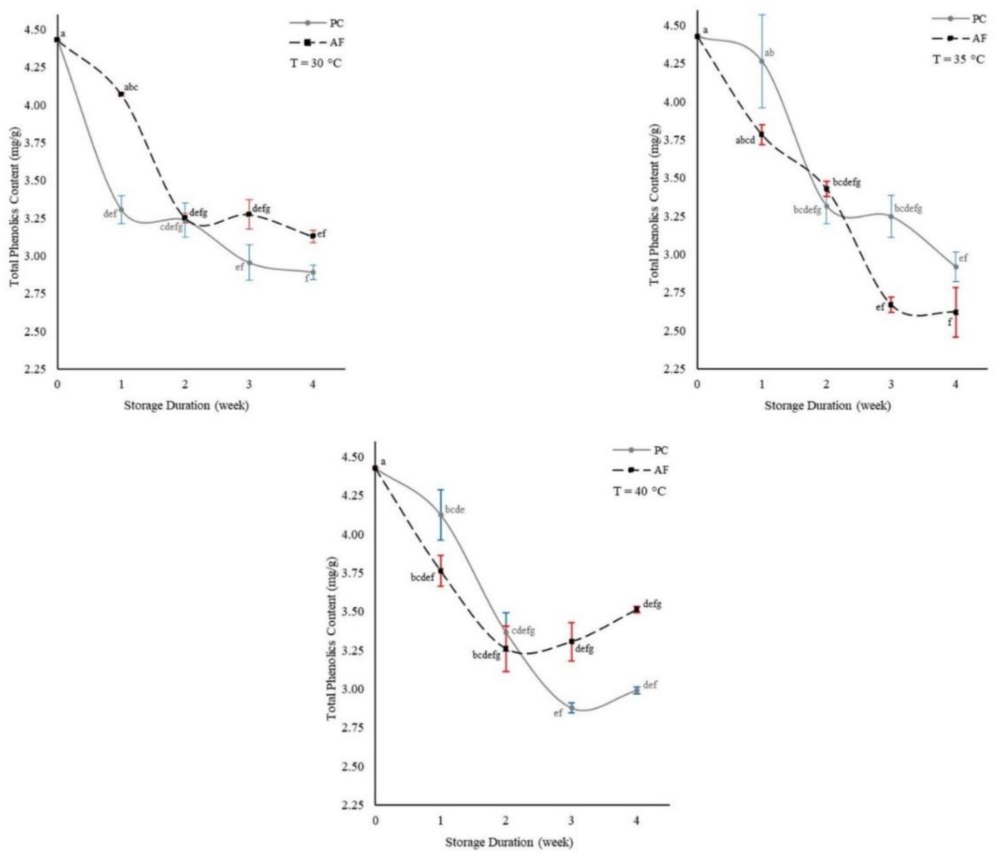

Figure 2. The trends of TPC on black garlic during storage at different temperature and packaging materials. Different letters indicate signifficant differences as determinde by the Duncan Multiple Range Test ($p < 0.05$).

compounds to undergo decomposition, hydrolysis, and oxidation reactions, resulting in the increased breakdown of heat-sensitive macromolecules and enhancing antioxidant capacity, particularly TPC (Vy Do *et al.*, 2024).

The use of different packaging materials, namely PC and AF, in this study did not significantly affect the stability of TPC values ($p = 0.58$). The results indicate that samples stored in AF packaging were generally more stable in retaining phenolic compounds than in PC packaging. However, at 35°C, PC packaging showed better retention of phenolic content than AF packaging. The decrease in TPC values for different packaging types can be attributed to the influence of light. Light exposure can degrade the quality of antioxidant compounds, particularly phenols, as the primary component of light—UV (ultraviolet)—possesses oxidative properties that react with oxygen and also trigger the formation of ROS (Reactive Oxygen Species): thereby damaging the existing phenolic compounds (Mahardani & Yuanita, 2021).

Changes in TFC during Storage

Figure 3 showed that the TFC in black garlic was lower than that of than TPC. The TFC trends indicate significant changes over the 4-week storage period. During the first week, an increase in TFC values was observed for PC and AF packaging at 35°C and for PC packaging at 40°C. TFC values were significantly influenced by the type of packaging used (p = 0.02). This finding is consistent with Giuffrè *et al.* (2019): which revealed that TFC is affected by packaging methods or interactions between film and storage days or months, but is strongly influenced by the storage period.

The TFC value at week 4 for PC packaging at 40°C was higher than the initial TFC value. Similar to the increase in TPC, the rise in TFC could also be attributed to the release of bioactive compounds under these conditions and temperatures (Adebo & Medina-Meza, 2020). On the other hand, the decrease in TFC may result from the breakdown or degradation of certain flavonoid compounds, leading to molecular chain scission and oxidation reactions at the hydroxyl groups, which form other volatile compounds (Sharma *et al.*, 2014).

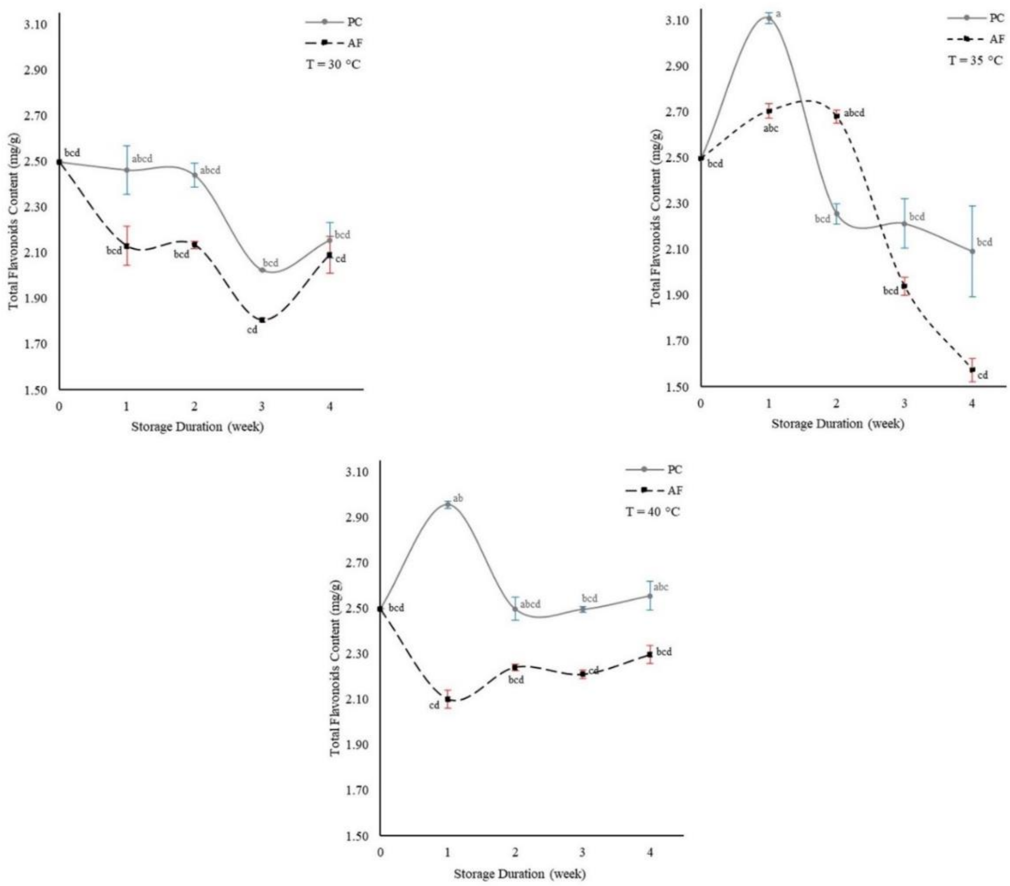

Figure 3. The trends of TFC on black garlic during storage at different temperature and packaging materials. Different letters indicate signifficant differences as determinde by the Duncan Multiple Range Test (p < 0.05).

Table 1. Comparison between this study with relevant previous results

Packaging	Temperature	Results	References
Kraft paper bag	4°C	The total phenolic content decreased by only 5% after 3 weeks of storage, from 19.26±0.48 mg/g.	Akan (2022)
PET jar	30–40°C	The total phenolic content decreased by 15% after 7 weeks of storage, from 0.20 mg/g. Additionally, a storage temperature of 35°C was the most effective in reducing the phenolic content in black garlic compared to temperatures of 30°C and 40°C.	Priadi *et al.* (2019)
PET bottle, kraft paper bag, and aluminium-PE bag	4–20°C	The use of aluminum-PE bag packaging better preserved the total phenolic and flavonoid contents compared to other packaging types, with reductions of 60% and 57% from the initial contents of 2.25 mg/g and 1.64 mg/g, respectively.	Ding *et al.* (2021)
PET jar and aluminium pouch	10–30°C	This study did not evaluate the total content of bioactive compounds in black garlic. However, it recommends the use of PET jars and storage at 10°C for optimal preservation of black garlic.	Sailah *et al.* (2024)
Paper core and aluminium foil pack	30–40°C	Different packaging types showed a significant effect on maintaining the total phenolic and flavonoid contents in black garlic. However, there was no significant effect from variations in storage temperature and duration. Based on the results, it is recommended to store black garlic using paper core packaging at 35°C, as this condition resulted in a reduction of only 34% in total phenolic content and 16% in total flavonoid content.	This study

Comparison Results with Relevant Previous Studies

The results presented in Table 1 demonstrate the complex interactions between packaging type, storage temperature, and the stability of phenolic and flavonoid compounds in black garlic. According to Priadi *et al.* (2019): storage at higher temperatures, such as 35°C, significantly decreased the total phenolic content of black garlic stored in a PET jar by approximately 15% after 7 weeks. This trend underscores the susceptibility of phenolic compounds to thermal degradation, which is in line with the findings of phenolics are particularly vulnerable to oxidation and polymerization at elevated temperatures, leading to a decline in their concentration (Albuquerque *et al.*, 2020). The current study supports this observation, as reductions in phenolic and flavonoid contents were also observed when black garlic was stored at 35°C, although the impact varied depending on the packaging type.

The effectiveness of different packaging materials in preserving phenolic and flavonoid contents is evident (Ding *et al.*, 2021). Their study showed that using an aluminum-PE bag resulted in a 60% reduction in total phenolic content and a 57% reduction in total flavonoid content after storage, compared to kraft paper bags and PET bottles. Puligundla & Mok (2018) stated the ability of the aluminum-PE bag to limit exposure to oxygen and light helps mitigate oxidative degradation of these compounds. This suggests that proper packaging selection can be more critical than temperature control alone in preserving the bioactive compound profile of black garlic during storage. Therefore, the results of this study reinforce the necessity for industry practitioners to choose packaging materials with superior barrier properties when aiming to retain the nutritional quality of black garlic.

In contrast, Akan (2022) reported a kraft paper bag at a low temperature of 4°C reported could maintained the total phenolic content with only a 5% reduction over 3 weeks of storage]. This result suggests that low-temperature storage can effectively suppress the degradation of bioactive compounds, even when using less protective packaging materials. However, for practical applications, using low temperatures may not always be feasible due to higher energy costs and increased environmental impacts associated with refrigeration (Du *et al.*, 2018). The findings of the present study suggest that using paper core packaging at a moderate temperature of 35°C is a more sustainable option, achieving only a 34% reduction in total phenolic content and a 16% reduction in total flavonoid content. This indicates that while refrigeration can provide the best preservation of phenolic and flavonoid contents, careful selection of packaging materials, such as paper core, can achieve comparable results without the need for extensive temperature control.

Moreover, Sailah *et al.* (2024) did not evaluate the total bioactive compound content directly but recommended PET jars and storage at 10°C for optimal preservation. These recommendations align with the findings of this study, which indicate that PET jars provide good protection against moisture and oxidation. However, in terms of practicality and sustainability, PET materials may not be ideal due to their environmental impact (Hahladakis *et al.*, 2017). The results of this study show that PC packaging is more effective in preserving the quality of black garlic compared to other options, while also being a more eco-friendly choice.

The findings of this study provide strong evidence that the type of packaging used plays a more critical role in maintaining the phenolic and flavonoid contents of black garlic than temperature control alone. Although higher storage temperatures can lead to significant losses in these compounds, selecting the appropriate packaging material, such as paper core, can mitigate these losses and preserve the functional properties of black garlic over extended periods. This study's outcomes are valuable for developing storage and packaging strategies in the food industry to enhance the shelf life and quality of functional foods like black garlic.

CONCLUSION

This study investigated the effects of different storage temperatures and packaging materials on the stability of phenolic and flavonoid contents in black garlic. The results indicate that higher storage temperatures (35°C and 40°C) led to significant reductions in these bioactive compounds, whereas moderate temperatures (30°C) were more effective in preserving their stability. Additionally, aluminum foil packaging showed better retention of phenolic and flavonoid contents compared to paper core packaging due to its superior barrier properties. These findings suggest that selecting appropriate storage conditions and packaging is essential for maintaining the functional properties of black garlic. Although refrigeration provides optimal preservation, using sustainable packaging options like paper core at moderate temperatures offers a balanced approach to quality retention and environmental impact. This research contributes to the development of storage strategies that optimize the shelf life and health benefits of black garlic, supporting its application as a high-value functional food product in the nutraceutical and food industries. Future studies should explore additional factors such as humidity control and alternative packaging materials to further enhance storage outcomes.

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