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Application of Black Garlic-Based Bioconservants as Natural Preservatives for Chicken Meat Through Antibacterial Studies

Ellen Stephanie Rumaseuw[1*], Monica Saptiningsih[2], Carissa Wityadarda[3]

[1] Diploma Three Program in Pharmacy, Santo Borromeus University, Padalarang, Indonesia.
[2] Diploma Three Program In Nursing, Santo Borromeus University, Padalarang, Indonesia.
[3] Bachelor Degree In Nutrition Science, Santo Borromeus University, Padalarang, Indonesia.

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Corresponding Author:
Ellen Stephanie Rumaseuw
ellen@ustb.ac.id

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Abstract: Black Garlic has great potential as a natural bioconservant, because of anti-microbial activity, anti-inflammation, anti-allergic, anti- diabetic, and anti-cancer. However, research on its application as a bioconservant for chicken meat, particularly against multi-drug-resistant pathogens (*E. coli, Salmonella* sp., and *Staphylococcus aureus*) remains limited. The potential benefits of combining black garlic application with cold storage are also underexplored. This study investigates the potential of Black Garlic (BG) extract as a natural bioconservant to preserves broiler chicken meat by inhibiting bacterial growth and maintaining sensory quality. A Randomized design was used with six treatments applied: control, sodium benzoate (0.1%), and BG extract at 5% ,10%, 15%, and 20%. The samples were marinated for 30 minutes, wrapped, and stored at ± 10°C for seven days. Microbiological analysis showed that the 20% BG treatment effectively suppressed *Salmonella* to undetectable levels and significantly reduce *E. Coli* and *Staphylococcus aureus*, comparable to sodium benzoate. Lower concentrations were less effective, particularly against S. aureus. The measurements of pH indicated stabilization around 5.00-5.5 in BG-treated samples, with the 20% concentration exhibiting the strongest acidifying effect. Sensory evaluation by 26 panellists indicated that 15% BG improved the freshness of aroma but negatively affected color and texture at higher concentrations. Moisture content slightly increased in BG treatments, suggesting improved water retention. These findings demonstrate that black garlic extract, especially 15%-20%, can serve as a natural preservative for chicken. It is offer antibacterial protection while maintaining acceptable of sensory attributes. Moreover, the result highlights the potential of BG as a safer alternative to synthetic preservatives in the poultry industry.

Keywords: Antibacterial; Bioconservant; Black Garlic; Chicken meat; Natural preservative

Introduction

Garlic (*Allium sativum* L.), a member of Alliaceae, is widely recognized as a medical plant with beneficial biological activities not only used as a cooking spice and condiment but also valued for anti-bacterial, anti-fungal, anti-oxidant, and immunoprotective properties (Bhatwalkar et al., 2021; Ramadani et al., 2021). This plant originated from Central Asia and spread throughout the world through cultural exchange and colonialism (Kassaw et al., 2021). The Distinctive aroma of garlic is caused by sulfur compounds such as *Allicin, Aliin, Dyallul Trisulfide*, and *S-Allyl Cystein* (Li et al., 2022; Wiczkowski, 2018). These compounds have various activities, including anti-inflammation, anti-apoptotic, and metabolic syndrome risk reducing effects (Al-Khayri et al., 2022; Fu et al., 2023).

However, people tend to avoid consumming raw garlic due to its strong odor and pungent tast (Juniantari & Susanti, 2023). As a solution, Garlic can undergo thermal fermentation to reduce its odor. The process is conducted at 60-90°C for 21-72 days with 80-90% humidity (Augustyńska-Prejsnar et al., 2024; Dampati et al., 2020). This Process triggers Maillard reaction, producing a new product, known as a black garlic (Javed

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et al., 2022; Yuan et al., 2018). This process alters the physical and chemical characteristics of garlic: the color turns black, the texture becomes soft and elastic, and the taste becomes sweet due to the formation of monosaccaharides such as glucose and fructose (Augustyńska-Prejsnar, Kačániová, Ormian, et al., 2024a). The strong odor decreases because of the Allicin is converted into water- soluble antioxidant such as S-Allyl Cystein (SAC), and S-Allyl-mervaptocystein (Juniantari & Susanti, 2023).

Black Garlic has great potential as a natural bioconservant, because of anti-microbial activity, anti-inflammation, anti-allergic, anti- diabetic, and anti-cancer (Orefice et al., 2022; Rumaseuw et al., 2024). The Use of black garlic can inhibit growth of microorganisms that caused of food spoilage such as putrefactive bacterial (Amir et al., 2022).The bioactive components forming during fermentation serve as the basis for food preservation especially for animal proteins such as broiler chicken meat which are easily spoiled (Ramadani et al., 2021). Broiler chicken meat is a strategic commodity in Indonesia's livestock sector. Chicken consumption per capital reached 7.46 kg/year with household demand reaching 2,08 million tons in 2023 (Badan Pangan Nasional, 2024). In addition, chicken meat accounts for more than 60% of total national chicken meat consumption due to its affordability and high acceptance among community. Based on nutritional data, broiler chicken meat contains 70.91% water content, protein ranging from 31-35 g/100g depending on the body part, and low-fat content (Afiyah, 2022).

However, the high water and protein content of chicken meat makes it highly perishable both of microbiologically and sensorially, especially when stored at room temperature. Observable changes include pale color, slimy texture and pungent odor. Contamination by microorganism such as; Pseudomonas, Micrococcus, Bacillus, and Clostridium can occur in less than 6 hours after slaughtering, causing accelerated spoilage (Pasya, 2025). Synthetic preservatives such as nitrites and benzoates are commonly used to slow spoilage; however, their use has been associated with potential health risks, including carcinogenicity (Devitria et al., 2023). This has led to increased interest in safe, natural preservation methods that can extend shelf life without compromising quality. Black garlic, with its rich bioactive profile, represents a promising natural alternative candidate as bioconservant.

Previous studies have mainly focused on black laboratory-scale characterization of black garlic. Its antioxidant activity, and limited in vivo applications (Oktari et al., 2020; Rumaseuw et al., 2022a; Saputra et al., 2023). However, research on its application as a bioconservant for chicken meat, particularly against multi-drug-resistant pathogens (*E. coli*, *Salmonella* sp., and *Staphylococcus aureus*) remains limited. The potential benefits of combining black garlic application with cold storage are also underexplored. Therefore, this study aims to evaluate the effectiveness of ethanol extracts of black garlic on broiler chicken meat to asses their sensory effects, and identify potential application as bacterial growth inhibitor, and explore potential application methods for industrial-scale perservation.

Method

Materials and Equipment

The main materials were six fresh chicken breast fillets of varying weights. Black garlic, obtained from fermented garlic (Allium sativum L.), was ground into a fine paste and diluted with distilled water at a 1:1 ratio equivalent to the meat weight. Sodium benzoate, acetic acid (CH_3COOH), and aquades were used as additional treatment materials. The equipment included an analytical balance, mortar and pestle, sterile containers, aluminium foil, plastic wrap, pH meter, rice cooker, rotary evaporator, incubator, autoclave, macerator, and refrigerator.

Experimental Design

A completely randomized design (CRD) was applied with duplo for each six treatments:

- A1 (Control): chicken breast (113 g) without treatment.
- A2 (Sodium benzoate 0.1%): chicken breast (119.93 g) marinated with 0.13 g sodium benzoate, 5 mL acetic acid (CH_3COOH), and 20 mL aquades.
- A3 (Black garlic 5%): chicken breast (125.92 g) marinated with 6.62 g black garlic paste diluted in aquades (1:1, w/v relative to meat weight).
- A4 (Black garlic 10%): chicken breast (134.36 g) marinated with 14.93 g black garlic (same procedure as A3).
- A5 (Black garlic 15%): chicken breast (178.59 g) marinated with 26.79 g black garlic (same procedure as A3).
- A6 (Black garlic 20%): chicken breast (155.22 g) marinated with 38.81 g black garlic (same procedure as A3).

Procedures

1. Sample preparation: chicken breast fillets were weighed, recorded, and placed in sterile containers.
2. Control: stored directly in a sterile container without treatment.
3. Sodium benzoate treatment: chicken breast was marinated for 30 minutes with sodium benzoate (0.1%), acetic acid (CH_3COOH), and aquades. After marination, the liquid was discarded, and the meat

was wrapped in aluminium foil and sealed with plastic wrap.

4. Black garlic treatments: black garlic was peeled, weighed according to the designated concentration, ground into a fine paste with mortar and pestle, then diluted with aquades (1:1 relative to meat weight) to obtain an aqueous black garlic homogenate. The homogenate was applied by marination for 30 minutes, after which the liquid was discarded and the meat was wrapped in aluminium foil and plastic wrap.

Storage

All samples, except the untreated control, were wrapped in aluminium foil and sealed with plastic wrap, then stored in a chiller at ±10 °C for 7 days. pH was measured daily, and samples were taken on day 0 and day 7 for microbiological analysis.

Microbiological Analysis

Total Plate Count (TPC): conducted on day 0 and day 7 to quantify the total microbial load of each treatment. Antibacterial activity: evaluated using the disc diffusion method against *Escherichia coli, Salmonella sp., and Staphylococcus aureus*. Inhibition zones were measured in millimeters and compared to a negative control (untreated sample) and a positive control (sodium benzoate).

Sensory Analysis

A sensory evaluation was performed on day 7 by 26 untrained panellists (students, and staff at Universitas Santo Borromeus). A 4-point hedonic scale was used to assess aroma, color, and texture:

Table 1. Hedonic Scale

Aspect	Scoring Description
Aroma	1 = putrid/fishy
	2 = slightly fishy
	3 = moderately pleasant
	4 = fresh and characteristic
Color	1= unattractive/pale
	2 = uneven
	3 = uniform
	4 = attractive and natural
Texture	1 = slimy/soft
	2 = slightly chewy
	3 = good chewiness
	4 = firm and elastic

Statistical Analysis

Data were tested for normality (*Kolmogorov-Smirnov/Shapiro-Wilk*) and homogeneity (*Levene's test*). Differences among treatments were analyzed using *Kruskal-Wallis* followed by *Mann-Whitney* for non-parametric data. Statistical significance was set at $p < 0.05$.

Result and Discussion

Microbiological Analysis

The microbiological analysis was performed to evaluate the effect of black garlic and sodium benzoate on the growth of *Salmonella, E.Coli, Staphylococcus aureus* in chicken meat. The results are presented in Table 2.

Table 2. Total Bacterial count (CFU/mL) of chicken meat treated with black garlic and sodium benzoate

Treatment	*Salmonella* (CFU/mL)	*E.coli* (CFU/mL)	*S.aureus* (CFU/mL)
Control	7.10×10^4	1.00×10^4	1.79×10^6
Na-benzoat	0	1.00×10^1	0
BG 5%	1.05×10^3	6.50×10^2	9.20×10^5
BG 10%	9.50×10^2	2.50×10^2	7.10×10^5
BG 15%	2.50×10^2	1.50×10^2	6.25×10^4
BG 20%	0	1.00×10^1	3.50×10^4

Value are mean counts from duplicate analysis.

As shown in Table 2, the untreated control showed the highest bacterial counts, especially for S. aureus (1.79×10^6 CFU/mL), indicating rapid spoilage of chicken meat without preservative treatment, in contrast, sodium benzoate completely inhibited Salmonella and S. Aureus, and reduced E. Coli to 1.00×10^1 CFU/mL. Black Garlic extract exhibited effects, with higher concentration (15% and 20%). Resulting in a grater reduction of bacterial load. Notably, the 20% treatment eliminated *Salmonella* and strongly suppressed *E. Coli and S. Aureus*. However, at 5% level, *S. aureus* remained relatively high (9.20×10^5 CFU/mL), suggesting that lower concentrations were less affective.

Moisture Content

The moisture content of chicken breast samples treated with black garlic and sodium benzoate is shown in Table 3. The moisture of untreated chicken breast (control) was 74.37%. Samples treated with black garlic extract showed slightly higher values, particularly at the 15%concetration (76.29%). The sodium benzoate treatment also resulted in an increased moisture content (75.21%) compared to the control.

Table 3. Moisture content (%) of chicken meat after treatment with black garlic and sodium benzoate

Treatment	Moisture Content (%)
Control	74.37
Na-benzoate	75.21
BG 5%	74.98
BG 10%	74.89
BG 15%	76.29
BG 20%	74.45

Overall, the treatments didn't cause drastic changes in water content, but the results suggest that black garlic extract, especially at 15%, may help retain water in chicken meat during storage. This effect could be associated with the bioactive compounds in black garlic, which potentially enhance water holding capacity by interacting with muscle proteins.

pH Value

The changes in pH value of chicken breast samples during 7 days of storage are presented in Table 4. During storage, the pH of chicken meat remained relatively stable across treatments, with slight fluctuations between 4.5 and 6.0. The control group maintained a constant pH of around 6.0, while samples treated with black garlic extract and sodium benzoate showed a tendency to stabilize at pH 5.0 after Day 2. Notably, the 20% black garlic treatment exhibited the lowest pH values (as low as 4.5), suggesting a stronger acidifying effect compared to other treatments. This trend indicates that black garlic extract may contribute to lowering the pH of meat, which can potentially inhibit microbial growth and prolong shelf life. The result from Kruskal-Wallis method, there are significantly different between pH of chicken that have been treated ($p<0,05$). Median of control still high (6,0). But the chicken that treated by black garlic 20% shown decline pH (5,0) . The decline of pH indicated that increases of black garlic concentration will give contribution about characteristic of meat acidification, that can help hold putrefactive bacterial growth.

Table 4. pH value of chicken meat treated with black garlic extract and sodium benzoate during storage

Treatment	Day 0 (Initial)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	6.0	6.0	6.0	5.5	6.0	6.0	6.0	6
Na-benzoate	6.0	5.0	5.0	5.0	5.0	5.0	5.0	5
BG 5%	6.0	5.5	5.0	5.5	5.0	5.0	5.5	5,5
BG 10%	6.0	5.5	5.5	5.5	5.5	5.0	5.0	5
BG 15%	6.0	5.0	5.5	5.0	5.0	5.0	5.0	5
BG 20%	6.0	4.5	5.0	4.5	5.0	5.0	5.0	5

Sensory Evaluation

The sensory evaluation result obtained from 26 untrained panellist are shown in figure 1-3. Panellist assessed aroma, color, and texture of chicken meat using a 4-point hedonic scale. The average of aroma on the table (Figure 1) was 2.5.

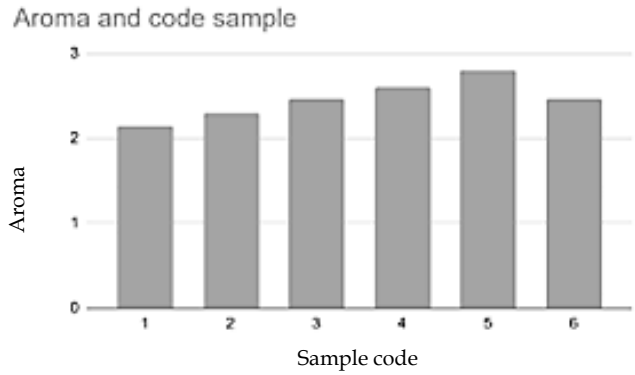

Figure 1. Mean sensory scores for aroma of chicken meat treated with black garlic and sodium benzoate after 7 days of storage (n=26 panellists, 4-poin hedonic scale)

The control meat was fishy and average panellist said that chicken as a control was fishy, the average of chicken that the panellist felt was 2.1. The freshest chicken that panellist felt was the chicken number 5 with a concentration of 15% black garlic connected with chickens given a concentration of black garlic of 10%. And level of fresh aroma of 5% and 20% concentrate is same. Kruskal-Wallis analytics shown the different between aroma aspect ($p<0.05$). Treated chicken with black garlic 10-15% show the highest median score (3). Compared to sodium benzoate and controlled chicken (2). This case shown that the used of black garlic in medium concentration will increase the fresh aromatic of chicken, even though, the high concentration (20%) the score was decreased again.

Color

The sensory evaluation results (Figure 2) show that the control sample obtained the highest score for color, while the samples treated with black garlic tended to receive lower scores as the concentration increased. This suggest that the addition of black garlic influenced the visual appearance of chicken meat, leading to a darker and less uniform color compared to control and sodium benzoate-treated samples. The Kruskal Wallis test demonstrated that the addition of black garlic extract had a significant effect on the color of chicken meat. Result of sensory test shown that black garlic with medium concentration (10-15%) gave a best balance between aroma, and texture, even the color be darker. This case show that out of color attribute, aroma and texture combination paid more for determinant panellist assessment about the chicken meat that be persevere by black garlic.

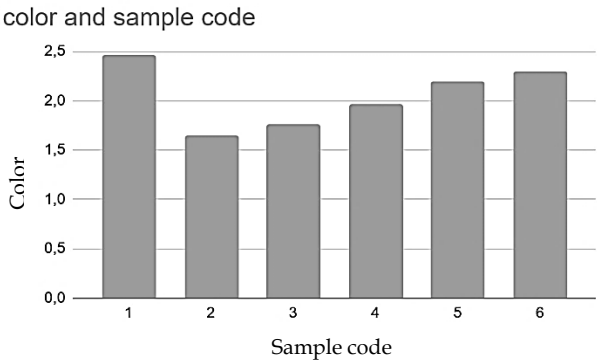

Figure 2. Mean color scores for measure color level of chicken meat treated with black garlic and sodium benzoate after 7 days of storage (n=26 panellists, 4-poin hedonic scale)

Texture

The texture given by sodium showing that the texture of chicken is more elastic than the others and the texture of chicken with a concentration of 15% has a slimy and soft texture, on average, panellist gave 2-4 points to chickens given sodium benzoate, and 1-2 for chicken with 10% concentration of black garlic. The evaluation of texture gave the different significally between any treated ($p<0,05$). The Kruskal–Wallis test results showed that the treatment with black garlic extract had a significant effect on the texture of chicken meat. Sodium benzoate produces more elastic texture and got the higher score than treated by black garlic. In this high concentration (15-20%), black garlic lowering the score of texture, black garlic makes the meat softer and less chewy.

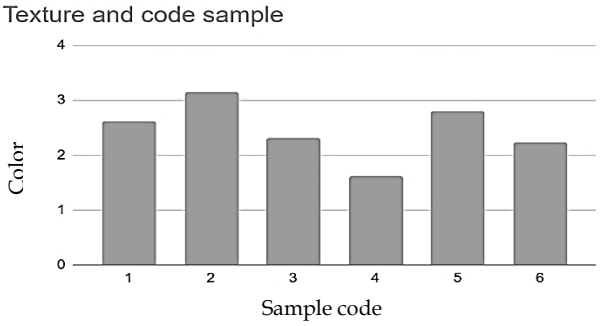

Figure 3. Mean texture scores for fingering of chicken meat treated with black garlic and sodium benzoate after 7 days of storage (n=26 panellists, 4-poin hedonic scale)

Discussion

Black garlic containing phenolic and antioxidant activity that can help slow down the decay prosses, it will maintain the texture of broiler chicken meat during storage (Qiu et al., 2020). The black garlic was cooked at ±70°C for 21 days with maceration method (Rumaseuw et al., 2022b). In Indonesia, the recommended pH is <6,05 (Ristanti *et al.,* 2017). We found that pH of chicken that we tested had a pH below 6.05, but after organoleptic testing, there were several things that needed to be considered besides pH resistance. Previous research, chicken saved in cold temperature storage chicken can be endure till seventh day (Addo et al., 2018). And if the chicken packed by polyethylene and nylon saved on ±4° C, it won't go stale until the eighth day (Silvia et al., 2022). With BG we found that all pH below 6,05. BG can press the bacterial (Proutière et al., 2021). We found it in our research that BG with 20% concentrate treatment can eliminated *Salmonella* sp. and strongly suppressed *E. coli and S. aureus*.

BG containing alkanoids flavonoids tannins and saponins which function as antibacterial the bacterial compounds inhibit the proteolytic enzymes from spoilage microorganism (Yu et al., 2023). Marinating with black garlic will add these antibacterial compounds. Similar mechanism, BG contains SAC and any sulphur that compounds antimicrobial activity (Juniantari & Susanti, 2023). The used of BG is affected into color parameters, lightness (L*), redness (a*), and yellowness (b*), (Augustyńska-Prejsnar et al., 2024). The color may not appear by the naked eye must use colorimeter. The BG effect would give darker product and the concentration would be increased (Soltani et al., 2021). These changes are attributed to the pigments formed during the maillard reaction and the phenolic compounds that adding black garlic powder to spent duck meat nuggets darkened the product's color but remained acceptable to panellists at lower concentrations (Augustyńska-Prejsnar et al., 2024) . It is written that more bacteria in chicken, would made the chicken inelastic and soft. This is caused by rotting by bacteria. The more bacteria there are, the less elastic the meat will be (Alhuur et al., 2020).

Conclusion

The BG can supress the potential presence of bacteria such as Salmonella, E.Coli, S. Aureus. In the marination experiment, a 20% percentage of black garlic can supress salmonella until zero for a period of approximately 8 days until the organoleptic test process. In this case, compare by sodium benzoate BG significantly reducing the bacterial. BG can be an option to protect chicken meat from bacteria that damage chicken protein. More than it, BG maintained pH stability (5-5,5) and slightly improved the water retention. Sensory evaluating revealed 15% of BG yielded the best balance of freshness and acceptability, the higher concentrations tended the darker color and soften texture. 15-20% of BG effectively preserve chicken meat. This presenting a promising and safer alternative to synthetic preservatives in the poultry industry.

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Author Contributions
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Conflicts of Interest
The authors declare that no conflict of interest. The funders had no role in the design of the study, in collection, analyses, or interpretation of data, writing of the manuscript, or in the decision to publish this result.

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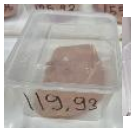
APENDIX

Table 5. Chicken margination picture table for 7 days

No	Weight of chicken we used (g)	Additional types of preservatives and percentage (%)	Mass of preservatives	Without any threats (0)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1.	113,52	-	-								
2.	119,93	$C_7H_5NaO_2$ (0,1%)	0,11mg								
3.	125,92	Black Garlic (5%)	6,62g								
4	134,36	Black Garlic (10%)	14,93g								

No	Weight of chicken we used (g)	Additional types of preservatives and percentage (%)	Mass of preservatives	Without any threats (0)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
5	151,77	Black Garlic (15%)	26,79g								
6	155,22	Black Garlic (20%)	38,81g