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Optimization of Bay Leaf and Garlic Extract Concentrations as Natural Preservatives in Tofu: Exploring a Novel Approach in Food Preservation

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Abstract
Introduction: *The use of synthetic preservatives such as formalin in food products, especially tofu, has raised serious health concerns, including the risk of cancer.* ***Objective:*** *As a solution, this study aims to evaluate the potential combination of bay leaf extract (Syzygium polyanthum) and garlic (Allium sativum) as a natural preservative to replace synthetic preservatives in tofu.* ***Method:*** *The study was conducted using a completely randomized block design (RCBD) with variations in extract concentration and tofu storage duration, as well as testing of physicochemical, microbiological, and sensory characteristics.* ***Results and Discussion:*** *The results showed that bay leaf extract produced a dark green color with a strong aroma, while garlic extract maintained a white color with a distinctive aroma without changes in texture. The combination of both extracts gave a complex aroma but showed black spots and mold growth on the 6th day of storage. Microbiologically, the combination of bay leaf and garlic extracts effectively reduced the number of Escherichia coli to below the detection limit, but slightly increased the total coliform count.* ***Conclusion:*** *The conclusion of the results is there the combination of bay leaf and garlic extracts can improve the sensory quality of tofu and reduce pathogenic bacteria, but further management is needed to ensure the microbiological safety of the product.*

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Introduction

The contemporary food industry is under increasing pressure to reduce the use of synthetic preservatives due to their potential adverse effects on human health (Hasrianti, Nururrahmah, & Nurasia, 2017). A notable example is the use of formaldehyde, which is frequently employed as a preservative in food products, including tofu (Berliana et al., 2021). The incorporation of formaldehyde in food has raised serious health concerns, as it has been linked to gastrointestinal irritation, internal organ damage, and even carcinogenesis (Sapoetri, Revina, & Muti, 2022). Consequently, there is a growing effort to identify safe and effective natural alternatives to synthetic preservatives. Previous studies have demonstrated the potential of certain natural substances as food preservatives. For instance, bay leaf extract (Syzygium polyanthum) and garlic extract (Allium sativum) are known to possess antimicrobial and antioxidant properties that help maintain the freshness of food products (Yanestria, Rahayu, Uru, & Chandra, 2020)

Bay leaf extract exhibits significant antimicrobial activity against various pathogenic microorganisms commonly associated with food spoilage. It contains essential oils that reduce odor and flavonoid compounds that contribute to preservation (Hyldgaard, Mygind, & Meyer, 2012). These compounds interfere with bacterial metabolism, leading to cell death by damaging the permeability of bacterial cell walls, lysosomes, and microsomes, thereby exerting toxic effects on the cells.

Similarly, garlic extract demonstrates antibacterial properties capable of inhibiting the proliferation of pathogenic bacteria such as Salmonella Typhimurium (De Greef et al., 2021). The presence of allicin in garlic extract is known to suppress the growth of spoilage-causing microbes by preventing the synthesis of microbial DNA and proteins, thus preserving the sensory quality of food products (Nadeem, Kazmi, Ullah, Muhammad, & Anwar, 2021); (Borlinghaus et al., 2021)

Despite existing research on the individual potential of bay leaf and garlic extracts as natural preservatives, studies investigating their combined application in tofu preservation remain limited. Prior investigations have predominantly focused on single-ingredient preservatives without exploring the synergistic effects of combining natural agents.

Therefore, the present study aims to evaluate the efficacy of a combination of bay leaf and garlic extracts as a natural preservative for tofu. The research seeks to answer the following questions: How effective is the combination of bay leaf and garlic extracts in maintaining tofu freshness and quality? Can this combination inhibit the growth of pathogenic microorganisms? Additionally, how do variations in extract concentration and storage duration affect the preservation efficacy in terms of physicochemical properties, microbiological safety, and sensory characteristics?. This study is expected to contribute to the development of safe, effective, and sustainable food preservation strategies.

Method

This study was conducted to determine the optimal concentration for the use of natural preservatives derived from bay leaves and garlic. The concentrations tested were 50 g/L, 100 g/L, 150 g/L, 200 g/L, and 250 g/L. Bay leaves (Syzygium polyanthum) and garlic were first sorted and then oven-dried until completely desiccated. The dried materials were then ground using a blender until a fine powder was obtained, followed by sieving to produce uniform powdered bay leaf and garlic.

Each powdered sample was weighed to 150 g. The bay leaf and garlic powders (150 g each) were subjected to maceration using 70% ethanol for 24 hours with continuous stirring. The resulting filtrate was filtered using filter paper to obtain a pure extract solution, which was then concentrated using a rotary vacuum evaporator to yield a viscous extract. The liquid extract obtained from this process was then used to soak tofu that had been pretreated by blanching at 100°C for 5 minutes, followed by draining. The blanched tofu was immersed in extract solutions of varying concentrations (50 g/L, 100 g/L, 150 g/L, 200 g/L, and 250 g/L) for 120 minutes. After immersion, the tofu samples were drained and placed in plastic containers for storage at ambient temperature from day 0 to day 3. Daily observations were conducted to assess the tofu's organoleptic properties, including color, aroma, and appearance.

Results and Discussion

Research Results Day 0, Day 1, Day 2 and Day 3

Table 1
Results of day 0 research

Treatment	Concentration (g/L)	Parameter		
		Color	Aroma	Texture
Control		White	Characteristic tofu aroma	Intact and soft
Bay Leaf + Garlic Extract	50 gr	White with a slight hue of bay leaf	Characteristic tofu aroma	Intact and soft
	100 gr	White with a slight hue of bay leaf	Characteristic tofu aroma	Intact and soft
	150 gr	White with a slight hue of bay leaf	Characteristic tofu aroma	Intact and soft
	200 gr	White with a slight hue of bay leaf	Mild garlic and bay leaf aroma	Intact and soft
	250 gr	White with a slight hue of bay leaf	Noticeable garlic and bay leaf aroma	Intact and soft

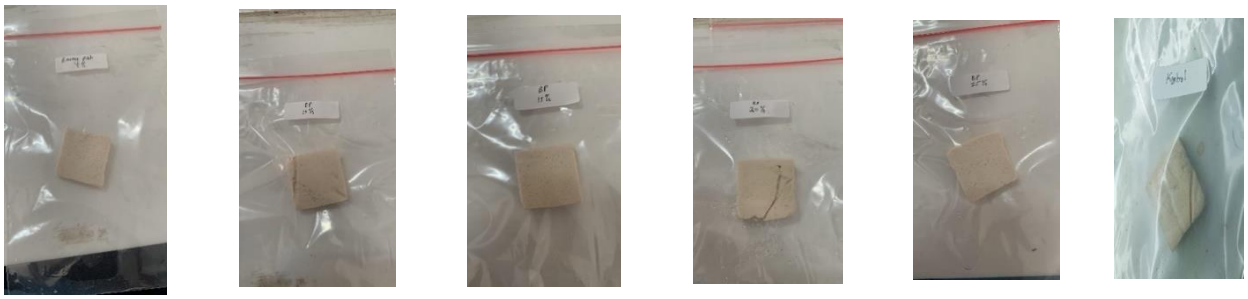
Based on the data presented in Table 1, the effects of the combined application of bay leaf and garlic extracts on tofu can be evaluated through three primary parameters: color, aroma, and texture. In the control group (untreated tofu), the tofu exhibited a yellowish-white color, a characteristic tofu aroma, and a soft texture.

Following treatment with varying concentrations of the bay leaf and garlic extract mixture (50 g/L, 100 g/L, 150 g/L, 200 g/L, and 250 g/L), the tofu maintained a white color with a slight bay leaf tint. This suggests that the bioactive compounds in bay leaves do not significantly alter the tofu's pigment, although a subtle visual change becomes more apparent with increasing concentrations. This color shift is likely attributable to the presence of flavonoids and tannins in bay leaves, which are known to be stable at low concentrations and exert only minimal influence on the appearance of tofu (Darni, 2022); (Hastuti & Mulangsri, 2022);(Salehi et al., 2019)

The tofu's characteristic aroma remained dominant up to a concentration of 150 g/L, indicating that at this level, the volatile compounds present in bay leaf and garlic were insufficient to alter the inherent tofu aroma. However, at 200 g/L, a mild mixed aroma of

garlic and bay leaf began to emerge, becoming more pronounced at 250 g/L. This can be attributed to allicin, a sulfur-containing volatile compound found in garlic, which is highly reactive and decomposes easily, producing a strong aroma when applied in higher concentrations (Guan et al., 2021)

In terms of texture, the tofu remained intact and soft across all treatments, suggesting that the combined extract of bay leaf and garlic did not adversely affect the tofu's physical consistency or structure. The maintenance of tofu's structural integrity may be attributed to the stability of soy protein networks, which appear unaffected by the chemical constituents of bay leaf and garlic at the tested concentrations (Ali, Tian, & Wang, 2021). An illustration of tofu immersed in the liquid extract of bay leaf and garlic on day 0 is presented in Figure 1 below.



A B C D E F

Figure 1. Tofu samples soaked in liquid extract of bay leaf and garlic on day 0: A (50 g/L water), B (100 g/L water), C (150 g/L water), D (200 g/L water), E (250 g/L water), and F (Control).

Table 2
Research Results on Day 1

Treatment	Concentration (g/L)	Parameter		
		Color	aroma	Texture
Control		Slightly yellowish white	Sour-smelling tofu	Intact and soft
Bay Leaf + Garlic Extract	50 gr	White with a slight bay leaf tint	Characteristic tofu aroma	Intact and soft
	100 gr	White with a slight bay leaf tint	Characteristic tofu aroma	Intact and soft
	150 gr	White with a slight bay leaf tint	Characteristic tofu aroma	Intact and soft
	200 gr	White with a slight bay leaf tint	Mild aroma of garlic and bay leaf	Intact and soft
	250 gr	White with a slight bay leaf tint	Pronounced aroma of garlic and bay leaf	Intact and soft

On the first day of observation, significant changes were observed in the color, aroma, and texture of tofu following immersion in the bay leaf and garlic extract combination. The control tofu, which was not subjected to any treatment, exhibited a slight change in color to yellowish white and began to emit a sour odor, indicating the onset of natural fermentation or possible microbial contamination. In contrast, tofu treated

with the bay leaf and garlic extract maintained a white appearance with a slight bay leaf hue, suggesting that the addition of bay leaf contributed to color stability in the tofu matrix (Tometri, Ahmady, Ariaii, & Soltani, 2020)

With regard to aroma, tofu samples treated with concentrations ranging from 50 g/L to 150 g/L retained their characteristic tofu odor, indicating that the volatile compounds in bay leaf and garlic were not present in sufficient quantities to alter the aroma significantly. However, starting from the 200 g/L concentration, the aroma of garlic and bay leaf became increasingly noticeable, and at 250 g/L, this aroma was more pronounced and dominant. This finding aligns with previous research indicating that allicin, a volatile sulfur compound in garlic, begins to dominate the aroma profile at higher concentrations (Guan et al., 2021)

A B C D E F

Figure 2. Tofu samples soaked in liquid extract of bay leaf and garlic on day 1: A (50 g/L water), B (100 g/L water), C (150 g/L water), D (200 g/L water), E (250 g/L water), and F (Control).

Table 3
Research Results on Day 2

Treatment	Concentration (g/L)	Parameter		
		Color	aroma	Texture
Control		Yellowish white	Sour tofu odor	Soft
Bay Leaf + Garlic Extract	50 gr	White with a slight bay leaf tint	Characteristic tofu aroma	Intact and soft
	100 gr	White with a slight bay leaf tint	Characteristic tofu aroma	Intact and soft
	150 gr	White with a slight bay leaf tint	Characteristic tofu aroma	Intact and soft
	200 gr	White with a slight bay leaf tint	Mild garlic and bay leaf aroma	Intact and soft
	250 gr	White with a slight bay leaf tint	Noticeable garlic and bay leaf aroma	Intact and soft

On the second day of observation, the control tofu exhibited a more pronounced change in color to yellowish white accompanied by a stronger sour odor compared to the previous day. This suggests ongoing microbial fermentation in the untreated tofu. The deterioration in tofu quality aligns with previous findings indicating that storage without protection from natural antimicrobials can accelerate microbial growth, resulting in changes in both color and odor (Benucci, Wang, Zhang, Bonito, & Yu, 2022); (Suhardini & Zubaidah, 2016)

In contrast, tofu samples treated with bay leaf and garlic extract retained a white color with a slight bay leaf hue, indicating that the addition of bay leaf extract effectively

preserved the natural appearance of the tofu. Bay leaves are known to contain tannins and flavonoids with antioxidant and antimicrobial properties that help inhibit the proliferation of microorganisms responsible for discoloration (Kristiananda et al., 2022)

In terms of aroma, the characteristic tofu scent remained dominant at extract concentrations of 50–150 g/L. However, at higher concentrations (200–250 g/L), the aromas of garlic and bay leaf became increasingly noticeable. Garlic contains allicin, a compound known for its antimicrobial activity, which contributes to food preservation without significantly altering the product's physical structure (Istiqomah & Jayuska, 2020). Additionally, the essential oils in bay leaf, particularly eugenol and methyl eugenol, are recognized for their natural preservative effects and contribute to a distinctive aroma that can prevent spoilage (Baptista, Horita, & Sant'Ana, 2020)

The texture of tofu remained intact and soft across all treatment concentrations, indicating that the addition of bay leaf and garlic extracts did not adversely affect tofu consistency. The ability of these extracts to preserve tofu quality without compromising texture can be attributed to the bioactive compounds' effectiveness in inhibiting microbial growth and oxidative processes, which are typically responsible for physical degradation in food products (Irkin & Korukluoglu, 2009)

A B C D E F

Figure 3. Tofu samples soaked in liquid extract of bay leaf and garlic on day 2: A (50 g/L water), B (100 g/L water), C (150 g/L water), D (200 g/L water), E (250 g/L water), and F (Control).

Table 4
Research Results on Day 3

Treatment	Concentration (g/L)	Parameter		
		Color	aroma	Textur
Control		Yellowish white	Sour tofu odor	Soft
Bay Leaf + Garlic Extract	50 gr	White with a slight bay leaf hue	Tofu aroma with a slight sour note	Intact and soft
	100 gr	White with a slight bay leaf hue	Tofu aroma with a slight sour note	Intact and soft
	150 gr	White with a slight bay leaf hue	Characteristic tofu aroma	Intact and soft
	200 gr	White with a slight bay leaf hue	Mild garlic and bay leaf aroma	Intact and soft
	250 gr	White with a slight bay leaf hue	Noticeable garlic and bay leaf aroma	Intact and soft

On the third day of observation, the results demonstrated that the combination of bay leaf and garlic extracts remained effective in preserving the color, aroma, and texture of tofu compared to the control. The control tofu showed a noticeable change in color to

yellowish white with a more pronounced sour odor, indicating that untreated tofu tends to deteriorate more rapidly. This deterioration is likely due to increased microbial activity over time, consistent with previous findings that tofu is highly susceptible to spoilage during storage without natural preservatives (Dorrigiv, Zareiyan, & Hosseinzadeh, 2020)

At extract concentrations of 50 g/L and 100 g/L, the tofu exhibited a slight change in aroma with a mild sour note, yet remained soft and intact. The presence of a light sour odor may reflect the initial stages of natural fermentation, although this process appeared to progress more slowly than in the control sample. Other studies have also demonstrated that the combination of natural antimicrobial agents, such as garlic and bay leaf, can delay the growth of pathogenic microorganisms in food products (Chahal, Bansal, & Kaur, 2016)

At concentrations of 150 g/L and above, the aroma of garlic and bay leaf became more dominant, indicating that the extract had a stronger inhibitory effect on the development of sour odor–causing microbes. The antimicrobial activity of garlic, attributed to sulfur-containing compounds such as allicin, acts synergistically with the essential oils in bay leaf—particularly eugenol—to protect tofu from microbial spoilage (Gokoglu, 2019);(Hartanti et al., 2019)

The texture of tofu remained intact and soft across all concentrations, both in the treated and control samples. This suggests that the addition of bay leaf and garlic extracts did not affect the physical structure of the tofu. The active compounds in these natural preservatives appear to contribute more to maintaining sensory quality rather than altering the mechanical properties of tofu, which aligns with previous research indicating that natural preservatives typically do not affect the texture of stored foods (Bravi, Perretti, Falconi, Marconi, & Fantozzi, 2017)

Overall, by the third day, the combination of bay leaf and garlic extracts effectively preserved the tofu's color, aroma, and structural integrity. These findings suggest that the use of natural preservatives can offer adequate protection without causing significant alterations to the physical characteristics of tofu.

A B C D E F

Figure 4. Tofu samples soaked in liquid extract of bay leaf and garlic on day 3: A (50 g/L water), B (100 g/L water), C (150 g/L water), D (200 g/L water), E (250 g/L water), and F (Control).

Conclusion

Based on the results of this study, it can be concluded that the combination of bay leaf and garlic extracts has a significant effect on the color, aroma, and physical integrity of tofu. Across all tested concentrations (50 g/L to 250 g/L), the tofu maintained a white color with a slight bay leaf tint, indicating that bay leaf extract does not significantly alter the visual appearance of tofu. In contrast, the control tofu (without treatment) began to exhibit a yellowish discoloration by the second and third days, suggesting spoilage or fermentation.

In terms of aroma, low concentrations (50 g/L and 100 g/L) resulted in only minor changes, with the addition of a mild sour note. However, at higher concentrations (200 g/L and 250 g/L), the aroma of garlic and bay leaf became more dominant, effectively suppressing the sour odor observed in the control samples. This indicates that higher concentrations of the bay leaf and garlic extract combination are more effective in delaying tofu spoilage.

Regarding texture, tofu remained intact and soft across all treatments, including the control, suggesting that the extract combination did not significantly affect the physical structure of the tofu. Overall, the optimal concentrations for preserving tofu quality in terms of color, aroma, and texture were found to be 200 g/L and 250 g/L.

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