

ORIGINAL ARTICLE

Feasibility of bay leaf (*Syzygium polyanthum* (Wight) Walp.) as a natural preservative for meatball

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Abstract

Meatball is a processed food product that can be produced from the basic ingredients of beef, chicken, or fish. High nutrient contents cause the meatball to be easily contaminated by bacteria, consequently decreasing their quality or shelf life. For this, it requires a preservative material to prevent the quality degradation of meatballs. Bay leaf extract (BLE) is one of the natural preservatives that can be used for coating material to inhibit bacterial growth on meatballs as it contains bioactive compounds. This work aims to evaluate the feasibility of BLE as a natural preservative for meatballs. Here, the TPC-based bacterial growth and shelf life are two important parameters to identify BLE's feasibility. This work was designed by using a completely randomized design (CRD) with 1 factor of BLE composition using various formulations of 0% (F0), 1%(F1), 1.5%(F2), and 2%(F3), respectively. The bacterial growth in the meatballs was evaluated based on the total plate count (TPC) values at different temperatures of 27 °C, 10 °C, and 5 °C for 1, 7, 14, 21, and 28 days of storage time. Meanwhile, the shelf life was estimated by using accelerated shelf-life testing (ASLT) based on the TPC values. The collected data were analyzed using Microsoft Excel software, one-way Analysis of Variance (ANOVA), and Duncan's test with SPSS 25.0. The BLE with a concentration of 2% showed good feasibility in preventing bacterial growth and improving the shelf life of meatballs, twice longer than that of without BLE.

Keywords: *Vegetable extract; Bay leaf; meatballs; shelf life; food preservative; natural preservative; ASLT.*

Highlights

- A natural preservative can be easily extracted from bay leaf
- Bay leaf extract has good potential as a natural preservative in meatball
- Meatball with bay leaf extract is more durable than those without one



1 Introduction

Meatball is one of the processed food products that commonly can be produced from meat (Mamuaja & Lumoindong, 2017). It has rich nutrients, good taste, low price, and easy to present, so it is preferred by many people. However, meatball is susceptible to damage because its nutrients and water content are suitable media for the growth of microorganisms. Currently, the spoilage of processed meat foods is prevented by using synthetic preservatives such as ascorbate acid and sodium benzoate. At the appropriate concentrations, these synthetic preservatives are permitted by law and safe for human. Unfortunately, a harmful synthetic preservative such as borax or formalin is used by producer to prevent the degradation of food quality. Since borax or formalin can cause some symptoms such as nausea, vomiting, and damage to the heart, kidneys, liver, and brain (Modeong et al., 2022), their use in food must strictly be prohibited. The Minister of Health of the Republic of Indonesia has banned the use of borax and formalin through Government Regulation Number 1168/MENKES/PER/X of 1999.

Based on the facts above, the use of natural preservatives has become an attractive option to replace harmful synthetic preservatives because they enable zero toxicity, are easy to collect, applicable, and have good activity for preventing microbiological contamination of food (Warnida et al., 2016). It is well known that spices or herbs have antimicrobial properties for containing bioactive components such as phenolic compounds, flavonoids, terpenes, and essential oils (Leite De Souza et al., 2005). Bioactive compounds can be found in leaves, stems, buds, seeds, fruits, and flowers of the bay plant (Liu et al., 2017). Bay leaf (*Shyzygium polianthum* (Wight) Walp.) is one of the herbal plants that are rich in bioactive compounds such as tannins, flavonoids, triterpenoids, and essential oil components of citral and eugenol, which act as anti-oxidants, anti-microbial, anti-inflammatory and anti-cancer (Tammi et al., 2018). In addition, the use of bay leaf is economically beneficial due to its affordable price, abundant availability, and bioactive compounds are easy to extract (Muhadi et al., 2007).

As mentioned above, meatball has high nutrient content, good taste, and low price but it is easy to spoil; therefore, a natural preservative is required to prevent damage to the meatball. Also, the bay leaf contains some bioactive compounds that can inhibit the microorganism's growth. This work attempts to explore the feasibility of BLE to prevent microbial growth in the meatballs. The parameter of total plate count (TPC) at different temperature and storage times were used to evaluate the shelf life and effectiveness of BLE.

2 Materials and methods

2.1 Materials

Some of the equipment used in this research included a cabinet dryer, Erlenmeyer, grinder, rotary evaporator, separating funnel, stove, analytical balance, blender, vacuum oven, and colony counter (Prio Colony Counter BCC116). Meanwhile, the ingredients used included bay leaf (*S. polyanthum*), tapioca flour, ground beef, fried garlic and shallots, salt, egg white, pepper, baking soda, and plastic purchased from the traditional market of Giwangan in Yogyakarta, Indonesia. Whereas, ethanol 96%, distilled water, and plate count agar (PCA) media were supplied by Merck.

2.2 Preparation of BLE

The preparation of BLE was performed by using a method as described by (Tammi et al., 2018). A total of 500g of sorted bay leaves were dried in a cabinet dryer at 50 °C for 24 hours. Then, the bay leaf samples were mashed with a blender and extracted by maceration method using ethanol 96% with a ratio of 1: 1.5 (bay leaf: ethanol). Subsequently, the maceration process was carried out in a 1000mL Erlenmeyer flask for 3 days. The extractants were filtered by using Whatman filter paper and concentrated using a rotary evaporator at 70 °C. The concentrated extract was stored in a 250mL Erlenmeyer and was used for further

experiments. Figure 1 shows the photograph of the extraction step of the bay leaf using the maceration method.

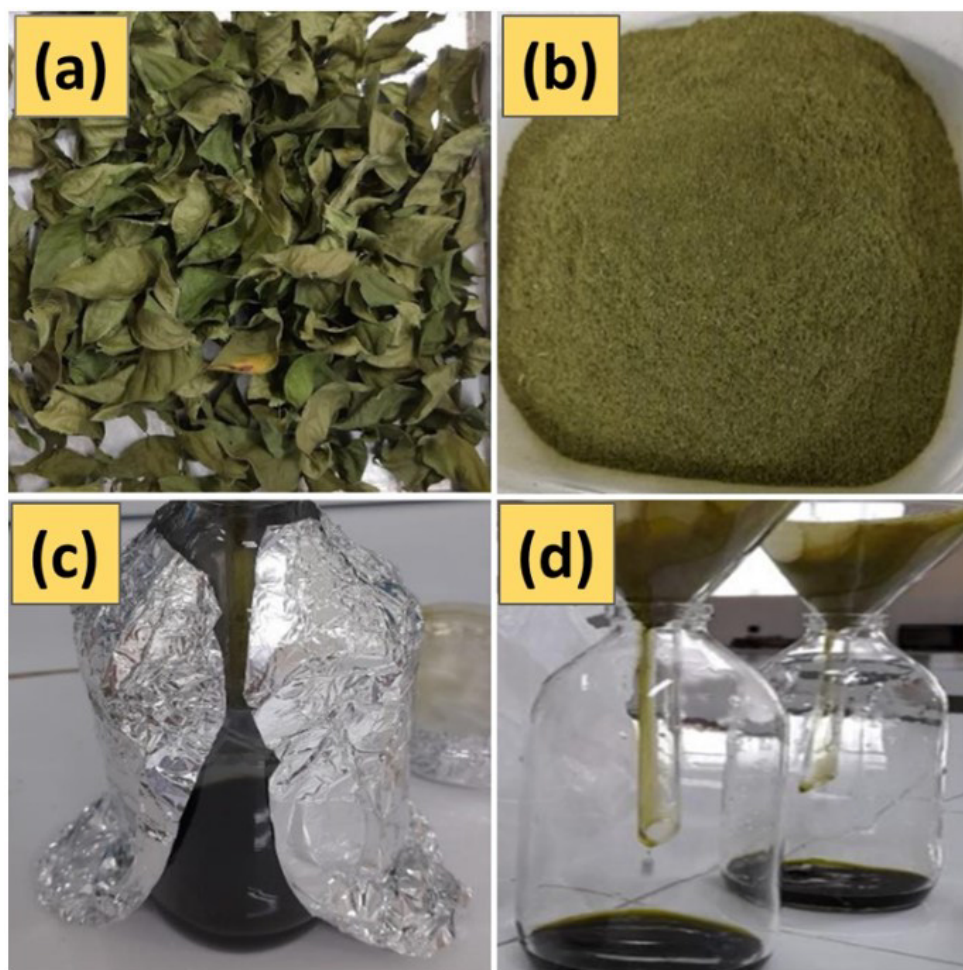


Figure 1. The photograph of (a) dried bay leaf, (b) bay leaf powder, (c) extracting bay leaf with ethanol, and (d) bay leaf extract (BLE) filtration.

2.3 Preparation of meatballs with BLE

The production of meatballs was conducted based on the method as described by (Aripin & Huda, 2018; Modeong et al., 2022). Table 1 presents the formulation used in detail. Once the meatballs were completely made, a total of 18 meatballs were dipped in the BLE with the concentrations of (all in v/w); 0% (F0), 1% (F1), 1.5% (F2) and 2.0% (F3) respectively. Then, the BLE-coated meatballs were dried and used for further experiments. Figure 2 depicts the photograph of meatballs coated with- and without BLE.

Table 1. Formulation of meatballs.

Ingredients	Formulations			
	F0	F1	F2	F3
BLE (% v/w)	0	1.0	1.5	2.0
Ground beef (g)	300	300	300	300
Tapioca flour (g)	60	60	60	60
Egg (g)	15	15	15	15
Other ingredients (g)	16	16	16	16



Figure 2. The selected figure of BLE-coated meatballs.

2.4 Total plate count (TPC) analysis

The TPC values were analyzed by using a method as described by (Sami et al., 2023). A total of 5 g of the outer layer of meatballs coated with and without BLE were scraped by using a sterilized knife, ground, mixed, homogenized, and put into an Erlenmeyer. Then, a total of 45 mL of sterile distilled water was added to the Erlenmeyer and shaken for 15 minutes (dilution factor of 10^{-1}). Next, the mixture was filtered and diluted in the dilution factors of 10^{-2} , 10^{-3} , 10^{-4} , and 10^{-5} , respectively. Each solution was put into a sterile petri dish, added with a total of 15 mL of PCA media, and incubated at different temperatures of 27 °C, 10 °C, and 5 °C, respectively. Each experiment was performed at different storage times of 1, 7, 14, 21, and 28 days. In general, the number of colonies is expressed as Total Plate Count (TPC), which can be calculated by using Equation 1 below.

$$TPC = \sum_{i=1}^n \left(\frac{\left(\frac{C}{F} \right)_1 + \left(\frac{C}{F} \right)_2 + \dots + \left(\frac{C}{F} \right)_n}{n} \right) \quad (1)$$

where *TPC* refers to the total number of colonies or total plate count (cfu/g sample), *C* is the number of colonies in a plate, *F* is the dilution factor, and *n* is the number of plates.

2.5 Determination of reaction order

The reaction order must be precisely determined before predicting the shelf life of the product. The order of reaction rate was chosen based on the highest value of R^2 of linear regression. The linear regression with the highest R^2 values (generally closest to 1) was used to predict the shelf life. To evaluate the reaction order of zero, the linear regression was generated from the TPC values at the end of treatment (A_t) plotted as the y-axis and *t* (storage time) plotted as the x-axis. Whereas, the linear regression of the reaction order of one (1) was produced from the $\ln A_t$ (plotted as the y-axis) and *t* (as the x-axis). The linear regression and R^2 can be expressed in Equation 2 below.

$$y = a + bx; R^2 = c \quad (2)$$

2.6 Estimation of shelf life

The estimation of shelf life was determined based on the total number of colonies (TPC) that grew on the tested samples (Sami et al., 2023). Meanwhile, the calculation of the shelf life was carried out by using an accelerated self-life testing (ASLT) approach as described by Kilcast & Subramaniam (2011). Based on the

linear regression (Equation 2), the slope (*b*) and intercept (*a*) values can be represented by the following Arrhenius formula (Equation 3).

$$\ln k = \ln k_0 - \left(\frac{E_a}{R} \times \frac{1}{T} \right) \tag{3}$$

where *ln k* refers to the *y* value, *ln k₀* refers to the intercept (*a*), *E_a/R* refers to the slope (*b*) and *1/T* refers to the *x* value. The *E_a* is the activation energy (cal), *R* is the ideal gas constant (1.986 cal/mol K) and *T* is the treatment temperature (K).

The shelf life for the reaction order of zero (0) can be calculated by using Equation 4 below.

$$t = \frac{(A_t - A_0)}{k} \tag{4}$$

whereas, the shelf life for the reaction order of one (1) can be calculated by using Equation 5, as follows:

$$t = \frac{(\ln A_t - \ln A_0)}{k} \tag{5}$$

where *t* is the shelf life (day), *A₀* and *A_t* are the TPC values at the beginning and end of treatment (cfu/g), and *k* is the quality reduction constant (or the constant of the bacterial growth rate).

2.7 Design of experiment

Experiments to estimate the shelf life of meatballs with and without the addition of BLE were designed by using the completely randomized design (CRD) method with 1 factor (Table 2). Treatment conditions were performed to identify the bacterial growth rate in meatballs at 27 °C (T1), 10 °C (T2) and 5 °C (T3) and 1 day (H1), 7 days (H2), 14 days (H3), 21 days (H4) and 28 days (H5). The total colonies in all samples were expressed as TPC and calculated using a colony counter.

Table 2. Design of experiment for evaluating the bacterial growth rate in meatballs with and without BLE. All treatments were conducted in three replications.

Formulations	Hours	Day	Temperature (°C)		
			27 °C (T1)	10 °C (T2)	5 °C (T3)
F0	0	1 (H1)	F0H1T1	F0H1T2	F0H1T3
	168	7 (H2)	F0H2T1	F0H2T2	F0H2T3
	336	14 (H3)	F0H3T1	F0H3T2	F0H3T3
	504	21 (H4)	F0H4T1	F0H4T2	F0H4T3
	672	28 (H5)	F0H5T1	F0H5T2	F0H5T3
F1	0	1 (H1)	F1H1T1	F1H1T2	F1H1T3
	168	7 (H2)	F1H2T1	F1H2T2	F1H2T3
	336	14 (H3)	F1H3T1	F1H3T2	F1H3T3
	504	21 (H4)	F1H4T1	F1H4T2	F1H4T3
	672	28 (H5)	F1H5T1	F1H5T2	F1H5T3
F2	0	1 (H1)	F2H1T1	F2H1T2	F2H1T3
	168	7 (H2)	F2H2T1	F2H2T2	F2H2T3
	336	14 (H3)	F2H3T1	F2H3T2	F2H3T3
	504	21 (H4)	F2H4T1	F2H4T2	F2H4T3
	672	28 (H5)	F2H5T1	F2H5T2	F2H5T3
F3	0	1 (H1)	F3H1T1	F3H1T2	F3H1T3
	168	7 (H2)	F3H2T1	F3H2T2	F3H2T3
	336	14 (H3)	F3H3T1	F3H3T2	F3H3T3
	504	21 (H4)	F3H4T1	F3H4T2	F3H4T3
	672	28 (H5)	F3H5T1	F3H5T2	F3H5T3

2.8 Statistical analysis

All data were analyzed by one-way Analysis of Variance (ANOVA) using SPSS 25.0 and Microsoft Excel 2016. If there was a significant difference between treatments at a significance level of $\alpha = 0.05$, then data would be further analyzed by using Duncan's test.

3 Results and discussion

3.1 Characteristics of bay leaf extract (BLE)

Table 3 presents the characteristics of BLE. Physically, the concentrated BLE showed the dark-green color with a typical bay leaf aroma. Whereas, the density and viscosity of BLE were obtained at 1.01 g/mL and 3.1 cP, respectively. So far, there is no specified requirement for the physical properties of the herbal solution when it is used as coat materials, but in general, based on the references, the viscosity and density were detected in the range of 3.03-3.83 cP (Stefani et al., 2023) and 0.89-1.17 g/mL (Shahtalebi et al., 2018). Ethanol composition in BLE must be evaluated to enable it to be used as a coating material in food. As mentioned above, ethanol solution was used for extracting bay leaf. According to the Indonesian government regulation, the maximum alcohol composition in food materials is not more than 0.5%. Based on the result, the alcohol composition of BLE was observed at 0.22%; thus, the BLE could be applied as a coating solution in food.

Table 3. The characteristics of concentrated BLE.

Parameters	Value	Unit
Density	1.01 ± 0.01	g/mL
Viscosity	3.10 ± 0.20	cP
Ethanol composition	0.22 ± 0.02	%
Aroma	Typical bay leaf	-
Color	Dark-green	-

3.2 Pattern of deterioration in the quality of meatballs during storage

According to the Indonesian National Standard (SNI 3818:2014), the maximum TPC value allowed for fast food products is about 10^5 cfu/g (Modeong et al., 2022). Table 4 shows the bacterial growth on 1st day (0 hours) for all meatballs with or without BLE that were obtained in the range of 0.52 - 1.81×10^4 cfu/g, which was lower than the maximum SNI limit. This fact might be due to the bacteria in the lag phase condition in which the bacteria still adapted to the sample conditions, making the bacterial growth slow. Furthermore, the TPC values in all samples exponentially increased with the increase of storage time. It is well known that the bacteria will grow when the condition of the sample is adequate for the bacteria's needs.

In addition to the nutrition content, the temperature storage also affects the bacterial growth rate. Except on the 1st day, the bacterial growth was extremely affected by storage temperature on the 7th, 14th, 21st, and 28th day. For example, based on the TPC value of F0 at 7 days of storage time, the bacterial growth rate at 27 °C were observed around two-fold and seven-fold faster compared to the bacterial growth rate at 10 °C and 5 °C. Similarly, the bacterial growth rate at 27 °C at 14 days of storage was obtained around eight-fold and 23-fold faster than that of 10 °C and 5 °C, respectively. Parallel to the research reports by (Qiu et al., 2022), the temperature improved the bacterial genera growth rate.

Moreover, storage time showed a significant effect on bacterial growth. As shown in Figure 3, the longer storage time led to the higher the TPC values. As shown in Figure 3a, the bacterial growth in meatballs of F0 at 27°C for 14 and 21 days of storage time was significantly higher than that of SNI standard (TPC > 10^5 cfu/g). Whereas, the TPC values in meatballs of F1 (Figure 3b), F2 (Figure 3c), and F3 (Figure 3d) were obtained lower than that of meatball control (F0). According to (Hoel et al., 2017), there is a tight correlation between bacterial growth and storage time. Thus, the quality of food products gradually decreases along with the longtime of storage. The shelf life of food products is also significantly determined by storage time.

Table 4. The total number of bacteria based on TPC in meatball samples.

Formulations	Hours	Day	Total plate count (cfu/g) × 10 ⁴		
			27 °C	10 °C	5 °C
F0	0	1	1.79 ± 0.22	1.79 ± 0.13	1.81 ± 0.13
	168	7	86.50 ± 2.71	45.15 ± 1.40	14.10 ± 1.20
	336	14	661.20 ± 10.12	77.08 ± 2.20	28.20 ± 2.15
	504	21	nc	943.50 ± 15.10	900.10 ± 7.10
	672	28	nc	3680.10 ± 17.30	1181.11 ± 8.10
F1	0	1	1.61 ± 0.11	1.62 ± 0.15	1.61 ± 0.12
	168	7	76.60 ± 3.23	40.15 ± 2.13	12.30 ± 0.23
	336	14	561.10 ± 9.10	70.11 ± 1.12	22.14 ± 1.12
	504	21	3230.17 ± 5.71	810.10 ± 9.10	491.23 ± 8.21
	672	28	nc	3160.30 ± 7.30	1027.77 ± 9.10
F2	0	1	1.59 ± 0.14	1.57 ± 0.11	1.57 ± 0.13
	168	7	16.81 ± 1.61	10.31 ± 0.31	9.12 ± 0.05
	336	14	381.10 ± 6.51	57.08 ± 0.20	12.05 ± 0.15
	504	21	2730.01 ± 9.12	520.11 ± 3.10	437.02 ± 6.07
	672	28	nc	2368.10 ± 12.01	908.11 ± 11.10
F3	0	1	0.52 ± 0.15	0.52 ± 0.15	0.52 ± 0.14
	168	7	4.36 ± 0.17	1.60 ± 0.02	0.77 ± 0.12
	336	14	107.57 ± 1.20	2.67 ± 0.05	1.65 ± 0.51
	504	21	200.81 ± 2.11	5.77 ± 0.05	3.18 ± 0.23
	672	28	671.60 ± 5.32	31.29 ± 0.11	22.80 ± 2.20

Note: nc (not calculated) = number of bacteria could not be precisely calculated.

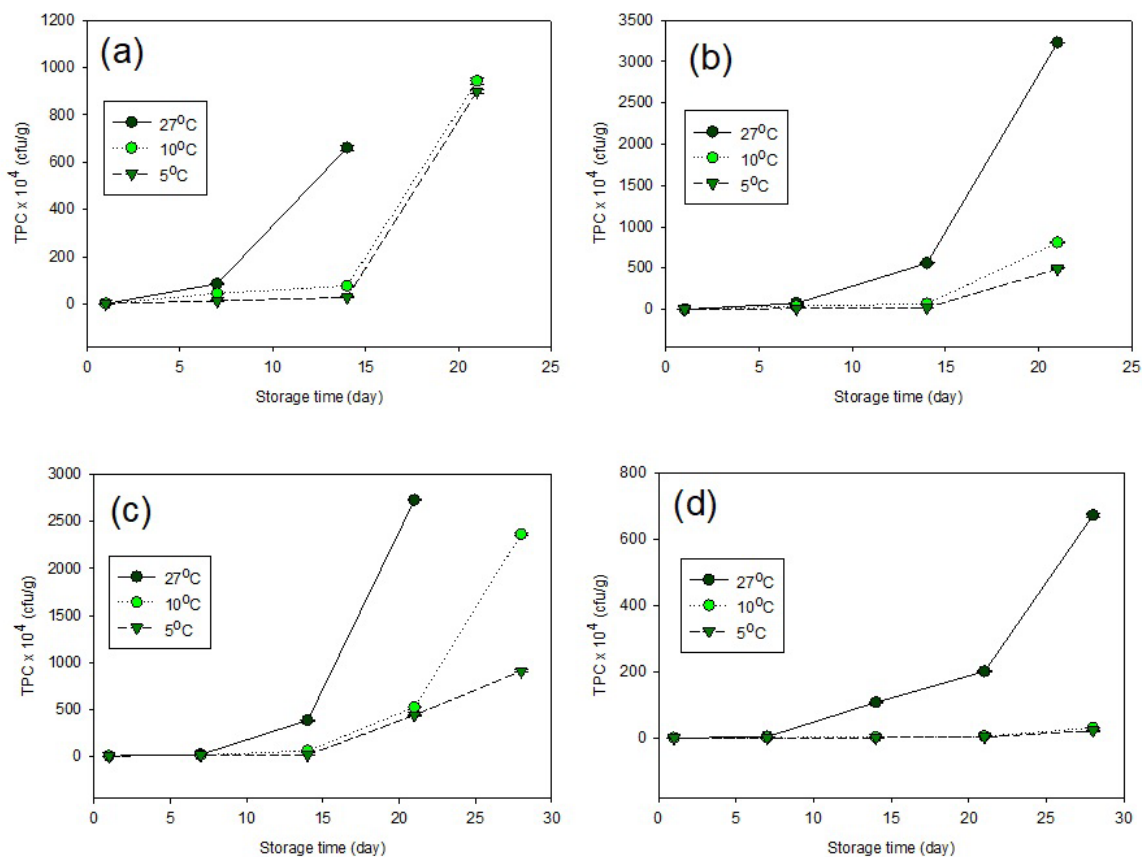


Figure 3. The TPC pattern of (a) meatballs without BLE (F0), (b) 1.0% of BLE (F1), (c) 1.5% of BLE (F2) and (d) 2.0% of BLE (F3) at 27 °C, 10 °C, and 5 °C during different storage time.

As presented in Figure 4 the bacterial growth in meatballs of F0, F1, and F2 at 27°C for 21 and 28 days of storage time was much higher (TPC spreader) compared to F3. From this fact, 2.0% of BLE was found as the most effective concentration to protect meatballs from bacterial contaminants. The low bacterial growth rate in meatballs with BLE, especially F3, might be due to the high antibacterial compounds such as eugenol and essential oils such as *β-caryophyllene*, *α-pinene*, and *β-pinene*, which were present in the sample (Boulila et al. 2015). Parallel with research conducted by (Tammi et al., 2018), BLE can suppress the growth of bacteria (such as *Escherichia coli* and *Staphylococcus aureus*) by about 92%.

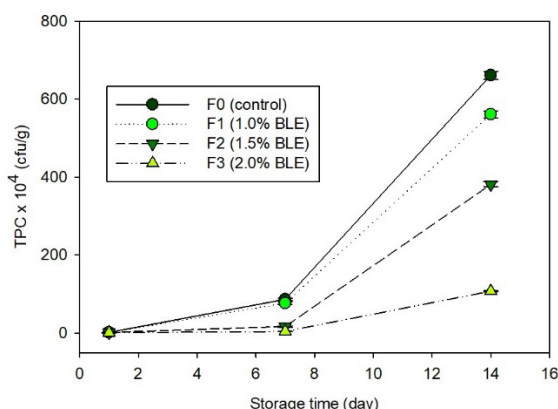


Figure 4. Trends of bacterial growth in meatballs coated with BLE and control for 14 days of storage time at 27 °C.

3.3 Determination of reaction order

The reaction order is an important factor in determining the shelf life of a food product. As mentioned above, the reaction order was determined based on the TPC value. Bacterial growth in samples involves the activation rate of microorganisms, enzyme production, and degradation of vitamins and other nutritional components in food (Sperber & Doyle. 2009). Generally, the reaction kinetics in food products have a reaction order of 0 or 1 depending on the reaction pattern that occurs (Labuza, 1984). For example, the reduction in the quality of frozen food is determined by using Equation 4 with a reaction order of 0. Meanwhile, the decrease of vitamins in food and the decrease in food quality caused by microbial growth can be evaluated by using Equation 5 with a reaction order of 1 (Van Boekel. 2009). The quality degradation of meatball was mainly caused by the bacterial growth, so the reaction order of 1 was used to determine the shelf life in this work.

As shown in Table 5, the high R² value of a linear regression indicated the adequate equation to determine the reaction order. The average value of R² at order 1 for all samples was greater than that of R² at order 0. Thus, the kinetics of the reaction in the meatballs could be categorized as a reaction order of 1.

Table 5. The R² value of each reaction order for meatballs with and without BLE.

Formulations	Temperature	R ²	Average R ²	R ²	Average R ²
	(°C)	(order 0)	(order 0)		(order 1)
F0	27	0.8439	0.3974	0.9692	0.7003
	10	0.1438		0.4145	
	5	0.2044		0.7172	
F1	27	0.7721	0.6479	0.9923	0.7787
	10	0.5561		0.4569	
	5	0.6156		0.8868	
F2	27	0.7271	0.6231	0.9573	0.7636
	10	0.5156		0.4569	
	5	0.6265		0.8765	
F3	27	0.7672	0.6441	0.9392	0.7274
	10	0.5614		0.3559	
	5	0.6036		0.8870	

3.4 Analysis of the quality degradation of meatballs

Based on the calculation of the reaction order, the estimation of the shelf life of meatballs with and without the addition of BLE was analyzed by using the reaction order 1. After the TPC values were plotted against storage time, several equations could be generated at the temperature of the treatment. As previously mentioned, the linear regression equation provides some information regarding the pattern of quality degradation under ideal conditions. Figure 5 shows the relationship curve between $\ln k$ and $1/T$. The rate of microbial growth at ambient temperature (27 °C) was found much faster than the low storage temperature (5 °C and 10 °C). A summary of degradation parameters as listed in Table 6 shows that the degradation of meatball quality refers to the value of k where a higher k value indicates a higher TPC value, meaning that the meatball was spoiled.

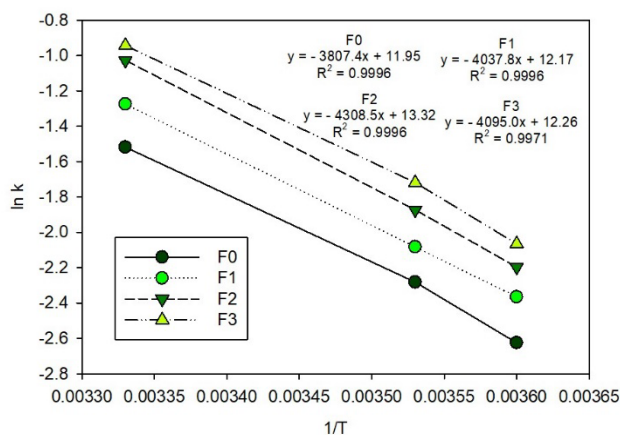


Figure 5. Linear regression of $\ln k$ and $1/T$ based on the microbial growth (TPC) in meatballs along with the duration of storage condition.

Table 6. Summary of degradation parameters of meatballs with and without BLE

Meatballs	T(K)	1/T	$\ln k_0$	Ea/R	$\ln k$	k
F0	300	0.00333	11.95	3807.4	-0.73	0.48
	283	0.00353	11.95	3807.4	-1.49	0.23
	278	0.00360	11.95	3807.4	-1.76	0.17
F1	300	0.00333	12.17	4037.8	-1.28	0.28
	283	0.00353	12.17	4037.8	-2.08	0.12
	278	0.00360	12.17	4037.8	-2.37	0.09
F2	300	0.00333	13.32	4308.5	-1.03	0.36
	283	0.00353	13.32	4308.5	-1.89	0.15
	278	0.00360	13.32	4308.5	-2.19	0.11
F3	300	0.00333	12.26	4096.0	-1.38	0.25
	283	0.00353	12.26	4096.0	-2.20	0.11
	278	0.00360	12.26	4096.0	-2.49	0.08

3.5 Shelf life of meatballs

Estimation of the shelf life of meatballs with and without BLE is presented in Table 7. The shelf life of the meatballs with BLE tended to be two-fold longer than the meatballs without BLE. For example, the shelf life of meatballs with BLE at 27 °C of storage temperature was obtained in the range of 1.70-2.33 days, while the meatballs without the BLE was around 1.36 day. Furthermore, the shelf life of meatball with BLE at 10 °C of storage temperature was observed in the range of 4.43-6.52 days, while meatballs without BLE was observed around 3.30 days. Similarly, meatballs with and without BLE storage at 5 °C had a shelf life observed in the range of 6.04-8.76 days and 4.61 days, respectively. The longest shelf life was shown by F3 (2% BLE), while the shortest one was shown by F0 (control). This fact clearly describes that the use of BLE has a significant effect ($p < 0.05$) on the bacterial growth rate in meatballs.

Table 7. Estimation of shelf life of meatballs with and without BLE at different storage temperatures.

Meatballs	Temperature (°C)	Shelf life (days)
F0 (control)	27	1.36 ± 0.05 ^a
	10	3.30 ± 0.08 ^b
	5	4.61 ± 0.17 ^c
F1	27	1.70 ± 0.03 ^d
	10	4.43 ± 0.03 ^e
	5	6.04 ± 0.05 ^e
F2	27	2.23 ± 0.06 ^f
	10	5.27 ± 0.02 ^g
	5	6.96 ± 0.08 ^f
F3	27	2.33 ± 0.05 ^f
	10	6.52 ± 0.09 ^f
	5	8.76 ± 0.17 ^h

Note: Different superscript notations indicated the significant effect of the use of BLE on the shelf life of meatballs. Statistical analysis was conducted at significant level of $\alpha = 0.05$.

It is well known that bacterial growth is strongly determined by several conditions such as temperature, humidity, oxygen availability, and storage time (Van Boekel, 2009). In general, the room temperature in the range of 25 and 28 °C is an optimal temperature for microbial growth. The increase in the total number of bacteria during storage at this temperature is related to the growth of mesophyll bacteria where these bacteria can grow well at temperatures around 20 °C to 40 °C. Therefore, the TPC value at 27 °C was higher compared to the lower temperature. The increasing activity of microorganisms can lead to a faster degradation reaction, change the physical and chemical characteristics, and ultimately damage the quality and freshness of the foods (Tamanna & Mahmood, 2015).

Apart from the potential of BLE in preventing bacterial growth, storage at low temperatures (such as 10 °C and 5 °C) can also extend the shelf life of meatballs. Storage carried out at 10 °C and 5 °C can make the rate of enzymatic reactions by bacteria slower. Thus, bacterial growth and decay processes can be inhibited significantly ($p < 0.05$). As discussed above, the shelf life of meatballs with the addition of BLE can be extended two-fold as meatballs without the addition of BLE. According to (Ordoudi et al., 2022), the phenomenon of reducing microbial growth is inhibited due to the presence of antibacterial components in bay leaves such as flavonoids, essential oils, alkaloids, tannins, and saponins. Flavonoids can denature proteins in permeable bacteria cell walls, essential oils have the potential to inhibit enzymes involved in converting food into energy, which will inhibit cell growth and alkaloids can disrupt the peptidoglycan building block in bacteria cells preventing the cell wall layer from fully forming and causing cell death. Meanwhile, tannins can stop bacterial growth by freezing the protoplasm and saponins interfere with the surface tension of the cell wall. Antibacterial agents will easily enter the cell and interfere with bacterial metabolism causing bacteria death (Boulila et al., 2015).

4 Conclusion

The evaluation of the potential of bay leaf extract (BLE) as a natural preservative for meatballs has been completed. The research results showed that 2% of BLE (F3) had the potential as a natural preservative, which could extend the shelf life of meatballs two-fold better than that without BLE. The effectiveness of 2% of BLE showed the longest shelf life of meatballs compared to other ones. Thus, BLE can be considered as an alternative natural preservative to prevent the spoilage of meatballs.

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References

- Aripin, N. H. M., & Huda, N. (2018). Quality characteristics of meatball prepared from different ratios of chicken and duck meat. *Asia Pacific Journal of Sustainable Agriculture Food and Energy (APJSAFE)*, 6(2), 6-9.
- Boulila, A., Hassen, I., Haouari, L., Mejri, F., Ben Amor, I., Casabianca, H., & Hosni, K. (2015). Enzyme-assisted extraction of bioactive compounds from bay leaves (*Laurus nobilis* L.). *Industrial Crops and Products*, 74, 485-493. <http://doi.org/10.1016/j.indcrop.2015.05.050>
- Hoel, S., Jakobsen, A. N., & Vadstein, O. (2017). Effects of storage temperature on bacterial growth rates and community structure in fresh retail sushi. *Journal of Applied Microbiology*, 123(3), 698-709. PMID:28654203. <http://doi.org/10.1111/jam.13527>
- Kilcast, D., & Subramaniam, P. (2011). *Food and beverage stability and shelf life*. Woodhead Publishing Limited.
- Labuza, T. P. (1984). Application of chemical kinetics to deterioration of foods. *Journal of Chemical Education*, 61(4), 348-358. <http://doi.org/10.1021/ed061p348>
- Liu, Q., Meng, X., Li, Y., Zhao, C. N., Tang, G. Y., & Li, H. (2017). Antibacterial and antifungal activities of spices. *International Journal of Molecular Sciences*, 18(6), 1283. PMID:28621716. <http://doi.org/10.3390/ijms18061283>
- Mamuaja, C. F., & Lumindong, F. (2017). Antimicrobial activity of Kluwek (*Pangium edule*) seed extract as natural preservatives of Tuna Fish Ball. *Jurnal Pengolahan Hasil Perikanan Indonesia*, 20(3), 592-601. <http://doi.org/10.17844/jphpi.v20i3.19815>
- Modeong, D. F., Buhari, F., & Arwati, N. L. (2022). Identification of borax and formalin content in wet noodles and meatballs in Gorontalo City. *Journal of Health, Technology and Science*, 3(1), 19-25. <https://doi.org/10.47918/jhts.v3i1.270>
- Muhadi, Suhayono, A. S., & Susilawati. (2007). Aktivitas antibakteri ekstrak daun salam (*Syzygium Polyanta*) dan daun pandan (*Pandanus Amaryllifliu*). *Jurnal Teknologi Dan Industri Pangan*, 18(1), 17-24.
- Ordoudi, S. A., Papapostolou, M., Nenadis, N., Mantzouridou, F. T., & Tsimidou, M. Z. (2022). Bay laurel (*Laurus nobilis* L.) essential oil as a food preservative source: Chemistry, quality control, activity assessment, and applications to olive industry products. *Foods*, 11(5), 752. PMID:35267385. <http://doi.org/10.3390/foods11050752>
- Qiu, Y., Zhou, Y., Chang, Y., Liang, X., Zhang, H., Lin, X., Qing, K., Zhou, X., & Luo, Z. (2022). The effects of ventilation, humidity, and temperature on bacterial growth and bacterial genera distribution. *International Journal of Environmental Research and Public Health*, 19(22), 15345. PMID:36430064. <http://doi.org/10.3390/ijerph192215345>
- Sami, A. N., Malaka, R., Hajrawati, H., & Tamal, M. A. (2023). Total plate count, *Staphylococcus aureus*, and pH of commercial beef meatball in Makassar. *AIP Conference Proceedings*, 2628, 050023. <https://doi.org/10.1063/5.0144064>
- Shahtalebi, M.A., Asghari, G.R., Rahmani, F., Shafiee, F., & Jahanian-Najafabadi, A. (2018). Formulation of herbal gel of antirrhinum majus extract and evaluation of its anti-propionibacterium acne effects. *Advanced Biomedical Research*, 7, 53. PMID:29657938. http://doi.org/10.4103/abr.abr_99_17
- Souza, E. L., Stamford, T. L. M., Lima, E. O., Trajano, V. N., & Barbosa Filho, J. M. (2005). Antimicrobial effectiveness of spices: An approach for use in food conservation systems. *Brazilian Archives of Biology and Technology*, 48(4), 549-558. <http://doi.org/10.1590/S1516-89132005000500007>
- Sperber, W. H., & Doyle, M. P. (2009). *Compendium of the microbiological spoilage of foods and beverages*. New York: Springer-Verlag. <http://doi.org/10.1007/978-1-4419-0826-1>
- Stefani, R., Vanessa, G. E., & Fibryanto, E. (2023). Evaluation of viscosity and pH of Zingiber officinale var. officinale juice in mouthwash formulation. *Majalah Kedokteran Gigi Indonesia*, 9(2), 163-170. <http://doi.org/10.22146/majkedgiind.82071>
- Tamanna, N., & Mahmood, N. (2015). Food processing and maillard reaction products: Effect on human health and nutrition. *International Journal of Food Science*, 2015, 526762. PMID:26904661. <http://doi.org/10.1155/2015/526762>
- Tammi, A., Apriliana, E., Umiana Sholeha, T., & Ramadhian, M. R. (2018). Potential of bay leaf extract (*Syzygium polyanthum* [Wight.] Walp.) as antibacterial to *Staphylococcus aureus* In Vitro. *J Agromedicine Unila*, 5(2), 562-564.
- Van Boekel, M. A. J. S. (2009). Kinetic modeling of food quality: A critical review. *Comprehensive Reviews in Food Science and Food Safety*, 7(1), 144-158. <http://doi.org/10.1111/j.1541-4337.2007.00036.x>
- Warnida, H., Sukawaty, Y., & Samarinda, A. F. (2016). Efektifitas ekstrak etanol daun salam (*Syzygium polyanthum* (Wight) Walp.) sebagai pengawet alami antimikroba. *Jurnal Ilmiah Ibnu Sina*, 1(2), 227-234. <https://doi.org/10.36387/jiis.v1i2.53>

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