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Bay leaf essential oils inhibited microbial growth and exerted potential preservation effects on tofu

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ABSTRACT

The antimicrobial activities of bay leaf [*Syzygium polyanthum* (Wight) Walp.] have been reported, in which various extracts of this plant are reported active against pathogenic and food spoilage microorganisms. As bay leaf is also commonly used as a spice in Indonesian culinary, the foods preserved with it are likely more acceptable. In this study, the microbial growth inhibitory activity, the physical characters of tofu, and subsequently the preservation potential of tofu by bay leaf water extract (WE) and essential oil (EO) during 10-day storage at the room temperature were evaluated. The bay leaf WEs and EOs were prepared by the infusion and steam-and-water distillation method, respectively. The microbial growth inhibitory activity was evaluated by the indirect microbial enumeration method. The physical characters of tofu during ten days of storage were organoleptically observed. The preservation potentials were calculated based on the comparison of changes in physical characters of tofu in treatment groups to those of negative control one. The results showed that bay leaf WEs weakly inhibited bacterial growth on tofu, generated unfavorable color and texture of tofu, and did not show preservation potential. In contrast, bay leaf EO at the optimum concentration of 1562.5 ppm significantly inhibited bacterial growth, maintained the initial color and texture of tofu, and demonstrated potential preservation of tofu up to 6 days. With these results, bay leaf EO is potentially to be developed further into a natural food preservative.

Introduction

Bay leaf [*Syzygium polyanthum* (Wight) Walp., synonym *Eugenia polyantha* Wight; Myrtaceae] is a plant native to Indonesia and Malaysia with the local name of *salam*. It is traditionally used for the treatment of various ailments, including diabetes mellitus, diarrhea, gastritis, hypertension, ulcers, skin problems, as well as infectious diseases (Ismail and Ahmad, 2019; Nasution et al., 2018). Among those traditional uses, the antimicrobial activities of this plant have been widely evaluated and resulted in promising results. The essential oils (EO) and non-polar extracts of bay leaf EO have shown inhibitory activity against a wide array of microorganisms (Dewijanti et al., 2019; Hamad et al., 2017; Umaru et al., 2020). The antimicrobial activities of the water- and other polar extracts of this plant against various bacteria and fungi have also been reported (Kusuma et al., 2011; Nordin et al., 2019; Ramadhania et al., 2018). In addition to

its medicinal uses, bay leaf is also commonly used as spices in Indonesian culinary. It is used in savory dishes, particularly for fish-, meat-, tempeh-, or tofu-based food (Ismail and Ahmad, 2019).

Tofu is the hardened curd obtained from the precipitation of whole soya beans water extract with addition of Calcium salt. It is mainly produced and consumed in East and Southeast Asian. Tofu contains high amounts of water and proteins, which enable the fast growing of microorganisms. The contamination of tofu by pathogenic microorganisms has been reported in Korea and Thailand (Ananchaipattana et al., 2012; Lee et al., 2017). Hence, the efforts for preventing contaminations need to be conducted.

Bay leaf is a potential candidate to be developed as a natural preservative for tofu. It has been experimentally proven to possess antimicrobial activities against the spoilage bacteria. Furthermore, the taste and aroma of bay leaf in the

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daily meals have been well known, so the sensorial aspects of bay leaf-preserved foods are likely more acceptable. The use of bay leaf for tofu preservative has been evaluated previously. Marinating tofu in the ethanolic extract from combination of bay leaf and betel leaf resulted in the lower total plate count (TPC) (Wahyuni et al., 2019). However, this study did not evaluate the physical characteristics of the tofu. Therefore, this study aimed to analyze the microbial growth inhibitory activity, the changes on the physical characteristics of tofu, and the preservation potential of both bay leaf water extract (WE) and EO during ten days of storage under ambient temperature.

Research Methods

Materials

The materials used in this study were the firm white tofu (obtained from a local market at Purwokerto, Indonesia), anhydrous sodium sulfate (Sigma-Aldrich, Germany), and nutrient broth (NB) (Oxoid, UK). The mature-stage bay leaves were collected from Banjarnegara and Purwokerto, Indonesia, and used for the preparation of bay leaf WE and EO, respectively. The leaves were dried by direct sun drying and pulverized into the fine powders. The specification of the plant was determined by the Laboratory of Botany, Jenderal Soedirman University, Purwokerto (ref. no 141 and 142/FB.Unsoed/TaksTumbVI/2015).

Preparation of Bay Leaf Water Extract

Bay leaf WE were prepared based on the procedure described in Hartanti et al. (2019). First, the powdered plant materials were weighed and put in a classical infusion pot. Then, distilled water was added to make the final concentrations of 5, 10, and 20% (w/v), respectively. The mixtures were boiled for 15 min and vacuum-filtered through a filter paper. The WE was freshly prepared for each experiment.

Preparation of Bay Leaf Essential Oil

Bay leaf EO were distilled following the procedure previously reported by Hamad et al. (2016). The powdered plant materials were put in a steam and water distillation apparatus, and each extraction process was run for 4 h. The trace of water in EO was dehydrated with anhydrous sodium sulfate. The obtained EO was stored in an air-tight amber glass vial at temperature of 4 °C. Prior the experiments, EO was manually mixed with distilled water to make the final concentrations of 62.5, 312.5, and 1562.5 µg/mL.

Preparation of tofu and storage condition

The tofu was prepared according to the previous study (Hamad et al., 2020), as follows: tofu was cut into cubes and immersed for 5 s in boiling water. Then, the cubes of tofu were put in an Erlenmeyer flask containing 100 mL of samples: bay leaf WEs (at concentrations of 5, 10, and 20%), EOs (at concentrations of 62.5, 312.5, and 1562.5 µg/mL), and sterile distilled water as control under aseptic condition. The flasks were tightly closed and stored at ambient temperature for ten days.

Microbial Analysis

The microbial growth on untreated and treated tofu was determined by the indirect enumeration method (Hamad et al., 2020). On day 2, 4, 6, 8, and 10, each cubed tofu was put in an Erlenmeyer flask containing 25 mL of sterile NB and then gently stirred for 1 min. One mL of media was transferred into 9 mL of sterile NB and incubated at 37°C for 24 h. The optical density (OD) of each cultured tofu suspension was recorded with a UV-Vis spectrophotometer (UV-1240, Shimadzu, Japan) at a wavelength of 600 nm.

Physical Characteristics Analysis

The physical characteristics of tofu were evaluated using the sensory method (Hamad et al., 2020). Color, odor, texture, and formation of slime on tofu were observed on day 2, 4, 6, 8, and 10. For each observation, the same fresh tofu as in the experiments was used as the reference.

Preservation Potential Analysis

The preservation potential of each treatment containing bay leaf WEs and EOs were calculated by comparing the time of any changes in tofu physical characteristics with that of in the control group.

Statistical Data Analysis

All experiments were prepared in triplicate. The effects of treatment group and storage time to the OD of tofu and the mean separation of each group were analyzed by two-way analysis of variance (ANOVA) followed by Duncan's tests. The significant differences were set for a *p*-value of less than 0.05. The statistical analyses were performed using IBM SPSS Statistics v. 20 (IBM, USA).

Results and Discussion

The higher OD is correlated to the more microbial growth on tofu. Both storage time (*p*=0.000) and treatment (*p*=0.000) significantly affected the OD values. The gradual increasing trends of OD were

observed with the time of storage from day 2 to the final day of storage. The tofu is reported as a food product easily contaminated by bacteria in both ambient and refrigerated- as well as during packaged and unpackaged storage, in which *Bacillus cereus*, *Staphylococcus* spp., *Listeria* spp., *Escherichia coli*, *Salmonella* spp., and *Enterococcus* spp. were commonly found as the contaminants (Ananchaipattana et al., 2012; Lee et al., 2017; Ribeiro et al., 2017). Previous studies found that the increase in the bacterial growth on tofu were detected from 4 h of storage and gradually increased until day 4 (Alqadeeri et al., 2020; Wahyuni et al., 2019).

The bay leaf WEs in all tested concentrations significantly decreased the OD of the cultured samples, with the best effect was shown from a concentration of 10%. The 20% bay leaf WE showed higher OD than those of the lower concentrations, which might be related to the nature of the obtained extract. A study reported that bay leaf WE contains flavonoids, tannins, and saponins, which effectively reduced the microbial growth on chicken meats during refrigerated storage at the optimum concentration of 10% (Hartanti et al., 2019). Tannins, as the large-molecule polyphenols, are brown in colour and have absorption with a wavelength of around 600 nm (Hassanpour et al., 2011). The 20% bay leaf WE contained the highest tannins compared to the lower ones, and hence showed the darkest brown color among all. The color was transferred to tofu and the culture media, which likely interfere with the OD reading. Hence, the OD of cultured tofu treated with 20% bay leaf WE was higher possibly due to false positive. In order to minimize the tannin interference, the use of direct enumeration of microbial growth, i.e., determination of colony-forming units, should have been conducted. This method has well-demonstrated the potential preservation of sea

breem fillets by kakadu plum leaves and fruits extracts (Wright et al., 2019).

On the other hand, bay leaf EOs at the highest concentration of 1562.5 $\mu\text{g/mL}$ showed a significant reduction in the OD compared to the control ($p=0.000$). The immiscibility of EO and water might underline the moderate microbial growth inhibitory activity of bay leaf EO in this study. The EO was quickly separated from the water after stirrer aided-mixing and did not effectively contact with tofu. Hence, the microbial growth inhibitory effects were not taken place. The use of co-solvents to homogenously solubilise EO in the water medium may provide an accurate and better result. Previous study mentioned that the use of DMSO as the co-solubilizing agent successfully improved the preservation effects of tailed pepper methanol extract on tofu. The encapsulation of EO was also shown to be a better option than the manual stirring, as indicated by a longer shelf-life of the strawberry treated with the alginate-encapsulated perilla essential oil (Alqadeeri et al., 2020; Li et al., 2018). Furthermore, the previous study also demonstrated that bay leaf EO used in this study exerted potential preservation on chicken meats, with the optimum concentration of 1562.5 $\mu\text{g/mL}$. The aldehydes, including cis-4-decanal, 1-decyl aldehyde, and capryl aldehyde, were considered as the antimicrobial constituents responsible for it (Hamad et al., 2016).

The comparison of bay leaf WE and EO, regardless of their respective control, showed there were two groups with microbial growth inhibitory activity on tofu, i.e., 10% bay leaf WE and 1562.5 $\mu\text{g/mL}$ of the EO. From all treatments, the lowest OD was resulted from bay leaf EO at a concentration of 1562.5 $\mu\text{g/mL}$, which indicating its best microbial growth inhibitory activity. The statistical analysis also confirmed this result, as shown in Table 1.

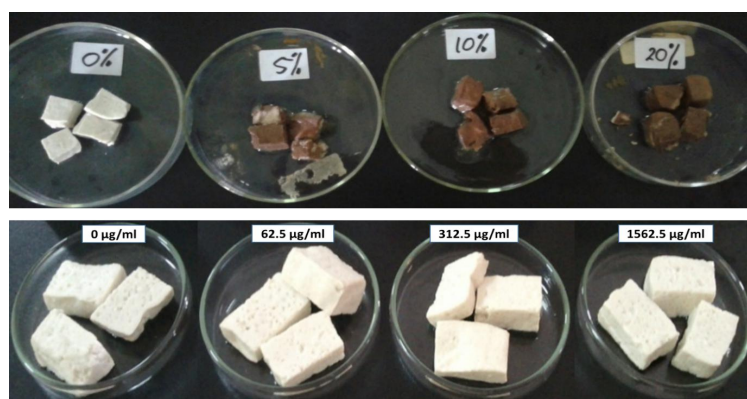


Figure 1. Tofu treated with Bay leaf WE (upper) and EO (lower) after 8-day storage under ambient temperature

Table 1. The profile of OD of the cultured tofu during 10-day storage treated with bay leaf WEs and EOs

Group	OD at 600 nm				
	Day 2	Day 4	Day 6	Day 8	Day 10
Bay leaf WE control	0.335±0.019 ^{Fa}	0.440±0.088 ^{Fb}	0.791±0.009 ^{Fc}	0.916±0.029 ^{Fd}	0.961±0.022 ^{Fe}
Bay leaf WE 5%	0.410±0.007 ^{Da}	0.550±0.016 ^{Db}	0.578±0.012 ^{Dc}	0.639±0.011 ^{Dd}	0.710±0.023 ^{De}
Bay leaf WE 10%	0.249±0.024 ^{Ba}	0.407±0.004 ^{Bb}	0.533±0.022 ^{Bc}	0.760±0.033 ^{Bd}	0.621±0.021 ^{Be}
Bay leaf WE 20%	0.448±0.008 ^{Ea}	0.497±0.000 ^{Eb}	0.744±0.034 ^{Ec}	0.760±0.033 ^{Ed}	0.753±0.043 ^{Ee}
Bay leaf EO control	0.047±0.001 ^{Ca}	0.628±0.012 ^{Cb}	0.605±0.074 ^{Cc}	0.717±0.048 ^{Cd}	0.725±0.090 ^{Ce}
Bay leaf EO 62.5 µg/ml	0.554±0.001 ^{Da}	0.545±0.066 ^{Db}	0.575±0.008 ^{Dc}	0.595±0.031 ^{Dd}	0.590±0.086 ^{De}
Bay leaf EO 312.5 µg/ml	0.461±0.002 ^{BCa}	0.496±0.009 ^{BCb}	0.529±0.018 ^{BCc}	0.542±0.033 ^{BCd}	0.660±0.006 ^{BCe}
Bay leaf EO 1562.5 µg/ml	0.430±0.008 ^{Aa}	0.455±0.029 ^{Ab}	0.343±0.027 ^{Ac}	0.443±0.041 ^{Ad}	0.627±0.066 ^{Ae}

Note: Different superscripted letters within the same column (A-F) and row (a-e) indicated significant differences of the OD between groups by two-way ANOVA and Duncan's test for $p < 0.05$

The moderate microbial growth inhibitory activity demonstrated in this study was in agreement with other studies on the antimicrobial activity-related preservation effects of bay leaf on various food matrixes. The extracts of bay leaf was reported to decrease the TPC on shrimp and the count of natural microflora on grapes at the concentrations up to 0.01 and 5%, respectively (Ramli et al., 2017; 2018). Similarly, the bay leaf WE at an optimum concentration of 10% significantly reduced the bacterial count on broiler chicken meats after 12-h storage. However, 20% of bay leaf WE was the concentration needed to obtain the least bacterial count after 15 min of marinating (Pura et al., 2015). Furthermore, combining bay leaf with other plant materials seemed to be an effective strategy for increasing the antimicrobial properties. Treating tofu with 9% of combined bay leaf and betel leaf ethanolic extracts at ratio of 1:1 for 96 h resulted in the least TPC. While the combined bay leaf and betel nut ethanolic extract at concentration ratio of 10:2.5 (in %) provided the best microbial growth inhibitory activity on minced broiler chicken meats during 6-day storage (Wahyuni et al., 2019; Parnanto et al., 2014).

The color of tofu treated with bay leaf WE was different from those treated with the bay leaf EO. Tofu in bay leaf WEs group was brown in colour, while that in the EO group was remained the same as most of EO is colourless at room temperature. Tannins were likely to be compounds that

contributed to the brown color in treated tofu. The colour changes were observed from day 2. The color intensity was gradually increased until the final day of storage. Figure 1 showed the comparison of tofu treated with bay leaf WEs and bay leaf EOs in day 8. Hence, the color change in treated tofu was likely caused by the color of the used bay leaf extract than the microbial activity. The presence of microorganisms on tofu was reported to cause changes in its physical characteristics. Spoiled tofu is characterised by a sour taste, an off-odor, an off-texture, and slime formation, which related to the metabolic activity of the microorganisms (Wang et al., 2019).

Table 2 shows that the changes in odor and texture, as well as slime formation, in a given treatment group, started to occur at the same time. The texture of tofu treated with bay leaf WE was varied from that of treated with bay leaf EO. Bay leaf WE treated tofu started to be firmer in day 6. While the texture of tofu treated with the EO at concentration of 62.5 µg/mL started to be friable on day 10 or remained unchanged at higher concentration groups. Tannins are known to interact with proteins, in which the interaction might affect the quality of protein-containing food (Wang and Heinonen, 2017). Thus, the contact between the proteins in tofu and tannins in bay leaf WEs during the storage might be responsible for the changes in the texture and color of treated tofu.

Treating tofu with bay leaf EO and bay leaf WE has different effects on its odor and texture. The off-odor of bay leaf WE treated tofu was observed since day 6 in all tested concentrations. While treated tofu with bay leaf EO at concentration of 62.5 µg/mL experienced a delayed off-odor, which started on day 10. At higher bay leaf EO concentration, no physical changes (i.e. odor and colour) were observed during 10-day

storage period. The bay leaf EO generated a stronger aromatic bay leaf odor in tofu compared to that of the bay leaf WE. The odor compounds associated with the aromatic bay leaf odor are mostly terpenoids, particularly monoterpenes and sesquiterpenes (Ismail and Ahmad, 2019). Those compounds were identified in bay leaf EO but not detected in the bay leaf WE (Hamad et al., 2016; Hartanti et al., 2019)

Table 2. The profile of physical characters of tofu during 10-day storage

Groups	Parameters	Storage (day)				
		2	4	6	8	10
Control	Color	White	White	White	White	White
	Odor	Fresh tofu	Fresh tofu	Off-odor	Off-odor +	Off-odor ++
	Texture	Firm	Firm	Friable +	Friable ++	Friable +++
	Slime formation	No	No	Yes	Yes	Yes
Bay leaf WE 5%	Color	Brown	Brown	Brown	Brown	Dark brown
	Odor	Aromatic bay leaf	Aromatic bay leaf	Aromatic bay leaf, off-odor	Off-odor	Off-odor +
	Texture	Firm	Firm	Firm +	Firm +	Firm ++
	Slime formation	No	No	Yes	Yes	Yes
Bay leaf WE 10%	Color	Brown	Brown	Brown	Brown	Dark brown
	Odor	Aromatic bay leaf	Aromatic bay leaf	Aromatic bay leaf, off-odor	Off-odor	Off-odor +
	Texture	Firm	Firm	Firm +	Firm +	Firm ++
	Slime formation	No	No	Yes	Yes	Yes
Bay leaf WE 20%	Color	Brown	Brown	Brown	Brown	Dark brown
	Odor	Aromatic bay leaf	Aromatic bay leaf	Aromatic bay leaf, off-odor	Off-odor	Off-odor +
	Texture	Firm	Firm	Firm +	Firm +	Firm ++
	Slime formation	No	No	Yes	Yes	Yes
Bay leaf EO 62.5 µg/mL	Color	White	White	White	White	White
	Odor	Fresh tofu	Fresh tofu	Fresh tofu	Fresh tofu	Off-odor
	Texture	Firm	Firm	Firm	Firm	Friable
	Slime formation	No	No	No	No	Yes
Bay leaf EO 312.5 µg/mL	Color	White	White	White	White	White
	Odor	Aromatic bay leaf +	Aromatic bay leaf +	Aromatic bay leaf +	Aromatic bay leaf +	Aromatic bay leaf
	Texture	Firm	Firm	Firm	Firm	Firm
	Slime formation	No	No	No	No	No
Bay leaf EO 1562.5 µg/mL	Color	White	White	White	White	White
	Odor	Aromatic bay leaf ++	Aromatic bay leaf ++	Aromatic bay leaf ++	Aromatic bay leaf ++	Aromatic bay leaf +
	Texture	Firm	Firm	Firm	Firm	Firm
	Slime formation	No	No	No	No	No

Note: Boldly printed words indicated the first changes in the physical characters of tofu in the respective treatment group.

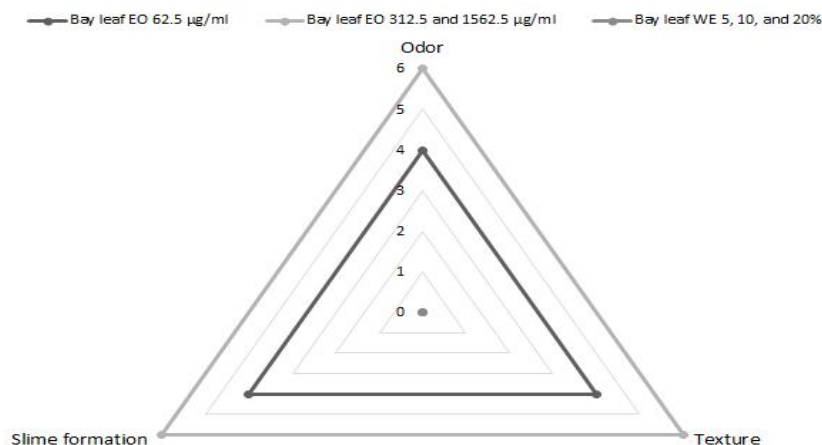


Figure 2. The profile of the preservation potential of bay leaf WE and EO

The treatment with various bay leaf extracts has shown to have different effects on the physical characteristics of food, which possibly due to the duration of contact and the nature of the food matrix. Washing the raw grapes with bay leaf ethanolic extract up to concentration of 5% did not significantly change the color, odor, and texture, and hence the fruits were accepted by the panelists (Ramli et al., 2017). The overall acceptability of chicken meat and shrimp soaked in 0.10% bay leaf methanol extract for 5 and 10 min were reported to be acceptable by the panelists (Ramli et al., 2018). Furthermore, marinating broiler chicken meats in 20% of bay leaf WE for 15 min did not affect the color, taste, and aroma (Pura et al., 2015).

The findings from this study demonstrated that bay leaf WE has no preservation potential as shown by significant physical changes on the treated tofu over the storage period. Figure 2 shows that the physical characteristics of bay leaf EO treated tofu at concentration of 312.5 and 1562.5 µg/mL were remained the same until day 10, with the preservation potential of up to 6 days. Previous studies reported that bay leaf extracts has preservation potential as shown by its ability to expand the food product's shelf life in various degrees. For example, bay leaf ethanolic extract at concentration of 3% and bay leaf WE at concentration of 15% were able to extend the shelf life of pork loin for 9 h under ambient temperature (Agustina et al., 2017).

Based on the overall parameters analysed from both bay leaf extracts, the results showed that bay leaf EO has superior performance than its counterpart. Thus, bay leaf EO is potential to be developed further as a natural food preservative. Yet, further studies on the quality of bay leaf EO treated tofu are suggested, include sensory analysis, optimisation of bay leaf EO concentration and contact time, as well as the process modelling.

Conclusion

Bay leaf WE was not suitable to be used as the natural preservative of tofu. Bay leaf EO at a concentration of 1562.5 µg/mL exerted a significant microbial growth inhibitory activity on the treated tofu, causing no changes in its color and texture for over 10-day storage period. The findings confirmed that bay leaf EO can potentially preserve tofu up to six days under the ambient temperature.

Conflict of interest

The author declares that there is no conflict of interest in this publication.

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