

The microbial growth inhibition profile of selected Indonesian spices essential oils on tofu during 8-day storage

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Abstract

This study evaluated the profile of microbial growth inhibition on tofu treated by the essential oil of the selected spices. The microbial growth on the tofu was visually observed by the pour plate method, while their number was enumerated by the indirect optical density (OD) method. All the tested essential oils were visually reduced the microbial growth on tofu during storage. Each spices oil showed a different inhibition pattern, with bay leaf, galangal, and ginger oils exerting favorable effects for the most prolonged period. The day-by-day comparison demonstrated that the best inhibition effect was on day-6 and -8, with bay leaf and galangal oils showing the most promising activity. The best profile of both essential oils might play a vital role in the tofu preservation process by natural products.

Keywords: spices, essential oil, microbial growth, natural food preservative

INTRODUCTION

Tofu is one of the most popular plant-based proteins, particularly in Asia. The hardened curd of whole soya beans extracts contained water and proteins in high levels that rapidly enabled microbial growth. The bacteria, i.e., *Bacillus cereus*, *Escherichia coli*, *Enterococcus* spp., *Listeria* spp., *Salmonella* spp., and *Staphylococcus* spp., were the common contaminants on tofu (Ananchaipattana *et al.*, 2012; Lee *et al.*, 2017; Ribeiro, Costa and Costa, 2017). In addition to the risk of food-borne disease spreading, the metabolism of microbial contaminants on tofu also causes a change in the color, odor, texture, and flavor of tofu. Hence, preservation efforts are needed.

The use of spices extracts to preserve tofu is an interesting subject to be explored. The essential oils of spices with potent antimicrobial activity are prospective natural tofu preservatives. The antimicrobial compounds in those spices essential oils might inhibit microbial growth on tofu, and hence, delay the tofu spoilage (Liu *et al.*, 2017). The use of

spices in the preservation effect might also offer better sensorial acceptability as they have been empirically used in the human diet for a long time.

Banyumas people ethnobotanically use bay leaf [*Syzygium polyanthum* (Wight) Walp., Myrtaceae], galangal [*Alpinia galanga* (L.) Willd., Zingiberaceae], ginger [*Zingiber officinale* Roscoe, Zingiberaceae], and lemongrass [*Cymbopogon citratus* (DC.) Stapf, Poaceae] as spices (Apriliani, Sukarsa and Hidayah, 2014). In addition, lemon basil [*Ocimum x africanum* Lour., Lamiaceae] is also popularly used as fresh leafy vegetables. Interestingly, the antimicrobial activity of the essential oils of those spices has been well reported elsewhere. The familiarity people experience with those five spices, and their antimicrobial activity warranted the evaluation for their preservation potential on tofu. This study evaluated the profile of microbial growth on tofu treated by various concentrations of spices essential oils during 8-day storage under room temperature

EXPERIMENTAL SECTION

Materials

The plant materials used in this study are presented in **Table 1**. Other materials included tofu (firm white variant, obtained from a fresh market at Purwokerto), anhydrous sodium sulfate (Sigma-Aldrich, Germany), deionized water, nutrient broth (NB) (Oxoid, UK), and nutrient agar (NA) (Oxoid, UK).

Table 1. Plant materials

Plant names	Plant parts	Origin	Authentication reference
Bay leaf	Leaves	Banyumas, Central Java	142/FB.Unsoed/TaksTumbVI
Galangal	Rhizomes	Banjarnegara, Central Java	140/FB.Unsoed/TaksTumbVI
Ginger	Rhizomes	Purbalingga, Central Java	137/FB.Unsoed/TaksTumbVI
Lemongrass	Leaves	Banyumas, Central Java	132/FB.Unsoed/TaksTumbVI
Lemon basil	Aerial parts	Banyumas, Central Java	133/FB.Unsoed/TaksTumbVI

Preparation of plant materials

Thinly sliced galangal and ginger rhizomes, about 10-cm chopped lemongrass leaves and lemon basil aerial parts, and bay leaves were dried by the direct sun-drying method. The dried plant materials were pulverized into fine powders and kept in an airtight container.

Extraction of essential oils

The powdered-dried plant materials were separately extracted by water and steam distillation following the previously reported method (Hamad *et al.*, 2017).

Preparation of essential oil medium mixture

The essential oils were individually mixed with sterile water and manually stirred to homogenize. The different series of concentrations of the mixture of each oil was

prepared according to their respective antimicrobial activity in our previous studies (Hamad *et al.*, 2017; Hamad, Nuritasari and Hartanti, 2017). The final concentrations for each essential oil are presented in **Table 2**.

Table 2. The concentration series of the prepared medium mixture

Essential oil	Concentration (mg/ml)		
	Lower	Intermediate	Higher
Bay leaf	0.063	0.313	1.563
Galangal	0.25	1.25	6.25
Ginger	0.25	1.25	6.25
Lemongrass	0.125	0.625	3.125
Lemon basil	0.125	0.625	3.125

Preparation, treatment, and storage of tofu

The cubed tofu was immersed for 5 seconds in boiling water to reduce the microbial load on the surface. They were further submerged in 100 ml of medium mixtures under aseptic conditions (Table 2). Sterile water was used as a control. The tofu was kept in the tightly closed flasks and stored at room temperature for eight days.

Visual observation of microbial growth on tofu

On day 2, -4, -6, and -8, 1 g tofu was suspended into 100 ml of sterile water. The suspension (100 μ l) was poured on the surface of 10 ml solidified NA under aseptic conditions. The petri dish was further incubated at 37°C for 24 hours. The microbial growth on the NA was visually observed and documented (Hartanti *et al.*, 2019).

Indirect enumeration of microbial growth on tofu

On day-2, -4, -6, and -8, the cubed tofu was immersed in 25 ml of NB and gently stirred for 1 minute. The suspension (1 ml) was diluted ten times in fresh NB and incubated at 37°C for 24 hours. The optical density (OD) of each cultured tofu suspension was recorded at a wavelength of 600 nm (Hamad *et al.*, 2020a).

Data analysis

The experiments were conducted in triplicate, and the results were presented as mean value \pm standard deviation (SD). The microbial growth inhibitory activity of each essential oil was reported as the OD reduction that was calculated according to Formula 1.

$$OD\ reduction\ (\%) = 100 \times \frac{(OD\ control - OD\ sample)}{OD\ control} \quad (1)$$

The OD reduction of each treatment group was compared by one-way ANOVA and Duncan's tests. The significant difference was assigned at a p-value lower than 0.05. The analysis was performed by the IBM SPSS Statistics v. 20 programs.

RESULTS AND DISCUSSION

The submerge of tofu in the various bay leaf essential oil concentrations resulted in the different microbial growth profiles. The presence of more bacteria on the tofu was observed on day-6 than on day 2. Bay leaf essential oil at 1.563 mg/ml in both observation times showed the least microbial growth. Quantitatively, all tested oil concentrations showed microbial growth inhibitory activity on day-4, -6, and -8. The concentration-dependent inhibitory activity was observed in day-8, with the equation and OD₅₀ value of $y = 0.0128x + 18.507$ and 2.46 mg/ml, respectively (**Figure 1**). Our previous study showed that bay leaf essential oil from Banyumas, Central Java, was strongly inhibited *Bacillus subtilis* with a MIC value of 31.25 µg/ml while inactive against other food spoilage bacteria (Hamad *et al.*, 2017). Further, the essential oil of bay leaf root barks collected in Sarawak, Malaysia strongly inhibited *Aspergillus flavin*, *Aspergillus niger*, *Candida tropicalis*, *Escherichia coli*, *Fusarium oxysporium*, *Klebsiella pneumonia*, *Salmonella typhi*, and *Staphylococcus aureus* at 500 µg/ml (Umaru, Umaru and Umaru, 2020).

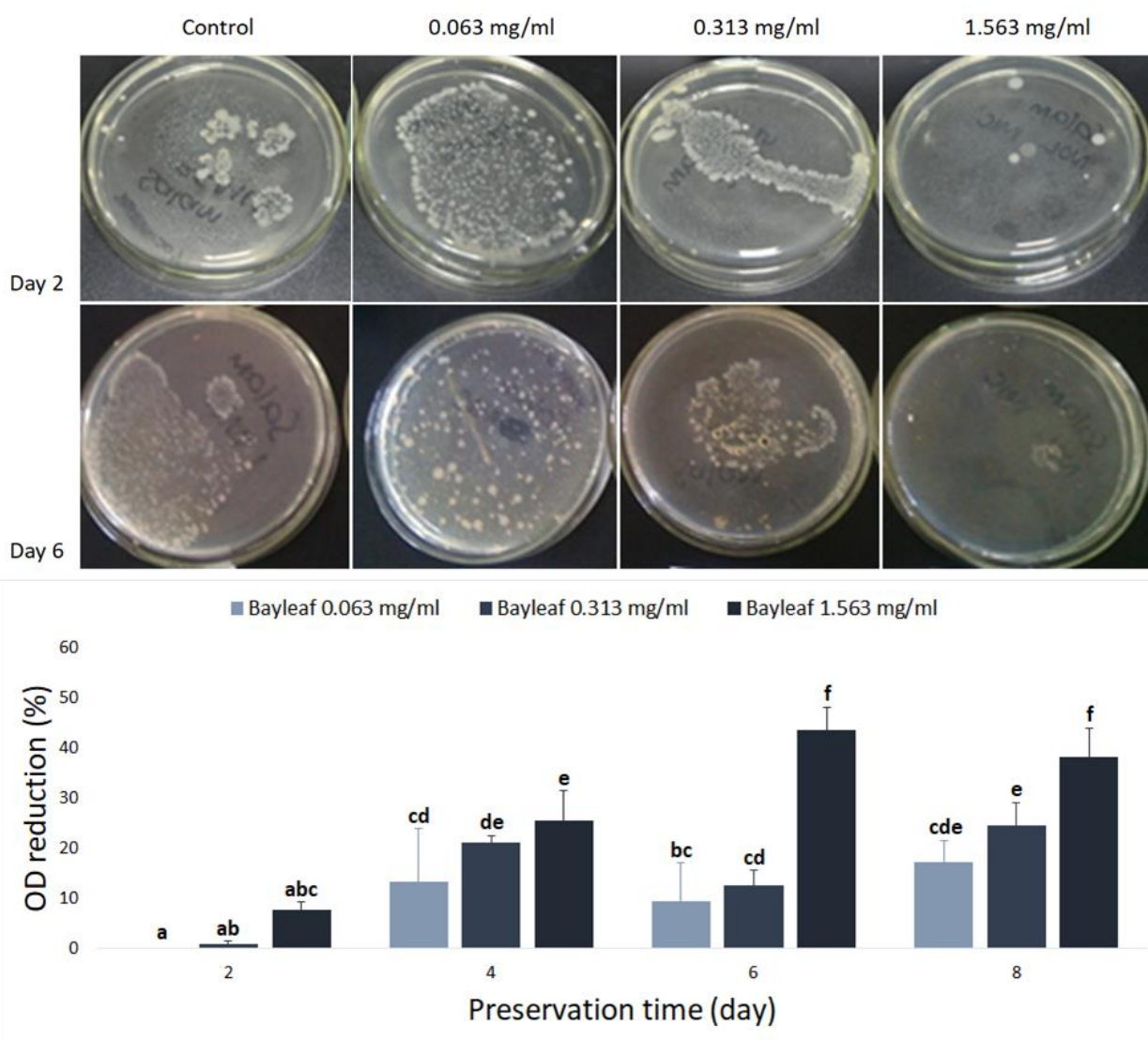


Figure 1. The effects of bay leaf essential oil on the microbial growth on tofu showed the comparison in control and those treated with various concentrations of the oil on day-2 (upper panel) and day-6 (middle panel), as well as the profile of the OD reduction during 8-day storage (lower panel). The different alphabets on different bars are statistically different.

The microbial growth on tofu treated with galangal essential oil on day-4 showed fewer colonies with the increased concentration. More colonies were observed on 6 in each tested concentration. The inhibitory activity was demonstrated by galangal oil on day-4 at 6.25 mg/ml, day-6 at 0.25 and 1.25 mg/ml, and day-8 at all tested concentrations (**Figure 2**). Galangal essential oil showed a considerably high antimicrobial activity in previous reports. One collected in Purbalingga strongly inhibited *Bacillus subtilis* with a MIC value of 0.063 mg/ml. It also showed moderate inhibitory activity against *Salmonella typhimurium*, *Staphylococcus aureus*, and *Vibrio cholera* with the MIC value of 1.0 mg/ml (Hamad *et al.*, 2016). It also showed strong bactericidal activity against *Escherichia coli*

O157:H7 (EHEC O157) by modulating membrane permeability, promoting the efflux of intracellular components, and disrupting the normal physiological metabolism (Zhou *et al.*, 2021).

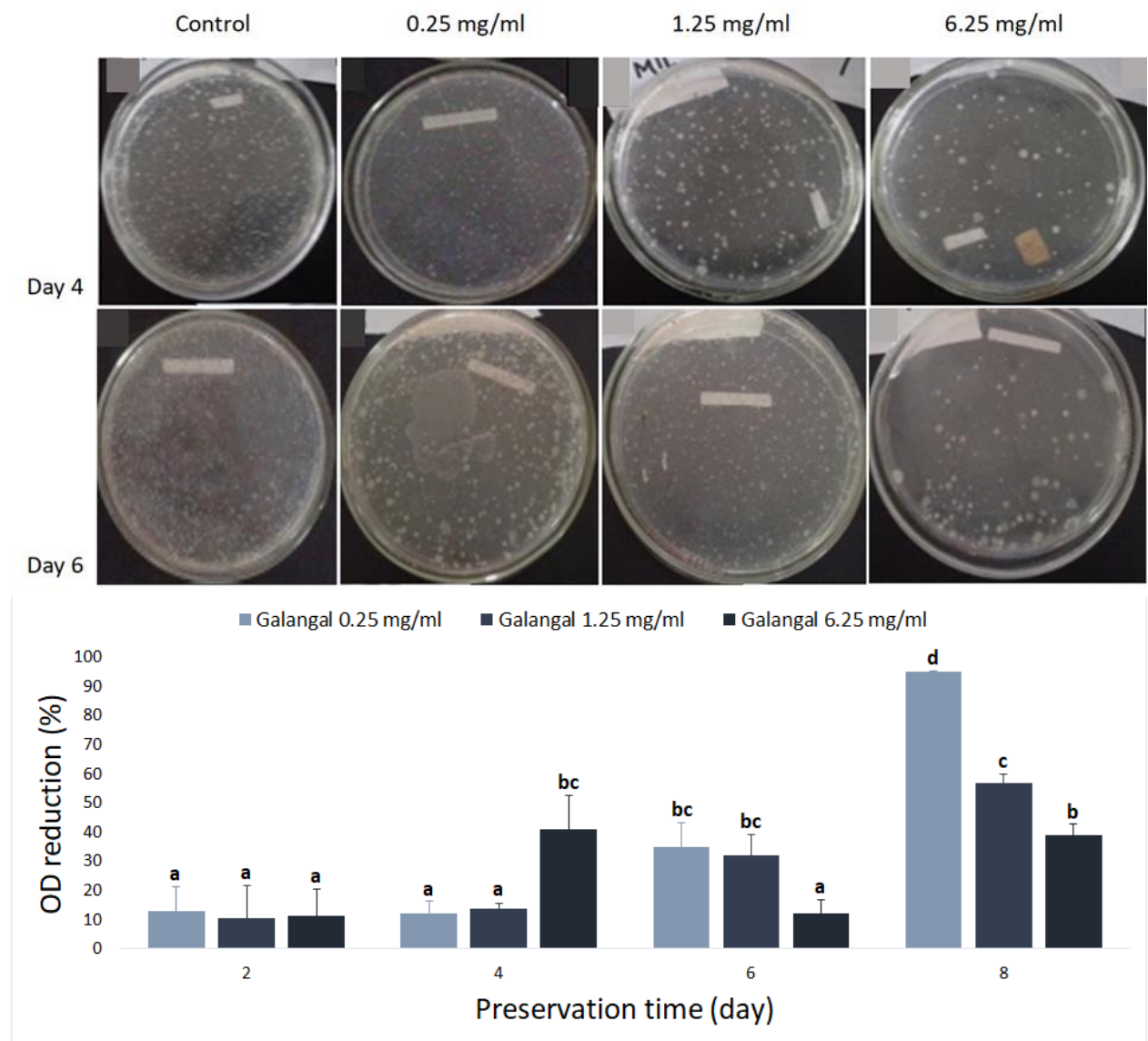


Figure 2. The effects of galangal essential oil on the microbial growth on tofu showed the comparison in control and those treated with various concentrations of the oil at on day-4 (upper panel) and day-6 (middle panel), and the profile of the OD reduction during 8-day storage (lower panel). The different alphabets on different bars are statistically different.

Many microbial colonies were visually observed on tofu treated with ginger essential oil in both day-2 and -6. Although the highest tested concentration showed a much lower number of colonies, no concentration-dependent OD reduction was observed. Quantitatively, the ginger essential oil on day-4 at 0.25 mg/ml, day-6 at 1.25 mg/ml, and day-8 at 1.25 mg/ml exerted the microbial growth inhibitory effects on tofu (Figure 3). Our results were similar to previous reports on the antimicrobial activity of

ginger essential oil. The oil obtained from ginger rhizomes collected in Purbalingga showed the antibacterial activity against *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, and *Vibrio cholera* with the MIC value of 0.5-1.0 mg/ml (Hamad *et al.*, 2016). Similarly, the Chinese and Saudi ginger oils showed a better inhibitory activity on *Staphylococcus aureus* than on *Escherichia coli* (Al-Dhahli *et al.*, 2020). The Brazilian ginger oil also showed moderate inhibitory activity against *Aeromonas* spp. (Monteiro *et al.*, 2021).

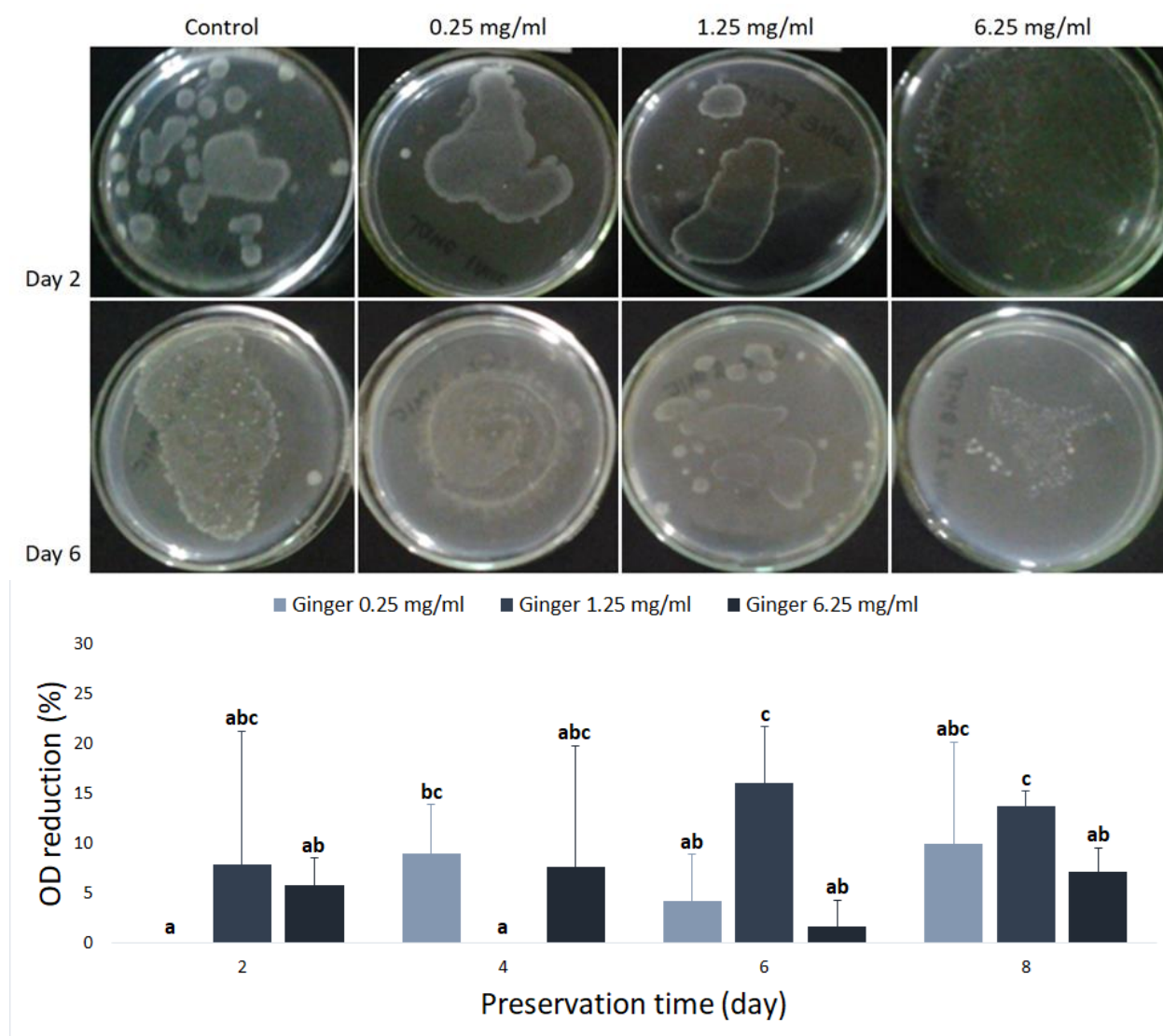


Figure 3. The effects of ginger essential oil on the microbial growth on tofu showed the comparison in control and those treated with various concentrations of the ginger oil at on day-2 (upper panel) and day-6 (middle panel), and the profile of the OD reduction during 8-day storage (lower panel). The different alphabets on different bars are statistically different.

The lemongrass essential oil visually inhibited the microbial growth on tofu until day-6 and -8, when only a few colonies were observed. The quantitative microbial growth inhibitory activity was shown by lemongrass oil on day-6 at 0.625 and 3.125 mg/ml and on day-8 at 3.125 mg/ml. The concentration-dependent inhibitory activity was observed in day-6, with the equation and OD₅₀ value of $y = 0.0107x + 16.737$ and 3.11 mg/ml, respectively (**Figure 4**). A previous study reported lemongrass oil strongly inhibited *Bacillus subtilis* and *Staphylococcus aureus* with MIC values of 0.032 and 0.125 mg/ml while moderately inhibited *Salmonella typhymurium*, *Staphylococcus aureus*, and *Vibrio cholera* at 1 mg/ml (Hamad, Nuritasari and Hartanti, 2017). Also, the Colombian lemongrass oil potentially eradicated the film formed by *Streptococcus mutans* while safe to the CHO ovary cell line (Tofiño-Rivera *et al.*, 2016).

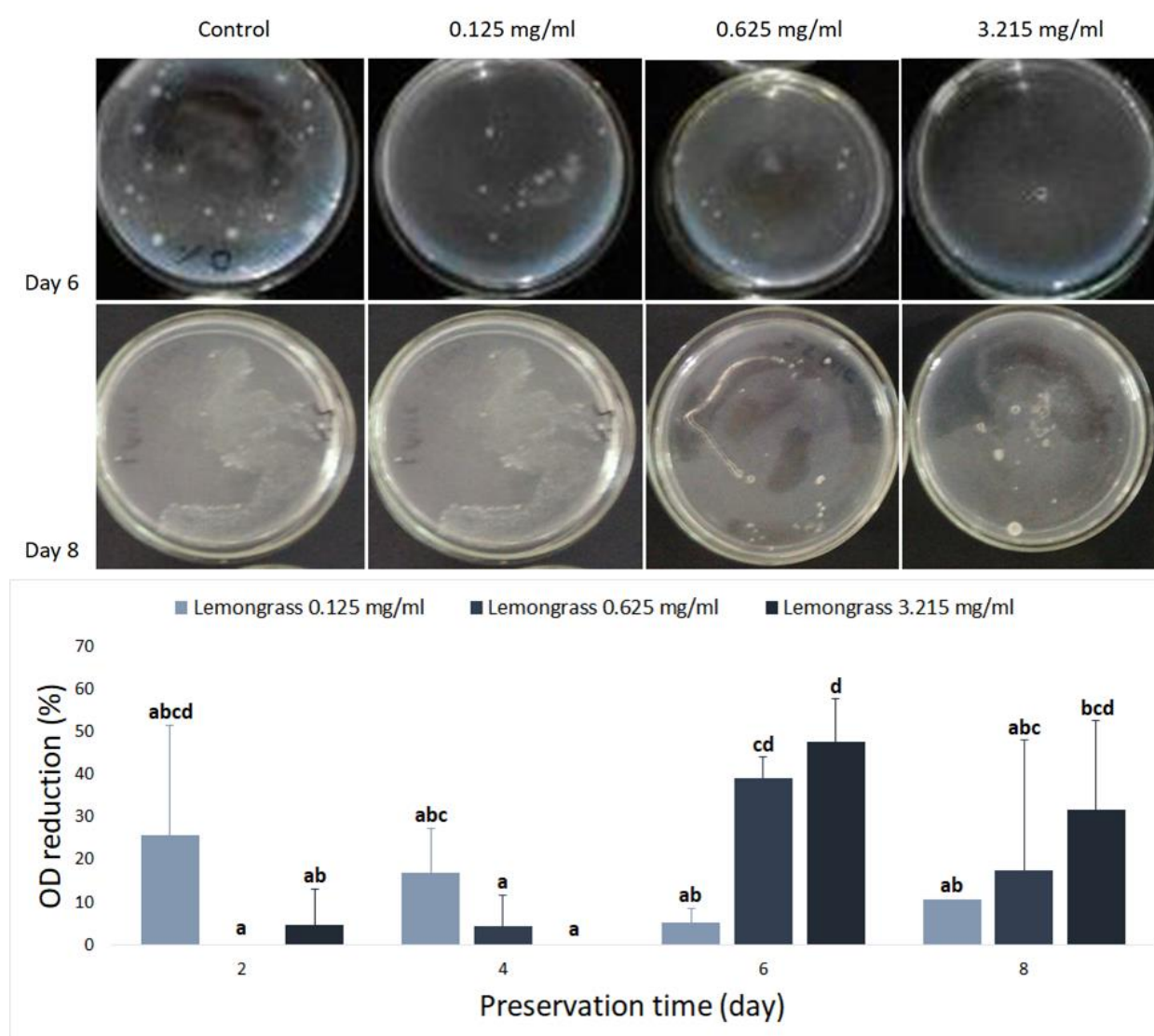


Figure 4. The effects of lemongrass essential oil on the microbial growth on tofu showed the comparison in control and those treated with various concentrations of lemongrass essential oil on day-6 (upper panel) and day-8 (middle panel), and the profile of the OD

reduction during 8-day storage (lower panel). The different alphabets on different bars are statistically different.

Albeit only showing a few microbial colonies on tofu on day-4 and -6, the lemon basil essential oil did not show any microbial growth inhibitory activity (**Figure 5**). A Croatian Ocimum study reported that lemon basil essential oil was less potent than other Ocimum plant oils. However, it still showed antimicrobial activity, particularly against *Enterococcus faecalis*, *Enterococcus faecium*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* (Carovic'-Stanko *et al.*, 2010).

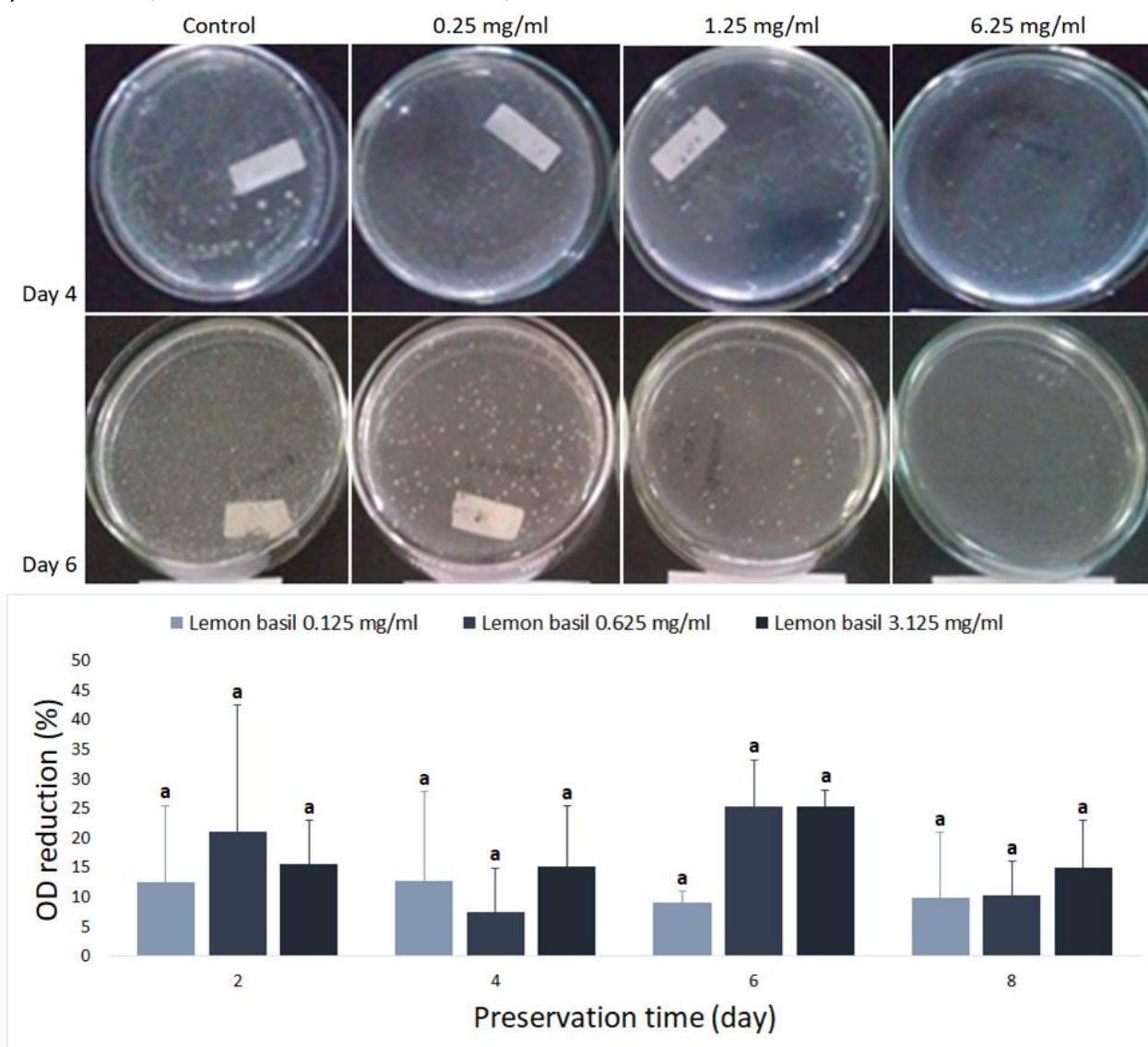


Figure 5. The effects of lemon basil essential oil on the microbial growth on tofu showed the comparison in control and those treated with various concentrations of the oil at on day-4 (upper panel) and day-6 (middle panel), and the profile of the OD reduction during 8-day storage (lower panel). The different alphabets on different bars are statistically different.

Overall, bay leaf, galangal, and ginger essential oils started to show microbial growth inhibitory at day-4, and the effect was consistently maintained until the final day of storage. Lemongrass inhibited the microbial growth on day-6 and -8, while lemon basil did not show inhibitory activity. The lowest concentration exerting the most extended period of inhibitory action of bay leaf, galangal, and ginger essential oils were 0.063, 0.25, and 1.25 mg/ml, respectively. Hence, from the overall inhibitory during 8-day storage, bay leaf oil was the most potent antimicrobial agent.

The comparison of the microbial growth inhibitory activity between the essential oils by day showed that on day-2, only lemongrass essential oil at 0.25 mg/ml exerted the inhibitory effects. On the other hand, on day-4, bay leaf essential oil at 0.313 and 1.563 mg/ml and all tested concentrations of galangal oil inhibited the microbial growth on tofu, with the better activity was exerted by galangal oil. Bay leaf oil at 1.563 mg/ml, galangal oil at 1.25 and 6.25 mg/ml, lemongrass oil at 0.65 and 3.125 mg/ml, and lemon basil oil at 0.65 3.125 mg/ml exerted the inhibitory activity on day-6. The nearly 100% inhibition was shown by galangal oil at 1.25 mg/ml. Further, on day-8, the inhibition effects were demonstrated by bay leaf oil at 1.563 mg/ml, galangal oil at all tested concentrations, and lemongrass oil at 3.125 mg/ml. Again, galangal oil at 0.25 mg/ml resulted in almost 100% inhibitory effects. Hence, from day-to-day comparison, the galangal showed the best overall microbial growth inhibitory activity (**Figure 6**).

The antimicrobial activity of a given essential oil can be linked to its chemical constituents. The phenols and aldehydes usually show the strongest individual antimicrobial activity; alcohols, esters, and ketones were intermediate, while hydrocarbons were inactive. However, the interaction between compounds of essential oils was commonly associated with the synergistic effects (Bassolé *et al.*, 2011). The presence of aldehydes in the essential oils used to preserve tofu in this study has been reported elsewhere. Bay leaf essential oil contained aldehydes, i.e., 1-decyl aldehyde, capryl aldehyde, and cis-4-decanal, while ginger, lemon basil, and lemongrass oils were rich with geranial and neral (Hamad, Saputri and Hartanti, 2016; Hamad *et al.*, 2019; A. Hamad *et al.*, 2020b).

Combining the overall inhibitory activity during 8-day storage and day-by-day comparison, bay leaf and galangal oils showed the most favorable inhibition effects on microbial growth. This might be beneficial for the overall preservation effects, as at 1.563 mg/ml, bay leaf oil prolonged the shelf life of tofu up to 6 days (Hamad *et al.*, 2020a).

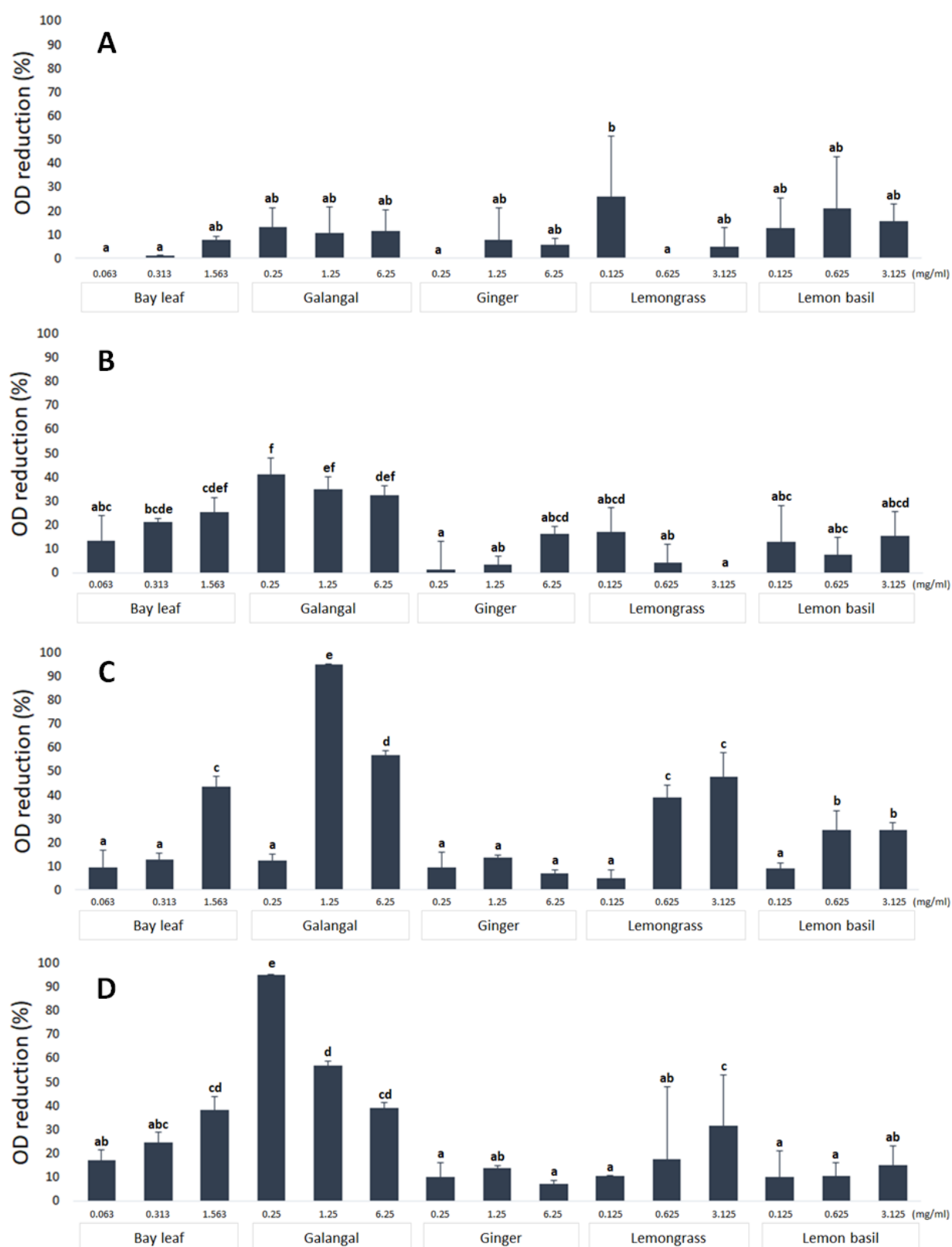


Figure 6. The profile of microbial growth inhibitory activity of selected spice essential oil on tofu at day-2 (A), day-4 (B), day-6 (C), and day-8 (B) showed that galangal and bay leaf oils exerting the most potent inhibition effects. The different alphabets on different bars are statistically different.

CONCLUSION

Essential oil of the spices showed the different microbial inhibition profiles on tofu during 8-day storage at room temperature. Bay leaf, galangal, and ginger oils inhibited microbial growth on day-4, -6, and -8; lemongrass showed inhibitory activity on day-4 and -8, while lemon basil did not show any inhibition effects. Day-by-day microbial inhibition effects confirmed the most favorable profile of bay leaf and galangal essential oils.

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