

Galangal and ginger essential oils exerted microbial growth inhibitory activity and preservation potential on tofu

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Abstract

Galangal and ginger are commonly used as spices. Interestingly, their antimicrobial activity has been reported against a wide array of microorganisms. This study aimed to evaluate the effects of essential oils of galangal and ginger on the microbial growth, physical characters, and preservation potential on tofu during 8-day storage at room temperature. The essential oils were prepared by steam-and-water distillation method, and their constituents were analyzed by Gas Chromatography-Mass Spectroscopy (GC-MS). The microbial growth on the tofu was evaluated by the indirect enumeration method. The physical characters of tofu were organoleptically observed. The preservation potentials were calculated based on the changes in tofu physical characters relative to the negative control. The main constituents of galangal essential oil were 1,8-cineole, β -farnesene, and α -pinene, while those of ginger were geranyl acetate, 1,8-cineole, and camphene. Galangal essential oil significantly inhibited microbial growth, maintained the texture of tofu until day eight, and exerted preservation potential for four days. The same preservation effect was shown by ginger essential oil at 6.26 mg/mL, however it generated a weaker microbial growth inhibitory activity and resulted in the unfavorable texture on tofu. Hence, galangal essential oil is a suitable candidate to be developed into a natural food preservative.

1. Introduction

Galangal (*Alpinia galanga* (L.) Willd., Zingiberaceae) has been long used for the traditional management of various ailments (Silalahi and Nisyawati, 2018; Hookheaw and Neamsuvan, 2019). The antimicrobial activity of galangal essential oil, among other bioactivities, has been well-described (Hamad, Alifah, Permadi *et al.*, 2016; Karunarathne *et al.*, 2020; Zhou *et al.*, 2021). Similarly, the traditional uses of ginger (*Zingiber officinale* Roscoe, Zingiberaceae) for medicinal purposes were reported in Thailand and West Java (Indonesia) (Phumthum and Balslev, 2020; Cita and Waluyo, 2021). The antimicrobial activity of ginger's essential oil was also prominently recorded (Abdullahi *et al.*, 2020; Al-Dhahli *et al.*, 2020; Monteiro *et al.*, 2021).

Both galangal and ginger are commonly used as spices in Asian countries. They are commonly used to

spice up protein-based savoury foods. Ginger is more versatile than galangal and commonly served as a refreshing herbal drink (Das *et al.*, 2020; Navia *et al.*, 2020; Antoniewicz *et al.*, 2021). Hence, most of us are familiar with their taste and aroma. The antimicrobial activity and familiarity with the sensorial aspects of a given herbal material contribute to the respective preservation and acceptability effects, which are essential for its development into a natural food preservative.

In this study, the preservation potential of galangal and ginger essential oils on the tofu was individually evaluated. Tofu is a well-known plant-based protein product that quickly deteriorates due to microbial activity (Ribeiro *et al.*, 2017). Specifically, the microbial growth inhibitory activity on tofu by both essential oils and the colour, odour, and texture of tofu in the course of 8-day storage at room temperature was determined.

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2. Materials and methods

2.1 Preparation of plant materials

The rhizomes of galangal and ginger were collected from Bawang and Pratin, Indonesia, respectively. The plants were authenticated in the Laboratory of Plant Taxonomy, Jenderal Soedirman University, Purwokerto, Indonesia, with the respective voucher reference of 137 and 140/FB.Unsoed/TaksTumbVI. The clean rhizomes were longitudinally sliced in about 0.3 cm of thickness and dried under the direct sun method for 40 hrs. The dried rhizomes were further pulverized into fine powders and kept in an airtight container until further use.

2.2 Isolation of essential oils

The essential oils of galangal and ginger were isolated from the powdered rhizomes following a previously reported by Hartanti *et al.* (2018). In brief, the plant materials were subjected to water and steam distillation for the average running time of 6 hrs.

2.3 The essential oil chemical constituent analysis

The composition of galangal and ginger essential oils was individually analyzed by Shimadzu QP2010 SE Gas Chromatography-Mass Spectroscopy (GC-MS). The separation was conducted over fused silica SH-Rxi-5Sil MS column (30 m × 0.25 mm × 0.25 μm) with helium as carrier gas at a flow rate of 1 mL/min. The temperature of the injector and detector was 280 and 230°C, respectively. A total of 1 μL of diluted essential oil (100 ppm in n-hexane) was injected, and the analysis was run for 60 mins. The fragments of the constituents of the essential oils were identified based on their similarity with those available on the Wiley 9.0.1 mass spectral database (Hamad, Djaliil, Saputri *et al.*, 2020).

2.4 Preservation potential evaluation

2.4.1 Treatment on tofu

The firm white tofu was purchased from a local market in Arcawinangun, Purwokerto. They were prepared for the experiments and stored as detailed in a previous report with modifications (Hamad, Ramadhan, Dewi *et al.*, 2020). In brief, the already-surface-cleaned cubed tofu was immersed in the sterilized water containing galangal and ginger essential oils at 0.25, 1.25, and 6.25 mg/mL. The tofu was further stored at room temperature for eight days with an evaluation time point of 2 days.

2.4.2 Microbial growth inhibitory activity analysis

The microbial growth on tofu during storage was evaluated indirectly by the previously described optical density (OD)-based enumeration method. At each evaluation time point, the bacterial growth on the tofu

was assessed by transferring a tofu cube into 25 mL of sterile nutrient broth (NB) and gently stirring them for a minute. One mL of suspension was transferred into 9 mL of sterile NB and incubated at 37°C for 24 hrs. The optical densities of cultured microbial suspension were recorded with a Shimadzu A109349 UV-Vis spectrophotometer at a wavelength of 600 nm (Hamad, Ramadhan, Dewi *et al.*, 2020). The OD-reduction of each sample was calculated according to Equation 1.

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

2.4.3 Preservation potential analysis

The physical characters of tofu, such as the color, odor, texture, and formation of slime, were subjectively observed at each evaluation time point. The fresh tofu of the same origin was used as the reference in each observation. The preservation potential of galangal and ginger essential oil was based on comparing the physical character changes on tofu in the sample and control groups as previously reported (Hamad, Ramadhan, Dewi *et al.*, 2020).

2.5 Statistical analysis

The effect of each essential oil concentration on the OD of the cultured tofu and their OD reduction was analyzed by one-way ANOVA. Mean comparison between concentrations was conducted by Duncan's post-hoc test. The difference was considered significant at a p-value > 0.05. All the analyses were performed on the IBM SPSS Statistic program ver. 20 (IBM, USA).

3. Results and discussion

There were nineteen compounds identified in galangal essential oil, with 1,8-cineole, β-farnesene, α-pinene, geranyl acetate, and 4-allylphenyl acetate as the major constituents. Galangal essential oil has a higher proportion of oxygenated compounds (69.41%) than that of hydrocarbons (29.77%) (Table 1). The oxygenated compounds showed a better antimicrobial potential over the hydrocarbons. Hence, 1,8-cineole, geranyl acetate, 4-allylphenyl acetate, and 3-phenylprop-2-enyl acetate might be responsible for the antibacterial activity of galangal essential oil. These result agreed with a report on galangal essential oil from Chiang Mai (Thailand) and Selangor (Malaysia), which mainly contained 1,8-cineole (37.43% and 61.9%, respectively) (Abdullah *et al.*, 2015; Khumpirapang *et al.*, 2018). However, constituents of galangal essential oil are greatly varied according to the sources of the distilled plant materials. Eucalyptol, carotol, camphor, and limonene were the major compounds in galangal collected in Odisha, India, while the oil collected earlier in Chiang Mai mainly contained piperitenone, limonene, and pentadecane

(Prakatthagomol *et al.*, 2011; Singh *et al.*, 2021).

2019; Al-Dhahli *et al.*, 2020).

Table 1. Chemical constituents of essential oils of galangal

Rt (min)	Constituents	Area (%)
12.934	α -Pinene	9.47
14.925	Sabinene	0.58
15.058	β -Pinene	1.72
15.919	β -Myrcene	0.69
17.972	1,8-Cineole	56.49
19.196	α -Terpinene	0.32
28.737	Bornyl acetate	0.95
31.038	4-Allylphenyl acetate	4.09
31.116	Citronellyl acetate	0.27
32.442	Geranyl acetate	4.33
32.988	Methyl eugenol	0.48
34.853	β -Farnesene	10.38
35.743	Calarene	0.26
36.11	Dodecane	3.38
36.47	α -Farnesene	1.05
37.031	Eugenol acetate	0.72
40.788	3-Phenylprop-2-enyl acetate	2.08
41.312	7-Hexadecene	0.42
41.534	1-Tetradecene	1.5
Total area		99.18
Hydrocarbons		29.77
Oxygenated compounds		69.41

The detailed constituents of ginger essential oil used for tofu preservation in this study have been reported earlier (Hamad, Mentari, Djalil *et al.*, 2016). In short, it contained 14 compounds and mainly were oxygenated compounds (60.42%), with some aldehydes as the primary antimicrobial compounds. Geranyl acetate, 1,8-cineole, camphene, geranial, and zingiberene were identified as the main constituents. The oil in this study contained similar major compounds as those of two cultivars grown in Sikkim, India, which also mainly constituted of geranyl acetate, zingiberene, and geranial (Sasidharan *et al.*, 2012). As in galangal essential oil, however, the composition of ginger essential oils is also widely varied across geographical regions. For example, the ginger collected in Cairo, Egypt, primarily contained curcumene and linalool. Ginger oil from China was mainly characterized by zingiberene, phellandrene, and curcumene, while that from Saudi was found to be rich in curcumene, zingiberene, and bisabolene (Fahmi *et al.*,

The concentration of galangal and ginger essential oils significantly affected the OD of the cultured tofu ($p = 0.000$). The OD in all groups was comparable on day two and indicated no bacterial growth inhibitory activity by all concentrations of galangal oil. The overall OD of the cultured tofu treated with galangal essential oil decreased on day four but gradually increased with time until the final day of storage. Galangal essential oils at the concentrations of 1.25 and 6.25 mg/mL showed a significantly lower OD than the control, which indicated their potential to reduce microbial growth on tofu. The decreased OD of the cultured tofu was in a concentration-dependent manner, in which a concentration of 6.25 mg/mL generated statistically lowest OD than the lower concentrations. On the other hand, the OD of tofu treated with ginger essential oils in all concentrations were equal to the control at day 2 and 4. On day six, the highest concentration of ginger oil showed the microbial growth inhibitory activity, and the same effect was also shown by all tested concentrations on day 8 (Table 2).

The OD reduction relative to the negative control of each essential oil was calculated to compare the antimicrobial efficacy of galangal and ginger oil. Galangal oil generally showed a better microbial growth inhibitory activity over that of ginger. The most prominent inhibitory activity by galangal essential oil was mainly shown on days four and six when the concentration-dependent antimicrobial activity was observable (Figure 1).

The higher antimicrobial activity of galangal essential oil over ginger was previously reported in Purbalingga, Indonesia (Hamad, Mentari, Djalil *et al.*, 2016). The bactericidal effects of this oil have also been demonstrated in various food-borne bacteria (Prakatthagomol *et al.*, 2011). The antibacterial effects of galangal were mediated by the impairment of the outer and inner membranes and coagulation cytoplasm, with MIC on *S. aureus* was 0.325 mg/mL. The disrupted intracellular physiological metabolism was reported to

Table 2. The profile of OD of cultured tofu during 8-day storage under ambience temperature

Treatment group	OD			
	Day 2	Day 4	Day 6	Day 8
Galangal negative control	0.570 \pm 0.049	0.318 \pm 0.042	0.499 \pm 0.033	0.679 \pm 0.024
Galangal 0.25 mg/mL	0.496 \pm 0.048	0.288 \pm 0.041	0.443 \pm 0.045	0.597 \pm 0.030
Galangal 1.25 mg/mL	0.491 \pm 0.011	0.187 \pm 0.036*	0.324 \pm 0.042*	0.461 \pm 0.049*
Galangal 6.25 mg/mL	0.500 \pm 0.027	0.016 \pm 0.001*	0.216 \pm 0.015*	0.416 \pm 0.028*
Ginger negative control	0.412 \pm 0.026	0.656 \pm 0.107	0.900 \pm 0.030	1.185 \pm 0.058
Ginger 0.25 mg/mL	0.486 \pm 0.010	0.670 \pm 0.167	0.854 \pm 0.033	1.096 \pm 0.098*
Ginger 1.25 mg/mL	0.424 \pm 0.000	0.648 \pm 0.133	0.871 \pm 0.054	0.981 \pm 0.066*
Ginger 6.25 mg/mL	0.411 \pm 0.018	0.594 \pm 0.071	0.777 \pm 0.014*	1.085 \pm 0.028*

The asterisk represented a significantly lower OD compared to the respective negative control, evaluated by one-way ANOVA and Duncan's test at p -value < 0.05

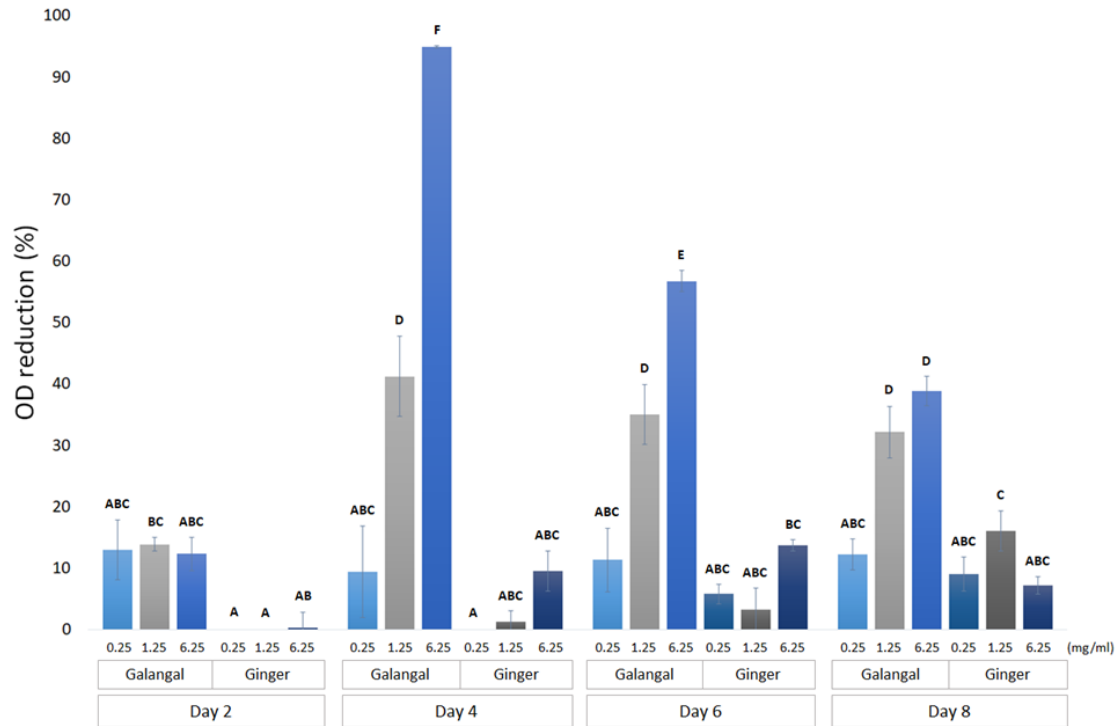


Figure 1. The profile of OD reduction in tofu treated with various concentrations of galangal and ginger essential oils. Bars with different notations are significantly different, evaluated by one-way ANOVA and Duncan's test at p -value < 0.05.

follow the morphological damage subsequently (Onmetta-aree *et al.*, 2006; Zhou *et al.*, 2021). A commercial ginger essential oil from Indonesia showed a weak antimicrobial activity against food-borne pathogens, with the MIC value of 0.39-6.25% (Budiaty *et al.*, 2018). However, China-originated ginger essential oil exerted antibacterial activity against food-borne *Escherichia coli* and *Staphylococcus aureus* by the mechanism of disrupting the cell membrane (Wang *et al.*, 2020). A similar effect was also demonstrated by ginger essential oil collected in Pahang, Malaysia, in which the oil suppressed the growth of phytopathogens at the concentration of 100 μ L (Abdullahi *et al.*, 2020).

The metabolic activity of the microorganisms broke down the available macromolecules in tofu and caused changes in taste, odor, and texture, as well as the formation of slime on the surface (Wang *et al.*, 2019). The physical properties of the stored tofu are presented in Table 3. Tofu in negative control started to show changes on day 4, in which the off-odour and slime were detected. They started to be friable on day 6. However, the colour of the tofu maintained white until the final day of storage. Tofu treated with galangal essential oil developed a specific galangal aroma that was noticeable since day 2. The galangal aroma on the tofu was stronger with the increased concentration. The colour of tofu remained white as those in the negative control. The off-texture and slime in tofu started to be noticeable on day 6 in those treated with galangal essential oil in the concentration of 0.25 mg/mL. However, both higher concentrations could maintain tofu from off-texture and

slime-free until the final day of storage.

As in galangal essential oil-treated tofu, the color of tofu treated with ginger essential oil was not changed. They were white at the beginning of the study and remained unchanged until the final day of storage. The use of ginger essential oil changed the fresh odor of tofu to the aromatic scent of ginger, in which the higher concentration generated a more pungent aroma. In the lower and medium concentration ginger essential oil-treated tofu, the off-odour was detected on day 8, while the highest concentration managed to maintain the initial aromatic odour in tofu until the final day of storage. The use of ginger essential oil delayed the start of off-texture in tofu, which was observable on days 6 and 8 in those treated with oils at concentrations of 0.25 and 1.25 mg/mL, respectively. Tofu treated with ginger oil at the highest concentration remained firm until day 8. The higher the ginger essential oil concentration, the longer time was needed to observe the presence of slime on tofu.

The preservation potential of essential oils was calculated based on a comparison between a given physical parameter change of a particular concentration of the oil to that of the negative control. The galangal essential oil in a 0.25 mg/mL concentration showed 2-day slime-free-based preservation potential, while other groups maintained it six days. All groups, however, showed equal texture-based preservation potential of 4 days. On the other hand, the highest concentration of ginger essential oil showed the best preservation potential of tofu in all evaluated parameters. The equal

Table 3. The physical characters of tofu during 8-day storage

Tofu treated with	Characters	Preservation time (day)			
		2	4	6	8
Sterile water	Color	White	White	White	White
	Odor	Fresh tofu	Off-odor	Off-odor	Off-odor
	Texture	Firm	Firm	Off-texture	Off-texture
	Slime	No	Yes	Yes	Yes
Galangal 0.25 mg/mL	Color	White	White	White	White
	Odor	Aromatic galangal	Aromatic galangal	Off-odor	Off-odor
	Texture	Firm	Firm	Off-texture	Off-texture
	Slime	No	No	Yes	Yes
Galangal 1.25 mg/mL	Color	White	White	White	White
	Odor	Aromatic galangal +	Aromatic galangal +	Aromatic galangal +	Aromatic galangal +
	Texture	Firm	Firm	Firm	Firm
	Slime	No	No	No	No
Galangal 6.25 mg/mL	Color	White	White	White	White
	Odor	Aromatic galangal ++	Aromatic galangal ++	Aromatic galangal ++	Aromatic galangal ++
	Texture	Firm	Firm	Firm	Firm
	Slime	No	No	No	No
Ginger 0.25 mg/mL	Color	White	White	White	White
	Odor	Aromatic ginger	Aromatic ginger	Aromatic ginger	Off-odor
	Texture	Firm	Firm	Off-texture	Off-texture
	Slime	No	Yes	Yes	Yes
Ginger 1.25 mg/mL	Color	White	White	White	White
	Odor	Aromatic ginger +	Aromatic ginger +	Aromatic ginger +	Off-odor
	Texture	Firm	Firm	Firm	Off-texture
	Slime	No	No	No	Yes
Ginger 6.25 mg/mL	Color	White	White	White	White
	Odor	Aromatic ginger ++	Aromatic ginger ++	Aromatic ginger ++	Aromatic ginger ++
	Texture	Firm	Firm	Firm	Firm
	Slime	No	No	No	No

preservation potential of 6 days was shown by those at 1.25 and 6.25 mg/mL concentrations. The lowest concentration of ginger EO did not show potential preservation in terms of slime formation and texture but could delay the start of off-odor for four days. When the first change in any parameters was taken into accounts, the best overall preservation potential was shown by the galangal essential oils in the concentration of 1.25 and 6.25 mg/mL and ginger at a concentration of 6.25 mg/mL (4 days), while the lowest concentration of both essential oils did not show any preservation effects (Figure 2).

Our result was in agreement with previously reported data on the preservation effectiveness of galangal on the various food matrix. Galangal water extracts at concentrations of 10% reduced the bacterial count on milkfish with smell, color, and texture liked by consumers. Other studies mentioned that galangal juice extended the shelf life of milkfish up to 46 hrs (Florensia *et al.*, 2012; Inayah and Bestari, 2018). In addition, galangal juice at a concentration of 20% showed a

preservation effect and could reduce the use of formalin without changing the sensorial aspects of white shrimp under cold storage (Jannah *et al.*, 2014). Also, galangal was the most promising Thai spice screened for natural food preservative, which exerted its preservation potential via antimicrobial and antioxidant activities (Wanangkarn *et al.*, 2018).

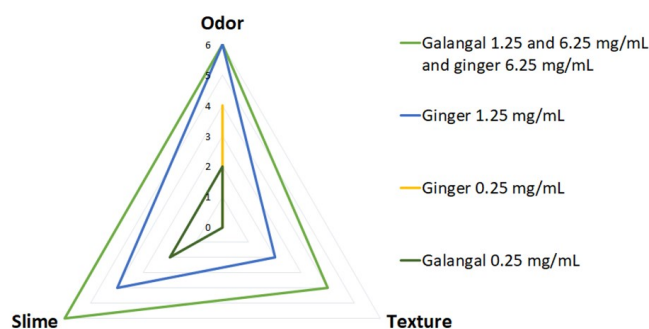


Figure 2. The profile of preservation potential of galangal and ginger essential oils

Ginger essential oil showed a weaker microbial growth inhibitory activity but was still able to preserve tofu. Other reports supported the positive effects of

ginger essential oil on the preservation of various foods, mainly when the study was set under refrigerated conditions. Ginger oleoresin prolonged the shelf life of the fresh sugarcane juice without any noticeable changes in the microbial load and sensorial aspects after 35-day refrigerated storage (Murthy *et al.*, 2015; Ramachandran *et al.*, 2017). The encapsulated ginger oleoresin also demonstrated similar effects on fresh milk (Krisanti *et al.*, 2017). The red ginger essential oil reduced the viability of the inoculated bacteria and improved the overall acceptance of the fortified fresh soft cheese (Ahmed *et al.*, 2021).

4. Conclusion

Our result suggested that galangal essential oil significantly inhibited microbial growth and exerted preservation potential on tofu for four days. Albeit showed the same preservation potential; ginger essential oil weakly inhibited microbial growth on tofu. The microbial growth inhibitory activity and preservation potential of both essential oils on tofu were likely related to the high concentrations of oxygenated compounds.

Conflict of interest

The authors declare no conflict of interest.

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