



## Combination of Clove and Lemon Basil Essential Oils for Preservation of Chicken Meat

D. Hartanti <sup>1\*</sup>, N.A. Septiyaningrum <sup>1</sup>, A. Hamad <sup>2</sup>

1. Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Muhammadiyah Purwokerto, Banyumas, Indonesia

2. Department of Chemical Engineering, Faculty of Engineering and Science, Universitas Muhammadiyah Purwokerto, Banyumas, Indonesia

### HIGHLIGHTS

- Eugenol,  $\beta$ -caryophyllene, and  $\alpha$ -humulene were the dominant constituents of clove Essential Oil (EO).
- Estragol, linalool, E-citral, and Z-citral were the main compounds of lemon basil EO.
- The combinations of clove and lemon basil EOs showed a better microbial inhibitory compared to the single use.

#### Article type

Original article

#### Keywords

Syzygium  
Ocimum  
Oils, Volatile  
Meat  
Food Preservation

#### Article history

Received: 1 Nov 2019  
Revised: 3 Mar 2020  
Accepted: 15 Mar 2020

#### Acronyms and abbreviations

EO=Essential Oil  
OD=Optical Density

### ABSTRACT

**Background:** Clove and lemon basil are widely used in Indonesian culinary and known for their antimicrobial properties. This study was designed to identify the chemical constituents of single clove and lemon basil Essential Oils (EOs) as well as determine the potential of the combinations of both EO for preserving chicken meats.

**Methods:** The compositions of clove and lemon basil EOs were evaluated with Gas Chromatography-Mass Spectrometer. Three different concentration ratios of the combination of clove and lemon basil EOs (2:0.2, 1:1, and 0.1:2% v/v) were prepared along with single clove and lemon basil EOs in a concentration of 1% v/v. Their potential preservation effect was evaluated by observing the reduction of the microbial growth on the meats by evaluating Optical Density (OD) of cultured bacterial suspensions during 15 days of refrigerated storage. Statistical analyses were conducted by SPSS Statistics v. 20.

**Results:** The major constituents of clove EO were eugenol,  $\beta$ -caryophyllene, and  $\alpha$ -humulene, while those of lemon basil were estragol, linalool, E-citral, and Z-citral. Both treatment groups and storage time affected significantly on ODs of the samples. Combination of these two EOs, particularly at the optimum ratio of 1:1%, showed the best microbial inhibitory activity, and delayed the sensorial changes of the meats for 12 days.

**Conclusion:** The combinations of clove and lemon basil EOs showed a better microbial growth inhibitory activity and preservation potential than those of the single use. This meat preservation effects might be related to the presence of high fractions of oxygenated compounds, mainly eugenol, Z-citral, and E-citral in both clove and lemon basil EOs.

© 2020, Shahid Sadoughi University of Medical Sciences. This is an open access article under the Creative Commons Attribution 4.0 International License.

### Introduction

Chicken meat is one of the most popular meats and commonly consumed worldwide (Erian and Phillips,

2017; González-García et al., 2018). However, it contains a high amount of water, proteins, and fats that enables the

\* Corresponding author (D. Hartanti)

✉ E-mail: dwihartanti@ump.ac.id

ORCID ID: <https://orcid.org/0000-0002-6600-7251>

**To cite:** Hartanti D., Septiyaningrum N.A., Hamad A. (2020). Combination of clove and lemon basil essential oils for preservation of chicken meat. *Journal of Food Quality and Hazards Control*. 7: 84-93.

growth of microorganisms (Wang et al., 2017). In order to prolong its shelf life and minimize the risk of food-borne illness, a preservation process is required for chicken meat (Dave and Ghaly, 2011).

Chemical preservation efforts on chicken meat have been evaluated by many researchers. The applications of chitosan, nisin, and Essential Oils (EOs) for the mentioned purpose have gained significant interests (Al-Hijazeen et al., 2016; Alnoman et al., 2017; Raeisi et al., 2016). EOs of spices and herbs with profound antimicrobial activity are engaging to be explored further for our familiarity with their taste and aroma in our daily meals. The antimicrobial activity of some plant EOs have been considered beneficial in reducing the growth of the spoilage microorganisms on the foods, and hence protect them from degradation process and eventually result in the improved shelf life (Hyldgaard et al., 2012; Pandey et al., 2017).

Clove (*Syzygium aromaticum* L., Myrtaceae) and lemon basil (*Ocimum x africanum* Lour., Lamiaceae) are commonly used in Indonesian culinary. Clove EO is well known as an antimicrobial agent against bacteria and fungi (Hamad et al., 2017; Naveed et al., 2013; Sethi et al., 2013). It has been evaluated in food preservation studies and resulted in moderate to strong efficacy (Hamad and Hartanti, 2015). On the other hand, the antimicrobial activities of lemon basil EO were reported against various microorganisms, including yeasts, common bacterial pathogens, and also the food spoilage ones (Carovic'-Stanko et al., 2010; Kaya et al., 2008; Khalil, 2013).

While the single-use of EOs generally resulted in the relatively low to moderate antimicrobial potencies, their uses as a combination were believed to generate the synergistic activities (Bassolé and Juliani, 2012). This synergistic effect is desirable in the application of EO for food preservatives since the lower concentration of respective EOs will not affect the flavor, aroma, and reception level of the food. Combining EOs is expected to increase their antimicrobial activity but also retain the character of the preserved foodstuffs (Rialita et al., 2015).

Although, the antimicrobial activities and further food preservation potentials of both clove and lemon basil EOs have been described before, most of the reports evaluated them in the single-use. There were no reports on their use in a combination yet for meat preservation. Thus, this study was conducted to analyze the chemical constituents of clove and lemon basil EOs as well as evaluate the potential of their combinations as the natural preservative of chicken meats.

## Materials and methods

### Preparation of plant materials

Leaves of clove and aerial parts of lemon basil were respectively collected from Pemalang and Banyumas, Central Java, and Indonesia. The plant materials were authenticated at the Laboratory of Plant Taxonomy, Universitas Jendral Soedirman, Purwokerto, Central Java, and Indonesia. Both plants were dried under the direct sunlight (8 am-3 pm) for 4 days.

### Extraction of EOs

Dried clove and lemon basil were separately distilled with steam and water distillation, as previously conducted by Hartanti et al. (2018). Each distillation process was run for the average time of 7 h. The remained water was absorbed with  $\text{Na}_2\text{SO}_4$  and stored in a tightly closed amber vial at a temperature of 4 °C until further analysis.

### Chemical constituents of EOs

EOs were analyzed using a hyphenated Gas Chromatography-Mass Spectrometer (GC-MS, QP2010 SE, Shimadzu, Japan) utilized a dimethylpolysiloxane column (SH-Rxi-5Sil MS, Shimadzu, Japan). The condition of the separation and ionization of the separated constituents followed a previously reported method described by Hartanti et al. (2018). The identification of constituents was conducted by comparing the mass spectrum of each constituent with those available in a spectral library (Wiley 6.0, Wiley Science Solution, USA).

### Preparation of experimental groups

The fresh chicken meats used in this study were obtained from a local market at Purwokerto, Indonesia. The breast meat cubes ( $1 \times 1 \times 1$  cm) were immersed in sterile water at a temperature of  $100 \pm 5$  °C for 5 s to reduce the number of microorganisms on their surface. The preservation potential of the combination of clove and lemon basil EOs were evaluated by following a previous report (Hartanti et al., 2018). In brief, three different concentration ratios of the combination of clove and lemon basil EOs (2:0.2, 1:1, and 0.1:2% v/v) were prepared along with single clove and lemon basil EOs in a concentration of 1% v/v. They were prepared by dissolving EOs in the equal volume of dimethyl sulfoxide (DMSO) and making the final volume of 500 ml with sterile water. DMSO in the water at the concentration of 2% v/v was used as the negative control. The cubed chicken meats were immersed in the EO preparations for 1 min and aseptically

kept under refrigerated storage with a temperature of 5±2 °C for 15 days. The indirect enumeration of bacterial growth on the meats and the physical characters of the preserved meats were carried out in 3-day interval in days 0, 3, 6, 9, 12, and 15.

#### *Microbial analysis*

On the respective observation days, the bacterial growth on meats was analyzed with an indirect enumeration method, that each cube of meats was put in an Erlenmeyer flask containing 25 ml of sterile nutrient broth medium (Difco, USA), and then manually homogenized by shaking for a min. One ml of suspension was transferred into 9 ml of sterile nutrient broth and then put in an incubator (KS40, IKA, Malaysia) at a temperature of 37 °C for 24 h. The Optical Density (OD) of cultured bacterial suspensions were recorded with the UV-Vis spectrophotometer (UV-1240, Shimadzu, Japan) at a wavelength of 600 nm.

#### *Sensorial analysis*

The sensorial characters of the preserved meats, including odor, texture, and formation of slime were subjectively evaluated by a panel of trained individuals. Those parameters were organoleptically observed soon after the meats were taken from the refrigerated storage. The fresh chicken meats were used as the reference in each observation.

#### *Theoretical relative volume calculation*

The theoretical relative volume of oxygenated compounds and aldehydes-phenols were calculated based on the area of the oxygenated compounds in the GC-MS chromatogram and the volume of each EOs in a given combination (Rialita et al., 2015). A formula as follow was used:

$$\text{Theoretical relative volume} = (Aa \times Va) + (Ab \times Vb)$$

Where  $Aa$  was the total area of peaks of given compounds in clove EOs,  $Va$  was the volume of clove EOs used in a particular combination;  $Ab$  was the total area of peaks of given compounds in lemon basil EOs,  $Vb$  was the volume of clove EOs used in a particular combination.

#### *Statistical analysis*

All experiments were done in triplicate. The effects of both treatment group and storage time to the OD of the samples were analyzed by two-way analysis of variance (ANOVA) followed by Duncan's tests. The  $p$  value less than 0.05 between parameter groups was considered as significant differences. The reduction in the microbial count of cultured bacterial suspensions in culture media

was calculated by subtracting the value of the ODs obtained from each treatment group with that of sterile water in culture media as the negative control on the same observation day. The differences between the mean of the OD reduction among the treatment groups were further analyzed by two-way analysis of variance followed by Duncan's tests, in which the significant difference was set at  $p<0.05$ . The OD reduction was denoted as 0 if the OD of a treatment group was higher than the negative control. All statistical analyses were conducted with the general procedures of IBM SPSS Statistics v. 20 (IBM, USA).

## **Results**

The steam and water distillation of clove and lemon basil dried plant materials produced EOs with a yield of 0.84 and 0.76%, respectively. In total, 17 chemical constituents were detected in the clove EO, mainly eugenol, trans-β-caryophyllene, and α-humulene. On the other hand, lemon basil EO constituted of 11 constituents with estragole, linalool L, E-citral, and Z-citral as major compounds. In addition, E-citral, eugenol, α-copaene, trans-β-caryophyllene, α-humulene, δ-cadinene, and also caryophyllene oxide were detected in both EOs (Table 1).

The bacterial growth on the meats during storage was enumerated by an indirect method utilizing OD of the culture of the meats. Our result showed that both treatment groups ( $p=0.000$ ) and storage time ( $p=0.000$ ) affected the ODs of the cultured samples. The simple main effects analysis regarding the treatment groups showed that the ODs of negative control were comparable to those of single clove and lemon basil EOs ( $p=0.612$ ). However, OD of the samples in day 0 was significantly lower than the other storage period, i.e. days 3, 9, and 15. The analysis of simple main effects showed that there were no differences between days 3, 9, and 15 ( $p=0.098$ ) (Table 2).

Sensorial characteristic of different meat groups during storage is shown in Table 3. The meats treated in the negative control started to be off-odor, softer; and showed the presence of slime on day 3. Regarding sensorial characteristics, the preservation potential of the combination of clove and lemon basil EOs in a ratio of 0.2:2% was 9 days, while those in the ratios of 2:0.2 and 1:1% were 12 days, respectively (Figure 1).

The theoretical relative volume of oxygenated compounds, as well as aldehydes and phenols in each concentration ratio of the combination of clove and lemon basil EOs were calculated in order to find the relationship between the preservation potentials with their chemical compounds. The theoretical relative volume of oxygenat-

ed compounds in combinations of both EOs at all ratios was found to be higher than in single use, with the highest value was shown by the ratio of 0.2:2 and 1:1%. The

highest theoretical relative volume of aldehydes and phenols were shown by the combination of EOs in the ratio of 2:0.2%, followed by 1:1% (Figure 2).

**Table 1:** Chemical constituents of clove and lemon basil essential oils

Chemical constituents	Retention time (min)	Area (%)	
		Clove	Lemon basil
3,3-Dimethylpentane	2.075	0.14	-
2-Methylhexane	2.136	0.22	-
2,3- Dimethylpentane	2.170	0.08	-
3- Methylhexane	2.209	0.21	-
3-Ethylpentane	2.301	0.17	-
n-Heptane	2.420	0.09	-
Methylcyclohexane	2.737	0.03	-
1-Ethyl butyl hydroperoxide	8.869	0.05	-
Linalool oxide	15.795	-	0.35
Linalool	17.830	-	18.9
Estragole	24.372	-	55.78
Z-Citral	27.028	-	6.54
E-Citral	28.916	0.05	10.06
$\alpha$ -Cubebene	32.604	0.56	-
Eugenol	32.772	67.0	0.64
$\alpha$ -Copaene	33.721	0.56	0.15
Trans- $\beta$ -caryophyllene	35.297	25.64	1.51
$\alpha$ -Humulene	36.505	2.66	0.45
$\Delta$ -Cadinene	37.063	0.07	0.11
$\beta$ -Farnesene	37.457	0.15	-
Caryophyllene oxide	40.270	0.86	0.26
Total area		98.54	94.75
Total hydrocarbon compounds		29.93	2.22
Total oxygenated compounds		68.61	92.53
Total phenol and aldehyde compounds		67.05	17.24

**Table 2:** The Optical Density (OD) of cultured chicken meats treated with clove and lemon basil essential oils during storage

Treatments	OD					
	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15
Negative control	0.681 $\pm$ 0.080 <sup>Ba</sup>	1.324 $\pm$ 0.053 <sup>Bc</sup>	0.947 $\pm$ 0.077 <sup>Bb</sup>	1.286 $\pm$ 0.051 <sup>Bc</sup>	0.811 $\pm$ 0.042 <sup>Bb</sup>	1.200 $\pm$ 0.212 <sup>Bc</sup>
Clove 1%	0.849 $\pm$ 0.182 <sup>Ba</sup>	1.074 $\pm$ 0.065 <sup>Bc</sup>	0.942 $\pm$ 0.012 <sup>Bb</sup>	1.191 $\pm$ 0.048 <sup>Bc</sup>	0.772 $\pm$ 0.047 <sup>Bb</sup>	1.300 $\pm$ 0.153 <sup>Bc</sup>
Lemon basil 1%	0.910 $\pm$ 0.177 <sup>Ba</sup>	1.222 $\pm$ 0.031 <sup>Bc</sup>	0.848 $\pm$ 0.059 <sup>Bb</sup>	1.136 $\pm$ 0.044 <sup>Bc</sup>	0.861 $\pm$ 0.043 <sup>Bb</sup>	1.250 $\pm$ 0.191 <sup>Bc</sup>
Clove-lemon basil (2:0.2%)	0.599 $\pm$ 0.156 <sup>Aa</sup>	0.884 $\pm$ 0.072 <sup>Ac</sup>	0.944 $\pm$ 0.062 <sup>Ab</sup>	1.068 $\pm$ 0.043 <sup>Ac</sup>	0.822 $\pm$ 0.024 <sup>Ab</sup>	1.127 $\pm$ 0.146 <sup>Ac</sup>
Clove-lemon basil (1:1%)	0.511 $\pm$ 0.040 <sup>Aa</sup>	1.106 $\pm$ 0.026 <sup>Ac</sup>	0.771 $\pm$ 0.006 <sup>Ab</sup>	0.970 $\pm$ 0.056 <sup>Ac</sup>	0.763 $\pm$ 0.016 <sup>Ab</sup>	1.116 $\pm$ 0.024 <sup>Ac</sup>
Clove-lemon basil (0.2:2%)	0.666 $\pm$ 0.097 <sup>Aa</sup>	0.974 $\pm$ 0.058 <sup>Ac</sup>	0.891 $\pm$ 0.022 <sup>Ab</sup>	1.044 $\pm$ 0.038 <sup>Ac</sup>	0.903 $\pm$ 0.043 <sup>Ab</sup>	0.913 $\pm$ 0.109 <sup>Ac</sup>

Different superscripted letters within the same column (A-B) and row (a-c) indicated significant differences, evaluated using two-way ANOVA and Duncan's test. Significant differences were set for  $p<0.05$

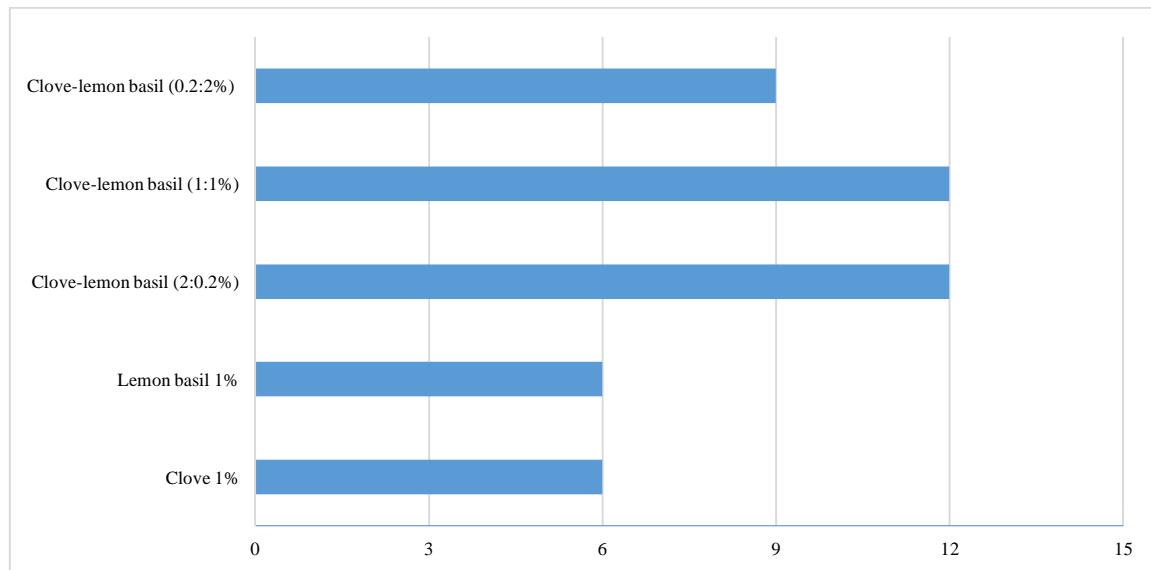
**Table 3:** Sensorial characteristic of chicken meats treated with clove and lemon basil essential oils during storage

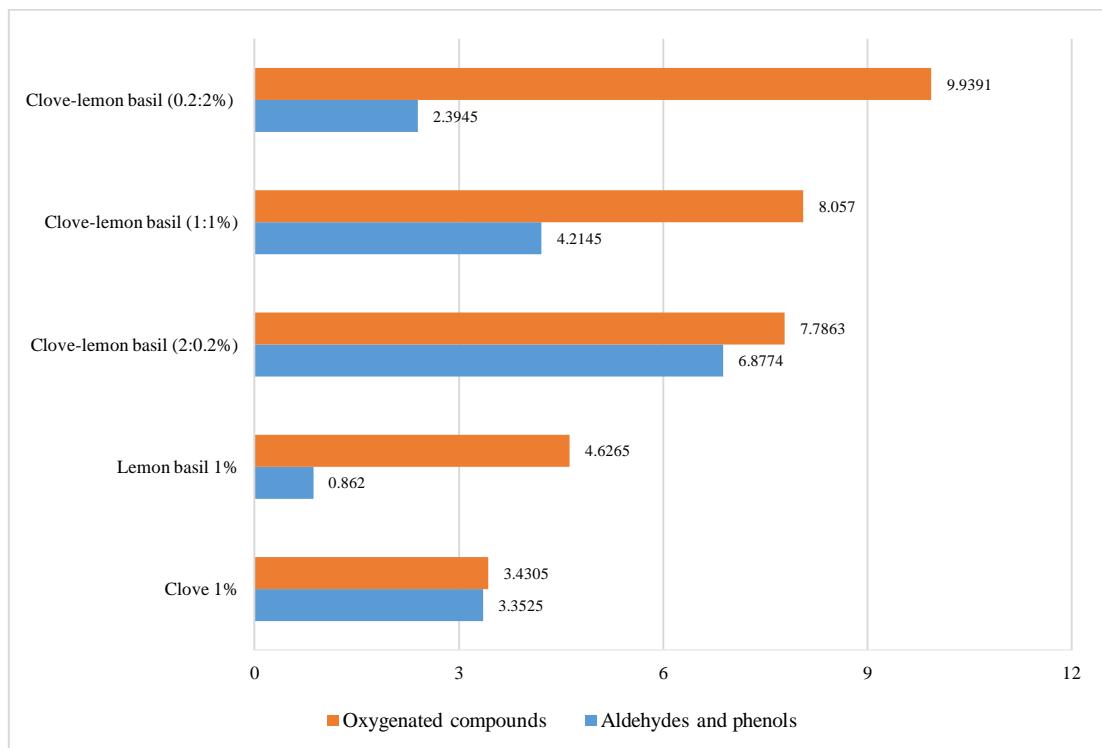
Treatment groups	Characters	Storage time (day)					
		0	3	6	9	12	15
Negative control	Odor	FC	Off-odor +	Off-odor +	Off-odor ++	Off-odor +++	Off-odor +++
	Texture	FC	Softer +	Softer +	Softer ++	Softer +++	Softer +++
	Slime formation	No	Yes +	Yes +	Yes ++	Yes ++	Yes +++
Clove 1%	Odor	AC	AC	AC	AC -	AC --	AC -, off-odor +
	Texture	FC	FC	FC	Firmer +	Firmer +	Firmer ++
	Slime formation	No	No	No	Yes +	Yes +	Yes ++
Lemon basil 1%	Odor	ALB	ALB	ALB	ALB -	ALB --, off-odor +	Off-odor ++
	Texture	FC	FC	FC	Softer +	Softer +	Softer ++
	Slime formation	No	No	No	Yes +	Yes ++	Yes ++
Clove-lemon basil (2:0.2%)	Odor	AC	AC	AC	AC	AC	AC -, off-odor +
	Texture	FC	FC	FC	FC	FC	Firmer +
	Slime formation	No	No	No	No	No	Yes +
Clove-lemon basil (1:1%)	Odor	AC	AC	AC	AC	AC	AC -, off-odor +
	Texture	FC	FC	FC	FC	FC	Firmer +
	Slime formation	No	No	No	No	No	Yes +
Clove-lemon basil (0.2:2%)	Odor	ALB	ALB	ALB	ALB	ALB --, off-odor +	ALB --, off-odor +
	Texture	FC	FC	FC	FC	Softer +	Softer ++
	Slime formation	No	No	No	No	Yes +	Yes ++

AC: Aromatic Clove

ALB: Aromatic Lemon Basil

FC: Fresh Chicken

**Figure 1:** The effect of combinations of clove and lemon basil essential oils to their preservation potential for the chicken meats during various storage time (0, 3, 6, 9, 12, and 15 days)



**Figure 2:** The profile of theoretical relative volume of oxygenated compounds as well as aldehydes and phenols in each concentration ratios of the combination of clove and lemon basil essential oils

## Discussion

The Indonesian clove EO mainly consisted of eugenol, which is typical as the most abundant constituent. However, the concentration of eugenol and composition of other less dominant constituents are varied based on the source of the clove. In agreement with our finding, De-Oliveira et al. (2013) stated that clove EO obtained from Brazil majorly contains eugenol (89.8%), trans-caryophyllene (5.9%), and  $\alpha$ -humulene (2.3%). However, a different composition of clove EO from Egypt was reported with eugenol (76.4%), camphene (3.0%), and lupeol (1.9%) as the main constituents (Mohamed et al., 2018). The major constituents of lemon basil EO in this study were similar with a report (Tangpao et al., 2018) in which estragole (68.5), E-citral (6.7%), and Z-citral (4.4%) were the principal components lemon basil EO collected in Chiang Mai, Thailand. Another variation in the composition of lemon basil EO was reported by Avetisyan et al. (2017) from Kotayk, Armenia, which contained major constituents of nerol (23.0%), E-citral (15.8%), and Z-citral (4.9%). Also, citrals (55.0-75.5%),  $\gamma$ -bisabolene (2.6-9.5%), nerol (1.7-8.9%), geraniol (1.5-6.5%), linalool (1.1-6.0%), and  $\beta$ -caryophyllene (0.7-3.2%) were identified the main constituents of lemon

basil EOs originated from the northern India (Padalia et al., 2018); all of those six compounds were also detected in our study. The variation in the composition of compounds in plant EOs is considered as the result of seasonal factors, geographical origin, as well as harvesting and post-harvest drying methods (Padalia et al., 2018; Toncer et al., 2017; Usai et al., 2016).

In this study, the microbial growth on the meats during storage was enumerated by an indirect method utilizing OD of the cultured meats. The varied initial microbial loading among treatment groups represented the insufficient surface microbial reducing process, as rinsing the meats in sterile water does not equally minimize the microbial counts (Bawcom et al., 1995). Those microbes were mainly from the skin, where the primary microbial contamination occurs and the normal bacterial flora lives. In order to minimize the excessive initial microbial loading, the use of skinless breast chicken fillet is suggested (Hinton and Cason, 2007; Hulankova et al., 2018).

The microbial growth in meats in single clove and lemon basil EOs groups was comparable to that in negative control groups, while those in combination EOs groups in all concentration ratios were significantly lower.

However, the erratic pattern of the microbial growth with the storage time in this study hindered us from concluding based on this data. Generally, the microbial growth of cultured food samples are increasing with time following the bacterial growth curve as reported previously in preserved hake fillets using *Photobacterium* sp. and *Shewanella* sp. as the models (Antunes-Rohling et al., 2019) and cooked ground beef using a mutant *Clostridium botulinum* as the model (Huang, 2018). The OD-based enumeration does not selectively measure the living microbial cells in the cultured medium; it also calculates the dead ones, as well as the friable particles and insoluble slime that are resulted from the putrefaction process. Hence, the samples tended to be more concentrated, in which the deviation of the Beer-Lambert Law took place and subsequently affected the discerned OD (Mäntele and Deniz, 2017).

As the erratic pattern of microbial growth was observed in this study, ODs alone could not be utilized as the parameter of the microbial growth inhibitory activity of the EOs. We used the reduction in OD as the parameter, which represented the capability of a given treatment group in reducing the microbial growth relative to that of the negative control. The more pronounced OD reduction of a given cultured sample indicated the better microbial growth inhibitory activity of the EOs used. In this study, both clove and lemon basil EOs showed a significantly lower microbial growth inhibitory activity on the meats compared to the combination ones. Our data could not describe the detailed mechanisms of antibacterial activity of both EOs, but they might induce microbial cell death by affecting the microbial membrane integrity as other studies reported. Individually, clove EO inhibits microbial growth by the mechanism of inducing cell lysis, which both cell wall and membrane of *Escherichia coli* and *Bacillus subtilis* were significantly damaged (Rhayour et al., 2003).

Besides, it was also mediated by oxidative stress, loss of motility, and also changes in the expression of virulence-associated genes, as observed in *Campylobacter jejuni* (Kovács et al., 2016). Until now, there is no proved evidence on the mechanisms of action of the antibacterial activity of lemon basil EO yet. However, it was recently reported that linalool, the main constituent of lemon basil EO, inhibits bacterial growth by disrupting the membrane integrity in *Pseudomonas aeruginosa* (Liu et al., 2020).

The current study result was similar to our previous work (Hamad et al., 2019) that both clove and lemon basil EOs, in combination with other plant EOs demonstrated a higher antibacterial effect compared to the single one. In the combination of lemon basil and lemongrass EOs, all given concentration ratios showed a significantly better microbial growth inhibitory activity on the chicken fillet during 9 days of refrigerated

preservation. Similarly, the vapor phase of a combination of clove and cinnamon EOs at the ratio of 1:1 showed more excellent antibacterial activity against food-borne *Yersinia enterocolitica*, *Listeria monocytogenes*, and *Enterococcus faecalis* (Goni et al., 2009). Besides, the combination of creeping blepharis and tosign thyme EOs in a ratio of 1:1 demonstrated the more pronounced antibacterial activities against methicillin-resistant *Staphylococcus aureus*, methicillin-susceptible *S. aureus*, antibiotic-resistant *Klebsiella pneumoniae* and *E. coli* strains than those of the respective single EO (Gadisa et al., 2019). In the food as the matrix model, the combination of lemongrass and onion EOs generated a more significant reduction in the growth of *E. coli*, *L. monocytogenes*, and *S. aureus* on the spinach and romaine lettuce leaves compared to the single-use of each EO (Ortega-Ramirez et al., 2017).

The superior antimicrobial activity of the combination of EOs might be related to the synergistic antimicrobial effects resulting from the interaction of the two EOs. However, our study did not evaluate the interaction effects between clove and lemon basil EOs. Generally, the synergistic effects are mediated by the mechanisms of inhibition of the common biochemical pathway, inhibition of protective enzymes, and also enhancement of the uptake of the EO (Bassolé and Juliani, 2012). Evaluated with the checkerboard method, the combination of lemongrass and Kachi grass EOs was proven to exert synergistic antibacterial effects against *S. aureus*, *L. monocytogenes*, *Enterobacter aerogenes*, and *Salmonella Typhimurium* (Bassolé et al., 2011). Another example of synergistic antibacterial activity against *S. aureus* was shown by the combination of basil and bergamot EOs, which killed the bacterium by damaging the cell membrane integrity (Lv et al., 2011).

The presence of microbes causes changes in the sensorial characters of the meats. These spoilage microorganisms can break proteins and lipids down, resulting in the changes in texture and odor, as well as the slime formation on the meats (Dave and Ghaly, 2011). Nonanal, octanal, heptanal, pentanal, hexanal, and dimethyl trisulfide were the compounds responsible for the off-odor of chicken meat produced by lipid oxidation process (Jayasena et al., 2013). Hence, the preservation potential of the EOs might have resulted from antimicrobial and antioxidant activities. Previously, clove EO was known for capable of increasing the oxidative stability of tilapia meat during cooking (Ramezani-Fard et al., 2016). Antioxidant activity of clove EO was shown in blue-fin tuna during refrigerated storage (Echeverría et al., 2018).

According to the current research, combination of clove and lemon basil EOs improved the sensorial properties of chicken meats during storage period of times. The preservation of bluefish (*Pomatomus saltatrix*) with 1%

of thyme and laurel EOs improved the fish shelf life by 3-4 days under iced storage (Erkan et al., 2011). Similar finding was revealed by Ghabraie et al. (2016) who evaluated the sensorial quality of ground meats treated by combination of Chinese cinnamon and common cinnamon EOs.

Each compound in a given EO contributes differently to its overall antimicrobial activity and further food preservation potential. The oxygenated compounds are generally recognized to have a superior antimicrobial activity compared to that of the hydrocarbons, while aldehydes and phenols are considered to demonstrate the best antimicrobial activity among other compounds (Chouhan et al., 2017; El-Shenawy et al., 2015). The theoretical relative volume of oxygenated compounds supported our findings in the enumeration of bacterial growth on the meats and was similar to our previous data (Hamad et al., 2019; Hartanti et al., 2018). The combination of clove and lemon basil EOs at the concentration ratios of 2:0.2 and 1:1% showed the highest theoretical relative volume of oxygenated compounds as well as the best microbial growth inhibitory activity. Nearly, all oxygenated compounds in clove EO were phenols (eugenol) and aldehydes (E-citral), while lemon basil EO only contained a much lower fractions eugenol, Z-citral, and E-citral. The potent antimicrobial activity of clove EO has been linked to its eugenol content; however, the interaction between eugenol with other phenolic and alcohol compounds was reported generating synergistic or additive antimicrobial effects (Bassolé and Juliani, 2012).

## Conclusion

The combinations of cloves and lemon basil EOs showed a better microbial growth inhibitory activity and preservation potential than those of the single use. At the optimum ratio of 1:1%, the combinations of cloves and lemon basil EOs reduced the microbial count on the meats and delaying the changes in sensorial characters of chicken meats for 12 days during refrigerated storage. This meat preservation effects might be related to the presence of high fractions of oxygenated compounds, mainly eugenol, Z-citral, and E-citral in both clove and lemon basil EOs.

## Author contributions

D.H., N.A.S., and A.H. designed the study and analyzed the data; N.A.S. and A.H. conducted the experimental work; D.H. and A.H. wrote the manuscript. All authors read and approved the final manuscript.

## Conflicts of interest

All the authors declared that this is no conflict of interest in this study.

## Acknowledgements

The authors would like to express their appreciation to the Directorate General of Higher Education, Ministry of Research, Technology, and Higher Education, the Republic of Indonesia, for financial support of this study.

## References

Al-Hijazeen M., Lee E.J., Mendonca A., Ahn D.U. (2016). Effect of oregano essential oil (*Origanum vulgare* subsp. *hirtum*) on the storage stability and quality parameters of ground chicken breast meat. *Antioxidant.* 5: 18. [DOI: 10.3390/antiox5020018]

Alnoman M., Udompijikitkul P., Sarker M.R. (2017). Chitosan inhibits enterotoxigenic *Clostridium perfringens* type A in growth medium and chicken meat. *Food Microbiology.* 64: 15-22. [DOI: 10.1016/j.fm.2016.11.019]

Antunes-Rohling A., Artaiz A., Calero S., Halaihel N., Guillén S., Raso J., Alvarez I., Cebrián G. (2019). Modelling microbial growth in modified-atmosphere-packed hake (*Merluccius merluccius*) fillets stored at different temperatures. *Food Research International.* 122: 506-516. [DOI: 10.1016/j.foodres.2019.05.018]

Avetisyan A., Markosian A., Petrosyan M., Sahakyan N., Babayan A., Aloyan S., Trchounian A. (2017). Chemical composition and some biological activities of the essential oils from basil *Ocimum* different cultivars. *BMC Complementary and Alternative Medicine.* 17: 60. [DOI: 10.1186/s12906-017-1587-5]

Bassolé I.H.N., Juliani H.R. (2012). Essential oils in combination and their antimicrobial properties. *Molecules.* 17: 3989-4006. [DOI: 10.3390/molecules17043989]

Bassolé I.H.N., Lamien-Meda A., Bayala B., Obame L.C., Ilboudo A.J., Franz C., Novak J., Nebié R.C., Dicko M.H. (2011). Chemical composition and antimicrobial activity of *Cymbopogon citratus* and *Cymbopogon giganteus* essential oils alone and in combination. *Phytomedicine.* 18: 1070-1074. [DOI: 10.1016/j.phymed.2011.05.009]

Bawcom D.W., Thompson L.D., Miller M.F., Ramsey C.B. (1995). Reduction of microorganisms on beef surfaces utilizing electricity. *Journal of Food Protection.* 58: 35-38. [DOI: 10.4315/0362-028X-58.1.35]

Carović-Stanko K., Orlić S., Politeo O., Strikić F., Kolak I., Milos M., Satović Z. (2010). Composition and antibacterial activities of essential oils of seven *Ocimum* taxa. *Food Chemistry.* 119: 196-201. [DOI: 10.1016/j.foodchem.2009.06.010]

Chouhan S., Sharma K., Guleria S. (2017). Antimicrobial activity of some essential oils-present status and future perspectives. *Medicines.* 4: 58. [DOI: 10.3390/medicines4030058]

Dave D., Ghaly A.E. (2011). Meat spoilage mechanisms and preservation techniques: a critical review. *American Journal of Agricultural and Biological Sciences.* 6: 486-510. [DOI: 10.3844/ajabssp.2011.486.510]

De-Oliveira T.L.C., Cardoso M.D.C., Soares R.D.A., Ramos E.M., Piccoli R.H., Tebaldi V.M.R. (2013). Inhibitory activity of *Syzygium aromaticum* and *Cymbopogon citratus* (DC.) Stapf. essential oils against *Listeria monocytogenes* inoculated in bovine ground meat. *Brazilian Journal of Microbiology.* 44: 357-365. [DOI: 10.1590/S1517-83822013005000040]

Echeverría I., López-Caballero M.E., Gómez-Guillén M.C., Mauri A.N., Montero M.P. (2018). Active nanocomposite films based on soy proteins-montmorillonite- clove essential oil for

the preservation of refrigerated bluefin tuna (*Thunnus thynnus*) fillets. *International Journal of Food Microbiology*. 266: 142-149. [DOI: 10.1016/j.ijfoodmicro.2017.10.003]

El-Shenawy M.A., Baghdadi H.H., El-Hosseiny L.S. (2015). Antibacterial activity of plants essential oils against some epidemiologically relevant food-borne pathogens. *The Open Public Health Journal*. 8: 30-34. [DOI: 10.2174/1874944501508010030]

Erian I., Phillips C.J.C. (2017). Public understanding and attitudes towards meat chicken production and relations to consumption. *Animals*. 7: 20. [DOI: 10.3390/ani7030020]

Erkan N., Tosun S.Y., Ulusoy S., Üretener G. (2011). The use of thyme and laurel essential oil treatments to extend the shelf life of bluefish (*Pomatomus saltatrix*) during storage in ice. *Journal für Verbraucherschutz und Lebensmittelsicherheit*. 6: 39-48. [DOI: 10.1007/s00003-010-0587-x]

Gadisa E., Weldearegay G., Desta K., Tsegaye G., Hailu S., Jote K., Takele A. (2019). Combined antibacterial effect of essential oils from three most commonly used Ethiopian traditional medicinal plants on multidrug resistant bacteria. *BMC Complementary and Alternative Medicine*. 19: 24. [DOI: 10.1186/s12906-019-2429-4]

Ghabraie M., Vu K.D., Tata L., Salmieri S., Lacroix M. (2016). Antimicrobial effect of essential oils in combinations against five bacteria and their effect on sensorial quality of ground meat. *LWT-Food Science and Technology*. 66: 332-339. [DOI: 10.1016/j.lwt.2015.10.055]

Goni P., Lopez P., Sanchez C., Gómez-Lus R., Becerril R., Nerín C. (2009). Antimicrobial activity in the vapour phase of a combination of cinnamon and clove essential oils. *Food Chemistry*. 116: 982-989. [DOI: 10.1016/j.foodchem.2009.03.058]

González-García S., Esteve-Llorens X., Moreira M.T., Feijoo G. (2018). Carbon footprint and nutritional quality of different human dietary choices. *Science of Total Environment*. 644: 77-94. [DOI: 10.1016/j.scitotenv.2018.06.339]

Hamad A., Hartanti D. (2015). The use of essential oil of clove (*Syzygium aromaticum*) as tofu's natural preservative. *Farmasains*. 2: 289-294.

Hamad A., Mahardika M.G.P., Yuliani I., Hartanti D. (2017). Chemical constituents and antimicrobial activities of essential oils of *Syzygium polyanthum* and *Syzygium aromaticum*. *Rasayan Journal of Chemistry*. 10: 564-569. [DOI: 10.7324/RJC.2017.1021693]

Hamad A., Nuritasari A., Hartanti D. (2019). A combination of lemongrass and lemon basil essential oils inhibited bacterial growth and improved the shelf life of chicken fillets. *Asia-Pacific Journal of Science and Technology*. 24: 1-9.

Hartanti D., Haqqi M.Z.U., Hamad A. (2018). Potency of combination of essential oils of ginger and lemongrass as fresh chicken meat natural preservative. *Advanced Science Letters*. 24: 91-94. [DOI: 10.1166/asl.2018.11929]

Hinton A., Cason J.A. (2007). Changes in the bacterial flora of skin of processed broiler chickens washed in solutions of salicylic acid. *International Journal of Poultry Sciences*. 6: 960-966. [DOI: 10.3923/ijps.2007.960.966]

Huang L. (2018). Growth of non-toxigenic *Clostridium botulinum* mutant LNT01 in cooked beef: one-step kinetic analysis and comparison with *C. sporogenes* and *C. perfringens*. *Food Research International*. 107: 248-256. [DOI: 10.1016/j.foodres.2018.02.028]

Hulankova R., Borilova G., Abdullah F.A.A., Buchtova H. (2018). Microbiological quality of organic chicken meat during refrigerated storage in air and modified atmospheres. *British Poultry Science*. 59: 506-513. [DOI: 10.1080/00071668.2018.1496399]

Hyldgaard M., Mygind T., Meyer R.L. (2012). Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. *Frontiers in Microbiology*. 3: 12. [DOI: 10.3389/fmicb.2012.00012]

Jayasena D.D., Ahn D.U., Nam K.C., Jo C. (2013). Flavour chemistry of chicken meat: a review. *Asian-Australasian Journal of Animal Sciences*. 26: 732-742. [DOI: 10.5713/ajas.2012.12619]

Kaya I., Yigit N., Benli M. (2008). Antimicrobial activity of various extracts of *Ocimum basilicum* L. and observation of the inhibition effect on bacterial cells by use of scanning electron microscopy. *African Journal of Traditional, Complementary and Alternative Medicines*. 5: 363-369.

Khalil A. (2013). Antimicrobial activity of ethanolic extracts of *Ocimum basilicum* leaf from Saudi Arabia. *Biotechnology*. 12: 61-64. [DOI: 10.3923/biotech.2013.61.64]

Kovács J.K., Felso P., Makszin L., Pápai Z., Horváth G., Ábrahám H., Palkovics T., Böszörmenyi A., Emödy L., Schneider G. (2016). Antimicrobial and virulence-modulating effects of clove essential oil on the foodborne pathogen *Campylobacter jejuni*. *Applied and Environmental Microbiology*. 82: 6158-6166. [DOI: 10.1128/AEM.01221-16]

Liu X., Cai J., Chen H., Zhong Q., Hou Y., Chen W., Chen W. (2020). Antibacterial activity and mechanism of linalool against *Pseudomonas aeruginosa*. *Microbial Pathogenesis*. 141: 103980. [DOI: 10.1016/j.micpath.2020.103980]

Lv F., Liang H., Yuan Q., Li C. (2011). *In vitro* antimicrobial effects and mechanism of action of selected plant essential oil combinations against four food-related microorganisms. *Food Research International*. 44: 3057-3064. [DOI: 10.1016/j.foodres.2011.07.030]

Mäntele W., Deniz E. (2017). UV-VIS absorption spectroscopy: Lambert-Beer reloaded. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*. 173: 965-968. [DOI: 10.1016/j.saa.2016.09.037]

Mohamed M.S.M., Abdallah A.A., Mahran M.H., Shalaby A.M. (2018). Potential alternative treatment of ocular bacterial infections by oil derived from *Syzygium aromaticum* flower (clove). *Current Eye Research*. 43: 873-881. [DOI: 10.1080/02713683.2018.1461907]

Naveed R., Hussain I., Tawab A., Tariq M., Rahman M., Hameed S., Mahmood M.S., Siddique A.B., Iqbal M. (2013). Antimicrobial activity of the bioactive components of essential oils from Pakistani spices against *Salmonella* and other multi-drug resistant bacteria. *BMC Complementary and Alternative Medicine*. 13: 265. [DOI: 10.1186/1472-6882-13-265]

Ortega-Ramirez L.A., Silva-Espinoza B.A., Vargas-Arispuro I., Gonzalez-Aguilar G.A., Cruz-Valenzuela M.R., Nazzaro F., Ayala-Zavala J.F. (2017). Combination of *Cymbopogon citratus* and *Allium cepa* essential oils increased antibacterial activity in leafy vegetables. *Journal of Science of Food and Agriculture*. 97: 2166-2173. [DOI: 10.1002/jsfa.8025]

Padalia R.C., Singh V.R., Bhatt G., Chauhan A., Upadhyay R.K., Verma R.S., Chanotiya C.S. (2018). Optimization of harvesting and post-harvest drying methods of *Ocimum africanum* Lour. for production of quality essential oil. *Journal of Essential Oil Research*. 30: 437-443. [DOI: 10.1080/10412905.2018.1495110]

Pandey A.K., Kumar P., Singh P., Tripathi N.N., Bajpai V.K. (2017). Essential oils: sources of antimicrobials and food preservatives. *Frontiers in Microbiology*. 7: 2161. [DOI: 10.3389/fmicb.2016.02161]

Raeisi M., Tabaraei A., Hashemi M., Behnampour N. (2016). Effect of sodium alginate coating incorporated with nisin, *Cinnamomum zeylanicum*, and rosemary essential oils on microbial quality of chicken meat and fate of *Listeria monocytogenes* during refrigeration. *International Journal of Food Microbiology*. 238: 139-145. [DOI: 10.1016/j.ijfoodmicro.2016.08.042]

Ramezani-Fard E., Romano N., Goh Y.M., Oskoueian E., Ehteshami F., Ebrahimi M. (2016). The effect of different cooking methods on fatty acid composition and antioxidant activity of n-3 fatty acids fortified tilapia meat with or without clove essential oil. *Journal of Environmental Biology*. 37: 775-784.

Rhayour K., Bouchikhi T., Tantaoui-Elaraki A., Sendine K., Remmal A. (2003). The mechanism of bactericidal action of oregano and clove essential oils and of their phenolic major components on *Escherichia coli* and *Bacillus subtilis*. *Journal of Essential Oil Research*. 15: 356-362. [DOI: 10.1080/10412905.2003.10475000]

10412905.2003.9698611]

Rialita T., Rahayu W.P., Nuraida L., Nurtama B. (2015). Aktivitas antimikroba minyak esensial jahe merah (*Zingiber officinale* var. *Rubrum*) dan lengkuas merah (*Alpinia purpurata* K. Schum) terhadap bakteri patogen dan perusak pangan. *Agritech*. 35: 43-52. [DOI: 10.22146/agritech.9418]

Sethi S., Dutta A., Gupta B.L., Gupta S. (2013). Antimicrobial activity of spices against isolated food borne pathogens. *International Journal of Pharmacy and Pharmaceutical Sciences*. 5: 260-262.

Tangpao T., Chung H.H., Sommano S.R. (2018). Aromatic profiles of essential oils from five commonly used Thai basil. *Foods*. 7: 175. [DOI: 10.3390/foods7110175]

Toncer O., Karaman S., Diraz E., Sogut T., Kizil S. (2017). Essential oil composition of *Thymus × citriodorus* (Pers.) Schreb. at different harvest stages. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*. 45: 185-189. [DOI: 10.15835/nbha45110672]

Usai M., Atzei A.D., Marchetti M. (2016). A comparative study on essential oil intraspecific and seasonal variations: *Melissa romana* Mill. and *Melissa officinalis* L. from Sardinia. *Chemistry and Biodiversity*. 13: 1076-1087. [DOI: 10.1002/cbdv.201500507]

Wang G.Y., Wang H.H., Han Y.W., Xing T., Ye K.P., Xu X.L., Zhou G.H. (2017). Evaluation of the spoilage potential of bacteria isolated from chilled chicken *in vitro* and *in situ*. *Food Microbiology*. 63: 139-146. [DOI: 10.1016/j.fm.2016.11.015]