Development of lemon basil essential oil as a natural chicken meat preservative

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Abstract. Lemon basil (Ocimum × africanum Lour.) is potentially developed into a natural food preservative as its antimicrobial activities have been proven against food-borne microorganisms. This study is designed to evaluate the preservation potential of lemon basil essential oil (EO) on chicken meat during 12 days of refrigerated storage. The steam-water distillation and Gas Chromatography-Mass Spectroscopy (GC-MS) techniques were used to extract and analyse chemical constituents of lemon basil EO, respectively. The microbial growth inhibitory activity was determined by the OD 600 nm-based indirect method, while the physical characters of the meat were organoleptically observed. A total yield of 0.20% lemon basil EO was obtained, which contained 38 compounds with neral, geranial, caryophyllene oxide, and α-bisabolene as main constituents. The optimum preservation effects were shown by lemon basil EO at a concentration of 625 ppm, which significantly decreased the microbial growth on the meat and delayed meats deterioration for six days. The preservation effects of lemon basil EO was likely related to the high fractions of aldehyde compounds in it.

1. Introduction
Lemon basil (Ocimum × africanum Lour., kemangi; Lamiaceae) is commonly consumed as fresh vegetable and leafy spices for protein-based savory meals in Southeast Asian cuisines. It is also utilized for medicinal purposes, i.e., for traditional treatment of cough and fever, as well as for wound healing [1]. The antimicrobial activity of this plant is particularly well-studied, in which extracts and essential oil (EO) showed promising antimicrobial effects against Gram-positive and Gram-negative bacteria, and also fungi [2–5]. Plant materials with a potent antimicrobial activity have proven to be a suitable candidate for natural food preservatives [6]. Our previous works have demonstrated that lemon basil water extracts having preservation potential on tofu and chicken meat, while the EO was showing a weak to moderate preservation effects on tofu [7, 8]. In this study, the preservation potential of lemon basil EO on chicken meat is evaluated.

The popularity of chicken meat as a protein source worldwide is attributed to the nutritional, economic, and environmental aspects [9, 10]. The limited shelf life of chicken meat and further contaminations by pathogenic microorganisms were reported elsewhere, hence, needed to be overcome [11–13]. Lemon basil is potentially used for the said purpose due to its antimicrobial activity and usage in chicken-based meals. The enhancement of shelf life and also better sensorial
acceptance are expected from this effort. There is no report on the use of lemon basil EO for the natural preservation of chicken meat until to date. In this study, the preservation potential of lemon basil EO is evaluated based on the inhibition of microbial growth and the physical characters of the meat during 12-day storage under refrigerated conditions.

2. Materials and method
2.1. Materials
The anhydrous sodium sulfate (Sigma-Aldrich, Germany), nutrient broth (NB) (Oxoid, UK), fresh chicken meats (bought from a local market at Purwokerto, Central Java), and aerial part of lemon basil (collected in Ajibarang, Central Java) were used in this study. The lemon basil specimen was identified in the Laboratory of Taxonomy, University of Jenderal Soedirman, Purwokerto, Central Java (ref no. 133/FB.Unsoed/TaksTumVI/2015).

2.2. Extraction of lemon basil EO
The general steam and water distillation method was used to extract lemon basil EO from the sun-dried lemon basil aerial parts, as previously described [14].

2.3. Identification of chemical constituents of lemon basil EO
Gas Chromatograph-Mass Spectrometry (GCMS) was used for separating the constituents in lemon basil EO, while the Wiley ver. 9.0. library was utilized to identify those compounds. The conditions of separation and identification followed those in a previous report [8].

2.4. Evaluation of microbial growth inhibitory activity on chicken meats
The microbial growth inhibitory activity of lemon basil EO on chicken meat was evaluated by a method previously described [8]. The meat was stored under refrigerated conditions, while observation time was modified to days 3, 6, 9, and 12. Other aspects were strictly followed.

2.5. Evaluation of physical characters of chicken meat
The evaluation of physical characters of chicken meats during storage followed a method as previously reported, with modified storage condition and observation time [8]. In this study, the meat was subjected to refrigerated storage, while the color, odor, texture, and slime formation on the stored meats were observed in days 3, 6, 9, and 12.

2.6. Data analysis
The triplicate samples were subjected to each experiment. The two-way analysis of variance (ANOVA) and subsequently Duncan’s tests were used to analyze the effect of treatment group and storage time to the OD 600 nm of the cultured meat and the mean separation of each group, respectively. A confidence level of 95% was applied. The statistical analyses were conducted with IBM SPSS Statistics v. 20 (IBM, USA).

3. Results and discussion
The yield of the steam and water distillation of lemon basil EO was 0.20%. There were 38 compounds in lemon basil EO, with seven dominant ones, separated by GC within 50 minutes (Figure 1). Those major constituents are neral, geranial, caryophyllene oxide, α-bisabolene, isocaryophyllene, methylheptenone, and α-bergamotene. In addition, β-bourbonene, α-cubebene, α-curcumene, and zerumbone were also detected with the total area of more than 1% (Table 1). Hence, lemon basil EO used in this study mainly consisted of terpenes, particularly aldehyde monoterpenes and hydrocarbon sesquiterpenes. It is well-understood that given EO constituents are varied, with intraspecific variation, seasonal condition, geographical origin, harvesting process, and post-harvest drying method as the factors [15–17].
Nevertheless, the typical main constituents of Southeast Asian lemon basil EO are geranial and neral. The previous distillation of lemon basil collected from Kedung Banteng, Banyumas showed geranial (22.79%), neral (15.60%), α-humulene (12.56%), and β-caryophyllene (11.74%) as the main constituents. Similarly, lemon basil from Purwalingga, Central Java contained major constituents of geranial (45.06%), neral (33.10%), β-myrcene (4.54%), and α-gurjunene (1.313%) [8, 18]. Lemon basil EO from East Java mainly consisted of geranial (30.55%), neral (23.16%), β-caryophyllene (9.38%), and farnesol (1.85%) [19]. A different profile of EO constituents was demonstrated in lemon basil originated from northern Thailand, with main constituents of neral (36.80%), geranial (33.40%), β-caryophyllene (3.50%), and linalool (3.10%) [20].

The profile of OD 600 nm of the culture of fresh chicken meat in NB during 12-day preservation is presented in Figure 2. Both treatment group and storage time statistically affected the OD 600 nm of the cultured samples, with a p-value of 0.001 and 0.000, respectively. The OD 600 nm of all groups increased with the time of storage, in which a significant OD difference between two consecutive times was observed. The relatively low gradient of OD 600 nm between days 3 to 6 represented the slow growth of the microorganisms and was considered as the lag phase of microbial growth. In contrast, the rapid microbial growth observed between days 6 to 12 was considered as the log phase. Since the higher OD 600 nm indicated the presence of more microorganisms in the culture, the microbial growth on the chicken meats in any group was increased as the function of preservation time. The OD 600 nm of the cultured meats treated with negative control was the highest and consecutively was followed by those treated by lemon basil EO at the concentrations of 3125, 125, and 625 ppm, respectively. Hence, the microbial growth inhibitory activity of the lemon basil EO on the meats during storage was not in a concentration-dependent manner. Besides, the highest concentration of lemon basil EO did not show inhibitory activity as its OD 600 nm was comparable to that of the negative control (p = 0.065). Overall, the best inhibitory activity was shown by lemon basil EO in a concentration of 625 ppm.
Table 1. The constituents of lemon basil EO

| Rt (min) | Compound name            | % Area | Rt (min) | Compound name            | % Area |
|---------|--------------------------|--------|----------|--------------------------|--------|
| 8.74    | Methylheptenone          | 4.143  | 31.04    | β-Cubebene               | 0.303  |
| 8.89    | Myrcene                  | 0.260  | 31.13    | Geranyl acetate          | 0.585  |
| 10.60   | Eucalyptol               | 0.272  | 31.21    | cis-3-Hexenyl butyrate   | 0.395  |
| 13.59   | (+)-Fenchone             | 0.442  | 32.08    | Isocaryophyllene         | 4.639  |
| 14.59   | Terpinolene              | 0.190  | 32.84    | α-Bergamotene            | 3.776  |
| 16.60   | 2-Octylfuran             | 0.379  | 33.09    | β-Funebrene              | 0.351  |
| 17.27   | trans-Chrysanthemal      | 0.717  | 33.29    | Humulene                 | 0.925  |
| 17.70   | Citronellal              | 0.529  | 33.67    | β-Bourbonene             | 0.314  |
| 18.44   | Verbenol                 | 0.531  | 34.42    | Germacre D               | 0.306  |
| 19.07   | Rosefuran epoxide        | 0.858  | 34.67    | α-Curcumene              | 1.291  |
| 19.57   | trans-Isopulegone        | 0.901  | 35.22    | γ-Selinene               | 0.223  |
| 20.18   | α-Terpineol              | 0.415  | 35.56    | β-Bisabolene             | 0.869  |
| 23.33   | Geranial                 | 25.936 | 35.60    | γ-Cadinene               | 0.563  |
| 25.32   | Neral                    | 30.915 | 36.24    | α-Bisabolene             | 5.922  |
| 28.32   | Methyl geranate          | 0.319  | 36.35    | α-Himachalene            | 0.254  |
| 28.99   | 13-Heptadecyn-1-ol       | 0.430  | 37.21    | Caryophyllene oxide      | 6.486  |
| 30.30   | Geranyl butyrate         | 0.327  | 37.89    | Zerumbone                | 1.099  |
| 30.39   | α-Cubebene               | 1.319  | 38.86    | Myrtenol                 | 0.231  |
| 30.72   | β-Bourbononene           | 1.947  | 48.47    | Diterpene                | 0.401  |

| Total area | 99.763 |
| Total hydrocarbon compounds | 23.853 |
| Total oxygenated compounds | 75.910 |
| Total aldehydes | 58.097 |

The microbial growth inhibitory activity of lemon basil on chicken meat might relate to its chemical constituents. More than three-quarter of the lemon basil EOs used in this study were oxygenated compounds, which were known for having a more potent antimicrobial activity compared to the hydrocarbons [21]. Among the oxygenated compounds, phenols and aldehydes were considered to have superior antimicrobial activity [22]. The studied lemon basil EO was rich with aldehydes but contained no phenols (Table 1). From this point of view, geranial, neral, trans-chrysanthemal, and citronellal were considered as the antimicrobial constituents responsible for their microbial growth inhibitory activity on chicken meats.

The microbial growth inhibitory activity of lemon basil in food models have been described. Our previous study suggested that lemon basil EO at an optimum concentration of 3.125 mg/ml significantly inhibited the microbial growth on the tofu during 10-day storage under ambient condition [8]. Further, the water extracts of this plant at the concentration of 10% were capable of inhibiting microbial growth on tofu stored in room temperature and chicken meat under refrigerated storage for 10 and 12 days, respectively [7]. Lemon basil water extracts at the concentrations of 25 and 30% were capable of maintaining the microbial growth on the fresh striped mackerel to comply with the Indonesian national standard (SNI) of fresh fish after 12 days of cold storage [23]. Also, steaming mackerel with a combination of lemon basil crude extract and salt in a ratio of 8%: 8% effectively decreased the microbial total plate count (TPC) on the fish to less than 250 colony-forming unit (CFU) [24].
Figure 2. The profile of OD 600 nm of the culture of chicken meat in NB during 12-day preservation

Figure 3. The profile of physical character changes in the meats during 12-day preservation. The plot on day 15 indicated that the given character was remained unchanged until the final day of storage.

The use of lemon basil EO changed the color of the meats. Without lemon basil EO, the meats were pinkish-white, but the addition of lemon basil EO made the meats paler, from white in the lower concentration to yellowish-white at the higher one. Once the colors of those meats changed, the new ones were retained until the final day of the preservation. The changes of the texture were observed in the meats in negative control and those treated with the lowest concentration of lemon basil EO. Meats
in both groups started to be softer on day 9. On the other hand, meats treated with higher concentrations of EO remained unchanged until the final day of preservation. Changes in meat texture resulted from the broken-down proteins and lipids by the metabolism of microorganisms. The breakdown of those two macromolecules also released metabolites causing off-odor in the meats [25]. In this study, lemon basil EO at concentrations of 625 and 3126 ppm changed the odor of the meats with their hint of specific aroma, while that at 125 ppm did not. The pattern of the observed off-odor of the meats was similar to that of texture changes. EO possibly masked the odor of the deteriorated chicken meats, but it also inhibited the microbial growth, so the production of metabolites from lipids and protein decomposition was diminished. The presence of slime was not detected on the meats in all groups until the final day of storage (Figure 3). According to the texture and odor aspects, lemon basil EO at the concentrations of 625 and 3126 ppm were capable of delaying the deterioration of the meats up to 6 days compared to the negative control.

The effects of lemon basil to the properties of the preserved food have been described as well. In tofu, lemon basil EO at a concentration of 3.125 mg/ml showed improvement of the shelf life up to 4 days under room temperature storage [8]. In Indonesia, the use of lemon basil water extracts for fish preservation efforts is evidenced. The immersion of fresh tilapia in water extract in a concentration up to 8% for 4 hours was effective for maintaining the fresh texture, color, odor, and eye condition of the fish [26]. Besides, immersing striped mackerel in lemon basil water extract at the optimum concentration of 30% for 2 hours was able to maintain the acceptable total volatile base nitrogen (TVBN) level, water activity, and pH of striped mackerel for day 12 days under cold storage [23]. The sensorial analysis of mackerel treated with the combination of lemon basil crude extract and salt showed that it was still acceptable up to the ratio of 8%: 8%, which was accompanied by a decrease in water content and pH of the meats [24].

4. Conclusion
Lemon basil EO at a concentration of 625 ppm showed an optimum preservation potential. It significantly inhibited microbial growth on the chicken meats during 12-day storage and delayed meats deterioration up to 6 days. The preservation potential of lemon basil EO was possibly the high content of oxygenated compounds, particularly aldehydes, i.e., geranial, neral, trans-chrysanthenal, and citronellal.

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