Preservation potential of lemon basil essential oil on tofu: Development of a natural food preservative

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Abstract. The antimicrobial activity of lemon basil (Ocimum × africanum Lour.) has been described. This activity can be elaborated further and possibly applied for natural foods preservative. In this study, the constituents of lemon basil essential oil were analysed, and its preservation potential on the tofu was evaluated. The essential oil was distilled from dried plant materials using a steam and water distillation process. The constituents of essential oil of lemon basil were analysed by Gas Chromatography-Mass Spectroscopy (GC-MS) technique. Its application for preservation of tofu was evaluated based on its capacity in inhibiting the growth of bacteria on the tofu as well as the physical observation of the tofu during ten days of preservation at room temperature. The lemon basil essential oil constituted of 21 constituents, with a high fraction of oxygenated compounds, including neral and geraniol, were detected. Lemon basil essential oil at an optimum concentration of 3.125 mg/ml inhibited the bacterial growth on the tofu during storage and improved its shelf life up to 4 days.

1. Introduction
Tofu is made from precipitation of water extract of whole soya beans and mainly consumed in East- and Southeast Asia. It typically contains high amounts of water (84.55%) and proteins (8.08%). This nature makes it is easily spoiled by microorganisms and hence has a relatively short shelf life [1]. A tofu preservation process is needed to prolong its shelf life as well as minimise the risk of food poisoning.

Spices and herbs, particularly those with profound antimicrobial activity, are the interesting sources of natural food preservative. The presence of antimicrobial compounds in those spices enabled the bacterial growth inhibition capacity toward a given food matrix [2]. Empirically, spices have been applied to food, and hence we have been familiar with their taste and aroma. This aspect might benefit us with better acceptability when they are used as a food preservative.

Lemon basil (Ocimum × africanum Lour., kemangi in Bahasa Indonesia; Lamiaceae) is a popular spice commonly used in savoury meals, including tofu-based ones. Lemon basil essential oil has demonstrated antimicrobial activities against both Gram-positive and Gram-negative bacteria [3–5]. In this study, the chemical constituents of lemon basil essential oil were analysed, and its application to be used as a natural tofu preservative was evaluated. The preservative potential of the oil was evaluated based on its capability in inhibiting the bacterial growth on the tofu as well as improving the shelf life of the tofu.
2. Materials and Method

2.1. Plant materials
Aerial parts of lemon basil were collected from Banyumas, Central Java. The plant materials were dried under direct sunlight. The identity of the plant was determined in the Laboratory of Taxonomy, Faculty of Biology, University of Jenderal Soedirman, Purwokerto, Indonesia.

2.2. Distillation of lemon basil essential oil
Lemon basil essential oil was distilled from the dried plant materials using a steam and water distillation process as previously described [6].

2.3. Identification of chemical constituents of lemon basil essential oil
Lemon basil essential oil was analysed using Shimadzu QP2010S SE Gas Chromatograph-Mass Spectrometer. The separation was performed on the AGILENT HP 5MS column (30mx0.25mmx0.25μm). The oven temperature was programmed from 50°C and raised to 260°C for 8 minutes, and then held for 9.5 minutes. The carrier gas was helium with a flow rate of 1ml/min. The injection ratio was 1:50. The injector and detector temperatures were 280 and 300°C, respectively. The volume of sample injection was 1μl at a concentration of 100ppm with n-hexane as the solvent. The solvent readout time was 2 minutes, and the sample analysis was run for 60 minutes. The ionisation voltage used was 70eV. The identification of the compounds in lemon basil essential oil was performed by comparing the mass spectrum of respective compounds with those available in Wiley ver. 9.0. Library.

2.4. Evaluation of the preservative potential of lemon basil essential oil
The preservative potential of lemon basil essential oil on the tofu was evaluated by following a previously described method with a minor modification [7]. In brief, after surface sterilisation and under sterile condition, the cubes of tofu were placed in the manually homogenised essential oil in water with to obtain the concentration of 0.125, 0.625, and 3.125 mg/ml, respectively. Sterilised water was used as a negative control. The preserved tofu was stored at room temperature. On day 2, 4, 6, 8, and 10, the bacterial growth on the tofu and their physical characteristic were evaluated. Each cube of tofu was put in 25 ml of sterile nutrient broth (NB) medium and then homogenised for a minute. One ml of suspension was transferred into 9 ml of sterile NB and then incubated in a temperature of 37°C for 24 hours. The enumeration of bacterial growth was conducted by recording the optical densities of cultured bacterial suspensions with UV-Vis spectrophotometer at a wavelength of 600 nm. The colour, odour, texture, and formation of slime on the surface of tofu were used as the evaluated physical characters to determine the prolonged shelf life of the tofu. All the works were replicated 3 times.

2.5. Data analysis
The total area under curve day 2-10 (AUC2-10) of the optical density of cultures of tofu preserved with lemon basil versus the preservation time was calculated using the trapezoid method. Means separation of the optical densities of cultured bacterial suspensions in NB and AUC of the curve, as mentioned above, were evaluated using by Duncan’s tests. Significance was evaluated at p-value <0.05. Statistical analysis was conducted by the general procedures of SPSS Statistics v.20 (SPSS Inc.)

3. Results and Discussion
There were 21 compounds identified in the lemon basil essential oil with major constituents of geranial, neral, α-humulene, β-caryophyllene, β-caryophyllene oxide, and α-bergamotene (Table 1). The constituents and their respective ratio in the essential oil of a given plant species are widely varied as the result of the phytochemical infraspecific variation. However, geranial and neral were found to be the typical main character constituents of lemon basil essential oil [8]. For example, the oil obtained from lemon basil collected in Chiang Mai, Thailand mainly constituted of estragole, geranial, and neral [9]. Another report mentioned linalool, α-bisabolene, β-caryophyllene, and geranial as the characteristic constituents of lemon basil essential oil originated from northern India [10].

An equal amount of oxidised and hydrocarbon compounds were found in the lemon basil essential oil, respectively (Table 1). Oxygenated compounds, including those with phenolic, aldehyde, ester, hydroxyl, and ether functional groups, are considered possessing a higher antimicrobial activity than
those of hydrocarbons [11]. Hence, geranial, neral, β-caryophyllene oxide, neryl acetate, methyl heptenone, nerol oxide, bisabolol oxide A, and geranyl acetate might be the compounds responsible for lemon basil essential oil antibacterial activity and further tofu preservative potential activity.

Table 1. Chemical constituents of lemon basil

| Retention time (min) | Compounds                      | Area (%) |
|----------------------|--------------------------------|----------|
| 15.685               | Methyl heptenone               | 2.25     |
| 23.433               | Nerol oxide                    | 1.17     |
| 24.619               | 2-Methyl-4,5-nonadiene         | 0.95     |
| 27.181               | Neral                          | 15.60    |
| 28.398               | Geranial                       | 22.79    |
| 31.506               | Geranyl acetate                | 0.41     |
| 32.065               | α-Copaene                      | 1.01     |
| 32.234               | Neryl acetate                  | 3.24     |
| 32.425               | β-Bourbonene                   | 0.45     |
| 33.747               | β-Caryophyllene                | 11.74    |
| 34.139               | α-Bergamotene                  | 6.11     |
| 34.717               | β-Farnesene                    | 2.19     |
| 34.850               | β-Selinene                     | 3.42     |
| 35.749               | Germacrene D                   | 4.40     |
| 36.037               | Zingiberene                    | 0.95     |
| 36.375               | α-Farnesene                    | 0.60     |
| 36.475               | β-Bisabolene                   | 1.14     |
| 36.993               | γ-Caryophyllene                | 1.54     |
| 37.660               | α-Humulene                     | 12.56    |
| 39.111               | β-Caryophyllene oxide          | 6.28     |
| 39.858               | Bisabolol oxide A              | 1.15     |
| **Total**            |                                 | 99.95    |
| **Total oxidised compounds** |                              | 52.89    |
| **Total hydrocarbon compounds** |                             | 47.12    |

The optical density of the culture of the tofu in NB was used as the parameter of the capability of lemon basil essential oil in inhibiting the growth of bacteria in tofu. The lower optical density indicated the presence of fewer bacteria on the tofu, and vice versa. In general, the optical densities of the culture of tofu were gradually increased from day 2 and reached the maximum value on the final day of observation. The optical densities of tofu cultures in day 2 and 4 were not significantly different and might indicate the lag phase of microbial growth, while the considerably highest optical density change observed from day 4 to 6 might refer to the log phase. The optical density of the culture of tofu treated with negative control was consistently higher than those treated with lemon basil essential oils. However, its pattern was only similar to that of lemon basil essential oil at a concentration of 0.125 mg/ml and different from those of essential oil in higher concentrations. Hence, particularly in day 2, 6, 8, and 10, lemon basil essential oil at a concentration of 0.625 and 3.125 mg/ml was capable of inhibiting the growth of bacteria on the preserved tofu (Figure 1).
Figure 1. The profile of the optical density of cultures of tofu preserved with lemon basil essential oil

AUC\textsubscript{2-10} of the optical density of cultures of tofu preserved with lemon basil versus the preservation time represented the overall bacterial growth in day 2-10. The lowest total AUC\textsubscript{2-10} value indicated the best inhibitory activity against the bacteria grown on the tofu during ten day-preservation. The total AUC\textsubscript{2-10} of tofu preserved with lemon basil essential oil was in a dose-dependent manner, which the higher concentration used resulted in, the lower total AUC\textsubscript{2-10}. However, only tofu treated with lemon basil essential oil at a concentration of 3.125 mg/ml demonstrated a significant difference in the value of total AUC\textsubscript{2-10} compared to the negative control (Figure 2). Hence, only the treatment with the highest concentration of the oil that was capable of inhibiting the growth of bacteria on the tofu during preservation.

Figure 2. The AUC\textsubscript{2-10} of the profile of the optical density of cultures of tofu preserved with lemon basil essential oil
Lemon basil essential oil has shown antimicrobial properties that support the result of our study. It inhibited the growth of Bacillus subtilis and Staphylococcus aureus with MIC value of 31.25 μg/mL and 125 μg/mL, respectively [3]. Another report mentioned that its minimum bactericidal (MBC) value against those two bacteria was 0.5 and 0.25% v/v, respectively [4]. The lemon basil essential oil was identified as the most active oils against Enterococcus faecalis, Enterococcus faecium, P. vulgaris, S. aureus and S. epidermis compared to 5 others essential oils from seven Ocimum taxa [5].

Physical characters of preserved tofu were evaluated to determine the capability of lemon basil essential oil in extending the shelf life of tofu. The bacterial growth on tofu caused the changes in the colour, odour, texture, and also slime formation on it. Improvement of tofu shelf life by treatment with the lemon basil essential oil was obtained from the comparison of time of each parameter changing between those treated with the oil and the negative control. The colour of the tofu was initially white. The colour of tofu treated with negative control was changed into yellowish in day 6, while those in lemon basil essential oil in all given concentrations started to be yellowish in day 10. The odour of unpreserved tofu started to be deteriorated in day 4, and the use of lemon basil essential oil effectively delayed it in a dose-dependent manner. The higher the concentration used, the stronger the hint of lemon basil aroma and the longer the first deteriorated odour time were observed. The same trend was also demonstrated in texture and slime formation parameters. At the concentration of 3.125 mg/ml, lemon basil essential oil was capable of maintaining the initial texture and inhibiting the slime formation until the final day of observation. The capability of lemon basil in improving the tofu shelf life was presented in Figure 3. The improvement of tofu shelf life was in a dose dependent manner in regards to odour, texture, and slime formation. When all parameters were taken into account, the improvement of tofu shelf life by treatment of lemon basil essential oil at lower, intermediate, and higher concentrations was 0, 2, and 4 days, respectively.

![Figure 3. Improvement of tofu shelf life based on the changing of physical characters](image)

However, the preservation potential of lemon basil essential oil in this study is considered low, that only the highest concentration showed the bacterial growth inhibitory activity. It might due to the unsuitability model used in this study. The essential oils were immiscible with the water as the medium, which minimize the contact between the essential oils with tofu. The essential oil would immediately separate from the water, form film in the upper surface of the water and was possibly evaporated from the medium afterwards [14]. For a better experimental model, the use of co-solvents or emulsifiers to increase the solubility of essential oil in medium might be applied. The use of
cetylpyridinium chloride to enhance the stability of cinnamon essential oil in the water had benefited the preservation of minimally processed basil leaves [15]. This strategy was also successfully applied to the preservation of Nile tilapia fillets, in which propylene glycol and tween were utilized to enhance the solubility of essential oils of rosemary and thyme in water medium [16].

4. Conclusion
Lemon basil essential oil at an optimum concentration of 3.125 mg/ml inhibited the bacterial growth on the tofu during storage and improved its shelf life up to 4 days. This preservation activity might be due to the presence of the oxygenated volatile compounds, including geranial and neral.

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