Bioactive Compounds of Lemongrass (Cymbopogon citratus) essential oil from different parts of the plant and distillation methods as natural antioxidant in broiler meat

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Abstract. The purpose of this study is to investigate bioactive compounds of lemongrass (Cymbopogon citratus) essential oil from different parts of the plant and distillation methods as natural antioxidant in broiler meat. Distillation of lemongrass leaves, stalks and whole plant was done by using steam water distillation and water distillation. Physical analysis conducted to lemongrass EO. Bioactive compounds identification by GC-MS and antioxidant activity was analyzed by DPPH radical scavenging test. The EO composition of lemongrass leaves, stalks and whole plant which been distilled using steam-water distillation and water distillation are very varied and have different inhibition. On steam water distillation, the EO was distilled from lemongrass stalk have the highest antioxidant activity up to 72.724% inhibition. While in water distillation method, the EO was distilled from whole plant of lemongrass have the highest antioxidant activity up to 70.113 % inhibition. Lemongrass oil can use as natural antioxidant in broiler meat.

Keywords: bioactive compounds, distillation method, GS-MS, lemongrass oil, natural antioxidant

1. Introduction

Meat and meat products including broiler meat are susceptible to quality deterioration due to their rich nutritional composition [1]. The quality deterioration is caused by chemical and microbial changes. The oxidation of meat lipids is the most important form of chemical deterioration. Oxidation of lipids leads to the formation of several other compounds which have negative effects on the quality of fresh meat and meat products. It causes changes in color, texture and flavor (sensory) and nutritional quality decreases [2][3]. The main factors that determine the loss of food quality and shelf-life reduction are lipid oxidation and bacterial contamination [4]. The short shelf-life of refrigerated packed meat cause these products commercialization more difficult [5]. Therefore, it happened postpone meat oxidation and preventing bacterial cross-contamination are highly to food processors. Lipid oxidation is an important determinant of shelf-life of meat and meat products, so that the control of lipid oxidation in fresh and meat products is the focus of meat scientists[6].

Lipid oxidation can be reduced by the using antioxidants in meat and meat product, so that the quality of product and shelf life can be increase [3][5]. The antioxidants can make from Synthetic or natural material. Synthetic antioxidant such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate (PG) and tert- butylhydroquinone (TBHQ) have been widely used in meat and poultry products [7][8][9][10]. Recently, consumers have refused synthetic antioxidants because of their carcinogenicity and this has resulted in increased demand for natural antioxidants, especially of plant origin. It has increased in the recent years due to growing worried of consumers about potential toxicological effect of synthetic antioxidants [11][12][13][5]. The main advantages of natural antioxidant in foods including in meat are high consumers preference and other reason is their save use but disadvantages are their higher production costs and low effectiveness [10]. So that the most of the reason investigations have been directed
towards the identification of natural antioxidants from various plant sources and plant materials. The plants that rich in phenolics are a good alternative to synthetic antioxidants [3]. The majority of natural antioxidants are phenolic compounds and the most important are the tocopherols, flavonoids and phenolic acids antioxidant [14].

Lemongrass is an aromatic plant and it belongs to the gramineae family [15]. Lemongrass (Cymbopogon citratus) is widely used herb in tropical countries especially in southeast Asia [16]. It has a citrus flavor and widely used as an herb in Asian cuisine, can be used fresh, dried or powdered. It is commonly used in soups, curries and teas, also is suitable for fish, beef, poultry and seafood. Moreover, it is used as a preservative. Lemongrass essential oil is applied for its medicinal value [17]. Lemongrass is so interesting due to its commercially valuable EOs and widely used in traditional medicine as well as in food technology [18]. The biologically active constituent of lemongrass is citral constituting more than 75% (w/w) of its essential oil [19]. The chemical composition of the essential oil Cymbopogon citratus varies according to the geographical origin [16]. Lemongrass grows in almost all tropical and subtropical countries [20]. In Indonesia, lemongrass (Cymbopogon citratus) is a plant commonly used as ingredient. Indonesians use lemongrass as spicy limited to the stalk only, while the leaves are just thrown away as garbage or waste and usually use in fresh form. The aim of this study was investigated the bioactive compounds of essential oil from lemongrass leaves, stalk and whole plant which extracted using steam-water distillation and water distillation as natural antioxidant in broiler meat.

2. Research Methods

2.1 Plant Materials and Preparation
Lemongrass (Cymbopogon citratus) plant used in the study were collected from Ngantang area, East Java, Indonesia. Research material is divided into 3 parts, namely leaves, stalks and whole plant (leaves + stalks). Lemongrass plant harvested at 6 months of age.

2.2 Extraction of Essential oil
Extraction of lemongrass essential oil was done by two methods of hydrodistillation are using steam-water distillation (indirect distillation) and water distillation (direct distillation). Distillation was done (carried out) by using extraction tool with a capacity of 5 kg. The were extracted from leaves, stalk and whole plant and lemongrass was extracted in fresh condition after harvesting. The extraction was performed for 4 h with the temperature maintained at approximately 100°C. Lemongrass oils were kept at room temperature in glass bottle away from sunlight.

2.3. Identification of lemongrass oil
The resulting lemongrass oils were identified to find out its appearance, color, odor, rendement, specific gravity, optical rotation, refractive index and solubility. Analysis procedure identification of lemongrass oil refers to SNI No. 06-3953-1995.

2.4 Determination of bioactive components
Bioactive component in lemongrass EO was analyzed using Gas chromatography- mass spectrometry (GC-MS) was performed using “GC-2010” first step, with the column oven temperature and injection temperature was set at 80°C and 250°C, respectively. The flow control mode used pressure 24.9 kPa with the total flow and column flow at 61.0 mL/min and 0.57 ml/min, respectively. Purge flow 3 ml/min and split ratio 100 were used. The oven temperature was programmed at 80.0°C for 3 minutes, and at 250°C for 5 minutes. For the second step using "MS-QP2010 Ultra", ion source temperature was set at 200°C and interface temperature 250°C, solvent cut time: 0.50 min, detector gain mode: relative to the tuning result, detector gain: 0.95 kV + 0.00 kV, with a threshold: 0. Start time: 1.00 min, end time: 29.25 min. ACQ mode used for the scan, with event time at 0.3 seconds, scan speed 3333, start m / z: 20 and end m / z: 800.
2.5 Antioxidant activity (DPPH radical scavenging activity)
DPPH scavenging activity of lemongrass oil was determined according the procedure of [21]. For assay, 1 gram sample was taken in test tube and 9 ml ethanol (96 %) was added to the sample tube, masses for 24 hours. Then 4 ml filtrate was taken in test tube and 1 ml of the fresh prepared solution of DPPH was added, let stand for 30 min. The absorbance of the sample was measured at 517 nm using spectrophotometer UV-VIS double bin (Shimadzu, Japan).

DPPH solution 1ml plus 4 ml ethanol was used as a control. All analyses were run in duplicates.

The DPPH radical scavenging effect was calculated as inhibition of percentage, according to the following formula:

\[
\text{Inhibition of percentage (\%) = } \left( \frac{\text{Ac}(o) \text{ - } \text{Aa}(t)}{\text{Ac}(o)} \right) \times 100
\]

where: \(\text{Ac}(o)\) is an absorbance of control DPPH solution at 0 min and \(\text{Aa}(t)\) is absorbance of test sample after 20 min.

The data of this research were analyzed using qualitative and quantitative description.

3. Results and Discussion
3.1 Characteristics of lemongrass oil
The results of identification to lemongrass oil are shown in Table 1.

| Method of hydro distillation | Characteristics of lemongrass oil |
|------------------------------|----------------------------------|
|                              | appearance | Color | Odor   | Relative Density | Refractive index | Optical rotation | Solubility tenement |
| Steam-water distillation     |            |       |        |                |                |                  |                      |
| Leaves                       | Liquid     | Yellow| Citrus | 0.8933         | 1.4773          | -0.06            | 1:2 0.05%          |
| Stalk                        | Liquid     | Yellow| citrus | 0.8712         | 1.4775          | +0.38            | 1:2 0.04%          |
| Whole plant                  | liquid     | yellow| citrus | 1.4780         | 1.4780          | +0.05            | 1:2 0.04%          |
| Water distillation           |            |       |        |                |                |                  |                      |
| Leaves                       | Liquid     | Yellow| Citrus | 0.8705         | 1.4725          | -0.01            | 1:2 0.23%          |
| Stalk                        | Liquid     | Yellow| Citrus | 0.8719         | 1.4745          | -0.03            | 1:2 0.07%          |
| Whole plant                  | Liquid     | Yellow| Citrus | 0.8876         | 1.4775          |                  | 1:2 0.04%          |

The results of the study showed the essential lemongrass oil distilled from leaves, stalk and whole plant liquid, yellow color, citrus odor, average relative density 0.8705 – 0.8933 and relative index 1.4725 -1.4780. This physical characteristic of lemongrass oil is in accordance with the requirement of lemongrass oil by SNI lemongrass oil no. 06-3953-1995.

3.2 Bioactive Compound of lemongrass oil by GCMS
The test results of GCMS method in leaves, stalk and whole plant lemongrass oil at steam- water distillation and water distillation are displayed in TABLE II. The compounds shown are compounds having concentrations greater than 1 percent.

The results of the study generally show the composition of EO of lemongrass leaves , stalks and whole plant which been extracted using steam-water distillation and water distillation are very varied. A total of compounds in oil from lemongrass’ leaves at steam-water distillation are more than lemongrass’ stalk and whole plant, but a total compounds lemongrass oil at water distillation are relatively the same. The compounds of lemongrass leave EO are different from lemongrass stalk EO.

Lemongrass is a rich source of citral. Citral is the main chemical component of lemongrass EO. Citral or 3,7-dimethyl-2,6-octadienal is the name given to a natural mixture of two isomeric acyclic
monoterpenoid aldehydes are geraniol (trans-citral, citral A) and neral (cis-citral, citral B). Chemically, citral is a mixture of two aldehydes that have the same molecular formula, C10H16O but different structures. In this study, citral in the leaves lemongrass oil was in either cis form (Z-citral) and trans form (E-citral), but in the stalk lemongrass oil was in cis form (Z-citral) and citral at both distillation methods. Besides that the leaves lemongrass oil contains trans-geraniol, 6-methyl-3-hepten-2-one, beta-myrcene and longipineneoxide in different amounts between steam-water distillation and water distillation methods [22] as reported the citral content of essential lemongrass oil made from leaves was higher than made from stalk at steam distillation method [18], which indicated that phenolic content of the essential oil extracted using hydro-distillation method from lemongrass stalk higher amount than extracted from lemongrass leaves.

It is noteworthy from several studies have shown that the composition of EOs are influence by climatic, seasonal, local and experimental conditions, etc. The maturity of lemongrass when harvesting influence, the quality essential oil and citral contents [23].

Table 2. Bioactive compounds of lemongrass oil with GC-MS

| Part of plant and Distillation method | Compounds | Retention Times | Con. Unit (%) |
|--------------------------------------|-----------|----------------|---------------|
| Leaves: Steam | 1. 6-Methyl-5-hepten-2-one | 5.745 | 14.137 |
| water distillation | 2. beta-Myrcene | 6.118 | 18.771 |
| 3. Linalool | 8.182 | 3.351 |
| 4. Citronella | 9.151 | 1.302 |
| 5. Longipineneoxide | 9.704 | 1.759 |
| 6. Z-citral | 10.965 | 19.225 |
| 7. Trans-geraniol | 11.323 | 10.686 |
| 8. E-Citral | 11.555 | 26.522 |
| 9. Geranyl acetat | 13.577 | 1.359 |
| 10. Patchouli alcohol | 18.507 | 1.060 |
| Stalk: Steam | 1. Z-Citral | 10.939 | 36.501 |
| water distillation | 2. Trans-Geraniol | 11.287 | 1.832 |
| 3. Z-Citral | 11.518 | 55.172 |
| 4. 1,2-Benzenedicarboxylic acid, diotyl ester | 21.989 | 8.949 |
| Whole plant Steam | 1. 6-Methyl-5-hepten-2-one | 5.739 | 1.252 |
| water distillation | 2. beta-Myrcene | 6.112 | 1.641 |
| 3. Z-Citral | 10.932 | 36.043 |
| 4. Trans-Geraniol | 11.276 | 2.272 |
| 5. Z-Citral | 11.506 | 52.846 |
| 6. 1,2-Benzenedicarboxylic acid, diotyl ester | 21.979 | 3.756 |
| Leaves: water distillation | 1. 6-Methyl-5-hepten-2-one | 5.740 | 1.614 |
| 2. beta-Myrcene | 6.115 | 3.000 |
| 3. Longipineneoxide | 9.705 | 1.071 |
| 4. Z-citral | 10.961 | 39.397 |
| 5. Trans-geraniol | 11.324 | 4.064 |
| 6. E-Citral | 11.531 | 48.730 |
| Stalk: water | 1. 6-Methyl-5-hepten-2-one | 12.063 | 2.986 |
| distillation | 2. L-LINALOOL | 16.150 | 1.217 |
| 3. TRANS-CARAN,4,5-EPOXI | 18.543 | 1.365 |
| 4. Z-Citral | 20.402 | 9.902 |
| 5. Citral | 21.318 | 72.896 |
| 6. Juniper camphor | 21.318 | 72.896 |
| 7. 1,2-Benzenedicarboxylic acid, diotyl phthalate (CAS) Dioctyl phthalate | 49.369 | 2.560 |
| Whole plant water distillation | 1. 6-Methyl-5-hepten-2-one | 12.057 | 2.447 |
| 2. Z-Citral | 20.368 | 24.042 |
| 3. Trans-geraniol | 21.056 | 11.670 |
| 4. E-Citral | 21.292 | 45.123 |
| 5. Geranyl acetat | 24.505 | 1.251 |
| 6. Juniper camphor | 30.793 | 1.714 |
| 7. 1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (CAS) Bis(2-ethylhexyl) phthalate | 49.401 | 5.622 |
3.2 Antioxidant Activity

The result of DPPH scavenging activity of lemongrass oils are shown in Table 3.

| Hydrodistillation method | Part of plant | Average of antioxidant activity (%) |
|-------------------------|--------------|-------------------------------------|
| Steam-water distillation| leaves       | 60.808                              |
|                         | Stalk        | 72.274                              |
|                         | Whole plant  | 48.026                              |
| Water distillation      | leaves       | 57.331                              |
|                         | Stalk        | 51.034                              |
|                         | Whole plant  | 70.113                              |

The results of this study of antioxidant activity with DPPH free radical scavenging test show that the EOs were distilled from lemongrass leaves, stalk and whole plant have different percentage inhibition, both on steam-water distillation and water distillation. On steam water distillation, the essential oil was distilled from lemongrass stalk have the highest antioxidant activity up to 72.724% inhibition. While in water distillation method, the essential oil was distilled from whole plant of lemongrass have highest antioxidant activity up to 70.113 % inhibition. Antioxidant activity of essential oil made from leaves was higher than made from stalk by using steam distillation method [22]. Other study shown that The highest percentage inhibition was obtained by EOs is distilled from lemongrass stalk [18] The application of the method is based on several considerations such as the type of plant raw material, oil characteristics, oil diffusion process with hot water, oil decomposition due to heat effects, production efficiency and reasons for economic value and production effectiveness.

4. Conclusion

The conclusion that the bioactive compounds of lemongrass (Cymbopogon citratus) essential Oil from different parts of the plant and different distillation method are very varied and have different percentage inhibition. On steam water distillation, the the highest value of antioxidant activity come from lemongrass stalk. While in water distillation method, the highest value of antioxidant activity come from whole plant of lemongrass. Based on average of antioxidant activity, Lemongrass oil can use as natural antioxidant in broiler meat.

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