Peppermint oil loaded on recycled paper as an antibacterial label for shrimp freshness

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Abstract. Peppermint oil was one of the essential oils with an antibacterial bioactive component and the potential to be used as an antibacterial label. The purposes of this research were preparing the antibacterial label and evaluate their activity in prolonging the storage of shrimp. Antibacterial label was created by loading the peppermint oil onto the recycled paper as a matrix. Peppermint oil was characterized with Gas Chromatography-Mass Spectrometry (GC-MS) to identified suspected antibacterial activity compounds. The results showed that peppermint oil contains 39.79\% of menthol and 35.69\% of menthone as the main component. Peppermint oil and antibacterial labels have been screened for Gram-positive bacteria Staphylococcus aureus NBRC 100910 and Gram-negative bacteria Escherichia coli NBRC 100910 using the paper disk diffusion method. Peppermint oil has antibacterial activity in both bacteria, and the label only have antibacterial activity in E. coli NBRC 100910. The antibacterial labels research on prolonging the storage of shrimp shown that the Total Volatile Basic Nitrogen (TVBN) value was lower than without using the label. The results on this study showed recycled paper filled with peppermint oil can be used as an antibacterial label for an active packaging system.

1. Introduction
An antibacterial label is a label containing an antibacterial agent that can be applied as an active packaging. Active packaging is an innovative technology developed to change the function of packaging from passive protection to active protection. The use of antibacterial label in packaging system is very interesting because it can maintain the quality of food products and ensure their safety.

An antibacterial label was made by using antibacterial agents combined in a matrix. The natural antibacterial agents like essential oils are very prospective to be used because they are safe, have a wide spectrum and biodegradable. Peppermint oil is one of essential oil which reported have antibacterial bioactivity against Gram positive bacteria and Gram negative bacteria [1]. There are some matrices have been used for supporting of peppermint oil as antibacterial label, like chitosan [2], cellulose [3], and synthetic polymer [4]. In this research, peppermint oil will be loaded in recycled paper for an antibacterial label. From studies on matrix for antibacterial label containing peppermint oil, no one has used recycled paper. The use of recycled paper will be a simple method to preparation antibacterial label from peppermint oil.

The aims of this research were to prepare antibacterial label using peppermint oil loaded in recycled paper, investigate its antimicrobial activity, and test its performance in shrimp storage.
2. Materials and Methods

2.1 Materials
Peppermint oil was obtained from Nusaroma, Indonesian essential oil company, and recycled paper from local recycled industry.

2.2 Methods
2.2.1 The Chemical Compound Identification of Peppermint Oil using GCMS. The chemical compounds in peppermint oil were characterized by Gas Chromatography Mass Spectrometer (GC-MS). The GC apparatus was Agilent 6890 series with a HP-5MS capillary column with length of 30 m, diameter of 0.25 mm and film thickness of 0.25 µm. The program of oven temperature was initiated 60°C, raised up to 240°C at rate of 3°C per minute [5]. This analysis used the Helium as carrier at 65 kPa (constant pressure). One µL of the peppermint oil was injected in 1:25 of split ratio.

2.2.2 Antibacterial Label Preparation. The antibacterial label was prepared by dropping 50 µL of peppermint oil in recycle paper circular shape with a diameter of 6 mm, then allowed to dry at room temperature for 5 minutes.

2.2.3 Antibacterial Activity Assay. Antibacterial assay was conducting using the paper disc diffusion method that carried out by Handayani [6]. This assay used the Staphylococcus aureus NBRC 100910 strain and Escherichia coli NBRC 3301 strain. Ten µl of molten media (The Mueller Hinton Agar) was poured into sterile Petri dish with diameter of 9 cm and allowed for 5 minutes until solidify. The bacteria culture 10⁶ CFU/mL of as much 10 µl was added with 10 µl of medium and mixed gently with the inoculate before poured on the top of molten media and allowed for 5 minutes to dry. The tetracycline 15 µg/mL as positive control, the sterile distilled water as negative control, and 1000 µg/mL of peppermint oil were loaded on 6 mm disc. The loaded paper disc placed on the medium surface then incubates for 18 hours at 32°C. When the incubation was end, a clear zone was formed around the paper disc and the diameter was measured.

2.2.4 Fourier Transform Infra Red (FTIR) Characterization. An FTIR spectra were collected for the antibacterial label using Thermo Nicolet iS5 to monitor label activity.

2.2.5 Total Volatile Basic Nitrogen (TVBN). The shrimp was weighed about 10 g, storage into a PVC square packaging and the label was affixed at the top of the PVC square packaging with the distance with shrimp of 10 mm. The PVC square packaging was then closed tightly. Shrimp freshness was observed for three days at room temperature and TVBN levels were measured every day. The method for measurement referred to the Commission Regulation (EC) No 2074/2005 [7]. As the control used shrimp that are packaged without using antibacterial label.

3. Results and Discussion
Characterization of peppermint oil using GC-MS showed the chromatogram profile in Figure 1. The chromatogram profile detected a lot of peaks representing the number of compounds in peppermint oil. The identifying of compounds was conducted based on spectrum of mass with reference data from the database of Wiley 9 and used the result with quality above 95% (Table 1). There were 17 compounds in peppermint oil. menthol (39.79%), menthone (35.69%) were the main component in peppermint oil followed by camphene (5.86%), 1,8 cineole (5.40%), trans caryophyllene (2.92%), α-terpinolene (2.13%), d limonene (2.04%), β-pinene (1.36%) and some others compound. Many studies reported menthol and menthone as the main component in peppermint oil [8] [9] [10].
Figure 1. The peppermint oil GCMS chromatogram

Table 1. The composition of chemical compounds in peppermint oil

| No. | Retention time | Compounds       | Abundance (%) |
|-----|----------------|-----------------|---------------|
| 1.  | 5.105          | α-pinene        | 0.98          |
| 2.  | 6.310          | β-pinene        | 1.36          |
| 3.  | 8.040          | d-limonene      | 2.04          |
| 4.  | 8.174          | 1,8 cineole     | 5.40          |
| 5.  | 9.137          | γ-terpine       | 0.22          |
| 6.  | 9.499          | γ-terpine       | 0.19          |
| 7.  | 13.306         | Menthone        | 23.25         |
| 8.  | 13.672         | Menthone        | 12.44         |
| 9.  | 14.431         | Menthol         | 39.79         |
| 10. | 14.877         | α-terpinolene   | 2.13          |
| 11. | 16.874         | Pulegone        | 1.22          |
| 12. | 17.520         | Piperitone      | 0.70          |
| 13. | 18.467         | Camphene        | 0.17          |
| 14. | 19.409         | Camphene        | 5.69          |
| 15. | 23.153         | β-bourbonene    | 0.30          |
| 16. | 24.666         | Trans Caryophyllene | 2.92  |
| 17. | 27.242         | D-germacrene    | 0.52          |

Menthol is a cyclic monoterpene alcohol with the minty taste and have antifungal and antibacterial activity [11] [12] [13]. Menthone is monoterpene with minty, structurally related to menthol, however, the alcohol group is oxidized to form ketones. Menthone have antifungal and antibacterial activity like menthol.

The antibacterial assay result showed that the inhibition zones were detected in the peppermint oil in *S. aureus* NBRC 100910 and *E. coli* NBRC 100910 (Figure 2). The clear zone diameter /the inhibition zone diameter in *E. coli* NBRC 100910 is higher than in *S. aureus* NBRC 100910 (Table 2). Gram negative bacteria is more resistant than Gram-positive bacteria against essential oil generally. Gram-negative bacteria have a rigid outer membrane which rich in lipopolysaccharides and more complex, thus limiting of the hydrophobic compound diffusion like essential oil. Gram-positive bacteria have a peptidoglycan wall but not dense enough and unable to hold small antibacterial molecules to through the cell membrane [14]. The peppermint oil is more active against *E. coli* NBRC 100910 in this experiment, contrary to that statement. In previous study showed that peppermint oil is more active in Gram negative bacteria *E.coli* than in Gram positive bacteria *S. aureus* NBRC 100910 [10], but in another study showed that peppermint oil is active in both Gram positive and in Gram
negative [9]. Many factors like chemical composition, the constituent of component, functional groups of component and synergistic effect between components influenced the antibacterial activity in essential oil [15]. The peppermint oil contains menthol with alcohol functional group and menthone with ketone functional group which have synergistic interactions in antibacterial activity.

![Figure 2. Peppermint oil antibacterial activities in S. aureus NBRC 100910 and E. coli NBRC 100910; A = control (-); B = control (+); C = sample.](image)

![Figure 3. Antibacterial activity of label](image)

| Table 2. Inhibition diameter zone of peppermint oil |
|-----------------------------------------------|
| Essential Oil | S. aureus NBRC 100910 (cm) | E. coli NBRC 100910 (cm) |
| Sample | Control (-) | Control (+) | Sample | Control (-) | Control (+) |
| Peppermint oil | 1.8 | 0 | 3.4 | 3.3 | 0 | 3.7 |

Antibacterial assay was also carried out on the antibacterial label and the results was showed in Figure 3. The inhibition zone was only formed in Gram-negative bacteria E. coli NBRC 100910 with a diameter of 1.92 cm. The inhibition zone was not detected in S. aureus NBRC 100910. The label has antibacterial activity on E. coli NBRC 100910 but not on S. aureus NBRC 100910. The amount of peppermint loaded to recycled paper is probably still insufficient, so the label did not show antibacterial activity on S. aureus NBRC 100910.

Antibacterial label performance was investigated in shrimp storage by monitoring of TVBN levels during storage. TVBN or volatile bases are compounds that are formed due to protein damage caused by enzymatic and biological activity to form compounds such as ammonia, trimethyl amine, dimethyl amine, and other volatile basic nitrogen compounds [16]. The measurement of TVBN can be used as a method for monitoring shrimp freshness [17]. TVBN determination was done to observe the freshness
of shrimp stored using label and without label. The result of TVBN during storage were shown in Figure 4. The TVBN value is increasing during storage, as in previous research which reported that TVBN levels will increase during the storage period[18]. The TVBN value on shrimp stored without antibacterial label is higher than shrimp stored with antibacterial label. It indicates that antibacterial label from peppermint oil prospective for maintain the freshness of shrimps. More research was necessary to conducted to optimize the concentration of peppermint oil filled in the recycled paper.

**Figure 4. TVBN of shrimp during storage**

FTIR spectroscopy was performed to observe the functional groups changes during the storage of shrimp. Figure 5 shows the antibacterial label FTIR spectra during storage. The IR characteristics of peppermint oil in 3,470 cm\(^{-1}\) and in the range 1,700—1,750 cm\(^{-1}\). In the wavenumber of 3,470 cm\(^{-1}\) there is the strong band that assigned to the \(-\text{OH}\) groups vibration revealed the presence of menthol, and the bands in the spectral range of 1,700—1,750 cm\(^{-1}\) is most possibility related to the vibration of C=O from menthone [19]. From the Figure 5, the \(-\text{OH}\) intensity of menthol and C=O intensity of menthone is decreasing indicates menthol and menthone have to be released from the label during storage.

**Figure 5. The antibacterial label FTIR spectra during the storage of shrimp**
4. Conclusion
The peppermint oil was used in this study contained 39.79% menthol and 35.69% menthone as the main component which have antibacterial activity. The peppermint oil has the antibacterial activity against S. aureus NBRC 100910 and E. coli NBRC 100910. The antibacterial performance indicated that the label prospective to be used to maintain the freshness of shrimp.

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