Simple Antimicrobial Labels from Cinnamon Oil Added to Recycled Paper

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Abstract: Essential oils are one of the antimicrobial agents that are safe for food, and thus can be used as an antimicrobial label to extend the shelf life of food products. This study aims to prepare antimicrobial labels and to investigate their activities in shrimp. Antimicrobial labels are made using cinnamon oil in the recycled paper as a simple matrix. Cinnamon oil was tested on Gram-positive Staphylococcus aureus and Gram-negative bacteria Escherichia coli using the paper disk diffusion method. From the results obtained, cinnamon oil has both antimicrobial activities. Cinnamon oil is also characterized using Gas Chromatography-Mass Spectrometry (GC-MS) to determine the level and presence of compounds suspected of having antimicrobial activity. Cinnamon oil has interactions with recycled paper functional groups as measured by Fourier Transform Infrared Spectroscopy (FTIR). Testing of antimicrobial labels on shrimp shows that the Total Volatile Basic Nitrogen (TVB-N) value is better than without the label. From the results of antimicrobial activity, can be seen that cinnamon oil applied to recycled paper has the potential to be used as a simple antimicrobial label.

1 INTRODUCTION

Fresh shrimp is very easy to damage. Many methods have been carried out to maintain the freshness and shelf life of shrimp. The use of synthetic preservatives to maintain the freshness and quality of shrimp can endanger health. At present natural preservatives with excellent antimicrobial properties have been searched and implemented as safe alternatives in seafood processing to extend shelf life. Natural preservatives commonly used include plant extracts, bacteriocins, bioactive peptides, chitosan and chitooligosaccharide, and essential oils (Olatunde and Benjakul, 2018). Essential oils from aromatic plants have antimicrobial properties and are safe to add to food or food packaging (Santos et al., 2017).

Currently, some researchers are developing the addition of essential oil as an antimicrobial to the paper matrix. Researches on adding essential oil as an antimicrobial and antifungal to paper matrix that have been carried out are carvacrol (Mascheroni et al., 2011) and cinnamon essential oil (Echegoyen and Nerin, 2014). From the research that has been done, the addition of essential oil to the paper matrix mostly uses a coating method that requires applicator coating equipment. Therefore, necessary to develop a preparation method that is simple, practical, can be used as an antimicrobial, and integrated with product packaging.

This research aims to develop a simple antimicrobial label by using cinnamon oil. As the matrix of this simple antimicrobial label is recycled paper. From studies on active paper packaging that have been done, no one has ever used a matrix of recycling paper. The use of recycled paper can increase the added value of the recycled paper. Also, the recycled paper easily absorbs essential oils compared to other types of paper. The preparation method of the label is simple, by dropping cinnamon oil on the circular shape of the recycled paper. The simple antimicrobial label is then tested to detect the

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characterization, antimicrobial activity, and TVB-N of the shrimp after applied by a label.

2 MATERIALS AND METHOD

2.1 Materials

The simple antimicrobial label was made using materials recycle paper purchased from local stationery shop and cinnamon oil purchased from Nusa Aroma local essential oils company in Indonesia.

2.2 Antimicrobial Label Preparation

The simple antimicrobial labels were composed of the cinnamon oil and the matrix made from recycled paper. The labels were prepared by dropping of 50 µL cinnamon oil on circular shape cutting of the recycle paper with a diameter of 6 mm. The label then dried in room temperature for 5 minutes ready for use.

2.3 Characterization

2.3.1 Cinnamon Oil Characterization using GCMS

Cinnamon oil was characterized using a mass spectrometer detector (GCMS), to find out the chemical compounds contained in cinnamon oil. The tools used are GC / MS with Agilent 6890 series specifications with capillary column HP-5MS, 30 m x 0.25 mm id x 0.25 µm film thickness. As the carrier gas was used helium gas at constant pressure. The essential oil was injected with a volume of 1 µL (split ratio of 25: 1). The oven temperature was programmed from 60 °C - 240 °C for an increase of 3 °C per minute until reaching 250 °C.

2.3.2 Characterization using FTIR

FTIR characterization is used to monitor label activity. Tests are carried out on blank paper and labels before and after storing shrimp and carried out every day. The blank and the label were measured on the Seri Nicolet iS5 FTIR spectrometer. All spectra were taken in the spectral range of 4000 cm^{-1} until 500 cm^{-1}.

2.4 Antimicrobial Activity Assay

2.4.1 Direct Contact Agar Diffusion Tests

Direct contact agar diffusion tests determined by the paper disk diffusion method using strain type of Staphylococcus aureus NBRC 100910 and Escherichia coli NBRC 3301 in The Mueller Hinton Agar. 10 mL of molten media was put into a sterile petri dish (d = 90 mm) until it became solid for 5 minutes. 10 µL bacterial culture 10^{-6} CFU / mL is added with 10 mL medium into the tube and mixed slowly with inoculating before pouring on the top surface of the molten media and allowed to dry for 5 minutes. The negative control (sterile distilled water), positive control (tetracycline 15 µg / mL), and cinnamon oil with a concentration of 1000 µg / mL are poured on 6 mm discs, where the volume for each disc is 10 µL. The disc is then placed on the surface of the medium then incubated at 35 °C for 18 hours. After completion of incubation, a clear zone is formed around the disc measured.

2.4.2 Vapour Phase Agar Diffusion Tests

This vapour phase agar diffusion uses the method used by (Wang et al., 2016). The vapour phase agar diffusion test technically has the same method as the direct contact method. The test uses a 6 cm diameter petri dish, bacterial culture, filter disc size, and cinnamon oil adding. The disk filter is placed in the middle of the lid of the petri dish. The dishes are then sealed using a paraffin laboratory to prevent evaporation of the test compound. Incubation ran at 32 °C for 24 hours. The clear zone diameter was measured.

2.5 Total Volatile Basic Nitrogen (TVB-N)

The shrimp used for this experiment were fresh obtained from the local market. Shrimp after being bought directly delivered to the laboratory and prepared as soon as possible for observation. The shrimp weighed as much as 10 g and then put into a PVC square packaging. Then a simple antimicrobial label was attached to the top of PVC square packaging containing the shrimp, placed indirectly in contact with the shrimp. The distance between the label and shrimp is about 1 cm. The PVC square packaging is then tightly closed. Observation of shrimp freshness was carried out at room temperature for three days. During this time, TVB-N levels were measured every day, as a control used

shrimp that are packaged without using simple antimicrobial label. Measurement of TVB-N levels in shrimp according to the Total Volatile Basic Nitrogen (TVB-N) method based on Commission Regulation (EC) No 2074/2005 (EC, 2005).

3 RESULT AND DISCUSSION

3.1 Chemical Compounds of the Cinnamon Oil

The method used to analyse volatile oils for many years is using gas chromatography. GC-MS is the most appropriate technique used to identify the compounds contained in essential oils. The chromatogram profile of cinnamon oil is shown in Figure 1, and the results of the characterization of the chemical compounds listed in cinnamon are shown in Table 1.

A total of four different components, with different retention times, were indicated by the chromatogram in Figure 1. Based on Table 1, GC-MS analysis revealed that different chemical compositions were identified in cinnamon oils, including cinnamaldehyde, iso-bornyl acetate, cinnamaldehyde dimethyl acetyl, and cynamil alcohol. The main component of cinnamon oil is cinnamaldehyde (83.87%). This result is the same as some of the results of previous studies conducted by

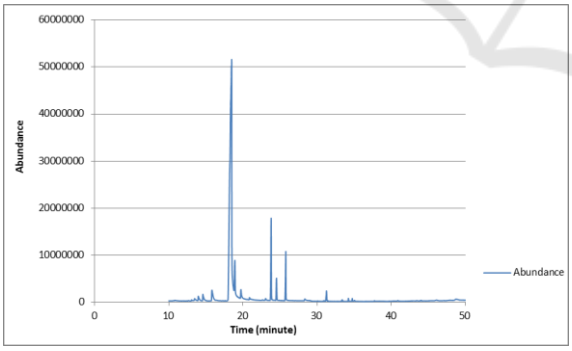

Figure 1: GCMS chromatogram of the cinnamon oil.

Table 1: Chemical component identified of cinnamon oil with GCMS.

Retention time	Identified compound	Molecular formula	Relative percentage area (%)
18.501	Cinnamaldehyde	C_9H_8O	83.87
18.913	Iso-bornyl acetate	$C_{12}H_{20}O_2$	4.71
23.817	Cinnamaldehyde dimethyl acetal	$C_{11}H_{14}O_2$	7.31
25.788	Cynamil alcohol	$C_{11}H_{12}O_2$	4.10

researchers that cinnamaldehyde is a major component of cinnamon oil (Gotmare and Tambe, 2019; Dwijatmoko, 2016; Li, Kong and Wu, 2013). Cinnamaldehyde is a compound containing aldehyde groups and conjugated double bonds outside the ring (Sachdeva et al., 2017). Cinnamaldehyde is an organic mixture that gives wood a sweet taste and smell (also known as cinnamic aldehyde). This organic compound is significant to inhibit bacterial growth (Ashakirin et al., 2017). Antimicrobial activity of cinnamaldehyde was found against E. coli and staphylococcus aureus. Cinnamaldehyde plays a role in disrupting bacterial cell membranes (Firmino et al., 2018).

3.2 Antimicrobial Activity of Cinnamon Oil

The antimicrobial activity of cinnamon oil was analysed for gram-positive bacteria (*S. aureus*) and gram-negative bacteria (*E. coli*). The results of the analysis of antimicrobial activity are shown in Figure 2 and Table 2.

Antimicrobial ability is shown from the diameter of the inhibition zone (measured the clear area) as shown in Figure 2. Based on Table 2, cinnamon oil has antimicrobial activity against Escherichia coli and Staphylococcus aureus. In the paper disc diffusion method, the area of inhibition depends on

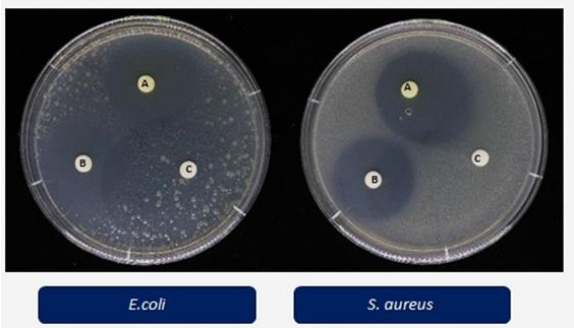

Figure 2: The inhibition zone cinnamon oil by the paper disc diffusion method (a : positive control, tetracycline; b : sample, cinnamon oil; c : negative control (sterile distilled water)).

Table 2: Antimicrobial activity of cinnamon oil.

Essential Oil	E. coli (mm)			S.aureus (mm)		
	Sample	Control (-)	Control (+)	Sample	Control (-)	Control (+)
Cinnamon oil	34	0	30	35	0	38

the ability of the essential oil to diffuse evenly to medium and also releases volatile compounds from essential oil. Inhibition zone of cinnamon oil against E. coli is 34 mm, and against a S. aureus is 35 mm. These results are similar with previous research conducted by (Adinew, 2014) reported that cinnamon oil shows an inhibitory effect against the gram-positive bacteria (Bacillus cereus, Micrococcus luteus, Staphylococcus aureus, and Enterococcus faecalis) and gram-negative bacteria (Alcaligenes faecalis, Enterobacter cloacae, and Escherichia coli).

3.3 Antimicrobial Activity of the Simple Antimicrobial Label

Cinnamon oil that has been added to the recycle paper is then analysed for its antimicrobial activity compared to the blank, to determine the antimicrobial ability of the label. Analysis of antimicrobial activity on labels is done by paper disk (direct contact) and vapour phase diffusion test because when applied to shrimp analysed using the phase diffusion vapour method. The analysis results are shown in Figure 3 and Table 3.

Based on Figure 3, the blank (only recycled paper) does not show the inhibition zone. The absence of the inhibition zone indicates that recycle paper does not have the antimicrobial ability.

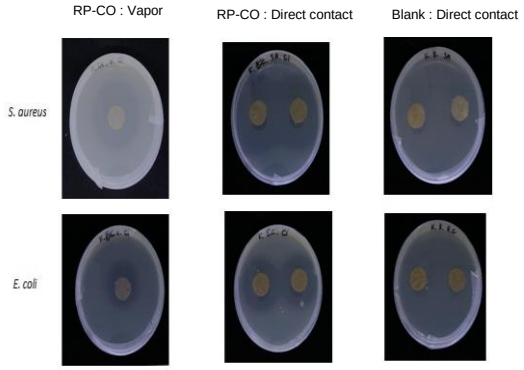

Figure 3: The inhibition zone simple antimicrobial label by the paper disc and vapour phase agar diffusion method.

Table 3: Antimicrobial activity of simple antimicrobial label.

	Direct contact		Vapor	
	E. coli (mm)	S.aureus (mm)	E. coli (mm)	S.aureus (mm)
Recycle Paper cinnamon oil	36.98	50.31	28.75	44.54

Meanwhile, after adding cinnamon oil to the recycle paper, the inhibition zone was seen, which stated that the label has the antimicrobial ability. The antimicrobial label has an antimicrobial ability against the gram-positive bacteria (Staphylococcus aureus) and gram-negative bacteria (Escherichia coli). It can be seen from Table 3 that a clear zone or diameter of the inhibition zone of 36.98 cm (E. coli) and 50.31 cm (S. aureus) for the direct contact method, while the vapour diffusion method is 28.75 cm (E. coli) and 44.54 cm (S. aureus). Antimicrobial labels have a better antimicrobial ability against S. aureus than E. coli, both from the test results using vapour or direct contact. This result is the same as the results of research conducted by (Zhang et al., 2016), which states that E. coli is more resistant to cinnamon oil than S. aureus. This phenomenon probably due to differences in the structure of the bacterial outer membrane. E. coli has a thick layer of lipopolysaccharide on its outer membrane that covers the cell wall, whereas S. aureus has only a single peptidoglycan layer structure. Therefore E. coli is more resistant to essential oils (hydrophobic substance) compared with S. aureus.

3.4 Total Volatile Basic Nitrogen (TVB-N)

The enzymatic and bacteriological activity can quickly reduce the protein content and quality of stale seafood, some ammonia, trimethylamine, dimethylamine, and other volatile basic nitrogen compounds are produced, which together are called TVB-N (Fallah et al., 2016). Total volatile basic nitrogen (TVB-N) is one method that is often used to measure seafood quality and, most commonly, as an indicator of chemical decay in marine products (Altissimi et al., 2017). TVB-N analysis was performed to find out the freshness of shrimp stored without or using simple antimicrobial labels. TVB-N analysis results are shown in Figure 4.

Based on the graph in Figure 4 shows that the value of TVB-N is increasing. It is consistent with the results of previous research conducted by (Chakrabortty et al., 2017), which states that the value of TVB-N increases with storage time. The low value of TVB-N is an indication of the quality of fresh shrimp, while the high value of TVB-N may be due to the action of the enzyme autolysis and spoilage bacteria. TVB-N values for "high quality" quality up to 25 mg / 100 g, "good quality" up to 30 mg / 100 g, "limit of acceptability" up to 35 mg / 100 g, and "spoiled" above 35 mg / 100 g (Jinadasa, 2014). From the graph in Figure 4 also shows that the value of TVB-N for shrimp stored using simple antimicrobial labels is lower than shrimp stored without using simple antimicrobial labels. It shows that simple antimicrobial labels can be used to maintain the freshness of shrimps, however further research is needed to determine the optimization of the addition of cinnamon oil to recycled paper.

3.5 Fourier Transform Infrared Spectroscopy

The functional group of the label was analysed using FTIR for three days to determine changes in functional groups that occur during that day. Figure 5 displays the spectra of the simple antimicrobial labels.

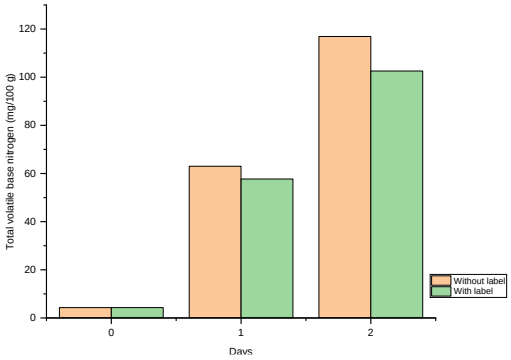

Figure 4: Total volatile base nitrogen (TVB-N) of shrimp.

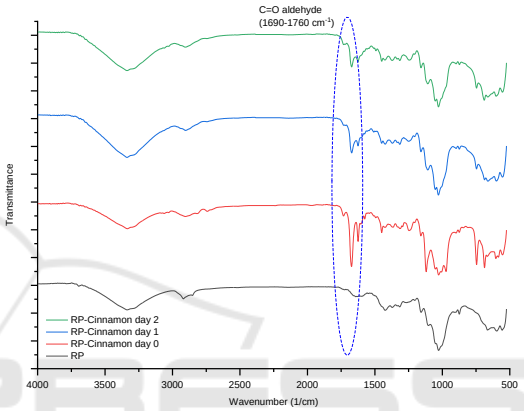

Figure 5: FTIR spectra of recycling paper and simple antimicrobial label.

Based on Figure 5, the IR characteristic fingerprint for cinnamon oil is mostly in the range of 1800 cm^{-1} - 600 cm^{-1} (Li et al., 2013). In the IR spectra, it is shown that the absorbance band at 1690 cm^{-1} - 1760 cm^{-1} revealed the presence of C = O bond for aldehyde from cinnamaldehyde (Adinew, 2014). These results are consistent with the results of the analysis using GCMS, which shows that the main component of cinnamon oil is cinnamaldehyde. The IR spectroscopy spectrum display characteristic bands corresponding to aromatic CH bonds (between 3000 cm^{-1} and 3100 cm^{-1}), CH alquenes (between 3020 cm^{-1} and 3080 cm^{-1}), and C = C (between 1640 cm^{-1} - 1680 cm^{-1}) (Singh et al., 2011). From the Figure, the C = O intensity of cinnamaldehyde is decreasing. It is because cinnamaldehyde has to be released from the label.

4 CONCLUSION

In this experiment, cinnamon oil has antimicrobial ability against the gram-positive bacteria (Staphylococcus aureus) and gram-negative bacteria (Escherichia coli). Simple antimicrobial labels from cinnamon oil added to the recycled paper also have antimicrobial ability against the gram-positive bacteria (Staphylococcus aureus) and gram-negative bacteria (Escherichia coli). The results obtained from this experiment indicated that this simple antimicrobial label could be used to maintain the freshness of shrimps. Further research is needed to determine the optimization of the addition of cinnamon oil to recycled paper.

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