The comparison of antimicrobial packaging properties with different applications incorporation method of active material

R W Anwar¹, Sugiarto¹ and E Warsiki*¹

¹Department of Agroindustrial Technology, Faculty of Agricultural Engineering and Technology, Bogor Agricultural University, Bogor, Indonesia

*Email : endang.warsiki@apps.ipb.ac.id

Abstract. Contamination after the processing of products during storage, distribution and marketing is one of the main causes of food safety issues. Handling of food products after processing can be done during the packaging process. Antimicrobial (AM) active packaging is one of the concept of packaging product development by utilize the interaction between the product and the packaging environment that can delay the bacterial damage by killing or reducing bacterial growth. The active system is formed by incorporating an antimicrobial agent against a packaging matrix that will function as a carrier. Many incorporation methods have been developed in this packaging-making concept which were direct mixing, polishing, and encapsulation. The aims of this research were to examine the different of the AM packaging performances including its stability and effectiveness of its function that would be produced by three different methods. The stability of the packaging function was analyzed by looking at the diffusivity of the active ingredient to the matrix using SEM. The effectiveness was analyzed by the ability of the packaging to prevent the growing of the microbial. The results showed that different incorporation methods resulted on different characteristics of the AM packaging.

1. Introduction
Active packaging sometimes referred to a smart packaging, are designed to safeguard food quality. The concept of active packaging is intended to sense internal environmental change and to respond by changing its own properties of package. Active packaging systems are developed with the goal of extending shelf life for foods and increasing the period of time that the food is still fresh. Active packaging technologies include some physical, chemical, or biological action which changes interactions between a package, product, and/or headspace of the package in order to get a desired outcome [1]. An exciting innovation in active packaging is antimicrobials packaging. Antimicrobials packaging systems based on the application of packaging materials with incorporated antimicrobial agents in the packaging matrix and/or using antimicrobial polymers. When the packaging system acquires antimicrobial activity, the packaging system (or material) limits or prevents microbial growth by extending the lag period and reducing the growth rate or decreases live counts of microorganisms [2]. The active agents could usually derive from chemical and nature compound, such as chili pepper. Chili pepper contains many biological active compounds, including capsaicinoids [3]. The famous compounds enlisted as capsaicinoids are capsacin, dihydrocapsaicin, nordihydrocapsaicin, dihydrocapsaicin and capsacin. Major part (greater than 90%) of chili pepper capsaicinoids consists of
two most potent compounds, capsaicin and dihydrocapsaicin [4]. Natural antimicrobials, such as chili peppers, are receiving a good deal of attention for a number of microorganism-control issues. Recent reports stated that the Capsicum genus, among other plant genera, was good source of antimicrobial and antifungal compounds [5]. Antimicrobial agents can be incorporated into a packaging system through simple blending with packaging materials, immobilization or coating differently depending on the characteristics of packaging system, antimicrobial agent and food [6].

These techniques are performed in an effort to maximize the potential for active ingredients used when it has been incorporated into the packaging matrix. The use of antimicrobial packing will be achieved if during the packaging process the microorganism’s inhibitory activity can lasted until the product is consumed. The effectiveness and stability of antimicrobial agents should further be known by the analytical approach to the things indicating that the incorporated active ingredients are maximized. This is the basis of the development of this research, which is expected to be a consideration for further antimicrobial packaging innovations. The aim of the study was to know the different of the AM packaging performances that would be produced by three different methods of incorporation of active agent into the matrix in order to know its stability and effectiveness of AM packaging function.

2. Materials and Methods

2.1. Material preparation

The material preparation included active compound and matrix. The active compound used was oleoresin from chili pepper containing capcaisin as an anti-microbial active compound. The use of this compound referred to the research of Panji [7] with the best result of using oleoresin of CapsicumTM. This compound would also be used in the step of anti-microbial film incorporation. The matrix was prepared based on the research of Lestari [8], which consisted of powder agar 2.0% (m/v), glycerol 1.0% (v/v), and tapioca powder 0.5% (b/v).

2.2. The incorporation of active compound

The production of anti-microbial film was carried out with three principles of the incorporation of active compound to film matrix i.e. direct mixing, spreading and encapsulating. The method of direct mixing was referred from Maharani [9], in which the active compound was incorporated into the film solution made in the previous step. At the end of film solution production (50°C), as much as 0.6% (v/v) chili pepper oleoresin was added to the solution with a 5-minute stirring. The film solution was then poured into a sterilized glass plate to form it. Then, it was left to dry at room temperature (ambient temperature) for 10 minutes prior to oven-drying at 50°C from 24 hours.

The spreading method followed Nofrida [10] with a modification. The same film solution was printed in the similar sterilized glass plate and was left to dry for 10 minutes in ambient temperature prior to the spread on the film with oleoresin to obtain an evenly spread anti-microbial film by visual mean. Next, it was left to dry at ambient temperature for 10 minutes before undergoing oven-drying at 50°C for 24 hours. The encapsulating method was done by encapsulate the active compound before incorporating it into the matrix. The encapsulation followed the coacervation method by using 3.0% alginate (m/v), 1.0% casein (m/v) and 1.0% active compound (v/v).

2.3. The measurement of incorporation respond

The measurements of film characteristics after three distinct incorporation methods were carried out, which were the measurement of film thickness, pH, water content and color as well as anti-microbial property test and microstructure using Scanning Electron Microscopy (SEM).
3. Results

3.1. The thickness of anti-microbial film

This parameter was measured by using digital thickness gauge to understand the influence of incorporation method to the thickness. The sample (A1) was made from direct mixing, (A2) was from spreading method and (A3) was the sample from encapsulating method. The amount of solution used in each sample was the same.

| Sample | Thickness (mm) |
|--------|----------------|
| A1     | 0.100 ± 0      |
| A2     | 0.086 ± 0      |
| A3     | 0.237 ± 0.01   |

Analysis of variance revealed that the incorporation method significantly influenced the thickness (α=5%). [9] investigated the influence of chili pepper variance to the thickness of the film, which resulted in a non-significant influence of it. The thickness of anti-microbial film influenced the effectiveness of the film to maintain the active compound incorporated in it. Owing to the active compound that can be released to function as an anti-microbial property, the thickness is then related to the theory of mass transfer which observes the kinetics of the absorption and release of a compound to a matrix. The thickness measurement in this research indicated that A3 resulted from encapsulation method had the highest thickness level. As encapsulation aimed to prevent a material/compound loss from its core, it might be assumed that encapsulation method prevent water loss during the drying process, hence it obtained a thicker film.

3.2. Anti-microbial film color by quantitative measurement

The quantitative method using colorimeter was employed to minimize the human error in organoleptic test (qualitative method).

| Sample | °Hue value      | Picture |
|--------|----------------|---------|
| A1     | 58.32 ± 0.15   | ![A1_color](image1) |
| A2     | 59.87 ± 1.72   | ![A2_color](image2) |
| A3     | 55.70 ± 0.18   | ![A3_color](image3) |

Based on the result and the chromatic value [11], the three of the samples had colors ranging from yellow to red with °hue value of 54-90. This was significantly affected by the addition of capsaicin which in fact had a natural color of red. Though they had a relatively similar color range, A2 had the highest range of °hue value amongst the others, followed by A1 and finally A3. The value of °hue below 54
means that the AM film has a color range of red, while the value of °hue above 90 describes a yellowish color. Encapsulated film had a more reddish color. This color is more identical to capsaicin natural color as the active compound. This could prove that the encapsulated process was able to maintain the active compound incorporated in it. Thus, our research corresponded to the research of [12], explained that the encapsulation was a technique to protect a core from its surrounding.

3.3. SEM Analysis of anti-microbial film

SEM analysis aimed to observe the surface or the microscopic structure of the film in detail. Figure 1 displayed the comparison of each sample as seen by SEM.

![SEM images](image)

**Figure 1.** The surface of the anti-microbial film made from the method of (a) direct mixing; (b) spreading; and (c) encapsulating

It can be seen in Figure 1(a), the surface of the sample consisted of white globes which spread unevenly but still physically bound to the matrix. This was probably due to the active compound – capsaicin, a volatile compound, had been suspended in the matrix could still evaporate. Thus the structure might be disturbed during the drying process. Meanwhile, Figure 1(b) showed the white globes appear on the matrix surface, clearly due to the process of incorporation which caused incomplete absorption of the active compound to the matrix. Moreover, this method might result a more evaporated active compound than other two methods which allowed the active compound to react with the solution directly. Figure 1(c) exhibited a more homogenous result compared to 1(a) and 1(b). Owing to the encapsulation process prior to the incorporation into the solution – which it would protect the active compound from the surrounding, thus the drying process might have little or no effect to the structure by means of visual microscopy.

3.4. The pH of anti-microbial film

The pH of the film was measured to investigate the influence of pH of the anti-microbial property, which was conducted with three repetition for each film.

| Sample | pH       |
|--------|----------|
| Control| 6.2 ± 0.11 |
| A1     | 4.8 ± 0.11 |
| A2     | 5.9 ± 0.10 |
| A3     | 5.1 ± 0.06 |

All of the sample including the control had pH values below 7.0 as showed in Table 3. It is known, the favorable pH for E. coli growth is in the range of 7.0 – 7.5 [13]. Our results showed that the film can provide an anti-microbial properties based on the pH test result. The control showed a higher pH compared to the capsaicin-incorporated sample, meaning the addition of the active compound resulted in pH decrease. Thus we can conclude that the lower the pH, the higher the anti-microbial activity. The anti-microbial compound with acidic condition could penetrate the cell of the microbe and destroy the
cell [14]. The mechanism of the anti-microbial property in preventing the growth of E. coli was described as follows. It is related to the mechanism of E. coli to maintain its internal pH to be neutral. If the pH is low, the high amount of proton will penetrate into the cytoplasm of the cell. This causes the cytoplasm pH decrease. The pH decrease induces the enzymes to return the pH into normal [15]. These protons should be removed out of the cell to prevent the denaturation of cell. The activity to return pH to normal requires such high energy that it can interfere the metabolism of the cell, which in longer period will kill the cell [13]. Describes that the extract of betel leaf at pH 4 was the most effective to prevent E. coli [16].

3.5. Anti-microbial property test of the film
The test was undergone using E. coli as subject culture and the testing method was distinguished for the active compound itself and for the produced anti-microbial film. The active compound anti-microbial test was conducted using well diffusion method, while the test to the produced film employed the agar diffusion method. The principle has a quite similar method in which an agar media was used where in well method, the agar media was holed, whereas in diffusion method the agar was not holed.

The activity of anti-microbial was measured by observing the surface area of clear zone, which indicated no bacterial growth in the zone, around the “well” (hole) and the anti-microbial film. If there is no clear zone, it is assumed that there was no anti-microbial activity. The results of both test (to the solution and to the film) showed that there was a clear zone on the sample. Furthermore, the test was conducted to the three sample of different incorporation method. Based on the result, there was significance (α=5%) in between the different method, as show in Table 4. This indicated that there was significant influence of the incorporation method to the anti-microbial activity.

| Sample | Clear zone (mm²) |
|--------|----------------|
| Control | 0 |
| A1      | 0.61 ± 0.03    |
| A2      | 0.68 ± 0.02    |
| A3      | 0.89 ± 0.03    |

The mechanism of the inhibition of bacterial growth is either (i) by interacting with cell membrane; (ii) by inactivating the essential enzymes; and (iii) by destructing or inactivating the genetic function and material [17]. Capsaicin is an anti-microbial compound associates with the film matrix. When the anti-microbial film has a contact with the E. coli-inoculated media, capsaicin will diffuse from the film to the cell wall of E. coli, as the cell wall is consisted of 80% fat content while capsaicin is a fat-soluble compound [18]. The dissolution of capsaicin to the cell wall of E. coli is called penetration. This penetration will inhibit the synthesis of protein and destroy the DNA band of the cell, causing the cell to stop from growing. Thus, the area around the film and on the film itself, E. coli cannot grow. That area is observable as a clear zone or no metallic green color because there is no E. coli growth [19].

4. Conclusions
In this research, it is proved that the incorporation method significantly influenced the thickness of the produced antimicrobial film. Each of the film had a chromatic color range of yellow-red as shown by the quantitative test of the film color. Particularly, the product from encapsulation method had the highest hue value which indicated its more reddish color than the other products, SEM analysis on the sample exhibited the difference of each’s surface structure in which the encapsulation method resulted a more homogenous structure. Meanwhile, the pH test revealed that all of the samples were acidic, indicating they has the property of antimicrobial activity. Moreover, the appearance of a clear zone on each sample in the antimicrobial test substantiated the presence of antimicrobial activity in any incorporation method.
5. References

[1] Yam K L, Takhistov P T and Miltz J 2005 Intelligent packaging: concepts and applications Journal of Food Science 70: R1-R10
[2] Han J H 2000 Antimicrobial food packaging Food Technol 54(3): 56-65
[3] Whiting S, Derbyshire E and Tiwari B K 2012 Appetite 59, 341
[4] Bernal M A, Calderon A A, Pedreno M A, Muñoz R and Ros Barceló A 1993 J. Agric Food Chem. 41: 1041
[5] Tajkarimi M M, Ibrahim S A and Cliver D O 2010 Antimicrobial herb and spice compounds in food J Food Control 21:1199-1218
[6] Ahvenainen R E, Hurme K, Randall and M Eilame 2000 The Effect of Leakage on the Quality of Gas-Packed Foodstuffs with the Leak Detection VTT Reasearch Note. Finland
[7] Panji C, Warsiki E, Rini P and Laras W S 2013 Oleoresin extraction techniques from various chili Proceeding of Seminar on Research Result LPPM IPB. Vol 1. Field of Food, Energy, Technology and Engineering: 177 – 187
[8] Lestari I A 2013 Making a Smart Label As Detector Escherichia coli (Bogor.ID: IPB)
[9] Maharani U 2015 Utilization of Chilli Oleoresin for Growth Inhibitor of Escherichia coli Antimicrobial Film (Bogor.ID: IPB)
[10] Nofrida R, E Warsiki and I Yuliasih 2013 The effect of storage temperature on the changing color of Erpa leave indicator smart label Agroindustrial Journal. Vol 23(3): 232 – 241
[11] Hutching J B 1999 Food Color and Appearance Second Edition (Maruland: US) Chapman Hall Food scl.
[12] Krasaekoopt W B, Bhandari H and Deeth H 2003 Evaluation of encapsulation techniques of probiotics for yoghurt Int. Dairy J. 13:3-13
[13] Fardiaz S 1992 Mikrobiologi Pangan 1 (Jakarta: Gramedia Pustaka Utama)
[14] Jay J M 1997 Modern Food Microbiology 5thed (New York: Chapman and hall)
[15] Booth I J 1985 Regulation of cytoplasmic pH in bacteria Microbial Rev. 49:359-378.
[16] Parhusip A J N, Anugrahati N A and Nathalia T 2005 Aktivitas Antimikroba Ekstrak Sereh (Cymbopogon citrarus DC Stapf) terhadap Bakteri Patogen Jurnal Ilmu dan Teknologi Pangan. 3(2):27-28
[17] Davidson P M and Branen A L 1993 Antimicrobial in Food (New York: Marcel Dekker)
[18] Jawetz, E, Melnick J L and Aldenberg E A 2005 Mikrobiologi Kedokteran (Jakarta: Salemba Medika)
[19] Krishna De and Amit 2003 Capsicum, the genus capsicum (New York: Tailor & Francis)