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Full Length Research Paper

Antimicrobial activity of whey protein based edible film incorporated with organic acids

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The effect of incorporating organic acids in whey protein based edible film, and its antimicrobial activity has been studied. Four organic acids namely acetic, lactic, propionic and benzoic acids (5% v/v each) were incorporated in whey protein based edible film, and their antimicrobial activity was tested using *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Eschericia coli* and *Salmonella* sp. There was a highly significant effect (P<0.01) of the organic acid on the inhibition zone of microorganisms, due to water vapor permeability (WVP) and protein solubility. A significant effect (P<0.05) was shown by different organic acids incorporated on the antimicrobial activity of the films. The highest inhibition zone was found at the *E. coli* tested sample (11.6 mm), whilst the lowest one was found at the *Salmonella* sp tested sample (9.77 mm). The highest inhibition zone was also found in the films that contained benzoic acid (11.76 mm), and the lowest was found in the film that contained lactic acid (10.44 mm). While the highest WVP value (0.0238 g.mm/m^2.kPa) was shown by the film that contained propionic acid, the lowest one was shown in the film that contained acetic acid (0.0116 g.mm/m^2.kPa). With regards to protein solubility, it was found that the film containing propionic acid had the highest value (44.4444%), while that with acetic acid showed the lowest protein solubility (35.5516%).

Key words: Organic acids, whey protein edible film, antimicrobial activity, WVP, protein solubility.

INTRODUCTION

In recent development, protein based edible film is the most interesting research object, although edible films can be prepared using protein, polysaccharide and lipid materials (Bourtoom, 2009). As this edible film is prepared from natural product, it is compatible to the environment issue due to the biodegradable characteristic of such films (Krochta and De Mulder-Johnson, 1997). The whey protein based edible film is usually prepared using whey protein with incorporation of plasticizer, crosslinking agent and lipid, before heat denaturation at 90°C for 30 min, then the pH was adjusted to 5.2 and cooled to room temperature before it was templated on Teflon plate and semi-vacuum oven for 24 h. The produced edible film had a soft, transparent and good aroma as well as oxygen resistant characteristics at low humidity (Galietta et al., 1998).

Wahyuni (2007) reported that the water vapor permeability value of whey protein based edible film heated at 90°C was 0.008561 g.mm/m^{2}h.kPa., whilst at similar denaturation temperature (90°C), if the proportion of protein and incorporated glycerol was 1.25:1.00, the product had the water vapor permeability value of 0.012100 g.mm/m^2 h.kPa. Furthermore, Manab (2008) found that, whey protein based edible film, incorporating glycerol at a proportion of 1.25:1.00, had water vapor permeability value of 0.011300 g.mm/m^2 h.kPa when added to 10% palm kernel oil from total solute.

Boutoom (2009) had reported that there were many studies carried out in improving the performance of protein based edible film to include the modification of using chemicals such as acids, alkali and crosslinking agents. According to Cagri et al. (2004), edible film functions as a mass transfer barrier and a carrier of

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organic acids such as acetic acid, lactic acid, propionic acid and benzoic acid which could improve the shelf life of the product as well as to prevent the growth of pathogen microorganisms on the surface of products.

Acetic acid and propionic acid have a significant effect on the growth inhibition of deterioration microbes in meat as reported by Ouattara et al. (2000). While whey protein based edible film at pH 5.2 and containing 0.5 to 1.5% (w/v), p-aminobenzoic acid (PARA) could inhibit the growth of *Listeria monocytogenes*, *Escherichia coli* (O157:H7) and *Salmonella typhimurium* (DT 104) (Cagri et al. 2003). Siragusa and Dickson (1993) noted that alginate based edible film incorporated lactic acid could inhibit the growth of *E. coli* O157:H7, *S. typhimurium* and *L. monocytogenes.*

The objectives of this research were to improve the whey protein based edible film performance by incorporating different organic acids, namely: acetic, lactic, propionic and benzoic acids; and to study the antimicrobial activities against *Lactobacillus bulgaricus, Streptococcus thermophilus, E. coli* and *Salmonella* sp.

MATERIALS AND METHODS

Materials

Whey powder (AMPEC, Australia); NaOH, HCl, $CaCl_2$, glycerol, Salmonella Shigella (SS) agar and MRS agar (merck); nutrient broth, nutrient agar, MRS broth, VRB agar yeast extract and pepton (Oxoid), were obtained from its agency in East Java.

Organisms and preparation of culture

S. thermophilus and *L. bulgaricus* were obtained from the Centre of Food and Nutrition Study, Gajah Mada University, Yogyakarta; whilst Salmonella sp and *E. coli* were obtained from Microbiology Laboratorium, Faculty of Medicine, Brawijaya University, Malang. *S. thermophilus* and *L. bulgaricus* were grown in MRS agar and cultured in MRS broth (Oxoid), while *Salmonella* sp and *E. coli* were grown in nutrient agar (Oxoid) and cultured in nutrient broth (Oxoid).

Whey protein isolation

Whey powder (500 g) was dissolved within 1 L aquadest (at ± 4°C), stirred until it was homogenized and then kept until 3 layers were obtained. The surface and middle layer were taken and then its pH was adjusted to 4.2 using 0.1 N HCl or 0.1 N NaOH, followed by centrifugation at 5000 rpm for 30 min. Consequently, a pellet form of whey protein was obtained as the final product.

Preparation of protein whey based edible film

Film forming solutions were prepared from a mixture of 15 ml whey protein added to 1.25% glycerol (w/v from whey protein) as the first solution, and then it was mixed with a solution of 30 ml aquadest and $CaCl_2$ 0.25% (from the first solution). Subsequently, 10% (v/v) palm oil and 10% lecithin were then added to the mixture and the pH was adjusted to 8 using NaOH 0.1 N before it was heated at 90°C on hot plate. Afterwards, it was stirred using magnetic stirrer for 30 min at 250 rpm. However, the mixture was then cooled down to 30°C, while 5% benzoic acid and 5% propionic acid (which formed the volume of edible film formula) were added as preservative and the pH was adjusted to 5.2 by 0.1 N HCl (Cagri et al., 2003).

Antimicrobial activity of whey protein based edible films

Antimicrobial activity test on films was carried out using the agar diffusion method according to Padgett et al. (1998), and the antimicrobial effects of films containing some kind of organic acids against *L. bulgaricus, S. thermophilus, E. coli* and *Salmonella sp* were carried out using zone of inhibition assay on solid media. The whey protein based edible films were cut into a circle form with 7.1 mm diameter and placed on the surface of the solid media which had been inoculated with 0.1 ml culture, approximately containing 106 to 107 cfu/ml. The plates were then incubated at 37°C for 24 h, and after incubation time, the inhibiting zone was measured on the film discs.

Water vapor permeability (WVP) test

The water vapor permeability test was carried out according to the method described by Perez-Gago and Krochta (1999) as follows: the whey protein based edible film was cut into 2.5 x 2.5 cm and then it was sealed in the base of a metal cup in the form of a ring using four symmetrical screw and placed on a 250 ml beaker glass containing 6 ml aquadest. The beaker glass was then placed in the dessicator with a fan that adjusted the RH value to 0% using calcium sulphate anhydrous. The weight of the edible film was periodically observed until a constant weight was reached, then it was used to calculate the RH value on the bottom surface of the film used to determine the WVP value. The formula for calculation was:

$$WVP = \frac{\text{Weight of film (g) x thickness of film (mm)}}{\text{Area of film (m}^2\text{) x time (h) x } \Delta \text{ P (kPa)}}$$

Protein solubility

About 500 g of dried samples were put into 150 ml measuring glass and 0.1 M NaCl were added and stirred until a smooth pasta was formed and until the dispersion volume reached 40 ml. Then the measuring glass was placed on the hot plate stirrer and insert magnetic stirrer (size 2.5 cm). Subsequently, the pH value was adjusted to 3.0 or 7.0 using 0.1 N HCl or 0.1 N NaOH. As such, the mixture was stirred continuously and the pH was monitored regularly during the stirring process. After this step, the mixture was moved to a 50 ml bottle and 0.1 M NaCl was added to it. It was shook, until homogeny was reached, and then centrifuged for 30 min at 20.000xg. Afterwards, the mixture was strained using the paper strainer (Whatman No. 1) until supernatant were obtained, and as such, the protein content was determined using Kjeldahl method (Morr et al., 1985). However, the formula for calculation is as follows:

$$\text{Protein solubility (\%)} = \frac{\text{Supernatant protein concentration (mg/ml) x 50}}{\text{Sample weight(mg) x Protein content of sample(\%)/ 100}}$$

Experimental design

This study was conducted using the factorial fully randomized

Table 1. Antimicrobial activity of whey protein based on edible film containing organic acids against *Lactobacillus bulgaricus, Streptococcus thermophilus, E. coli* and *Salmonella* sp.

Microorganisms	Acetic acid	Lactic acid	Propionic acid	Benzoic acid	Means
	Inhibition zone(mm)	Inhibition zone(mm)	Inhibition zone(mm)	Inhibition zone(mm)	
Lactobacillus bulgaricus	10.53±0.31	11.10±1.39	10.74±1.09	11.80±0.20	11.04±0.56^{b}
Streptococcus thermophilus	10.60±0.81	11.48±0.72	11.34±0.65	11.29±0.44	11.17±0.39^{b}
Escherichia coli	10.75±0.79	10.27±0.77	11.52±2.19	13.85±1.96	11.60±1.59^{b}
Salmonella sp.	9.92±0.63	8.91±0.42	10.15±0.95	10.10±0.10	9.77±0.58^{a}
Means	10.45±0.37^{x}	10.44±1.14^{x}	10.94±0.62^{x}	11.76±1.56^{y}	

- Superscript (a and b) in the same column of means of inhibition zone using different species of microorganisms showed a highly significant effect (P<0.01) on means of organic acids inhibition zone. Superscript (x and y) in the same row of means of organic acids inhibition zone using different kind of organic acids showed a significant effect (P<0.05) on organic acids inhibition zone.

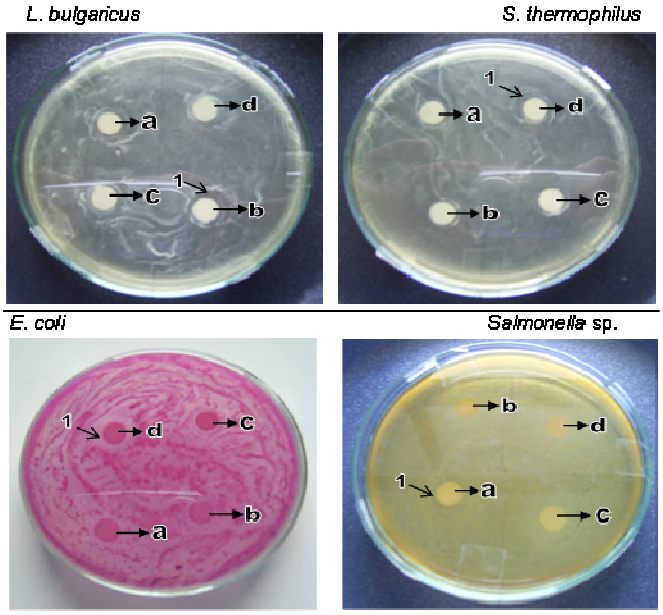

Figure 1. Inhibition of microbial growth in whey protein based edible films containing acids against different species of microorganisms. (1) Inhibition zone of whey protein based edible films containing (a) acetic acid, (b) lactic acid, (c) propionic acid and (d) benzoic acid.

design where the first factor comprised an addition of 4 kinds of organic acids (5% v/v), namely: acetic acid, lactic acid, propionic acid and *benzoic acid*. The second factor comprised 4 tested microorganisms, namely: *L. bulgaricus, S. thermophilus, E. coli* and *Salmonella* sp. The treatments were replicated three times and the data were analysed using ANOVA and if there was significant difference, the analysis was continued using least significant difference according to Sastrosupadi (2000).

RESULTS AND DISCUSSION

Antimicrobial activity

It was found that there was highly significant (P<0.01) effect on the inhibition zone of whey protein based edible film containing organic acids against those four microorganisms tested. A significant difference (P<0.05) was also found in the different kind of organic acids used on the inhibition zone against those microorganisms. However, the interaction between species of microorganisms tested and the kind of organic acids did not give significant effect (P>0.05) on the inhibition zone as shown in Table 1. Figure 1 showed the inhibition zone of whey protein based edible films containing organic acids against four microorganisms tested.

Data in Table 1 showed that *E. coli* was the most sensitive bacteria against the whey protein based edible film containing organic acids, especially the one containing acetic, propionic and benzoic acids. This condition indicated that there was an adaptation effect of *E. coli* against the organic acids when added to the whey protein based edible films. Tosun (2005) noted that *E. coli* basically had an adaptation capacity against the negative effect of organic acids on the addition of acid. Hill et al. (1995) stated that the buffering capacity of cytoplasm, that is, low proton permeability and proton excretion from cytoplasm through proton membrane, play an important role in the mechanism of *E. coli* adaptation against acid. *Salmonella* sp. was the most resistant microorganism to whey protein based films containing acetic acid, lactic acid, propionic acid and benzoic acid, and this is possibly due to the adaptation capacity of this microorganism against organic acids. According to Hill et al. (1995), *Salmonella* sp. needs a protein synthesis and genetic change to have an adaptation response against organic acid. Thus, Russel (1991) and Nikaido and Vara (1985) noted that, in general, gram negative bacteria are more resistant to organic acid compared to gram positive bacteria, and the difference of the adaptation response between bacteria is affected by different structures and chemical compositions of cell membrane.

The data in Table 1 also showed that whey protein based edible film containing benzoic acid had the biggest inhibition zone against those four microorganisms tested (that is, 11.76 mm), while the one containing lactic acid

Table 2. Means of WVP values of whey protein based edible films containing different kinds of organic acids.

Organic acids	WVP value (g.mm/m^2.h.kPa.)
Acetic	0.0116 ± 0.0017a
Lactic	0.0117 ± 0.0023a
Propionic	0.0238 ± 0.0041b
Benzoic	0.0144 ± 0.0010a

Superscript (a and b) at the same column showed a highly significant effect (P<0.01) on the means value of WVP.

showed the smallest inhibition zone (that is, 10.44 mm). The inhibition zone of the film containing propionic acid was 10.94 mm and was smaller than the one containing benzoic acid; however this inhibition zone was still bigger than the one shown by the film containing acetic acid (10.45 mm). This condition could be as a result of the difference of organic acids' effectiveness against the microorganisms tested. Brown and Brooth (1991) stated that organic acid could prevent the essential molecule reactions within the cell by the addition of H^+ ion, which decrease the intracellular pH value. If the pKa value of a certain organic acid is bigger, then the amount of un-dissociated molecules is also bigger, hence its inhibition effectiveness will also be lower when compared to the one with lower pKa value. Supardi and Sukamto (1999) also noted that the microbial membrane cell was only permeable to the un-dissociated acid molecules.

According to Brown and Brooth (1991), in Beales (2004), the effectiveness of organic acids did not only depend on the pKa values, but also had different effectiveness against different microorganisms. However, Hui (1991) stated that the organic acids action was through the depression of intracellular pH value by the un-dissociated acid molecules or by separating the substrate transportation via changing the permeability of the cell membrane. Thus, Herrero (1983) noted that the role of organic acids in inhibiting the growth of microorganisms was by affecting the homeostatic pH value. As a result, the unstability of this pH value might change the utilization of energy which should be used for growth, and which now play the role of sustaining the homeostatic pH value. Nonetheless, Eklund (1983) reported that the growth inhibition activity by organic acids was mainly due to the release of proton into cytoplasm and the accumulation of anion had the ability to decrease the speed of macromolecule synthesis, which thus affected the transportation of nutrient into the cell membrane.

The inhibition zone of whey protein based edible films, containing organic acids against *L. bulgaricus* (11.04 mm), was very similar to the one of *S. thermophilus* (11.17 mm) and this was possibly due to the similarity of their properties as they are gram positive bacteria. According to Holt et al. (1994), both bacteria are gram positive, catalase negative, no spores, unicellular, anaerobe, hetrotrophic and they grow well in media containing carbohydrate and yeast extract. Although both of them could ferment lactose into lactic acid which is an organic acid, in this study, the organic acid inhibition zone against *L. bulgaricus* and *S. thermophilus* are relatively big.

Water vapour permeability (WVP)

The data in Table 2 showed that the highly significant difference (P<0.01) effect occurred by using different kinds of organic acids in the preparation of whey protein based edible films on the WVP value of those films. The highest WVP value was found in the film containing propionic acid (0.0238 g.mm/m^2.h.kPa) and the lowest was found in the film containing acetic acid (0.116 g.mm/m^2.h.kPa). The organic acids within the whey protein based edible films will be dissociated from becoming a carboxyl ion group ($RCOO^-$) and hydrogen ion (H^+) as reported by Hart et al. (2003). It is believed that $RCOO^-$ will bind the amino ion (NH_3^+) protein, hence the bridging salt which is already formed between protein-Ca-protein will be changed into organic acid-protein-Ca-protein and this condition might be affected by the WVP value of the films.

The highest WVP value found in whey protein based edible film, containing propionic acid, is possibly due to the high pKa value and only 57% of the molecule were dissociated and bounded to the NH_3^+ ion of protein in the preparation of organic acid containing the film. Luck and Joger (1997) stated that the pKa value of propionic acid was 4.88 and this value is believed to affect the effectiveness of its antimicrobial properties. Hence, the incorporation of this acid in film preparation could improve its antimicrobial activity, but not on its WVP value. WVP values of films, containing acetic, lactic and benzoic acids, showed no difference effect (Table 2) and this is possibly due to the pKa values of those organic acids that were low when compared to pKa value of propionic acid. Although the WVP values of these films were lower than the one containing propionic acid, these WVP values were still higher compared to the one from the previous study as reported by Manab et al. (2007). In their study, Manab et al. (2007) found that, the edible film sample prepared at denaturation temperature of 90°C, protein and glycerol ratio of 1.25:1.00, incorporation of 10% palm oil (v/v total solution) and 0.25% $CaCl_2$ from total solution gave a WVP value of 0.0113 g.mm/m^2.h.kPa.

In general, incorporation of organic acids could increase the WVP value of whey protein based edible film; however, the one incorporating the acetic acid showed the lowest WVP value, although its pKa value was 4.75. It is assumed that there is another factor influencing this phenomenon, and as such, further study

Table 3. Means of protein solubility of whey protein based edible film samples incorporating different kinds of organic acids.

Organic acid	Protein solubility (%)
Acetic	35.55 ± 2.40a
Lactic	38.83 ± 1.53a
Propionic	44.44 ± 2.33b
Benzoic	40.26 ± 1.51ab

Superscript (a and b) at the same column showed a highly significant difference (P<0.01) effect on means of protein solubility percentage.

is crucially needed. It is interesting to note that Sothornvit et al. (2003) found the WVP values of compressed molding edible whey protein, with an addition of 30, 40 and 50%, glycerol to have the WVP values of 15.8, 15.0 and 14.2 g mm/h kPa m^2, respectively. This result is differs from the results obtained in this study and is possibly due to different roles played by the incorporated glycerol when compared to the organic acids used. Also, it could possibly be due to the fact that thicker compression molded film encountered higher RH on the inside of the WVP cup than the thinner solvent-cast film.

Protein solubility

The data in Table 3 showed corporation of different organic acids in whey protein based edible film preparation which gave a highly significant difference (P<0.01) effect on protein solubility. It was found that the one containing propionic acid had the highest protein solubility and the lowest one was found in the sample containing acetic acid. Walstra et al. (1999) stated that in some proteins, there were only small amounts of salt needed to decrease its solubility; however, on the contrary, a bigger amount of salt could increase the solubility. Manab et al. (2007) found in their study that the film sample incorporating 0.25% $CaCl_2$ from the total solution had its protein solubility of 39.60% and this figure was lower when compared to the protein solubility of samples containing propionic (44.44%) and benzoic acids (40.26%), but slightly higher than the one containing acetic (35.55%) and lactic acids (38.83%). Accetic and lactic acids are commonly used as crosslinking agents that could reduce edible films solubility.

Cagri et al. (2004) also reported that lactic acid could be used as an acidulant which modified the loosen strength and antimicrobial activity of the films; whilst Marquie et al. (1995) in Galietta et al. (1998) noted that crosslinking agent was used to improve water resistant, cohesiveness, toughness, mechanical strength and barrier properties of the material. Sothornvit et al. (2003) reported that protein solubility, in compression-molded whey protein films, increased with the amount of glycerol added, such as 30% glycerol incorporated only at 11.1 and 50% glycerol incorporated at 21.3%. Thus, the difference of solubility protein values are affected by the amount of water or glycerol or organic acids added to the mixture when it was first started. Results in this study, as well as results obtained from other researchers, suggested that the presence of water or glycerol, or organic acids interferes with the crosslinking of proteins.

Conclusion

The results showed that incorporation of acetic, propionic and benzoic acids in whey protein edible film samples, respectively, were the most effective in inhibiting the growth of *E. coli*, while *Salmonella* sp. was found as the most resistant bacteria against the organic acids that incorporated edible films. The film sample containing propionic acid had the highest WVP value as well as the highest protein solubility, while the lowest WVP value and protein solubility were shown by the samples containing acetic acid.

REFERENCES

Beales N (2004). Adaptation of microorganisms to cold temperatures,weak acid preservatives,low pH and osmotic stress: A Review. Food Sci., 3: 1-15.

Bourtoom T (2009). Review article: Protein edible film:Properties enhancement. Int. Food Res., J., 16: 1- 9.

Cagri A, Osburn W, Ryser ET (2003). Inhibition of Listeria monocytogenes on hot dogs using antimicrobial whey protein based edible casings. J. Food Sci., 66(1): 291-298.

Cagri A,Ustunol Z ,Ryser ET (2004). Antimicrobial edible films and coating. J. Food Protec., 67 (40): 833-848.

Eklund T (1983). The antimicrobial effect of dissociated and undissociated sorbic acid at different pH levels. Appl. Bacteriol., 54: 383-389.

Galietta G, Di Golia LD, Guilbert S, Cuq B (1998). Mechanical and thermomechanical properties of films based on whey protein as affected by plasticizer and crosslinking agents, J. Dairy Sci., 81: 3132-3130.

Hart H, Craine LE, Hart DJ (2003). Organic Chemistry (kimia Organik) translated by Ahmadi SS, Erlangga, Jakarta,

Herrero AA (1983). End product inhibition in anaerobic fermentation. Trends Biotechnol., 1: 49-53.

Hill C, O'driscoll, Booth IR (1995). Acid adaptation and food poisoning microorganism. J. Food Microbiol., 28: 245-254.

Holt JG, Kreig NR, Smeath HA, Stanley JT, William ST (1994). Bergey's Manual of Determinative Bacteriology. 9th Edition. lliam and Wilkins,New York,

Hui YH (1991). Encyclopedia of Food Science and Technology. Wiley Interscience Publication. John Wiley and Sons Inc., New York, pp. 89-96

Luck E, Joger M (1997). Antimicrobial Food Additives Characteristics, Uses and Effect. Springer verlagge, New York.

Krochta JM, De Mulder-Johnson CD (1997). Edible and biodegradable polymer films: Challenges and opportunities. Food Technol., 51: 61-74.

Manab A (2008). Effect of incorporating palm kernel oil on whey protein based edible film characteristics.(Pengaruh penambahan minyak kelapa sawit terhadap karakteristik edible film protein whey., 3(2): 5-16.

Manab M, Sawitri ME, Zanah SK (2007). Effect of the addition of CaCl2 as cross linked agent in whey protein edible film on its physical and chemical properties, 3(2): 47-53.

Morr CV, German B, Kinsella JE, Regenstein JM, Van Buren JP, Kilara A, Lewis BA , and Mangino ME (1985). A collaborative study to develop a standardized food protein solubility procedure. J. Food Sci., 50: 1715-1721.

Ouattara BRE, Simard G, Piette A, Begin RA Holley (2000). Diffusion of Acetic and Propionic Acids from Chitosan-Based Antimicrobial Packaging Films. *In* Cagri A, Ustunol Z, Ryser ET (2004). Antimicrobial Edible Films and Coating. J. Food Protec., 67(4): 833-848.

Nikaido H, Varra T (1985). Molecular Basis of Bcteria Outer Membrane Permeability. Microbiol Review, In Beales N, 2004. Adaptation of Microorganism to Cold Temperatures, Weak Acid Preservatives, Low pH, and Osmotic Strees: A Review. Compre. Rev. Food Sci. Food Saf., 3: 1-15.

Padget T, Han LJY, Dawson PL (1998). Incorporation of food grade antimicrobial compounds into biodegradable packaging films. J. Food Protec., 61(10):1330-1335.

Perez-gago MB , Krochta JM (1999). Water vapor permeability of whey protein emulsion film as affected by pH. J. Food Sci., 64(4): 695-698.

Russell AD (1991). Mechanism of bacterial resistance to non-antibiotics: Food additives and pharmaceutical preservatives, J. Appl Bacteriol., 71: 191-201.

Sastrosupadi A (2000). Practical Experimental Design in Agriculture(Rancangan Percobaan Praktis Bidang Pertanian). Kanisius, Yogyakarta.

Siragusa GR, Dickson JS (1993). Inhibition of *Listeria monocytogenes*, *Salmonella Typhimurium* and *Escherichia coli* O157:H7 on Beef Muscle Tissue by Lactic Acid or Acetic Acid Contained in Calcium Alginate Gels. *In* Cagri AZ, Ustunol, ET, Ryser , Antimicrobial Edible Films and Coating. J. Food Protec., 67(4): 833-848.

Sothornvit R, Olsen CW, McHugh TH, Krochta JM (2003). Formation conditions, water-vapor permeability and solubility of compression-molded whey protein films. J. Food Sci., 68: 1985-1989.

Supardi T, Sukamto M (1999). Microbiology in Food Processing and Food safety (Mikrobiologi dalam Pengolahan dan Keamanan Pangan), Penerbit Alumni, Bandung,

Tosun H (2005). The effect of acid adaptation condition and acid tolerance reponse of Eschericia coli O157:H7. Tur. J. Biol., 29: 197-202.

Wahyuni E (2007). The preparation of whey protein based edible film: Study on the ratio of protein and glycerol on its physic-chemical characteristics. (Pembuatan edible film protein whey: Kajian rasio protein dan gliserol terhadap sifat fisik dan kimia.) Bachelor thesis, Faculty of Animal Husbandry,Brawijaya University,Malang,

Walstra P, Geurst TJ, Noomen A, Jellema A , van Boekel MAJS (1999). Dairy Technology: Principles of Milk Properties and Process. Marcel Dekker Inc.,New York,