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Physical, Mechanical, and Antimicrobial Properties of Edible Film Produced from Defatted Soybean Meal Fermented by *Bacillus subtilis*

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Abstract In order to extend the shelf-life of packaged or coated foods, an antibacterial edible film was developed from soybean meal that had been fermented with *Bacillus subtilis* under the optimum condition of pH 7.0–7.5 and 33°C for 33 h. The water vapor permeability of the fermented film ($86.0\ mg/cm^2{\cdot}h$) was higher than those of normal soybean films ($66.9\ mg/cm^2{\cdot}h$). Protein solubility of the fermented film was also higher than ordinary soy protein film at the pH range of 3–10. The fermented soybean film had higher tensile strength and lower % elongation (elongation rate) than the ordinary soybean film, mainly because partial hydrolysis of proteins in the soybean film occurred during fermentation. Antimicrobial properties of the fermented film on foodstuffs were measured by placing the films on surime, jerked beef, and mashed sausage media, containing 10^2–10^3 CFU/plate of foodborne pathogenic bacteria, and showed significantly higher inhibitory effects on the growths of all the indicating bacteria. The film could be used as a packaging material in the food industry. However, before direct application of the fermented film to the commercial food industry, its poor mechanical and antibacterial properties need to be improved.

Key words: Bacteriocin-like substance, fermented soybean film, antimicrobial film, physical and mechanical properties of film, functional film

Soy protein is a viable and renewable resource for producing environmentally safe industrial products. Therefore, a few attempts have been made to develop and utilize the biodegradable soy protein films as packaging and coating materials [1, 5]. However, utilization of soy protein films to extend the shelf-life and guarantee the safety of food is limited, because the packaging and coating with soy protein film can prevent only from moisture migration and cross-contamination of foods. Therefore, the application of effective preservatives to the films in packaging has been considered to extend shelf-lives of foods, that are not feasible for direct addition of preservatives. However, most people are presently reluctant to use synthetic preservatives in foods. In our previous study [11], therefore, we economically developed an antimicrobial edible film from defatted soybean meal, which is a type of waste product, by inoculating with bacteriocin-like substance (BLS)-producing bacteria. However, the potential use of the previously developed film as packaging materials depends on its mechanical and moisture barrier properties. Although the mechanical and moisture barrier properties of ordinary soy protein films are already well known, it is necessary to clarify those properties of the newly developed fermented film, which was probably influenced by hydrolytic enzymes produced during fermentation. Furthermore, it is commercially necessary to investigate the antibacterial properties of the film against common pathogens, when it is used as a packaging material for genuine foodstuffs.

In the present study, therefore, we determined the physical and mechanical properties of the edible film developed from defatted soybean meal and the antibacterial properties of the film in a real food system by inoculating with BLS-producing bacteria.

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MATERIALS AND METHODS

Materials

Defatted soybean meal used in this study was supplied by Shindongbang Co. (Seoul, Korea).

Film Preparation

The fermented soybean solution for film formation was prepared from soybean meal (15 g/100 ml of water) by inoculation with *Bacillus subtilis* and fermentation under optimum conditions of 33°C and pH 7.0–7.5 for 33 h. The pH of the fermented soybean solution was adjusted to 9 [11]. After heating for 20 min at 75°C in a constant temperature heat in magnetic stirrer, the solutions were strained through five layers of cotton sieve to remove any minuscule particles. Two ml or 3 ml of glycerol were added to the film, forming solutions as plasticizers, to overcome film brittleness and to obtain freestanding films in this solution. Addition of 2 g of glycerol to the soybean solution led to cracking of the resulting fermented film, therefore, an additional 1 g of glycerol was added to the fermented solution to form the fermented film. The film-forming solutions containing glycerol were spread onto a PVC petri dish (diameter=15 cm). The casting films were dried for 48 h at 23°C in a ventilated oven before peeling off from the dish [4, 6, 11, 20, 22]. Control film, the ordinary soybean film, was also prepared by the same procedures except with no fermentation.

Measurements of Physical Properties

Film thickness was measured using a 0–25-mm micrometer screw gauge (Mitutoyo, Tokyo, Japan). Mean thickness of films was determined from the average of measurements at 10 locations [7]. The water vapor permeability of the films was determined as described in ASTM [2]. After equilibration, films were trimmed to an 8.0-cm diameter and sealed with lubricant grease over test cups (diameter= 7.0 cm). Each cup contained distilled water, which created internal humidity. All films were oriented with the side that faced the petri dish during drying, facing the lowest humidity. The whole device was weighed, and then placed in a climatically controlled chamber (25°C and 50±5% of relative humidity). The weight changes of the cups were periodically recorded and described as the % of moisture evaporated. Forced air movement was not provided and performed by ASTM method [2, 17, 24, 29]. The protein solubility of films was also determined by ASTM method [2]. Film samples (2 cm×2 cm) were weighed and transferred into test tubes containing 10 ml of distilled water. The tubes were mildly shaken for 12 h at ambient temperature (25°C). After centrifugation, protein concentrations of the supernatants were measured using Lowry's methods. Protein solubility was expressed in percentage of dissolved protein [6, 14, 27].

Measurements of Mechanical Properties

Tensile strengths and % elongation of the films were measured by ASTM method [1] using a texture analyzer (TA Plus, Lloyd Instruments, England). The films were cut into strips of 20 mm width and 50 mm length. The film samples were clamped into the metal grips of the tensile geometry and stretched at an overhead crosshead speed (120 mm/min). Tensile strength at the maximum and % elongation at break were calculated [1, 14, 18].

Measurement of Antimicrobial Properties of the Fermented Film on Foodstuffs

The antimicrobial activities of BLS-producing bacteria were determined against natural microflora of jerked beef, surimi, and mashed sausage, including *E. coli* and the 3 foodborne pathogenic bacteria *Staphylococcus aureus*, *Salmonella typhimurium*, and *Listeria monocytogenes*. All 4 bacteria were purchased from KCTC (Korean Collection for Type Cultures, Daejeon, Korea). These organisms were maintained in frozen stocks with 20% (w/v) glycerol at -20°C and propagated twice from single colony prior to experimental uses [23]. Each bacterial strain was cultivated in their optimum media and temperatures.

Antimicrobial properties of the fermented film on foodstuffs were measured by placing the films on surimi, jerked beef, and mashed sausage media, containing 10^2–10^3 CFU/ml of foodborne pathogenic bacteria as indicator. Cultivation temperatures were adjusted, depending on each medium (15°C for surimi medium, 25°C for jerked beef and mashed sausage media). Colonies were enumerated every 6 h under a light microscope until 60 h. The same procedures were also applied to the control soybean film [11].

RESULTS AND DISCUSSION

Physical and Mechanical Properties of the Films

To produce fermented soybean film, the film-forming solution was heated at 75°C for 20 min to unfold protein chains and initiate pasteurization. The pH of the fermented soybean solution was adjusted to 9, because alkalinity of the film-forming solution helps to disperse the protein thoroughly in the solution [13, 19]. Heating of film-forming solutions cleaves disulfide bonds and exposes the sulfhydryl and hydrophobic groups of soy proteins [15]. Formation of new disulfide, hydrophobic, and hydrogen bonds during heat treatment and drying of the film-forming solution are considered to be important in the formation of soy protein film structure [19]. Moreover, proteins undergoing structural changes in the presence of plasticizers affect the mechanical properties of films. Plasteins, the proteins in the plasticizers-containing solution, have higher surface hydrophobicity than the original peptide mixture and tend to aggregate at relatively low temperature

[15, 26]. When the film-forming solution is cast, the reformed disulfide bonds link the polypeptide chains together to produce the film structure with the aid of noncovalent interactions, resulting in the formation of soybean film. The antimicrobial activity of the fermented film was still retained after heat treatment. The film thicknesses were measured to determine the mechanical properties and water vapor permeability. The central part of the films was thinner than the edges due to the drying behaviors, in accordance with other reports [19, 27].

Water vapor permeability is a barrier property that has most commonly been investigated to assess the ability of edible films to protect foods from the environment and adjacent food components with different water activity. As shown in Fig. 1, the water vapor permeability of the fermented film was higher than those of the control soybean films. The control and fermented soybean films permeated 66.9 and 86.0 mg/cm^2·h of moisture, respectively. Considering the amount of moisture evaporated from the unsealed cup (133.8 mg/cm^2·h), the control and fermented soybean films permeated 50% and 64% of evaporated moisture, respectively. The higher water vapor permeability of the fermented film was probably due to a large amount of glycerol added and fragmentation of soybean proteins during the fermentation. The increases in water vapor permeability of protein films with increasing amounts of plasticizers have been observed by others [17, 28], and are due to an increase in the free volume between the protein chains. Since most of the plasticizers are hydrophilic, an increase in their concentration favors the absorption of water by the network, thus resulting in a higher level of vapor transfer. The hydrolysis of peptide bonds by the hydrolytic enzymes produced by *Bacillus subtilis* during the fermentation might also cause the changes in water

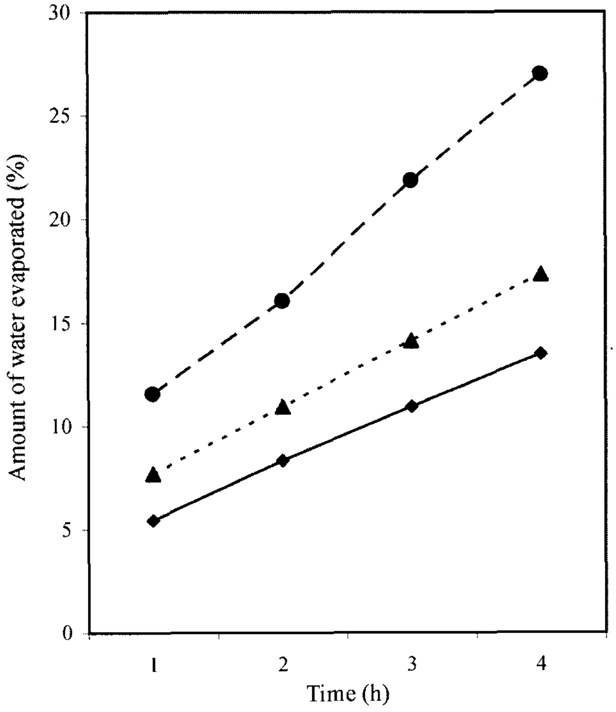

Fig. 1. Amounts of evaporated water in the cup covered with the fermented soybean film (-▲-) and the control soybean film (-◆-), and without cover (-●-).

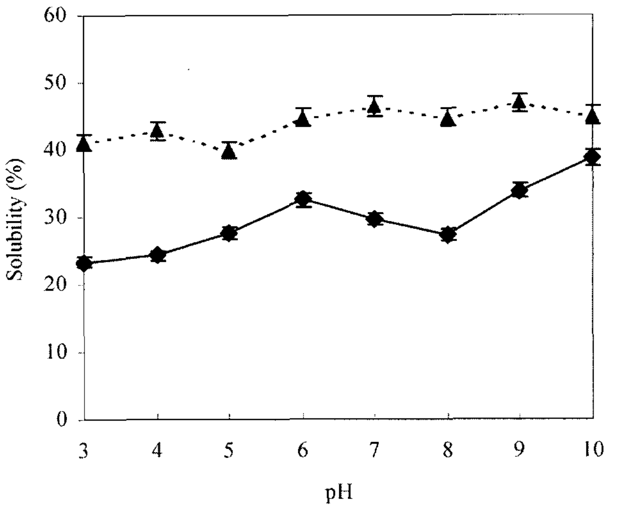

Fig. 2. Protein solubility profiles of the fermented soybean film (-▲-) and the control soybean film (-◆-).
* Within errors of less than 5%.

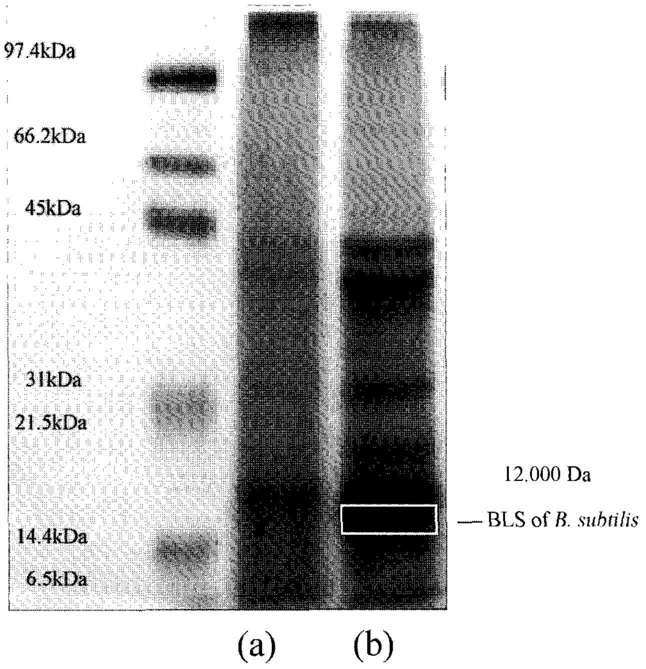

Fig. 3. SDS-PAGE patterns of (a) the control soybean film and (b) the fermented soybean film.
Each of the side lanes contained molecular weight protein standards (sigma).

vapor permeability of the film. Changes in water vapor permeability of the film due to hydrolysis of peptide bonds at high pHs have also been noted in some other reports [6, 17, 27, 28]. The hydrolysis of peptide chain increases the solubility of soy protein, the affinity to water, and consequently, water vapor permeability of the fermented film.

Protein solubility profiles of films are presented in Fig. 2. Protein solubility of the fermented film was higher than the control soy protein film at the pH ranges of 3–10. The increase in protein solubility of the fermented film resulted from hydrolytic enzymes produced by BLS-producing strain during the fermentation. Manti and Jost [21] investigated the ability of trypsin, papain, and a neutral protease of *Bacillus subtilis* to break down whey protein aggregates formed during film production. Small protein fragments were observed on SDS-PAGE of the fermented film (Fig. 3) [11]. Hydrolysis of 2.0% and 6.7% of casein with *Staphylococcus aureus* protease increased the solubility by 25% and 50%, respectively [7]. Therefore, partial hydrolysis of globular proteins by the enzyme increased the protein solubility of the fermented films at a wide pH range, in accordance with other reports [13].

The mechanical properties of the films were measured after 48 h of equilibration in a controlled environment at 50±5% relative humidity (RH) and temperature of 23±2°C. The control soybean film had higher tensile strength than the fermented film containing partially hydrolyzed proteins (Table 1). The molecular weight of protein mainly affected the mechanical properties of soy protein films [6]. Aging time for film formation has also to be taken into consideration prior to characterization of the mechanical properties of film [20]. Formation of ordinary soybean film took only 30 h, while the fermented film formation took 45 h due to the high content of glycerol in the fermented film. On the contrary, % elongation of the fermented film was higher than the control soybean film. The negative correlation between tensile strength and % elongation of films has generally been observed by many researchers [18, 22, 27–29]. Increasing amount of plasticizer in the fermented film also leads to a decrease in tensile strength and an increase in % elongation of the film.

Generally, the mechanical properties of protein films are inferior to those of synthetic films. Nevertheless, because of increasing concern over the environmental safety of nondegradable synthetic packaging materials, there is a demand for natural degradable products from renewable sources as an alternative to synthetic polymers [19]. The

Table 1. Tensile strengths and % elongations of the control soybean film and the fermented soybean films at 25°C and 50±5% of RH.

Soybean films	Film thickness (mm)	Tensile strength (Mpa)	% elongation
Control film	0.2184±0.024	1.6±0.5	137±5
Fermented film	0.2769±0.016	1.3±0.5	164±5

Reported values are means of 5 replicates±standard deviation.

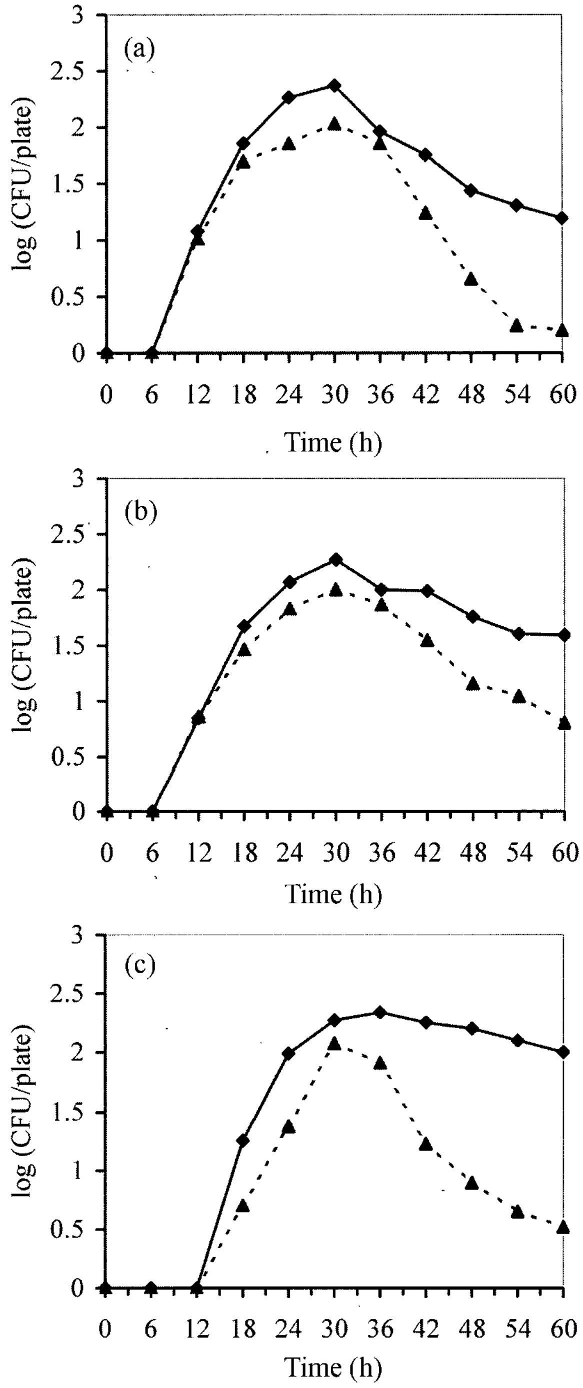

Fig. 4. Growth of natural microflora on the surfaces of (a) jerked beef, (b) surimi, and (c) mashed sausage media covered with the fermented soybean film (-▲-) and the control soybean film (-◆-).

The initial cell number was 10^2–10^3 CFU/plate.

fermented soybean film has an adequate durability to be utilized in small food pouches or coatings, although not enough.

Resistance of the Fermented Film to Indicator Strains on Foodstuffs

The fermented film could effectively inhibit both pathogenic and spoilage organisms in a wide variety of foods, which might provide a viable microbial strategy for reducing the incidence of pathogens on foods, even when these packaged products had been opened and contaminated by the consumer. Figures 4–8 show the growth of natural microflora, *E. coli*, and foodborne pathogenic bacteria *S. typhimurium*, *L. monocytogenes*, and *Sta. aureus*, on various foods like

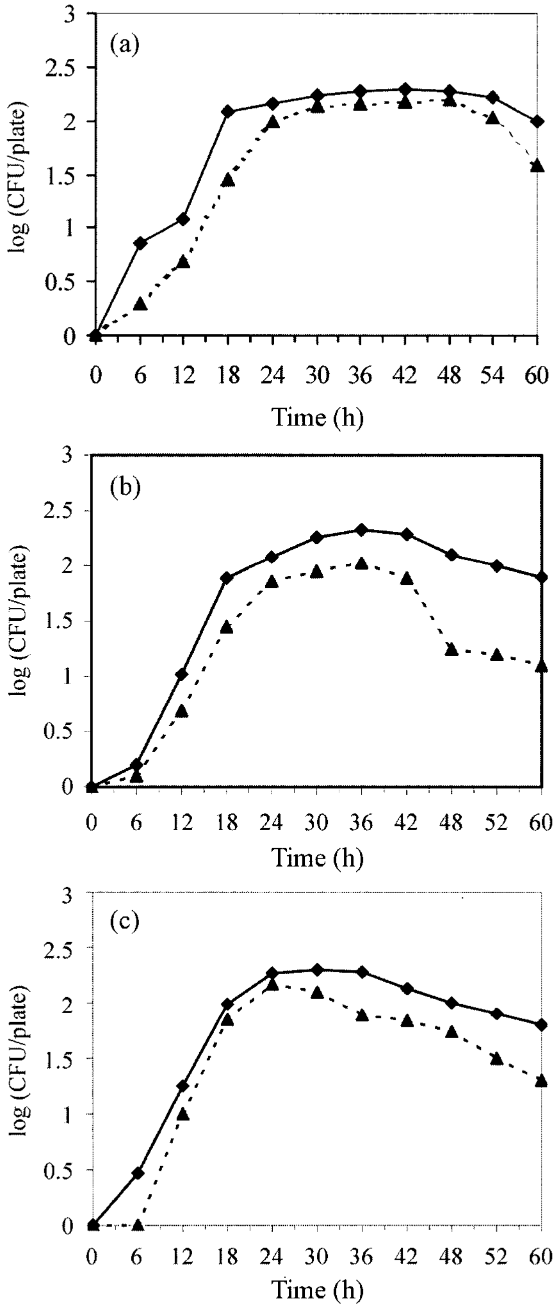

Fig. 5. Growth of *E. coli* on the surfaces of (a) jerked beef, (b) surimi, and (c) mashed sausage media covered with the fermented soybean film (-▲-) and the control soybean film (-◆-).
The initial cell number was 10^2–10^3 CFU/plate.

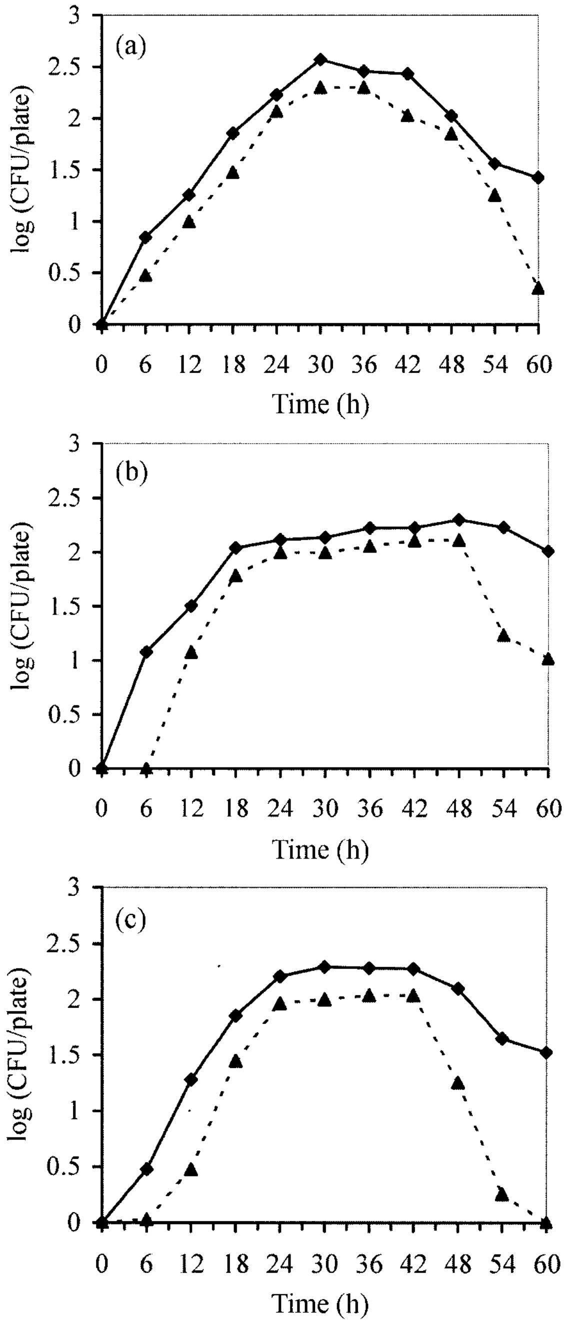

Fig. 6. Growth of *Salmonella typhimurium* on the surfaces of (a) jerked beef, (b) surimi, and (c) mashed sausage media covered with the fermented soybean film (-▲-) and the control soybean film (-◆-).
The initial cell number was 10^2–10^3 CFU/plate.

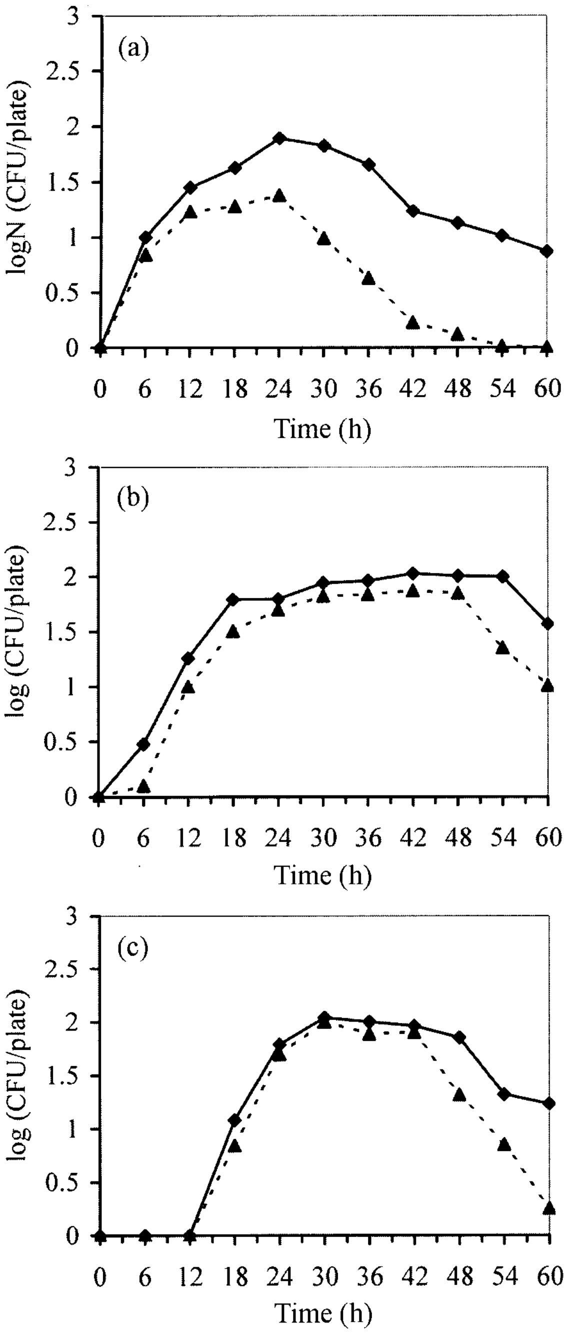

Fig. 7. Growth of *Listeria monocytogenes* on the surfaces of (a) jerked beef, (b) surimi, and (c) mashed sausage media covered with the fermented soybean film (-▲-) and the control soybean film (-◆-).
The initial cell number was 10^2–10^3 CFU/plate.

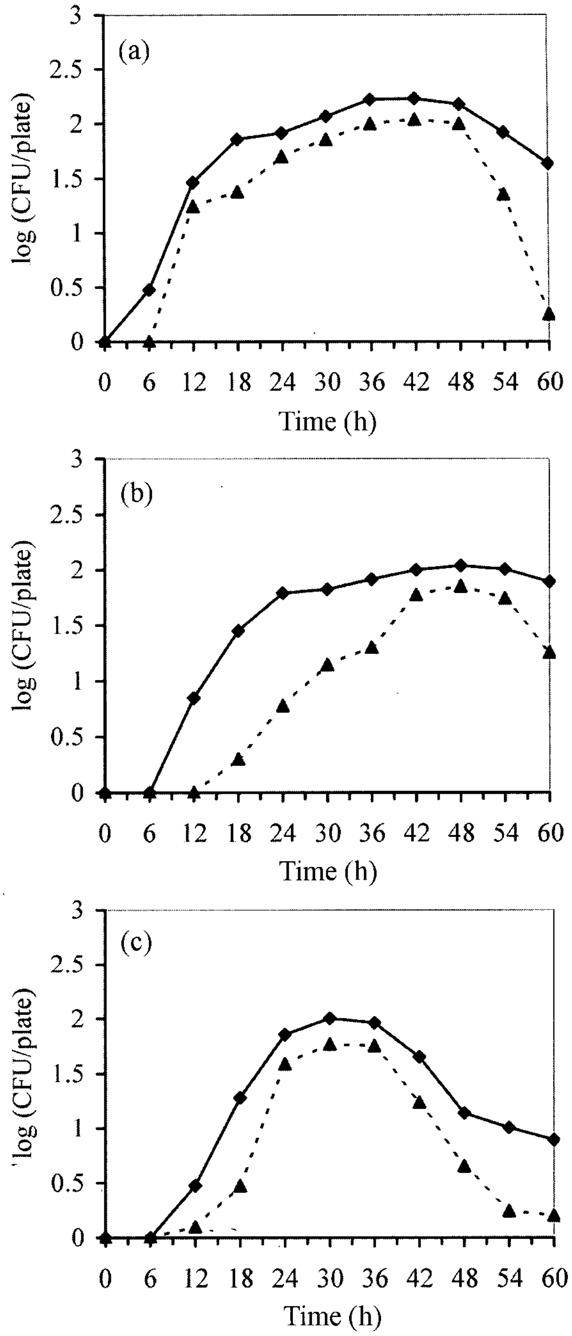

Fig. 8. Growth of *Staphylococcus aureus* on the surfaces of (a) jerked beef, (b) surimi, and (c) mashed sausage media covered with the fermented soybean film (-▲-) and the control soybean film (-◆-).
The initial cell number was 10^2–10^3 CFU/plate.

jerked beef, surimi, and mashed sausage media that had previously been covered with the control soybean film or the fermented film. The growth rates of all five kinds of microorganism in all food media covered with the fermented films were significantly lower than those of control film. Furthermore, the lag phases of all these microorganisms with the fermented films were also significantly longer than those of control film, and the death rates after reaching the peak were also faster with the fermented film than the control film. This might be due to gradual diffusion of BLS from the film. These results imply that packaging foods with the fermented film can slightly extend the shelf-life of foods. The advantage of antimicrobial edible film is that the antimicrobial agents in this film can specifically be targeted to post-processing contaminants on the food surface [4, 5]. The diffusion rate of antimicrobial into the product is incorporated into the film and the film properties. Diffusion of the antimicrobial through an edible film is influenced by the type, procedure for production, hydrophilic characteristics, storage temperature, and duration [5, 8]. In the present study, we evaluated the physical, mechanical, and antibacterial properties of the fermented soybean film containing BLS, and attempted to verify whether the film could be used as a packaging material in the food industry. However, direct application of the fermented film to the commercial food industry still remains questionable due to the film's poor mechanical and antibacterial properties. Therefore, it is necessary to increase the BLS content in the fermented film, because the BLS content in the present fermented film is only 0.32% [11]. However, although the antibacterial property could be enhanced by increasing the fermentation period to produce more BLS, the mechanical properties would become poorer due to the hydrolytic enzymes produced during the prolonged fermentation. Therefore, these problems that interfere with commercial uses of the fermented film should be genetically solved by producing bacterial strains with more BLS production and less hydrolytic enzymes.

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REFERENCES

1. ASTM (American Society for Testing Methods). 1995. Standard test method for tensile properties of thin plastic sheeting (D882-95a). In *Annual Book of American standards Testing Methods*. Philadelphia, U.S.A.
2. ASTM (American Society for Testing Methods). 1995. Standard test method for water vapor transmission of materials (E96-95b). In *Annual Book of American standards Testing Methods*. Philadelphia, U.S.A.
3. Blacido, D. T., J. S. Paulo, and C. M. Florencia. 2004. Development and characterization of biofilms based on Amaranth flour (*Amaranthus caudatus*). *J. Food Eng.* **67:** 215–223.
4. Cagri, A., Z. E. Ustunol, and T. Ryser. 2003. Antimicrobial edible film and coatings. *J. Food Protect.* **67:** 833–848.
5. Kim, H. J., J. H. Kim, J. H. Son, H. J. Seo, S. J. Park, N. S. Paek, and S. K. Kim. 2004. Characterization of bacteriocin produced by *Lactobacillus bulgaricus*. *J. Microbiol. Biotechnol.* **14:** 503–508.
6. Cho, S. Y. and C. Rhee. 2004. Mechanical properties and water vapor permeability of edible films made from fractionated soy proteins with ultrafiltration. *Lebensm.-Wiss. u.-Technol.* **37:** 833–839.
7. Chobert, J. M. and M. Z. Zitohy. 1988. Solubility and emulsifying properties of caseins modified enzymatically by *Streptococcus aureus* V8 protease. *J. Agric. Food Chem.* **36:** 220.
8. Emna, M., V. Caroline, and L. Monique. 2000. Bacterial use of biofilms cross-linked by gamma irradiation. *Radiat. Phys. Chem.* **58:** 203–205.
9. Eugenio, P., B. Carla, M. Marcella, and R. Annamaria. 1995. A comparison of methods for measurement of bacteriocin activity. *J. Microbiol. Methods* **22:** 95–108.
10. Khouti, Z. and J. P. simon. 1997. Detection and partial characterization of a bacteriocin produced by *Carnobacterium piscicola* 213. *J. Industr. Microbiol. Biotechnol.* **19:** 28–33.
11. Kim, H. W., K. M. Kim, E. J. Ko, S. K. Lee, S. D. Ha, K. B. Song, S. K. Park, K. S. Kwon, and D. H. Bae. 2004. Development of antimicrobial edible film from defatted soybean meal fermented by *Bacillus subtilis*. *J. Microbiol. Biotechnol.* **14:** 1303–1309.
12. Kim, K. M., L. W. Curtis, A. H. Milford, and A. Gennadios. 2002. Heat curing of soy protein films at selected temperatures and pressures. *Lebensm.-Wiss. u.-Technol.* **35:** 140–145.
13. Kumar, C. G. and S. K. Anand. 1998. Significance of microbial biofilms in food industry: Review. *Int. J. Food Microbiol.* **42:** 9–27.
14. Liu, C. C., M. Angela, T. Garay, and M. E. C. Perez. 2004. Physical and mechanical properties of peanuts protein films. *Lebensm.-Wiss. u.-Technol.* **37:** 731–738.
15. Longares, A., F. J. Monahan, E. D. O'Riordan, and M. O'Sullivan. 2004. Physical properties and sensory evaluation of WPI films of varying thickness. *Lebensm.-Wiss. u.-Technol.* **37:** 545–550.
16. Oh, S. J., M. H. Kim, J. C. John, and W. W. Randy. 2003. Purification and characterization of an antilisterial bacteriocin produced by *Leuconostoc sp.* W65. *J. Microbiol. Biotechnol.* **13:** 680–686.
17. Mauer, L. J., D. E. Smith, T. P. Labuza. 2000. Water vapor permeability, mechanical, and structural properties of edible β-casein films. *Int. Dairy J.* **10:** 353–358.
18. Mariniello, L., P. D. Pierro, C. Esposito, S. Angela, M. Paolo, and P. Raffaele. 2003. Preparation and mechanical properties of edible pectin-soy flour films obtained in the absence or presence of transglutaminase. *J. Biotechnol.* **102:** 191–198.
19. Maria, P. and S. Mats. 2005. Water vapor permeability and mechanical properties of mixed starch-monoglyceride films and effect of forming conditions. *Food Hydrocolloids* **19:** 123–132.
20. Micard, V., R. Belamri, M. H. Morel, and S. Guilbert. 2000. Properties of chemically and physically treated wheat gluten. *J. Agric. Food Chem.* **48:** 2948–2953.
21. Monti, J. C. and R. Jost. 1978. Enzymatic solubilization of heat-denatured cheese whey protein. *J. Dairy Sci.* **61:** 1233.

22. Orliac, O., A. Rouilly, S. Francoise, and R. Luc. 2003. Effect of virus plasticizers on the mechanical properties, water resistance and aging of thermo-moulded films made from sunflower proteins. *Industr. Crops Prod.* **18:** 91–100.
23. Moon, G. S., C. H. Kang, Y. R. Pyun, and W. J. Kim. 2004. Isolation, identification, and characterization of a bacteriocin-producing *Enterococcus* sp. from kimchi and its application to kimchi fermentation. *J. Microbiol. Biotechnol.* **14:** 924–931.
24. Park, H. J. and M. S. Chinnan. 1995. Gas and water vapor barrier properties of edible films from protein and cellulosic materials. *J. Food Eng.* **25:** 497–507.
25. Park, S. K., C. O. Rhee, D. H. Bae, and S. Hetiarachchy. 2001. Mechanical properties and water-vapor permeability of soy protein film affected by calcium salts and glucono-d-lactone. *J. Agric. Food Chem.* **49:** 2308–2312.
26. Paik, H. D., S. K. Lee, S. Heo, S. Y. Kim, T. J. Kwon, and H. H. Lee. 2004. Purification and characterization of the fibrinolytic enzyme produced by *Bacillus subtilis* KCK-7 from Chungkookjang. *J. Microbiol. Biotechnol.* **14:** 829–835.
27. Rhim, J. W., A. Gennadios, H. Akihiro, L. C. Weller, and A. H. Milford. 2000. Solubility, tensile, and color properties of modified soy protein isolate films. *J. Agric. Food Chem.* **48:** 4937–4941.
28. Sabato, S. F., B. Ouattara, H. Yu, G. D'Aprano, C. Le Tien, M. A. Mateescu, and M. Lacroix. 2001. Mechanical and barrier properties of cross-linked soy and whey protein based films. *J. Agric. Food Chem.* **49:** 1397–1403.
29. Schou, M., A. Longares, C. H. Montesinos, F. J. Monahan, D. O'Riordan, and M. O'Sullivan. 2004. Properties of edible sodium caseinate film and their application as food wrapping. *Lebensm.-Wiss. u.-Technol.* **24:** 234–246.