*Journal of Food Protection, Vol. 69, No. 4, 2006, Pages 784–793*


# Inhibition of *Salmonella enterica* and *Escherichia coli* O157:H7 on Roasted Turkey by Edible Whey Protein Coatings Incorporating the Lactoperoxidase System

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MS 05-410: Received 11 August 2005/Accepted 13 November 2005

## ABSTRACT

The effects of whey protein isolate (WPI) coatings incorporating a lactoperoxidase system (LPOS) on the inhibition of *Salmonella enterica* and *Escherichia coli* O157:H7 on roasted turkey were studied by testing the initial inhibition as well as the inhibition during storage. The initial antimicrobial effects of WPI coatings incorporating LPOS (LPOS-WPI coatings) were examined with various inoculation levels and LPOS concentrations. LPOS-WPI coatings with 7 and 4% of LPOS demonstrated initial 3- and 2-log CFU/g reductions of *S. enterica* and *E. coli* O157:H7, respectively. The antimicrobial effect was observed regardless of whether the turkey was inoculated before or after coating. Storage studies were conducted for 42 days at 4 and 10°C with *S. enterica* (6.0 log CFU/g)– or *E. coli* O157:H7 (5.6 log CFU/g)–inoculated sliced turkey. LPOS concentrations for the storage studies of *S. enterica* and *E. coli* O157:H7 were 5 and 3% (wt/wt), respectively, in the coating solution and in an LPOS solution for spreading. LPOS-WPI coatings inhibited the growth of both *S. enterica* and *E. coli* O157:H7 in turkey at both 4 and 10°C for 42 days. The inhibition was more pronounced when the coating was formed on the surface of the turkey prior to inoculation than when the coating was formed on the inoculated surface. More effective inhibition of *S. enterica* and *E. coli* O157:H7 was observed with the LPOS-WPI coatings than with the LPOS solution-spreading treatment. LPOS-WPI coatings also retarded the growth of total aerobes during storage.

*Salmonella* is estimated to cause between 2 and 4 million cases of salmonellosis annually in the United States *(20)*. In 2000, economic costs due to salmonellosis were estimated at $2.4 billion, and *Escherichia coli* O157:H7 was implicated in >62,000 cases of illness, at an estimated cost of $700 million in medical expenses and lost productivity *(41)*. Foodborne diseases caused by *Salmonella* and *E. coli* O157:H7 have been associated with the consumption of various products, including poultry *(2, 7)*.

Antimicrobial edible coatings can be applied to ready-to-eat (RTE) products to prevent postprocessing contamination by undesirable microorganisms *(9, 31)*. Many researchers have demonstrated the potential of applying these coatings to control surface contamination of foods with respect to *Salmonella* and *E. coli* O157:H7 *(8, 18, 32, 44)*. Antimicrobial edible coatings have an advantage over the direct application of antibacterial agents because the coatings can be designed to slow the diffusion of antimicrobial components into the food surface *(39, 40)*.

The food industry has shown interest in using natural or biologically derived antimicrobial materials as preservatives *(19)*. Some research efforts have focused on the incorporation of these materials in various edible coatings *(11, 12)*. The lactoperoxidase system (LPOS) is a natural antimicrobial system found in human secretions such as saliva, tear fluid, and milk *(23)*. Lactoperoxidase (LPO) in the LPOS catalyzes the oxidation of the thiocyanate ion ($SCN^-$), generating products such as hypothiocyanite and hypothiocyanous acid that oxidize the sulfydryl groups of microbial enzymes and other proteins. The oxidation of sulfydryl groups results in altered cellular structure and function, such as in the cells' membrane integrity and transport systems, inhibition of metabolic enzymes, and eventual death *(15, 23, 37)*.

LPOS has been suggested for use as a preservative in foods and pharmaceuticals *(6, 23, 37)*. The antibacterial properties of LPOS and its generally recognized as safe status have resulted in numerous publications demonstrating its potential use as a natural food preservative *(15)*. Antimicrobial activities of LPOS against *Salmonella* and *E. coli* O157:H7 have been reported *(15, 22, 43)*. However, to our knowledge, no research has been reported on the antimicrobial effects of edible coatings incorporating LPOS against *Salmonella* and *E. coli* O157:H7 in a food system.

Whey protein isolate (WPI) can form transparent, flexible, colorless, and odorless coatings that provide excellent oxygen, aroma, and oil barrier properties *(18, 25, 26)*. LPO is a component of whey protein that can be separated and concentrated *(42)*. Thus, an edible coating based on WPI could be a compatible matrix for LPO.

The objectives of this research were to (i) evaluate the initial inhibition of *Salmonella enterica* and *E. coli* O157:H7, inoculated on sliced roasted turkey, by edible WPI coatings incorporating LPOS (LPOS-WPI coatings); (ii) study the growth patterns of *S. enterica*, *E. coli* O157:H7, and total aerobic microorganisms during aerobic storage of

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the inoculated turkey treated by either LPOS-WPI coating or LPOS solution spreading; (iii) compare the antimicrobial effects of the LPOS-WPI coating with those of the LPOS solution-spreading treatment during aerobic storage; and (iv) compare the effectiveness of the coatings and solutions applied before or after inoculation.

## MATERIALS AND METHODS

**Bacterial strains.** Dr. Larry Beuchat (University of Georgia, Griffin) provided the bacterial strains used in this study. Cocktails were prepared with the following five strains of each pathogen (sources indicated): *S. enterica* serotypes Agona (alfalfa sprouts), Enteritidis (patient in an egg-associated outbreak), Gaminara (orange juice), Michigan (cantaloupe-associated outbreak), and Montevideo (patient in a tomato-associated outbreak); and enterohemorrhagic *E. coli* O157:H7 strains E0019 (calf feces), H1730 (patient in a lettuce-associated outbreak), F4546 (patient in an alfalfa sprout–associated outbreak), 932 (human feces), and 994 (fermented salami). All strains were adapted to grow in the presence of 50 μg of nalidixic acid per ml and were stored in a freezer at −80°C *(24).*

**Media.** Tryptic soy agar (Difco, Becton Dickinson, Sparks, Md.) supplemented with 50 μg of nalidixic acid per ml (TSAN; Sigma-Aldrich, St. Louis, Mo.) was used as growth and recovery media for both *S. enterica* and *E. coli* O157:H7. Bismuth sulfite agar and sorbitol MacConkey agar supplemented with 50 μg of nalidixic acid per ml (BSAN and SMAN, respectively) were used as selective agar media for enumerating *S. enterica* and *E. coli* O157:H7, respectively. Nalidixic acid was filtered (0.22 μm) and added to basal media after autoclaving and cooling to approximately 50°C. Nalidixic acid was added to minimize colonies of bacteria present on roasted turkey (the model food), thus facilitating the detection of *S. enterica* and *E. coli* O157:H7. Plate count agar (Difco, Becton Dickinson) was used to enumerate total aerobic organisms, and dichloran rose-bengal chloramphenicol agar (DRBC; Merck, Darmstadt, Germany) was used to enumerate yeasts and molds.

**Inoculum preparation.** Inocula of each pathogen were prepared following the method of Lang et al. *(24).* Frozen stock cultures of *S. enterica* and *E. coli* O157:H7 were streaked on TSAN. Cultures were incubated at 37°C for 24 h, and an isolated colony of each strain of each pathogen was transferred to 10 ml of tryptic soy broth (TSB; Difco, Becton Dickinson). At two consecutive 24-h intervals, further transfers were made in TSB with a sterile loop (approximately 10 μl). Cells of each strain in 1.5 ml of brain heart infusion broth were collected by centrifugation (4,000 × *g,* 2 min, 22°C) and washed twice in 750 ml of sterile 0.1% peptone water. Cocktails of each pathogen were prepared by combining equal portions of each strain to produce an inoculum of approximately $10^9$ CFU/ml. The cocktail was diluted in 0.1% peptone water to produce the desired inoculum concentration. Samples of the cocktail were plated onto TSAN to determine inoculum levels.

**Enzymes and other chemicals.** The LPOS was composed of LPO (102 U/mg), glucose oxidase (235 U/mg), α-D-glucose, potassium thiocyanate, and hydrogen peroxide ($H_2O_2$). LPO, glucose oxidase, α-D-glucose, and potassium thiocyanate were purchased from Sigma-Aldrich; $H_2O_2$ was purchased from EM Science (Gibbstown, N.J.); and WPI was supplied by Davisco Foods International (Le Sueur, Minn.). Glycerol, which was used as a plasticizer to improve coating flexibility, was purchased from Fisher Scientific Inc. (Fair Lawn, N.J.).

**Preparation of LPOS solution.** The weight ratios of the LPOS components were 1.00:0.35:108.70:1.09:2.17, in the order of LPO, glucose oxidase, α-D-glucose, potassium thiocyanate, and $H_2O_2$. This composition was selected on the basis of the results obtained from previous research *(30).* The components were dissolved separately in Butterfield's buffer (Weber Scientific, Hamilton, N.J.), and a ratio of 1.00:2.84 was used for the weight of LPOS to buffer. The LPOS-dissolved solution was incubated at 23 ± 2°C for 24 h, with shaking at 160 rpm by a water bath shaker (model G76, New Brunswick Scientific, Edison, N.J.) to increase the antimicrobial activity of LPOS *(6).* LPO constituted 0.9% (wt/wt) of the LPOS on a dry weight basis. After incubation, the LPOS-buffer mixture was used immediately for coating or solution preparation.

**Preparation of turkey samples.** Oven-roasted turkey products with sodium diacetate as an antimicrobial agent (Healthy Choice, ConAgra Foods, Downers Grove, Ill.) and without sodium diacetate (Boar's Head Brand, Brunckhorst Co., New York, N.Y.) were purchased at a local supermarket. The turkey with sodium diacetate contained poultry ingredients (turkey breast and white turkey), water, potassium lactate, 2% or less of celery juice, dextrose, salt, modified food starch, sodium phosphate, sodium diacetate, and flavorings. The turkey product without sodium diacetate contained turkey breast, water, less than 1.5% of salt, sugar, sodium phosphate, and natural flavor; this product was labeled ''no preservatives.'' The turkey product with sodium diacetate was presliced, and the product without sodium diacetate was sliced at the supermarket; the thickness of all slices was approximately 0.25 cm. Turkey slices were stored at 4°C for a maximum of 1 day before use in experiments. Each slice of turkey was trimmed to 7 by 7 by 0.25 cm, for a weight of 10 ± 0.4 g.

**Preparation of LPOS-WPI–coated turkey samples.** WPI coating solutions were prepared by following the method of McHugh and Krochta *(26).* A WPI solution (10%, wt/wt) was prepared in distilled water; glycerol (weight equal to that of WPI) was added, and the WPI-glycerol solution was degassed under vacuum. The solution was maintained at 90°C for 30 min in a water bath and then cooled on ice to 5°C; once cooled, the solution was degassed again. Various concentrations of LPOS (0 to 7%, wt/wt) were added to the degassed solution to prepare LPOS-WPI coating solutions.

Turkey samples were placed in a single layer on a wire screen in a laminar flow biohazard hood (SterilGARD Hood, The Baker Company, Inc., Sanford, Maine) at 28 ± 2°C and 30% relative humidity for coating and inoculation. Coating solution (1 ml) was applied to the surface of each turkey sample after (I+C) or before (C+I) inoculation with *S. enterica* or *E. coli* O157:H7. For I+C, *S. enterica* or *E. coli* O157:H7 was first inoculated onto the surface of turkey slices. The inoculated turkey slices were dried for 0.5 h and then coated inside the biohazard hood at 28 ± 2°C and 30% relative humidity. For C+I, coated turkey slices were dried for 1 h; then, the coated surfaces of turkey slices were inoculated inside the hood. Inoculum was spotted either directly onto the turkey sample or onto the top of the coating previously applied to the turkey sample, with approximately equal volumes, at 25 to 30 locations per turkey sample. The total inoculum volume was 100 μl. The inoculum spots were spread evenly with a presterilized disposable plating hockey stick (Fisher). Coating thickness was estimated to be 0.2 mm, based on the dimensions of the turkey sample and the volume of coating solution applied. A coating solution containing a blue dye (FD&C Blue No. 1, Sensient Technologies Corp., St. Louis, Mo.) (7%, wt/wt) was spread on the surfaces of turkey samples ($n = 10$), and the resulting coated

surfaces were visually examined. This test confirmed that a continuous coating of uniform thickness was achieved by the technique described *(29).*

**Preparation of LPOS solution-spread turkey samples.** Solution-spread samples were prepared in a manner similar to the coated samples, but instead of incorporating LPOS into the WPI coating solution, LPOS was mixed with sterile deionized water to prepare the solution for spreading. One milliliter of LPOS solution was applied to the top surface of each turkey sample, either before (S+I) or after (I+S) inoculation with *S. enterica* or *E. coli* O157:H7, and then spread with a presterilized disposable plating hockey stick. The inoculum (100 μl) was applied as described for the coated samples. Drying times after inoculation and spreading were the same as those used in the previous experiment after inoculation and coating.

**Initial inhibition by LPOS-WPI coatings.** The levels of prepared inocula ranged from 3 to 6 log CFU/g. The concentrations of LPOS in the coating solutions ranged from 0 to 7% (wt/wt). Coated turkey samples (I+C and C+I), as well as inoculated samples without coating, were placed in stomacher bags (19 by 30 cm; Nasco WHIRL-PAK, Fort Atkinson, Wis.). Samples were diluted 10-fold with 0.1% peptone water and stomached for 2 min at normal speed (230 ± 5% rpm) (Stomacher 400, Seward Ltd., London, UK). The homogenate was diluted serially and plated (100 μl or 1 ml) on BSAN or SMAN. The BSAN and SMAN plates were incubated at 37°C for 48 and 20 h, respectively, before colonies of presumptive cells were counted.

**Storage studies.** Only the turkey product without sodium diacetate was used for the storage studies to avoid compounding antimicrobial effects. Turkey slices were coated (I+C and C+I) or spread with the LPOS solution (I+S and S+I), as described previously. The *S. enterica* storage study used an inoculum of 6.0 log CFU/g and a 5% (wt/wt) LPOS concentration in both the LPOS-WPI coating solution and LPOS solution for spreading. The *E. coli* O157:H7 storage study used an inoculum of 5.6 log CFU/g and a 3% (wt/wt) LPOS concentration in both the LPOS-WPI coating solution and LPOS solution for spreading. The inoculum levels and LPOS concentrations were selected on the basis of the results obtained from the initial inhibition study, for a desired initial cell number between 4.0 and 4.5 log CFU/g for treated turkey samples (I+C, C+I, I+S, and S+I). Inoculated control samples were prepared by inoculating turkey slices that had no coating or spreading treatment. Uninoculated control samples without coating or spreading were included only in the experimental plan for the *E. coli* O157:H7 study. The treated samples (I+C, C+I, I+S, and S+I) and the control samples were individually sealed without vacuum in sterile bags (18 oz [532 $cm^3$], Nasco WHIRL-PAK) and stored at 4 or 10°C for up to 42 days. Storage temperatures were monitored by a temperature datalogger (TempTaleH, Sensitech Inc., Beverly, Mass.). At each sampling time during storage, samples were diluted 10-fold with 0.1% (wt/vol) peptone water and homogenized for 2 min at a medium speed in the stomacher. The homogenate was serially diluted and plated (100 μl or 1 ml) on BSAN, SMAN, plate count agar, and dichloran rose-bengal chloramphenicol agar to enumerate *S. enterica*, *E. coli* O157:H7, total aerobes, and yeasts and molds, respectively. Before colony counting, BSAN and SMAN plates were incubated at 37°C for 48 and 20 h, respectively; plate count agar plates were incubated at 35°C for 48 h; DRBC plates were incubated at 22°C for 5 days. Turkey samples were analyzed for water activity ($a_w$) immediately after unpacking, before storage, and after 28 and 42 days of storage with an $a_w$ meter (AquaLab CX-2, Decagon Devices, Inc., Pullman, Wash.). Solutions obtained from stomaching the turkey samples and 0.1% peptone water (1:10) in stomacher bags were collected for pH measurement at 22°C with a pH meter (model 370, Orion, Beverly, Mass.).

**Statistical analysis.** The numbers ($n$) of each treatment sample were four for all experiments. The SAS software program (version 8.1, SAS Institute Inc., Cary, N.C.) was used to analyze the data. Microbiological data were analyzed by the General Linear Models procedure and Duncan's multiple range tests, with examination for significant differences ($\alpha = 0.05$) at each storage interval for individual treatments.

## RESULTS AND DISCUSSION

**Initial inhibition studies.** Oven-roasted turkey served as the model food to test the antimicrobial efficacy of LPOS-WPI coatings. Presumptive *Salmonella* was not detected (detection limit, 10 CFU/g) from turkey slices, either with or without sodium diacetate. The LPOS-WPI coatings influenced the inhibition of *S. enterica* at all inoculation levels on the sliced turkey, either with or without sodium diacetate, as shown in Table 1a and 1b, respectively. As the concentration of LPOS increased, the number of cells that survived at each inoculum level decreased. An approximately 3-log CFU/g amount of *S. enterica* cells, inoculated on the turkey samples without sodium diacetate, was initially inhibited by LPOS-WPI coatings at 7% (wt/wt) in both I+C and C+I (Table 1b). At other LPOS concentrations, antimicrobial effectiveness was not significantly affected ($P > 0.05$) by the order of coating and inoculation (I+C versus C+I).

There was no difference between estimated inoculum levels and initial cell levels recovered from the inoculated control samples (no coating), suggesting that the level of sodium diacetate present was insufficient to reduce *Salmonella* (Table 1a). Sodium diacetate is widely used as an antimicrobial in certain foods, including turkey products, at levels of 0.05 to 0.4% *(28).* Previous research has shown that the numbers of *E. coli* and *Salmonella* Enteritidis (3 log CFU/5 ml of broth) in a nutrient broth with 0.45% sodium diacetate increased by less than 3 log after 48 h at 35°C, but initial inhibition of the cells by sodium diacetate was not observed *(38).* Sodium diacetate–based antimicrobial systems may not sufficiently protect turkey and other RTE products from postprocessing contamination by *Salmonella.*

The turkey samples used for tests of the initial inhibition of *E. coli* O157:H7 did not contain sodium diacetate. The number of cells that survived at each inoculum level generally decreased as the concentration of LPOS increased (Table 2). A reduction of approximately 2 log of *E. coli* O157:H7 was observed with LPOS-WPI coatings at 4% (wt/wt) (Table 2). The order of coating and inoculation did not significantly affect effectiveness ($P > 0.05$).

For both the *S. enterica* and *E. coli* O157:H7 initial inhibition studies, there was no significant difference between the inoculum level and the number of cells recovered from inoculated samples without coating ($P > 0.05$), indicating no significant loss of cells during inoculation and drying ($P > 0.05$) (Tables 1a and 1b and 2). The WPI

TABLE 1. *Initial inhibition of* Salmonella enterica *at different inoculum levels on roasted turkey with (a) and without (b) sodium diacetate preservative by whey protein isolate coatings incorporating the lactoperoxidase system (LPOS-WPI) with concentrations of 0, 3, 5, and 7% (wt/wt) in the coating solution*

**a. With sodium diacetate**

| Concn of lactoperoxidase system (%, wt/wt)[a] | Treatment | No. of *S. enterica* (log CFU/g) recovered following an initial inoculum (log CFU/g) of[b]: 3.3 | 4.3 | 5.3 |
|---|---|---|---|---|
| 0 | Inoculation but no coating | 3.2 ± 0.3 C A | 4.0 ± 0.3 B A | 4.9 ± 0.3 A A |
| | I+C[c] | 2.6 ± 0.3 C A | 3.5 ± 0.2 B A | 4.7 ± 0.3 A A |
| | C+I[d] | 2.7 ± 0.2 C A | 3.6 ± 0.3 B A | 4.5 ± 0.4 A A |
| 3 | I+C | 2.0 ± 0.2 C B | 3.0 ± 0.4 B AB | 3.7 ± 0.2 A B |
| | C+I | 1.9 ± 0.2 C B | 2.7 ± 0.3 B B | 3.3 ± 0.2 A BC |
| 5 | I+C | 1.6 ± 0.2 C BC | 2.4 ± 0.2 B B | 3.5 ± 0.2 A BC |
| | C+I | 1.2 ± 0.2 C C | 2.4 ± 0.3 B B | 3.1 ± 0.2 A C |

**b. Without sodium diacetate**

| Concn of lactoperoxidase system (%, wt/wt) | Treatment | No. of *S. enterica* (log CFU/g) recovered following an initial inoculum (log CFU/g) of: 3.2 | 4.2 | 5.2 | 6.2 |
|---|---|---|---|---|---|
| 0 | Inoculation but no coating | 3.2 ± 0.2 D A | 3.9 ± 0.3 C A | 4.8 ± 0.2 B A | 5.6 ± 0.3 A A |
| | I+C | 2.7 ± 0.4 D A | 3.6 ± 0.2 C A | 4.8 ± 0.2 B A | 5.5 ± 0.4 A AB |
| | C+I | 2.6 ± 0.3 D A | 3.6 ± 0.3 C A | 4.6 ± 0.1 B A | 5.4 ± 0.3 A AB |
| 3 | I+C | 1.9 ± 0.2 D B | 2.8 ± 0.4 C B | 3.9 ± 0.4 B B | 4.9 ± 0.3 A B |
| | C+I | 1.7 ± 0.3 D BC | 2.3 ± 0.2 C B | 3.6 ± 0.2 B B | 4.7 ± 0.2 A B |
| 5 | I+C | 1.5 ± 0.2 D BC | 2.5 ± 0.2 C B | 3.3 ± 0.2 B BC | 4.2 ± 0.2 A C |
| | C+I | 1.2 ± 0.2 D C | 2.2 ± 0.2 C B | 3.2 ± 0.2 B C | 4.1 ± 0.2 A C |
| 7 | I+C | <1.0[e] D D | 1.0 ± 0.5 C C | 2.2 ± 0.2 B D | 3.1 ± 0.1 A D |
| | C+1 | >1.0 C D | >1.0 C D | 1.7 ± 0.2 B E | 2.6 ± 0.2 A E |

[a] Lactoperoxidase constituted 0.9% (wt/wt) of the lactoperoxidase system dry weight.

[b] Values are means ± standard deviations, $n = 4$. Mean values (log CFU per gram) in columns were analyzed for significant differences ($\alpha = 0.05$). First letter: within concentration of lactoperoxidase system; values not followed by the same letter are significantly different. Second letter: within inoculum level; values not followed by the same letter are significantly different.

[c] Inoculation before coating.

[d] Coating before inoculation.

[e] Under detection limit, <1.0 log CFU/g (estimated).

coating itself (0% LPOS samples) did not have a significant effect on the numbers of *S. enterica* and *E. coli* O157:H7 ($P > 0.05$).

Bactericidal activity is defined as a reduction in the original number of bacteria determined by plating on a recovery agar medium *(17).* Bactericidal effects of LPOS against *Salmonella* Typhimurium have previously been reported *(43),* in which all cells at 2 to 5 log CFU/ml in TSB were killed by the LPOS containing LPO at 1 ppm. The antimicrobial activity of LPOS against *S. enterica* inoculated on beef was demonstrated with an LPOS of 2 to 10 ppm, which prevented the growth of *S. enterica* (4.7 log CFU/cm$^2$) for 7 days at 12°C and for 42 days at 12 to −1°C *(15).* Our results demonstrated that the LPOS-WPI coating possessed bactericidal activities against both *S. enterica* and *E. coli* O157:H7.

In other studies in which $H_2O_2$ was supplied exogenously, coliforms and *Salmonellae* were not only inhibited, but were also possibly killed *(5, 34).* Other researchers have suggested that very short-lived oxidation products from LPOS, such as $O_2SCN^-$ and $O_3SCN^-$, are formed when $H_2O_2$ is present at higher than equimolar concentration with $SCN^-$; these oxyacids may be very strong antimicrobial agents against *E. coli (33, 37).* The LPOS used in our research, which was prepared with added $H_2O_2$, was strongly effective against both *S. enterica* and *E. coli* O157:H7.

Epidemiological investigations of outbreaks of foodborne illnesses have shown that *Salmonella* and *E. coli* O157:H7 were frequently present due to postprocessing recontamination of the foods consumed *(36).* Results from the I+C samples can be used to predict what would happen if LPOS-WPI coatings were formed over *S. enterica*– or *E. coli* O157:H7–contaminated food surfaces. Similarly, the results from the C+I samples predict what would happen if *S. enterica* or *E. coli* O157:H7 postprocessing contamination occurred on LPOS-WPI–coated foods. This study indicates that LPOS-WPI coatings have the potential to inhibit those microorganisms already present on food products, as well as to control microbial growth from contamination after the wrapping or coating of the food products.

TABLE 2. *Initial inhibition of* Escherichia coli *O157:H7 at inoculum levels of 4.0, 5.0, and 6.0 log CFU/g on roasted turkey without sodium diacetate preservative by whey protein isolate coatings incorporating the lactoperoxidase system at 0, 0.1, 0.15, and 0.2 g/g in the coating solution*

| Concn of lactoperoxidase system (%, wt/wt)[a] | Treatment | No. of *E. coli* O157:H7 (log CFU/g) recovered following an initial inoculum (log CFU/g) of[b]: 3.4 | 4.4 | 5.4 |
|---|---|---|---|---|
| 0 | Inoculation but no coating | 3.3 ± 0.1 C A | 4.4 ± 0.1 B A | 5.1 ± 0.2 A A |
| | I+C[c] | 3.4 ± 0.3 B A | 4.4 ± 0.2 A A | 4.8 ± 0.4 A A |
| | C+I[d] | 3.1 ± 0.3 B AB | 4.0 ± 0.2 A AB | 4.5 ± 0.4 A AB |
| 2 | I+C | 3.3 ± 0.2 C A | 4.2 ± 0.2 B AB | 4.7 ± 0.2 A A |
| | C+I | 2.7 ± 0.1 B B | 3.8 ± 0.2 A B | 4.2 ± 0.2 A B |
| 3 | I+C | 2.4 ± 0.4 B BC | 3.8 ± 0.2 A B | 4.1 ± 0.2 A B |
| | C+I | 1.9 ± 0.4 C C | 2.9 ± 0.3 B C | 3.8 ± 0.2 A B |
| 4 | I+C | 1.5 ± 0.3 C D | 2.7 ± 0.3 B CD | 3.6 ± 0.4 A B |
| | C+I | 1.1 ± 0.1 C D | 2.2 ± 0.2 B D | 2.9 ± 0.2 A C |

[a] Lactoperoxidase constituted 0.9% (wt/wt) of the lactoperoxidase system dry weight.
[b] Values are means ± standard deviations, $n = 4$. Mean values (log CFU per gram) in columns were analyzed for significant differences ($\alpha = 0.05$). First letter: within concentration of lactoperoxidase system; values not followed by the same letter are significantly different. Second letter: within inoculum level; values not followed by the same letter are significantly different.
[c] Inoculation before coating.
[d] Coating before inoculation.

**Storage studies.** The growth or survival patterns of *S. enterica* in inoculated LPOS-treated turkey samples (no sodium diacetate) were studied at 4 and 10°C. Both coating and solution-spreading treatments (5% LPOS, wt/wt) reduced the initial number of *S. enterica* by approximately 1 log from 5.2 log CFU/g (Fig. 1a and 1b), which is consistent with the result from the initial inhibition study. No significant differences in microbial counts ($P > 0.05$) were observed on day 0 between coated and solution-spread samples (I+C versus I+S, or C+I versus S+I) or between samples treated before or after inoculation (I+C versus C+I, or I+S versus S+I).

At 4°C, the number of *S. enterica* in control samples decreased slightly during storage (Fig. 1a). The number of *S. enterica* in the samples treated before inoculation (both C+I and S+I) was further reduced significantly from the initially reduced numbers ($P < 0.05$). Viable counts of *S. enterica* in the sample coated before inoculation (C+I) were smaller than those of the corresponding inoculated control sample by 3.4 log on day 21. Both treatments (coating and solution spreading) applied to turkey slices before inoculation were more effective than those made after inoculation. The difference between the numbers of *S. enterica* in I+C and C+I samples increased with increased storage time at 4°C. On day 28, the number of *S. enterica* in I+C samples was 3.3 log CFU/g but <1.0 log CFU/g in C+I samples. A difference in the antimicrobial effectiveness between I+S and S+I treatments was also observed. Some *Salmonella* exhibit psychrotrophic properties by their ability to grow in food stored at 2 to 4°C, which has raised

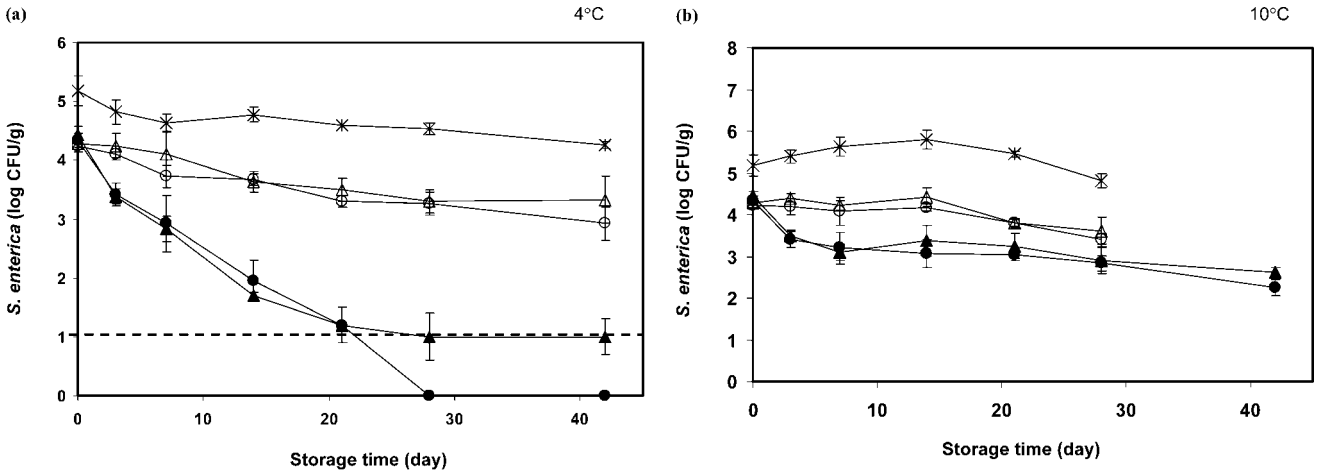


FIGURE 1. *Effects of a whey protein isolate coating incorporating the lactoperoxidase system (LPOS) (C+I and I+C) and LPOS solution spreading (S+I and I+S) on the number of* S. enterica *of* S. enterica*–inoculated roasted turkey during storage at 4°C (a) and 10°C (b) for up to 42 days. *, Inoculated control; △, I+S; ▲, S+I; ○, I+C; ●, C+I. The inoculated control (C) was neither coated nor solution spread. The detection limit (1.0 log CFU/g) is indicated with a dotted line, and zero counts on the plates are plotted as zero on the vertical axis. Data represent means ± standard errors of the means (*n = 4*).*

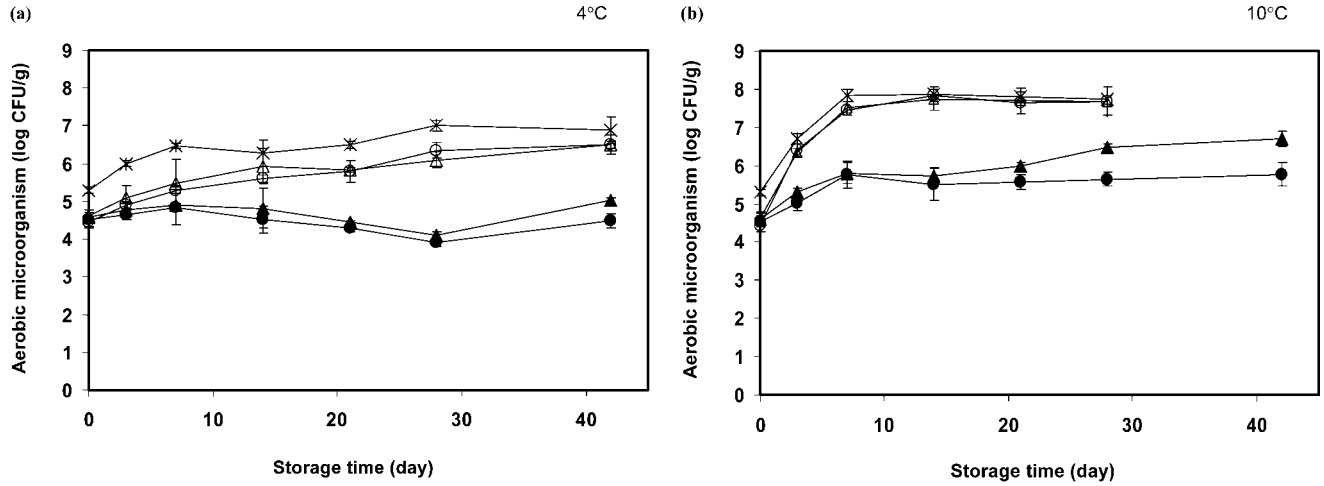


FIGURE 2. *Effects of a whey protein isolate coating incorporating the lactoperoxidase system (LPOS) (C+I and I+C) and LPOS solution spreading (S+I and I+S) on the number of total aerobic microorganisms of* S. enterica*–inoculated roasted turkey during storage at 4°C (a) and 10°C (b) for up to 42 days. *, Inoculated control; △, I+S; ▲, S+I; ○, I+C; ●, C+I. The inoculated control (C) was neither coated nor solution spread. Data represent means ± standard errors of the means (*n = 4*).*

concerns regarding the efficacy of chill temperatures to ensure food safety *(10)*. LPOS in WPI coatings or in solution-spreading treatments on foods appears to provide a safety hurdle for protection against *Salmonella* at refrigeration temperatures.

At 10°C, the number of *S. enterica* in the control samples did not change significantly during storage ($P > 0.05$) (Fig. 1b). Sampling of control, I+C, and I+S samples was discontinued after 28 days because they were visibly spoiled. Cell densities in I+C and I+S samples were constant for 14 days and then decreased slightly. Differences between I+C and I+S samples were not observed ($P > 0.05$). The number of *S. enterica* in the C+I and S+I samples was reduced significantly for 3 days from the initially reduced numbers ($P < 0.05$). The number of cells in C+I samples decreased further until day 42 and was significantly different from the number of cells in S+I samples on day 42.

The number of total aerobic microorganisms was initially reduced from 5.3 to between 4.4 and 4.6 log CFU/g on day 0 by the coatings (I+C and C+I) and the solution spreading (I+S and S+I) (Fig. 2a and 2b). When the numbers of *S. enterica* that survived initially in the control, coated, and solution-spread samples (5.2, 4.3, and 4.4 log CFU/g, respectively) were considered (Fig. 1a and 1b), the coating and the solution spreading initially reduced the number of total aerobic microorganisms, excluding that of *S. enterica*, by approximately 4.5 and 4.4 log, respectively, from 5.3 log CFU/g. LPOS has been reported to inhibit the growth of total aerobes and other foodborne bacteria in cubed and ground beef *(15, 22)*. After 42 days at 12 to −1°C, the number of total aerobes in LPOS (LPO, 2 to 10 ppm)–dipped cubed beef was 5.9 log CFU/cm$^2$, whereas cubed beef without LPOS dipping reached 8.3 log CFU/cm$^2$ *(15)*. At 4°C, the number of total aerobes in the C+I samples did not change significantly for 21 days ($P > 0.05$), whereas that of total aerobes in the I+C samples increased with storage time (Fig. 2a). Similar results were observed in the solution-spread samples, indicating that treatments made before inoculation were more effective against total aerobic microbes than were those made after inoculation. This phenomenon was also observed in samples stored at 10°C (Fig. 2b). When inoculation followed treatment, the coating was more effective than the solution spreading, which was shown between C+I and S+I samples on day 42 at 4°C ($P < 0.05$) (Fig. 2a). This difference was also observed from day 21 at 10°C (Fig. 2b).

Both LPOS-WPI coating and LPOS solution-spreading treatments (3% LPOS, wt/wt) reduced the initial number of *E. coli* O157:H7 on inoculated turkey samples by more than 1 log CFU/g (Fig. 3a and 3b). No significant differences were observed on day 0 in microbial counts between coated and solution-spread samples (I+C versus I+S and C+I versus S+I) or between samples treated before or after inoculation (I+C versus C+I or I+S versus S+I) ($P > 0.05$). Although nonpathogenic *E. coli* will not grow under normal refrigeration conditions (3 to 7°C), pathogenic *E. coli* is reported to survive well at these temperatures *(1)*. In this study at 4°C, the number of *E. coli* O157:H7 cells in the control samples did not change significantly between days 0 and 7 ($P > 0.05$), whereas high rates of cell reduction (2.3 to 2.7 log CFU/g/7 days) were observed in all treated turkey samples during this period (Fig. 3a). Thus, the inhibition during storage at 4°C, shown in the treated samples, was caused by the antimicrobial activity of LPOS from the coating and the solution-spreading treatments. The numbers of *E. coli* O157:H7 in C+I and I+C samples were further reduced and reached the detection limit (<10 CFU/g) on days 14 and 28, respectively. The cell numbers in I+S and S+I samples also decreased continuously, and the cell numbers in S+I samples fell below the detection limit on day 28. Significant differences between the numbers of *E. coli* O157:H7 in I+C and C+I samples were found from days 3 to 21 and also between the numbers in I+S and S+I samples from day 14 ($P < 0.05$). Significant differences between the numbers of *E. coli* O157:H7 in coated and

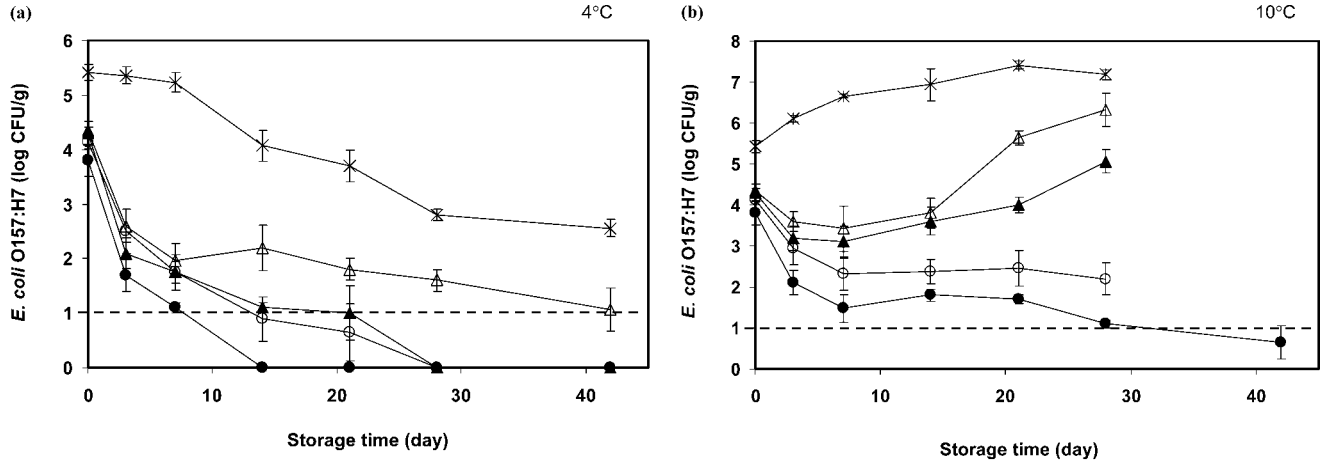


FIGURE 3. *Effects of a whey protein isolate coating incorporating the lactoperoxidase system (LPOS) (C+I and I+C) and LPOS solution spreading (S+I and I+S) on the number of* E. coli *O157:H7 of* E. coli *O157:H7–inoculated roasted turkey during storage at 4°C (a) and 10°C (b) for up to 42 days. *, Inoculated control; △, I+S; ▲, S+I; ○, I+C; ●, C+I. The inoculated control (C) was neither coated nor solution spread. The detection limit (1.0 log CFU/g) is indicated with a dotted line, and zero counts on the plates are plotted as zero on the vertical axis. Data represent means ± standard errors of the means (*n = 4*).*

solution-spread samples were shown ($P < 0.05$). Differences between I+C and I+S samples and between C+I and S+I samples were observed from days 14 to 42 and from days 7 to 21, respectively (Fig. 3a).

At 10°C, the number of *E. coli* O157:H7 cells in controls increased from 5.4 to 7.2 log CFU/g during storage (Fig. 3b). The LPOS solution-spreading treatment (LPO, 35 ppm) reduced *E. coli* O157:H7 cells in the samples by 2.0 to 2.3 from 5.4 log CFU/g. Agreement can be found in previous reports. Antimicrobial properties of LPOS against *E. coli* O157:H7 inoculated on ground beef and cubed beef by LPOS have previously been reported *(15, 22).* An LPOS (LPO, 2 to 10 ppm) dipping decreased the number of *E. coli* O157:H7 in the cubed beef by 1 log from 4.5 log CFU/cm$^2$ after 7 days at 12°C *(15).* In our study, the growth patterns of the cells on coated and solution-spread samples were also different. The number of cells in solution-spread samples (I+S and S+I) decreased for 7 days and then increased significantly, especially in the I+S samples ($P < 0.05$). The I+S samples of day 21 contained as many *E. coli* O157:H7 cells as the control samples measured on day 0. On the other hand, the numbers in I+C and C+I samples decreased from 4.1 to 2.5 log CFU/g and from 3.8 to 1.7 log CFU/g, respectively, by day 21. The numbers were reduced for 7 days in coated samples (I+C and C+I) and did not increase to the day 0 values. The number of cells in C+I samples decreased further, and cells in some C+I samples were not detected on day 42 (Fig. 3b). Significant differences in the numbers of *E. coli* O157:H7 were found between the samples of I+C and C+I from day 3 and also between the samples of I+S and S+I from day 21 ($P < 0.05$). The difference between I+C and I+S samples was observed from day 7, and the difference between C+I and S+I samples was shown from day 3.

The number of total aerobic microorganisms was initially reduced from 5.7 to between 4.3 and 4.6 log CFU/g by coatings (I+C and C+I) or solution spreading (I+S and S+I) (Fig. 4a and 4b). At 4°C, the number of total aerobes in the uninoculated control samples increased significantly ($P < 0.05$) from 3.2 log CFU/g at day 0 and reached the number in inoculated control samples on day 7 (Fig. 4a). Significantly higher numbers were counted in I+S samples than in S+I samples after 14 days ($P < 0.05$). Significantly smaller numbers were counted in C+I samples than in I+C samples after 3 days ($P < 0.05$). Smaller numbers of total aerobes were obtained from coated samples compared to solution-spread samples. A difference between the numbers of total aerobes in I+C and I+S samples was observed from day 14, and a significant difference between C+I and S+I samples was observed from day 7 ($P < 0.05$). The effectiveness was higher when the treatments (both coating and solution spreading) were applied before inoculation. At 10°C, the difference between the numbers of total aerobes in inoculated control and I+C samples or C+I samples decreased as the incubation time increased (Fig. 4b). Significant differences between the effectiveness of the coating and the solution spreading (I+C versus I+S and C+I versus S+I) were observed during storage at 10°C, except on day 28 ($P < 0.05$).

The yeast and mold count (1.0 ± 0.5 log CFU/g) in the turkey samples was not significantly reduced initially by the LPOS-WPI coating or solution spreading ($P > 0.05$) (data not shown). At 4°C, the yeast and mold counts from treated samples by I+C, C+I, and S+I were 6.9, 5.1, and 7.0 log CFU/g and were significantly smaller than those of the *E. coli* O157:H7–inoculated control (7.8 log CFU/g) on day 42 ($P < 0.05$). A 2.5-log difference was observed between the counts of uninoculated and C+I samples (7.6 and 5.1 log CFU/g, respectively) on day 42, indicating the growth of yeasts and molds was noticeably inhibited by the LPOS-WPI coating and then inoculation. At 10°C, the yeast and mold counts in the coated samples (I+C and C+I) were 6.4 to 6.5 log CFU/g at day 28, whereas those in the control and solution-spread samples were 7.1 log CFU/g. The growth of yeasts and molds at 10°C in the coated turkey samples was significantly inhibited for 28 days, com-

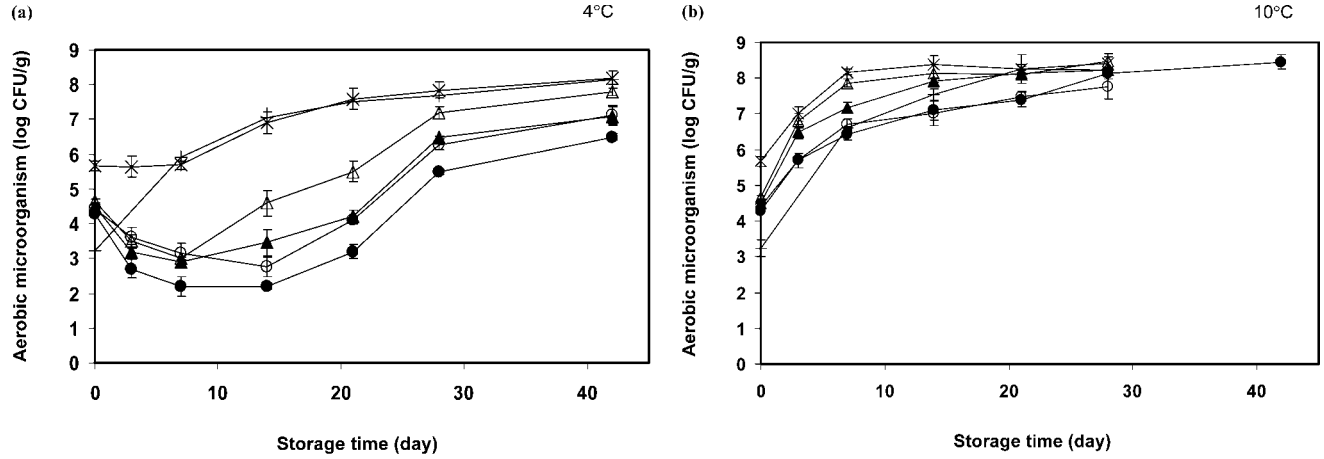


FIGURE 4. *Effects of a whey protein isolate coating incorporating the lactoperoxidase system (LPOS) (C+I and I+C) and LPOS solution spreading (S+I and I+S) on the number of total aerobic microorganisms of* E. coli *O157:H7–inoculated roasted turkey during storage at 4°C (a) and 10°C (b) for up to 42 days.* |, *Uninoculated control; *, inoculated control;* △, *I+S;* ▲, *S+I;* ○, *I+C;* ●, *C+I. Uninoculated and inoculated controls (C) were neither coated nor solution spread. Data represent means ± standard errors of the means (*n = *4).*

pared to that in the control and solution-spread samples ($P < 0.05$). The growth of yeasts and molds was more effectively controlled by coating than by solution spreading at 4 and 10°C.

The initial microbial counts of the turkey sample obtained with BSAN, SMAN, plate count agar, and DRBC were <10 (estimated), <10 (estimated), 3.3, and 1.0 log CFU/g, respectively. The selective media, BSAN and SMAN, can prevent the recovery of injured cells, leading to an underestimation of viable cell numbers of *S. enterica* and *E. coli* O157:H7. However, the numbers recovered from BSAN and SMAN in a preliminary study were not significantly different from those recovered from TSAN ($P > 0.05$) (data not shown).

The LPOS concentrations used in the storage studies were selected to allow growth, rather than to completely inhibit the pathogens during storage, so that growth patterns on the treated turkey slices could be compared. The LPOS used in this research contained glucose and glucose oxidase for the continuous generation of oxidizing products (e.g., hypothiocyanite, hypothiocyanous acid) *(13, 14).* By continuously generating these oxidizing products, the LPOS would have strong and prolonged antimicrobial effects *(21).* The concentrations of LPO in the coatings used for the storage studies of *S. enterica* and *E. coli* O157:H7 were 58 and 35 ppm, respectively, based on the weight of the turkey sample (10 g). These concentrations are comparable to the LPO levels naturally present in bovine milk (30 ppm) *(3)* and those suggested for food applications (100 ppm) *(4).* If the coating were applied to food with a larger volume/surface area ratio than our turkey sample, the concentration would be reduced significantly *(22).*

Significant differences were observed between the numbers of both *S. enterica* and *E. coli* O157:H7 in I+C and C+I samples during storage at both 4 and 10°C (Figs. 1 and 3) ($P < 0.05$). The effectiveness of antimicrobial treatments depends on many factors associated with the food product, storage environment, handling, and target microorganisms *(10, 27).* Those factors include nutrient availability and food morphology, specifically nutrients required for the recovery of the cells from the antimicrobial treatment, and the surface morphology where the microorganisms contaminates the food. Cells injured by the LPOS treatments might recover better if they were on the turkey surface rather than on the WPI coatings, because of possibly higher availability of nutrients on turkey than on WPI coatings. It can also be hypothesized that the cells on turkey would survive and grow better if they were protected from antimicrobials generated from LPOS (e.g., hypothiocyanite, hypothiocyanous acid) by residing within the rough turkey surface. In contrast, there may be a relatively uniform contact between the coating and the cells when cells are inoculated onto the coating (C+I), resulting in greater antimicrobial effects. Thus, the lower availability of nutrients and greater contact efficiency on the coating surface may explain why the C+I treatment was generally more effective than the I+C treatment.

A difference in antimicrobial effectiveness between I+S and S+I treatments was observed in the *E. coli* O157:H7–inoculated samples, which may also be attributed to the rough morphology of the turkey surface. Cells may have been more protected from the solution treatment when inoculated prior to solution spreading, as described for the coating treatment.

The coating treatments (I+C and C+I) were generally more effective than the solution-spreading treatments (I+S and S+I) for inhibiting both pathogens, with greater effects observed for *E. coli* O157:H7 (Figs. 1 and 3). The number of *E. coli* O157:H7 in the solution-spread samples decreased during the early stage of storage but increased significantly after day 7 at 10°C. This may show the ability of *E. coli* O157:H7 to recover from the LPOS treatments at 10°C. However, the cells in the coated samples were inhibited continuously, even at 10°C. The results may suggest that the antimicrobials from LPOS were continuously avail-

able at the coating interface by their sustained release from the coating interior.

The $a_w$ of turkey from the commercial package as well as the initial control, coated (I+C and C+I), and solution-spread (I+S and S+I) turkey samples was 0.98. Thus, the $a_w$ of turkey samples was not significantly affected by the different treatments ($P > 0.05$). The initial $a_w$ value did not significantly change during storage at either 4 or 10°C in either storage study ($P > 0.05$).

The pH values of turkey from the commercial package and control, coated (I+C and C+I), and solution-spread (I+S and S+I) samples ranged from 6.0 to 6.4. The coating and the solution spreading with each solution (pH 6.7) did not affect the initial pH of turkey samples significantly ($P > 0.05$). The pH values of the uninoculated controls stored at 4°C increased from 6.3 to 7.0 after 42 days, and those of the uninoculated controls at 10°C increased from 6.3 to 7.2 after 28 days. In the *S. enterica* storage study at 4°C, the pH values of the coated samples (I+C and C+I) and of the samples spread with the LPOS solution before inoculation (S+I) were consistent (6.0 ± 0.2) for 42 days ($P > 0.05$), whereas the pH values of the control samples and the samples spread after inoculation (I+S) increased from 6.2 ± 0.1 to 6.7 ± 0.2. Similar results were observed at 10°C for 14 days. However, the pH values of C+I and S+I at 10°C, measured on day 0 or 14 (6.2 ± 0.2), were significantly different from the values on day 42 (7.7 ± 0.3 and 7.4 ± 0.5, respectively). Spoilage of aerobically stored meat products is accompanied by a rise in pH, which is caused by metabolites of microorganisms such as ammonia and amines *(38)*. The results may imply that the growth inhibition against microorganisms in turkey was efficiently achieved by I+C, C+I, and S+I at 4°C but not effectively at 10°C for 42 days. In the *E. coli* O157:H7 storage study at 4°C, the pH values of the samples coated before inoculation (C+I) were consistent (6.2 ± 0.2) for 42 days ($P > 0.05$), whereas the pH values of the other treated samples (I+C, I+S, and S+I) increased from 6.2 ± 0.2 to 6.9 ± 0.3 ($P < 0.05$). The pH values of the coated samples (I+C and C+I) were consistent (6.4 ± 0.2) for 28 days at 10°C, whereas those of the solution-spread samples increased from 6.3 ± 0.1 to 6.9 ± 0.2 ($P < 0.05$). Yeasts and molds can grow inside RTE meat products under aerobic conditions, causing off-flavor, discoloration, and pH changes *(35)*. The yeast and mold growth in the samples coated before inoculation (C+I) might be effectively inhibited, which is consistent with the results obtained from the yeast and mold counts during storage.

The preservation of meat relies on combinations of temperature control, vacuum or modified atmosphere packaging, and preservation addition (e.g., nitrates or sorbates) *(22)*. The application of an LPOS to meat has been suggested to provide an additional hurdle to preventing the proliferation of pathogenic bacteria, including *S. enterica* and *E. coli* O157:H7 at growth-permissive temperatures *(15)*. A storage temperature of 10°C was chosen in this study to simulate the temperature abuse that may occur during the transport or retail display of refrigerated products. The antimicrobial activity of LPOS-WPI coatings against the growth of the pathogens *S. enterica* and *E. coli* O157:H7 at 10°C was demonstrated in this study. LPOS-WPI coatings could be used to protect against the temperature abuse effects that cause the proliferation of *Salmonella* and *E. coli* O157:H7 on foods. The turkey products purchased for this research were vacuum packed; the turkey product containing sodium diacetate had a recommended shelf life of 4 weeks at refrigeration temperatures, whereas the turkey product without the sodium diacetate antimicrobial agent had a recommended shelf life of 2 weeks. On the basis of our findings, LPOS-WPI coatings show potential for extending microbial stability, even under aerobic conditions, and also for inhibiting postprocessing contamination pathogens on roasted turkey and other RTE products, thus improving the shelf life and microbial safety of these foods.

## ACKNOWLEDGMENTS

This research was supported by the University of California (UC) under UC Discovery grant bio03-10379, which included matching funds from the California Dairy Research Foundation (CDRF). We thank group members of the UC Davis Biopolymer Film and Food Safety Laboratories for their support. We acknowledge and thank Davisco Foods International for supplying us with WPI for the research.

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