Antimicrobial, Mechanical, and Moisture Barrier Properties of Low pH Whey Protein-based Edible Films Containing p-Aminobenzoic or Sorbic Acids

A. CAGRI, Z. USTUNOL, AND E.T. RYSER

ABSTRACT: Low pH (5.2) whey protein isolate–based edible films containing p-aminobenzoic acid (PABA) or sorbic acid (SA) were developed and assessed for inhibition of *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Salmonella* Typhimurium DT104 in a disc diffusion assay. Water vapor permeability (WVP), tensile strength (TS), and percent elongation (%E) were also determined. Using 1.5% PABA and SA, average inhibition zone diameters were 21.8, 14.6, 13.9, and 26.7, 10.5, 9.7 mm for *L. monocytogenes*, *E. coli* O157:H7, and *S.* Typhimurium DT104, respectively. Three strains of *S.* Typhimurium DT104 were resistant to 0.5% SA. Addition of PABA and SA increased %E, but decreased TS. WVP was not affected by 0.5% and 0.75% SA; however, PABA increased WVP.

Key Words: edible film, antimicrobials, *Listeria, E. coli* O157:H7, *S. Typhimurium* DT104

Introduction

MICROBIAL STABILITY OF A FOOD SURFACE IS A MAJOR DETERMInant of product quality and safety during storage and distribution since most Class I product recalls in the U.S. result from post-processing contamination during handling and packaging. In December 1998, new food safety concerns were raised when consumption of hot dogs was traced to over 100 cases of listeriosis, including 21 fatalities, in 22 states (CDC1999). A nationwide recall was subsequently issued for 35 million pounds of contaminated product. *Listeria monocytogenes* continues to threaten the processed meat industry. Sixty-three of 97 microbiologically related Class I recalls issued from January 1999 to October 2000 involved a total of more than 3.5 million pounds of cooked/ready-to-eat meats contaminated with *L. monocytogenes* (USDA-FSIS 2000). Two additional foodborne pathogens, namely *Escherichia coli* O157:H7 and *Salmonella* Typhimurium DT104 are also raising considerable public health concerns. *E. coli* O157:H7 has been responsible for many widely publicized outbreaks involving ground beef (Bell and others 1994), fermented meat products (Tilden and others 1996), and fresh produce (Besser and others 1993). In addition, over 30 million pounds of raw ground beef have been recalled since 1995 due to *E. coli* O157:H7 contamination (USDA-FSIS 2000). *S.* Typhimurium DT104, a multiantibiotic resistant strain, is also emerging as a serious foodborne pathogen of public health concern with 103 of 306 (34%) *S.* Typhimurium isolates serotyped at the Centers for Disease Control and Prevention resistant to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracyclines (Glynn and others 1998).

Incorporating antimicrobial compounds into edible films or coatings provides a novel means for enhancing the safety and shelf life of ready-to-eat foods. Dawson and others (1997) and Padget and others (1998) used nisin and pediocin in soy protein and corn zein films to inhibit *Lactobacillus plantarum* and *E. coli* on laboratory media. Antimicrobial edible films are receiving attention as a potential pathogen intervention strategy for various muscle foods. Sirugusa and Dickson (1993) demonstrated that calcium alginate coatings containing organic acids were marginally effective on beef carcasses, reducing levels of *L. monocytogenes*, *S.* Typhimurium, and *E. coli* O157:H7 by 1.80, 2.11, and 0.74 logs, respectively. According to Ming and others (1997), pediocin-coated cellulosic casings inhibited *L. monocytogenes* on ham, turkey breast meat, and beef. In addition, McDade and others (1999) reported that dipping frankfurters in an aqueous whey protein solution (pH 5.2) containing propionic/sorbic acid prevented growth of *L. monocytogenes* on the product during the 1st 2 to 3 wk of storage at 4 °C.

Sorbic acid, p-aminobenzoic acid, lactic acid, and acetic acid have a long history as generally recognized as safe (GRAS) food preservatives. The World Health Organization has set the acceptable daily intake for sorbic and p-aminobenzoic acid at 25 and 30 ppm, respectively (Kabara and others 1991). When used in combination with lactic and/or acetic acid, sorbic acid can inhibit the growth of *L. monocytogenes*, *S.* Typhimurium, and *E. coli* O157:H7 in many low acid foods including cold-pack cheese (Ryser and Marth 1988), bologna (Wederquist and others 1994), beaker sausage (Hu and Shelef 1996), and apple cider (Zhao and others 1993; Uljas and Ingham 1999). p-Aminobenzoic acid reportedly exhibited greater inhibitory activity against *L. monocytogenes*, *E. coli,* and *Salmonella enteritidis* than formic, propionic, acetic, lactic or citric acids (Richards and others 1995).

Use of whey protein antimicrobial-containing films as a casing for frankfurters appears to be a promising means of retarding surface microbial growth, thereby enhancing product safety and extending shelf life. However, if used as a sausage casing, the mechanical properties of such an edible film (that is tensile strength, percentage elongation, and water vapor permeability) are of equal importance if the film is to function properly and provide adequate physical protection for the product during production and storage. Consequently, our objectives were (1) to develop an edible film (pH 5.2) from whey protein isolate (WPI) containing p-aminobenzoic acid (PABA) or sorbic acid (SA) that is inhibitory to *L. monocytogenes, E. coli* O157:H7, and *S.* Typhimurium DT104 and (2) to assess the film for water vapor permeability (WVP), tensile strength (TS), and percentage elongation (%E) at break.

Materials and Methods

Film preparation

Whey protein isolate (WPI, Alacen 895) (New Zealand Milk Products, North America, Inc., Santa Rosa, Calif., U.S.A.) (5% w/v) and glycerol (Sigma Chemical Co., St. Louis, Mo., U.S.A.) (2% w/v) were dissolved in distilled water containing 0.04% $CaCl_2$ (w/v) (Sigma). After mixing and adjusting the pH to 8.0 with 1.0 N NaOH, the solution was heated at 90 °C for 30 min in a shaking water bath (170 Marcel Drive water bath, Precision Scientific, Winchester, Va., U.S.A.). Following the addition of candelilla wax (Stahl Pash Inc., New York, N.Y., U.S.A.) (0.4%, w/v) during the last 5 min of heating, the solution was homogenized for 2 min in a SD-45 homogenizer (Tekmar Co., Cincinnati, Ohio, U.S.A.), filtered through cheesecloth and cooled to 23 ± 2 °C. After incorporating 0.5%, 0.75%, 1.0%, or 1.5% (w/v) sorbic acid (SA) or p-aminobenzoic acid (PABA), the pH was adjusted to 5.2 using 3 different solutions of lactic acid and acetic acid at ratios of 1:0, 1:1, and 7:3 lactic acid (1.0 N): acetic acid (1.0 N). Following degassing by vacuum, the whey protein solution (40 ml/plate) was cast by pipetting the solution into sterile 17-cm-dia Teflon plates. The solutions were dried for approximately 24 h at 23 ± 2 °C/50% ± 5% relative humidity (RH), after which the films were peeled from the plates and stored at 23 ± 2 °C/50% ± 5% RH until used.

Bacterial strains

Four strains of *Listeria monocytogenes* (CWD 95 and CWD 249 from silage, CWD 201 from raw milk, and CWD 1503 from ground turkey) and 3 strains of *Escherichia coli* O157:H7 (AR, AD 305, AD 317) were obtained from C.W. Donnelly (Dept. of Nutrition and Food Sciences, Univ. of Vermont, Burlington, Vt., U.S.A.). Five strains of *Salmonella* Typhimurium DT104 (G01074, G11601, G10931, G10601, G10127) were obtained from B. Swaminathan (Centers for Disease Control and Prevention, Atlanta, Ga., U.S.A.). All strains were maintained at –70 °C in trypticase soy broth containing 10% (v/v) glycerol and subcultured twice in trypticase soy broth containing 0.6% (w/v) yeast extract (Difco Laboratories, Detroit, Mich., U.S.A.) at 35 °C/18 to 24 h before use.

Diffusion-type assay

WPI films were aseptically cut into 16-mm-dia discs using a sterile cork borer. The discs were then aseptically transferred to pour plates containing exactly 15 ml of either trypticase soy agar + 0.6% yeast extract (TSAYE) (pH approximately 6.5) or TSAYE acidified to pH 5.2 with 1.0 N lactic acid (Difco), which had been previously seeded with 0.1 ml of an 18- to 24-h culture of the test organism. After 24 h of incubation at 35 °C, the dia of the inhibition zone around the edible film disc was measured perpendicularly to the nearest millimeter. The end result was the average of 2 measurements.

Film thickness

A model M micrometer (Testing Machine Inc., Amityville, N.Y., U.S.A.) was used to determine film thickness. Measurements were taken at 5 different locations, and the mean value was used in further calculations for moisture barrier and mechanical properties.

Mechanical properties

Films were cut into strips measuring 101.6 mm by 25.4 mm using a Precision Sample Cutter (Thawing Albert Instrument Co., Philadelphia, Pa., U.S.A.). All films were conditioned for 48 h at 23 ± 2 °C/50% ± 5% RH before testing. Tensile strength (TS) and percent elongation at break (%E) were determined according to standard D-882-91 (ASTM 1992). The test was run using the Instron Universal Testing Machine Model 2401 (Canton, Mass., USA) at 23 ± 2 °C/50% ± 5% RH with a static load cell of 1 kN and a cross head speed of 50.8 cm/min. TS was calculated in MPa from the following equation:

TS = load/sample × sample thickness

% Elongation at break was determined by the following equation:

% E = (distance sample stretched/original length of sample) × 100

Water vapor permeability

Standard Method E96 to 80 (ASTM 1992) was used; the film was sealed on top of an aluminum test cup containing desiccant (calcium sulfate) and then placed in a chamber at 37 °C/85% RH. The area of the cup mouth was 54 cm^2 , and the cup well depth was 1.1 cm. Cups were weighed at 2-h intervals during ~12 h of controlled storage. Square edible film samples having a surface area of 9 cm^2 were placed in the chamber to examine moisture absorption. WVP was calculated from the water vapor transmission rate through film, the partial vapor pressure difference between the 2 sides of the film, and the thickness according to McHugh and others (1993).

Statistical analysis

All experiments were replicated 3 times using a complete randomized design. Two-way analysis of variance (ANOVA) was performed using the SAS Statistical Analysis System (SAS Institute Inc. 1990). Means were compared using the Duncan Grouping test at $p = 0.05$.

Results and Discussion

Antimicrobial properties

Increasing the concentration of PABA and SA in the film discs increased the diameter of inhibition zones for *L. monocytogenes* (4 strains), *E. coli* O157:H7 (3 strains), and *S.* Typhimurium (5 strains) on TSAYE (pH 5.2) ($p < 0.05$). SA and PABA are weak acids and are most effective in the undissociated form (Luck 1980) due to their increased ability to penetrate the cytoplasmic membrane of bacteria (Chichester and Tanner 1972). At pH 5.2 and 6.5, 28.48% and 1.25% and 26.18% and 1.11% of SA (pKa = 4.75) and PABA (pKa = 4.8) is undissociated. Hence, no inhibition was observed in TSAYE adjusted to pH 6.5 (results not shown). Control films (pH 5.2) without antimicrobials were non-inhibitory. Therefore, the antimicrobial-containing films developed in our study would be best suited for foods that have pH values near 5.2, such as meats and cheeses.

All *L. monocytogenes* strains were inhibited using WPI film discs (pH 5.2) containing SA or PABA at levels of 0.5%, 0.75%, 1.0%, or 1.5% with inhibition zones ranging from 12.0 to 32.0 and 4.0 to 27.0 mm, respectively (Table 1). El-Shenawy and Marth (1988) also showed that *L. monocytogenes* was inhibited when 0.2% to 0.3% potassium sorbate was added to trypticase soy broth at pH 5.0. Films containing SA were generally more inhibitory to *L. monocytogenes* than films containing PABA. McDade and others (1999) reported that growth of *L. monocytogenes* was inhibited on frankfurters during 2 to 3 wk of storage at 4 °C by coating the frankfurters with a whey protein film-forming solution that contained propionic/sor-

Table 1—Antimicrobial activities of whey protein-based edible films containing sorbic acid (SA) or p-aminobenzoic acid (PABA) against 4 strains of *L. monocytogenes*

Strains		Dia of Inhibition Zone (mm)							
Antimic.		CWD 95		CWD 249		CWD 201		CWD 1503	
(%)(w/v)	LA:AA*	SA	PABA	SA	PABA	SA	PABA	SA	PABA
0	1:0	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	7:3	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	1:1	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
0.50	1:0	19.0 ± 2.3^{b}	13.3 ± 2.6^{bcd}	19.3 ± 5.7^{bc}	8.7 ± 2.3^{bc}	12.3 ± 2.1^{bc}	15.0 ± 1.7^{cd}	13.7 ± 3.8^{a}	11.7 ± 2.6^{cd}
	7:3	18.0 ± 1.3^{b}	9.3 ± 1.5^{bc}	12.3 ± 3.1^{ab}	8.0 ± 2.2^{bc}	21.3 ± 3.7^{cd}	8.3 ± 3.7^{def}	12.7 ± 3.2^{a}	17.0 ± 3.2^{def}
	1:1	17.0 ± 1.3^{b}	8.0 ± 0.8^{ab}	17.0 ± 1.5^{bc}	4.3 ± 1.2^{bc}	15.3 ± 0.1^{bcd}	5.3 ± 0.1^{ab}	18.0 ± 1.2^{ab}	4.0 ± 0.2^{ab}
0.75	1:0	21.0 ± 1.9^{bc}	18.0 ± 0.6^{def}	20.0 ± 0.2^{bc}	15.0 ± 1.6^{ab}	12.0 ± 0.7^{b}	20.0 ± 0.5^{cde}	23.0 ± 0.5^{abc}	13.0 ± 1.2^{cde}
	7:3	24.7 ± 4.2^{bc}	13.0 ± 0.4^{bcd}	16.7 ± 3.4^{bc}	12.0 ± 0.9^{cd}	21.7 ± 1.4^{cd}	17.0 ± 1.8^{cde}	25.7 ± 2.5^{bc}	14.0 ± 1.5^{cde}
	1:1	27.3 ± 0.6^{bc}	14.0 ± 3.2^{cde}	21.7 ± 3.0^{bc}	8.7 ± 0.4^{bcd}	20.3 ± 1.1^{cd}	12.3 ± 1.1^{bc}	28.3 ± 2.5^{c}	9.0 ± 3.5^{bc}
1.00	1:0	27.0 ± 3.3^{bc}	21.0 ± 0.4^{ef}	23.3 ± 1.9^{bc}	17.0 ± 5.3^{bc}	26.3 ± 1.2^{e}	22.3 ± 0.5^{def}	27.7 ± 3.3^{bc}	19.3 ± 2.3^{def}
	7:3	29.7 ± 2.5^{bc}	18.3 ± 2.9^{def}	25.7 ± 1.3^{bc}	19.3 ± 4.9^{de}	24.0 ± 0.8^{de}	16.0 ± 0.6^{ef}	28.3 ± 1.5^{bc}	20.3 ± 3.5^{ef}
	1:1	30.0 ± 2.8^{bc}	18.7 ± 2.3^{def}	25.7 ± 3.2^{bc}	13.0 ± 5.7^{de}	23.3 ± 1.2^{de}	18.3 ± 2.1^{def}	30.0 ± 3.8^{c}	17.3 ± 3.8^{def}
1.50	1:0	31.3 ± 2.5^{c}	24.7 ± 4.6^{f}	30.3 ± 0.4^{c}	19.7 ± 3.3^{cd}	22.3 ± 3.8^{de}	25.0 ± 3.8^{f}	30.7 ± 3.1^{c}	22.3 ± 4.1^{f}
	7:3	27.3 ± 3.1^{bc}	22.8 ± 0.1^{ef}	13.7 ± 0.9^{ab}	22.8 ± 0.9^{de}	20.7 ± 1.1^{cd}	22.7 ± 0.1^{ef}	30.0 ± 2.0^{c}	16.7 ± 3.2^{def}
	1:1	25.3 ± 4.0^{b}	20.3 ± 2.3^{def}	30.0 ± 4.8^{c}	18.1 ± 1.1^{e}	27.7 ± 1.8^{e}	27.0 ± 0.7^{ef}	32.0 ± 3.8^{c}	20.3 ± 5.8^{ef}

Mean ± standard deviation (n = 3). Means in same column with different superscript are significantly different ($p < 0.05$).
*Ratio of lactic acid to acetic acid.

Table 2—Antimicrobial activities of whey protein-based edible films containing sorbic acid (SA) or p-aminobenzoic acid (PABA) against 3 strains of *Escherichia coli* O157:H7

Strains		`Dia of Inhibition zone (mm)					
		AR (Acid Resistant)		AD 305		AD 317	
Antimic (%)(w/v)	LA: AA*	SA	PABA	SA	PABA	SA	PABA
0	1:0	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	7:3	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	1:1	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
0.50	1:0	1.7 ± 0.2^{ab}	5.7 ± 1.2^{cb}	0.7 ± 0.3^{a}	7.7 ± 0.5^{b}	0.7 ± 0.2^{a}	6.7 ± 1.2^{ab}
	7:3	1.7 ± 0.5^{ab}	7.0 ± 0.8^{bcd}	2.3 ± 0.3^{a}	9.7 ± 0.5^{bc}	1.7 ± 0.4^{a}	8.7 ± 1.4^{b}
	1:1	2.3 ± 0.3^{abc}	5.3 ± 1.2^{b}	4.0 ± 0.4^{abc}	10.0 ± 1.6^{bc}	4.0 ± 0.2^{a}	6.7 ± 1.3^{ab}
0.75	1:0	2.0 ± 0.2^{abc}	7.3 ± 2.2^{bcd}	2.7 ± 0.2^{ab}	9.2 ± 1.3^{b}	3.0 ± 1.3^{a}	8.0 ± 0.7^{ab}
	7:3	4.3 ± 1.3^{abc}	7.7 ± 1.1^{bcd}	4.3 ± 0.5^{abc}	12.8 ± 1.2^{bc}	2.0 ± 0.2^{a}	9.8 ± 0.5^{b}
	1:1	2.3 ± 0.3^{abc}	7.5 ± 0.2^{bcd}	1.7 ± 0.4^{a}	11.8 ± 0.9^{bc}	2.7 ± 0.3^{a}	7.8 ± 2.3^{ab}
1.00	1:0	5.0 ± 1.2^{bcd}	10.8 ± 0.2^{ef}	5.0 ± 1.1^{abc}	12.5 ± 0.5^{bc}	3.0 ± 0.2^{a}	12.0 ± 1.9^{b}
	7:3	5.3 ± 1.2^{bcd}	10.3 ± 2.3^{def}	4.5 ± 0.4^{abc}	12.3 ± 3.0^{bc}	5.3 ± 0.1^{ab}	10.0 ± 0.5^{b}
	1:1	8.3 ± 0.5^{cde}	9.0 ± 0.6^{def}	7.5 ± 1.2^{bc}	12.5 ± 1.2^{bc}	3.0 ± 0.7^{a}	9.8 ± 0.8^{b}
1.50	1:0	9.0 ± 3.2^{cde}	13.2 ± 2.2^{f}	8.3 ± 2.1^{cd}	15.8 ± 0.2^{cd}	9.5 ± 0.9^{bc}	13.7 ± 1.2^{b}
	7:3	11.3 ± 1.5^{de}	13.0 ± 1.0^{f}	10.0 ± 0.7^{d}	15.8 ± 1.2^{cd}	10.3 ± 1.5^{bc}	10.3 ± 0.7^{b}
1:1		13.3 ± 0.9^{e}	13.2 ± 0.4^{f}	12.3 ± 0.3^{d}	21.3 ± 1.8^{cd}	11.0 ± 1.0^{c}	14.8 ± 2.6^{b}

Mean ± standard deviation (n = 3). Means in same column with different superscript are significantly different ($p < 0.05$).
*Ratio of lactic acid to acetic acid.

bic acid (pH 5.2). However, non-uniformity of the antimicrobial coating on frankfurters after dipping, draining, and drying would likely produce a less effective antimicrobial barrier as compared to pre-casted films. In our study, all antimicrobial edible films were uniform in thickness. Consequently, these films would be better suited to inhibit post-processing surface contaminants such as *L. monocytogenes*.

Film discs containing SA or PABA also were inhibitory to *E. coli* O157:H7 with inhibition zones ranging from 0.7 to 13.3 mm and 5.3 to 21.3 mm, respectively (Table 2). When used at concentrations of 0.5%, 0.75%, or 1.0%, PABA was more effective against *E. coli* than SA. Richards and others (1994) and Tsai and Chou (1996) showed similar inhibition of *E. coli* O157:H7 on laboratory media using PABA and SA, respectively.

Using SA and PABA, inhibition zones for *S.* Typhimurium DT104 ranged from 0 to 12.2 mm and 3.0 to 16.3 mm, respectively (Table 3). Films containing PABA inhibited all strains of *S.* Typhimurium DT104 on TSAYE, whereas film discs containing 0.5% and 0.75% SA and lactic acid:acetic acid (1:0 and 7:3) failed to inhibit 3 strains of *S.* Typhimurium DT104 (G10127, G10931, and G10601). While laboratory media containing 0.2% or 0.5% SA is reportedly bacteriostatic to *Salmonella* at pH 5.5 (Restaino and others 1981; Elliot and Gray 1981), the amount of SA released from our film discs containing 0.5% or 0.75% SA was presumably too low to inhibit these 3 strains.

In accordance with previously published data (Ahamad and Marth 1989; Richards and others 1995), acetic acid is more inhibitory to *L. monocytogenes, E. coli* O157:H7, and *Salmonella* in laboratory media at the same pH value than lactic acid. Three ratios of lactic acid:acetic acid (1:0, 7:3, or 1:1) were used to adjust the pH of our film solutions. We expected that the solution containing more acetic acid (1:1) would be most inhibitory based on the aforementioned studies. However, incorporating 3 different ratios of lactic acid:acetic acid in films containing PABA or SA did not synergistically alter inhibition of the 3 test pathogens on TSAYE at pH 5.2.

Table 3—Antimicrobial activities of whey protein-based edible films containing sorbic acid (SA) or p-aminobenzoic acid (PABA) against 5 strains of *Salmonella* Typhimurium DT104

Strains		Dia of Inhibition Zone (mm)									
Antimic.		G10931		G10127		G01074		G10601		G11601	
(%)(w/v)	LA:AA*	SA	PABA	SA	PABA	SA	PABA	SA	PABA	SA	PABA
0	1:0	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	7:3	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	1:1	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
0.50	1:0	0^{a}	6.8 ± 0.3^{b}	0^{a}	6.7 ± 0.5^{bc}	1.7 ± 0.1^{ab}	6.7 ± 1.52^{bc}	2.0 ± 0.3^{ab}	7.3 ± 1.6^{bc}	3.2 ± 0.7^{abc}	4.7 ± 0.8^{b}
	7:3	0^{a}	9.2 ± 0.2^{bcd}	0^{a}	8.3 ± 0.4^{c}	5.3 ± 0.7^{abc}	7.7 ± 2.5^{bcd}	4.0 ± 0.8^{bcd}	6.8 ± 2.4^{bcd}	0^{a}	6.3 ± 0.1^{bc}
	1:1	3.3 ± 0.1^{a}	7.0 ± 0.6^{bc}	0.7 ± 0.2^{a}	3.0 ± 0.5^{ab}	1.0 ± 0.2^{ab}	4.7 ± 1.5^{b}	4.5 ± 1.5^{bcd}	7.7 ± 1.2^{b}	3.0 ± 0.5^{abc}	6.2 ± 1.6^{bc}
0.75	1:0	4.9 ± 0.4^{ab}	9.7 ± 1.5^{bcd}	0^{a}	8.7 ± 0.2^{c}	1.7 ± 0.5^{ab}	7.7 ± 0.6^{bcd}	2.7 ± 0.9^{abc}	8.5 ± 2.0^{bcd}	1.7 ± 0.3^{ab}	4.7 ± 2.8^{b}
	7:3	6.3 ± 0.5^{bcd}	11.3 ± 1.5^{cde}	2.0 ± 0.2^{a}	7.7 ± 0.4^{c}	7.3 ± 0.9^{abc}	8.3 ± 1.5^{cd}	7.7 ± 2.3^{def}	10.7 ± 0.5^{bcd}	2.0 ± 0.8^{abc}	8.5 ± 0.5^{bc}
	1:1	5.3 ± 1.4^{bcd}	8.5 ± 2.3^{bcd}	2.7 ± 0.5^{ab}	3.0 ± 0.7^{ab}	5.7 ± 1.4^{abc}	7.5 ± 2.9^{bcd}	5.7 ± 0.3^{cde}	9.2 ± 2.0^{cd}	2.0 ± 0.1^{abc}	7.8 ± 1.3^{bc}
1.00	1:0	7.5 ± 1.1^{bcd}	12.0 ± 2.8^{bcd}	5.0 ± 0.2^{abc}	9.8 ± 2.3^{cd}	5.3 ± 0.3^{abc}	9.8 ± 2.3^{cd}	5.3 ± 1.5^{cde}	10.0 ± 2.1^{cd}	6.2 ± 0.4^{bcd}	5.0 ± 2.5^{b}
	7:3	5.3 ± 0.9^{bcd}	13.7 ± 1.2^{de}	4.7 ± 0.3^{abc}	10.2 ± 3.6^{cd}	6.2 ± 1.4^{bcd}	10.2 ± 3.6^{de}	7.2 ± 2.7^{de}	11.2 ± 1.3^{de}	6.0 ± 1.2^{bcd}	10.5 ± 2.6^{c}
	1:1	8.3 ± 1.5^{cd}	11.0 ± 1.5^{bcd}	8.0 ± 1.8^{bcd}	6.0 ± 0.5^{bc}	8.0 ± 2.2^{cd}	9.0 ± 1.0^{cd}	7.5 ± 3.7^{def}	11.7 ± 1.1^{cd}	6.5 ± 2.4^{bcd}	10.2 ± 0.5^{c}
1.50	1:0	9.3 ± 0.6^{d}	16.3 ± 1.0^{e}	9.7 ± 0.6^{cd}	14.0 ± 1.8^{d}	7.3 ± 2.7^{bcd}	15.3 ± 2.5^{f}	8.3 ± 3.1^{def}	14.0 ± 3.0^{f}	9.7 ± 1.7^{cd}	14.0 ± 2.4^{d}
	7:3	8.0 ± 0.3^{cd}	14.5 ± 2.3^{bcd}	9.3 ± 1.2^{cd}	13.7 ± 3.2^{d}	9.7 ± 1.4^{cd}	13.8 ± 5.1^{f}	9.7 ± 1.9^{ef}	13.7 ± 2.1^{f}	8.0 ± 0.8^{bcd}	13.7 ± 1.9^{cd}
	1:1	11.0 ± 1.5^{d}	15.0 ± 1.8^{cde}	11.3 ± 1.8^{d}	10.3 ± 0.6^{cd}	11.6 ± 3.4^{d}	13.0 ± 3.2^{ef}	12.2 ± 4.8^{f}	15.3 ± 0.6^{ef}	11.0 ± 1.8^{de}	14.3 ± 0.1^{d}

Mean ± standard deviations (n = 3). Means in same column with different superscript are significantly different ($p < 0.05$).
*Ratio of lactic acid to acetic acid.

The antimicrobial findings in this study are based on the measurement of clear inhibition zones surrounding film disks where growth of the pathogen was inhibited. Diffusion of antimicrobials from the film disc depends on the size, shape, and polarity of the diffusing molecule, as well as the chemical structure of the film and the degree of molecular crosslinking (Guilbert 1986). According to Michaels and others (1962), the shape of the diffusing molecule (linear, branched, or cyclic) may impact the diffusion rate. When Chen and others (1996) measured diffusion rates for SA and benzoic acid from chitosan film in a water-glycerol solution ($a_w = 0.8$), more SA (57%) was released than benzoic acid (65%). The different interactions of SA and PABA in our WPI-based edible films likely resulted in different diffusion rates leading to varying degrees of inhibition.

Mechanical properties

Average film thickness was 127.11 μm (± 35.39) with no significant differences observed between films (Table 4). When SA and PABA concentrations increased from 0% to 1.5%, % E increased from 6.37% to 74.28% and 42.16%, respectively (Table 4). While TS of WPI films significantly decreased with increasing levels of SA ($p < 0.05$) (Table 4), TS of films containing 1.5% PABA (5.7 MPa) was similar to the control (5.92 MPa). Films containing SA exhibited lower TS and higher %E as compared to films containing PABA. The reason for this phenomenon could be that the straight chain of SA can more easily penetrate into WPI chains than PABA, which has a benzene ring. Consequently, SA may have allowed more mobility between protein chains, thereby producing films of lower TS and greater flexibility. The various organic acid mixtures used to adjust the pH of the film solutions did not significantly alter % E or TS.

Increasing the amount of additives other than crosslinking agents generally produced films with lower TS and greater elongation, since these molecules insert between protein chains to form hydrogen bonds with amide groups of proteins (Guilbert 1986; Kester and Fennema 1986). Reduced interactions between these protein chains lead to increased flexibility and movement. In our study, SA, PABA, acetic acid, and lactic acid might be functioning as plasticizers to increase elongation and decrease TS as was previously suggested for lactic acid (Krull and Inglett 1971).

$CaCl_2$ was incorporated into our film solution as a crosslinking agent to improve the mechanical and water vapor permeability properties of the low pH films as previously suggested by others (Guilbert 1986; Avena-Bustillos and Krochta 1993). As a divalent cation, calcium crosslinks between negatively charged groups on proteins, thereby increasing cohesion between protein chains, reducing protein polymer segmental mobility and improving both the mechanical properties and water vapor permeability (Krochta and others 1990). Jeyarajah and Allen (1994) reported that $CaCl_2$ induced a change in β-lactoglobulin conformation, which facilitated polymerization during heating. Calcium ions also increased the reactivity of SH groups at low pH. Although the SH – S-S interchange reaction is not possible at pH < 6.5, aggregation of most whey proteins can still occur in the presence of calcium (de Wit 1981).

Whey protein films are formed by heat-catalyzed protein-protein interactions that involve disulfide, hydrogen, and hydrophobic bonds. Heating denatures the protein and exposes internal SH and hydrophobic groups (Watanabe and Klostermeyer 1976; Shimada and Cheftel 1998), which promote intermolecular S-S and hydrophobic bonding upon drying (McHugh and Krochta 1994b). Film formation is favored in more alkaline film solutions since SH reactivity increases at pH > 8 (Kella and Kinsella 1988; Banerjee and Chen 1995). In the present study, the film solution was at pH 8.0 during heating at 90 °C, after which the pH was decreased to 5.2 using lactic and acetic acid. A low pH environment would likely prevent S-S bond formation in the protein matrix, thereby weakening the film structure. Thus, tensile strength of the low-pH film (5.92 MPa) was substantially lower than that reported for high-pH film (13.9 MPa) (McHugh and Krochta 1994a). However, tensile strength of our low-pH film was higher than that reported for corn zein (0.4 MPa) (Aydt and others 1991), soy protein (4.5 MPa) (Gennadios and Weller 1991), and wheat gluten based edible films (1.9 to 4.4 MPa) (Gennadios and others 1993) when tested at 23 °C/50% RH.

Water vapor permeability

Films containing 0%, 0.5%, 0.75%, 1.0%, or 1.5% PABA exhibited average WVP values of 27.24, 53.73, 53.90, 55.34, and

Table 4—Thickness, tensile strength (TS), percent elongation (%E), and water vapor permeability (WVP) of whey protein isolate-based films containing sorbic acid (SA) and p-aminobenzoic acid (PABA)

Antimicrobial (%)(w/v)	LA:AA*	Thickness (μm)	%E	TS (Mpa)	WVP (g. mm/m^2.d.kPa)
Control	1:0	128.41 ± 24.40^a	6.37 ± 3.28^a	5.88 ± 1.38^a	27.24 ± 12.01^a
SA (0.50)	1:0	121.07 ± 36.62^a	20.00 ± 14.64^b	4.85 ± 2.70^b	27.25 ± 9.81^a
	7:3	126.36 ± 24.46^a	23.48 ± 1.07^b	4.45 ± 0.15^b	21.32 ± 16.57^a
	1:1	127.63 ± 40.23^a	31.63 ± 1.85^c	4.59 ± 0.74^b	31.52 ± 2.07^a
SA (0.75)	1:0	137.26 ± 30.33^a	26.58 ± 3.24^c	4.87 ± 0.54^b	28.62 ± 6.15^a
	7:3	134.56 ± 23.12^a	27.11 ± 0.87^c	4.83 ± 1.37^b	27.53 ± 1.32^a
	1:1	112.85 ± 48.23^a	24.57 ± 8.73^c	4.36 ± 1.38^b	32.96 ± 12.44^a
SA (1.00)	1:0	130.54 ± 40.40^a	67.78 ± 6.40^e	3.75 ± 0.18^c	43.51 ± 5.23^b
	7:3	132.85 ± 22.34^a	73.54 ± 1.35^e	3.83 ± 0.12^c	41.84 ± 16.32^b
	1:1	120.36 ± 19.45^a	70.67 ± 1.31^e	3.85 ± 0.34^c	41.53 ± 7.99^b
SA (1.50)	1:0	118.90 ± 43.12^a	73.01 ± 2.07^e	3.05 ± 0.45^d	43.76 ± 6.71^b
	7:3	123.74 ± 23.45^a	74.28 ± 4.53^e	2.60 ± 1.04^d	45.55 ± 1.86^b
	1:1	133.44 ± 27.33^a	73.33 ± 5.32^e	2.73 ± 0.94^d	44.06 ± 8.76^b
PABA (0.50)	1:0	123.71 ± 22.32^a	18.32 ± 5.58^b	5.38 ± 0.97^a	51.76 ± 2.16^b
	7:3	145.42 ± 45.67^a	19.87 ± 6.46^b	5.41 ± 0.70^a	55.16 ± 3.11^b
	1:1	120.45 ± 27.33^a	16.15 ± 3.85^b	4.42 ± 1.14^a	54.29 ± 1.93^b
PABA (0.75)	1:0	134.09 ± 23.45^a	20.85 ± 8.47^b	5.23 ± 2.84^a	56.43 ± 9.54^b
	7:3	119.07 ± 45.12^a	30.82 ± 3.63^c	5.15 ± 3.37^a	50.19 ± 6.57^b
	1:1	136.26 ± 56.23^a	28.50 ± 15.16^c	5.15 ± 2.44^a	55.08 ± 1.60^b
PABA (1.00)	1:0	133.17 ± 43.12^a	34.73 ± 6.27^c	4.36 ± 0.18^a	59.79 ± 3.85^b
	7:3	111.78 ± 29.30^a	33.25 ± 2.47^c	5.28 ± 2.22^a	47.08 ± 8.05^b
	1:1	120.67 ± 39.34^a	30.08 ± 8.27^c	5.81 ± 1.77^a	59.17 ± 11.14^b
PABA (1.50)	1:0	123.37 ± 57.65^a	34.98 ±10.56^d	5.31 ± 0.14^a	56.11 ± 2.01^b
	7:3	131.74 ± 34.90^a	38.67 ± 5.11^d	5.80 ± 1.39^a	56.95 ± 5.84^b
	1:1	130.04 ± 43.13^a	42.16 ± 10.09^d	6.00 ± 1.63^a	48.96 ± 2.92^b

* Ratio of lactic acid to acetic acid.

54.00 g.mm/m^2.d.kPa, respectively (Table 4). Increasing the concentration of PABA from 0.5% to 1.5% did not significantly alter WVP ($p > 0.05$). Average WVP values for films containing 0.5% and 0.75% SA were 26.69 and 29.70 g.mm/m^2.d.kPa, respectively, and were not significantly different from the control (27.24 g.mm/m^2.d.kPa) ($p > 0.05$). However, addition of 1.0% and 1.5% SA significantly increased WVP to 42.29 and 44.46 g.mm/m^2.d.kPa, respectively ($p < 0.05$).

WVP is a measure of the ease with which a material can be penetrated by water vapor. WPI edible films tend to be poor moisture barriers due to abundant hydrophilic groups in proteins. Their moisture barrier properties can be improved by adding nonpolar compounds such as lipids (McHugh and Krochta 1994b). We incorporated candelilla wax into the film solution to reduce WVP. In preliminary experiments, diffusion of SA and PABA as demonstrated by inhibition zones was similar for films prepared with and without candelilla wax (results not shown). Adding SA and PABA to the film solution increased WVP because both antimicrobials are hydrophilic compounds. Addition of polar additives may increase the hydrophilic character and the solubility coefficient of the film (McHugh and others 1994). Moreover, additives such as SA or PABA weaken chain packing in the film to produce a looser structure, which increases water mobility.

Conclusion

INCORPORATING 0.5% TO 1.5% OF SA OR PABA INTO WPI films (pH 5.2) led to inhibition of *L. monocytogenes, E. coli* O157:H7, and *S.* Typhimurium DT104 on TSAYE at pH 5.2. Addition of PABA and SA increased %E and WVP, but decreased TS. Given our current work involving ready-to-eat meat products, which will be reported elsewhere, these films may prove useful for inactivating post-processing contaminants on ready-to-eat foods such as processed meats.

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Authors are with the Dept. of Food Science and Human Nutrition, Michigan State Univ., East Lansing, MI 48824-1224, U.S.A.. Direct inquiries to author Ryser (E-mail: ryser@msu.edu).