

INTRODUCTION

LISTERIA MONOCYTOGENES IS A FOOD-borne pathogen of great concern to the food industry because its ubiquitous occurrence in the environment can lead to its presence in foods (Armstrong, 1985; Brackett, 1988). Published studies have examined the incidence and fate of *L. monocytogenes* in fresh vegetables (Beuchat, 1996; Petran et al., 1988). In two surveys of fresh market produce, *L. monocytogenes* was detected on 27.1% (Heisick et al., 1989a) and 25.8% (Heisick et al., 1989b) of potato samples tested. The organism has been isolated from the feces of animals (Weis and Seeliger, 1975) which may be a potential source of fresh produce contamination (Schlech et al., 1983).

Methods of controlling enzymatic browning in minimally processed produce involve chemical treatment with browning inhibitors. The most widely used browning inhibitor for fresh, pre-peeled potatoes is sulfite. Over 1 million people in the U.S. are sensitive to sulfites (FDA, 1987), but the use of sulfites on pre-peeled potatoes is still authorized. However, the possibility of future regulatory action against the use of sulfites and a decrease in consumer acceptance of sulfite-treated products (McEvily et al., 1992) have increased the need for alternative browning inhibitors. Several alternatives have been developed including combinations of organic acids, phosphates, preservatives, and other adjuncts (Duxbury, 1987; Langdon, 1987; Santerre et al., 1991). To extend the shelf-

life of pre-peeled potatoes, reduction of oxygen levels at the surface by packaging under vacuum or modified atmosphere, and storage at refrigeration temperatures, are necessary (Langdon, 1987; O'Beirne and Ballantyne, 1987; O'Beirne, 1988). As a facultative anaerobe, *L. monocytogenes* can thrive in an environment with little oxygen, such as that of a vacuum package.

While antimicrobial activity of sulfites in many foods is well documented (Wedzicha, 1981), the activity of sulfite alternatives is not known. Accordingly, our study was undertaken to examine the fate of *L. monocytogenes* on the surface of fresh peeled potatoes, treated with sulfite or a commercial sulfite alternative, packaged under vacuum and stored at 4°, 15° and 28°C. Our objective was to correlate the pathogen levels with background microflora (aerobic and anaerobic), and to determine whether treatment of fresh, vacuum-packaged pre-peeled potatoes with sulfiting agents or a commercial browning inhibitor would inhibit growth of *L. monocytogenes*.

MATERIALS & METHODS

Test organism

Listeria monocytogenes strains Scott A (serotype 4b; clinical isolate from Food and Drug Administration, Cincinnati, OH) and Murray B (serotype 4a; clinical isolate from Centers for Disease Control, Atlanta, GA) from our in-house culture collection, maintained in 15% glycerol at -80°C, were used. Cells were grown on tryptic soy agar (Difco Laboratories, Detroit, MI) slants at 35°C for 24h and stored at 4°C. Cultures were transferred periodically to maintain viability and used as stock cultures. Brain Heart Infusion broth (BHI; Difco) was used to grow cultures

for inoculum preparation prior to all experiments.

Preparation of inoculum

Inoculated BHI was incubated at 37°C for 18h. The cells were harvested by centrifugation at room temperature for 10 min at 7,700 × g, the cell pellet washed twice, resuspended and diluted in sterile 0.1% peptone water (w/v) (Difco).

Sample preparation and inoculation

Fresh, raw Russet potatoes (80-110g each) were obtained from a commercial potato distributor. Potatoes were abrasion-peeled for 2 min with a Vegetable Peeler (Model A1-15, Toledo Scale Co., Toledo, OH) and held under water up to 30 min prior to treatment. Potatoes received one of 4 different treatments: (1) immersion in 1.2% (w/v) sodium bisulfite for 2 min, (2) immersion in a 2% (w/v) solution of a commercial browning inhibitor (CBI) consisting of citric acid, ascorbic acid, sodium acid pyrophosphate and L-cysteine HCl for 5 min, (3) immersion in a 2% solution of the CBI for 5 min, followed by a brief rinse with tap water (30 mL rinse/kg potatoes), or (4) untreated control. Residual sulfite levels were determined in uninoculated control samples by the Optimized Monier-Williams Method (Hillary et al., 1989).

Duplicate sets of two potatoes (100g each) were weighed into filter stomacher bags (SFB-0410; Spiral Biotech., Bethesda, MD) and inoculated with 1 mL of an appropriate dilution of *L. monocytogenes* cell suspension to yield a final concentration of 1-2 log₁₀ CFU/g. Thereafter, the bags were manually massaged to ensure even distribution of organisms on the surface of the potatoes. The bags were placed in 17.8 × 20.3 cm barrier bags (Koch Model 01 46 09, Kansas City, MO). The oxygen transmission rate (by manufacturer) of the nylon/polyethylene film was 54.2 cc/m² in 24h measured at 24°C and 75% relative humidity. The bags were evacuated to a negative pressure of 1000 millibars and heat sealed using a Multivac Model A300/16 gas packaging machine (Germany). Two replications were performed for each treatment. For each replicate experiment, two bags of each treatment were prepared for each sampling time and temperature. Controls consisted of inoculated, untreated potatoes stored under vacuum.

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Storage and sampling

The inoculated vacuum-packaged potato samples were stored at 4°, 15° and 28°C. Samples stored at 4°C were analyzed on days 0, 8, 14, and 21. Samples stored at 15°C were analyzed on days 0, 3, 6, 9, and 12; and those stored at 28°C were analyzed at 0, 4, 8, 12, 18, 24, 48, and 72h. Samples were observed for visual evidence of spoilage or presence of off-odor.

Bacterial enumeration

At the scheduled sampling time, sterile 0.1% peptone water (w/v) was added to each bag to give a 1:1 (w/v) dilution and the contents were homogenized for 1 min with a stomacher Lab-blender (Model 400, Spiral Systems, Inc). Further serial dilutions of each sample were made using the same diluent as required. This was followed by spiral plating (Spiral Systems Model D plating instruments; Cincinnati, OH) of each dilution in duplicate onto modified Modified Vogel Johnson agar (MMVJ), Tryptic Soy agar (TSA; Difco), or Crystal Violet Tetrazolium agar (CVT; Difco) dishes. Also, 0.1 or 0.5 mL of undiluted suspension was surface plated, where relevant. The MMVJ agar plates were incubated for 48h at 37°C for *L. monocytogenes* enumeration, and CVT agar plates were incubated at 28°C for 24h for psychrotrophic Gram-negative bacterial counts. The "total" aerobic and anaerobic colony forming units (cfu) were determined after aerobic and anaerobic incubation of TSA plates at 37°C for 1 and 2 days, respectively.

RESULTS & DISCUSSION

AT 4°C, *L. MONOCYTOGENES* GREW RAPIDLY in controls to almost 4 log₁₀ CFU/g as early as Day 8, but the organism did not grow at that temperature in all samples treated with sulfite (residual sulfite levels 237 ppm ± 55 ppm) or the Commercial Browning Inhibitor (CBI), with or without a post-treatment rinse, in 21 days (Fig. 1). The population

Table 1—Growth of background bacterial populations* as affected by treatments in vacuum-packaged pre-peeled potatoes stored at 4°C

Treatment		Day			
		0	8	14	21
Aerobic Plate Counts	Sulfite	2.82 ± 0.15	6.36 ± 0.31	6.31 ± 0.36	6.27 ± 0.28
	CBI	2.82 ± 0.15	6.29 ± 0.26	6.92 ± 0.27	7.74 ± 0.14
	CBI-RINSE	2.82 ± 0.15	6.27 ± 0.37 ^a	6.22 ± 0.14 ^b	6.13 ± 0.25 ^b
	Control	2.82 ± 0.15	6.46 ± 0.21	7.65 ± 0.37 ^b	9.60 ± 0.28 ^b
Anaerobic Plate Counts	Sulfite	2.69 ± 0.22	6.74 ± 0.15	6.47 ± 0.28	5.53 ± 0.28
	CBI	2.69 ± 0.22	6.29 ± 0.40	5.67 ± 0.16	7.54 ± 0.17
	CBI-RINSE	2.69 ± 0.22	7.83 ± 0.26	6.40 ± 0.36	5.99 ± 0.33
	Control	2.69 ± 0.22	7.46 ± 0.36	8.28 ± 0.28	8.91 ± 0.32
CVT Plate Counts	Sulfite	2.47 ± 0.19	7.29 ± 0.23	8.32 ± 0.43	5.25 ± 0.16
	CBI	2.47 ± 0.19	6.48 ± 0.23	7.34 ± 0.26	7.68 ± 0.25
	CBI-RINSE	2.47 ± 0.19	7.41 ± 0.15	7.75 ± 0.33	7.95 ± 0.27
	Control	2.47 ± 0.19	7.31 ± 0.27	7.86 ± 0.14	8.89 ± 0.32

densities of aerobic, anaerobic and psychrotrophic bacteria in treated and control samples were greater than 6 log₁₀ CFU/g by Day 8 (Table 1). However, only samples treated with the CBI followed by a rinse showed marked evidence of spoilage (undesirable odor and appearance) on this day of observation. Hence, lacking or showing only slight indications of spoilage, the potentially hazardous controls might be perceived to be edible on day 8. The levels of naturally occurring microflora were consistently high in all samples at the end of the 21-day storage; all samples except those treated with sulfite and the CBI (unrinsed) exhibited evidence of spoilage. At 15°C, *L. monocytogenes* rapidly grew to >6 log₁₀ CFU/g in the controls by day 3 (Fig. 2). The aerobic, anaerobic, and gram-negative psychrotroph levels were 8 log₁₀ CFU/g and there was visual evidence of spoilage at day 3 (Table 2). *L. monocytogenes* grew in all treated samples, but growth was slower than in controls. By day 3, growth of the pathogen had not exceeded 4.5 log₁₀ CFU/g (only 2.5 log₁₀ CFU/g increase) in any of the treated potato sam-

ples. While the aerobic, anaerobic and psychrotrophic bacteria plate counts were >6 log₁₀ CFU/g, all treated potato samples were considered to be of normal odor and color. On day 6, *L. monocytogenes* counts were >5 log₁₀ CFU/g in all samples, and the levels of aerobic, anaerobic and Gram-negative psychrotrophic bacteria were >8 log₁₀ CFU/g. However, only controls and those samples treated with the CBI followed by a rinse had developed spoilage odors and appearance. Neither off odor nor discoloration was noted in samples treated with sulfite or the CBI (unrinsed) by Day 12, although growth of the pathogen exceeded 7 log₁₀ CFU/g and background bacterial populations were extremely high. Hence, use of sulfite or the CBI in combination with vacuum packaging created the same potentially hazardous situation when held at the abusive temperature of 15°C, as found in controls at 4°C.

At 28°C, *L. monocytogenes* levels were below the limit of detection (1 CFU/g) in all treated samples by 12h (Fig. 3). In contrast, the population density increased in the control potatoes to 5 log₁₀ CFU/g within 12h.

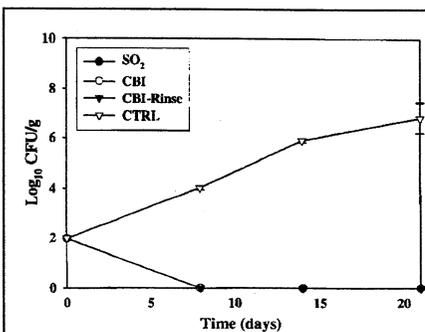


Fig. 1—Growth of *L. monocytogenes* in treated vacuum-packaged pre-peeled potatoes stored at 4°C. Log₁₀ CFU/g of the organism below the limit of detection (1 CFU/g) were considered at 0.

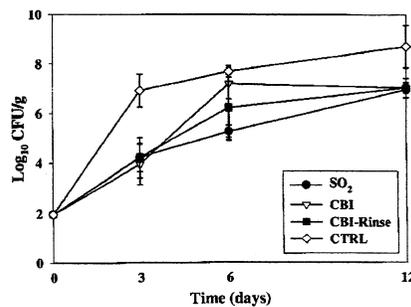


Fig. 2—Growth of *L. monocytogenes* in treated vacuum-packaged pre-peeled potatoes stored at 15°C.

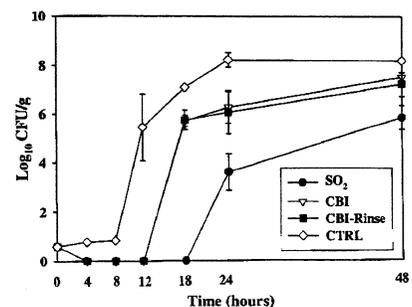


Fig. 3—Growth of *L. monocytogenes* in treated vacuum-packaged pre-peeled potatoes stored at 28°C. Log₁₀ CFU/g of the organism below the limit of detection (1 CFU/g) were considered at 0.

Table 2—Growth of background bacterial populations* as affected by treatments in vacuum-packaged pre-peeled potatoes stored at 15°C

Treatment		Day				
		0	3	6	9	12
Aerobic Plate Counts	Sulfite	2.82 ± 0.22	5.92 ± 0.24	9.59 ± 0.48	10.37 ± 0.28	10.41 ± 0.32
	CBI	2.82 ± 0.22	7.02 ± 0.31	9.54 ± 0.51	10.54 ± 0.33	9.42 ± 0.20
	CBI-RINSE	2.82 ± 0.22	6.89 ± 0.32	10.15 ± 0.33 ^b	11.16 ± 0.17 ^b	9.35 ± 0.38 ^b
	Control	2.82 ± 0.22	8.25 ± 0.13 ^b	10.08 ± 0.28 ^b	11.29 ± 0.28 ^b	9.81 ± 0.13 ^b
Anaerobic Plate Counts	Sulfite	2.69 ± 0.17	5.99 ± 0.12	8.38 ± 0.32	9.22 ± 0.16	9.50 ± 0.13
	CBI	2.69 ± 0.17	6.83 ± 0.37	9.60 ± 0.22	10.99 ± 0.14	9.44 ± 0.57
	CBI-RINSE	2.69 ± 0.17	7.87 ± 0.26	9.73 ± 0.29	10.68 ± 0.12	9.41 ± 0.17
	Control	2.69 ± 0.17	7.94 ± 0.31	10.30 ± 0.13	11.14 ± 0.32	10.34 ± 0.11
CVT Plate Counts	Sulfite	2.47 ± 0.13	7.15 ± 0.32	10.22 ± 0.28	10.36 ± 0.41	9.77 ± 0.27
	CBI	2.47 ± 0.13	6.51 ± 0.35	9.50 ± 0.26	11.04 ± 0.42	8.70 ± 0.16
	CBI-RINSE	2.47 ± 0.13	6.89 ± 0.41	9.82 ± 0.13	10.87 ± 0.26	8.59 ± 0.31
	Control	2.47 ± 0.13	7.87 ± 0.16	9.35 ± 0.11	10.47 ± 0.19	9.74 ± 0.32

The population was greater than 5 log₁₀ CFU/g in samples treated with CBI (unrinsed and rinsed) at 18h, although these samples did not appear to be spoiled. Initial levels of aerobic, anaerobic, and gram-negative psychrotrophic bacteria were >3 log₁₀ CFU/g, and ranged from 4 to 10 log₁₀ CFU/g at 24h (Table 3). All samples except those treated with sulfite appeared to be spoiled by 24h. Note that *L. monocytogenes* grew (3 log₁₀ CFU/g by 24h) in sulfite-treated potatoes that were perceived to be unspoiled.

The antimicrobial effects of sulfites, as well as that of the citric acid found in the CBI formulation, are well-known (Davidson and Juneja, 1989). The most important factor affecting the antimicrobial activity of sulfites is pH. Increased effectiveness at low pH is likely due to the ability of un-ionized sulfur dioxide to pass across the cell membrane (Rose and Pilkington, 1989). Regarding citric acid, the mechanism of inhibition by citrate has been hypothesized to be related to its ability to chelate metal ions, in addition to its pH-lowering effects (Davidson and Juneja, 1989).

When pre-peeled potatoes were dipped in a solution of 2% ascorbic acid and 1% citric acid prior to vacuum-packaging and cooking, growth and toxin production of proteolytic *Clostridium botulinum* type B was inhibited for 70 days at 15°C and at least 28 days at 20°C compared to controls which were toxic after 60 and 14 days at 15 and 20°C, respectively (Notermans et al. 1985). In our results, treatment with sulfites and the CBI (with or without a rinse) reduced growth of *L. monocytogenes* compared to controls at all storage temperatures. Complete inhibition for the duration of storage was observed in the treated samples at 4°C. Therefore, samples treated with sulfites or the CBI (with or without a rinse) are unlikely to have dangerous levels of *L. monocytogenes* if the product at low contamination levels is kept under low refrigeration temperatures. At 15°C, however, *L. monocytogenes* grew >3 log₁₀ CFU/g in all samples and as high as 7 log₁₀ CFU/g in some samples before obvious product spoilage. Therefore, the pathogen could present a danger if this product received prolonged, extreme temperature

abuse, especially since the product might still exhibit normal odor, color, and texture. Temperature abuse could occur during transportation, distribution, storage or handling in supermarkets or by consumers, primarily due to equipment malfunction or electrical outage.

Institutional foodservice organizations, catering establishments and retail consumers who demand high quality, convenient foods may be consumers of vacuum-packaged, pre-peeled potatoes. Consumers are most likely to accept or reject such a product based primarily on appearance and odor. Results indicate that potentially hazardous situations could occur in vacuum-packaged and temperature abused potatoes in the absence of apparent spoilage signs. While the cooking procedures for raw pre-peeled potatoes would be expected to inactivate *L. monocytogenes*, high levels of this pathogen on raw product present the risk of cross-contamination with other foods during handling. Food processors should be aware that treatments in addition to vacuum-packaging are needed to control *L. monocytogenes* in pre-peeled potatoes. The CBI we tested (both with and without a post-treatment rinse) provided a similar level of safety (at 4 and 15°C) for *L. monocytogenes* control as the sulfite treatment. It may therefore be considered as a replacement for sulfites, particularly if employed in conjunction with refrigeration. However, sufficient evidence exists to document temperature abuse as a common occurrence at both the retail and consumer levels. Manufacturers should assume that temperature abuse will probably occur at some point during the distribution of a refrigerated food product (NFPA, 1988). Surveys of retail food stores and consumer refrigeration units have revealed that holding temperatures of >10°C are common (Anonymous, 1989; Bryan et al., 1978; Daniels, 1991; van Grade and Woodburn, 1987; Wyatt and Guy, 1980). Therefore, it is unrealistic to rely on refrig-

Table 3—Growth of background bacterial populations* as affected by treatments in vacuum-packaged pre-peeled potatoes stored at 28°C

Treatment		Time (h)						
		0	4	8	12	18	24	48
Aerobic Plate Counts	Sulfite	4.22 ± 0.09	2.98 ± 0.11	3.65 ± 0.46	2.76 ± 0.28	3.77 ± 0.33	6.53 ± 0.19	8.37 ± 0.53 ^b
	CBI	4.22 ± 0.09	2.86 ± 0.24	3.03 ± 0.37	5.38 ± 0.28	6.17 ± 0.19	8.54 ± 0.14 ^b	9.03 ± 0.27 ^b
	CBI-RINSE	4.22 ± 0.09	2.79 ± 0.35	3.32 ± 0.26	5.08 ± 0.42	5.32 ± 0.27	7.26 ± 0.21 ^b	8.32 ± 0.16 ^b
	Control	4.22 ± 0.09	3.71 ± 0.26	4.29 ± 0.26	7.12 ± 0.05	7.80 ± 0.08	10.35 ± 0.19 ^b	8.82 ± 0.42 ^b
Anaerobic Plate Counts	Sulfite	3.36 ± 0.15	5.20 ± 0.28	5.41 ± 0.39	4.28 ± 0.43	3.44 ± 0.32	4.33 ± 0.59	7.28 ± 0.31
	CBI	3.36 ± 0.15	5.28 ± 0.17	4.94 ± 0.32	4.24 ± 0.21	6.12 ± 0.37	9.67 ± 0.13	8.83 ± 0.29
	CBI-RINSE	3.36 ± 0.15	3.74 ± 0.11	3.34 ± 0.13	4.45 ± 0.28	5.35 ± 0.33	6.13 ± 0.28	8.32 ± 0.44
	Control	3.36 ± 0.15	5.22 ± 0.27	4.84 ± 0.37	7.15 ± 0.35	8.45 ± 0.06	8.93 ± 0.19	8.81 ± 0.43
CVT Plate Counts	Sulfite	3.59 ± 0.12	3.54 ± 0.04	3.96 ± 0.11	3.91 ± 0.32	3.82 ± 0.19	5.12 ± 0.51	7.12 ± 0.23
	CBI	3.59 ± 0.12	3.48 ± 0.24	3.97 ± 0.26	4.12 ± 0.17	6.44 ± 0.36	7.33 ± 0.07	9.34 ± 0.43
	CBI-RINSE	3.59 ± 0.12	4.19 ± 0.08	3.47 ± 0.08	4.97 ± 0.33	4.66 ± 0.08	6.96 ± 0.33	7.85 ± 0.34
	Control	3.59 ± 0.12	3.95 ± 0.31	3.87 ± 0.17	6.64 ± 0.31	7.30 ± 0.50	8.59 ± 0.53	8.61 ± 0.41