

Bacterial Spore Inhibition and Inactivation in Foods by Pressure, Chemical Preservatives, and Mild Heat

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ABSTRACT

Sucrose laurates, sucrose palmitate, sucrose stearates, and monolaurin (Lauricidin) were evaluated for inhibitory effects against spores of *Bacillus* sp., *Clostridium sporogenes* PA3679, and *Alicyclobacillus* sp. in a model agar system. The combined treatment of sucrose laurate, high hydrostatic pressure, and mild heat was evaluated on spores of *Bacillus* and *Alicyclobacillus* in foods. The minimum inhibitory concentrations of the sucrose esters were higher than that of Lauricidin for all spores tested in the model agar system, but Lauricidin was not the most readily suspended in the test media. The sucrose laurates and sucrose palmitate were more effective and more readily suspended than the sucrose stearates. A combined treatment of sucrose laurate ($\leq 1.0\%$), 392 megaPascals (MPa) at 45°C for 10 to 15 min provided 3- to 5.5- \log_{10} CFU/ml reductions from initial populations of 10^6 CFU/ml for *Bacillus subtilis* 168 in milk, *Bacillus cereus* 14579 in beef, *Bacillus coagulans* 7050 in tomato juice (pH 4.5), *Alicyclobacillus* sp. N1089 in tomato juice (pH 4.5), and *Alicyclobacillus* sp. N1098 in apple juice. The most notable change in the appearance of the products was temporary foaming during mixing of the sucrose laurate in the foods. The effect of sucrose laurate appeared to be inhibitory rather than lethal to the spores. The inhibitory effects observed on *Bacillus* and *Alicyclobacillus* spores by the combined treatment of pressure, mild heat, and sucrose laurate appear promising for food applications where alternatives to high heat processing are desired.

Thermal processing of foods is a broadly effective and relatively inexpensive method of rendering foods safe from human pathogens and deterioration by spoilage microorganisms and undesirable enzymatic reactions; however, heat processing can also reduce nutrient content, modify the freshlike organoleptic qualities of foods, and limit the types of usable packaging materials to those that can withstand high processing temperatures. Alternative processes that provide comparable control of microorganisms with minimal changes to the freshlike characteristics of foods are being developed.

High hydrostatic pressure processing (HPP) is generally effective at reducing or eliminating vegetative bacteria at 300 to 700 MPa (21), but bacterial spores have demonstrated resistance to pressures of 981 MPa for 40 min and 588 MPa for 120 min (15). Studies have shown that the antimicrobial effect of HPP against spores is enhanced when used in conjunction with other treatments including heat (20, 22), acidification (22), carbon dioxide (4), and antimicrobial substances such as nisin (22) and sucrose palmitic acid ester (6). The efficacy of HPP is also improved with pressure oscillations to induce germination of spores at low pressures followed by slightly elevated pressures to destroy the remaining nondormant cells (6, 7); however, no pressure treatment, alone or in conjunction with other treatments, has been found adequate to inhibit or inactivate bacterial spores in foods.

Monoglycerides have been used as emulsifiers by the food industry since 1932 (10). Some medium chain length monoglycerides, particularly monolaurin, also have antimicrobial activity. Monolaurin (Lauricidin) has antifungal (11, 13), antiviral (8), and antibacterial (1, 27) properties, including cells adhering to surfaces (19) and spores (12). The antimicrobial effect of monolaurin is enhanced when used in conjunction with eugenol (2), heat (26), certain acids (17), and at pH values below 6 (18). Monolaurin is generally unperceptible by most taste panelists at 500 ppm or less (9); however, it has some limitations as a solo antimicrobial agent. These issues include its minimal effect on gram-negative bacteria, its low solubility in aqueous solutions, and the apparent competitive interactions with food lipids, starches, and proteins and subsequent loss of antimicrobial activity in foods (10, 24).

Sucrose esters of fatty acids are approved for use as emulsifiers internationally, but less has been published on their antimicrobial activity than that of monolaurin. Monk et al. (14) demonstrated antimicrobial activity of sucrose monolaurate against *Listeria monocytogenes*, including a synergistic effect with EDTA. A synergistic antimicrobial effect was observed with sucrose palmitic acid ester and HPP on *Bacillus stearothermophilus* (7). Synergy between HPP and sucrose laurate for the inhibition of *Bacillus subtilis* in a buffer system has also been reported (25).

The practice of combining preservation technologies (hurdle concept) to compensate for individual process limitations and to minimize the use of extreme levels of any one treatment has proven most effective for applications of

TABLE 1. Chemical and physical characteristics of inhibitors (sucrose esters and Lauricidin) and concentrations tested on spores

Inhibitor ^a	Fatty acid no. carbons	Monoester content (%)	HLB value ^b	Form	Concentrations tested (%)
Sucrose stearate (S570)	18	30	5	Powder	0.5
Sucrose stearate (S970)	18	50	9	Powder	0.5
Sucrose stearate (S1570)	18	70	15	Powder	0.5
Sucrose stearate (S1670)	18	75	16	Powder	0.5
Sucrose palmitate (P1670)	16	80	16	Powder	0.1, 0.5
Sucrose laurate (LWA1570)	12	70	15	Liquid ^c	0.01, 0.05, 0.1, 0.5
Sucrose laurate (L1695)	12	80	16	Crystal-powder	0.005, 0.01, 0.05, 0.1, 0.5, 1.0
Lauricidin	12	>94	4-4.5	Pellet	0.001, 0.005, 0.01, 0.05, 0.1, 0.5

^a Sucrose esters from Ryoto Sugar Ester, Mitsubishi Chemical Corporation, White Plains, N.Y.; Lauricidin from Med-Chem Labs, Inc., Galena, Ill.

^b Hydrophilic to lipophilic balance.

^c Mixture of 40% L-1570 or O-570, 4% ethanol, and 56% water.

HPP as an alternative to traditional thermal processing of foods. The objective of this study was to evaluate the inhibitory effects of a variety of sucrose esters on bacterial spores with and without HPP and mild heat in a model system and in foods.

MATERIALS AND METHODS

Production of spore crops. Spore crops of *B. subtilis* 168, *B. subtilis* 6051, *Bacillus coagulans* 7050, *B. stearothermophilus* 10149, *Bacillus cereus* ATCC 14579, *Clostridium sporogenes* PA3679, *Alicyclobacillus acidoterrestris* N1096, and two other *Alicyclobacillus* spp. (isolates of canned tomatoes [N1089] and apple juice concentrate [N1098]) were prepared as follows. Spores of *Bacillus* spp. were prepared by spread-plating a 24- to 48-h nutrient broth culture onto nutrient agar (NA, Difco Laboratories, Detroit, Mich.). *B. cereus* 14579 was plated onto Schaeffer's sporulation agar (5). Plates were incubated for approximately 1 week at 37 or 55°C for *B. stearothermophilus* 10149 and 32°C for *B. cereus*. Sporulation was assessed by phase-contrast microscopy, and spores were harvested when predominant (>90%).

Spores of *C. sporogenes* PA3679 were prepared by spread-plating a 24- to 48-h reinforced clostridial medium culture onto reinforced clostridial agar (RCA, Difco). RCA plates were incubated anaerobically at 37°C for approximately 1 week until spores were predominant (>90%) as determined by phase-contrast microscopy.

Alicyclobacillus spp. were grown at 42°C for 5 to 7 days in K medium (per liter, 2.5 g yeast extract, 5.0 g peptone, 1.0 g dextrose, 1.0 ml Tween 80, pH-adjusted to 3.9 with 1.0 M malic acid). Spread plates of potato dextrose agar acidified to pH 3.6 with malic acid were inoculated with growth of *Alicyclobacillus* spp. in K medium. Potato dextrose agar plates were incubated aerobically at 42°C for 10 to 19 days until spores predominated as determined by phase-contrast microscopy.

Spores of *Bacillus*, *Clostridium*, and *Alicyclobacillus* were harvested from NA, RCA, and potato dextrose agar, respectively, with sterile distilled water and a bent-glass rod. Spore crops of

Bacillus and *Clostridium* were washed according to the method of Nicholson and Setlow (16). Spores were cleaned by alternate centrifugation and washing of the pellets with 1.0 M KCl/0.5 M NaCl, 10 ml Tris.Cl (pH 7.2) containing 50 µg/ml lysozyme (incubated 1 h at 37°C), 1.0 M NaCl, sterile distilled water, 0.05% sodium dodecyl sulfate, TEP buffer (50 mM Tris-Cl buffer, pH 7.2, with 10 mM EDTA and 2 mM phenylmethylsulfonyl fluoride), and three washes of sterile distilled water. Spores of *Alicyclobacillus* were cleaned by alternate centrifugation and washing of the pellets by sterile distilled water three times. Spore crops were stored in sterile distilled water at 4°C. The spore crops were evaluated by phase-contrast microscopy and enumerated by pour plating with NA, RCA, and K agar (KA) for *Bacillus*, *Clostridium*, and *Alicyclobacillus*, respectively.

Exposure of spores to sucrose esters and Lauricidin in model system. Spores of *B. subtilis* 168, *B. subtilis* 6051, *B. coagulans* 7050, *B. cereus* 14579, *B. stearothermophilus* 10149, *C. sporogenes* PA3679, *A. acidoterrestris* N1096, *Alicyclobacillus* spp. N1098 and N1089 (10⁶ CFU/ml) were pour-plated in duplicate in NA (for *Bacillus*), KA (for *Alicyclobacillus*), or RCA (for *C. sporogenes*) amended with various concentrations of sucrose esters (Table 1) depending on culture sensitivity. The sucrose esters (Ryoto Sugar Ester, Mitsubishi Chemical Corporation, White Plains, N.Y.) evaluated for inhibitory effect were four types of sucrose stearate (manufacturer product codes S570, S970, S1570, and S1670), sucrose palmitate (P1670), and two types of sucrose laurate (L1695 and LWA1570). The sucrose esters varied by monoester content, fatty-acid chain length, hydrophilic to lipophilic balance, and physical form (Table 1). The antimicrobial activity of the monolaurin, Lauricidin (Med-Chem Labs, Inc., Galena, Ill.), was tested in comparison to the sucrose esters (Table 1).

Treatment by pressure, inhibitors, and mild heat. The most inhibitory of the sucrose esters as determined during screening studies against *Bacillus*, *Clostridium*, and *Alicyclobacillus* spp. in model agar systems were evaluated for individual and combined effects with hydrostatic pressure and mild heat against select

TABLE 2. Experimental conditions for treatment of spores in foods at 392 MPa at 45°C for 10 min

Microorganism	Food product	Sucrose laurate L1695 (%)	Recovery media	Incubation temper- ature (°C)	Incubation period (days)
<i>B. subtilis</i> 168	Milk	0.1, 0.5	NA	37	5
<i>B. cereus</i> ATCC 14579	Beef in gravy	0.01	NA	30	5
<i>B. coagulans</i> 7050	Tomato juice, pH 4.5	1.0	TJA, pH 4.5	37	7
<i>Alicyclobacillus</i> N1089	Tomato juice, pH 4.5	0.01, 0.005	TJA, pH 4.5	42	21
<i>Alicyclobacillus</i> N1098	Apple juice	0.025, 0.04, 0.045	KA, pH 3.8	42	21

sporeformers in foods with which they are commonly associated. *B. subtilis* 168, *B. cereus* 14579, and *B. coagulans* 7050 were treated in milk, beef in gravy, and tomato juice, respectively. *Alicyclobacillus* N1089 and N1098 were treated in tomato juice and apple juice, respectively. This was done in three phases as follows.

I. Evaluation of sucrose laurates and Lauricidin in milk.

In the first phase, we studied the effectiveness of sucrose laurates L1695 and LWA1570 and Lauricidin in conjunction with high-pressure treatment at various times and temperatures against *B. subtilis* 168 in milk. Results from this phase further enabled the selection of appropriate treatment conditions and inhibitor for subsequent studies.

Ultrahigh temperature processed milk (2% milkfat, Parmalat, Teaneck, N.J.) was inoculated with *B. subtilis* (10⁶ CFU/ml). The appropriate inhibitor was added to the milk at various concentrations (L1695 at 0.1, 0.5, and 1.0%; LWA1570 at 0.05 and 0.1%; and Lauricidin at 0.001 and 0.0025%). Ten milliliters of milk were transferred to a polyester/polyethylene pouch, and the pouches were heat-sealed (both by Kapak Corp., Minneapolis, Minn.). The milk was pressurized at 392 MPa at 25, 45, 50, or 55°C for 10 or 15 min (Autoclave Engineers Isostatic Press model IP2-22-60, Erie, Pa.). Come-up time was approximately 2.75 min and depressurization time was approximately 40 s. Neither come-up nor depressurization times were considered as part of the total 10- or 15-min treatment times. Milk samples treated at temperatures greater than 25°C were immediately cooled in water to ambient temperature after treatment. The pouches were washed and opened aseptically. Surviving spores were enumerated by pour plating in NA amended with the same type and concentration of inhibitor as was added to the milk. The plating diluent was 0.1% peptone water. Plates were incubated at 37°C for a minimum of 5 days, and colonies were counted manually. Negative controls were plated to assure commercial sterility of the milk. Positive controls were plated of the inoculated milk prior to addition of the inhibitor and pour-plated in unamended NA to determine the initial spore count. All experiments were duplicated.

II. Sporistatic effects of sucrose laurate. Sucrose laurate L1695 was selected among the inhibitors for further evaluation in food systems for reasons presented in the Results. In phase II, we studied the effects of individual treatments as well as the combined effects of pressure, mild heat, and sucrose laurate on inhibition of *B. subtilis* 168 in milk. The sporistatic effects of sucrose laurate were evaluated by altering the enumeration media.

Milk was inoculated with *B. subtilis* 168 (10⁶ CFU/ml) as described in phase I. Negative controls were plated to verify commercial sterility of the milk prior to inoculation, and the initial spore count of the inoculated milk was determined as in phase I. Ten-milliliter samples were treated by the following processes:

P/H in NA: Pressure and mild heat treatment. Spore enumeration in nutrient agar.

P/H in NA/SL: Pressure and mild heat treatment. Spore enumeration in nutrient agar amended with sucrose laurate L1695.

P/SL/H in NA: Pressure, sucrose laurate added to milk, and mild heat treatment. Spore enumeration in nutrient agar.

P/SL/H in NA/SL: Pressure, sucrose laurate added to milk, mild heat treatment. Spores enumerated in nutrient agar amended with sucrose laurate.

SL/H in NA: Sucrose laurate added to milk and mild heat treatment. Spore enumeration in nutrient agar.

SL/H in NA/SL: Sucrose laurate and mild heat treatment of milk. Spore enumeration in nutrient agar amended with sucrose laurate.

Sucrose laurate L1695 was tested at concentrations of 0.1 and 0.5%. The spores were pressurized at 392 MPa for 10 min at 45°C as previously described for phase I. Recovery conditions of the spores after treatment were the same as in phase I with the exception of plating in NA both with and without sucrose laurate. All experiments were duplicated.

III. Effect of individual and combined processes on spores in various foods. The need for continuous exposure of the spores to sucrose laurate in the plating medium was apparent from the results of phase II studies; therefore, the following treatments were used in subsequent studies with a variety of sporeformers in foods with which they are associated. The systems, sucrose laurate concentrations, and recovery media varied. Details are provided below and a summary of the foods tested is presented in Table 2.

Treatments.

P/H in (plating medium): Pressure and mild heat treatment. Spore enumeration in appropriate agar.

P/SL/H in (plating medium)/SL: Pressure, sucrose laurate added to milk, mild heat treatment. Spores enumerated in appropriate plating agar amended with sucrose laurate.

SL in (plating medium)/SL: Sucrose laurate in food. Spore enumeration in appropriate plating agar amended with sucrose laurate.

SL/H in (plating medium)/SL: Sucrose laurate and mild heat treatment of milk. Spore enumeration in appropriate plating agar amended with sucrose laurate.

Commercial sterility of uninoculated foods was verified, and initial spore counts of the inoculated food were determined as in phase I and plated in the appropriate medium. The pressure treatment (392 MPa, 45°C, 10 min) was as described in phase I. Sucrose laurate was added to the recovery media at the same concentration as in the foods. All experiments were duplicated with the exception of *B. cereus* in beef for which four trials were done.

Food systems. *B. cereus* 14579 was treated in beef in gravy baby food (10 g) (Gerber Products, Fremont, Mich.; containing beef, water, corn starch, lemon juice concentrate) with sucrose

TABLE 3. MICs of sucrose esters and Lauricidin for spore outgrowth of *Bacillus* spp., *Alicyclobacillus* spp., and *C. sporogenes*

Sporeformer	MIC (%) ^a				
	Sucrose stearate S1670	Sucrose palmitate P1670	Sucrose laurate L1695	Sucrose laurate LWA1570	Lauricidin
<i>B. subtilis</i> 168	>0.5	>0.5	>1.0		
<i>B. subtilis</i> 6051	>0.5	>0.5	>1.0	0.5	0.005
<i>B. coagulans</i> 7050	>0.5	>0.5	>1.0	0.5	0.005
<i>B. cereus</i> 14579	>0.5	>0.5	>1.0	0.5	0.01
<i>B. stearothermophilus</i> 10149	0.5	>0.5	0.1	0.1	0.01
<i>Alicyclobacillus</i> sp. N1089	>0.5	0.5	0.5	0.5	0.005
<i>A. acidoterrestis</i> N1096	>0.5	0.5	0.05	0.1	0.01
<i>Alicyclobacillus</i> sp. N1098	>0.5	>0.5	0.05	0.1	0.01
<i>C. sporogenes</i> PA3679	0.5	≤0.1	0.05	0.1	0.01
			0.05	0.5	0.01

^a In NA for *Bacillus* spp., KA for *Alicyclobacillus* spp., RCA for *C. sporogenes*.

laurate L1695 at 0.01%. Outgrowth of *B. cereus* 14579 survivors was enumerated on NA/SL. Plates were incubated at 30°C for approximately 5 days.

B. coagulans 7050 was treated in tomato juice (Campbell Soup Company, Camden, N.J., containing tomato juice from concentrate, salt and vitamin C) adjusted to pH 4.5 with 1.0 N NaOH from an initial pH of 4.2. The sucrose laurate concentration tested was 1.0%. Outgrowth of *B. coagulans* survivors was enumerated on tomato juice agar (TJA, Difco) with SL and pH-adjusted to 4.5 with filter-sterilized 1.0 M citric acid (Fisher, Fair Lawn, N.J.). Plates were incubated at 37°C for approximately 1 week.

Alicyclobacillus sp. N1089 was treated in tomato juice (as in the *B. coagulans* study) with SL concentrations of 0.005 and 0.01%. Outgrowth of surviving *Alicyclobacillus* spores was enumerated on TJA/SL at pH 4.5. Plates were incubated at 42°C for approximately 3 weeks.

Alicyclobacillus sp. N1098 was treated in apple juice (America's Choice, Baltimore, Md., from concentrate) with SL (0.025, 0.04, and 0.045%). Outgrowth of *Alicyclobacillus* survivors was enumerated on KA/SL pH-adjusted to approximately 3.8 with filter-sterilized 1.0 M malic acid (Acros Organics, Fair Lawn, N.J.). Plates were incubated approximately 3 weeks at 42°C.

Statistical analyses. The standard deviations for the trials within each treatment were calculated and are illustrated with error bars in the figures. Analysis of variance for the various treatments was calculated with the general linear models procedure of SAS (23). For significant *F* tests, differences in the means among treatments considering all replications were determined according to a *t* test with a significance level of 5%.

RESULTS

Inhibition by sucrose esters and Lauricidin in the model agar system. The general order of inhibitory action against outgrowth of spores in the model agar systems from greatest to least was Lauricidin > sucrose laurate > sucrose palmitate ≥ sucrose stearates (Table 3). The minimum inhibitory concentrations (MICs) of sucrose stearates S570, S970, and S1570 were greater than 0.5% for all sporeformers tested (data not shown). *B. stearothermophilus* 10149 was generally the most susceptible to the inhibitors. Of the sucrose laurates, *Alicyclobacillus* spp. and *C. sporogenes* PA3679 were more susceptible to L1695 (at 0.05%) than to LWA1570 (≥0.1%), while *Bacillus* spp. (with the ex-

ception of *B. cereus* 14579) were more susceptible to LWA1570 (at 0.5%) than L1695 (>1.0%). The sucrose stearates were insoluble in agar until suspended by boiling but precipitated upon tempering of the agar to 45°C. Although Lauricidin had lower MICs than sucrose laurates against the sporeformers, Lauricidin was less readily suspended in agar at room temperature than the sucrose laurates.

Sucrose laurates and Lauricidin in milk. Lauricidin and the sucrose laurates, the most effective of the sucrose esters, were evaluated for effectiveness when used in conjunction with pressure and mild heat on *B. subtilis* 168 in milk. Because LWA1570 and Lauricidin were effective alone at 0.5 and 0.01%, respectively, against *Bacillus* spp. (Table 3), these two inhibitors were tested at room temperature with HPP at 392 MPa. With Lauricidin (0.001%) and 392 MPa pressure applied for 10 min, there was no reduction in spore count at 25°C; however, at 45°C, a 3-log₁₀ reduction was observed for *B. subtilis* 168 from an initial population of 10⁶ CFU/ml (Table 4). When the Lauricidin concentration was increased to 0.0025%, the inhibitory effect was the same at 25 and 45°C with or without pressure (Table 4). Because of the low concentration and small volumes of milk tested, Lauricidin was added by serial dilution where appropriate. Prior dissolution of Lauricidin with mild heat, ethanol, or propylene glycol would probably be necessary because of the uncertain solubility in milk at room temperature.

LWA1570 (0.05 and 0.1%) and 10 min of 392 MPa provided approximately 4- to 5-log₁₀ reductions, respectively, of *B. subtilis* 168 in milk at room temperature (data not shown); however, the plate counts were erratic in that 10-fold reductions were not apparent in the dilution series and the plates were countable only when the milk was diluted adequately (10⁻² dilution). This would suggest that the apparent effectiveness of LWA1570 might have been less had the spores been incubated in the milk rather than plated in agar. The effectiveness of LWA1570 is worth further investigation, but the recovery methods should be modified to increase confidence in the results.

Higher concentrations of L1695 were needed to

TABLE 4. Effect of 392 MPa, inhibitors, and mild heat on *B. subtilis* 168 in milk

Temp (°C)	Inhibitor (%)	Time (min)	Initial population (log ₁₀ CFU/ml) (standard deviation)	Final population (log ₁₀ CFU/ml) (standard deviation)
45	L1695 (0.1)	10	6.53 (0.02)	3.25 (0.45)
45	L1695 (0.5)	10	6.40 (0.16)	2.89 (0.06)
45	L1695 (1.0)	10	6.59 (0.06)	2.64 (0.22)
45	L1695 (1.0)	15	6.42 (0.01)	1.78 (0.02)
45	Lauricidin (0.001)	10	6.59 (0.06)	3.65 (0.38)
25	Lauricidin (0.001)	10	6.52 (0.16)	6.05 (0.00)
45	Lauricidin (0.001)	15	6.42 (0.01)	3.30 (0.16)
25	Lauricidin (0.0025) ^a	10	6.42 (0.01)	2.47 (0.76)
45	Lauricidin (0.0025) ^a	10	6.42 (0.01)	2.66 (0.16)

^a Effect was the same without pressure.

achieve the same reduction in *B. subtilis* 168 spore counts in milk than Lauricidin (Table 4). Solubility of L1695 was greater than observed with Lauricidin, and the results were more reliable by plate counts than those observed with LWA1570. Concentrations of 0.1, 0.5, and 1.0% L1695 and 392 MPa applied for 10 min at 45°C provided 3-, 3.5-, and 4-log₁₀ reductions, respectively (Table 4) from an initial inoculum of 10⁶ CFU/ml of *B. subtilis* 168. Increasing the pressurization time to 15 min at 45°C with 1.0% L1695 provided a 5-log₁₀ reduction (Table 4). Increasing the temperature to 50 and 55°C for 15 min of treatment at 392 MPa with 1.0% L1695 provided no additional reduction in *B. subtilis* 168 spores when compared to treatment at 45°C under the same conditions (data not shown).

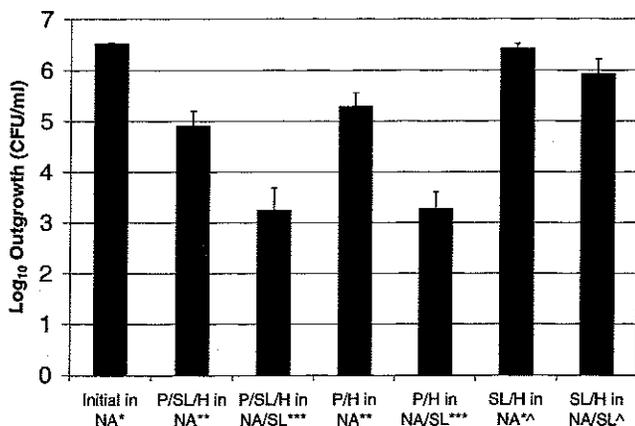


FIGURE 1. Inhibition of *B. subtilis* 168 in milk by individual and combined treatments of 392 MPa (P) for 10 min at 45°C (H) and sucrose laurate (SL) L1695 (0.1%), plated in NA and NA with SL (NA/SL). Error bars represent standard deviations of replicates within treatments. Treatments labeled with different superscripts are significantly different.

Sporistatic effects of sucrose laurate. To quantify the inactivation of spores versus the inhibition of outgrowth, *B. subtilis* 168 was treated in milk with 0.1 and 0.5% L1695 and 392 MPa at 45°C for 10 min and plated with and without sucrose laurate in the media (Fig. 1). Pressure with mild heat (P/H in NA) or sucrose laurate with mild heat (SL/H in NA/SL and NA) provided ≤ 1 -log₁₀ reductions. Limiting exposure to the sucrose laurate only during pressurization of the milk (P/SL/H in NA) yielded approximately a 1.5-log₁₀ reduction in spore counts for both concentrations tested, but continuous exposure to the sucrose laurate in the plating medium (P/SL/H in NA/SL) provided approximately a 3-log₁₀ reduction. The effect was the same whether the sucrose laurate was added before or after pressurization (Fig. 1, P/SL/H in NA/SL vs. P/H in NA/SL).

Individual and combined processes on spores in various foods: *B. cereus* 14579 in beef. *B. cereus* 14579 was less resistant to sucrose laurate L1695 than *B. subtilis* 168 and 6051 in the screening studies in agar (Table 3). Similarly, the concentration of L1695 needed to achieve approximately a 5-log₁₀ reduction of *B. cereus* 14579 in beef was only 0.01% when combined with pressure at 45°C for 10 min (Fig. 2A), as compared to 1.0% required for *B. subtilis* 168 in milk (Table 4). The contribution of pressure to the inhibition and inactivation of *B. cereus* was greater than that provided by sucrose laurate alone or sucrose laurate with mild heat, neither of which had an effect on spore counts; however, when sucrose laurate was used in conjunction with pressure and mild heat, an additional 1-log₁₀ reduction was observed as compared to use of pressure and mild heat alone.

***Bacillus coagulans* 7050 in tomato juice (pH 4.5).** Sucrose laurate L1695 at 1.0% with 10 min at 392 MPa

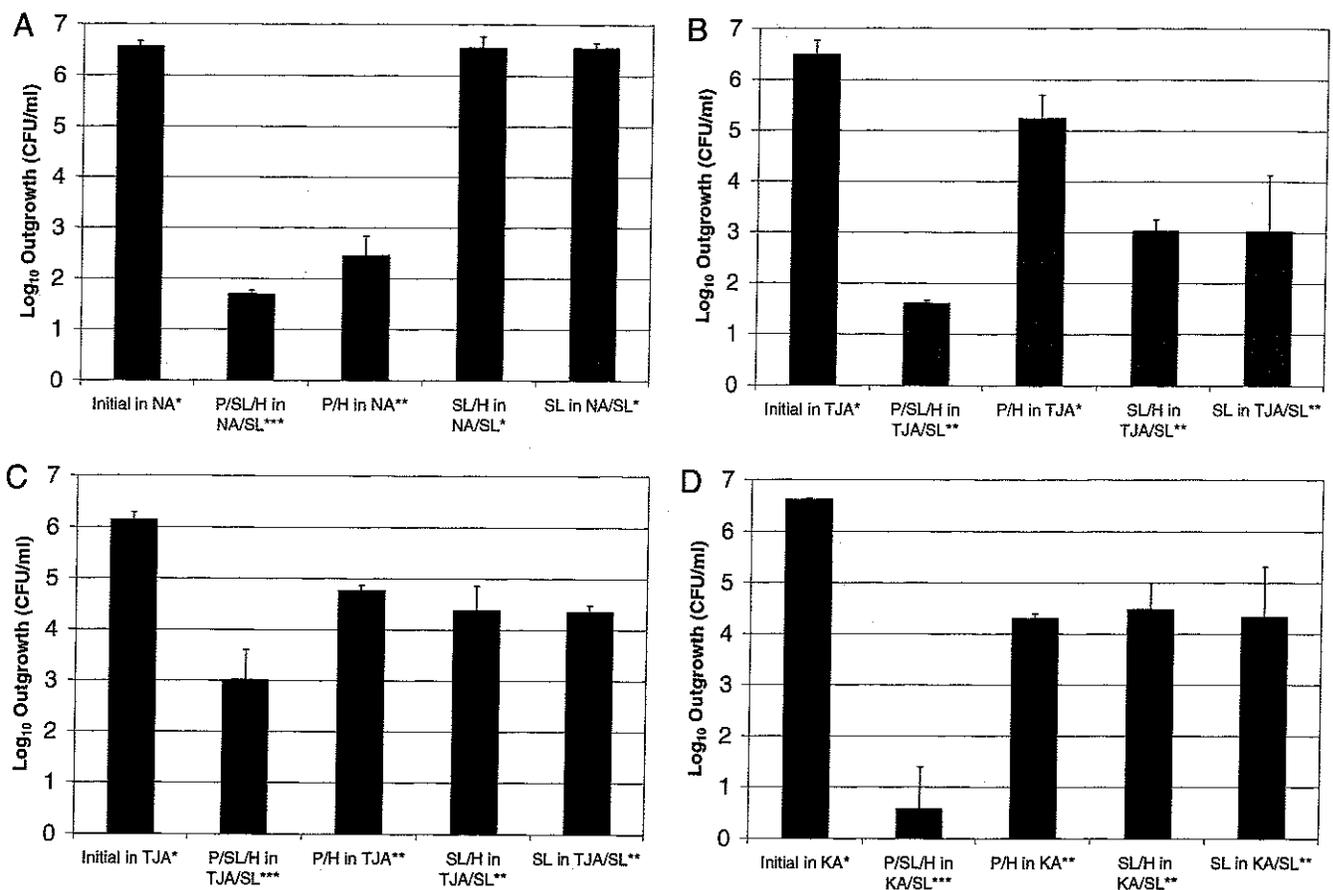


FIGURE 2. Inhibition of sporeformers in foods by individual and combined treatments of 392 MPa (P) for 10 min at 45°C (H) and sucrose laurate (SL) L1695: (A) *B. cereus* 14579 in beef baby food, 0.01% L1695, plated in NA or NA amended with 0.01% SL (NA/SL); (B) *B. coagulans* 7050 in tomato juice, 1.0% L1695, plated in TJA or TJA with 1.0% SL (TJA/SL); (C) *Alicyclobacillus* N1089 in tomato juice with 0.005% L1695, plated in TJA or TJA with 0.005% SL (TJA/SL); and (D) *Alicyclobacillus* N1098 in apple juice with 0.045% L1695, plated in KA or KA with 0.045% SL (KA/SL). Error bars represent standard deviations of replicates within treatments. Treatments labeled with different superscripts are significantly different.

and 45°C reduced a 10^6 -CFU/ml initial inocula of *B. coagulans* 7050 by nearly 5 log₁₀ (Fig. 2B). In contrast to observations with *B. cereus* 14579, the contributions of sucrose laurate alone and with heat were greater (approximately 2 log₁₀) on *B. coagulans* than treatments employing just pressure and mild heat.

***Alicyclobacillus* N1089 in tomato juice (pH 4.5).** A 3-log₁₀ reduction of *Alicyclobacillus* N1089 was observed with the combined treatment of 0.005% L1695 with 10 min at 392 MPa and 45°C (Fig. 2C). At this concentration of L1695, the combined effects of pressure/mild heat and sucrose laurate/mild heat were nearly cumulative; synergy was not observed in this particular system. When the concentration of L1695 was increased to 0.01%, no outgrowth of spores was observed with the combined treatment of pressure/sucrose laurate/mild heat; the effect was the same with L1695 at 0.01% without pressure (data not shown). Interestingly, the effect of 0.01% L1695 was greater in tomato juice and tomato juice agar (pH 4.5) than when MICs were determined in KA at pH 3.8. In KA, spore outgrowth was observed at 0.01% L1695 (Table 3).

***Alicyclobacillus* N1098 in apple juice.** Sucrose laurate L1695 at 0.025% in apple juice with or without treatment

at 45°C had no inhibitory effect on *Alicyclobacillus* N1098 (data not shown). Treatments of 392 MPa for 10 min at 45°C showed reductions of approximately 2-log₁₀ CFU/ml with no additional reduction from the addition of 0.025% L1695. L1695 at 0.04% with pressure and mild heat treatment reduced *Alicyclobacillus* N1098 spore counts in apple juice by just 2.5 log₁₀ (data not shown). A slight increase in the L1695 concentration to 0.045% with pressure and mild heat gave 5.5-log₁₀ reductions of *Alicyclobacillus* spp. N1098 (Fig. 2D).

DISCUSSION

The greater inhibitory effect observed with sucrose laurates (C₁₂ fatty-acid chain length) and sucrose palmitate (C₁₆) as compared to the sucrose stearates is consistent with the typically greater inhibitory effect of saturated lauric acid and unsaturated palmitoleic acid over other acid esters (10). The fatty acid used to esterify polyols seems to determine the potency of the ester; however, antimicrobial activity is also a function of the monoester content and degree of saturation of the fatty acid chain (10, 12). In contrast to glycerides, the diester of sucrose rather than the monoester, has been reported to be more active (10). Among the sucrose stearates tested, the greater the monoester content, the

greater the antimicrobial activity observed against *C. sporogenes* PA3679 and *B. stearothermophilus* 10149; however, the hydrophilic to lipophilic balance values also varied so activity could have been as much a function of solubility.

The mechanism of antimicrobial activity of monoglycerides and sucrose esters is uncertain; however, perturbation at the cell membrane of vegetative cells is believed to be involved for fatty-acid antimicrobials (10). The mechanism against spores is also unclear, but our results indicate the effect is inhibitory rather than lethal as indicated by the need for continuous exposure to the sucrose ester in the plating medium. This observation is consistent with the findings of Chaibi et al. (3) who studied bacterial spore inhibition by glycerides and that of Hayakawa et al. (6). Hayakawa et al. (6) observed no bacteriostatic effect by pressure treatment of spores exposed to stearic acid ester; however, pressure-treated spores exposed to sucrose palmitic acid ester were vulnerable. They hypothesized that the application of high pressures helped deposit sucrose esters on the spore coat and thereby changed the surface hydrophobicity and water permeability during pressurization (6). Synergistic antimicrobial effects were observed with HPP and sucrose laurate against several sporeformers in various foods (Figs. 1, 2A, 2B, and 2D). The effect against *B. subtilis* 168 in milk was the same when treated by 0.1% L1695, 392 MPa for 10 min at 45°C whether the sucrose laurate was present during pressurization or merely added after pressurization (Fig. 1). This suggested that the synergy observed was more a function of increased susceptibility of *B. subtilis* 168 to sucrose laurate consequent of pressurization rather than an increased susceptibility to pressure due to the presence of sucrose laurate.

The addition of sucrose laurate L1695 at $\leq 1.0\%$ had no obvious effect on the appearance or odor of the food products, but caused foaming during mixing. Lauricidin is generally not detectable by taste at 500 ppm (9); the taste threshold of sucrose laurate in the various products tested must be further evaluated. The combination of all three processing treatments of sucrose laurate, HPP, and mild heat appeared to be necessary to inactivate and inhibit spores of *Bacillus* in this study. The combination of sucrose laurate and mild heat provided no additional effect on any spore-former over the application of sucrose laurate alone. Of the two sporeformers of concern in acid foods, the level of L1695 necessary to partially inhibit *B. coagulans* in tomato juice at pH 4.5 was approximately 100 times greater than the level needed to inhibit *Alicyclobacillus* N1089 in the same product. *B. stearothermophilus* 10149 was the least resistant among the bacilli tested against the sucrose esters. *B. cereus* 14579 in beef was the least pressure-resistant among all spores tested in this study. Further research is needed to elucidate the mechanisms of inhibition and inactivation in order to explain the differences in susceptibility among the genera and species to the treatments. Because the aim of this study was to evaluate the effectiveness of treatments in a variety of systems, few direct comparisons can be made among the sporeformers to suggest which parameters have the greatest impact on the different results observed. The foods evaluated varied in composition and

pH, both of which may have influenced the efficacy of HPP and sucrose esters. Additionally, varying conditions in temperature or growth media for optimal sporulation during crop preparation may have influenced the differences in resistances of the sporeformers to the various treatments.

The inhibitory effects on sporeformers by the combined treatment of HPP, mild heat, and sucrose laurate appear promising for food applications where alternatives to high heat processing are desired. The results warrant further investigation and adjustment of the processing parameters to attempt to achieve complete spore inhibition in specific food products.

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