

Bactericidal Effect of The SIAD TGIR Nonthermal Pasteurizer on Bacterial Contaminants in Liquids

Aim of the study:

The purpose of this study was to examine the effect of exposure time of the SIAD TGIR Nonthermal Pasteurizer on viable cell counts of previously reported bacterial contaminants in liquids.

Materials and Methods:

Included in the study were *Salmonella choleraesuis subsp. choleraesuis serotype enteritidis* ATCC strain 13076, *Escherichia coli* ATCC strain #43895 (O157:H7) and ATCC strain #47056, all acid tolerant, gram-negative, non-spore forming enteric rods. *E. coli* strain #47056 was used in some cases as a surrogate for ATCC #43895 the more pathogenic strain of *E. coli*. In addition, *Bacillus cereus* (ATCC #11778), a facultative, gram positive, spore forming rod, often used as a surrogate for *Bacillus anthracis*, was included.

Test strains maintained on the surface of Tryptic Soy Agar (TSA) were used to inoculate two to three liters of 1/2 strength Brain Heart Infusion broth. After 24 hours of incubation at 37° C in air. The cells were harvested aseptically by centrifugation (15000 rpm for 20 min.), resuspended in sterile 1/4 strength Ringer's solution and used to inoculate seven liters of water, cider or orange juice. After the SIAD TGIR Nonthermal Pasteurizer lamp reached a voltage of approximately 525 volts (~ 1 minute) aliquots were aseptically removed at predetermined times (Table 1 - 6), diluted to concentrations of 10⁻², 10⁻⁴ and 10⁻⁶, then plated onto TSA using a Spiral Plater (Spiral Systems Inc.) and incubated at 37° C in air. Maximum voltage and liquid temperature were recorded at each sample time and the speed of the mixer was maintained at 530 rpm. After 24 hrs, recoverable cell count was determined and compared to that of an equivalent, unexposed cell suspension of each strain.

Water

Ringer's tablets were added to sterile distilled water to minimize cell lysis of the test strains due to the increased osmotic from the water. Enough tablets were added to give a final concentration 1/4th that of physiological saline.

Test strains: *Escherichia coli* (ATCC #47056)
Bacillus cereus (ATCC #11778)

Orange Juice

Freshly squeezed as well as juice with heavy pulp content (Tropicana) were used in the study

Test strains: *Escherichia coli* (ATCC #47056)
Salmonella choleraesuis subsp. choleraesuis serotype enteritidis (ATCC #13076)

Apple Cider

Fresh squeezed

Test strain: *Escherichia coli* [ATCC #43895 (O157:H7)]

Results:

Water

Table 1

Effect of SIAD TGIR Nonthermal Pasteurizer with constant stirring on 24hr Culture of *Escherichia coli* (#47056) in 1/4 strength Ringer's solution

Exposure (min.)	Volts	Max.Temp In Degrees Centigrade	Cfu's/ml	Reduction in Log Units
0	N/A	26.5	8.33E+08	N/A
2	530	30.2	2.03E+01	7.61
4	529	32.1	0.00E+00	8.92
6	529	34.9	0.00E+00	8.92
8	529	37.5	0.00E+00	8.92
10	529	39.9	0.00E+00	8.92
15	529	48.9	0.00E+00	8.92

Table 2

Effect of SIAD TGIR Nonthermal Pasteurizer with constant stirring on 24hr Culture of *Bacillus cereus* (ATCC #11778) in 1/4 strength Ringer's solution

Exposure (min.)	Volts	Max.Temp In Degrees Centigrade	ML/ml	Reduction in Log Units
0	N/A	23	8.33E+08	N/A
2	525	25	1.63E+08	0.71
4	525	29	0.00E+00	8.92
6	530	30	0.00E+00	8.92
8	531	33	0.00E+00	8.92
10	533	35	0.00E+00	8.92
30	534	51	0.00E+00	8.92

Orange Juice

Table 3

Effect of SIAD TGIR Nonthermal Pasteurizer with constant stirring on 24hr Culture of *Escherichia coli* (ATCC #47056) in Orange Juice with Heavy Pulp

Exposure (min.)	Volts	Max.Temp In Degrees		Reduction in Log Units
		Centigrade	ML/ml	
0	N/A	7	4.39E+07	N/A
20	529	25	8.13E+03	3.73
30	529	33	1.22E+02	5.56
40	531	41	0.00E+00	7.64
50	529	48	0.00E+00	7.64
60	529	55	0.00E+00	7.64

Table 4

Effect of SIAD TGIR Nonthermal Pasteurizer with constant stirring on 24hr Culture of *Salmonella choleraesuis* subsp. *choleraesuis* serotype *enteritidis* (ATCC #13076) in orange juice with heavy pulp

Exposure (min.)	Volts	Max.Temp In Degrees		Reduction in Log Units
		Centigrade	ML/ml	
0	N/A	8	1.20E+07	N/A
10	527	19	2.03E+05	1.77
20	529	28	1.63E+04	2.87
30	530	35	1.34E+03	3.95
40	530	43	2.64E+02	4.66
50	530	52	0.00E+00	7.08
60	528	60	0.00E+00	7.08

Table 5

Effect of SIAD TGIR Nonthermal Pasteurizer with constant stirring and bubbling carbon dioxide gas on 24hr Culture of *Salmonella choleraesuis* subsp. *choleraesuis* serotype *enteritidis* (ATCC #13076) fresh squeezed orange juice

Exposure (min.)	Volts	Max.Temp In Degrees		Reduction in Log Units
		Centigrade	ML/ml	
0	N/A	2	3.66E+06	N/A
5	529	10	1.02E+06	0.56
10	530	16	4.27E+04	1.93
15	529	20	1.22E+04	2.48
20	529	24	1.04E+03	3.55
25	527	29	2.44E+02	4.18
30	527	33	4.07E+01	4.95
40	525	43	0.00E+00	6.56
50	524	51	0.00E+00	6.56
60	525	60	0.00E+00	6.56

Apple Cider

Table 6

Effect of SIAD TGIR Nonthermal Pasteurizer with constant stirring a on 24hr Culture of *Escherichia coli* [ATCC #43895 (O157:H7)] in Apple Cider

Exposure (min.)	Volts	Max.Temp In Degrees Centigrade	ML/ml	Reduction in Log Units
0	N/A	7	1.82E+08	N/A
15	545	22	2.03E+01	6.95
20	546	29	4.27E+02	5.63
30	547	37	1.83E+02	6.00
40	544	46	2.03E+01	6.95
50	545	55	0.00E+00	8.26
60	544	64	0.00E+00	8.26

Escherichia coli (Table 1) and *Salmonella choleraesuis subsp. choleraesuis serotype enteritidis* (Table 2) levels in water (1/4 strength Ringer's solution) were reduced by more than 5 log units after two minutes of exposure to the SIAD TGIR Nonthermal Pasteurizer and viable cells could not be recovered after four minutes. *Escherichia coli* (Table 3) and *Salmonella choleraesuis subsp. choleraesuis serotype enteritidis* (Table 4) levels in orange juice with heavy pulp (1/4 strength Ringer's solution) were reduced 5.56 and 3.95 Log units respectfully after thirty minutes of exposure to the SIAD TGIR Nonthermal Pasteurizer and viable cells could not be recovered after forty and fifty minutes respectfully. Carbon dioxide gas was bubble through fresh squeezed orange juice to preserve nutritional content and to improve the taste of the juice. The data in table 5 demonstrates that the addition of CO₂ to the process had little if any change in bactericidal effect.

The level of the pathogenic strain of *Escherichia coli* (ATCC #43895) in apple cider was reduced by more than 5 Log units after 15 minutes of exposure and was not recoverable after fifty minutes of exposure.

Conclusions:

The SIAD TGIR Nonthermal Pasteurizer process of Synergina, Inc. achieved a 5 Log reduction in viable cell count with *Escherichia coli* (ATCC #47056) in orange juice and water, with *Escherichia coli* [ATCC #43895 (O157:H7)] in apple cider, with *Salmonella choleraesuis subsp. choleraesuis serotype enteritidis* (ATCC #13076) in orange juice, and with *Bacillus cereus* (ATCC #11778) in water and was able to reduce the level of all test organisms to undetectable levels over time.

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