

Efficacy of acidified sodium chlorite (ASC) and tri-sodium phosphate (TSP) in decontaminating chicken carcass against *Campylobacter coli*

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Food safety against pathogenic microflora is drawing attention of researchers globally to address public health concern from food-borne diseases. Among the pathogens, *Campylobacter* have been linked to poultry in many outbreaks, primarily due to the consumption of raw or undercooked chicken (Tauxe *et al.* 1988). Thermophilic *Campylobacter* spp. particularly *C. jejuni* and *C. coli* have emerged as major cause of diarrheal illness globally and are also responsible for the mortality and morbidity, especially among children. The most visible vehicle for *Campylobacter* are human; though vermin and insects are the other sources of infection to human beings (Ridley *et al.* 2008) and cross contamination is the dominant route of exposure (Nauta *et al.* 2008).

Though poultry processors have used chlorinated water for washing carcasses to reduce *Salmonella* and *Escherichia coli*, such measures may have little effect in reducing *Campylobacter* populations (Morrison and Fleet 1985). Limited research has been done to demonstrate interventions that are effective for reducing, both the prevalence and level of contamination by this pathogen, hence, the present study was carried out to study the efficacy of acidified sodium chlorite (ASC) and tri-sodium phosphate, the two USDA approved generally recognized as safe (GRAS) chemical agents.

Standard culture: Standard culture of *Campylobacter coli* MTCC 1126 was procured from Institute of Microbial Technology (IMTECH), Chandigarh, and working culture of *Campylobacter coli* MTCC 1126 was prepared. The working culture so obtained was stored in egg based medium recommended by Nair *et al.* (1984). The *Campylobacter coli* MTCC 1126 was then subcultured and maintained for further

use. The enriched culture was serially diluted in the sterile normal saline solution (NSS) onto 10⁻¹⁰ dilution and the concentration of the bacteria was determined by turbidimetric method (560 nm). Then 0.1 ml of each dilution was cultured on Park and Sander's agar base by spread plate method and incubated for 24 h at 42–43°C under microaerophilic conditions. After incubation, the colonies were counted using colony counter and a concentration of 10⁶ bacterial cells/ml was obtained by adjusting the dilution with sterile NSS.

Preparation of acidified sodium chlorite (ASC) and tri-sodium phosphate (TSP): In 10 ml of sterile distilled water 1 g of citric acid and 0.8 g of sodium chlorite was dissolved and kept for ripening for 30 min at room temperature (26°C). After ripening, different concentrations of ASC were prepared in distilled water. Like wise, 10% (w/v) solution of TSP was prepared in sterilized distilled water and kept in sterile containers.

Inoculation of *Campylobacter* spp. on skin samples: Chicken breast skin samples (25 g) were collected within 20 min of slaughter from a poultry dressing plant after being removed manually under sterile conditions. The pieces were selected only if there was no visible damage on the skin surface. The skins were transported in an ice box and kept refrigerated till used. To eliminate the contaminated microorganisms present in the samples, skin samples were treated with ultra-violet radiation for 15 min on a clean bench (Min *et al.* 2007). Pre-calibrated standard strain standard strain of *Campylobacter coli* MTCC 1126 (0.1 ml) was inoculated on the skin samples to final concentration of 10⁶ CFU/cm² by drip inoculation method. Skin samples were left undisturbed at 21±1°C for 10 min to allow the bacterial attachment (Conner and Bilgili 1994).

Application of ASC and TSP on skin samples: After inoculation, the skin samples were aseptically placed in the sterile petri dish. The skin samples were subjected to treatment with water at a temperature of 21°C (group 1) and 50°C (group 2), 10% TSP (group 3) and 0.1% ASC (group 4) with different contact times as shown in Table 1. After the

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Table 1. Chemical treatment and various contact times for inhibiting *Campylobacter coli*

Contact time	<i>Campylobacter coli</i> MTCC 1126 10 ⁶ cfu/ml			
	Group 1	Group 2	Group 3	Group 4
0.5	Water @ 21°C (control)	Water @ 50°C	10% TSP	0.1% ASC
1.0				
3.0				

*Group 2 treatment with water 50°C is taken since it is the usual scalding temperature for broilers.

treatment, the skin samples were taken in 10 ml of sterile NSS and triturated in a sterile pestle and mortar. Then 0.1 ml of the triturate was spread onto Park and Sander's selective medium containing 2% agar, 5% sterile defibrinated lysed sheep blood and reconstituted contents of Park and Sander's selective supplement 1 and supplement 2 by surface spread method.

Incubation and enumeration methods: All the inoculated plates were placed into anaerobic containers and flushed with a microaerobic gas mixture of approximately 5% oxygen, 10% carbon dioxide, and 85% nitrogen at 42°C for 48 h. Characteristic *Campylobacter* colonies were counted and subjected to presumptive identification (Park and Sanders 1992). These isolates were further subjected to other biochemical tests for confirmation and species differentiation (Skirrow and Benjamin 1980). These tests were performed at random intervals during the experiments and immediately if any outside contamination or abnormal colony growth was suspected. The data obtained were subjected for statistical analysis (Snedecor and Cochran 1980).

The antimicrobial effect of different organic antimicrobials was evaluated against *Campylobacter coli* individually at different contact time. For this purpose, *Campylobacter coli* MTCC 1126 was applied to the skin and subjected for thermal and chemical treatments at different contact times as shown in Table 1. Individual numerical results of antimicrobial effect of different organic antimicrobials are presented in Table 2.

There was no significant reduction in *Campylobacter coli* counts between group 1 (control) and group 2 (water @ 50°C) at different contact times. But the counts of *Campylobacter*

coli reduced significantly ($P<0.05$) in group 3 (10% trisodium phosphate) and group 4 (0.1% acidified sodium chlorite) at the rate of $\log_{10}$ 2.27 and $\log_{10}$ 1.11 cfu/cm², respectively, at different contact times in comparison to group 1 (control). When the overall means were compared between group 3 and 4, the counts of *Campylobacter coli* reduced significantly ($P<0.05$) at the rate of $\log_{10}$ 1.16/cm². The overall results have shown that the treatment with 10% TSP with a contact time of 1 min was most effective in decontaminating *Campylobacter coli* on the poultry carcasses. Similar to our findings, Slavik *et al.* (1994) dipped post-chilled chicken carcasses in 10% solution of TSP at 50°C for 15 min and found a reduction of 1.5 and 1.2 $\log_{10}$ cfu/cm² in TSP treated carcasses. TSP exerts antimicrobial effect by combination of several factors. Firstly, the high pH (12–13) of TSP solutions leading to high alkalinity could have helped to remove fat films and allow the chemical to contact more bacteria as well as disrupt fatty molecules in the cell membrane causing the cells to leak intracellular fluid (Giese 1993). Secondly, TSP must have caused detachment of bacteria from the surfaces (Kim *et al.* 2007) thereby causing reduction in *Campylobacter* numbers on the surface where it was applied. The high pH of the chemical causes problems for the bacteria to survive and this mechanism led to destruction of bacteria on poultry carcasses (USDA 2002).

When the chicken skin samples were treated with 0.1% ASC, the counts of *Campylobacter coli* reduced significantly ($P<0.05$) at the rate of $\log_{10}$ 1.11/cm² with different contact times in comparison to Group I (control). Kemp *et al.* (2000) conducted similar experiments and reported that citric acid is more effective than phosphoric acid on *Campylobacter* when used to activate ASC. They also reported $\log_{10}$ 2.6 cfu/ml reduction, however, did not mention contact time, spray pressure or duration. ASC chemical action is the result of sodium chlorite (NaClO₂) acidification which when applied to organic matter, a number of organic intermediates are formed (Gordon *et al.* 1972). These intermediates cause disruption of oxidative bonds on the cell membrane making them broad-spectrum germicides (Kross 1984). Due to this non-specific chemical action, it is thought that formation of resistant strains following long exposure are reduced (Kemp *et al.* 2000). When the overall means were compared between

Table 2. *Campylobacter* counts ($\log_{10}$ cfu/cm²) of chicken breast samples treated with different organic antimicrobials

Time (Min)	<i>Campylobacter coli</i> MTCC 1126 $\log_{10}$ cfu/cm ²			
	Group 1	Group 2	Group 3	Group 4
0.5	5.30±0.08 ^{aA}	5.46±0.12 ^{aA}	3.23±0.07 ^{aC}	4.15±0.07 ^{aB}
1.0	5.03±0.08 ^{bA}	5.25±0.06 ^{abA}	2.60±0.17 ^{bC}	4.13±0.16 ^{aB}
3.0	4.93±0.05 ^{bA}	4.91±0.19 ^{bA}	2.61±0.25 ^{bC}	3.63±0.08 ^{bB}
Overall mean±SE	5.08±0.05 ^A	5.21±0.09 ^A	5.21±0.09 ^A	3.97±0.09 ^B

Means bearing at least one common small letter superscript (a and b) in a column and capital letter superscript (A, B, C) in a row do not differ significantly ($p<0.5$); Group 1, Control (water @ 21°C); group 2 (water @ 50°C); group 3, 10% TSP; group 4: 0.1% ASC.

group 3 (10% TSP) and 4 (0.1% ASC), the counts of *Campylobacter coli* reduced significantly ($P < 0.05$) at the rate of $\log_{10}$ 1.16 cfu/cm² in group 3.

The results revealed that treatment with 10% TSP at a contact time of at least 1 min was most effective in decontaminating *Campylobacter coli* on the poultry carcasses which could be due to the reason that at this contact time the chemical exhibited attachment prevention properties. The study also concluded that the treatment with 0.1% ASC with at least 3 min contact time was most effective in decontaminating *Campylobacter* on poultry carcasses.

SUMMARY

In the present study attempts were made to decontaminate carcasses against *Campylobacter coli* by acidified sodium chlorite (ASC) and trisodium phosphate (TSP). Pre-calibrated standard strain of *Campylobacter coli* MTCC 1126 (0.1 ml) was inoculated on the chicken skin samples to final concentration of 10^6 cfu/cm² and treated with different antimicrobials by dipping them for different contact time. A total of four groups were taken in this experiment with treatment groups of water @ 21°C (group 1), water @ 50°C (group 2), 10% TSP (group 3) and 0.1% ASC (group 4) at different contact time of 0.5 min, 1 min and 3 min. The counts of *Campylobacter coli* reduced significantly in group 3 and group 4 @ $\log_{10}$ 2.27 and $\log_{10}$ 1.11, respectively, at different contact times in comparison to group 1. When the overall means were compared between contact times in comparison to group 1. When the overall means were compared between group 3 and group 4, the count of *Campylobacter coli* reduced significantly @ $\log_{10}$ 1.16 reductions, while compared to other groups. The overall results have shown that the treatment with 10% TSP at the contact time of at least 1 min was most effective in decontaminating *Campylobacter coli* on poultry carcasses.

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