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การลดโอกาสเสี่ยงจากการปนเปื้อนข้ามของ *Salmonella typhimurium* ในการเตรียมผักสดพร้อมบริโภค
Reduction of Possible Risk from Cross-contamination of *Salmonella typhimurium* during Preparation Ready-to-eat Fresh Produce

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Abstract

The study on reduction of *Salmonella typhimurium* in artifically cross-contaminated from unsanitary cutting board transferred on sliced cucumber, sliced tomatoes, shredded cabbage ,and shredded carrot were conducted. The cutting boards were contaminated as 10^8 – 10^9 CFU/mL and 10^3 – 10^4 CFU/mL, respectively. Two type of chlorinated sanitizers, sodium hypochlorite (NaOCl) 25, 50, 100 and 200 ppm and sodium chlorite ($NaClO_2$) 25, 50 and 100 ppm adjusted at pH 4 with acetic acid were used to inactivate *S. typhimurium* contaminated on these vegetables . Using $NaClO_2$ at concentration of 50 ppm for 15 min was effectively destroyed high contamination prepared produce as sliced cucumber, sliced tomatoes, shredded cabbage ,and shredded carrot decreased by 2.9, 2.0, 2.2 and 2.8 log (or 99.9, 99.0,99.3 and 99.8% reduction). Lower concentration of $NaClO_2$ at 25 ppm for 15 min was completely destroyed low contaminated prepared produce as 2.5, 2.7 and 3.0 log (or 100, 100 and 100% reduction) except sliced cucumber which reduced by 2.3 log (97.9%). Survival and growth of *S. typhimurium* on the model prepared salads stored at 5 °C and 10 °C were examined. After inoculating either normal cells of *S. typhimurium* , acid-stressed cell (pH 5.8, 1 hr.), cold–stressed cell (10 °C, 30 min) and sanitizer-stressed cell (NaOCl 30 ppm, 10 min) 10^3-10^4 CFU/mL on prepared vegetables. Without applying sanitizer, all cells decreased by 2-3 log and 1.5-2 log unit on sliced cucumber and shredded carrot during storage for 14 days at 5 °C. At higher temperature 10 °C both control and stressed cells showed better resistant to adverse conditions, cells decreased by 0.5-2 log during 7 days. After washing sliced cucumber and shredded carrot with $NaClO_2$ 50 ppm 15 min, none of those cells were founded on Xylose Lysine Desoxycholate agar. However enrichment step by modified Trypticase Soy broth could recovered injured cells. This study indicates that unsanitary cutting board cause cross-contaminated which transferred foodborned pathogen to ready-to-eat fresh vegetables but bacterial hazard could be reduced by washing with suitable type of sanitizers combination with low temperature. Low temperature as 5 °C could inhibit pathogenic bacterial growth, therefore, enhance safety of ready-to-eat fresh produce consumption.

บทคัดย่อ

การศึกษาการลดปริมาณแบคทีเรีย *Salmonella typhimurium* โดยสร้างสภาวะจำลองการปนเปื้อนข้ามระหว่างการเตรียมผักสลัดได้แก่แตงกวาหั่นแว่น มะเขือเทศหั่นแว่น กะหล่ำปลีและแครอทหั่นฝอย ปนเปื้อนข้ามจากเขียงที่มีปริมาณเชื้อปริมาณสูงและปริมาณต่ำคือ 10^8 – 10^9 CFU/ml และ 10^3-10^4 CFU/ml ล้างผักที่ปนเปื้อนเหล่านั้นด้วยสารละลายโซเดียมไฮโปคลอไรท์ (NaOCl) 25 50 100 และ 200 ppm และสารละลายโซเดียมคลอไรท์ ($NaClO_2$) 25 50 และ 100 ppm ในน้ำประปาปรับพีเอช 4 ด้วยกรดแอซีติก พบว่าสารละลาย $NaClO_2$ ลดจำนวนเซลล์ *S. typhimurium* ที่ปนเปื้อนบนผักทั้ง 4 ชนิดได้ดีกว่าสารละลาย NaOCl สารละลาย $NaClO_2$ 50 ppm 15 นาที ทำลายแบคทีเรียที่ปนเปื้อนปริมาณสูงในแตงกวาหั่นแว่น มะเขือเทศหั่นแว่น กะหล่ำปลีหั่นฝอย และ แครอทหั่นฝอยได้ 2.9, 2.0, 2.2 และ 2.8 log (หรือ 99.9, 99.0, 99.3 และ 99.8%) ที่ความเข้มข้นต่ำกว่า 25 ppm 15 นาที สามารถทำลายแบคทีเรีย *S. typhimurium* ที่ปนเปื้อนปริมาณต่ำในผักทั้ง 3 ชนิดได้ 2.5, 2.7 และ 3.0 log (หรือ 100, 100 และ 100%) ยกเว้นแตงกวาหั่นแว่นซึ่งเซลล์ลดลง 2.3 log (97.9%) ต่อมาเก็บผักที่ปนเปื้อนแต่ไม่ล้างด้วยสารฆ่าเชื้อที่อุณหภูมิ 5 °C และ 10 °C เพื่อศึกษาการเจริญและการรอดชีวิตของ *S. typhimurium* ในผักสลัด พบว่า *S. typhimurium* ชนิดเซลล์ปกติและเซลล์ที่ผ่านความเครียดของกรดพีเอช 5.8 (1 ชั่วโมง) หรือความเย็น 10 °C (30 นาที) หรือสารฆ่าเชื้อ NaOCl 30 ppm (10 นาที) ในแตงกวาหั่นแว่นจำนวนเริ่มต้น 10^3-10^4 CFU/mL ที่อุณหภูมิ 5 °C มีจำนวนลดลง 2-3 log ส่วนแครอทหั่นฝอยมีจำนวนลดลง 1.5-2 log ระหว่างเก็บรักษา 14 วัน ขณะที่แตงกวาหั่นแว่นเก็บที่ 10 °C มีจำนวนเซลล์ลดลง 1-2 log และแครอทหั่นฝอยลดลงเพียง 0.5 log ระหว่างการเก็บ 7 วัน หลังจากล้างผักด้วย $NaClO_2$ 50 ppm 15 นาที เมื่อเพาะเลี้ยงด้วย Xylose Lysine Desoxycholate agar ตรวจไม่พบ *S. typhimurium* ทั้งเซลล์ปกติและเซลล์ผ่านสภาพเครียดทั้ง 3 ชนิด แต่ตรวจพบแบคทีเรียนี้หากเพาะเลี้ยงด้วย modified Trypticase Soy broth การศึกษานี้แสดงให้เห็นว่าเขียงที่ไม่ถูกสุขลักษณะทำให้เกิดการปนเปื้อนข้ามของแบคทีเรียที่

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ก่อให้เกิดโรคสู่ผักโดยเฉพาะผักสดพร้อมบริโภค แต่สามารถลดโอกาสเสี่ยงได้โดยใช้การล้างด้วยสารฆ่าเชื้อที่เหมาะสมร่วมกับการเก็บรักษาที่อุณหภูมิ 5 °C จะช่วยยับยั้งการเจริญของแบคทีเรียและช่วยเพิ่มความปลอดภัยในการบริโภคผักสด

Introduction

Freshly prepared salads become a popular food item according to health and diet trends of today's consumer. Generally, consumer have considered fresh fruit or vegetable salads as safe foods until foodborne illnesses have been traced to fresh salads. Several of the pathogens of concerns have associated in fresh salads include *Salmonella* spp. (Beuchat, 1996; Fain, 1996) Cross-contamiantion, due to improper hygiene or food handling practices, certainly plays a role in foodborne transmission of pathogens (Bryan, 1988).

Washing step have been known to reduce the number of viable pathogenic bacteria on produce, however water in that step needs to add chlorine compounds as sanitizer due to its effectiveness antimicrobial agent. Storage at low temperature could also extend shelf life and enhance food safety. Since low temperatures as refrigeration limits the growth of most foodborne pathogens, so product have to maintain at refrigeration temperatures during storage.

The objectives of this study were to determine the effective of the chlorine compounds in reducing bacterial contamination on fresh produce by simulating cross-contamination during preparation foods on unsanitary cutting board. Since the temperature was crucial on growth and survival of *S.* Typhimurium, in order to determine growth charactetistics of this bacteria. Normal and stressed cell was inoculated on model fresh produce. The efficancy of chlorine on reduction of normal and stressed cell were determined.

Materials and Methods

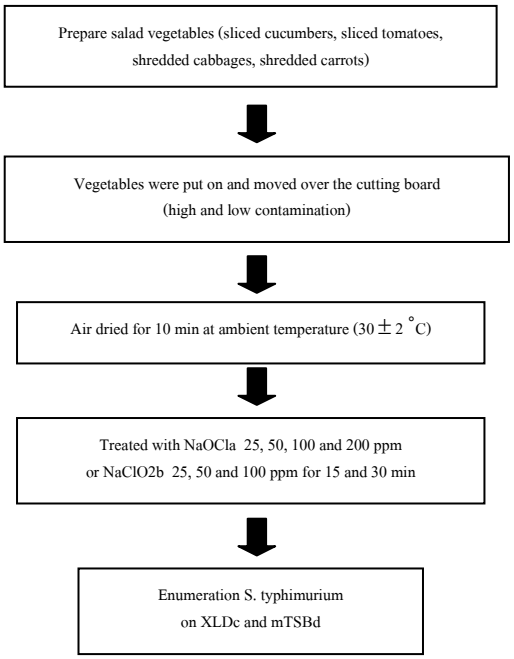
- **Preparation of the *S. typhimurium* suspension**

A culture of *S. typhimurium* (strain ATCC 13311) was obtained from Bangkok MERCEN . Stock culture on Trypticase Soy Agar (TSA; Merck Laboratories, Germany) was store at 5 °C. Prior to use, the culture was subjected to two successive transfer by loop inocula to 9 mL was made into 100 mL Trypticase Soy Broth (TSB; pH 7.3; Merck Laboratories, Germany) incubated at 37 °C for 18 h, and used as inocula. Dilution was made and gain the final cell concentration high and low as 7.5-8 $\log_{10}$CFU/mL and 2.5-3 $\log_{10}$CFU/mL.

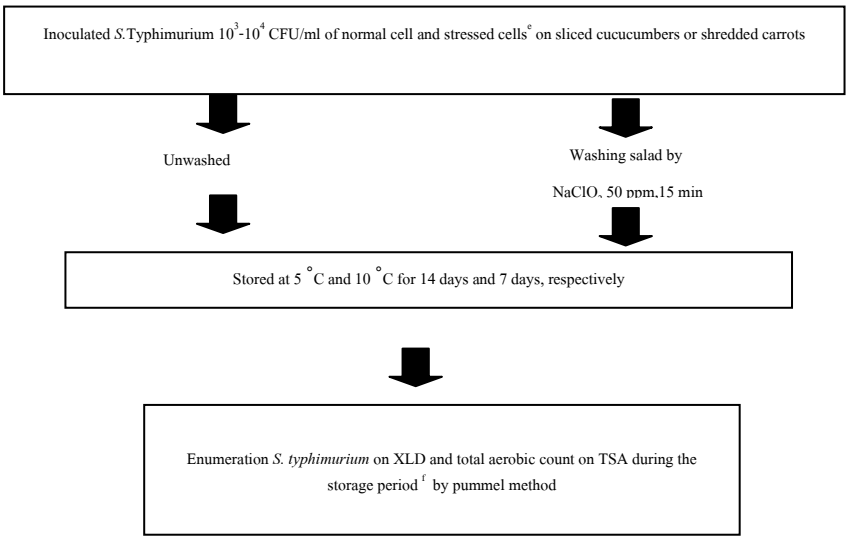
- **Simulating bacterial transfer from contaminated cutting board to vegetables**

To simulate cross-contamination via cutting board, prepared salad vegetables were placed on and moved over the inoculated cutting board. They were then removed and allowed to dry for 10 min at ambient temperature (30 ± 2 °C). The concentration of *S. typhimurium* on inoculated on each vegetables were determined on 5 trials with two replicates.

- **Treatment of salad vegetables with chlorinated water**



- **Survival and growth of *S. typhimurium* in salad vegetables**



Note [a] XLD = Xylose Lysine Desoxychlolate Agar

[b] NaOCl = Sodium hypochlorite solution adjusted at pH 4 with 5M acetic acid

[c] $NaClO_2$ = Sodium chlorite solution adjusted at pH 4 with 5M acetic acid

[d] mTSB = modified Trypticase Soy Broth; samples were enriched with mTSB to recover *S. typhimurium* cells

[e] stressed cell = acid-stressed cell (pH 5.8, 1 h) , cold stressed cell (10 °C, 30 min) and sanitizer stressed cell (NaOCl 30 ppm, 10 min)

[f] samples were taken to enumerate at 0, 2, 4, 7 and 14 days at 5 °C and 0, 2, 4 and 7 days at 10 °C

Results and Discussion

The population of *S. typhimurium* in artificially contaminated from cutting board that inoculated at high level (10^9-10^9 CFU/ml) and low level (10^3-10^4 CFU/ml) on sliced cucumbers, sliced tomatoes, shredded cabbages and shredded carrots were ranged 7.6-8.2 $\log_{10}$CFU/g and 2.4-3.0 $\log_{10}$CFU/g, respectively.

The efficacy of washing with NaOCl and $NaClO_2$ in killing *S. typhimurium* on contaminated sliced or shredded vegetables are shown in Table 1. Population of *S. typhimurium* on sliced cucumber and sliced tomatoes were reduced by 2.0 log (99%) and 1.6 log (98%), respectively, as result of 200 ppm NaOCl at 30 min. Treated produce with 200 ppm for up to 30 min could reduce cells more on shredded cabbages and shredded carrots by 2.6 and 3.3 log, however , at these conditions caused these prepared produce wilt and pale.

The best condition to reduce cells using of $NaClO_2$ was 50 ppm 30 min. This condition resulted in reductions of 3.3 log (99.9%) for sliced cucumber and 3.7 log (99.9%) for shredded carrots. The maximum reduction on sliced tomatoes showed 3.1 log (99.9%) by washing them with 100 ppm of $NaClO_2$ at 30 min. Any changes on their texture and color was observed. Efficiency of $NaClO_2$ in eliminating *S. typhimurium* on salad vegetables showed significantly higher than NaOCl. Although chlorine did reduce microbial populations, it was not completely effective. Not only the bactericidal properties of sanitizer itself, effectiveness depends on chemical and physical properties of each produce such as tissue surface. (Takeuchi and Frank, 2001). Thus, in this study the efficiency of $NaClO_2$ on bacterial reduction was different in sliced tomatoes compared to other vegetables.

At low level contamination on sliced cucumber exposed to NaOCl solution concentrate of 25 ppm completely killed *S. typhimurium* cells within 30 min, while the same concentration of NaOCl completely destroyed *S. typhimurium* cells on shredded cabbage within 15 min. The treatment with higher NaOCl at 50 ppm eliminated *S. typhimurium* on both sliced tomatoes and shredded carrots 100% within 15 min. (Table 2)

Using of $NaClO_2$ solution at concentrate of 25 ppm was also completely killed low contamianated *S. typhimurium* cells on sliced tomatoes, shredded cabbage and shredded carrots within 15 min while reduced cells on sliced cucumbers 99.5% and completely killed cells at 25 ppm at longer period 30 min.

Table 1 Population of *S typhimurium* detected on high level inoculated salad vegetables treated with chlorinated water.

Products	Treatment		Population of *S.* Typhimurium[a] (log_{10}CFU/g) at time (min)	
			15	30
Sliced cucumbers	NaOCl	50 ppm	6.18 ± 0.48[b]	6.03 ± 0.30
		100 ppm	6.21 ± 0.22	5.87 ± 0.06
		200 ppm	5.78 ± 0.35	5.71 ± 0.28
	$NaClO_2$	25 ppm	5.26 ± 0.04	5.05 ± 0.59
		50 ppm	4.85 ± 0.25	4.41 ± 0.23
		100 ppm	5.10 ± 0.22	5.13 ± 0.15
Sliced tomatoes	NaOCl	50 ppm	6.58 ± 0.13	6.40 ± 0.00
		100 ppm	6.42 ± 0.12	6.04 ± 0.23
		200 ppm	6.39 ± 0.14	6.01 ± 0.24
	$NaClO_2$	25 ppm	6.09 ± 0.20	5.96 ± 0.18
		50 ppm	5.66 ± 0.02	5.60 ± 0.36
		100 ppm	5.49 ± 0.70	4.59 ± 0.81
Shredded cabbages	NaOCl	50 ppm	6.26 ± 0.47	6.02 ± 0.23
		100 ppm	6.32 ± 0.02	6.21 ± 0.19
		200 ppm	6.12 ± 0.22	5.06 ± 0.08
	$NaClO_2$	25 ppm	5.55 ± 0.21	5.15 ± 0.32
		50 ppm	5.50 ± 0.54	5.15 ± 0.32
		100 ppm	5.00 ± 0.08	5.00 ± 0.06
Shredded carrots	NaOCl	50 ppm	5.78 ± 0.37	5.75 ± 0.42
		100 ppm	4.78 ± 0.53	4.44 ± 0.22
		200 ppm	5.02 ± 0.64	4.87 ± 0.31
	$NaClO_2$	25 ppm	6.15 ± 0.30	5.37 ± 0.23
		50 ppm	5.35 ± 0.28	4.49 ± 0.46
		100 ppm	5.80 ± 1.03	5.04 ± 0.20

Note [a] = Population of *S. typhimurium* cell enumerated by XLD; [b] = standard deviation; n=2

Table 2 Population of *S.*Typhimurium detected on low level inoculated salad vegetables treated with chlorinated water.

Products	Treatment		Population of *S.* Typhimurium[a] (log_{10}CFU/g)at time (min)	
			15	30
Sliced cucumbers	NaOCl	50 ppm	1.30 ± 0.00	0.00 ± 0.00
		100 ppm	0.00 ± 0.00	0.00 ± 0.00
	$NaClO_2$	25 ppm	0.05 ± 0.71	0.00 ± 0.00
		50 ppm	0.00 ± 0.00	0.00 ± 0.00
		100 ppm	0.00 ± 0.00	0.00 ± 0.00
Sliced tomatoes	NaOCl	50 ppm	0.80 ± 1.13	1.60 ± 0.00
		100 ppm	0.00 ± 0.00	0.65 ± 0.92
	$NaClO_2$	25 ppm	0.00 ± 0.00	0.00 ± 0.00
		50 ppm	0.00 ± 0.00	0.00 ± 0.00
		100 ppm	0.00 ± 0.00	0.00 ± 0.00
Shredded cabbages	NaOCl	50 ppm	1.00 ± 0.00	0.00 ± 0.00
		100 ppm	0.65 ± 0.92	1.50 ± 0.28
	$NaClO_2$	25 ppm	0.00 ± 0.00	0.00 ± 0.00
		50 ppm	0.00 ± 0.00	0.00 ± 0.00
		100 ppm	0.00 ± 0.00	0.00 ± 0.00
Shredded carrots	NaOCl	50 ppm	1.78 ± 0.25	1.62 ± 0.46
		100 ppm	0.00 ± 0.00	0.65 ± 0.92
	$NaClO_2$	25 ppm	0.00 ± 0.00	0.00 ± 0.00
		50 ppm	0.00 ± 0.00	0.00 ± 0.00
		100 ppm	0.00 ± 0.00	0.00 ± 0.00

Note [a] = Population of *S. typhimurium* cell enumerated by XLD

[b] = standard deviation; n=2

Survival and growth of *S. typhimurium* on salad vegetables as affected by temperature was conducted. Populations of *S. typhimurium* in sliced cucumbers inoculated normal cells and stressed cells at 10^3-10^4 CFU/mL and stored at 5 °C and 10 °C for up to 14 days and 7 days. Reduction of population on unwashed sliced cucumber, inoculated with normal cells and stressed cells of *S.* Typhimurium, decreased by 2-3 log during the 14 days for held at 5 °C, whereas populations stored at 10 °C decreased lower approximately 1-1.4 log during 7 days. (Figure 1). *S. typhimurium* was mesophilic pathogen, although it could survive under refrigeration and slowly grow, cells may grow faster during any temperature abuse of food. (Marth, 1998) Total aerobic count decreased by 1.5-3 $\log_{10}$CFU/g on unwashed shredded carrots stored at 5 °C within 14 days (Figure 2), whereas total cells decreased only 0.5 log when they were kept at 10 °C during 7 days. Stressed cells survived better than normal cells when kept at 10 °C compared to result obtained at 5 °C. After washing sliced cucumber and shredded carrots with $NaClO_2$ 50 ppm 15 min, both normal cells and stressed cells of *S. typhimurium* cells were not detected on XLD (during storage period at 5 °C and 10 °C) but could be recovered in modified Soy broth (mTSB).

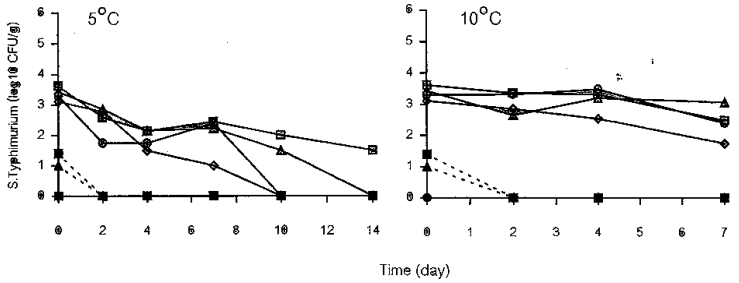


Figure 1 Population changes of *S.typhimurium* inoculated on unwashed or washed sliced cucumbers to storage at 5 °C and 10 °C; unwashed sliced cucumbers: (🡐) normal cells; (Y) acid-stressed cells; (↖) cold stressed cells; (↓) sanitizer stressed cells; washed sliced cucumbers: (◆) normal cells; (■) acid stressed cells; (▲) cold stressed cells; (●) sanitizer stressed cells.

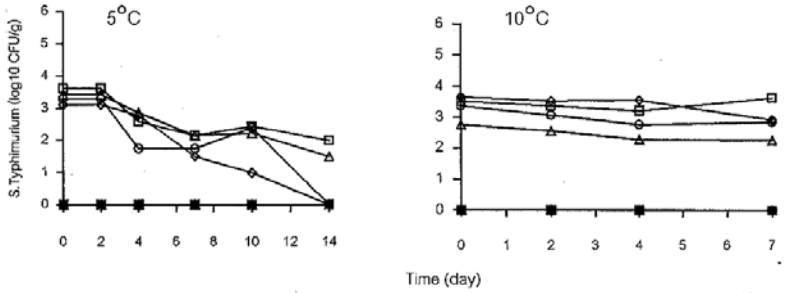


Figure 2 Population changes of *S. typhimurium* inoculated on unwashed or washed shredded carrots to storage at 5 °C and 10 °C; unwashed shredded carrots: (🡐) normal cells; (Y) acid stressed cells.

Conclusion

Cross-contamination experiments showed that *S.*Typhimurium can be transferred from cutting boards during food preparation to salad vegetables. Treatment with $NaClO_2$ to decontaminate fresh produce was more effective than NaOCl. $NaClO_2$ concentration at least 25 ppm was effectively killed highly contaminated on sliced cucumber, shredded cabbage and shredded carrot 2.0-2.8 log whereas destroyed bacterial cell only 1.6-1.7 log on sliced tomatoes. Using NaOCl 50 ppm reduced 1.0-2.4 log on salad vegetables. Although the concentration at 200 ppm was the most effectiveness but it cause off-flavor, off-odor and soft texture. Treated low contaminated on salad vegetables with $NaClO_2$ inactivated cell by 100% at concentration 25 ppm except on sliced cucumber. The results showed that similarly reduction when using the same concentration of NaOCl, except on shredded carrot. The study of survival of *S.*Typhimurium on unwashed cucumber and shredded carrot stored at 5 °C and 10 °C revealed that normal cell and stressed cell decresed for the time of storage. Stressed cells showed the ability to survive than normal cell when kept at 10 °C. Washing produces with $NaClO_2$ 50 ppm 15 min and storage at both temperatures indicated that this bacterium could not grow but be able to survive during storage since it was found positive after enrichment.

คำขอบคุณ

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