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EFFECT OF USING SOME CHEMICAL SANITIZERS ON THE REMOVAL OF SURFACE MICROORGANISMS FROM FRESH CUCUMBER AND TOMATO FRUITS

EL.Makhzangy, A.

Agro-Industry Dept., Institute of Efficient Productivity, Zagazig Univ., Zagazig, Egypt.

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ABSTRACT: This work was carried out to study the effect of using some chemical sanitizers as sodium hypochlorite, acetic acid or hydrogen peroxide (H_2O_2) on the reduction of the natural surface microorganisms found on cucumber and tomato fruits as well as artificially *E.coli* infected fruits in comparison to fruits washed by warm or hot water, mild soap or potassium permanganate.

Treatments of cucumbers showed that: (a) The use of 2% acetic acid (50°C)/3 min removed 88-93.4% of the initial surface microorganisms; (b) The use of 3% hydrogen peroxide (H_2O_2) removed 75.6-89.3% of microbial groups and; (c)Washing by mild soap at room temperature/ 3 min removed 78.7-81.6% of the initial microorganisms

Treatments of tomato fruits showed that: (a) Immersing in 150ppm sodium hypochlorite (50°C)/3 min removed 80.8-92.2% of the initial microorganisms. (b) Washing by mild soap at room temperature/3 min removed 70.5-89.5% of the initial microorganisms whereas, the rest of the used treatments did not organoleptically accepted.

The obtained results may be used in the packaging houses and in application of HACCP programs for agricultural goods that marketed or consumed fresh.

Key words: sanitizers, microbial removal, cucumber, tomatoes.

INTRODUCTION

Fruits and vegetables are frequently in contact with soil, insects and animals during growth and harvesting in the field. Thus, their surfaces are not free from natural contaminants (Pao and Brown, 1998). The presence of coliform bacteria including fecal coliforms on fresh vegetables don't usually represent a public health concern and not allowed from FDA and WHO agences (Andrews *et al.*, 1995). Thus, the reduction of fruit surface microflora is important to improve safety and quality of fresh fruits.

Food and Drug Administration (FDA) proposed rules requiring the application of hazard analysis of critical control points (HACCP) for the production of fresh fruits and vegetables products. Many researchers were reported on the use of washing and chemical treatments such as; sodium hypochlorite, acetic acid, peroxyacetie acid, phosphoric acid, sodium hydroxide, hydrogen peroxide and ammonium compounds to reduce some pathogenic microorganisms from fruits and vegetables surfaces (Beuchat *et al.*, 1998; Sapers *et al.*, 1999; Takeuchi & Frank, 2000 and Flesichman *et al.*, 2001).

Some of these studies showed that the organism E.coli can survive for prolonged periods on freshly peeled orange, water melon, cantaloupe, strawberry, broccoli, cucumber, green pepper and lettuce (Pao and Brown, 1998 and Yu *et al.*, 2001).

Brackett (1994) showed that improper handling of fruits and thier products can serve as vehicles for both spoilage and pathogenic microorganisms.

The increase of illnesses associated with consumption of fresh vegetables may be attributed to changes in agricultural practices, handling, distribution, processing and consumption patterns (FDA, 2000).

Understanding the nature of microbial attachment and the role of disinfecting chemicals would facilitate the development of washing treatment that will effectively reduce the natural microorganisms of fresh products and may therefore minimize the risk of food borne illnesses (Takeuchi and Frank, 2000).

Chemical santizers have been widely used in food processing to reduce undesirable microorganisms. However, the efficiency of common chemical

sanitizers on the surface of fruits and vegetables may be limited or unpredictable for example, Reina *et al.* (1995) reported that the use of chlorine dioxde in hydrocooling water of cucumbers was effective in the controlling of microbial population in the water but had little effect on microorganisms on the fruits surface. Similary, Pio & Davis (1999) and Karen & Holt (2000) found that chlorine treatment had little effect on the surface microflora of tomatoes and oranges during the packing operation.

The present work aimed to study the effect of using some sanitizers on reducing natural microorganisms from fresh cucumbers and tomatoes surface as well as contaminated with *E. coli*.

MATERIALS AND METHODS

Fresh cucumber variety "score" and Fresh tomato variety "Luxor" used in this study were obtained from El-Salhia Farm, Sharkia Gavornorate, Egypt. These vegetables were stored at 4°C for one day until used.

E.coli Culture Preparation

A non pathogenic pure culture of *E.coli* was obtained as a gift from Microbiology Lab., Fac. Medicine, Mansura University. Consecutive propagation and maintaing of the culture were carried out on tryptic soy agar (Difico) then kept at 4°C until used. The active culture was streaked on tryptic soy agar, incubated for 24 hrs. at 37°C then transferred to flasks containing 100ml sterile saline solution. This suspension was count by plating on Mackonky agar, it contained 8.0×10^6 cfu/ml and used as a stock suspension.

Infection with *E. coli*

Each 100ml of the stock suspention was diluted using 3 litres of sterile saline solution then poured on the fruits in volume enough to cover them for 5min., then drained.

Determination of the initial number of microorganisms: the infected and non infected fruits were individually soaked in a sterile saline solution with volume equal to the total area of the fruit surface and shaked for 10min., thus the total count of microorganisms groups found in one ml of soaking suspension equally expresses the total count of microorganisms group present on one cm2 of the fruit surface Senter

et al. (1985). The obtained number of microorganisms groups are the initial number of the untreated fruits.

Treatment with Different Sanitizers

The infected or non-infected fruits were dipped in aqueous solutions made in tap water to simulate the conditions in food factories of the following sanitizers for 2 or 3 min. at 25±2 or 50°C,

1- Sodium hypochlorite (NaOCl, Agropharm, UK) at 50, 100 or 150 ppm.

2- Acetic acid (CH_3CooH. Merck) at 1 or 2% (V/V).

3- Hydrogen peroxide (H_2O_2; Merck) at 1 or 2 or 3% (V/V).

In comparison to the traditional washing with either, warm water (50°C), hot water (70°C), mild soap (FEBA, household detergent, Alex.) or Potassium Permanganate (100 ppm) for 2 or 3 min after these treatments, the fruits were immediately rinsed twice for 2min with distilled water to remove the chemical residues. The residue of the microorganisms groups were counted as above mentioned for the initial number determination. Standard plate count agar and potato dextrose agar are the media used for determination of total plate count and mould & yeast; respectively.

Consumer Acceptability

Colour surface, appearance and taste are the freshness criteria which expresses as a consumer acceptability were run with the aid of five stuff member (Gorny *et al.*, 2000).

RESULTS AND DISCUSSION

Firstly, the initial numbers of microorganisms and infected *E.coli* on the surfaces of cucumbers and tomatoes were differed, this differences may be reflecting differences in adhesion characteristics, competitive mycoflora or type of mycoflora on the surfaces of two type of fruits.

Chlorinated Water

Date from Table (1) showed that soaking cucumbers for 3min of 150 ppm sodium hypochlorite at room temperature had a high removal percentage (75.5%) of infected *E.col.* and the fruits had a good consumer acceptability. Meanwhile the immersion for 3min in 100ppm sodium hypochlorite at 50°C removed about 71.5-75.2% of the initial numbers of both natural microorganisms and infected

Table 1: Effect of immersing fresh cucumbers and tomatoes in chlorinated water on the removal of surface microorganisms

Immersing treatments	Natural microorganisms				Infected *E.coli*		Consumer acceptability
	bacteria		mold & yeast		log cfu/ cm2	% removal	
	log cfu/ cm^2	% removal	log cfu/ cm^2	% removal			
Cucumbers							
Untreated:	4.15		3.97		4.27		Good
In 50ppm sodium hypochlorite(25±2°C) for:							
2 min	2.84	31.6	2.43	38.8	3.00	29.7	Good
3 min	2.80	32.5	2.41	39.3	2.62	38.6	Good
In 50ppm sodium hypochlorite(50°C) for:							
2 min	2.17	47.7	2.42	39.1	2.23	47.8	Good
3 min	2.10	49.4	2.40	39.5	2.00	53.2	Good
In 100ppm sodium hypochlorite(25±2°C) for:							
2 min	2.49	40.0	2.29	42.3	1.25	70.7	Good
3 min	2.47	40.5	2.17	45.3	1.15	72.8	Good
Immersing in 100ppm sodium hypochlorite (50°C) for:							
2 min	1.46	64.8	1.14	71.3	1.17	72.6	Good
3 min	1.16	72.1	1.13	71.5	1.06	75.2	Good
In 150ppm sodium hypochlorite(25±2°C) for:							
2 min	2.46	40.7	1.93	51.3	1.16	72.8	Good
3 min	2.26	45.5	1.82	54.2	1.05	75.5	Good
In 150ppm sodium hypochlorite (50°C) for:							
2 min	0.85	79.5	0.71	82.0	0.77	82.0	Good
3 min	0.54	87.0	0.28	93.0	0.62	85.4	Rejected
Tomatoes							
Untreated:	4.69		2.95		2.86		Good
In 50ppm sodium hypochlorite(25±2°C) for:							
2 min	3.69	21.3	1.49	49.5	2.14	25.5	Good
3 min	3.14	33.0	1.34	54.6	2.07	27.6	Good
In 50ppm sodium hypochlorite(50°C) for:							
2 min	3.51	25.2	1.32	55.3	2.03	29.0	Good
3 min	3.06	34.8	1.12	62.0	1.79	37.4	Good
In 100ppm sodium hypochlorite(25±2°C) for:							
2 min	3.19	32.0	1.16	60.7	1.87	34.6	Good
3 min	2.08	55.7	0.87	70.5	1.24	56.6	Good
In 100ppm sodium hypochlorite(50°C) for:							
2 min	1.12	76.1	0.63	78.6	0.86	69.9	Good
3 min	0.92	80.4	0.42	85.8	0.80	72.0	Good
In 150ppm sodium hypochlorite(25±2°C) for:							
2 min	1.77	62.3	0.83	71.9	0.89	68.9	Good
3 min	1.00	78.7	0.62	78.9	0.73	74.5	Good
In 150ppm sodium hypochlorite(50°C) for:							
2 min	1.23	74.8	0.59	80.0	0.79	72.4	Good
3 min	0.53	88.7	0.23	92.2	0.55	80.8	Good

E.coli and the fruits also had a good consumer acceptability. On the other hand, off flavour (chlorine odour) was noticed when the cucumber were immersed in 150 ppm sodium hypochlorite at 50°C for 3min and the fruits were rejected.

As shown in Table (1), all tomatoes samples which treated with sodium hypochlorite till 150ppm at 50°C for 3min had a good characteristics for consumer acceptability, and reduced approximately 81-92% of the initial microorganisms. This indicating that microorganisms adhered less tenaciously to the tomato surface as compared to the cucumber surface. These findings are in agreement with both Beuchat *et al.* (1998) who stated that the effectiveness of 200 or 2.000 ppm of chlorine in killing *E.coli*.O_{157}:H_7 on tomatoes was similar; and Yu *et al.* (2001) who indicated that the treatment of the inoculated strawberry fruit with pathogenic strains of *E.coli* using 100 or 200ppm of NaOCl were equally effective and causes approximately a 1.3 log reduction compared to the initial inoculation levels.

Hydrogen Peroxide

Results from Table (2) revealed that the removal percent of natural surface microorganisms and infected *E.coli* were increased gradually with increasing of hydrogen peroxide concentration from 1 to 3% and exposure time from 2 to 3 min. The removal percent ranged between 71-78% in case of cucumber fruits without any adversely effects on the consumer acceptability. On the other side, H_2O_2 led to increase the removal of all natural microorganisms and infected *E.coli* from tomato surfaces in the order as cucumbers but the consumer acceptability of tomotoes were rejected when 3% concentration of H_2O_2 was used because the pallid colour and dryness of tomato skin was occurred. Our results are in line with findings of *Park and Beuchat (1999)* who indicated that bacterial population decreased on cantaloupes when H_2O_2 concentration increased from 1 to 3% and it was the only tested compound that was clearly more effective at the higher concentration.

Aectic Acid

From data in Table (3), it was found that the use of 1% or 2% acetic acid in immersing raw cucumbers or tomato for 2 or 3 min either in 25± 2°C or 50°C removed partially the number of

Table 2: Effect of immersing fresh cucumbers and tomatoes in hydrogen peroxide water on the removal of surface microorganisms

Immersing treatments	Natural microorganisms				Infected *E.coli*		Consumer acceptability
	bacteria		mold & yeast				
	log cfu/ cm2	% removal	log cfu/ cm2	% removal	log cfu/ cm2	% removal	
Cucumbers							
Untreated:	4.11		3.92		4.27		Good
In 1% H2O2 for:							
2 min	3.55	13.6	1.14	70.9	2.58	39.6	Good
3 min	2.14	47.9	1.08	72.5	2.58	39.6	Good
In 2% H2O2 for:							
2 min	2.02	50.9	1.00	74.5	1.33	68.9	Good
3 min	1.73	57.9	0.83	78.8	1.20	71.9	Good
In 3% H2O2 for:							
2 min	1.09	73.5	0.81	79.3	1.34	68.6	Good
3 min	0.87	78.8	0.42	89.3	1.04	75.6	Good
Tomatoes							
Untreated:	4.15		3.00		2.44		Good
In 1% H2O2 for:							
2min	2.18	47.5	1.59	47.0	1.82	25.4	Good
3min	2.00	51.8	1.42	52.7	1.65	32.4	Pass
In 2% H2O2 for:							
2 min	1.64	60.5	1.02	66.0	1.43	41.4	Pass
3 min	1.17	71.8	0.97	67.7	1.26	48.4	Pass
In 3% H2O2 for:							
2 min	1.14	72.5	0.69	77.0	0.72	70.5	Reject
3 min	1.02	75.4	0.52	82.7	0.30	87.7	Reject

both natural microorganisms and infected *E.coil.* from their surfaces. The removal percent increased with increasing concentration, temperature and time exposure. The removal percentage from cucumber are higher (88-93%) than those obtained in case of tomato (71-74%). Also, immersion in acetic acid till 2% did not diversely affect the consumer acceptability of treated cucumbers. Meanwhile, the immersed tomatoes in 1% acetic acid at 50°C for 3 min and in 2% acetic acid at 25°C or 50°C for 2 or 3 min were rejected because an obvious effects on flavour (acid flavour) as well as weakness of the texture were noticed. Wright *et al.* (2000) found that 5% acetic acid caused a 3-log cfu/cm^2 reduction in infected apples with pathogen compared to the initial number (control).

Traditional Washing with Water, Mild Soap or Potassium Permanganate

Data in Table (4) shows the Effect of traditional washing with (warm water 50°C, hot water; 70°C, mild soap or 100 ppm potassium permanganate) on the removal of cucumber and tomato surface microorganisms. It was found that washing cucumber with warm water for 3 min removed about 66.5% of the initial *E.coli* and 52.7% of standard plate count while the hot water removed 77% and 69.5% respectively, meanwhile, mild soap washing at room temperature for 3 min removed 78.7. 81.6 and 80.5% of the initial numbers of standard plate count, molds & yeasts and *E.coli* respectively with a good consumer acceptability. Charbonneau *et al.* (2000) showed that washing hands with a mild soap and water for 20s was more effective than applying a 70% alcohol hand sanitizer.

Washing with potassium permanganate showed a lower removal percent of all microorganisms from cucumber or tomato fruits than other treatments and the consumer acceptability was rejected because the blue color spots were found. A similar results were noticed when tomato washed with mild soap. While, tomato washed with hot water (70°C) both at 2 min or 3 min were rejected.

Generally, immersion in 2% acetic acid at 50°C for 3 min; or immersion in 3% H_2O_2 at room temperature for 3 min; or washing with mild soap at room temperature for 3 min; then rinsed with potable water were found to

Table 3: Effect of immersing fresh cucumbers and tomatoes in acetic acid solution on the removal of surface microorganisms

Immersing treatments	Natural microorganisms				Infected E.coli		Consumer acceptability
	bacteria		mold & yeast				
	log cfu/ cm2	% removal	log cfu/ cm2	% removal	log cfu/ cm2	% removal	
Cucumbers							
Untreated:	4.23		3.92		4.00		Good
In 1% acetic acid (25±2°C) for:							
2 min	2.90	31.4	1.73	55.9	2.00	50.0	Good
3 min	2.72	35.7	1.50	61.7	1.89	52.8	Good
In 1% acetic acid (50°C) for:							
2 min	1.97	53.4	1.03	73.7	1.72	57.0	Good
3 min	1.47	65.2	0.92	76.5	0.91	77.3	Good
In 2% acetic acid (25±2°C) for:							
2 min	1.08	74.5	0.97	75.3	1.70	57.5	Good
3 min	0.89	78.9	0.78	80.1	1.19	70.3	Good
In 2% acetic acid (50°C) for:							
2 min	0.87	79.4	0.43	89.0	1.18	70.5	Good
3 min	0.28	93.4	0.30	92.3	0.48	88.0	Good
Tomatoes							
Untreated:	4.69		2.95		2.86		Good
In 1% acetic acid (25±2°C) for:							
2min	2.19	53.3	1.41	52.2	1.39	51.4	Good
3min	2.04	56.5	1.23	58.3	1.17	59.1	Good
In 1% acetic acid (50°C) for:							
2min	1.67	64.4	1.13	61.7	1.09	61.9	Good
3min	1.53	67.4	1.00	66.1	0.92	67.8	Pass
In 2% acetic acid (25±2°C) for:							
2 min	2.02	56.9	1.40	52.5	1.13	60.5	Pass
3 min	1.87	60.1	1.09	64.1	0.97	66.1	Pass
In 2% acetic acid (50°C) for:							
2 min	1.50	68.0	1.08	63.4	0.98	65.7	Reject
3 min	1.23	73.8	1.02	65.4	0.82	71.3	Reject

Table 4: Effect of traditional washing of fresh cucumbers and tomatoes on the removal of surface microorganisms

Washing treatments	Natural microorganisms				Infected *E.coli*		Consumer acceptability
	bacteria		mold & yeast		log cfu/ cm2	% removal	
	log cfu/ cm2	% removal	log cfu/ cm2	% removal			
Cucumbers							
Untreated:	4.23		3.92		4.00		Good
With warm water (50°C) for:							
2 min	2.85	32.6	2.60	33.7	1.77	55.8	Good
3 min	2.00	52.7	2.27	42.1	1.34	66.5	Good
With hot water (70°C) for:							
2 min	2.78	34.3	2.16	44.9	1.75	56.3	Pass
3 min	1.29	69.5	2.03	48.2	0.92	77.0	Reject
Mild soap (20±5°C) for:							
2 min	0.97	77.1	1.16	70.4	0.90	77.5	Good
3 min	0.90	78.7	0.72	81.6	0.78	80.5	Good
With 100ppm potassium permanganate at room temp. for:							
2min	0.95	77.5	1.79	54.3	1.61	59.8	Reject
3min	0.83	80.1	1.62	58.7	1.00	75.0	Reject
Tomatoes							
Untreated:	4.69		2.95		2.86		Good
With warm water (50°C) for:							
2 min	2.00	57.4	1.14	61.4	1.30	54.5	Good
3 min	1.13	76.0	1.08	63.4	1.04	64.6	Good
With hot water (70°C) for:							
2 min	2.98	36.5	1.00	66.1	1.16	59.4	Pass
3 min	2.10	55.2	0.92	68.8	1.05	63.0	Reject
Mild soap (20±5°C) for:							
2 min	1.19	74.6	1.00	66.1	0.39	86.4	Good
3 min	0.82	82.5	0.87	70.5	0.30	89.5	Good
With 100ppm potassium permanganate at room temp. for:							
2min	0.97	79.3	0.92	68.8	1.02	64.3	Pass
3min	0.82	82.5	0.70	76.3	0.89	68.9	Reject

be the optimum treatments to remove most of the natural surface microorganisms and infected *E.coli* from fresh cucumbers. On the other hand, immersion in 150 ppm of sodium hypochlorite for 3 min at 50°C or washing with mild soap for 3 min at room temperature then rinsed with potable water could be considered optimum treatments to remove most of the natural surface microorganisms and infected *E.coli* from fresh tomato.

Treatments with selected chemical sanitizers as mentioned above or traditional washing with mild soap to reduce initial bacterial contamination from cucumber or tomato fruits will be ineffective if these fruits were re-contaminate during handling or cutting as for salad dishes preparation. So, more care should be taken by using clean and sanitized utensils and surfaces to minimize contamination followed by immediate consumption.

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تأثير استخدام بعض المطهرات الكيميائية على إزالة الميكروبات من أسطح ثمار الخيار والطماطم

عطية محمد عطية المخزنجى

قسم التصنيع الزراعى – معهد الكفاية الإنتاجية – جامعة الزقازيق –ج.م.ع

أجريت هذه الدراسة بغرض معرفة تأثير استخدام بعض المطهرات الكيميائية مثل هيبوكلوريت الصوديوم وفوق أكسيد الهيدروجين وحامض الخليك على إزالة الميكروبات الطبيعية من على أسطح ثمار الخيار والطماطم وكذلك قدرتها على قتل ميكروبات *E.coli* من على الثمار الملقحة بها. ومقارنة هذه المطهرات بالطرق التقليدية مثل غسيل الثمار بالماء الدافئ أو الساخن أو باستخدام المنظفات السائلة أو باستخدام برمنجنات البوتاسيوم.

وكانت أفضل النتائج على ثمار الخيار هى المعاملات التالية على الترتيب:-

- أدى الغمر فى ٢% حامض الخليك (٥٠°م) لمدة ٣ دقائق إلى إزالة ٨٨ – ٩٣,٤% من المجاميع الميكروبية المدروسة.
- بينما أدى استخدام ٣% فوق أكسيد الهيدروجين لمدة ٣ ق إلى إزالة ٧٥,٦ – ٨٩,٣% من المجاميع الميكروبية المدروسة.
- فى حين أدى الغسيل بالماء العادى والمنظف السائل إلى إزالة ٧٨,٧ – ٨١,٦% من الميكروبات.

وكانت أفضل المعاملات بالنسبة لثمار الطماطم هى كالتالى:-

- الغمر فى محلول هيبوكلوريت الصوديوم ١٥٠ جزء فى المليون (٥٠م) لمدة ٣ ق إلى إزالة ٨٠,٨ – ٩٢,٢% من الميكروبات السطحية.
- أدى الغسيل بالمنظفات السائلة على الحرارة العادية لمدة ٣ق إزالة ٧٠,٥ – ٨٩,٥٠% من الميكروبات السطحية. بينما لم تحظى باقى المعاملات المذكورة فى الدراسة قبولاً من الناحية الحسية بغض النظر على كفاءتها فى إزالة الميكروبات.
- يمكن استخدام النتائج المتحصل عليها فى هذه الدراسة فى معاملة الثمار فى بيوت التعبئة بغرض الإنتاج الجيد (GMP) وتطبيق برامج (HACCP) على الحاصلات الزراعية التى تسوق أو تستهلك بحالة طازجة.