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Efficacy of Decontaminating Agents for Raw Vegetable Consumption and Sensory Screening

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Sanitization of vegetables consumed raw has tremendous importance due to health risks associated with food-borne pathogens. This study elaborated the effectiveness of varied concentrations of disinfectants used for washing fresh carrot vegetable against five potential pathogens. The pathogens were identified during the course study and compared with MTCC standard cultures. It was found that induction of 50percent killing against five pathogens viz. *Aeromonas hydrophila*, *Shigella flexneri*, *Staphylococcus aureus*, *Escherichia coli* and *Listeria monocytogenes* required concentration (LC_{50}) of Sodium hypochlorite upto 100ppm, Citric acid upto 1000ppm, Lactic acid 724ppm, CaO 2570ppm, Tannic acid 794ppm and Cineole 100ppm with 5minutes washing period. Of the entire chemical tested, sensory scoring for vegetable taste, acidic taste the overall appreciation of citric acid corroborates its use as an effective sanitizing agent to treat vegetables. Cineole on the other hand has high response value at 69ppm but loose appreciation due to poor odour and taste. The purpose of the study is to establish current state of intervention for maintaining the high microbial safety of fresh vegetables consumed raw as intended health benefits. Organic acids like citric and lactic acid can be used in food industry as emerging sanitation strategy with minimum contact time and consumers palatability.

Keywords: Cineole, Disinfectant, Food borne pathogen, LC_{50}, Organic acid, Vegetable.

The fresh produce consumed raw is often associated with food borne illness due to presence of human pathogens. There is high demand of fresh produce in daily household and imminence of illness associated with its raw consumption goes by it. Freshly consumed food is considered as contamination vehicles which can potentially disseminate human pathogens (CDC 2006). Among bacterial pathogens, *Salmonella* and *Listeria monocytogenes* have been ranked as the most important pathogens associated with foodborne illness[1] while others are *Escherichia coli, Bacillus cereus, Staphylococcus aureus, Aeromonas hydrophila* and *E. coli* O157:H7 [2,3,4]. Growth of human bacterial pathogens is encouraged in the freshly cut salad vegetables as the later exudes abundant nutrients for bacterial growth. The penetration and internalization of bacteria into plant tissue make it further difficult to strategise sanitation with variety of chemicals [5].

Good sanitation prior to consumption can deliver safety and fulfil the goal of intended health benefits. Thorough washing of the minimally processed salads eliminates the inhabitant microbial flora to certain extent, hence pivotal in processing chain. Wash water systems may also pose a risk of further contamination to untreated product if the water is recycled without disinfecting it. So there

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is need to introduce decontaminating agents to the wash water to reduce the microbial load [6,7]. In addition to eliminating the indigenous microbial load, it also establishes a chemical barrier to diminish the rate of cross contamination. The wash water system in combination with chemicals is effective during subsequent batches processed in same circulated washing system.

There are number of chemical additives such as chlorine [8,9] and hydrogen peroxide [10] and certain organic acids have also been used as effective natural disinfectants as well as flavouring agents, Citric, acetic and lactic acids are the most common acids applied in the food industry[8,11] and hold GRAS status and are FDA and EC (European Commission) approved. However, these disinfectants may drag along possible disadvantages that could be the change in the flavor of the product which influence its sensorial acceptability.

Besides these, plant essential oils[12] and calcium based solutions[13] are getting interest for their potential as decontaminating agents against various bacterial pathogens such as *Escherichia coli*, *Clostridium perfringens*, bacteria of the genera *Staphylococcus, Enterococcus, Pseudomonas* and other Gram-negative aerobic bacteria.

The indigenous bacterial population diversifies its survival strategy against stress conditions like osmotic stress, pH, temperature, and apparent safety using commercial disinfectants may not prove effective. The objective of the present study was to evaluate the relevance of various categories of disinfectants on fresh carrot produce consumed as raw and put forth an effective remedial measure to counter the risk of health hazards associated with it without any toxic effects, high log reduction of microbial load while retaining its sensorial properties.

MATERIALS AND METHODS

Bacterial strains and inoculum preparation

Three Gram negative bacteria, *Escherichia coli* (MTCC443), *Aeromonas hydrophila* subsp. *Hydrophila* (MTCC1739) and *Shigella flexneri* (MTCC1457); and two Gram positive bacteria, *Staphylococcus aureus* subsp. *aureus* (MTCC96) and *Listeria monocytogenes* (MTCC657) (Table 1) were procured from Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, India as reference cultures. In addition, each of the respective indigenous pathogenic strain isolated from carrot (*Daucus carota*) samples grown in village fields around Buddha Nallah – a tributary of the Sutlej River. It was concluded highly polluted due to the addition of contaminants from the industrial and urban areas around Ludhiana which makes the water brackish. This water merges in the clean water of Sutlej[14]. Fresh vegetable, 25grams of sample was taken and washed with autoclaved water so as to omit any environmental contamination. Vegetable sample was chopped with the help of sterile knife into 2-3cm pieces and transferred to the 225ml water blank. It was shaken vigorously for uniformity and streaked for isolation onto the selective medium such as Eosin Methylene Blue Agar base, Aeromonas isolation HiVeg media base (Supplemented with Ampicillin at 0.0005%), SS Agar (Modified), Baired Parker Agar (Supplemented with 1% Potassium tellurite) and Gum Listeria medium. The dried culture media were sourced from HiMedia(TM) Laboratories unless otherwise mentioned.

All selected organisms were resuscitated in Luria-Bertani broth and transferred to the nutrient agar slants. A loopful of each pure bacterial culture was transferred to 500ml steam sterilized (15 psi for 20 min) nutrient broth in 1 litre Erlenmeyer flask and incubated at 37±2^{0}C for 24 hours. The final culture was diluted to set the initial microbial load of each culture in 500ml nutrient broth from 10^7-10^9 cfu/ml by standard optical density method. The cultures were used individually for inoculation of fresh cut carrot samples.

Preparation of vegetable model product

Fresh Carrot samples were sourced from the local vegetable market and processed on same day. A total of 1200 grams of carrot sample was taken for each decontaminating agent with different concentration treatments at different contact times. The samples were rinsed with autoclaved water and surface sterilized with 70% ethanol. Twenty five grams of carrot sample taken for each treatment was cut aseptically into 2-3cm pieces with heat sterilized stainless steel knife. Cutting was applied in order to simulate the procedures of inoculation and disinfection. The cut vegetables were aseptically dipped into individual bacterial suspensions targeting an initial population of approximately 10^7-10^9 CFU/g for 2 min contact time.

Decontaminating agents

Decontamination agents included solutions of single antimicrobial agent (chemical) and natural antimicrobial plant extract. The concentration of each antibacterial was varied with uniformity in contact time of 5, 10, 20 and 30 min followed by rinsing with autoclaved water for 2min (Table 2). Due to diversity of the decontaminants in chemical structure, composition and mode of action, solutions were not compared on the basis of their absolute concentration. All treatments were given at room temperature 29±2^{0}C to draw out the best place value of disinfectant for routine household use.

Microbiological analysis

Microbiological analysis was conducted for each disinfectant treatment on a 25 g sample (i) before treatment to estimate the attached viable count; (ii) after treatment, to assess the immediate antimicrobial effect of the washing solutions. Samples were transferred aseptically to stomacher bags, diluted 1:10 with water and homogenized for 60s. Serial dilutions were prepared and total plate count was enumerated on nutrient agar plates after incubation at 37 °C for 24-48h using GeneSnap software on Syngene G-Box. All experiments were performed in triplicates.

Dose response statistics

Log CFU reduction per gram was calculated directly from initial and final count of bacteria before and after treatment. Probit values were determined using Finney's table[15] to compare the amount of decontaminating agent required to create the same response in each of the various decontaminating treatments. The LC_{50} represent the concentration (LC_{50}) or dose that kills 50 per cent of the population. Regression graphs were drawn using probit value versus log of concentrations. Inverse log of concentration (x-axis) corresponding to probit 5 (y-axis) was calculated to deduce LC_{50}.

Sensory analysis

Sensory screening was performed with the carrot cut produce by a 9-member expert panel of individuals of 25 to 40 years age. The sensory parameters were scored on a descriptive scale of 1 to 9. Descriptions for each score are given in Table 5. The sensory attributes recorded were (i) vegetable aroma, (ii) herbal odour, (iii) acidic odour, (iv) chemical odour, (v) other odours, (vi) vegetable taste, (vii) Acidic taste, (viii) other tastes, (ix) overall acceptability, and (x) overall appreciation on 9- Point

Hedonic Scale[16]. Sensory analyses were carried out in the industrial and clinical Microbiology laboratory in Department of Microbiology, PAU, Ludhiana under appropriate uniform lightening conditions at room temperature (29±2)^{0}C.

RESULTS

The indigenous isolates in the present study were biochemically identified using standards methods shown in Table 1 (Online resource Table 1. doc).

Effect of decontaminating agents against pathogenic bacteria

Citric Acid

Citric acid at a concentration of <500ppm was required to achieve 50 percent cell death of atleast three bacterial pathogens viz. *Aeromonas*

Table 1. Bacterial pathogens and their source used in this study

S. No.	Organism	Source	Remarks
1	*Escherichia coli*	MTCC 443	Clinical isolate
2	*Escherichia coli*	This study	Carrot produce
3	*Aeromonas hydrophila* subsp. *hydrophila*	MTCC 1739	Tin of milk with fishy odour
4	*Aeromonas hydrophila*	This study	Carrot produce
5	*Shigella flexneri*	MTCC 1457	Not documented
6	*Shigella spp.*	This study	Carrot produce
7	*Staphylococcus aureus* subsp. *aureus*	MTCC 96	Not documented
8	*Staphylococcus aureus*	This study	Carrot produce
9	*Listeria monocytogenes*	MTCC 657	Poultry
10	*Listeria monocytogenes*	This study	Carrot produce

hydrophila, *Shigella flexneri* and *Listeria monocytogenes* whereas, 0.05-0.1% Citric acid was required to reduce *Staphylococcus aureus* and *E.coli* populations to half (Table 3). Furthermore, it has been observed that the time treatment more than 5 min did not further reduce the pathogenic load significantly indicating the threshold for concentration time of 1000ppm and 5 min contact time proves effective for 50 per cent reduction in populations of different pathogens (Table 3).

Sodium hypochlorite (NaOCl)

The population of three bacterial pathogens viz. *Shigella* spp., *Staphylococcus aureus* and *Listeria monocytogenes* could be reduced by 50 per cent using 5min rinse with wash solution containing sodium hypochlorite at <50 ppm (Table 3). For *E. coli* and *Aeromonas hydrophila*, LC50 was observed in range of 50-115ppm for different exposure durations. There is significant difference observed in concentrations needed to inhibit *E. coli* MTCC 443 (81ppm) and *E.coli* CR EXT 6 (115ppm, Table 3). The highest log reduction (4.63) was achieved with 200ppm concentration of NaOCl after 30 min of washing shown in Table 2 (Online resource Table 2.2.xls).

Lactic acid

Lactic acid significantly declined the population of three pathogens viz. *Aeromonas hydrophila*, *Listeria monocytogenes* and *E.coli* to half in number with < 500ppm concentration and 5 min of washing time (Table 3). For *Shigella flexneri* and *Staphylococcus aureus*, LC_{50} was observed to be 724ppm and 549ppm respectively (Table 3). Lactic acid reduced 2-4log value in all pathogens except for *Listeria monocytogenes* (<1log) both in reference strains and indigenous isolates in 5min of contact period shown in Table 2 (Online resource Table 2.xls).

Calcium Oxide (CaO)

In this study, CaO was found highly effective against *Aeromonas hydrophila*, *Shigella flexneri*, *Staphylococcus aureus* and *E.coli* with

Table 2. Decontamination treatment for carrot samples at varied concentrations of wash solution.

S. No.	Wash Solution	Working concentration (ppm)	Respective pH value
1	Sodium Hypochlorite	50	7.9
		100	8.2
		150	8.3
		200	8.4
2	Lactic Acid	500	2.6
		1000	2.3
		2000	2.0
		2500	1.9
3	Citric Acid	500	2.5
		1000	2.3
		2000	2.0
		2500	1.9
4	Tannic Acid	500	3.6
		1000	3.5
		2000	3.2
		2500	3.1
5	Cineole (Eucalyptus Oil)	50	7.3
		100	7.4
		150	7.4
		200	7.4
6	Calcium oxide	500	11.4
		1000	11.5
		2000	11.7
		2500	11.7

Table 3. Fifty percent response value of pathogens against sanitizers at different washing time

LC50(ppm) Disinfectant	Time Treatment	*Aeromonas hydrophila* MTCC 1739	*Aeromonas hydrophila* CR EXT9	*Shigella flexneri* MTCC 1457	*Shigella flexneri* CR EXT 29	*Staphylococcus aureus* MTCC 96	*Staphylococcus aureus* CR EXT 14	*Escherichia coli* MTCC 443	*Escherichia coli* CR EXT6	*Listeria monocytogenes* MTCC 657	*Listeria monocytogenes* CR EXT 7
Citric acid	5 min	<500	<500	<500	<500	645	645	912	933	<500	<500
	10 min	<500	<500	<500	<500	524	525	989	977	<500	<500
	20 min	<500	<500	<500	<500	501	512	945	993	<500	<500
	30 min	<500	<500	<500	<500	549	501	1005	997	<500	<500
Sodium hypochlorite	5 min	75	93	<50	<50	<50	<50	81	115	<50	<50
	10 min	72	75	<50	<50	<50	<50	74	115	<50	<50
	20 min	74	79	<50	<50	<50	<50	70	100	<50	<50
	30 min	70	75	<50	<50	<50	<50	77	94	<50	<50
Lactic acid	5 min	<500	<500	758	724	549	550	<500	<500	<500	<500
	10 min	<500	<500	758	741	549	549	<500	<500	<500	<500
	20 min	<500	<500	616	691	513	513	<500	<500	<500	<500
	30 min	<500	<500	513	630	<500	489	<500	<500	<500	<500
Calcium Oxide	5 min	<500	<500	<500	<500	<500	<500	<500	<500	2398	2570
	10 min	<500	<500	<500	<500	<500	<500	<500	<500	2455	2511
	20 min	<500	<500	<500	<500	<500	<500	<500	<500	2398	2454
	30 min	<500	<500	<500	<500	<500	<500	602	602	2409	5211
Tannic Acid	5 min	707	794	<500	<500	<500	<500	<500	<500	<500	<500
	10 min	724	758	<500	<500	<500	<500	<500	<500	<500	<500
	20 min	687	691	<500	<500	<500	<500	<500	<500	<500	<500
	30 min	557	562	<500	<500	<500	<500	<500	<500	<500	<500
Cineole	5 min	<50	<50	53	51	69	63	98	89	<50	<50
	10 min	<50	<50	53	54	66	73	87	85	<50	<50
	20 min	54	54	63	74	72	87	93	100	<50	85
	30 min	<50	<50	75	79	95	95	91	95	89	89

LC_{50} of <500ppm within 5 min (Table 3). However, significantly higher concentration of CaO was needed (2398ppm for MTCC 657 and 2570ppm for CRT EXT 7) to inhibit the growth of *Listeria monocytogenes*.

Tannic Acid

Tannic acid was observed to inhibit growth of all pathogens except *Aeromonas hydrophila* with 50 percent cell death at concentrations below 500ppm with contact time of 5 min (Table 3). Tannic acid showed killing action on *Aeromonas hydrophila* at a significantly higher concentration of 794ppm for isolate from carrot produce and 707 ppm for MTCC 1739 with similar contact period (Table 3).

Cineole

Except NaOCl, cineole was found effective at significantly lower concentration than other decontaminants in this study. During 5 min of washing time, less than 50ppm concentration was observed effective against *A. hydrophila* (MTCC 1739 and CR EXT 9), *L. monocytogenes* (MTCC 657 and CR EXT7) (Table 3). For *Shigella flexneri*, *S. aureus*, and *E. coli*, cineole concentration of 50-100ppm was required for 50 per cent reduction in bacterial population within 5 min. It has also been observed that there is 20-40ppm higher concentration needed if the contact washing was of longer period (30min) (Table 3).

Immediately after treatment of the contaminated vegetables, antimicrobial solutions were tested for remaining surviving populations by plating on nutrient agar plates and no bacterial populations were detected.

Sensory evaluation

The means sensory score fell between "like slightly" and "like moderately" on the hedonic scale for samples treated with organic acids (lactic acid, citric acid and tannic acid). However, there is no significant difference found in sensory attribute of vegetables aroma and herbal odour among all chemical decontaminants whereas significant variability at the level of 5% was observed among all other attributes viz. acidic odour, chemical odour, vegetable taste, acidic taste, other tastes, overall acceptability and overall appreciation.

In case of Sodium hypochlorite, vegetable taste was appreciated on hedonic score of 6 (like slightly) which was higher among all the antimicrobials tested (Table 4). CaO was least appreciated by the panel of experts which ranged from 4.77 to 5.66 score on hedonic scale with neutral review to vegetables taste (Neither like nor dislike).

The scores presented for acidic odour were significantly lower than the control only in vegetables exposure to cineole. Vegetables exposed to citric acid and tannic acid for chemical odour obtained scores significantly higher than control. The pH of tannic acid (3.1-3.6) did not have sensory

Table 4. Comparison of scores sensory attributes from carrot treated with most active sanitizers

	Mean (±SD) sensory scores [a]							
Sensory attribute	Control	Lactic acid	Calcium oxide	Citric acid	Cineole	Tannic acid	Sodium hypochlorite	CD at 5%
Vegetable aroma	5.44±1.24	6.22±1.20	5.66±1.22	6.22±1.39	5.22±1.30	6.22±1.30	5.66±1.32	NS
Herbal odour	5.77±1.09	5.11±2.02	5.11±0.78	5.33±0.86	5.33±1.22	6.11±1.05	5.88±1.27	NS
Acidic odour	5.44±0.88	6.00±1.32	5.33±0.87	6.11±1.05	4.44±1.13	6.00±1.12	6.00±1.00	1.00
Chemical odour	5.44±1.51	5.55±1.42	4.77±1.20	6.33±1.32	4.44±1.33	6.22±1.39	5.77±1.09	1.26
Vegetable taste	5.44±0.72	6.44±1.13	5.22±1.39	7.11±0.92	4.00±1.87	5.88±1.54	6.66±0.87	1.19
Acidic taste	5.44±1.01	5.88±1.27	5.44±0.73	7.00±1.58	3.88±1.83	5.44±1.33	6.11±1.27	1.26
Other tastes	5.66±0.71	6.22±1.09	5.55±1.33	5.66±1.80	3.77±1.30	4.77±0.97	5.88±1.27	1.18
Overall acceptability	5.66±0.71	6.33±1.22	5.22±1.71	6.55±1.33	3.88±2.31	5.44±1.67	6.44±1.13	1.43
Overall appreciation	5.88±0.60	6.22±1.09	5.22±1.71	7.00±1.41	3.77±2.39	5.44±1.67	6.33±1.00	1.42

[a] sensory parameters were scored on descriptive scale: 9,Like Extremely;8, Like very much;7,Like moderately;6,Like slightly;5,Neither like nor dislike;4,dislike slightly;3,dislike moderately;2, dislike very much;1,dislike Extremely.

impact and lost the overall appreciation below level of control.

DISCUSSION

This study selected the five food borne pathogens which are persistently causing large outbreaks of food borne illness associated with fresh produce[17].

Effect of decontaminating agents against pathogenic bacteria

Effect of citric acid

It was expected initially that the resistance might have been developed against these decontaminants but no significant differences were observed within concentration demand against the indigenous as well as standard cultures indicating that citric acid is equally effective for killing of pathogens irrespective of their colonization potential. The mode of action of citric acid is based on the acidification of the cytoplasm, disruption of proton motive force, osmotic stress and inhibition of macromolecule synthesis[18]. A commercial formulation of citric acid, Citrox was reported to significantly reduce the *E. coli* O157:H7 in spinach and lettuce samples[19], while citric acid @0.5% for 2 and 5 min could reduce *E. Coli* and *L. monocytogenes* populations by 2log cfu/g and 0.9 log cfu/g sample respectively[20]. Also, <1 log reduction was reported with citric acid based sanitizer as similar to regular tap water[21]. Various investigations have mentioned post treatment storage of the vegetable is also determining factor for increase or static pathogenic load. Citric acid has been found effective during long term storage and it may further cause killing of the survival pathogens during initial exposure.

Sodium hypochlorite

E. coli resistance to alkalinity of sodium hypochlorite has been reported[22]. Resistance to multiple antibiotics has been observed for *E. coli* CR EXT 6 (MAR index 0.15) (Data not shown). NaOCl causes membrane disintegration, degradation of proteins and halt protein synthesis. The cell division in *Escherichia coli* was inhibited by 5 min exposure to HOCl and this effect cannot be reverted with extensive washing afterwards [23]. Chlorinated solutions have been observed to significantly reduce the *E. coli* populations in lettuce leaves by 1.42 to 2 log concentrations[24, 20]. NaOCl was reported to reduce at least 2logcfu/g of *Escherichia coli* O157:H7, *Salmonella,* and *Listeria monocytogenes* when it is applied in range 50-200ppm to use as washing solution[25]. However, it has potential adverse effects due to formation of chlorine by-products and hence it is also banned in some European countries [8, 26].

Lactic Acid

Since lactic acid as citric acid has significant effect on inhibiting the growth of all the pathogens (carrot isolates and MTCC), organic acids can be proven effective and an alternative to disinfectants having potent effects on human.

Lactic acid at 2% concentration reduced count of *E.coli* O157:H7 on spinach from 5.4 log cfu/g to 1.8 log cfu/g within 5 min of treatment[19]. Additionally, it has also reduced pathogen population on lettuce samples at same concentration from 7.4 log cfu/g to 4.4log cfu/g. Further, about 1.9 log cfu/g in *E. coli* and 1.5 log cfu/g reduction in *L. monocytogenes* on fresh cut iceberg lettuce leaves after washing with 0.5% lactic acid for 2 and 5 min were observed[20]. Organic acids are known to kill bacteria by disturbing their ionic permeability across the membrane and anion accumulation, thereby decrease the cellular pH[26].

Combined action of lactic acid and H_2O_2 on artificially inoculated lettuce leaves with *E.coli* O157:H7 and *Salmonella enteritidis* reduced the count to logrithimic value of 4, whereas 3 log reduction in case of *Listeria monocytogenes*[27]. Chlorine is still being used in food industry as most effective biocide but this study has provided insight of using organic acid (GRAS status) as alternative to sodium hypochlorite due to high microbial killing action, non-toxicity, flavour enhancement and consumer acceptance.

Calcium oxide

Calcium oxide leads to formation of calcium hydroxide with water and oxidant free radicals thus released have lethal effect on bacterial cells[28]. Calcium oxide produces active oxygen species upon degradation and its alkalinity imparts the bactericidal effect to its property, however *Listeria monocytogenes* has been known to partially resist the action of active oxygen species[29]. CaO has been reportedly used as a decontaminant for various food items and has been reported to be highly effective against various food borne pathogens[30, 31, 32]. However treatment of food items with CaO are not without associated risks. CaO can cause skin irritation, eye burns and chronic respiratory diseases that limits its widespread use (CDC1995).

Tannic Acid

Tannins are the bioactive phenolic compounds which are known for the plant defence against pathogens or stress. They posses certain hydroxyl groups which make them toxic and this property can be exploited for microbiological safety of fresh produce[33]. Tannin has been reported to act against bacterial adhesion by membrane damage and prevents biofilm formation by inhibiting the matrix production[34].

Tannic acid 3 % was reported to exhibit high antimicrobial activity against *Salmonella typhimurium* CCM5445, *E. coli* ATTC 25922 and *Staphylococus aureus* ATCC 6538 [35].

Antibacterial activity of tannic acid is effective due to bacterial membrane disruption by forming complex with its proteins[33]. However, there are some health concerns posed by use tannic acid due to its carcinogenic activity[36].

Cineole

Cineole, an active ingredient of *Eucalyptus* oil has been reported as non-toxic and raises no concern to human health during *in-vivo* studies[37]. However, in other studies high concentrations of cineole were required. The minimum inhibitory concentration (MIC) value of cineole was reported to be 500 ppm for *Aeromonas hydrophila* INCQS 7966 and 2000ppm for *L. monocytogenes* ATCC 7644[38]. The MIC range for Eucalyptus oil against *E.coli* O157:H7, *Salmonella typhimurium*, *Listeria monocytogenes* and *Staphylococcus aureus* was observed in range between 0.23 - 0.27% (v/v) and found best along with the Cilantro[39]. IC_{50} values for essential oils have illustrated their effectiveness in past studies that of Oregano (*Origanum vulgare*), thyme[40] and 1,8-cineol[41,42] against food borne pathogens. Transmission electron microscopy has revealed its killing effect due to its hydrophobic entity which damages the cell membrane and cause reduction in cytoplasmic content of the cell[43]. The need of 20-40ppm higher concentration of cineole during 30min contact time could be due to the volatile nature of the essential oil which looses it potentiality if kept open for longer duration. Contact time of 5 min appeared to be the case for all chemical compounds and any increase in washing time from 5 min upto 30 min had no significant effect on microbial load reduction. This suggested that time treatment can be shortened without compromising the quality and microbiological level of fresh vegetables as has been reported by other researchers also[44, 45].

The investigations of current study have provided the use of decontaminating agents to maintain the microbiological quality and safety of the fresh vegetables. The entire six chemicals destined for eradicating five known pathogens were examined to be effective at different concentrations of their solutions. However, Sodium hypochlorite upto 100ppm, citric acid upto 1000ppm, Lactic acid 724ppm, CaO 2570ppm, Tannic acid 794ppm and Cineole 100ppm were found effective at LC_{50} scale for all six pathogen tested.

Sensory evaluation

The acidic odour and acidic taste of cineole minimized its level of acceptance. Similar result was observed when vegetables were treated with carvacrol[46]. Vegetable taste and acidic taste attribute was scored higher in case of citric acid treatment probably due to its low pH value followed by sodium hypochlorite and lactic acid. Citric acid significantly influences the overall acceptability and appreciation. Sensory analysis of carrot slices treated with Basil, lemon balm and thyme were score poorly due to acidic taste, chemical aroma and herbal aroma [40].

Because the sensory analysis depicted overall appreciation of lactic acid and citric acid significantly comparable to that of sodium hypochlorite, these chemicals can be the alternatives for decontamination to minimize the risk associated with the by-products of chlorine. There is no difference in visual quality of the product was observed inferring that the concentration used, pH and chemical composition of the decontaminating agent did not cause browning, pigment leaching or structure deformity in vegetable sample. On the sensory scale, citric acid was scored high on 9 point hedonic scale with retention of all quality attributes of the fresh cut carrot produce. This corroborates its safe routine use in households for washing vegetables prior to raw consumption.

CONCLUSION

The investigations of current study have provided the use of decontaminating agents to maintain the microbiological quality and safety of the fresh vegetables. The entire six chemicals destined for eradicating five known pathogens were examined to be effective at different concentrations of their solutions. However, Sodium hypochlorite upto 100ppm, citric acid upto 1000ppm, Lactic acid 724ppm, CaO 2570ppm, Tannic acid 794ppm and Cineole 100ppm were found effective at LC_{50} scale for all six pathogen tested. On the sensory scale, citric acid was scored high on 9 point hedonic scale with retention of all quality attributes of the fresh cut carrot produce. This corroborates its safe routine use in households for washing vegetables prior to raw consumption Chlorine is still being used in food industry as most effective biocide but this study has provided insight of using organic acid (GRAS status) as alternative to sodium hypochlorite due to high microbial killing action, non-toxicity, flavour enhancement and consumer acceptance.

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