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Optimization of Disinfection Treatment for Shelf Life Extension of Fresh Cut Salad Vegetables

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Abstract: In the present study, disinfectant pre-treatment conditions were optimized for Fresh cut salad vegetables after evaluating bactericidal efficacy of the five different disinfectants viz. Sodium hypochlorite, potassium metabisulfite, citric acid, acetic acid and benzoic acid. Sodium hypochlorite @ 100ppm was selected for disinfectant pretreatment of fresh cut vegetables viz. cucumber, carrot and tomato through physicochemical and microbial analysis. Optimization of pre-treatment conditions viz. temperature, pH and contact time was statistically designed and analyzed using response surface methodology. Numerical optimization of results revealed a temperature of 6.5, 10.0 and 6.5°C; pH 6.0, 7.0 and 7.0; contact time 23.0, 8.0 and 30.0 minutes was found to be optimized for fresh cut cucumber, carrot and tomato disinfectant pretreatment with a desirability level of 94, 94 and 92%, respectively. Shelf life analysis of pre-treated (under optimized conditions) and untreated fresh cut vegetables stored under refrigeration conditions (5-7°C) revealed enhancement in shelf life of treated fresh cut vegetables from 6, 9 and 6 days to 9, 12 and 9 days of fresh cut cucumber, carrot and tomato, respectively. During shelf life period, all physicochemical parameters of pre-treated fresh cut vegetables differed non-significantly along with microbial load under acceptable limits and a good sensory score.
Keywords: Fresh cut salad vegetables; Numerical optimization; Pretreatment; Response Surface Methodology; Shelf life; Sodium hypochlorite.

1. Introduction

India holds the second position in the vegetable production with a yield of 191769 (‘000MT) under an area of 10353 (‘000Ha). Presently, Punjab produces 5207.36 (‘000MT) of vegetables under an area of 249.32 (‘000Ha) [1] and it is also estimated that an area of 66550 Ha will be brought under vegetable crops in the next five years. The term “fresh cut” indicates the raw vegetables and fruits that have undergone various physical operations such as cutting, shredding, peeling, and abrasions to prepare convenient ready-to-cook or ready-to-eat products. According to International Fresh Cut Produce Association [2], the most important criteria for fresh cut vegetables and fruits is that it should have 100% usable material and also the tissue intact should be present in a living and respiring physiological state [3].Usage of fresh cut products is very advantageous for the restaurants and foodservice industry because of the need of very less manpower in the preparation of food, also there is a reduction in the use of special systems that handle waste, thereby increasing the possibility to provide fresh cut commodities and its specific forms in a short period [4, 5]. But all these benefits are for a short period, at later stages rapid deterioration of the product can be seen that causes short shelf life of the product in the market leading to various potential health hazards associated with the spoilage of the fresh cut product. So, understanding the physiological processes exhibited by the fresh cut vegetables is very important to retain the wholesomeness of the product along with maintaining the nutritional quality and developing the best innovative new technologies, various handling procedures that can maintain the product quality and extending its shelf life. The growth of spoilage microorganisms which cause contamination of the fresh cut vegetables are of major concern for the fresh cut industry. The extent of the injury caused during the process of cutting is directly related to the potential growth of microorganisms present in the product [6,7]. The initial process before pretreatment of the fresh cut produce involves various steps such as peeling, trimming and deseeding of the fresh produce if needed and then cutting it into equal sizes. The process of initial washing by potable water that involves the thorough removal of any kind of dirt present on the surface of the produce. Washing of a whole vegetable in potable water leads to a very less reduction in the population of microorganisms [8, 9]. It reduces the risk of the transfer of microorganisms present during the processing operations. In the process of production, fresh-cut vegetables come in contact with various microorganisms present in the environment of the processing unit. Reduction in the rate and level of contamination depends on the usage of the appropriate chemical disinfectant. A chemical disinfectant is an antimicrobial agent that is applied to reduce the count of microorganisms that are of public concern, without causing any effect to the quality of the product including the physicochemical parameters such as (TSS, total sugars, total phenols, titratable acidity, pH and firmness). The disinfectant used for the pretreatment of fresh cut vegetables should be used under the optimized concentration and pretreatment conditions like pH, temperature and contact time to reduce maximum microbiota over fresh cut vegetables. Washing of a whole vegetable in potable water leads to a very less reduction in the population of microorganisms. Use of disinfectants like chlorine, hydrogen peroxide, ozone, peroxyacetic acid or acidified sodium chloride can lead to an additional reduction of 1-2 log in the initial count [6, 8] of microorganisms that are present on the surface of the product. Therefore, a disinfectant pretreatment under standardized conditions is a pre-requisite for fresh cut vegetable industry. So, the present study was conducted to optimize the concentration of the disinfectant and, pretreatment conditions and shelf life analysis of fresh cut vegetables under refrigeration conditions.

2. Materials and methods

2.1 Selection of optimum disinfectant

Cucumber, carrot and tomato vegetables were procured from the local vegetable market. Healthy, ripened and even sized vegetables were selected and washed with potable water. The vegetables were peeled (if needed), calyxes were removed and then were cut into small equal sized pieces with sterilized sharp knife on a cutting board, and both were wiped with 90% ethanol. In order to avoid cross contamination during sample preparation, cutting boards, knives and other equipments coming in contact with vegetables were sanitized separately. Disposable gloves and head covers were worn by the handlers during the washing and cutting of vegetables.

250 grams of fresh cut vegetables (FCV) viz. cucumber, carrot and tomato were taken for each treatment. Five different chemical disinfectants viz. sodium hypochlorite (NaOCl), potassium metabisulfite (KMS), citric acid, acetic acid and benzoic acid were chosen depending upon the different chemical nature and mechanism of action were taken for the study. All three FCV were immersed in different chemical disinfectant solution of 100 ppm concentration in a container for 15 minutes [10]. After 15 minutes of immersion, FCV were taken out, drained and allowed to air dry. Untreated FCV washed with potable water only were taken as control sample. Chemical disinfectant that showed the maximum % reduction in microflora for Total Plate Count (TPC), yeast & mould count (Y&M) and coliforms count over the selective media plates of Total Plate Count agar, Yeast & Mould agar, Eosin Methylene Blue agar was selected for the further studies of optimization.

2.2 Experimental design

In order to optimize the disinfection pretreatment conditions viz. temperature, pH and contact time, Response Surface methodology (RSM) was opted using Design expert 12.0 software. A five-level two-factor central composite rotatable design (CCRD) was employed. The independent variables were the temperature (X1), pH (X2), and contact time (X3). The three independent variables were coded as −1.682 (lowest level) −1, 0, 1 (middle level) and +1.682 (highest level). Levels taken for different process variables for the pretreatment are presented in (Table 1). The statistical analysis of the data and three-dimensional (3D) plotting were performed using Design Expert software 12.0. An experimental set of 20 runs having different combinations was carried out using 250gm of each fresh cut vegetables and 100ppm concentration of selected pretreatment disinfectant.

Physicochemical and microbial parameters were taken as responses for analysis of the each combination produced by the RSM. An optimized condition in terms of temperature, pH and contact time that reduces maximum microbial load over the FCV was selected for the disinfectant pretreatment.

Table 1. Actual level of different process variables for pretreatment of fresh cut salad vegetables

Independent variables	Levels	
	Low	High
Temperature (°C)	3	10
pH	4	7
Contact time (min)	8	30

2.3 Validation of optimized conditions

After optimization of disinfectant pretreatment conditions, a validation experiment was conducted at 3 kg scale for each fresh cut vegetable under optimized conditions.

2.4 Shelf life study of treated fresh cut salad vegetables

Peeled, pretreated (under optimized conditions) /untreated (control), even sliced FCV were packed in air tight food grade containers and were stored under refrigeration conditions (5-7℃). Physicochemical and microbial parameters were analyzed at a regular interval of 3 days for 15 days or till the sample was deteriorated.

2.5 Physical parameters

Firmness was measured with Penetrometer device (Labpro). It is a handy probe with convex tip that pierce into the piece of the vegetable. Vegetable piece is held on the firm surface and the probe is pushed into the piece to a depth of 8mm, corresponding reading is marked as firmness level on the meter.

2.6 Chemical parameters

2.6.1 pH

The pH of the sample was determined with pocket size pH meter (Hanna HI96017).

2.6.2 Total soluble solids

Total soluble solids (% TSS) of the vegetable sample were determined by using Erma hand refractometer of 0-32ºB.

2.6.3 Titratable acidity

Titratable acidity was analyzed using the method of Amerine *et al* [11]. Titratable acidity was determined by titrating known quantity of extract of fresh vegetable juice against standardized 0.2 N NaOH using a few drops of 1%phenolphthalein solution as indicator to pink end point which should persist for 15 seconds.

$$\text{Titratable Acidity (\%)} = \frac{\text{Volume of 0.2 N NaOH used} \times 0.2 \times 6}{\text{5 g (weight of sample taken)}} \times 100$$

2.6.4 Total phenols

Total phenols were extracted by adding 2g of the solid sample to 50% of 50ml methanol and heat for few minutes. Total phenols were estimated by method of Malik and Singh [12] to 1ml of extract prepared, add 5ml each of Folin-Ciocalteu reagent (diluted 1:9 with distilled water) and sodium carbonate reagent (10.6 grams of sodium carbonate dissolved in 100ml distilled water) was added and then mixed. After 20 min, intensity of color was recorded at 765 nm against reagent blank. The concentration of total phenols was calculated from the standard curve prepared by using gallic acid (10-100µg/ml).

2.6.5 Total sugars

Total sugars of the treated samples were estimated by the Dubois method [13]. 1ml of fruits juice was taken and was diluted 100 times. From that volume 1ml was taken. To that, 5ml of 5% phenol reagent and 5ml conc. Sulphuric acid was added. Mixture was mixed on the vortex mixture and let the mixture cool. Finally intensity of the color was recorded at 490nm against reagent blank. The concentration of total sugars was calculated from the standard curve prepared by using glucose (10-100 mg/ml).

2.7 Microbial count

The quantitative assay of the microbial count of samples was carried out by following different IS methods for respective cultures. Samples (250g) were properly homogenized before microbial analysis. Plate count agar, yeast & mould agar and EMB agar, SS agar, MacConkey agar was used for the enumeration of selective microflora on the fresh cut vegetable pieces.

10g of fresh cut (treated and untreated) homogenized vegetable sample was suspended into the 90ml of sterile water blank to make 10^{-1} dilution. Afterwards, 1ml of supernatant was accurately pipette out into the 9ml test tube to get 10^{-2} dilution and in the same way the sample were serially diluted to10^{-5} dilution. 1ml of the sample was taken from desired dilution in the Petri plate and selective media was pour onto it. Microbial count was taken after 24-48h of incubation. Plates kept at an incubation temperature of 30°C, 28°C and 37°C for TPC, Y&M and coliforms, respectively.

$$\text{No. of colony forming units per gram of sample (cfu/g)} = \frac{\text{Total No. of colony formed} \times \text{dilution factor}}{\text{Aliquot taken}}$$

Percentage microbial reduction was calculated by using following formulae:

$$\text{\% reduction} = \frac{A - B}{B} \times 100$$

where, A = Number of initial microbial colonies; B = Number of final microbial colonies.

2.8 Sensory analysis

A semi trained panel of 10 judges evaluated the sensory attributes of fresh cut vegetables (at different intervals) on the basis of aroma, flavor, appearance, color, texture and taste on a 9 point hedonic scale. The scores given by the 10 judges were statistically analyzed and mean values were taken for all the quality parameters.

3. Results and discussion

Fresh cut vegetables (FCV) (viz. cucumber, carrot and tomato) were immersed in five different disinfectant solutions [sodium hypochlorite (NaOCl), potassium metabisulfite (KMS), citric acid, acetic acid and benzoic acid] of 100ppm concentration for 15 minutes [10]. After evaluating different disinfectants and optimizing the concentration of NaOCl @ 100ppm [14], further optimization of disinfectant pretreatment parameters viz. temperature, pH and contact time for the pretreatment was done opting Response Surface Methodology (RSM) which designed the experimental plan statistically. Chlorine has been successfully used at concentrations ranging from 50 to 200 parts per million (ppm) to wash vegetables in fresh cut vegetable studies with a typical contact time of about 15 minutes [15]. On treatment of a sample with 200 ppm of sodium hypochlorite, the total bacterial count reduced to the values of less than 5 log (cfu/g) [16] but at higher concentrations of chlorine, release of chlorine vapours occurs in addition with formation of some of the chlorinated by products, which are hazardous for human health [15, 16]. RSM design combinations along with responses taken are presented in Table 2, 3 and 4 for FC cucumber, carrot and tomato, respectively. Results were analysed using response parameters in terms of % microbial reduction along with physicochemical parameters including TSS, total sugars, total phenols, titratable acidity, pH and firmness (Table 5). Use of some of the disinfectants like sodium hypochlorite, potassium metabisulfite, hydrogen peroxide, ozone, peroxyacetic acid or acidified sodium chloride can lead to an additional reduction of 1-2 log in the initial count [6, 7]. Currently, sodium hypochlorite is the most commonly used chemical disinfectantin produce wash water because of its high bactericidal efficacy, low cost, easy availability and a long history of use [16].

Table 2. Effect of temperature, pH and contact time combinations on physicochemical and microbiological quality of fresh cut cucumber

Run	Temperature (°C)	pH	Contact time (min)	R1	R2	R3	R4	R5	R6	R7	R8	R9
1	6.5	5.5	19	4.7	2.9	5.65	3.57	0.14	7.30	47.35	50.00	64.28
2	0.613725	5.5	19	4.7	2.7	5.88	3.69	0.07	5.86	17.64	12.50	40.30
3	6.5	5.5	19	4.6	2.8	5.64	3.58	0.13	7.20	44.11	68.50	71.42
4	6.5	2.97731	19	4.5	2.8	5.78	3.06	0.10	6.92	32.35	25.00	35.71
5	3	4	30	4.8	2.7	5.93	3.35	0.12	6.31	38.23	25.00	42.85
6	12.3863	5.5	19	4.7	2.9	5.69	3.91	0.09	6.60	32.35	12.50	28.57
7	6.5	5.5	19	4.6	2.9	5.65	3.57	0.14	7.30	57.05	69.00	64.28
8	3	7	8	4.2	2.8	5.73	3.46	0.06	6.34	14.70	25.00	21.42
9	6.5	5.5	37.4997	4.6	3.1	5.78	3.14	0.12	6.59	41.17	37.50	57.14
10	10	7	30	4.4	3.1	5.79	3.35	0.08	6.90	38.23	25.00	42.85
11	10	4	8	4.7	2.8	5.68	3.43	0.11	6.89	26.47	12.50	15.28
12	3	7	30	4.7	3.0	5.95	3.23	0.08	6.35	40.86	25.00	49.71
13	6.5	8.02269	19	4.2	2.9	5.90	3.23	0.07	7.10	25.52	37.50	39.16
14	10	4	30	4.4	3.0	5.62	3.33	0.10	6.49	38.23	37.50	32.62
15	6.5	5.5	0.500279	4.6	2.7	5.66	3.39	0.10	6.96	17.64	12.50	21.42
16	6.5	5.5	19	4.6	2.8	5.64	3.58	0.13	7.20	57.05	69.50	71.42
17	6.5	5.5	19	4.7	2.9	5.64	3.57	0.14	7.30	56.05	68.00	64.28
18	10	7	8	4.7	2.7	5.82	3.71	0.09	7.17	14.70	12.50	21.42
19	6.5	5.5	19	4.6	2.8	5.65	3.58	0.13	7.20	49.11	64.50	71.42
20	3	4	8	4.5	2.7	5.78	3.26	0.08	6.58	17.64	12.50	22.21

* R1- TSS, R2- Firmness, R3- pH, R4- Total sugars (g/100g), R5- Titratable acidity (%), R6- Total phenols (mg/100g), R7- Total Plate Count (% reduction) R8- yeast &mould count (% reduction), R9- coliforms count (% reduction)
* Control sample count- 5.1 log (cfu/g)TPC, 3.1 log (cfu/g) Y&M and 3.2 log (cfu/g) coliforms count

Table 3. Effect of temperature, pH and contact time combinations on physicochemical and microbiological quality of fresh cut carrot

Run	Temperature (°C)	pH	Contact time (min)	R1	R2	R3	R4	R5	R6	R7	R8	R9
1	10	4	30	7.1	26.9	5.50	6.01	0.14	8.13	51.77	33.47	44.27
2	3	4	8	7.2	27.1	5.50	6.21	0.16	7.91	16.45	20.00	10.00
3	6.5	2.9773	19	7.4	27.0	5.50	5.92	0.15	7.96	46.58	20.00	30.00
4	10	7	8	7.0	27.2	5.50	6.20	0.14	8.04	63.63	59.47	70.00
5	0.613725	5.5	19	7.1	27.1	5.30	5.89	0.17	7.96	27.27	20.00	10.00
6	10	7	30	7.0	27.1	5.60	6.06	0.14	7.99	48.52	40.00	50.00
7	3	7	30	7.1	27.0	5.50	5.94	0.17	7.90	54.54	28.55	50.40
8	3	7	8	7.0	27.1	5.30	6.11	0.15	8.07	31.81	20.00	10.00
9	10	4	8	7.2	27.0	5.60	6.14	0.15	7.98	38.07	27.61	39.40
10	12.3863	5.5	19	7.0	27.1	5.50	5.91	0.14	8.10	38.25	40.00	44.72
11	6.5	5.5	19	7.0	27.0	5.40	6.10	0.15	8.21	40.90	20.00	30.00
12	6.5	5.5	19	7.1	27.0	5.40	6.20	0.14	8.20	36.36	40.00	34.83
13	3	4	30	7.2	27.0	5.40	6.02	0.16	7.94	54.54	40.00	54.03
14	6.5	5.5	19	7.0	27.0	5.40	6.10	0.15	8.30	40.09	20.00	30.00
15	6.5	5.5	19	7.0	27.0	5.50	6.10	0.13	8.30	40.90	40.00	30.00
16	6.5	5.5	37.4997	7.0	27.0	5.50	6.43	0.14	8.01	59.09	47.63	66.84
17	6.5	8.0227	19	7.1	27.1	5.50	5.93	0.15	7.97	59.16	20.00	45.29
18	6.5	5.5	19	7.1	27.0	5.40	6.20	0.14	8.20	36.36	20.00	40.00
19	6.5	5.5	0.500279	7.0	27.1	5.50	6.85	0.13	8.03	25.11	40.00	31.24
20	6.5	5.5	19	7.1	27.1	5.50	6.20	0.13	8.20	36.36	20.00	40.00

* R1- TSS, R2- Firmness, R3- pH, R4- Total sugars (g/100g), R5- Titratable acidity (%), R6- Total phenols (mg/100g), R7- Total Plate Count (% reduction) R8- yeast &mould count (% reduction), R9- coliforms count (% reduction)
* Control sample count- 5.3 log (cfu/g) TPC, 3.1 log (cfu/g) Y&M, 3.1 log (cfu/g) coliforms count

Table 4. Effect of temperature, pH and contact time combinations on physicochemical and microbiological quality of fresh cut tomato

Run	Temperature (°C)	pH	Contact time (min)	R1	R2	R3	R4	R5	R6	R7	R8	R9
1	12.3863	5.5	19	4.9	4.7	4.08	4.54	0.49	11.63	49.43	25.00	61.13
2	6.5	5.5	19	5.4	4.7	4.18	5.10	0.42	12.10	50.00	41.66	65.00
3	6.5	5.5	19	5.5	4.8	4.19	5.20	0.44	12.20	52.08	33.33	60.00
4	3	4	30	4.8	4.5	4.04	4.23	0.46	10.26	60.41	50.00	70.00
5	10	4	8	5.2	4.8	4.12	4.95	0.52	10.50	42.69	16.66	53.32
6	6.5	5.5	19	5.4	4.7	4.18	5.10	0.42	12.10	50.00	37.54	65.00
7	6.5	8.02269	19	5.1	4.7	4.2	4.81	0.50	10.41	62.50	58.33	75.00
8	3	4	8	5.0	4.8	4.05	4.89	0.43	10.66	43.75	16.66	51.62
9	6.5	5.5	37.4997	4.9	4.4	4.06	4.45	0.40	11.08	60.41	50.00	70.00
10	3	7	8	5.0	4.9	4.07	4.62	0.44	10.75	44.44	30.92	55.00
11	6.5	2.97731	19	5.2	4.6	4.12	4.98	0.48	10.19	53.00	35.31	66.59
12	6.5	5.5	0.500279	5.0	4.9	4.09	4.68	0.43	10.83	37.50	13.26	46.06
13	10	4	30	5.0	4.5	4.08	4.53	0.43	10.97	55.83	41.66	66.56
14	6.5	5.5	19	5.5	4.8	4.19	5.20	0.46	12.20	52.08	33.33	60.00
15	10	7	8	4.8	4.8	4.14	4.36	0.53	10.98	46.11	29.00	65.00
16	10	7	30	5.0	4.5	4.13	4.70	0.43	11.28	58.33	41.66	70.00
17	0.613725	5.5	19	5.0	4.7	4.00	4.73	0.46	10.79	52.08	35.69	60.00
18	6.5	5.5	19	5.4	4.7	4.18	5.10	0.42	12.10	50.00	41.66	65.00
19	3	7	30	5.1	4.6	4.09	4.78	0.48	10.43	59.67	54.23	68.56
20	6.5	5.5	19	5.5	4.7	4.19	5.20	0.43	12.20	52.08	37.54	60.00

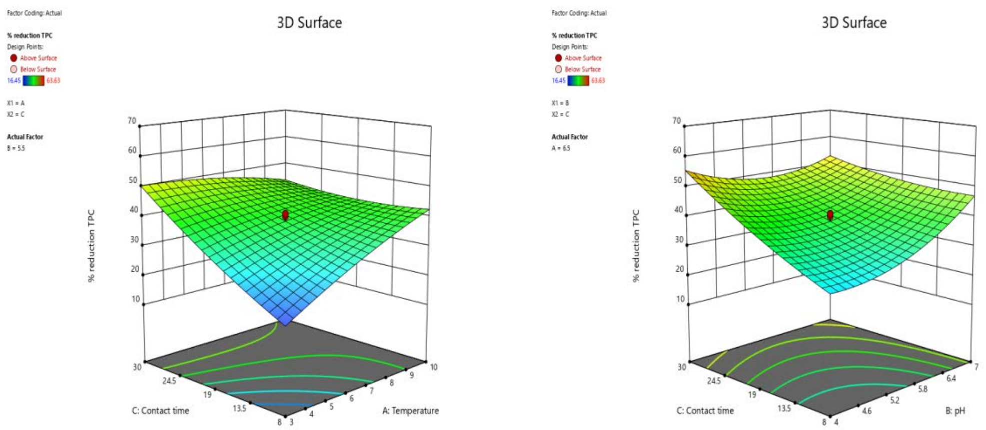
* R1- TSS, R2- Firmness, R3- pH, R4- Total sugars (g/100g), R5- Titratable acidity (%), R6- Total phenols (mg/100g), R7- Total Plate Count (% reduction) R8- yeast &mould count (% reduction), R9- coliforms count (% reduction)
* Control Sample- 5.6 log (cfu/g)TPC 3.2 log (cfu/g)Y&M and 3.4 log (cfu/g) coliforms count

Table 5. RSM responses in terms of model values, adjusted R^2 and predicted R^2 for physicochemical parameters of fresh cut cucumber, carrot and tomato

Parameters	Model value			Adjusted R^2			Predicted R^2		
	Cucumber	Carrot	Tomato	Cucumber	Carrot	Tomato	Cucumber	Carrot	Tomato
Total soluble solids	25.48	481.21	47.90	0.9206	0.9956	0.9569	0.8325	0.9862	0.9126
Total sugars	1117.95	24.92	49.36	0.9981	0.9189	0.9582	0.9925	0.7796	0.8890
Total phenols	187.72	25.89	186.60	0.9888	0.9218	0.9888	0.9823	0.9399	0.9629
Titratable acidity	51.08	4.99	16.73	0.9595	0.6541	0.8817	0.9132	0.5383	0.8442
pH	591.07	7.20	178.28	0.9964	0.7460	0.9882	0.9885	0.6233	0.9663
Firmness	18.19	8.27	23.79	0.8906	0.7751	0.9152	0.8607	0.6181	0.8904
% TPC reduction	9.80	28.61	34.98	0.8066	0.9290	0.9415	0.6210	0.7627	0.8139
%Y&M reduction	23.50	3.52	28.48	0.9142	0.5441	0.9286	0.8834	0.5546	0.8653
% coliforms Reduction	52.77	25.89	22.59	0.9608	0.9218	0.9109	0.9107	0.8129	0.8784

FCV (250g) of each vegetable was taken and immersed in the NaOCl solution (100ppm) having desired pH and kept at required temperature and contact time under controlled conditions according to the RSM design. Samples were taken out and drained for further analysis. Maximum % reduction of TPC,Y&M and coliforms count was reported at 6.5°C, 10.0°C and 6.5°C temperature; 5.5, 7.0 and 8.0 pH and 19.0, 8.0 and 19.0 minutes contact time for FC cucumber, carrot and tomato, respectively.

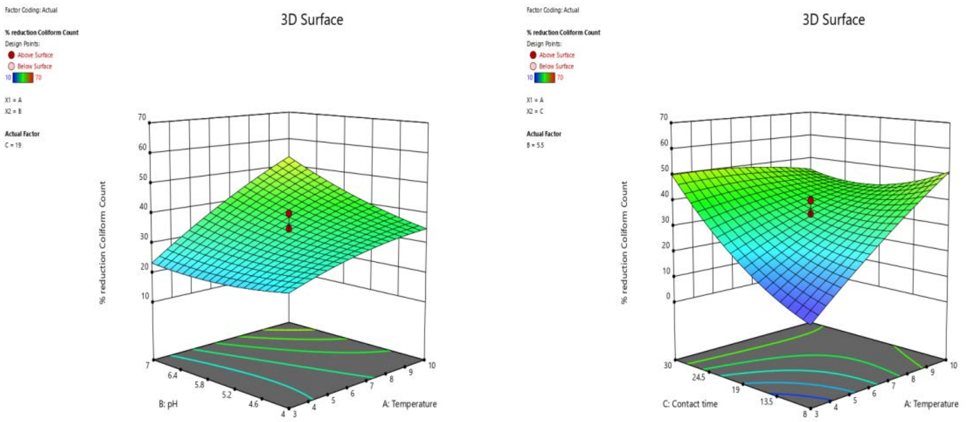
Fig 1 (a) (b) shows the 3D significant interaction between temperature, pH and contact time for % reduction of TPC in FC carrot. Fig 2 (a) (b) shows the 3D significant interaction between temperature, pH and contact time for % reduction of coliforms count in FC carrot. Fig 3 (a) shows the 3D significant interaction between temperature, pH and contact time for % reduction of Y&M in FC tomato. Fig 4 (a) shows the 3D significant interaction between temperature, pH and contact time for % reduction of coliforms count in fresh cut tomato.



(b)

Fig1. 3D surface interaction graphs between variables for FC carrot: (a) Interaction of contact time and temperature for % TPC reduction; (b) Interaction of contact time and pH for % TPC reduction

3D surface interaction graphs showed that there is a positive interaction between temperature-contact time and pH-contact time to reduce the microbial count over FCV. Figure 1 (a) Figure 2 (a), (b) and Figure 4 (a) depicts that within the temperature range of 6- 10°C significant higher percent microbial reduction was observed. In case of FC cucumber and tomato, a low temperature is found to be more preferred for the pretreatment probably due to their high water content. So to maintain the physicochemical parameters (especially firmness and phenols) of the FC cucumber and tomato, low temperature was found to be optimum. Survival of microorganisms on the surface of vegetables depends upon the temperature [17,18], chlorine based disinfectants are more effective at low temperature because of its maximum solubility at a temperature range of 4 to 10°C [19]. Heat and low temperature are both used in the pretreatment of fruits and vegetables in fresh cut processing in order to increase shelf life [17,19].



(a) **(b)**

Fig 2. 3D surface interaction graphs between variables for FC carrot: (a) Interaction of pH and temperature for % coliforms count reduction; (b) Interaction of contact time and temperature for % coliforms count reduction

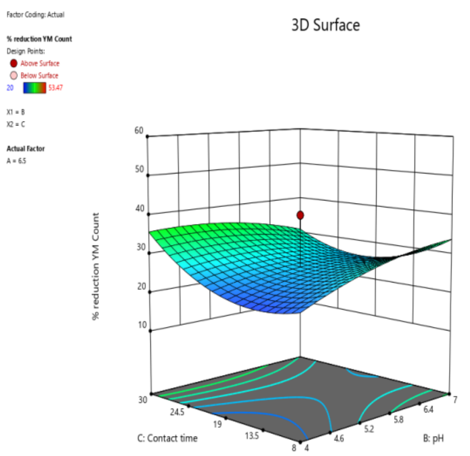


Fig3. 3D surface interaction of contact time and pH for % Y&M reduction for fresh cut carrot

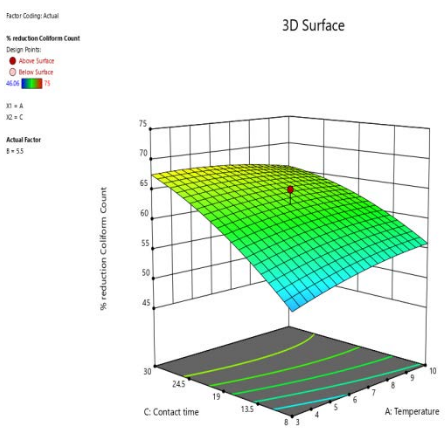


Fig4. 3D surface interaction graphs between contact time and temperature for % coliforms count reduction variables for FC tomato

A variety of antimicrobial wash solutions have been used to reduce populations of microorganisms on fresh produce. The effectiveness of washing or antimicrobial dipping depends on a number of factors including: Type of treatment, Type number, physiological growth phase, and stress resistance of the target microorganism(s), Product type, Antimicrobial concentration, pH of the solution, Contact time, Temperature of washing water, and General sanitation of plant and equipment [19, 20]. pH also plays an important role in determining the bactericidal efficacy of NaOCl as pH effects the release of OCl^- ions [21]. Figure 1 (b), Figure 2 (a) and Figure 3 (a) showed the effect of pH to reduce the microbial count over FCV, it represents that maximum % microbial reduction was observed in the range of pH 6-7 because at low pH range of less than 6, chlorine gas may be formed. At higher pH levels (more than 7.5) the activity of NaOCl decreases because of more inactive hypochlorite (OCl^-) ions formation. Measurement and recording of the chlorine level and the pH of wash water is a critical element of any quality assurance programme. Due to its low pH and available chlorine concentration, the product also carries no hazard and thus requires no specialised PPE or disposal consideration. It is non corrosive and current testing shows good compatibility with clean room materials. Many operations inject chlorine as a disinfectant along with acid in order to maintain a pH range of 4.5 -5.5 and assure the effectiveness of chlorine during washing operations [21, 22]. Contact time of 8- 19 minutes is required for the disinfection pretreatment of FCV. It has been seen that after contact time of 25-30 minutes the percent microbial reduction does not increase. The temperature, contact time, pH and chlorine concentration should be monitored for their effectiveness during washing [23, 24].

Numerical optimization of the RSM data was carried out keeping the fact that % microbial reduction should be maximum and other six physicochemical parameters namely TSS, total sugars, total phenols, titratable acidity, pH and firmness in range. Results indicated that the pretreatment disinfectant solution kept at temperature 6.5°C, pH 6.0 and contact time 23.0 minutes for FC cucumber; temperature 10°C, pH 7.0 and contact time 8.0 minutes for FC carrot and at temperature 6.5°C, pH 7.0 and contact time 30.0 minutes for FC tomato showing the desirability of 94 %, 94 % and 92 % for cucumber, carrot and tomato, respectively.

Optimized results were validated for 3 kg vegetables (viz. cucumber, carrot and tomato) that result TSS 4.70, 7.00, 5.18 (°Brix); firmness 13.35, 120.15,19.49 (Newton); pH 5.78, 5.31, 4.09; total sugars 3.61, 6.19, 4.82 (g/100g); titratable acidity 0.15, 0.12, 0.41(%); total phenols 7.40, 8.01, 11.06 (mg/100g); TPC 56.31, 62.91, 60.21 (% reduction) Y&M 68.20, 58.80, 57.71 (% reduction) coliforms count 70.98, 69.20,74.80 (% reduction) in FC cucumber, carrot and tomato, respectively showed that the results are in accordance to the desirability range of optimized results. Shelf life analysis of different FCV (viz. cucumber, carrot and tomato) was studied by treating them under optimized conditions of disinfectant concentration, pH, and temperature and contact time followed by storage under refrigeration conditions (5-7°C). Maintaining low temperature during transport and storage of fresh cut produce is a very critical aspect of produce quality due to the impact of low temperature on metabolic reactions [19, 20, 21]. The study revealed that all the physicochemical parameters were non-significantly differ over the period of 9-14 days for each FCV. However, total phenols were found decrease significantly during the storage of pretreated FC cucumber, carrot and tomato (Table 6, 8 and 10). Microbial analysis of untreated sample has the count of TPC, Y&M and coliforms count in order of 3.54, 2.20 and 2.27 log (cfu/g) in cucumber; 3.37, 1.99 and 2.14 log (cfu/g) in carrot; 3.68, 2.05 and 2.39 log (cfu/g) in tomato, respectively (Table 7, 9 and 11).

Table 6. Shelf life analysis of pretreated fresh cut cucumber under refrigeration conditions

Parameter	Control	Treated 0 day	Storage under refrigeration at 5-7°C (days) 3 day	6 day	9 day	12 day	CD (5%)
TSS (◦B)	5.0±0.5	4.5±0.3	4.7±0.2	5.0±0.8	5.2±0.4	5.4±0.2	NS
Total Sugars (g/100g)	4.12±0.31	3.85±0.25	3.94±0.28	4.10±0.27	4.19±0.21	4.26±0.26	NS
Total Phenols (mg/100g)	8.6±2.5	7.2±2.5	7.6±2.7	7.9±1.9	8.1±1.9	8.2±2.0	NS
Titratable acidity (%)	0.17±0.05	0.14±0.02	0.12±0.05	0.10±0.06	0.09±0.01	0.09±0.03	NS
pH	5.94±0.2	5.78±0.1	5.81±0.3	5.94±0.2	6.03±0.4	6.10±0.2	NS
Firmness (lb)	3.1±0.4	2.8±0.3	2.6±0.1	2.5±0.4	2.4±0.5	2.3±0.4	NS
Sensory score (out of 9)*	8	8	7	7	6	5	1.23

NS-Non significant

Table 7. Microbiological analysis of untreated and pretreated fresh cut cucumber during shelf life studies

Parameter	Storage under refrigeration at 5-7°C (days) Control 0 day	3 day	6 day	9 day	CD (5%)	Pretreated 0 day	3 day	6 day	9 day	12 day	CD (5%)
TPC (log cfu/g)	3.54	4.30	4.86	5.12	0.196	3.07	3.55	3.76	3.91	4.04	0.253
Yeast &Mould count (log cfu/g)	2.20	2.48	2.81	3.28	0.112	1.68	1.83	2.24	2.95	3.00	0.108
Coliforms count (log cfu/g)	2.27	2.42	2.76	3.18	0.752	1.86	1.98	2.25	2.99	3.04	0.956

Table 8. Shelf life analysis of pretreated fresh cut carrot under refrigeration conditions

Parameter	Control	Treated 0 day	Storage under refrigeration at 5-7°C (days) 3 day	6 day	9 day	12 day	14 day	CD (5%)
TSS (◦brix)	7.3±0.5	7.4±0.4	7.6±0.3	7.8±0.2	8.0±0.2	8.2±0.3	8.4±0.4	NS
Total Sugars (g/100g)	6.20±0.35	6.31±0.32	6.40±0.35	6.56±0.29	6.78±0.31	6.89±0.34	6.96±0.34	NS
Total Phenols (mg/100g)	8.60±1.9	8.16±2	8.30±2.4	8.49±2.4	8.61±2.6	8.88±2.1	8.94±2.5	NS
Titratable acidity (%)	0.12±0.03	0.16±0.04	0.14±0.01	0.12±0.04	0.09±0.02	0.08±0.05	0.07±0.05	NS
pH	5.7±0.4	5.5±0.5	5.7±0.3	5.8±0.1	5.9±0.4	6.2±0.3	6.3±0.2	NS
Firmness (lb)	27.1±0.5	27.0±0.4	28.8±0.6	28.5±0.2	28.4±0.4	28.2±0.4	28.1±0.3	NS
Sensory score (out of 9)*	8	8	7	7	7	6	5	1.23

NS-Non significant

Table 9. Microbiological analysis of untreated and pretreated fresh cut carrot during shelf life studies

Parameter	Storage under refrigeration at 5-7°C (days)												
	Control						Pretreated						
	0 day	3 day	6 day	9 day	12 day	CD (5%)	0 day	3 day	6 day	9 day	12day	14 day	CD (5%)
TPC (log cfu/g)	3.89	4.12	4.56	4.86	5.18	0.210	3.37	3.34	3.68	3.85	3.97	4.11	0.449
Yeast &Mould count (log cfu/g)	1.99	2.23	2.56	2.76	3.09	0.156	1.38	1.76	1.83	2.35	2.95	3.00	0.125
Coliforms count (log cfu/g)	2.14	2.36	2.58	2.82	3.16	0.144	1.83	1.89	2.35	2.59	2.98	3.04	0.80

Table 10. Shelf life analysis of pretreated fresh cut tomato under refrigeration conditions

Parameter	Control	Treated 0 day	Storage under refrigeration at 5-7°C (days) 3 day	6 day	9 day	12 day	CD (5%)
TSS (◦brix)	5.5±0.5	5.7±0.7	5.8±0.3	5.8±0.2	5.9±0.1	6.2±0.4	NS
Total Sugars (g/100g)	4.12±0.39	5.20±0.36	5.36±0.29	5.42±0.2	5.54±0.34	5.61±0.3	NS
Total Phenols (mg/100g)	13.16±2.2	12.05±2.4	12.15±1.9	12.18±2.4	12.26±2.0	12.29±2.1	NS
Titrable acidity (%)	0.80±0.02	0.43±0.03	0.39±0.04	0.37±0.01	0.36±0.02	0.31±0.01	NS
pH	4.20±0.4	4.16±0.3	4.21±0.2	4.25±0.2	4.29±0.1	4.32±0.1	NS
Firmness (lb)	4.5±0.3	4.8±0.5	4.7±0.4	4.7±0.2	4.5±0.5	4.4±0.2	NS
Sensory score (out of 9)*	8	8	7	7	6	5	1.23

Table 11. Microbiological analysis of untreated and pretreated fresh cut tomato during shelf life studies

Parameter	Storage under refrigeration at 5-7°C (days)										
	Control					Pretreated					
	0 day	3 day	6 day	9 day	CD (5%)	0 day	3day	6 day	9 day	12 day	CD (5%)
TPC (log cfu/g)	3.68	4.12	4.96	5.28	0.128	3.37	3.71	4.03	4.17	4.29	0.166
Yeast &Mould count (log cfu/g)	2.05	2.39	2.92	3.08	0.110	1.68	1.89	2.25	2.99	3.04	0.100
Coliforms count (log cfu/g)	2.39	2.48	2.99	3.18	0.103	1.58	2.18	2.36	2.89	3.07	0.136

Disinfectant treatment under optimized conditions of FC cucumber led to decrease in the microbial count form 3.54 to 3.07 log (cfu/g) TPC, 2.20 to 1.68 log (cfu/g) Y&M and 2.27 to 1.86 log (cfu/g) coliforms count. Disinfection pretreatment decreased the microbial count under acceptable limits in accordance with USFDA and FSSAI guidelines. Untreated FC cucumber reported increase in microbial count during storage and exceed the acceptable limits after 6th day. However, treated FC cucumber's microbial count remained under the acceptable limits till 9th day. Physicochemical parameters showed non-significant difference in values of TSS 5-5.4 (°B), total sugars 4.12- 4.26 (g/100g), total phenols 8.6- 8.2 (mg/100g), titratable acidity 0.17- 0.09 (%), pH 5.94-6.10, firmness 3.1- 2.3 (lb) in pretreated FC cucumber. Taking into consideration microbial and physicochemical parameters, it was found that upto 9th day samples of pretreated FC cucumber were fit for consumption. Sensory analysis of pretreated and untreated samples was carried out by semi trained panel of ten judges. Sensory score of both pretreated and untreated FC cucumber at 0 day got 9 points score on the hedonic scale that showed treatment does not affect the sensory characteristics of the sample. At 9th day of storage treated FC cucumber scored 6 points that indicates the samples are in liking category of the consumers (Fig. 5, 6 and 7).

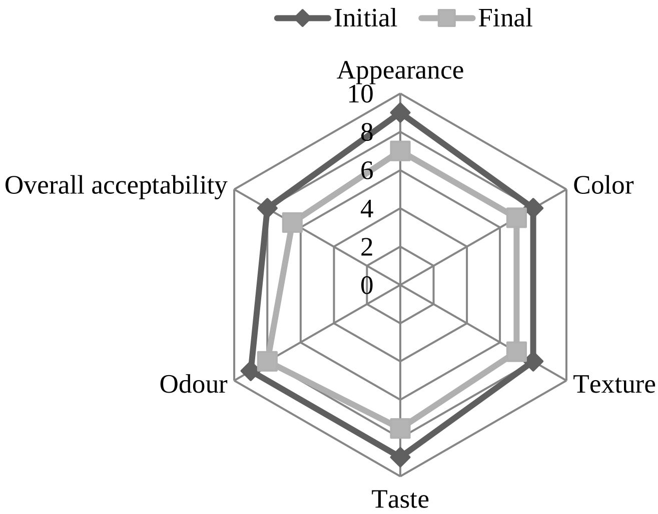


Fig 5. Radar Graph depicting the evaluation of sensory attributes of pretreated fresh cut cucumber

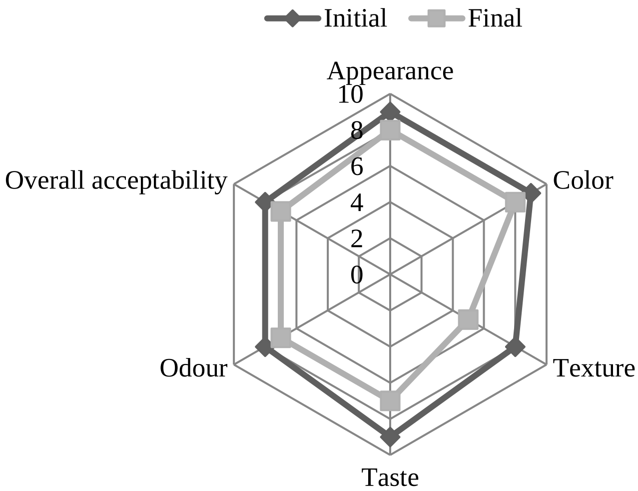


Fig 6. Graph depicting the evaluation of sensory attributes of pretreated fresh cut carrot

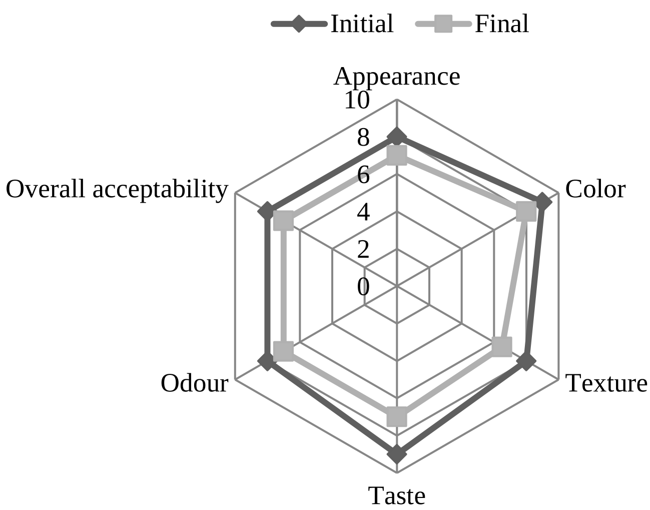


Fig 7. Graph depicting the evaluation of sensory attributes of pretreated fresh cut tomato

4. Conclusions

The present study revealed that disinfectant pretreatment of NaOCl @ 100 ppm at pH (6.0, 7.0, 8.0), temperature (6.5, 10.0, 6.5°C) for (23.0, 8.0, 30.0 minutes) of contact time under refrigeration conditions (5-7°C) with disinfectant reduce the microflora over FC cucumber, carrot and tomato, respectively. Pretreatment of FCV with NaOCl results in reduction of microflora to acceptable levels that resulted in an increase in the shelf life of the final product retaining its sensory attributes. Pretreatment with disinfection showed an increase in the shelf life of FCV to 9 days as compared to 6 days of untreated FCV cucumber, 12 days from 9 days of FCV carrot and 9 days from 5 days in case of FCV tomato. Thus, the standardized pretreatment given to FCV led to increase in the shelf life of the product that can help to keep the product for a longer period of time and to develop the fresh cut market in the North Indian Conditions.

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