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Standardization of UV-C treatment, Ozonization and chlorination for reducing microbial growth in carrot under laboratory conditions

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Abstract
The *in-vitro* lab experiment was conducted at the Department of Food Process Engineering, Agricultural Engineering College & Research Institute, Tamil Nadu Agricultural University, Coimbatore to find out the effect of physical (UV-C treatment) and chemical treatments (Ozonization and Chlorination) on inactivation of microbial growth in freshly harvested new carrot variety korda collected from farmer Mr. Saji in Hanumanthapuram, Ooty, Tamil Nadu. From the results it was observed that by UV-C irradiation there was one log reduction in microbial growth even at 0.050 kg/m^2. Hence, the chosen UV-C dosages were not sufficient for inactivation of total microbial load and were not taken for further study. In case of chemical treatment ozonation and chlorination, treatment with different concentration of chlorine *viz.*, 50, 100, 150 and 200 ppm, and Ozonization for 10, 20 and 30 min. It were concluded that the carrot washed in 200 ppm chlorine concentrated water and in 30 min treated ozone water showed 3 log reductions in bacterial and 2 log reduction in fungal population.

Keywords: Carrot, Korda, UV-C, Chlorine, ozone, bacteria and fungi

1. Introduction
UV-C treatment is a non-thermal, non-toxic disinfection method which removes certain organic contaminants when performed at a low temperature. UV-C treatment is mainly used as a surface sterilizer. The mechanism of microbial inactivation by UV-C radiation when the radiation is applied at 253.7 nm (highest germicidal effect) over the produce, it gets largely absorbed by the DNA of microorganism. This prevents both DNA transcription and translation through adjacent pyrimidine bases bonding each other on the same strand of DNA. The treatment method is found to maintain the nutritional characteristics, colour and aroma of the product without changing its chemical composition.

Gogo *et al*. (2017) [10] conducted an experiment to extend the shelf life of African indigenous leafy vegetables using UV-C treatment. Solanum scabrum and Amaranthus cruentus were treated with a UV-C dosage of either 1.7 kJ m-2 or 3.4 kJ m-2 and stored for a period of 14 days. The results showed an increase in microbial reduction and suggested to be an essential step in reducing food losses in developing countries. Ojaghian *et al*. (2017) [21] studied the efficacy of UV-C radiation in inducing systematic acquired resistance against storage carrot rot by Sclerotinia sclerotorium. In the study four isolates of Sclerotorium including 44-2, 7-3, SSCL1 and SZ257 were inoculated into the carrot roots and exposed to UV-C light. Seven germicidal tubes emitting UV-C c with a peak of 254 nm were kept at 40 cm above the roots. UV-C was exposed evenly to the roots by rotating them at an interval of 1 min for a period of 5 min. The treatments were given in three different form including (1) WT: the whole surface of carrots with a dosage of 0.88 kJ/m-2, (2) the crown and (3) the lower half of the carrots by covering the rest of surfaces with aluminum foil. The results have showed an increase in the systematic acquired resistance in treated carrots and also a marked decrease in microbes when treated for 5 min with UV-C radiation.

Stevens *et al*. (1997) [35] used ultraviolet (UV-C) light for controlling yeast in postharvest storage of fruits and vegetables. UV-C was combined with a biocontrol agent, Debaryomyces hansenii, or postharvest fungicides to test their ability to reduce the incidences of brown rot caused by Monilinia fructicola of peach, green mold (Penicillium digitatum) of tangerine, and Rhizopus soft rot (Rhizopus stolonifer) of tomato and sweet-potato that resulted from both field infections and artificial inoculations. It was found that biocontrol agents were more effective in controlling the postharvest spoilage than when UV-C was used alone. But when both the treatments were combined, the produce was able to store for more days without spoilage.

Liew and Prange (1994) [18] studied the effect of ozone and storage temperature on postharvest diseases and physiology of carrots. In this study, pathogens like Botrytis cinerea and Sclerotinia sclerotiorum were investigated. The carrot were exposed to an ozone concentration of 0, 7.5, 15, 30 and 60 µl/litre in a flow rate of 0.5 litre/min, and was stored at 2, 8 and 16°C. It was found that, at a concentration of 10 to 22 µl/litre at 2°C, there was an inhibitory effect on Botrytis cinerea and Sclerotinia sclerotiorum.

Ozone can be applied at almost any step in the fruit supply chain, from the orchard to the display case at local grocery stores. The gas can be easily produced commercially and used in either gaseous or aqueous states. In addition to improving fruit safety and extending product shelf-life, the treatment ozone may also be selected to enhance the nutritional quality of food or remove residues of pesticide applied to fruits in the field. Ozone inactivates microorganisms by its strong biocidal property which is a combination of high oxidation potential and its ability to diffuse through biological membrane (Hunt and Mariñas, 1997) [14]. Ozone is 1.5 times stronger to chlorine and other disinfectants (Xu, 1999) [39].

Ozone is a triatomic form of oxygen characterized by a high oxidation potential that conveys bactericidal properties (KIM *et al*., 1999) [16]. The inactivation mechanism of ozone in microorganisms is through oxidation and residual ozone decomposes to non-toxic products as oxygen, making it environmental friendly antimicrobial agent in food industry. It is a widely used disinfectant in drinking water and waste water treatment (KIM *et al*., 1999) [16].

Ozone solubility in water is 13 times higher to oxygen at 0-30o C and it progresses in cool water (Rice, 1986) [29]. Increased ozone concentration causes saturation leading to ineffective treatment which requires longer time of exposure to achieve the same log- reduction values (Patil *et al*., 2009) [27]. The solubility ratio for ozone increases as the temperature of water deceases (Langlais *et al*., 1991) [17]. Ozone can be produced from photochemical (UV radiation), electric discharge methods and also by chemical, thermal chemo-nuclear and electrolytic methods.

Hassenberg *et al*. (2008) [12] studied the effect of ozonized wash water for inhibition of pectobacterium carotovorum on carrots and in the physiological behavior of produce. In this study, carrots were washed with ozonized water in a concentration of 4 ppm for 2 min. The results showed that there were changes on physiological activity without producing any hazardous residues. The treatment also reduced the potential infection with human pathogen like Bacterial soft rot. The result showed a complete reduction of suspended bacteria by ozonizing the washing water.

Hildebrand *et al*. (2008) [13] studied the effects of a continuous low ozone exposure on decay and quality of stored carrots caused by Sclerotinia sclerotorium and Botrytis cinerea. The carrots were inoculated with microbes and then treated with a continuous ozone concentration of 50 ±10 nLL-1 and stored for a period of 6 months at 0.50C and >95% relative humidity. Ozone treatment was found to reduce the lesions incited by both Sclerotinia sclerotorium and Botrytis cinerea during the first month following inoculation. Ozone was able to kill or restrict the surface mycelium which resulted in smaller lesions compared to the untreated carrots.

Kim *et al*. (1999) [16] studied the usage of ozone in inactivating microorganisms on lettuce. Ozone at 1.3 ppm was bubbled for 3 min in a mixture of shredded lettuce and water. Counts of mesophilic and psychrotrophic bacteria decreased to 1.4 and 1.8 log cfu/g respectively. When ozone was introduced in bubbling gaseous form into water with a high-speed stir, it produced the most effective form of ozonation for reducing the microbial growth in lettuce.

Chlorinated water treatment for vegetables is one of the most widely practiced sanitizing techniques. It is commonly used in food industry and for drinking water disinfection. Chlorinated water kill pathogens and stop their further spreading from infecte decaying fruit or vegetables to healthy products (Palou *et al*., 2002) [24]. Generally for commercial disinfection purposes, chlorine gas or aqueous solutions of sodium hypochlorite (NaOCl) solutions, or calcium hypochlorite (CaCl2O2) are generally used (Suslow, 1997) [38]. Edible waxes in fruits and vegetables have been reported to provide protection to the product, hindering the effectiveness of chlorine (Saranraj *et al*., 2012) [32]. Produce sterilized using chlorine under recommended dosage do not pose any health hazard to consumers, (Ölmez *et al*., 2009) [22]

NaOCl (50–200 mg/L), with a contact time of 1-2 min is often used to reduce microbial populations in the water used during washing operations (Beuchat and Ryu, 1997) [3]. Its antimicrobial effect is due to the attack of amino groups in nucleic acids, (Denyer and Stewart, 1998) [8]. Since chlorine reacts with organic matter, its use may lead to the formation of some potentially hazardous by-products like tri-halo-methanes and use of NaOCl were found effective (Sapers, 2001) [31] in reduction of b acterial concentration around 1-2 log units (90-99%) in both water and produce.

Ong *et al*. (2014) [23] studied the effect of different concentrations of ozone on physiological changes associated to gas exchange, fruit ripening, fruit surface quality and defense related enzymes levels in papaya fruit in ambient storage. In the study, papaya fruit were exposed continuously to ozone at concentration levels of 0, 1.5, 2.5, 3.5 and5.0 µLL-1 for a period of 96 h prior to ambient storage at 25 ± 3oC and 70 ± 5% RH. The results showed a positive effect when fruits were treated with ozone at concentrations lower than 5 ppm having lower respiration rate and delayed ripening compared to the control. Enzyme activities were found higher in papaya when ozone was >5 ppm than in untreated fruit throughout the storage period.

Sarig *et al*. (1996) [33] studied the ability of ozone in controlling the postharvest decay caused by Rhizopus stolonifera in table grapes. The study showed that ozone concentrations depend upon the quantity of organic matter present, the amount of grape berries used and the microflora on the surface of produce used. The effectiveness of ozone can be increased by the periods of exposure. The results showed a rapid decrease in the number of colony forming units (CFU) of fungi, yeasts and bacteria naturally present on the berry surface when exposed for 20 min to ozone. Ozone treatments significantly reduced the berry decay caused by fungi following cold storage and extended the shelf-life. The study suggests the usage of ozone treatments as a possible substitute for SO2 fumigation for the control of postharvest fungal decay among fruits and vegetables.

The major losses in carrots due to fungal attack like Sclerotinia rot, Botrytis rot, Erwinia Bacterial soft rot, Geotrichum Sour rot reducing the shelf life of carrots. Spoilage microorganisms reduces the shelf life of carrots by degrading the important bio-components in carrots reducing its market value. So far, there are no treatment methods followed in Nilgiris to reduce the microbial spoilage in carrots. The three types of treatments that can be used were as follows; i) physical treatment - UV-C radiation ii) Chemical treatment: Chlorine and Ozone. Use of this potent strong

oxidizer requires careful consideration and process optimization so that the goal of the treatment is accomplished. Therefore in this project, a study on different techniques like UV-C, chlorination and Ozonized water treatments were undertaken with the following objectives:

1. To standardize UV-C treatments at different concentration levels that reduces the microbial load in carrots *viz*., 0.025, 0.038 and 0.050 kJ/m2.
2. To standardize chlorination at different concentration levels to reduce the microbial load in carrots *viz*., 50, 100,150 and 200 ppm.
3. Ozonation of the surface disinfection of the fruit and the vegetable samples at various time intervals *viz*., 10, 20 and 30 min.

2. Materials and Methods

2.1 Collection of raw materials for the study

Freshly harvested carrots (New Korda variety) were collected from a carrot machinery plant run by a farmer Mr.Saji in Hanumapuram, Ooty, Tamilnadu, India. The collected samples were stored under cold storage until used for analysis.

2.2 Design layout

The experiments on physical, chemical and biological treatments were conducted by varying the concentration and time of exposure to different treatments. A two factor completely randomized block design (CRD) was followed to determine the effects of treatments in quality of postharvest carrots at varying concentration and time of exposure. The design layout of the experiment with four independent variables and their levels is as follows:

Independent variables	Variable levels
1. Physical treatment a. Ultraviolet- C (253 nm)	2. Levels ▪ Distance- 0.4 cm ▪ Time exposure–30, 45 and 60 s
2. Chemical treatment	**2 levels**
1. Chlorination 2. Ozonization	▪ Time exposure-30, 45 and 60 s ▪ Dosage- 50, 100, 150 and 200ppm 3. Time exposure-10, 20 and 30 min
4. Storage condition A. Ambient 5. Temperature – 22 ºC to 32 ºC Mean relative humidiy - 61.1%	
Dependent variables a. Microbial load b. Total carotene content c. Percent weight loss d. Color e. Hardness	

2.3 Standardization of postharvest treatments for controlling postharvest spoilage under laboratory conditions

2.3.1 Physical treatment

2.3.1.1 Experimental setup and UV-C surface application

A chamber of size 1.2 m × 0.6 m × 0.6 m with a UV-C lamp (TUV 30W/G30 TS, Holland) of 253 nm fitted to it was used to perform the surface sterilization of carrots. The total surface area of the chamber is 0.36 m2. The UV-C dosage was achieved with a mercury vapor discharge lamp of length 0.9 m mounted on the top of the chamber. The carrots were surface sterilized by placing them at a distance of 0.4 m from the lamp with varying time of exposure namely 30, 45, 60 s. In order to expose UV-C radiation on all sides, carrots were rotated for every half a period of total exposure time.

Plate 1: UV-C chamber used for sterilization of carrots

Plate 2: Hand held digital lux-meter

The total dosage imposed over carrots was calculated from the product of exposure time and irradiance as measured by a portable hand held digital lux-meter (Lutron LX-101 A, Taiwan). Based on this, three different dosage were applied, i.e. 0.025 kJ/m2, 0.038 kJ/ m2, 0.050 kJ/ m2 for a period of 30, 45 and 60 s respectively. The dosage was chosen based on the preliminary experiments and commonly applied UV-C dosages on vegetables (Chairat *et al*., 2013) [4]. The untreated carrots served as control. After UV-C application, carrots were collected and transferred to sterile bags with 0.1% peptone solution and processed for microbial counts.

2.3.2 Chemical treatments

2.3.2.1 Chlorinated water treatment

Sodium hypochlorite, ultra-germicidal bleach was mixed in 1000 ml sterile water to have a final concentration of 50, 100, 150 and 200 ppm. Carrots were washed in chlorinated solution for 30, 45 and 60 s. The unwashed carrots remained as control. After each test, carrots were collected and transferred to sterile bags with 0.1% peptone solution and processed for microbial counts.

2.3.2.2 Ozonized water treatment

Ozone was generated in an ozone generator with air cooled corona discharge (Model- E2G, Faraday Ozone, India)

producing an ozone output of 2 g/h and flow rate of 12-14 litres per minute. The ozone generator was connected to a diffuser through a Teflon tube. This diffuser was inserted in sterile water contained in a cylindrical tank made of SS 304 containing 100 l sterile water. A concentration of 0.3 ppm of ozone was made to diffuse in water for 10, 20 and 30 min. The ozone concentration was checked using an ozone concentration water test kit, showing different color variation according to the concentration produced. The different ozone treated water was used to treat carrots and untreated carrots were kept as control. The initial temperature and pH of the water used for ozonization were observed

Plate 3: Ozone generator

Plate 4: Micro-diffuser

Plate 5: Ozone water

2.4 Enumeration of microbial population in carrots after treatments

The population density of bacteria, fungi, actinomycetes and viability of probiotic culture were enumerated by serial dilution plate technique (Parkinson *et al*., 1971) [26].

2.4.1 Serial dilution of carrot sample

A known quantity of whole carrot sample was transferred to peptone water to get 10-1 dilution. After thoroughly mixing it, one ml of this dilution was transferred to 9 ml peptone water to get 10-2 dilution. Likewise, sample was diluted serially with 9 ml peptone water till appropriate dilution was obtained.

2.4.2 Heterotrophic bacteria

The heterotrophic bacterial population was enumerated by plating one ml of 10-3 dilution in sterile petri plates using nutrient glucose agar medium (Allen, 1958) [2]. The bacterial colonies appearing on the plates after 48 h of incubation at 300C were counted and expressed per g of carrot used.

2.4.3 Fungi

The fungal population were enumerated by plating one ml 10-5 dilution in sterile petri plates using Martin's Rose Bengal Agar (Martin, 1950) [19]. After 72 h of incubation at 300C, fungal colonies were counted and expressed per g of carrot used.

3. Result and Discussion

3.1 Physical Treatment

3.1.1 Effect of UV-C treatment on carrots at different dosage

The carrots were exposed to UV-C radiation for a dosage level of 0.025, 0.038 and 0.050 kJ/m2. The total microbial population at different dosage level is given in Table 1. The change in bacterial and fungal population after UV-C treatment is shown in Fig. 1. The dosage applied was not effective in total elimination of bacteria and fungus in carrots. The bacterial and fungal population was reduced to less than one log cycle even when higher dosage was provided. The bacterial population in untreated sample was 4.29×106 CFU/ml while carrots treated in 0.050 kJ/m2 dosage had a population 3.42×106 CFU/ml. The fungal population in untreated sample was 1.48×104 CFU/ml that reduced gradually to 0.56×104 CFU/ml when treated at 0.050 kJ/m2 dosage. UV-C treatment had decreased the bacterial and fungal population, but not more than one log reduction in microbes was achieved. The UV-C treatment was not found significant at $p< 0.05$ and was not used for further studies.

Table 1: Effect of UV-C treatment on microbial load in carrots

Treatments	Bacterial population (× 106 CFU/ml)			Fungal population (× 104 CFU/ml)		
T0	4.29			1.48		
	(5.6330)			(4.1721)		
T1	3.99			1.29		
	(5.6010)			(4.1108)		
T2	3.77			1.16		
	(5.5766)			(4.0658)		
T3	3.42			0.5681		
	(5.5343)			(3.7544)		
	S.Ed	CD(0.05)	CV(%)	S.Ed	CD(0.05)	CV(%)
	0.2391	0.5327	5.05	0.3023	0.6735	9.13

Note: Values in parenthesis are log-transformed values
T0- Control T1- 0.025 kJ/m2 T2- 0.038 kJ/m2 T3- 0.050 kJ/m2

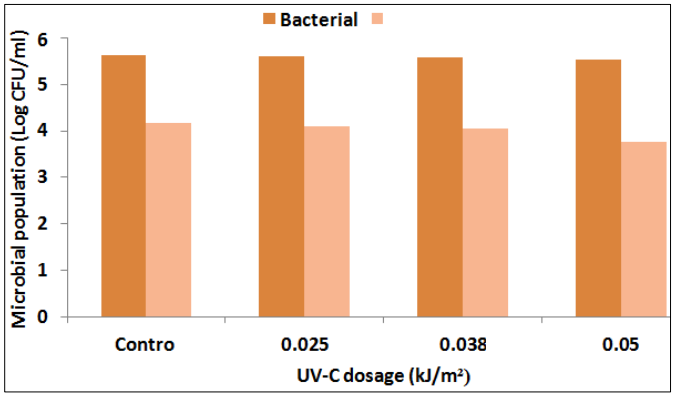

Fig 1: Effect of UV-C treatment on microbial population of carrots

Similar findings were reported by Taze *et al*. (2015) [39] in the study on the effect of UV- C irradiation to inactivate the spoilage microorganisms in orange juice. The spoilage microorganisms showed higher resistance at 1.32 kJ/m2 irradiation level. Our results are contradict with the findings of Santhirasegaram *et al*., (2015) [30] where a longer exposure of mangoes to UV-C resulted in higher reduction of microbial load during storage. Longer exposure of UV-C radiation can reduce the microbial load in vegetables and increase its shelf life (Pinheiro *et al*., 2015) [28].

3.2 Chemical Treatment

3.2.1 Effect of chlorine treatment on carrots at different dosage

Carrots washed in sodium hypochlorite treated water at different concentration had a greater effect on microbial population. The total microbial load at different concentration of chlorine treatment is given in Table 2. The population of bacterial and fungal population after the treatment is presented in Fig. 2. Carrots treated in chlorine water having a concentration of 200 ppm for 60 s had the maximum microbial reduction. The bacterial population of untreated carrots were 34.56×105 CFU/ml and when treated with 200 ppm showed a population of 0.00045×105 CFU/ml.

The fungal population of untreated carrot samples were 3.53×104 CFU/ml and when treated with 200 ppm of chlorinated water showed a reduced population to 0.0016 ×104 CFU/ml. Both the microbial population had more than one log reduction when compared to the untreated samples. Bacterial population was reduced by 3 log reduction and fungal growth reduced to 2 logs after washing in 200 ppm chlorinated water. Hence, the chlorine treatment at a concentration of 200 ppm was selected for further studies.

Table 2: Effect of chlorine treatment on microbial load in carrots

Treatments	Bacterial population (× 105 CFU/ml)			Fungal Population (×104 CFU/ml)		
T0	34.56 (6.5385)			3.53 (4.5413)		
T1	5.73 (5.7582)			1.25 (4.0922)		
T2	0.316 (4.5000)			0.74 (3.8718)		
T3	0.101 (4.0062)			0.23 (3.3718)		
T4	0.045 (3.9798)			0.0016 (2.9508)		
	S.Ed	CD(0.05)	CV(%)	S.Ed	CD(0.05)	CV(%)
	0.2391	0.5327	5.09	0.0816	0.1819	2.47

Note: Values in parenthesis are log-transformed values
T0- Control T1- 50 ppm T2- 100 ppm T3- 150 ppm T4- 200 ppm

Similar results were reported by Chung *et al*. (2011) [6] in carrots by showing a 5 logs (CFU/g) reduction in total bacterial count when treated in 200 ppm of sodium hypochlorite. The present study also supports the findings of Gómez-López *et al*., 2007 [11] that ClO2 is highly effective in preservation of carrot and vegetables washed in chlorine (Pan and Nakano, 2014) [25] had a 2 log reduction on microbial population compared to normal water. NaOCl (50–200 ppm), with a contact time of 1–2 min is often used to reduce microbial populations in the water used during washing operations (Beuchat and Ryu, 1997) [3].

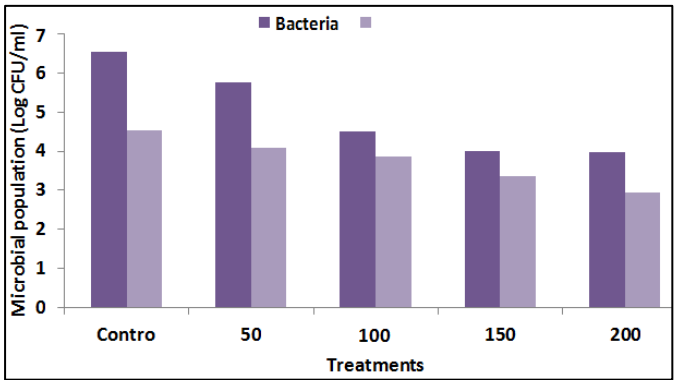

Fig 2: Effect of chlorine treatment on microbial load in carrots

3.2.2 Effect of ozone treatment on carrots at different time exposure

Carrots were treated in ozonized water having 0.3 ppm concentration for varying time period of 0, 10, 20 and 30 min. The total microbial population after ozone treatment is given in Table 3. The population of bacterial and fungal population after ozone exposure for varing time period is presented in Fig. 3. The total bacterial population in untreated carrot samples were 36.54×105 CFU/ml that gradually reduced to 0.023×105 CFU/ml on exposing to ozonized water for 30 min. The fungal population in carrots before treating with ozone were 5.36×104 CFU/ml that reduced to 0.0265×104 CFU/ml when washed in 30 min treated ozone water. The total bacterial load in carrots had a 3 log reduction while fungal population reduced to 2 logs after washing the 30 min treated ozone water.

The study suggests that increased ozone exposure to water can improve the quality of water. Carrots washed in 30 min treated water had the reduced microbial population. Hence, ozone at 0.3 ppm in 30 min treated water was selected for further analysis.

Table 3: Effect of ozone at 0.3 ppm on microbial load

Treatments	Bacterial population (× 105 CFU/ml)			Fungal Population (×104 CFU/ml)		
T0	36.54			5.36		
	(6.5627)			(4.72)		
T1	9.78			0.881		
	(5.990)			(3.94)		
T2	0.56			0.0934		
	(4.750)			(2.9703)		
T3	0.023			0.0265		
	(3.3699)			(2.4232)		
	S.Ed	CD(0.05)	CV(%)	S.Ed	CD(0.05)	CV(%)
	0.3274	0.7551	7.37	0.07 85	0.1810	1.77

Note: Values in parenthesis are log-transformed values

Similar results were reported in carrots when exposed to 0.45 ppm ozone for 48 h had increased the shelf life to 12 days by reducing the microbial load by 4 log cycle (Sharpe *et al*., 2009) [34]. Carrots by immersing in baskets containing 1 ppm ozonized water reduced the bacterial population by 3 logs

(Alegria *et al*., 2009) [1]. An increased reduction in E. coli O157: H7 in carrots was reported when washed in water having ozone concentration 5.2, 9.7 and 16.5 ppm for varying time period (Singh *et al*., 2002) [36].

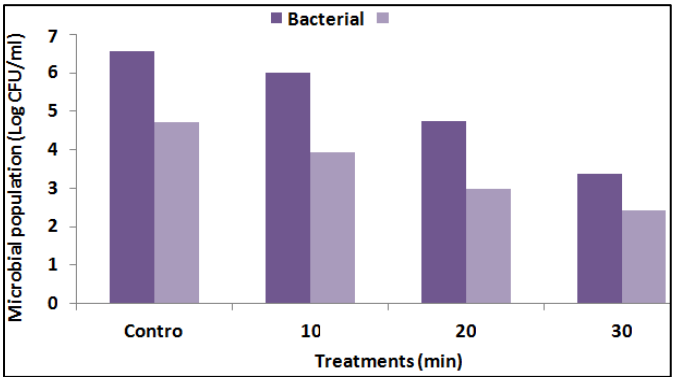

Fig 3: Effect of ozone treatment at 0.3 ppm on microbial load in carrots

4. Conclusion

Conventionally in carrot wshing industries, the harvested carrots were washed in carrot washing machineries using water that have a high chance of contamination. Carrots those washed in washing centers have a reduced shelf life for a period of 48 hr. However existing methods do not possess any techniques that can reduce the microbial load of harvested carrots. Recently the effect of biological - Probiotic microorganism in preserving the microbiological property and nutritional quality in carrots had already been found out. Hence the experiments were done on the physical, and chemical treatments to enhance the shelf life of carrots.

A comparative study between physical, and chemical treatments were carried out to minimise the microbial load in postharvest carrots in Nilgiris reveals that physical treatment of UV-C at 253 nm there was less than one log reduction in both bacterial and fungal growth even at 0.050 kJ/m2. Hence, the chosen UV-C dosages were not sufficient for inactivation of total microbial load and were not taken for further study. In case of chemical treatments; find out the effective dosage of uv-c, chlorine and ozone in increasing the shelf life of carrot. Carrots washed in 200 ppm of chlorine concentrated water and carrots washed in 30 min treated ozone water both reduced the bacterial load by 3 logs and fungal load by 2 logs. Hence, therefore chlorine at 200 ppm and ozone treatment for 30 min were used for further studies. The uses of chlorine in washing vegetables were already in practice for some vegetables, but it is not mandatory. The potential benefits of ozone in fruit processing seem very vast. Adoption of ozone is likely to continue in the future by the fresh vegetable and fruit industry; however, the rate at which this occurs will depend on how well researchers provide consistent measurements and results, and how well publications help us understand details that effects ozone processing.

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